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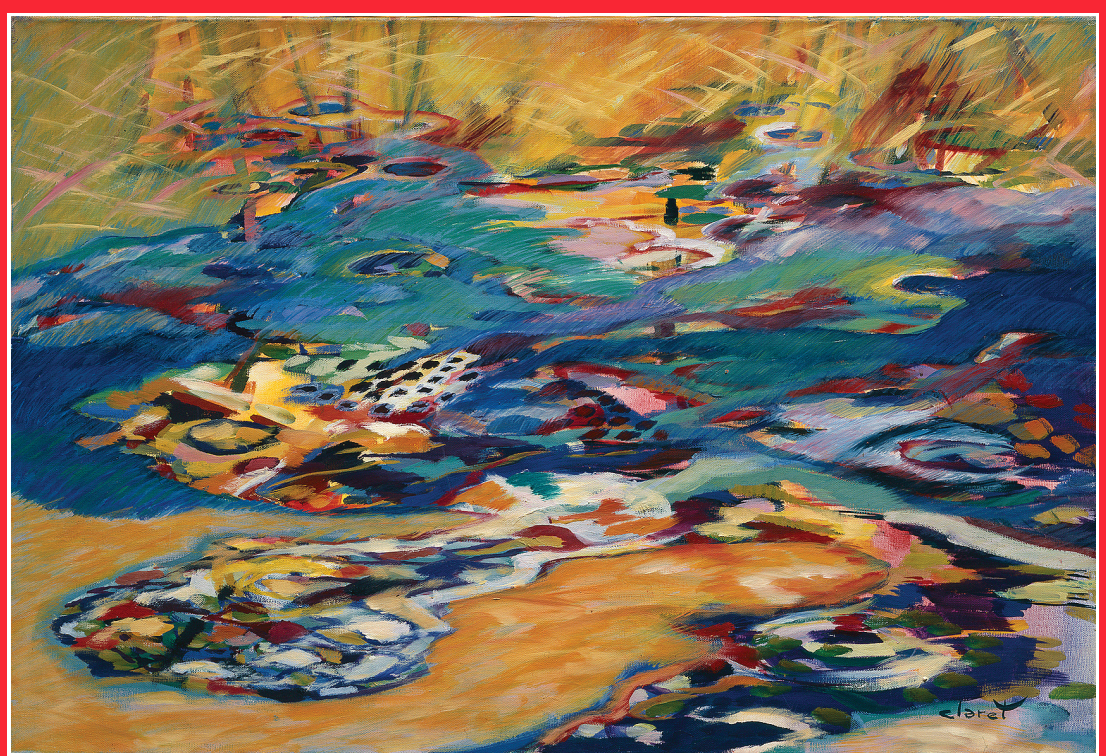

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La Tapa (Ver p. IV)
Esteros, 1989
Susana Claret

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LXI REUNIÓN ANUAL DE LA SOCIEDAD ARGENTINA DE INVESTIGACIÓN CLÍNICA (SAIC)

LXIV REUNIÓN ANUAL DE LA SOCIEDAD ARGENTINA DE INMUNOLOGÍA (SAI)

XLVIII REUNIÓN ANUAL DE LA SOCIEDAD ARGENTINA DE FARMACOLOGÍA EXPERIMENTAL (SAFE)

VII REUNIÓN ANUAL DE LA SOCIEDAD ARGENTINA DE NANOMEDICINA (NANOMEDAR)

V CONGRESO NACIONAL DE LA ASOCIACIÓN ARGENTINA DE CIENCIA Y TECNOLOGÍA DE ANIMALES DE LABORATORIO (AACYTAL)

15-19 de noviembre de 2016
Hotel 13 de Julio – Mar del Plata

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LXI ANNUAL MEETING
ARGENTINE SOCIETY FOR CLINICAL INVESTIGATION
(SAIC)

LXIV ANNUAL MEETING
ARGENTINE SOCIETY OF IMMUNOLOGY
(SAI)

XLVIII ANNUAL MEETING
ARGENTINE SOCIETY OF EXPERIMENTAL PHARMACOLOGY
(SAFE)

VII ANNUAL MEETING
ARGENTINE SOCIETY OF NANOMEDICINE
(NANOMEDAR)

V NATIONAL CONGRESS
ARGENTINE ASSOCIATION FOR SCIENCE AND TECHNOLOGY OF LABORATORY ANIMALS
(AACYTAL)

November 15-19, 2016
13 de Julio Hotel – Mar del Plata

- - - -

LA TAPA

Susana Claret. **Esteros**, 1989

Óleo sobre tela. 80 × 120 cm. Cortesía de la Comisión Nacional de Energía Atómica, Predio TANDAR, Centro Atómico Constituyentes. Comisión Organizadora de la Exposición Permanente: Dr. A.J.G.Maroto, Dr. H. Ceva.

Susana Claret nació en Buenos Aires. Profesora Superior de Pintura. Estudió con Batlle Planas y M. Dávila. Realizó numerosas exposiciones individuales (Wildenstein, Art and Arch. y Erko, en Ámsterdam, entre otras) y muestras colectivas. Obtuvo numerosas menciones y premios, entre ellos: Gran Premio Museo de Bellas Artes de Luján, 1ra Mención LXXI Salón Nacional, Gran Premio Salón de Otoño, etc. Poseen obras suyas los museos de Entre Ríos, Quilmes, Luján, Bernal, Uruguay, Río Negro y colecciones particulares en el país y en el exterior[1].

[1]Comisión Nacional de Energía Atómica. Artistas Plásticos con la CIENCIA, 102 Centro Atómico Constituyentes, Predio TANDAR, Buenos Aires, 1999; p 22. En: *www.medicinabuenosaires.com*, *link: http://www2.cnea.gov.ar/xxi/artistas/artistasplasticos.htm*

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MENSAJE DE BIENVENIDA SAIC-SAI-SAFE

MEDICINA (Buenos Aires) 2016; 76 (Supl. I): 1

Estimados colegas y amigos,

Nos complace darles la bienvenida a la Reunión Conjunta 2016 entre la **Sociedad Argentina de Investigación Clínica (SAIC), la Sociedad Argentina de Inmunología (SAI) y la Sociedad Argentina de Farmacología Experimental (SAFE)** que, además, cuenta con la participación de la **Asociación Argentina de Nanomedicinas (NANOMEDar)** y la **Asociación Argentina de Ciencia y Tecnología de Animales de Laboratorio (AACYTAL)**. Como es tradicional, el programa científico incluye Conferencias y Simposios con participantes nacionales e internacionales de reconocido prestigio. Una vez más, becarios e investigadores especialmente seleccionados tendrán la oportunidad de presentar sus trabajos en forma oral en diversos simposios y/o compitiendo por premios en distintas disciplinas y, como todos los años, se llevarán a cabo sesiones de posters en todas las especialidades abarcadas por las sociedades científicas participantes. Es nuestro propósito para esta reunión favorecer un fluído y productivo intercambio entre todos los asistentes, facilitando la interacción entre participantes de distintas nacionalidades, edades y especialidades. Hemos logrado, en esta oportunidad, convocar renombrados científicos argentinos y extranjeros del campo de la Biomedicina, así como jóvenes investigadores, becarios, médicos residentes y estudiantes de grado no sólo de nuestro país sino también de otros países de Latinoamérica.

Las sociedades convocantes nos proponemos incentivar el análisis y discusión de nuevas opciones de diagnóstico y tratamiento basados en los más recientes descubrimientos de los mecanismos que subyacen al inicio y avance de distintas patologías. Particularmente se llevarán a cabo sesiones dedicadas discutir estos hallazgos y sus posibilidades en el diagnóstico y tratamiento de enfermedades oncológicas, neurodegenerativas, cardiovasculares, endócrinas, así como infecciosas de origen bacteriano, viral y parasitario. Además, se discutirán las bases de la resistencia a fármacos, la neurofarmacología, farmacovigilancia, toxicología, fitofarmacología, farmacoepidemiología, farmacoterapéuica e inmunofarmacología, y se abordarán tópicos relacionados con la memoria inmunológica y sus implicancias en el desarrollo de vacunas.

Como organizadores de la Reunión Conjunta 2016, consideramos que es nuestro deber generar un ambiente propicio para la presentación y discusión de los avances realizados en las áreas de investigación que nos incumben. Creemos que esto nos permitirá conocer, interpretar y contextualizar los avances que se realizan en nuestro país, así como debatir mecanismos para incrementar el aporte que hacemos los investigadores a nuestra realidad científica y social. Los invitamos a participar activamente de esta reunión, en la que los paradigmas principales serán la rigurosidad científica y la crítica constructiva.

Finalmente, aprovechamos esta oportunidad para expresar nuestro más sincero y profundo agradecimiento a las entidades oficiales y empresas que dieron apoyo a este congreso y, particularmente, queremos destacar el esfuerzo, voluntad y dedicación puesto en su organización por todos los integrantes de las Comisiones y Consejos Directivos de las Sociedades Científicas participantes. Sin su aporte invalorable, especialmente de las Secretarias y Tesoreras de cada una de ellas, este congreso no hubiera sido posible.

Dra. Edith Kordon
Presidente SAIC

Dr. Norberto Zwirner
Presidente SAI

Dr. Sergio Sánchez
Presidente SAFE

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SAIC LECTURE I

OPENING LECTURE

INSIGHTS INTO MECHANISMS OF BREAST CANCER DEVELOPMENT

NANCY HYNES, ALESSIA BOTTOS AND ALBANA GATTELLI

Friedrich Miescher institute for Biomedical Research, Basel, Switzerland

Breast carcinoma is the most frequent cancer in women with more than 1 million new cases reported in the USA each year. Although current therapeutic interventions have increased the 5-year survival rate for women with the disease, there is still an urgent need to uncover therapeutic targets, in particular for metastatic breast cancer. Our lab has taken different approaches to tackle this problem. On the one hand, we have studied breast cancer metastasis models in order to directly target disseminated disease; on the other hand, we have searched for new breast cancer driver genes. My talk will cover recent work from both areas.

Metastasis is a complex process whereby tumor cells acquire various properties allowing them to colonize and grow at distant sites. We have been studying important pathways involved in metastasis. In my presentation I will discuss targeting the Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) pathway in breast cancer models of lung and bone metastasis. Activation of STATs through JAKs is known to contribute to tumor initiation and progression at several levels and JAK inhibitors (JAKi) are currently in clinical trials for breast cancer. We showed in cancer patient biopsies that STAT proteins are active in the primary tumor and in matched metastasis, reinforcing the rational of employing JAKi to block STATs and, potentially, metastatic growth. We investigated the effect of a JAK1-JAK2i, ruxolitinib, *in vivo* by employing preclinical models of breast cancer metastasis that show activation of STAT proteins. Surprisingly, we found a significant increase in metastatic burden in mice treated with ruxolitinib, compared to vehicle treated mice, despite the fact that JAKi did block the JAK/STAT signaling pathway in the metastatic lesions. This result was unexpected. The potential clinical relevance of our finding prompted us to further investigate the biological mechanisms underlying the detrimental effect of JAKi observed in these models Reasoning that in addition to the cancer cells, other cells in the tumor environment might be affected by JAK inhibition, we designed experiments to test this hypothesis. We demonstrated that the pro-metastatic effect of JAKi is due to their immunosuppressive activity, leading to an impairment of natural killer (NK) cell-mediated anti-tumor immunity. Our study indicates that in the breast cancer models we studied, JAKi-mediated inhibition of NK cell tumor surveillance prevails over potential anti-cancer effects of blocking the JAK/STAT pathway, and that this inhibition causes enhancement of metastasis. Our findings highlight the importance of evaluating the effect of targeted therapy on the tumor environment, in addition to the cancer cells, in order to predict and potentially prevent undesired bystander effects. This work was recently published in Bottos et al 2016 Nature Comm.

In the second part of my presentation, I will discuss our work on Ret, a member of the receptor tyrosine kinase superfamily. Ret is known as the key oncoprotein in thyroid carcinomas due to gain-of-function mutations and Ret has recently been implicated in other cancer types. Indeed, Ret mutations have been reported at low frequencies in lung and breast tumors. Furthermore, we and others have reported that Ret is overexpressed in about 40% of human tumors and that this correlates with poor patient prognosis. Ret activation regulates numerous intracellular pathways related to proliferation, differentiation and inflammation, but it is not known whether abnormal Ret expression is sufficient to induce mammary carcinomas in mice. Using the doxycycline-inducible transgenic mouse model with the MMTV promoter controlling Ret expression, we show that overexpression of wild type Ret in the mammary epithelium produces hyperplasia and evokes mammary tumors displaying a morphology that recapitulates features of human ductal carcinoma in situ. Importantly, tumors rapidly regress after doxycycline withdrawal indicating that Ret is the driving oncoprotein. Using next generation sequencing we examined levels of transcripts in these tumors and have evidence that STAT signaling might contribute to Ret-driven tumorigenesis. Data on other signaling pathways that are controlled by aberrant Ret expression will be presented Ret-evoked tumors can be passaged in mice and are now being used to test novel therapeutic approaches (Gattelli et al, in preparation).

SAIC LECTURE II

THE CALYX OF HELD SYNAPSE. A MODEL FOR CHEMICAL TRANSMISSION

OSVALDO D. UCHITEL

IFIBYNE-UBA-CONICET. DFBMC, School of Natural and Exact Sciences, University of Buenos Aires, Argentina

Chemical synapses are the fundamental units that mediate communication between neurons in the mammalian brain. Classically, the basic concept of chemical synaptic transmission was established at the frog neuromuscular junction, and at the squid giant synapse. More recently, recordings from the calyx of Held in rodent brainstem slices have extended the classical concept to mammalian synapses providing new insights into the mechanisms underlying strength and precision of neurotransmission.

The calyx of Held synapse plays an important role in the auditory system, relaying information about sound localization via fast and precise synaptic transmission, which is achieved by its specialized structure and giant size. During development, the calyx of Held undergoes anatomical, morphological, and physiological changes necessary for performing its functions. The large dimensions of the calyx of Held nerve terminal are well suited for direct electrophysiological recording of many presynaptic events that are difficult, if not impossible to record at small conventional synapses. This unique accessibility has been used to investigate presynaptic ion channels, transmitter release, and short-term plasticity, providing invaluable information about basic presynaptic mechanisms of transmission at a central synapse.

I plan to summarize findings from our laboratory and others on the interplay between the waveform of action potentials, $Ca2^{+}$ currents and transmitter release in normal and pathological conditions.

I also plan to present our recent findings that evoked release protons are neurotransmitters and neuromodulators acting via activation of acid sensing ion channels (ASICs).

ASICs are voltage-independent, proton-gated cation-selective channels mostly permeable to Na^{+} ion. They belong to the degenerin/epithelial Na^{+} channel (DEG/ENaC) superfamily.

Protons are important signals for neuronal function. In the central nervous system (CNS), proton concentrations change locally when synaptic vesicles release their acidic contents into the synaptic cleft, and globally in ischemia, seizures, traumatic brain injury, and other neurological disorders due to lactic acid accumulation. The finding that protons gate a distinct family of ion channels, the ASIC channels, has shed new light on the mechanism of acid signaling and acidosis-associated neuronal injury.

At the calyx of Held postsynaptic neuron, ASIC channels can be activated by a drop in extracellular pH. Their activation induces transient inward currents (I_{ASIC}) in postsynaptic neurons from wild type mice. The inhibition of I_{ASIC} by the specific blocker psalmotoxin-1 (PcTx1) and the absence of these currents in *knock out* mice for ASIC-1a subunit ($ASIC1a^{-/-}$) suggest that homomeric ASIC-1a channels are mediating these currents. Furthermore, after blocking all postsynaptic glutamate receptors we detect nerve evoked ASIC1a-dependent currents strong enough to generate postsynaptic action potential suggesting an acidification of the synaptic cleft due to the co-release of glutamate and H^{+} from synaptic vesicles. A significant characteristic of these homomeric ASIC-1a channels is their permeability to Ca^{2+}. Activation of ASIC-1a by exogenous H^{+} induces an increase in intracellular Ca^{2+}. Furthermore, the activation of postsynaptic ASIC-1a channels during high frequency stimulation (HFS) of the presynaptic nerve terminal leads to a PcTx1-sensitive increase in intracellular Ca^{2+} in which is independent of glutamate receptors and is absent in neurons from $ASIC1a^{-/-}$ mice. During HFS, the lack of functional ASICs in synaptic transmission results in an enhanced short term depression of glutamatergic excitatory postsynaptic currents. These results strongly support the hypothesis of protons as neurotransmitters and demonstrate that presynaptic released protons modulate synaptic transmission by activating ASIC-1a channels at the calyx of Held synapse.

SAIC LECTURE III

CONFERENCIA PLENARIA "ALFREDO LANARI"

LO ESENCIAL NO ES INVISIBLE A LOS OJOS

RUTH E. ROSENSTEIN

Laboratorio de Neuroquímica Retiniana y Oftalmología Experimental, Dpto. de Bioquímica Humana, Facultad de Medicina/ CEFyBO, Universidad de Buenos Aires/CONICET, Argentina

El objetivo central de mi grupo de trabajo es el estudio de la retina, tanto en condiciones fisiológicas como patológicas. Particularmente en los últimos años, nos concentramos en el estudio de distintas enfermedades visuales

prevalentes que constituyen causas de ceguera, tales como retinopatía diabética, uveítis, neuritis óptica, glaucoma e isquemia retiniana, para las cuales aún no existen terapias suficientemente efectivas. Para cumplimentar este objetivo, desarrollamos nuevos modelos experimentales o validamos modelos pre-existentes, y analizamos la viabilidad de nuevas estrategias terapéuticas. En este sentido, demostramos que el condicionamiento isquémico previene la disfunción retiniana, la pérdida de integridad de la barrera hemato-ocular y el aumento en los niveles del factor de crecimiento vascular endotelial inducidos por diabetes experimental de tipo 1 y evita la progresión de la retinopatía diabética en ratas. La uveítis es una enfermedad inflamatoria intraocular que involucra al tracto uveal (iris, cuerpo ciliar y coroides), y las estructuras oculares adyacentes (retina y vítreo). Desarrollamos un modelo experimental de panuveítis en hámster, a través de la inyección intravítrea de lipopolisacárido bacteriano (LPS) y analizamos el efecto terapéutico de la melatonina sobre las alteraciones funcionales y estructurales inducidas por uveítis experimental. En este contexto, demostramos que el tratamiento con melatonina previene las alteraciones clínicas, bioquímicas, funcionales y ultraestructurales inducidas por la inyección intravítrea de LPS. Considerando la efectividad de la melatonina en la inflamación ocular, decidimos examinar el efecto de la melatonina en otra enfermedad inflamatoria ocular que afecta primariamente al nervio óptico, la neuritis óptica. La neuritis óptica es una neuropatía aguda inflamatoria y desmielinizante del nervio óptico. Dado que no existen modelos experimentales para la forma primaria de esta enfermedad, desarrollamos un modelo a través de la microinyección de LPS directamente en el nervio óptico de ratas *Wistar*, que reproduce aspectos centrales de la neuritis óptica primaria humana y demostramos que la melatonina previene las alteraciones axogliales del nervio óptico y de la retina y evita la progresión del daño funcional inducido por neuritis óptica experimental.

El glaucoma es una disfunción ocular de alta prevalencia que se caracteriza por la pérdida progresiva de las funciones visuales, asociada a la muerte de células ganglionares retinianas (CGR) y axones del nervio óptico. En los últimos años, hemos logrado avances de consideración en cuanto a la elucidación de los mecanismos patogénicos involucrados en el desarrollo de la enfermedad y la búsqueda de nuevas estrategias terapéuticas, utilizando un modelo de glaucoma experimental desarrollado en nuestro laboratorio a través de inyecciones intracamerales de glicosaminoglicanos. En forma más reciente, analizamos un aspecto del glaucoma que ha recibido escasa atención, como es su efecto sobre el sistema visual no formador de imagen. En este contexto, demostramos que el glaucoma experimental en ratas provoca una pérdida significativa de CGR intrínsecamente fotosensibles, una disminución en el reflejo pupilar y alteraciones en el ritmo diario de actividad locomotora. En base a estos antecedentes, analizamos el ritmo diario de actividad en pacientes con glaucoma avanzado y demostramos que el glaucoma induce una disminución significativa en la calidad del sueño. En conjunto, estos resultados demuestran que el glaucoma afecta no sólo las funciones visuales formadoras de imagen, sino también las funciones visuales no formadoras de imagen, como el control de los ritmos circadianos, lo que constituye un riesgo adicional para la calidad de vida de pacientes con glaucoma. En la búsqueda de estrategias terapéuticas no invasivas, analizamos el efecto de la exposición a ambiente enriquecido sobre el daño isquémico retiniano. Para ello, luego de una isquemia retiniana deletérea, los animales fueron albergados en ambiente estándar o ambiente enriquecido por 3 semanas. La exposición a ambiente enriquecido previno significativamente el daño funcional y estructural inducido por isquemia retiniana.

Epílogo

Dice (y con razón) el saber popular que hay dos formas de "ver el vaso": medio lleno o medio vacío. A lo largo de todos estos años no hemos logrado obtener evidencias en favor de una u otra de estas opciones, pero en cambio, hemos pretendido contribuir a mejorar la visión de aquellos que padecen por no tenerla, porque después de todo, parafraseando al poeta español Ramón de Campoamor: "*Y es que en este mundo traidor, no hay verdad ni hay mentira: todo es según el color del cristal con que se mira*".

SAIC LECTURE IV

RAS-RELATED PROTEINS AS DIRECT ONCOGENIC DRIVERS

XOSÉ R. BUSTELO[1], JAVIER ROBLES-VALERO[1], BALBINO ALARCÓN[2], JESÚS M. PARAMIO[3], MARÍA I. FERNÁNDEZ-PISONERO[1]

[1]Salamanca Cancer Research Center, CSIC-Univ. Salamanca, 37007 Salamanca, Spain. [2]Centro de Biología Molecular Severo Ochoa, CSIC-Univ. Autónoma de Madrid, 28049 Madrid, Spain. [3]CIEMAT, 28040 Madrid, Spain.

Given the large number of mutations found in tumor genomes, it is of paramount importance to specifically pinpoint those that play driving roles during the initiation, progression, and/or posttreatment response of tumors.

Unfortunately, this task is as challenging as looking for a needle in a haystack due to the complex genetic make-up of most cancer genomes as well as the low frequency in which most mutations are found in them. Due to this, it is important to devise new genetic ways to identify genes playing proactive functions in this malignant process.

R-Ras2 (also known as TC21) is a GTPase that shows high structural similarity with the three members of the Ras subfamily. This protein attracted attention upon its discovery due to its signaling similarity with Ras proteins, high oncogenic potential in cell culture, and localization in a mutation "hotspot" in a variety of tumors. Despite this, little information exists about its physiological roles and, more importantly, about its ability to contribute to tumor development, progression, and/or maintenance in vivo. In the same context, we do not know as yet whether the mutations found in human tumors have clinical relevance or are just passenger ones. To address these issues, we have used a number of genetic (shRNA interference, ectopic expression, knockout and tamoxifen-inducible knock-in mice) and signaling techniques to address the effect of the loss-of- and gain-of-function of this GTPase in in vivo tumorigenic processes.

Using loss-of-function approaches, we have found that the elimination of endogenous R-Ras2 can abate breast cancer primary tumorigenesis and the metastatic dissemination of cancer cells. These effects are even seen when such an inactivation takes place in cancer cells bearing oncogenic mutations in classical Ras proteins. Interestingly, the contribution of R-Ras2 to this process seems to be through a PI3K-dependent route that mediates efficient protein synthesis rather than distal transcriptional events. Perhaps more importantly, we have found using a new inducible *R-Ras2* knock-in mouse strain that the somatic replacement of endogenous wild-type R-Ras2 by a mutant version found in human tumors leads to the death of the animals in a 2-3 month period. This rapid lethality derives from the development of quite aggressive T cell acute lymphoblastic leukemia that exhibit both constitutive Notch1 and PI3K signaling. We also have found the frequent development of noninvasive marginal zone splenic lymphomas, Harderian gland adenomas, skin papillomas, and several subtypes of genitourinary tumors. Collectively, these data indicate that this Ras-like protein promotes signaling events that are overlapping, but not identical, to those usually triggered by the three Ras subfamily proteins. They also demonstrate, for the first time, that genetic alterations in this gene probably act as oncogenic drivers for specific types of hematological and solid tumors. These observations suggest that the presence of such mutations in human tumors may have diagnostic, clinical, and therapeutic interest.

This work has been supported by grants from the Spanish Ministry of Economy and Competitiveness (RD12/0036/0002), Worldwide Cancer Research (14-1248), The Spanish Association against Cancer (GC-16173472GARC), and a Research Action supported by the Castilla-León Autonomous Government-European Social Fund (CSI049U16). Address correspondence to: xbustelo@usal.es.

SAIC LECTURE V

CONFERENCIA PLENARIA "ALBERTO TAQUINI"

LIGHTS AND SHADOWS IN CARDIAC REGENERATION

ALBERTO J. CROTTOGINI

Instituto de Medicina Traslacional, Trasplante y Bioingeniería (IMETTYB-Universidad Favaloro-CONICET), Buenos Aires, Argentina

Ischemic heart disease, the leading cause of mortality worldwide, often results in myocardial infarction, after which the surviving tissue undergoes a process termed remodelling. This process consists of myocardial hypertrophy, myocyte death, defective regeneration and progressive replacement of contractile myocytes by fibrotic tissue. The progressive loss of myocytes and its replacement by non contractile tissue leads to heart failure. Although considerable progress has been made in its pharmacological management, heart failure continues to be associated with a high mortality. Once overt heart failure develops, about 30% to 45% of patients die within 1 year, unless they receive a heart transplant. The extent of remodeling and the chances of evolving towards contractile failure are largely dependent on infarct size. Small infarcts do not lead to substantial remodelling whereas large ones do. Therefore, reducing the size of the initial infarct is of utmost therapeutic and prognostic relevance.

Currently, the most investigated strategy to meet this goal is the myocardial implantation of stem cells of diverse origin and differentiation potential including, among others, embryonic stem cells, skeletal myoblasts, bone marrow mononuclear cells, endothelial progenitor cells, mesenchymal stromal cells and, more recently, cardiac progenitor cells. Although promising achievements have been observed in small and large animal models, results from the few controlled trials in humans have been considerably poorer or even undetectable.

On account of their easy isolation and ex-vivo expansion, their amenability for genetic modification and their immunosuppressive properties which allow for allogeneic utilization, bone marrow mesenchymal stromal cells (MSCs) constitute the cell type most consistently used in pre-clinical and clinical studies of cardiac regeneration.

Although MSCs may delineate in vitro a cardiomyocyte phenotype based on morphology, automatic beating and expression of sarcomeric α-actinin, troponin I and connexin 43, the most accepted mechanism by which they afford regeneration is the paracrine angiogenic, anti-apoptotic, anti-fibrotic and pro-mitotic effect of the multiple growth factors and cytokines that they produce.

Additionally, some of these molecules may display a pro-mitotic effect on the adult cardiomyocyte, favouring not only their re-entry in the cell cycle, but also inducing advancement into mitosis.

Given that the myocardium behaves as an electromechanical syncytium, myocardial regeneration is not just a matter of repopulating the heart with cells, but also, and most importantly, to promote establishing electromechanical connections between the new cells and the remaining viable ones. Therefore, strategies aimed at encouraging the resident cardiomyocytes to exit the post-mitotic phenotype and divide into daughter cells would not only produce cardiomyocyte hyperplasia but also guarantee the physiological connection between cells that supports myocardial function.

One of such strategies would consist of genetically modifying MSCs to overexpress pro-mitotic factors that would not only increase angiogenesis but also encourage the adult cardiomyocyte to re-enter the cell cycle.

On account that in large mammals the adult cardiomyocyte can polyploidize but not advance into mitosis, an alternative approach would be removing the brake that prevents the adult cardiomyocyte to overcome de G2/M checkpoint of the cell cycle. So far, the *meis1* gene has been identified in mice as a transcription factor of inhibitor proteins, but more studies are needed both to confirm that this gene acts similarly in large mammals and to find new cell cycle-inhibiting molecules that can become targets of therapies aimed at promoting adult cardiomyocyte hyperplasia.

Despite the scientific progress achieved during the last two decades, the goal of building a new heart, or at least mending the old one, still seems remote. While new approaches have emerged, such as the use of inducible progenitor stem cells, bioresorbable scaffolds, decellularized matrices, etc., many questions remain to be answered, including how cardiomyocyte proliferation is regulated during heart development, how the adult cardiomyocytes and cardiac progenitor cells interact with the extracellular matrix and how we can attenuate the limitations of animal models of human ischemic heart disease. Only advancing our knowledge about these and other important issues will enhance the chance of partially regenerating the human heart.

SAIC LECTURE VI

CLOSING LECTURE

TARGETING SIGNALING CIRCUITRIES: NEW PRECISION CANCER TREATMENTS AND IMMUNOTHERAPIES

J. SILVIO GUTKIND

Department of Pharmacology and UC San Diego Moores Cancer Center, La Jolla, CA 92093

The goal of our program is to exploit the emerging information on dysregulated signaling circuitries and individual genomic and molecular alterations to develop new precision therapies to prevent and treat cancer. Specifically, we have focused on the study of growth-promoting signal transduction pathways, the nature of the dysregulated signaling networks in cancer, and on the use of genomic, proteomic, and system biology approaches to study cancer initiation and progression. We have shown that human and virally-encoded G proteins and G protein coupled receptors (GPCRs) can display potent oncogenic activity. Strikingly, our recent analysis of human cancer genomes revealed an unanticipated high frequency of mutations in G proteins and GPCRs in most tumor types. Among them, mutually exclusive activating mutations in *GNAQ* or *GNA11* (encoding $G\alpha_q$ family members) occur in 5.6% of tumors, including >90% of ocular melanomas, thus providing a clear example of a human malignancy that is initiated by gain of function mutations in $G\alpha_q$ and $G\alpha_{11}$ proteins. How $G\alpha_q$ and its coupled receptors transduce mitogenic signals is still unclear, due to the complexity of signaling events perturbed upon G_q activation. Evidence will be presented that while many biological responses elicited by G_q depend on the transient activation of second messenger system, G_q utilizes a hardwired protein-protein interaction-based signaling network to achieve the sustained stimulation of proliferative pathways, thereby controlling normal and aberrant cell growth. In parallel, our team has focused on the study of oncogenic signaling circuitries driving the initiation and progression of head and neck cancer (HNSCC), a disease that results in 300,000 deaths each year worldwide, aimed at identifying novel

druggable targets for prevention and treatment. There is an urgent need to develop new precision therapies to prevent and treat HNSCC. A striking finding from the recent deep sequencing of the HNSCC genomic landscape was the multiplicity and diversity of genetic alterations in this malignancy. The emerging picture, however, is that most fall within only a few major driver biological processes, including mitogenic signaling with particular emphasis on aberrant activation of the PI3K/mTOR pathway. Remarkably, this is aligned with our early discovery that the persistent activation of the PI3K/mTOR signaling circuitry is the most frequent dysregulated signaling mechanism in HNSCC, and that PI3K/mTOR inhibition exerts potent antitumor activity in a large series of genetically-defined and chemically-induced HNSCC models, including those involving HPV-associated HNSCC. These findings provided the rationale for launching a multi-institutional Phase II clinical trial (NCT01195922), targeting mTOR in HNSCC, which was recently completed and achieved encouraging results. Emerging results from this trial and the molecular mechanisms underlying the remarkable effects of mTOR inhibitors in HNSCC and other cancer types will be discussed. A recently initiated clinical trial for HNSCC precision prevention using metformin, which decreases mTOR activity in oral premalignant lesions and their cancer initiating cells, will be presented. Recently launched efforts aimed at harnessing the full potential of immune oncology agents, including checkpoint inhibitors, to achieve durable responses (cure) in HNSCC patients will be also discussed.

SAI OPENING LECTURE

DEVELOPMENT OF AN ATTENUATED DENGUE VACCINE

PROF. JORGE KALIL

Center of Toxins, Immune-response and Cell Signaling

Faculdade de Medicina da Universidade de São Paulo, Incor, Laboratório de Imunologia, São Paulo, Brasil.

SAI LECTURE II

REGULATORY T CELLS AND NOVEL IMMUNOMODULATORS

PROF. TIM SPARWASSER, MD.

Institute of Infection Immunology, Twincore, Centre for Experimental and Clinical Infection Research, Hannover School of Medicine, Hannover, Germany

SAI LECTURE III

FUNCTIONAL REPROGRAMMING OF MYELOID CELLS IN CANCER: MECHANISMS AND CLINICAL SIGNIFICANCE "DR. LEONARDO SATZ CONFERENCE"

ANTONIO SICA, PHD.

Humanitas Research Hospital Milano, Italy

SAI LECTURE IV

T HELP VERSUS REGULATION BY $CD4^+$ T CELLS

STEPHEN SCHOENBERGER, PHD.

Division of Developmental Immunology, La Jolla Institute for Allergy and Immunology, La Jolla, California, USA.

SAI LECTURE V

TUMOR AND HOST FACTORS REGULATING ANTI-TUMOR IMMUNITY AND IMMUNOTHERAPY EFFICACY

TOM GAJEWSKI, MD, PHD.

Department of Pathology, The University of Chicago, Chicago, Illinois, USA.

SAI CLOSING LECTURE

NATURAL KILLER CELLS IN HOST IMMUNITY AGAINST VIRAL INFECTION

JOSEPH SUN, PHD.

Memorial Sloan Kettering Cancer Center, Weill Cornell Medical College, New York, USA

SAFE OPENING LECTURE

MOLECULAR AND FUNCTIONAL GENETICS OF APPETITIVE BEHAVIORS IN GENETICALLY MODIFIED MICE

MARCELO RUBINSTEIN

Instituto de Investigaciones en Ingeniería Genética y Biología Molecular, CONICET and Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina

A spectre is haunting the world, the spectre of obesity. This pandemia has been steadilyincreasing for the last 40 years and during the last decade its prevalence has skyrocketedparticularly in infants and adolescents, as well as in lower income families from most countries worldwide. Although pandemic obesity has been purely driven by a large increase in the consumption of ultra processed edibles of poor nutritional value, the obesogenic changes in contemporary human lifestyle do not affect all individuals at the same level revealing the importance of genetic predisposition for hyperphagia and increased adiposity. In this lecture I will present genetic and functional results that are contributing to understand how the hypothalamus orchestrates food intake and, in particular, the role of a satiety system led by a group of neurons expressing the proopiomelanocortin (*Pomc*) gene. During the past several years my group has been working in the identification of the molecular components responsible for hypothalamic *Pomc* expression. By using a large variety of transgenic and mutant mouse strains we were able to dissect the neuronal transcriptional code of Pomc, define with unanticipated resolution the cis-acting regulatory elements and the transcription factors involved in the selective expression this gene and characterize their functional importance in the regulation of food intake and body weight. I believe that these results are helping us to better understand the power and limitations of the mammalian central satiety pathways and probably will help us to improve the individual and collective strategies to reduce the overwhelming increase of this insatiable human-induced pandemia.

SAFE LECTURE II

ETHICAL ASPECTS OF HIGH COSTS OF ONCOLOGICAL DRUGS: WHEN LIFE HAS A PRICE

ERNESTO GIL DEZA

Instituto Oncológico Henry Moore. Carrera de Oncología Clínica, Universidad del Salvador, Buenos Aires, Argentina

The cost of drugs in oncology is the biggest obstacle patients face to access new treatments, and this is an ethical challenge we cannot avoid. It is so important, we use the term “financial toxicity” when talking about new drugs.

When we analyze the most common reasons given for these costs, we can see that they are unjustifiable:

a) **Inflation**: from 1970 until today, pharmaceutical products have increased their prices by a hundredfold (from an average of one hundred dollars to an average of ten thousand) in countries with stable economies (theU.S.A.), whereas other products only had a tenfold increase in that period.

b) **Breakthrough costs**: most common breakthroughs take place in universities financed by taxes and not as a consequence of money invested in pharmaceutical companies.

c) **Production costs**: most of today’s new drugs are monoclonal antibodies, discovered by César Milstein, who never patented them. Laboratories freely use the technique, but they patent their products, which results in billions of dollars of profit for their companies.

d) **Research costs:** only 15% of a company’s profits are devoted to research.

e) **Drug efficacy or security**: the cost of new drugs is similar, no matter how much safer or more efficient they are. The cost derives from novelty

f) **None of these variables (alone or combined) can explain drug prices**: this is demonstrated by the large variance in price for the same medication in different parts of the world. So much so that a medication may be up to six times cheaper in one place than in another.

g) This is even more notable when pharmaceutical companies are willing to offer **secret discounts** (allegedly, up to 90% discounts) so that the drug is used by the British NHS, or other such services in developed countries, while they refuse to do so for poorer countries, thus ensuring that the latter subsidize the former.

The only truth is that drug prices are as high as they are because someone is willing to pay those prices.

The new treatments in the US have such elevated costs that, as of 2014, there has been a move to quantify financial toxicity, since it is well known that half of all oncological patients in the US end up bankrupt due to this toxicity. Others simply abandon treatments, gain acess at a later stage, or significantly reduce the dosage.

It is necessary to shine a light on this kind of extortion, to which states, health services and patients are exposed on a daily basis. We must understand that what is happening to poor patients in rich countries is but a prelude of what will happen to the health services of poor countries if we do not act.

This idea that medication is a luxury good goes against the ethical foundations of medicine. The idea that the market will self-regulate and thus drop prices is childish, to say the least. It is the duty of society as a whole (politicians, scientific societies, doctors and patients) to preserve the rights of patients rather than the greed of pharmaceutical companies.

SAFE LECTURE III

SIGNAL TRANSDUCTION BY G PROTEINS: FROM RECEPTOR TO EFFECTOR – HISTORICAL AND STRUCTURAL VIEWS

LUTZ BIRNBAUMER

Institute of Biomedical Research (BIOMED.CONICET-UCA), Pontificia Catholic University of Argentina, C1107AFF Buenos Aires CABA.

The Royal Society of Chemistry's web site, under Drug Development, states: *"Cell-surface receptors,such as G-protein-coupled receptors (GPCRs), are the targets of over half the drugs currently used*". Many of them "happen" to be of this kind and were developed independently of any knowledge about their mechanisms of action. But, independently of their importance, the molecular and atomic mechanism by which the GPCRs "do their thing" is fascinating and happens to involve much of my life as a scientist. This lecture is structured around the key discoveries that have led to our current understanding of how occupancy of receptors by their ligands is transduced by the G protein signal transducing machine into intracellular signals picked up by a heterogeneous mix of enzymes: 9 types of adenylyl cyclase, 4 phospholipases Cbeta, the visual phosphodiesterase, 3 guanine nucleotide exchange factors for RhoGTPases, voltage-gated Ca channels, and the GIRK K channels. The field spawned two Nobel Prices: one in Physiology (or Medicine) in 1994 to Martin Rodbell and Al Gilman for the discovery of G Proteins as Signal Transducers, and the other in Chemistry in 2012 to Robert Lefkowitz and Brian Kobilka for their work on discovery and structure elucidation of GPCRs. I participated in some of the seminal findings that led to the 1994 Nobel Price, as I was Martin Rodbell's first postdoctoral fellow and happened to pipet the ingredients he had written out that led to the discovery of the GTP-dependent activation of adenylyl cyclase, a finding that in turn led to the discovery of the GTP-binding regulatory component, separate from receptor and adenylyl cyclase, and eventually to the realization that the regulatory GTP-binding component is a GTPase (inhibited by cholera toxin) which in its GTP-bound form is active as an activator of adenylyl cyclase and loses this capacity when it hydrolyzes the resident GTP to GDP (Cassel and Selinger). The main and fundamental contribution of Gilman's laboratory to the field was to have purified the regulatory component and discovering that it was a dimer that dissociated into alpha and beta subunits upon GTP binding, which re-associated upon GTP to GDP hydrolysis. Parallel studies discovered that there were more than one GTP-binding alpha-beta protein (Sternweis and Robishaw in Dallas and Eva Neer at Harvard). At this point, 1984, we discovered that yet another G protein, this one purified by us and a substrate for the ADP-ribosylating activity of pertussis toxin (an inhibitor of inhibitory regulation of adenylyl cyclase, a form of regulation discovered by us several years earlier) was an alpha-beta/gamma heterotrimer, and that the three G proteins (Gs, Go and Gi) underwent upon GTP binding not only a conformational change but also the subunit dissociation. The three proteins shared a common pool of beta/gamma dimers. Both arms, the GTP-alpha and the beta/gamma dimer regulate effectors. It was between 1984 and 1990 that it became clear that not only adenylyl cylcase (Gs and Gi) but also the other effectors mentioned above were regulated by G protein alphas, of which there are 16, some biochemically isolated (Gq/G11 – Exton, Sternweis), the others cloned based on their structural similarity (Simon, Numa, Gilman, Kaziro, also us). Beta/gammas were found also to regulate effectors, acting on ion channels (Clapham and Neer the GIRK channels, Catterall the Ca channel, Schultz, Jakobs and Gierschik the PLC betas). An interesting curiosity in this field was that prior to the elucidation of the alpha/beta/gamma and GTPase nature of Gs and Gi in the adenylyl cyclase field, in the vision field, a light activated GTPase of subunit composition alpha/beta/gamma had been

found to be responsible for activation of the visual PDE by light-stimulated rhodopsin (Bitensky at Yale, Kuehn in Bochum, Germany), and that the parallelism, and in fact identity between the signal transduction architecture in adenylyl cyclase regulation and that of the signal transduction machinery converting light into cGMP hydrolysis had been missed by so many of us.

In my presentation I shall summarize these historical initial developments, concentrate on presenting the molecular structures of the players in adenylyl cyclase regulation, and describe in detail the importance of the Mg ion in the double GTPase and subunit dissociation-re-association cycles, at the atomic level as deduced from x-ray crystallography.

AACYTAL LECTURE I

THE CRISPR/CAS9 REVOLUTION: RAISING THE LIMITS OF FUNCTIONAL GENETICS WHILE THREATENING THE 3RS.

MARCELO RUBINSTEIN

INGEBI, CONICET, and FCEyN, Universidad de Buenos Aires, Argentina

Genetic engineering in live organisms is experiencing a novel revolutionary wave, the latest of the very many that have been occurring since recombinant DNA technology started almost 50 years ago. The use of molecular components of a recently discovered adaptive immune system present in many prokaryotes, known as CRISPR/Cas, is pushing forward the frontiers of transgenesis and genome editing at great strength and promise. Different to the landscape of a few years ago, it is now possible to modify the genome of a large variety of animals, plants and microorganisms, a technological breakthrough with unanticipated consequences. Animal facilities are already experiencing fast changes with more transgenic and mutant species being made, reproduced and maintained and a lot more rat and mouse models that are, once again, challenging the aims of the 3Rs, especially Reduction. During my talk I will discuss how this new CRISPR/Cas technology is changing the present and future of laboratory and farm animals.

AACYTAL LECTURE II

ANIMALES DE EXPERIMENTACIÓN: CONSIDERACIONES FRENTE A UN NUEVO PARADIGMA

CECILIA CARBONE

La ciencia de los animales de laboratorio se puede definir como una rama multidisciplinaria de la ciencia que se ocupa del estudio de todos aquellos aspectos que contribuyen al empleo humanitario de los animales en investigaciones biomédicas y a la obtención de resultados válidos, reproducibles y comparables. En ella se incluye el estudio de la biología de los animales de experimentación, su manejo y requerimientos de alojamiento como también su condición sanitaria, características genéticas y la prevención y tratamiento de enfermedades.

Los aspectos éticos y morales han sido un blanco de atención importante, sin embargo, en los últimos años se han producido cambios que conducen a reflexionar sobre las necesidades y demandas actuales para el uso y cuidado de los animales de experimentación.

A estos cambios nos enfrentamos cuando hablamos de un nuevo paradigma. Se define como paradigma a un patrón, modelo o ejemplo.

El físico, historiador y filosofo Thomas Kuhn, en su libro “La estructura de las revoluciones científicas” lo define como lo que se debe observar y escrutar, considerando a los paradigmas como realizaciones científicas universalmente reconocidas que durante cierto tiempo proporcionan modelos de problemas y soluciones a la comunidad científica.

El bienestar animal constituye uno de los conceptos que actualmente se deben considerar, irrefutablemente, a la hora de diseñar y programar investigaciones o ensayos con animales. El reconocimiento de que los animales son seres sensibles susceptibles de sentir angustia, dolor y estrés ha provocado un impacto en lo referente al uso, manejo y cuidado de los mismos. Las

variaciones que puede causar la alteración del bienestar de los modelos animales en los resultados de las investigaciones y ensayos conducen al desarrollo de estudios sobre el comportamiento, las emociones y la conciencia de dichos individuos.

A su vez, el hecho de prestar atención a la condición de bienestar de los animales de experimentación, ha hecho no solo necesario el entrenamiento y la formación del personal técnico y científico sino que también ha conducido a optimizar y, en algunos casos, cambiar las condiciones de alojamiento, alimentación y los procedimientos que se realizan.

A esto se añade el desarrollo e incremento significativo de modelos animales genéticamente modificados con los consiguientes planteos y debates éticos referidos a su creación y uso.

AACYTAL LECTURE III

RATONES CONSANGUÍNEOS: IGUALES PERO DIFERENTES. LA DIFERENCIA ESTÁ EN LOS DETALLES...

FERNANDO BENAVIDES

The University of Texas - M.D. Anderson Cancer Center Department of Epigenetics and Molecular Carcinogenesis, Smithville, Texas, USA

En estos tiempos dónde la genómica y la manipulación genética del ratón y la rata de laboratorio progresan día a día, se hace imprescindible estandarizar los animales utilizados en investigación. No sólo el control y la preservación de la calidad genética deben ser prioritarios, sino también concientizar a los investigadores sobre la influencia del fondo genético en sus modelos y la existencia de diferencias genéticas marcadas entre sub-cepas. Ya a principios de los años 1970 se publicaron artículos que mostraban una gran diferencia en el fenotipo diabético de las mutaciones *diabetes* (*Lepr*^{db}) y *obese* (*Lep*^{ob}), según estuvieran en fondo C57BLKS/J o C57BL/6J. Otro caso de modificación del fenotipo según la cepa de fondo fue observado para la mutación espontánea inmunodeficiente *Prkdc*^{scid}, donde la tendencia a producir linfocitos B y T funcionales con la edad es muy variable: alta en BALB/c, baja en C3H, y muy baja en NOD. En base a estos hallazgos, empezó a prestarse más atención a la influencia que pueden tener los distintos fondos genéticos en los fenotipos, particularmente en los modelos de ratones transgénicos, KO y KI. Desde finales de la década de 1990 se han publicado muchos reportes sobre esta influencia, los cuales discutiré en mi presentación, incluyendo diferencias importantes entre sub-cepas, por ejemplo C57BL/6**N** (NIH) versus C57BL/6**J** (Jax), y la presencia de mutaciones “pasajeras” que pueden afectar el fenotipo. Finalmente, presentaré distintas formas de prevenir y solucionar esta situación.

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SAIC SATELLITE SYMPOSIUM

SIGNAL TRANSDUCTION

TARGETED INHIBITION OF ONCOGENIC DRIVERS IN GLIOMA AND BREAST CANCER CELLS: NEW INSIGHTS INTO THE MECHANISMS OF THE DEVELOPMENT OF DRUG RESISTANCE

VIKTORIA VON MANSTEIN AND BERND GRONER

Georg-Speyer-Haus, Institute for Tumor Biology and Experimental Therapy, Paul-Ehrlich-Straße 42, 60596 Frankfurt/Main, Germany

Tumor cell resistance to drug treatment severely limits the therapeutic success of treatment. Tumor cells, exposed to chemotherapeutic drugs, have developed intricate strategies to escape the cytotoxic effects and adapt to adverse conditions. The molecular mechanisms causing drug resistance can be based upon modifications of drug transport or metabolism, structural alterations of drug targets or the adaptation cellular signaling. An important component in the transformation of cells and the emergence of drug resistance is the activation of the transcription factor Stat3. The persistent, inappropriate activation of Stat3 causes the expression of target genes which promote tumor cell proliferation, survival, invasion and immune suppression; and it is instrumental in the process of the emergence of resistance to both conventional chemotherapeutic agents and novel targeted compounds. For these reasons, Stat3 inhibition is being pursued as a promising therapeutic strategy.

We investigated and compared the effects of the tyrosine kinase inhibitor canertinib and the down regulation of the transcription factor Stat3 on signaling pathways and the cellular phenotypes of Tu-2449 glioma and MDA-MB-468 breast cancer cells. Tu-2449 are glioma cells in which the v-Src and Bmx tyrosine kinases, and the downstream effectors Akt and Stat3, are persistently activated. MDA-MB-468 are triple negative breast cancer cells in which the EGFR functions as oncogenic driver and the downstream effectors, the Mek and Erk kinases, Akt and Stat3 are persistently activated. Exposure of Tu-2449 cells to canertinib inhibited the activation of the Bmx kinase and the subsequent phosphorylation of Stat3, but had no effect on the activation of v-Src.

A single dose of 5 μM canertinib caused a transient G1 arrest, whereas prolonged administration of canertinib proved cytotoxic. The down regulation of Stat3 by specific shRNA did not affect cell viability and normal cell cycle progression *in vitro*, but single cell infiltration and tumor growth were inhibited *in vivo*. We conclude that the cytotoxic effects of canertinib in Tu-2449 cells are not solely mediated by Stat3 inhibition, but probably require the simultaneous inhibition of the Bmx and Akt kinases. Canertinib treatment of MDA-MB-468 breast cancer cells caused inhibition of the EGFR, Erk1/2 and Mek1/2 kinases and of the downstream effectors Stat3 and Akt. In contrast to the glioma cells, the down regulation of Stat3 was sufficient to kill MDA-MB-468 cells.

Canertinib exposure of Tu-2449 cells rapidly caused the inhibition of the Bmx kinase and the deactivation of Stat3 and prolonged exposure of the cells to canertinib caused the death of the large majority of the cells. Only a few cells became drug resistant and survived in tight clusters. When the canertinib resistant cells were expanded and cultured at lower cell densities, they regained their sensitivity towards canertinib. We measured the extent of Stat3 activation as a function of cell density and found that higher cell densities accompanied by increased Stat3 activation and a higher expression of Stat3 target genes. We suggest that Stat3 induction through tight cell-cell interactions, most likely through the engagement of cadherins, can counteract the inhibitory effects exerted by canertinib on Bmx. Cell-cell interactions induced Stat3 and compensated for the suppression of Stat3 by canertinib, thus transiently protecting the cells from the cytotoxic effects of the inhibitor.

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STUDY OF HORMONE REGULATION OF MITOCHONDRIAL FUSION/FISSION AS A PLATFORM FOR SUBCELLULAR COMPARTMENTALIZATION AND PROTEIN LOCALIZATION IN ENDOCRINE SYSTEMS

CECILIA PODEROSO

INBIOMED-UBA-CONICET, Facultad de Medicina, Universidad de Buenos Aires, Argentina.

The mitochondrion is a dynamic organelle that responds to fluctuations in cellular metabolic demand by changing its shape, number and even its intracellular distribution. These changes are the result of the balance between the activity of different processes such as fusion and fission, known as mitochondrial dynamics. Alterations in dynamics are involved in the evolution of many diseases, as neurodegenerative and immunological diseases, cancer, diabetes, etc. Mitochondria are tightly associated with the endoplasmic reticulum (ER) forming a subcellular domain per se, called mitochondria-associated membranes (MAM). Despite the importance of mitochondrial dynamics, little is known about its regulation in endocrine systems.

Steroid producing cells/tissues are very interesting models to study these mechanisms as steroid synthesis requires a very close interaction between membranes, through which cholesterol and its derivatives must transit.

Recently, we have shown that hormone stimulation increases mitochondrial fusion through the induction of Mitofusin 2 (Mfn2), a key fusion protein, in MA-10 Leydig cells. By immunofluorescence we observed a morphological shift of mitochondria from punctuated to elongated/tubular shape. Increased mitochondrial fusion is dependent of PKA activity, which mediates LH/hCG signaling pathway. We have also determined that fusion of mitochondria is necessary for the localization in mitochondria of a MAPK family member, ERK and also for the proper localization in MAM of an Acyl-CoA synthetase 4 (ACSL4), key enzyme in steroid production. By the downregulation of Mfn2 with an RNAi, we determined that Mfn2 plays a role in the regulation of the expression of the Steroidogenic Acute Regulatory protein (StAR), an essential protein for cholesterol transport to the mitochondria. We observed that phosphorylation by mitochondrial ERK of StAR promotes a lasting longer retention of this protein at the mitochondria to achieve a higher efficiency in cholesterol transfer. Our results suggest that mitochondrial fusion hormone stimulated can regulate the assembly of key proteins in mitochondria and concomitantly promote their functionality. Then, mitochondrial fusion can settle out the choreography used by cells to give specificity to the spatial and temporal response, in both physiological and pathological situations.

(286) TNF ALPHA DOWN-REGULATION BY TRISTETRAPROLIN (TTP) IS RELEVANT FOR MOUSE LACTATION MAINTENANCE

MARÍA VICTORIA GODDIO[1], ALBANA GATTELLI[1], LOURDES PÉREZ CUERVO[1], JOHANNA TOCCI[1], NANCY HYNES[2], ROBERTO MEISS[3], EDITH KORDON[1]

[1]IFIBYNE-UBA-CONICET, Buenos Aires, Argentina. [2]FMI, Basel, Switzerland. [3]Academia Nacional de Medicina, Buenos Aires, Argentina

From pregnancy to lactation, lobulo-alveolar growth is followed by the complete differentiation of mammary epithelium, which allows the production and secretion of milk proteins. At weaning, a rapid switch from survival to death signaling occurs, leading to involution, which involves extensive remodeling and an innate immune response. TTP is a RNA-binding protein that leads to degradation of messenger RNA of pro-inflammatory cytokine and invasiveness-associated genes. We have previously shown that in the mouse mammary gland, TTP expression is induced during lactation and repressed after weaning. We have also determined, using conditional KO mice (TTP-MGKO), in which TTP expression is specifically down-regulated in the mammary secretory cells, that TTP prevents early cell death during lactation. Then, we found (by cytochemistry, immunohistochemistry, western blot and RT-qPCR analysis of mammary tissue) that TTP-MGKO glands show signs of involution at mid-lactation, like the presence of apoptotic epithelial cells, increased levels of cleft caspase-3, inflammatory cytokines (TNFα, LIF and IL-6) and STAT3 phosphorylation, as well as a decrease in AKT phosphorylation. As TNFα mRNA is a main target for TTP destabilization activity, we speculated that the increase of this specific cytokine would play a primordial role in the development of the phenotype in the transgenic animals. To test this hypothesis, TTP-MGKO mice were inoculated with TNFα blocking antibody "Etanercept" during the first 15 days of lactation (n=4 in each group). This caused a significant reduction in mammary cell death, without modifying the high cytokine expression and Stat3 phosphorylation levels observed in the lactating TTP-MGKO placebo treated or untreated mice. These observations confirm that TTP significantly contributes to lactation maintenance, and shows that keeping TNFα levels low is fundamental for mammary cell survival during that period.

(1022) AP-1 IS MODULATED BY FK506-BINDING PROTEIN 52

MARÍA FERNANDA CAMISAY[1], SONIA DE LEO[1], VANINA FONTANA[1], DANIELA CONVERSO[1], MARIO GALIGNIANA[1,2], ALEJANDRA ERLEJMAN[1]

[1]Departamento de Química Biológica/ IQUIBICEN, FCEN, UBA and [2]IBYME, Buenos Aires, Argentina

The FK506 binding protein 52 (FKBP52) is an Hsp90-binding protein with important regulatory functions on steroid receptor and others transcription factors such as NF-kappaB and p53. FKBP52 has two key domains: the tetratricopeptide repeat (TPR) domain, where Hsp90 binds, and the peptidylprolyl-isomerase (PPIase) domain, where its catalytic site is located. In view of the features shown by the Hsp90 cochaperone on the above-mentioned factors, we hypothesized that FKBP52 could also regulate the activity of AP-1 (activator protein 1). Transcriptional activity of AP-1 was evaluated by luciferase assays in HEK293T cells co-transfected with p-AP1-Luc and either expression vector: pCI-neo-FKBP52wt, pCI-neo-FKBP52K354A (a point mutant in the TPR domain, which does not bind Hsp90), or one of the PPIase domain mutants, pCI-neo-FKBP52F67Y or pCI-neo-FKBPP52F130Y. When cells were stimulated with 100 ng/ml PMA, AP-1 transcriptional activity was increased in an FKBP52-dependent manner, whereas it was abolished by the overexpression of the mutants. In human pregnancy, trophoblast cells invade into the uterine wall by an AP-1 mediated mechanism. Therefore, we analyzed AP-1 modulation by FKBP52 in an in vitro choriocarcinoma model (BeWo cells). ERK1/2 activation, which leads to AP-1 activation, was evaluated at protein level. FKBP52 stabilized ERK1/2 phosphorylation over time. To analyze the effects of these regulatory events, IL-6 secretion (by ELISA assay) and MMP-2 proteolytic activity (by zymography) were analyzed. Both IL-6 secretions to the medium and MMP-2 enzymatic activity were greatly increased by FKBP52 overexpression, whereas they were abrogated by the PPIase mutants. In summary, in this study we demonstrate for the first time that FKBP52 enhances ERK1/2-signalling and AP-1 transcription activity. FKBP52 effect requires binding to Hsp90 via TPR domains, and also the PPIase enzymatic activity. We conclude that FKBP52 is a novel positive regulator of AP-1.

LONG TERM OVARIAN HORMONE DEPRIVATION INDUCES MITOCHONDRIAL BIOENERGETIC DECAY AS WELL AS CHANGES IN MITOCHONDRIAL MEMBRANE LIPID PROFILE AND DNA REPAIR MECHANISMS IN THE HIPPOCAMPUS

SANDRA ZÁRATE

Universidad de Buenos Aires, Consejo Nacional de Investigaciones Científicas y Técnicas, Instituto de Investigaciones Biomédicas (INBIOMED), Facultad de Medicina, Buenos Aires, Argentina.

Mitochondrial dysfunction is a common hallmark in aging. In the female, reproductive senescence is characterized by loss of ovarian hormones, many of whose neuroprotective effects converge upon mitochondria. Mitochondria functional integrity is dependent on membrane fatty acid and phospholipid composition as well as mitochondrial DNA (mtDNA) stability, parameters also affected during aging. The aim of this work was to study the effect of long-term ovarian hormone deprivation upon mitochondrial function and its putative association with changes in mitochondrial membrane lipid profile and in mtDNA repair mechanisms the hippocampus, an area primarily affected during aging and highly responsive to ovarian hormones. To this aim, Wistar adult female rats were ovariectomized or sham-operated. Twelve weeks later, different parameters of mitochondrial function (O_2 uptake, ATP production, membrane potential, respiratory complex activities), membrane phospholipid content and composition as well as single steps in the main mtDNA repair pathway were evaluated in hippocampal mitochondria. Our results show that chronic ovariectomy reduced mitochondrial O_2 uptake and ATP production rates and induced membrane depolarization during active respiration without altering the activity of respiratory complexes. Mitochondrial membrane lipid profile from ovariectomized rats showed no changes in cholesterol levels but higher levels of unsaturated fatty acids, rendering them more prone to peroxidation. Interestingly, chronic ovariectomy also reduced cardiolipin content and altered cardiolipin fatty acid profile. mtDNA repair pathway was also altered in hippocampal mitochondria from ovariectomized rats. Our results show that chronic ovarian hormone deprivation induces mitochondrial bioenergetics dysfunction and changes in the mitochondrial membrane lipid profile and mtDNA repair mechanisms comparable to an aging phenotype. The maintenance of membrane properties and mtDNA integrity emerge as putative therapeutic targets worth exploring to avoid early impairments in mitochondrial energy expenditure that affects the high-energy demanding brain after ovarian hormone natural or surgical loss.

(1031) EFFECT OF TANKYRASE INHIBITION IN OVARIAN CANCER CELL LINES AND NOTCH SYSTEM CROSSTALK

SEBASTIAN BOCCHICCHIO[1], MARTA TESONE[1], GRISELDA IRUSTA[1]

[1]Laboratorio de Fisiología y Biología Tumoral del Ovario. Instituto de Biología y Medicina Experimental (IBYME - CONICET), Buenos Aires, Argentina

Notch and Wnt/β-catenin are highly conserved pathways which regulate proliferation, apoptosis and differentiation. While Notch system has widely been demonstrated to be involved in ovarian cancer, Wnt/β-catenin pathway has been poorly studied in these tumors. Besides, there is little evidence that suggests a crosstalk between them. We analyzed the effect of inhibiting these two pathways and their interaction in ovarian cancer cell lines. Two human ovarian tumor cell lines, a human granulosa-like tumor cell line (KGN) and a human ovarian adenocarcinoma cell line (IGROV-1) were incubated in the presence of a Wnt inhibitor (XAV939: 1, 10, 20 and 50 μM), a Notch inhibitor (DAPT: 15, 20 μM) or both. We evaluated the involvement of Wnt/β-catenin pathway and a crosstalk with Notch system in cellular proliferation. Our results show a significant decrease in proliferation when IGROV-1 cells were incubated in the presence of XAV939 (10, 20 and 50 μM) or DAPT (15, 20 μM). There was also a significant decrease in β-Catenin and Cyclin D1 levels together with an increase of total Axin when treated with XAV939. KGN cells also showed a significant decrease in proliferation after incubation with XAV939 (50μM). Most importantly, when IGROV-1 and KGN cells were incubated in the presence of both inhibitors, there was a synergistic decrease in proliferation suggesting a novel crosstalk between these pathways in ovarian cancer cell lines. We also tested a Wnt/β-Catenin pathway activator: LiCl. At low concentrations (10, 100 μM), KGN cells proliferation increased while β-Catenin levels remained constant. On the contrary, when KGN cells were incubated in the presence of high concentrations of LiCl (5, 10 mM), cell proliferation decreased significantly as well as total Nf-κβ, while β-Catenin levels increased. In conclusion, we demonstrate a clear involvement of Wnt/β-catenin pathway in ovarian tumor cell proliferation and suggest an interaction between this pathway and Notch system.

CONTROL OF PIP3 LEVELS BY PI3K ALPHA IN HEALTH AND DISEASE

IGNACIA ECHEVERRIA[a,b], EVAN BROWER[c,d], DANIELE CHAVES MOREIRA[a,e], YUNGLONG LIU[a], MICHELLE MILLER[a,f], B. VOGELSTEIN[c], S. B. GABELLI[a,g], AND L. M. AMZEL[a]

[a] Department of Biophysics and Biophysical Chemistry, Hopkins University School of Medicine, Baltimore, MD 21205, USA, [b] Department of Chemistry and Biochemistry, University of Maryland, College Park, Maryland 20742, USA, [c] Ludwig Center for Cancer Genetics and Therapeutics and Howard Hughes Medical Institute at the Hopkins-Kimmel Cancer Center, University School of Medicine, Baltimore, MD 21231, USA, [d] Present address: Paragon Bioservices, Baltimore, MD, USA, e Present address: Universidade Federal do Paraná, Department of Cell Biology, Brazil, [f] Medicinal Chemistry, Monash Institute of Pharmaceutical Sciences, 381 Royal Parade, Parkville, Victoria 3052, Australia, [g] Department of Medicine and Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21287, USA.

Phosphatidylinositide-3-kinase-a (PI3Ka) is a lipid kinase that catalyzes the phosphorylation of PIP2 to produce PIP3 in response to phosphorylated receptor tyrosine kinases (RTK) or their substrates. The increased levels of PIP3 initiate a number of signaling pathways by recruiting other kinases, such as Akt, to the plasma membrane leading to increased cell survival, cell motility, cell metabolism, and cell cycle progression. Levels of PIP3 are control by a balance between its production by PI3K and his hydrolysis back to PIP2 by the phosphatase PTEM, a tumor suppressor factor.

PI3Kα is composed of two subunits, p110 and p85, each comprising five domains. It is frequently mutated in many cancer types and the mutations increase PI3K kinase activity and provide growth advantages to the respective clones.

Several atomic resolution structures of the enzyme reveal that the enzyme has a complex architecture in which each domain interacts with several domains of the same or the other subunit. Structural and biochemical data show that physiological activation, as well as activation by some oncogenic mutations, involves relief of autoinhibition by dislodging the inhibitory nSH2 domain of the regulatory subunit p85 from its inhibitory position. Computational studies show that most of these effects involve, in addition to structural changes, modifications of the dynamics of the protein that alter the relative stabilities of the different states accessible to the enzyme.

Recent progress toward determining the mechanism of activation benefited from two developments: the determination of the structure PI3K bound to short chain phosphoinositides, and the characterization of the conformations accessible to the activation loop in molecular dynamics simulations.

SAIC SYMOPOSIUM I

SOLID TUMORS
THE PATHOGENESIS OF CASTRATE RESISTANT PROGRESSION OF PROSTATE CANCER IN BONE

NORA M. NAVONE

Dept. Genitourinary Medical Oncology, MD Anderson Cancer Center, Houston, Texas USA

Bone metastases typically develop in patients with advanced prostate cancer (PCa). These metastases are osteoblastic (bone-forming) and constitute the main cause of morbidity and mortality of PCa. Androgen deprivation is commonly used to treat bone metastases of PCa, but responses to such therapy are short, and eventually, the disease progresses to a castration-resistant form. Bone is the primary site of castration-resistant PCa progression, and clinical and laboratory investigation suggests that the PCa induced new bone formation contributes to PCa growth. Further development of therapies for bone metastases of PCa requires an understanding of the mechanisms underlying the growth of CRPC in bone.

The fibroblast growth factor (FGF)/FGF receptor (FGFR) complex, a signaling axis that typically mediates epithelial–stromal cell interactions (FGF axis), is central to prostate development, and is commonly altered during PCa progression. Recent studies by our group and others have implicated the FGF axis in the pathogenesis of PCa progression in bone, identified the FGF axis as a candidate target for therapy. Blockade of FGFRs with dovitinib (TK1258, Novartis Pharma), a receptor tyrosine kinase inhibitor (TKI) with potent activity against FGFR and vascular endothelial growth factor receptor has clinical activity in a subset of men with castration-resistant PCa and bone metastases. Therapy responses in these patients are associated with improvements in bone scan findings, lymphadenopathy, and tumor-specific symptoms without a proportional decline in PSA level. In fact, only a reduction in bone-specific alkaline phosphatase level was predictive of increased median treatment durations (23.6 weeks versus 10.6 weeks in those without a reduction in this level; P=0.056 [Wilcoxon rank sum test]). Integrated analyses of clinical and preclinical studies suggest that FGF signaling mediates a positive feedback loop between PCa cells and bone cells and that blockade of FGFR1 in osteoblasts partially mediates the antitumor activity of dovitinib by improving bone quality and by blocking PCa cell–bone cell interaction

Similar therapeutic activity without proportional reduction in PSA blood levels was observed with Alpharadin (Radium-223 dichloride), a bone seeking alpha emitter (FDA-Approved for castration-resistant PCa with bone metastases). These clinical findings support our hypothesis that a specific interaction between PCa and bone cells favors PCa growth in bone. Understanding the mechanism underlying the interaction between PCa cells and bone cells will help identify markers of progression and more effective targets for therapy.

GENOMICS-BASED IDENTIFICATION OF CLINICALLY-INFORMATIVE LUNG CANCER BIOMARKERS

ANA I. ROBLES

Laboratory of Human Carcinogenesis, National Cancer Institute, NIH, Bethesda, Maryland, USA

Lung cancer is the leading cause of cancer-associated deaths worldwide, despite a slow but continuous decline in incidence and mortality in Western countries over the past two decades. Global variations in lung cancer incidence largely follow historical patterns of smoking, and incidence and mortality rates are still on the rise in Asia and some countries in Latin America and Africa, where the smoking epidemic began later. Most lung cancer patients are diagnosed with locally advanced or metastatic disease, with few therapeutic options and a dismal survival rate. Cigarette smoking is the major risk factor for lung cancer and other smoking-related diseases. Even as this risk gradually decreases after smoking cessation, former smokers account for most new lung cancer diagnoses. Thus, lung cancer screening efforts have focused on older individuals with a history of heavy smoking. Implementation of lung cancer screening using Low-Dose Computed Tomography (LDCT) is expected to result in a greater proportion of lung cancers being diagnosed at an early, operable, stage. Still, the overall rate of recurrence for surgically treated Stage I lung cancer patients is up to 30% within 5 years of diagnosis. Clinically validated biomarkers for lung cancer detection, and prediction of patient prognosis and response to therapy may help patient management.

Comprehensive genomic characterization has advanced our understanding of the complex changes as-

sociated with cancer development. For lung cancer, these data have revealed vast genetic heterogeneity that poses at the same time a challenge and an opportunity to selectively target specific molecular alterations and disease subtypes. The development of molecularly targeted therapies against oncogenes that are somatically activated or translocated in tumors has revolutionized the treatment paradigm for lung cancer patients. Clinical management is increasingly based on molecular parameters in addition to histological classification, despite the fact that over 50% of lung adenocarcinomas show no clinically actionable DNA alterations. In addition, recent promising results of T cell-based immunotherapy associated high mutational burden in lung cancer patients suggest that exome-guided neoantigen identification may improve treatment responses. Thus, the implementation of targeted therapies and immunotherapy rely on accurate stratification of patients based on molecular data.

Biomarkers of lung cancer detection, patient prognosis and response to therapy have been identified through bioinformatics and statistical analyses of exome sequencing, DNA methylation, gene and miRNA expression data. Beyond a more comprehensive understanding of the molecular taxonomy of lung cancer, these biomarkers can have clinical utility for the management of early stage lung cancer patients. First, tumor-derived circulating biomarkers associated with lung cancer risk could help prioritize individuals for LDCT screening. Second, biomarkers could aid in detection of lung cancer or help discriminate malignant nodules from benign or indolent lesions. Third, biomarkers that molecularly categorize Stage I patients after tumor resection could help identify high-risk patients who may benefit from adjuvant chemotherapy or innovative immunotherapy.

SAIC SYMOPOSIUM II

ENDOCRINOLOGY, *IN MEMORIAM* DR. CARLOS LANTOS

ROLE OF MICRORNAS IN CARDIAC INJURY AND DYSFUNCTION IN PRIMARY ALDOSTERONISM

DAMIAN G. ROMERO

Department of Biochemistry, Cardio-Renal Research Center, and Women's Health Research Center, University of Mississippi Medical Center, Jackson, MS, USA

Primary aldosteronism is characterized by excess autonomous secretion of aldosterone (ALDO) independent of the renin-angiotensin system and accounts for ~10% of hypertensive patients. Excess ALDO, inappropriate for the salt intake status, causes hypertension and cardiac hypertrophy, inflammation and fibrosis that lead to cardiac dysfunction. The molecular mechanisms that trigger the onset and progression of ALDO-mediated cardiac injury and dysfunction are poorly understood.

MicroRNAs (miRNAs) are endogenous, small, non-coding RNAs that have important roles in development and cell proliferation and differentiation. miRNAs downregulate the expression levels of specific proteins by translational repression or mRNA degradation. Several miRNAs have been implicated in diverse cardiac pathologies in humans and animal experimental models, yet very little is known about their regulation and role in ALDO-mediated cardiac injury and dysfunction.

To analyze the regulation of miRNAs in ALDO-mediated cardiac injury, we performed a time-series analysis of left ventricle (LV) miRNA expression. Eight-week old uninephrectomized male Sprague Dawley rats were treated with ALDO (0.75 µg/h) infusion and SALT (1.0% NaCl/0.3% KCl) in the drinking water for up to 8 weeks. miRNA expression was analyzed by miRNA microarrays followed up by qRT-PCR and Northern-blot validation. ALDO/SALT time-dependently modulated the expression of 96 miRNAs in the LV. microRNA-21 (miR-21) was the most upregulated miRNA after 2 weeks of treatment and remained elevated until the end of the study. LV ALDO/SALT-mediated miR-21 upregulation was specific to the LV cardiac chamber and prevented by co-treatment with a triple antihypertensive therapy suggesting that such increase is blood pressure-dependent.

To elucidate the role of miR-21 in ALDO/SALT-mediated cardiac injury, miR-21 was downregulated using chemically modified antisense oligonucleotides (antagomirs) in ALDO/SALT-treated rats. miR-21 downregulation exacerbated ALDO/SALT-mediated cardiac and LV hypertrophy and cardiac dimensions determined by echocardiography. Furthermore, miR-21 downregulation increase cardiac fibrosis markers (collagen I AND III) gene expression, interstitial and perivascular fibrosis, and OH-proline content. On the other hand, miR-21 downregulation attenuated ALDO/SALT-mediated cardiac inflammation markers (Tgfb2, IL-1β, MCP-1) gene expression. miR-21 downregulation exacerbated ALDO/SALT-mediated cardiac dysfunction as observed by a further decrease in cardiac output and fractional shortening.

In summary, these results suggest that ALDO/SALT-mediated cardiac miR-21 upregulation may be a compensatory mechanism that mitigates ALDO/SALT-mediated cardiac deleterious effects. We speculate that miR-21 supplementation would have beneficial effects in reverting or mitigating cardiac injury and dysfunction in patients with primary aldosteronism.

IMPACT OF THE ENDOCRINE SYSTEM ON AGEING

JESÚS TRESGUERRES

Dept of Physiology Medical School, University Complutense of Madrid, Spain

The ageing process is apparently due to the accumulation of oxidative damage in cells and molecules that in turn are leading to vascular alterations, infections and degenerative alterations of the Central Nervous System (CNS). Skin has been shown to be also affected .The age related reduction in the activity of the immune system is linked with an enhanced susceptibility to infections, autoimmune disorders and cancer Aging is also associated with the reduction in the secretion of several hormones including GH, melatonin and estrogens, and some of the aging associated alterations could be at least partially due to this fact.

Growth hormone (GH) exerts effects on the CNS, the immune system, the skin and on the vascular endothelium. In addition, the fall in estrogens induced by ovariectomy in females or menopause in women is able to induce further deleterious effects on different organs and systems. Melatonin shows also effects on all the above mentioned organs and systems and shows also a reduction with age. The aim of our study has been to investigate the effect of chronic replacement therapy with physiological doses of GH, melatonin, estrogens and phytoestrogens on vascular function and structure, bone physiology, the immune system and on some parameters related to oxidative stress and inflammation in the CNS and liver using both a rat model of aging and a mouse model of accelerated senescence.

An increase in the aortic media thickness was seen in old animals, which showed also a reduction in the vasodilatory response to isoprenaline ($p<0.001$) as compared to young animals. GH treatment partially restored both. A reduction in the total number of neurones in old animals of both genders ($p<0.005$) has been detected together with a marked reduction of neurogenesis. Treatment with GH increased the total number of neurones without affecting neurogenesis whereas melatonin administration was able to enhance neurogenesis without affecting total number of neurones in the same way as estrogens and phytoestrogens. Markers of oxidative stress, inflammation and apoptosis have been determined in the CNS and also in the liver, showing a marked increase with age. Measurements of TNF alpha, several interleukins, iNOS, eNOS, NFkB,and parameters of apoptosis such as BCL2, BAX, BAD and others, were performed by ELISA , but also using molecular biology (PCR and Western blot) GH, melatonin and estrogen treatments were able to reduce all pro inflammatory, pro oxidative stress and pro apoptotic substances and to stimulate anti-inflammatory and anti apoptotic markers. All these actions lead in the liver to an increase in ATP formation. Age linked skin alterations, such as increased fat dermis content and reduced epidermal thickness showed a marked improvement after GH, melatonin and estrogen treatments. Keratinocytes in culture obtained from old animals presented enhanced oxidative stress and apoptosis and reduced BCl2 that were restored with GH, melatonin and estrogens.

In conclusion, chronic GH replacement to old experimental animals showed beneficial effects on body composition, vascular function and morphology, CNS and immune parameters**.** Melatonin has also shown beneficial effects on the skin, immune parameters and bone. Estrogens and isoflavones given to ovariectomized females were able to prevent oxidative changes in liver tissue and hepatocytes and also in keratinocytes in culture. All these results can be used in some of the age associated alterations, also in the human, as will be discussed during the presentation.

SAIC SYMPOSIUM III

NEURODEGENERATION AND NEUROPROTECTION

ROLE OF ASTROCYTES IN BRAIN ISCHEMIA: ACTIVE PLAYERS OR MERELY BYSTANDERS OF THE INNATE IMMUNE SYSTEM?

ALBERTO JAVIER RAMOS

Laboratorio de Neuropatología Molecular, IBCN UBA-CONICET, Facultad de Medicina, Universidad de Buenos Aires.

For almost a century astrocytes were disregarded when studying the Central Nervous System (CNS) neuronal complexity. The prevalent vision during that time simply considered astrocytes to be the brain *glue*, and

their function to give metabolic and physical support to neurons. It was not until the last decade that the glia field, and specifically the study of astroglial functions and heterogeneity, has been further explored using state-of-the-art tools. Various astroglial roles have been uncovered; including their ability to modulate neuronal physiology, synaptic transmission, neurogenesis, microcirculation, CNS development, and interaction with the innate immune system. Several of these recently described astroglial roles are very important; not only are they essential for the development and function of the healthy CNS, but they are also deeply involved in the processes occurring in the injured or diseased CNS.

Since the CNS is believed to have its own professional immune cells –microglia–, with only a limited participation of peripheral blood-derived immune cells, astroglial direct participation in the innate immunity response is still today under controversy. Innate immunity activation in the absence of infection relies on the Damage Associated Molecular Patterns (DAMP) release, which behave as ligands of the Pattern Recognition Receptors (PRR), such as Toll-like (TLR), RAGE and others. During the last years our work has been focused in studying the astroglial participation in the innate immunity response after focal brain ischemia. We have shown that astrocytes essentially behave as facultative cells of the innate immunity response that classically follows brain ischemia. Following experimental brain ischemia or *in vitro* OGD, astrocytes upregulate the expression of the PRR, including TLR2, TLR4 and RAGE. This effect parallels the well known reactive gliosis, a phenomenon largely observed by pathologists in the injured brain. Using gain- and loss-of-function studies achieved with plasmid transfection and knockout mice, we have shown that TLR expression in astrocytes facilitates astroglial response to PAMP and DAMP. TLR4 overexpression and activation by ligand also produces a sustained NF-κB activation with increased expression of the proinflammatory cytokines IL-1β and TNFα, indicative of a proinflammatory polarization of reactive astrocytes. Furthermore, we have recently shown that Triggering Receptor Expressed on Myeloid cells (TREM-2) is also expressed in astrocytes after OGD, and that TREM-2 activation by antibody crosslinking limits LPS-induced NF-κB activation. This is another typical response of cells committed to the innate immune response.

Taken together, our results show that brain ischemia induces reactive gliosis, which involves the expression of a large number of genes. These include the PRR, a family of receptors that enable astroglial direct participation in the innate immunity response, by sensing the DAMP released by the necrotic ischemic core. In this scenario, astrocytes and microglia act both as sensors but also as effectors of the innate immunity response following ischemia. Having in mind the important role of brain inflammation in the injured brain, the DAMP/PRR/NF-κB pathway emerges as a tempting target to develop new treatment strategies.

PROTECTIVE ROLE OF SEX STEROIDS AND DERIVATIVES IN MOTONEURON DEGENERATION: A TRANSLATIONAL PERSPECTIVE

MARIA CLAUDIA GONZÁLEZ DENISELLE

Instituto de Biología y Medicina Experimental (IBYME), CONICET y Departamento de Ciencias Fisiológicas, Facultad de Medicina, Universidad de Buenos Aires (UBA)

Amyotrophic lateral sclerosis (ALS) is a fatal degenerative disorder with onset in adulthood, characterized by the selective and progressive death of upper and lower motoneurons, leading to progressive paralysis of voluntary muscles. Respiratory impairment is a major cause of morbidity and mortality in these patients and respiratory function is directly related to survival time. Epidemiological studies have shown male predominance in ALS, suggesting the participation of hormonal factors in disease development. Changes of the hypothalamic-pituitary-adrenal (HPA) axis have been observed in ALS patients (Gargiulo Monachelli et, 2011; Spataro et al, 2015). The Wobbler mouse has successfully been used as an animal model for ALS in the investigation of both pathology and therapeutic treatment. Previous limited studies have also reported steroidal hormone deregulation in Wobblers. Clinically, Wobblers develop forelimb muscle atrophy and gait disturbances. In common with other rodent models and ALS, Wobbler present abnormalities of TDP43 (transactive response DNA binding protein) and ubiquitination, upregulation of the tumor necrosis factor alpha and cortical hyperexcitability (Bigini et al. 2008; Dennis and Citron 2009). Sex steroids such as progesterone have neuroprotective effects and others such as glucocorticoids at sustained high levels have neurotoxic effects. Progesterone therapy provides beneficial effects in Wobbler and superoxide dismutase 1 (SOD1) transgenic mice, reducing molecular and functional abnormalities of motoneurons (Gonzalez Deniselle et al, 2012; Kim et al, 2013).

Since progesterone is metabolized in the central nervous system (CNS) into 5 alpha-dihydroprogesterone (DHP) and 3a, 5a tetrahydroprogesterone or allopregnanolone (ALLO), the effects of this steroid may be partly mediated by its reduced derivatives. In this regard, ALLO is also neuroprotective in nerve regeneration and myelination, excitotoxic damage, ischemic stroke and disorders such as Alzheimer disease (Guennoun et al. 2015; Irwin et al 2014). We studied if ALLO, a reduced metabolite endowed with gabaergic activity, also prevents neuropa-

thology in Wobbler mice after an acute or chronic therapy. Untreated Wobblers showed increased serum levels of progesterone and its reduced derivatives DHP and ALLO vs. control animals. Treatment with ALLO elevated its levels in serum without changing the concentration of progesterone and DHP. Parameters measured in the spinal cord included brain-derived neurotrophic factor (BDNF) mRNA, p75 neurotrophin receptor (p75NTR) and TrkB receptors, the phosphorylation of the downstream AKT and the stress activated kinase JNK. Untreated Wobblers showed reduction of BDNF, TrkB, and pAKT and high expression of pJNK and p75NTR. With the exception of BDNF, these alterations were prevented by an acute ALLO treatment. On the other hand, chronic administration of ALLO enhanced BDNF mRNA and attenuated pJNK and muscle weakness, Thus, ALLO decreased motoneuron pathology and delayed disease progression. Downregulation of p75NTR may provide adequate neuroprotection at early stages of the disease. Since long-term steroid treatment also increased BDNF mRNA and reduced pJNK, both ALLO-treatment protocols should be combined in order to provide neuroprotection in motoneuron disease. In the animal model, our data shows that ALLO, a $GABA_A$ receptor agonist, might directly delay the progression of the Wobbler disease, without being metabolized into progesterone receptor agonists, DHP and progesterone.

Changes in serum concentration of sex steroid hormones and glucocorticoids also occur in ALS patients. Increased serum cortisol levels have been found in these patients, suggesting HPA axis dysfunction. Regarding sex steroids, patients with a slow and intermediate disease course showed higher progesterone serum levels than those with a rapid disease course. On the other hand, the finding of a low index-to-ring finger length ratio (2D:4D ratio) in ALS suggested an increased prenatal exposure to androgens, which might play a role in motoneuron vulnerability in adulthood. In this regard, we searched for the relationship between the circulating levels of gonadal and adrenal steroids and respiratory function, considering that respiratory failure is one frequent cause of death in ALS. Testosterone serum levels declined with age in controls but not in ALS patients. Higher dehydroepiandrosterone sulfate (DHEAS)/cortisol ratio showed a positive correlation with respiratory function, whereas elevation of circulating testosterone, as well as low progesterone/free testosterone ratio were associated with a rapid worsening of the respiratory function (Gargiulo Monachelli et al, 2014). In ALS patients, circulating gonadal and adrenal steroids are differentially expressed relative to controls, which might influence respiratory function and outcome. DHEAS or progesterone may provide a protective function, while other steroids like testosterone or cortisol probably have a negative influence.

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(1004) PROGESTERONE HAS A NEUROPROTECTIVE EFFECT AND PREVENTS DEPRESSION SIGNS IN A MALE RAT DEPRESSION LIKE MODEL

VANINA ANABEL VILLEGAS, MARÍA BELÉN MULLE BERNEDO, SEBASTIÁN GARCÍA, ANTONELLA ROSARIO RAMONA CÁCERES GIMENEZ, ROBERTO YUNES, RICARDO CABRERA

Instituto de Investigaciones Biomédicas - Facultad de Ciencias de la Salud- IMBECU-CONICET

Depression is one of the psychiatric disorders with the highest incidence in the recent decades. It has been recently related to different neurodegenerative diseases such as Parkinson. These pathologies often appear associated to a premotor sign such as depression and decreased cognitive performance.

The objective was to study and evaluate if the treatment with the neuroactive steroid progesterone could prevent the development of premotor early signs of neurodegenerative diseases in a model of catecholaminergic depletion by reserpine. Sprague-Dawley male rats (250-350 g), between 60 and 90 days old were used. The experimental groups were: C (saline), R (reserpine 0.1 mg/kg/sc, 10 injection over the course of 20 days), P (progesterone 4 mg/kg/sc) alone, 5 days after de experiments and PP + R (reserpine 5 days after a previous dose of progesterone). During the course of reserpine treatment the animals were evaluated in the catalepsy test. Forced swimming and novel object recognition were tested before the appearance of the motor signs. Data were analysed by ANOVA-1 and Tukey`s post hoc.

A significant decrease ($p<0.05$) was observed between the R vs C groups in all evaluated behavioural parameters. There were no significant differences in the catalepsy time in the PP+R vs C. Although PP+R vs R, showed a significant increase in the time spend swimming on the

force swimming test ($p<0.05$) and in the discrimination index ($p <0.05$) in novel object recognition.

We conclude that a previous progesterone treatment can avoid depression like behaviour and improves short time memory and the on/ off effect on locomotor activity. Progesterone exerts a neuroprotective effect against the reserpine treatment, preventing cognitive and depression premotor disorders induced by catecholamine depletion.

STRUCTURAL COMPOSITION AND FUNCTIONAL CHARACTERISTICS OF THE GUT MICROBIOME IN AUTOIMMUNE DISEASES

SERGIO E. BARANZINI

Department of Neurology, University of California San Francisco

A major role of the human gut microbiota is to regulate both innate and adaptive immune responses during health and disease. While most studies of the human microbiome to date have focused on analyzing microbial population structures, it is equally important to investigate how variation in microbial abundance and composition affects host functions. Responses by primary human immune cells to gut microbiota is one model for studying microbial immunoregulation. Growing evidence of microbiome alterations in multiple human autoimmune diseases and specifically of microbial regulation of immune responses in experimental autoimmune encephalomyelitis (EAE) led us to investigate changes in intestinal microbiota as a potential pathogenenic mechanism in multiple sclerosis (MS), an autoimmune disorder of the central nervous system.

MS-like symptoms in EAE can be exacerbated by T helper 1 and 17 (Th1 and Th17) responses, and modulated by Tregs. Recent studies compared the microbiota of MS patients to healthy controls, and while these studies were performed with small sample sizes and did not discriminate for treatment with disease modifying drugs, a consistent pattern of modest dysbiosis emerged.

We performed microbiome analysis by 16S rRNA gene sequencing of stool samples from 68 untreated MS patients and 64 healthy controls. Although no major shifts in community structure were found when compared to healthy controls, we identified specific microbial taxa significantly associated with MS. We found that *Akkermansia muciniphila* and *Acinetobacter calcoaceticus*, which are increased in MS, induce a proinflammatory response from human PBMCs. In contrast, *Parabacteroides distasonis*, which is reduced in MS, promotes regulatory T cell (Tregs) differentiation.

In addition, physical fractionation of bacterial communities resulted in the selection of certain populations whose abundance clearly discriminate samples of cases and controls.

Finally, microbiota transplants from MS patients into germ-free mice results in more severe experimental autoimmune encephalomyelitis and reduced Tregs compared to controls. This study identifies specific human gut bacteria that regulate adaptive autoimmune responses, suggesting therapeutic targeting of the microbiota as a novel treatment for MS.

SAIC-SAFE SYMPOSIUM (IV)

CARDIAC FUNCTION AND REGENERATION

ROLE OF P38MAPK PATHWAY IN CARDIAC POSTNATAL DEVELOPMENT

GUADALUPE SABIO

CNIC, Instituto de Salud Carlos III, Madrid, Spain

Cardiac growth is tightly regulated to ensure that the heart reaches its appropriate size. Cardiomyocytes rapidly proliferate during fetal life, but soon after birth differentiated cardiomyocytes enter a postmitotic state and the ability to proliferate is lost. Postnatal cardiac growth is therefore mainly achieved through increases in cell size (physiological hypertrophy) associated with increased protein synthesis together with the expansion of non-myocyte populations. Hypertrophic growth is also the adaptive response of cardiomyocytes to stress stimuli, including cardiac pressure or volume overload, cytoskeletal abnormalities, and intrinsic contractility defects.

Disrupted organ growth underlies the development of several diseases. Hypertrophy underlies postnatal heart growth and is activated after stress. Here we will discuss the implication of p38 pathway in this process. The relative involved of the upstream kinases MKKs in this process.

MESENCHYMAL AND CARDIAC DIFFERENTIATION OF PLURIPOTENT STEM CELLS: OVERCOMING HURDLES TO CLINICAL APPLICATION

SANTIAGO MIRIUKA

FLENI Foundation and CONICET, Buenos Aires, Argentina

Pluripotent stem cells are anchored at an early developmental stage. Once signals that keep them in an undifferentiated stage are withdrawn, they rapidly differentiate into any cell of the three germinal layers. This property is widely used for research in potential regenerative therapies, understanding the early differentiation processes in human development, and to in vitro modeling disease. The discovery ten year ago of cell reprogramming has foster many laboratories to work with pluripotent stem cells.

Our lab has been interested in mesoderm differentiation, particularly in cardiomyocyte development starting from pluripotent stem cells. Our latest research is focused in the microRNA regulation of mesoderm and cardiac differentiation. By using several bioinformatic tools, we have build a comprehensive mirnome of the regulating microRNA in this landscape. This mirnome shows that several clusters and family microRNA forms a complex network for mRNA expression. Examining the mirnome, it is possible to identify different behaviors in the expression of the individual microRNA. Interesting, families and clusters of microRNA behave in a similar way. Gene ontology analysis of these families and clusters reveals subset of microRNA that specifically regulates major signaling pathways of pluripotent stem cell and mesoderm and cardiac differentiation.

The mirnome is, however, a part of the complex regulation of mesoderm and cardiac specification. We face now two challenges. First, to analyze microRNA families as a whole, and not individual microRNAs. Second, to apply a broader view in order to understand the regulation of mesoderm and cardiac differentiation. We are therefore on the way to analyze the lncRNA population and to reveal how it relates to the mirnome.

(218) DIFFERENCES IN THE PROTECTION MECHANISMS OF PRECONDITIONING AND POSTCONDITIONING INDUCED BY VAGAL STIMULATION IN MYOCARDIAL INFARCTION IN MICE.

JAZMÍN KELLY[1], BRUNO BUCHHOLZ[1], MARINA MUÑOZ[2], EDUARDO BERNATENÉ[1], NAHUEL MÉNDEZ DIODATI[1], DANIEL GONZÁLEZ MAGLIO[3], FERNANDO P. DOMINICI[2], RICARDO J. GELPI[1]

[1]Institute of Cardiovascular Physiopathology, Faculty of Medicine, University of Buenos Aires. [2]Institute of Chemistry and Biological Physicochemistry (IQUIFIB), Faculty of Pharmacy and Biochemistry University of Buenos Aires. [3] Department of Immunology, Institute of Studies of Humoral Immunity (IDEHU UBA-CONICET), Faculty of Pharmacy and Biochemistry, University of Buenos Aires.

We have previously proven that vagal stimulation (VS) decreases infarct size both when applied before ischemia and during reperfusion. However, their mechanisms are yet unknown. Thus, our objective was to study the molecular pathways involved in the protection of preischemic VS (pVS) and reperfusion VS (rVS). Mice were randomly assigned to the following groups: Sham (n=6); 30 min of regional myocardial ischemia and 15 min of reperfusion without VS (I/R, n=6); with 10 min of pVS with (n=6) and without (n=6) muscarinic blockade with atropine; 10 min of rVS with (n=6) and without (n=6) alpha-7 nicotinic blockade with MLA. Left ventricle samples were taken for Western blotting at the end of reperfusion. IL-6 levels were assessed by ELISA in myocardium and plasma after 2 h of reperfusion. pVS increased Akt phosphorylation in comparison to the I/R and Sham group (4.2±0.64; 2.15±0.17 and 0.80±0.13 respectively). This was reversed with administration of atropine (1.13±0.34). GSK-3β had a similar increase (4.65±0.52; 2.91±0.36 and 1.05±0.05; respectively) and reversed as well with atropine (1.44±0.33). There were no significant differences in ERK1/2, JAK2 and STAT3 phosphorylation. rVS produced a non significant rise in JAK2 phosphorylation compared to I/R (2.36±0.40 and 1.64±0.2; respectively). There were no significant differences between I/R, rVS and rVS+MLA groups in Akt, GSK-3β, ERK1/2 and STAT3 phosphorylation. Additionally, rVS did not reverse the increase in IL-6 produced during ischemia/reperfusion. In conclusion, pVS reduces infarct size in mice by muscarinic activation of the Akt/GSK-3β pathway. Conversely, rVS protection seems to be mediated by alpha-7 nicotinic activation of the JAK2 pathway, independently of local myocardial and systemic anti-inflammatory responses.

REGULATION OF THE CARDIAC SODIUM CHANNEL NAV1.5 BY CALMODULIN AND CALCIUM

JESSE YODER[A], GORDON TOMASELLI[B], SANDRA GABELLI[A,B] AND L. MARIO AMZEL[A]
Departments of Biophysics and Biophysical Chemistry[a] and Medicine[b]
Johns Hopkins University School of Medicine, Baltimore, MD, USA

Voltage gated sodium channels (Nav) are membrane bound proteins that allow rapid Na^+ influx during the leading edge of action potentials in neural, cardiac, and muscle cells. Activation of the channels in response to membrane depolarization leads to the opening of their central pore allowing Na^+ influx, followed by a fast inactivation mechanism that occludes the still-open pore, ablating ion flow. The biological regulation of the channels is complex. It includes transitions from the resting-state to inactivation states such as steady-state inactivation, and the state resulting from Ca^{2+}-dependent inactivation (CDI) and FHF (a co-protein) dependent inactivation.

The molecular mechanisms of activation and inactivation are thought to involve in part the Nav cytosolic C-terminal tail (CT-Nav) and calmodulin (CaM). CT-Nav, the cytsolic portion of the channel that follows the final trans-membrane helix, contains an EF-hand like domain (EFL) with no apparent Ca^{2+}-binding ability, followed by a long helix that contains an IQ-motif. Calmodulin (CaM) binds to the IQ-motif in the presence and absence of Ca^{2+}. Ca^{2+}-free CaM enhances activation of both Nav1.4 (the skeletal muscle isoform) and Nav1.5 (the cardiac isoform). Addition of calcium and the formation of Ca^{2+}-CaM, however, inactivates Nav1.4 but not Nav1.5.

The three dimensional structure of CT-Nav1.5 in complex with apo-CaM provides insights into possible mechanisms regulating the activation of the cardiac channels. Thermodynamic data obtained using Isothermal Titration Calorimetry (ITC) provide clues about the different responses to Ca^{2+} (CDIs) of the cardiac (Nav1.5) and the muscle (Nav1.4) channels.

SAIC SYMPOSIUM V

EPIGENETICS

EARLY LIFE ADVERSITIES AND THE EPIGENETIC PROGRAMMING OF GENE EXPRESSION

EDUARDO CÁNEPA
Department of Biological Chemistry, School of Exact and Natural Sciences,
University of Buenos Aires, Argentina

The quality of brain architecture is established early in life through a series of dynamic interactions in which environmental conditions and personal experiences have a significant impact on the establishment of genetic programming. Brain architecture is constructed over a succession of ''sensitive periods'', each of which is associated with the formation of specific circuits that underlie specific abilities. The prenatal period and the first few years of life are particularly important because vital development occurs in all domains. The brain develops rapidly through neurogenesis, axonal and dendritic growth, synaptogenesis, cell death, synaptic pruning, myelination, and gliogenesis. These ontogenetic events happen at different times and build on each other, such that small perturbations in these processes can have long-term effects on the brain's structural and functional capacity. Several longitudinal studies suggest that adverse childhood experiences are associated with changes in biological systems responsible for maintaining physiological stability through environmental changes. Children exposed to maltreatment showed smaller volume of the prefrontal cortex, greater activation of the HPA axis, and elevation in inflammation levels compared to non-maltreated children. Animal research shows that environmental toxins, stress, and poor stimulation and social interaction can affect brain structure and function, and have lasting cognitive and emotional effects. In humans and animals, variations in the quality of maternal care can produce lasting changes in stress reactivity, anxiety, and memory function in the off spring. These findings raise the intriguing question of how these experiences become incorporated at the cellular and molecular level in the brain architecture leading to long-term alterations in various functions ultimately culminating in an increased risk to mental disease. Current work suggests that epigenetic mechanisms of gene regulation could explain how early life experiences can leave indelible chemical marks on the brain and influence both physical and mental health later in life even when the initial trigger is long gone. Epigenetic regulation of gene expression therefore allows theintegration of intrinsic and environmental signals in the genome, thus facilitating the adaptation of an organism to changing environment through alterations in gene activity. In this way, epigenetics could be thought of as conferring addi-

tional plasticity to the hard-coded genome. In the context of the early life environment, epigenetic changes offer a plausible mechanism by which early experiences could be integrated into the genome to program adult hormonal and behavioral responses.

Maternal malnutrition, due to its widespread incidence, remains one of the major early life adversities affecting the development of newborn's brain. An increasing number of studies point out that the effects of early-life nutritional inadequacy are persistent and lead to permanent deficits in learning and behavior. While there is no doubt that maternal malnutrition is a principal cause of perturbed development of the fetal brain and that all nutrients have certain influence on brain maturation, proteins appear to be one of the most critical for the development of neurological functions. Studies carried out by our group in recent years demonstrated that mice subjected to perinatal protein malnutrition show a delay in their physical and neurological development and present deficiencies in their learning and memory capacities and display abnormal emotional behavior, such as anxious and depressive-like disorders. Likewise, we demonstrated that these cognitive deficiencies and behavioral modifications persist during later stages of life. Currently, we investigate the molecular bases of these cognitive and behavioral deficiencies with special emphasis on epigenetic mechanisms. We focus on the hippocampus because as part of the limbic system has a major role in cognition and mood regulation. The mice that were subjected to protein malnutrition during pre- and postnatal development have a diminished expression of immediate early genes and calcineurin when confronted with an acute stress in the adulthood and display an altered miRNA-expression profile. Additionally, these mice exhibit a fewer number of neurons in the hippocampus, especially in CA3 and dentate gyrus, regions that are critical for stress response. The deep and persistent consequences of malnutrition on the intellectual and social skills of individuals affected throughout their lives represent a huge human and economic cost. Research in this area could provide knowledge for the design of suitable social or pharmacological interventions that reverse deleterious epigenetic programming triggered by adverse conditions during early life.

TOWARDS A BETTER UNDERSTANDING OF THE EPIGENETIC MECHANISMS UNDERLYING INTELLECTUAL DISABILITY: FUNCTIONAL CHARACTERIZATION OF THE HISTONE DEMETHYLASE PHF8

MARIAN MARTÍNEZ-BALBÁS

Instituto de Biología Molecular de Barcelona (IBMB), Spanish Research Council (CSIC), Barcelona Science Park (PCB), Barcelona 08028, Spain.

Histone methylation is a regulatory mark that serves to control the transcriptional programs. In the last years several histone demethylases (HDM) have been identified as important players in neural development and function and their molecular mechanisms of action are starting to be underscored. PHF8 is a recently identified HDM that removes H4K20me and H3K9me2 marks. Interestingly, mutations on the PHF8 catalytic domain lead to mental retardation and autism. Although the activity of PHF8 is well characterized in vitro, the molecular mechanisms responsible for its role in nervous system development and function are not clearly established. In this talk we will discuss a new function of PHF8 fine tuning the transcriptional activity of genes to properly respond to external signals.

EPIGENETIC REGULATION IN THE HEMATOPOIETIC SYSTEM

MARIA E. FIGUEROA

University of Miami Miller School of Medicine, Florida, USA

Maintenance of the hematopoietic stem cell (HSC) pool is crucial for the production of mature blood and bone marrow cells. With age, there is loss of HSC function, exemplified by a decreased homing ability and an increased predisposition to differentiate into myeloid rather than lymphoid cells. This age-associated loss of HSC function contributes to an impaired hematopoietic system; elderly individuals have increased rates of anemia, loss of adaptive immunity, and an increased risk to develop myeloid malignancies. One such disorder is Myelodysplastic Syndromes (MDS), a heterogeneous group of malignancies seem most frequently in elderly individuals. Mutations in proteins involved in alternative splicing and epigenetic modifiers such as DNMT3A and TET2, which are frequently observed in MDS, can also occur in otherwise healthy elderly individuals. However, little is known about epigenetic deregulation in the human hematopoietic system with aging, and whether such deregulation predisposes for MDS. In order to investigate this, we examined epigenetic profiles in FACS purified HSC/HSPCs isolated from bone marrow from young (18-30 yo) and elderly (65-75 yo) healthy donors. For each age group

we performed 5-6 biological replicates of genome-wide chromatin immunoprecipitation followed by sequencing (ChIP-seq) for promoter-associated chromatin marks (HeK4me3 and H3K27me3), enhancer-associated histone modifications (H3K4me1 and H3K27ac), as well as genome-wide cytosine modifications (5-methylcytosine and 5-hydroxymethylcytosine) and gene expression profiled by next-generation RNA sequencing. We have found marked epigenetic differences during normal aging that target enhancer and promoter regions of genes involved in key pathways in development and disease. These differences consist primarily of a loss of activation marks. A subset of bivalent promoters targeting the WNT, cadherin and hedgehog signaling pathways display loss of bivalent potential with aging. Analysis of cytosine modification profiles revealed only moderate changes in 5-methylcytosine while 5 hydroxymethylcytosine was markedly increased with aging. Finally, RNA-seq analysis revealed downregulation of gene transcription and aberrant patterns of alternative splicing with aging, which targeted important hematopoietic transcription factors and epigenetic modifiers. Our findings demonstrate that even in the absence of disease, aged HSCs shows massive epigenetic reprogramming targeting important pathways in development and hematopoiesis.

SAIC-SAFE SYMPOSIUM VI

MOLECULAR PARASITOLOGY

TOXOPLASMA HISTONE VARIANTS AND THEIR ROLE IN DIFFERENT PARASITE PROCESSES

SERGIO O. ANGEL, LAURA VANAGAS, SILVINA S. BOGADO

IIB-INTECH, CONICET/UNSAM, Argentina

Toxoplasma gondii is a coccidian protozoan parasite that belongs to the phylum Apicomplexa. It is estimated that toxoplasmosis exists as a chronic asymptomatic form in 5 hundred million to 1 billion of the world human population. Although infection with *T. gondii* is usually asymptomatic in most individuals, it is of great medical significance for pregnant women and immunocompromised patients. In humans, *T. gondii* infection is characterized by two stages, the rapidly growing tachyzoites, and the latent bradyzoite tissue cysts. Tachyzoites are responsible for acute illness and congenital birth defects. *T. gondii* tachyzoites contain basal levels of γH2A.X, a marker of double strand break (DSB) damage, even in normal conditions lacking a DNA damaging stress. This parallels what is seen due to fork collapse under replication-associated DNA stress in cancer cells. Bradyzoites form cysts that remain latent for many years but are still capable of converting into the destructive tachyzoite form if host immunity decreases. These two developmental stages are essential for cause and propagation of disease. Tachyzoite to bradyzoite conversion, and vice-versa, includes a high number of gene expression modifications. It is believed that the epigenetic control of gene regulation is crucial for parasite development, a process that relies on the post-translational modification (PTM) of histones and histone variant exchange. *T. gondii* possess the four canonical histones H2A, H2B, H3 and H4 and variant histones of H3 and H2A families. Concerning the H2A family, the parasite has H2AZ and H2A.X variants, a feature that is not shared by other apicomplexan parasites in which H2AX is not present. In higher eukaryotes, the role of H2A.Z is associated to transcriptional regulation, genome stability, and blocking the spread of heterochromatin, whereas H2A.X is involved in DNA repair being recruited at double strand break (DSB) site, a process that requires the phosphorylation of the serine present at C-terminal motif SQEY/F. Interestingly, *T. gondii* has a variant of H2B, that has been named H2B.Z since it forms dimers mainly with H2A.Z. Double variant H2A.Z/H2B.Z nucleosome and H2A.X/H2Ba are not present in the same nucleosome as it was observed by ChIP-qPCR and ChIP-seq. These findings reveal that nucleosomal arrangements are not random in protozoa, highlighting their relevance in chromatin composition and regulation. H2A.Z and H2B.Z have shown to be highly acetylated at their N-terminal tails, a marker of active chromatin. *H2A.Z* and *H2B.Z* genes have shown to be essential. The over-expression of different H2B.Z mutants, that are unable to acetylate the N-tail, has shown little effect in tachyzoite replication rate but an important alteration in the differentiation process. On the other hand, proteomic analysis confirms the presence of gH2A.X in normal conditions suggesting that tachyzoites may be subjected to fork collapse and DSB, situations that activate the homologous recombination repair machinery. H2A.X is phosphorylated at its SQE motif by ATM kinase at the initial step of HRR pathway. Based on that, the impact on tachyzoite replication of CPT, which generates DSB during DNA replication, and KU55933, a highly specific ATM inhibitor was analyzed. Both, CPT and KU55933 produced a significant effect on parasite replication suggesting their inhibition effect may be blocking *T. gondii* DNA replication and/or activating cell cycle checkpoints either affecting *T. gondii* ATM directly or through HFF ATM inhibition. As expected, the combination of both

drugs generated a serious blocking of replication rates with a large number of tachyzoites that were not able to carry out the first event of replication. Taken together the results show that histone variants and their PTM are important epigenetic regulators in different processes of the parasite life cycle.

NEW THERAPEUTIC STRATEGIES FOR ECHINOCOCCOSIS: MODIFICATION ON THE DRUG RELEASE TO INCREASE BIOAVAILABILITY AND EFFICACY

MARÍA CELINA ELISSONDO

Laboratorio de Zoonosis Parasitarias, Facultad de Ciencias Exactas y Naturales
CONICET y Universidad Nacional de Mar del Plata, Argentina.

Echinocccosis, also known as hydatid disease or hydatidosis, is a parasitic zoonoses caused by infection with the larval stage of the cestode *Echinococcus* spp. The World Health Organization has recently included human echinococcosis within the group of neglected tropical diseases, and recommends a veterinary public health strategy as part of an effective control approach.

Depending on different factors such as cyst number, size and location, viability status, the involved organ and location, the interaction between the expanding parasite and the adjacent host tissue and bacterial and fungal infection, there are different treatment and management options human echinococcosis.

In the last 30 years, an increase in the use of anthelmintic drugs for the medical treatment of echinococcosis was observed. The only two drugs licensed to date are the benzimidazole carbamate derivatives albendazole and mebendazole. Albendazole belongs to Class II of the biopharmaceutical classification system, with high permeability and low aqueous solubility. Approximately a third of the patients treated with benzimidazole drugs have been cured, 30–50% develop some evidence of a therapeutic response while between 20 and 40% of cases do not respond favourably. Therapeutic failures attributed to medical management of echinococcosis with albendazole have been primarily linked to the poor drug absorption rate (<5%) resulting in low drug level in plasma and cysts. On the other hand, the poor water solubility of albendazole offers only few formulation possibilities, limiting the administration routes.

Novel and improved therapeutical tools are needed in order to optimize treatment of human echinococcosis. Unfortunately, the pharmaceutical industry is not developing novel treatment options besides benzimidazoles against these neglected diseases. Novel chemotherapeutics have to be identified by one of the following strategies (1) *in vitro* and *in vivo* testing of broad-spectrum anti-infective drugs and drugs inhibiting proliferation of cancer cells; (2) new drug targets are being identified from the knowledge of the genome, transcriptome, proteome and metabolome of the parasite; (3) the use of pharmacotechnical strategies to optimize the use of existing drugs.

In this presentation, some of the new technologies applied to the experimental treatment of echinococcosis will be discussed. During the last 17 years, the Parasitic Zoonoses Research Group (Faculty of Natural and Exact Sciences, Mar del Plata National University) has been working in the experimental chemotherapy of hydatid disease. Since 2011, in a joint effort with the Laboratory of Pharmacotechnics (Faculty of Chemistry, National University of Cordoba), different albendazole drug delivery systems are being studied with the aim to improve the treatment against the murine model of echinococcosis. After evaluating the clinical and chemoprophylactic efficacy of a solid dispersion, nanocrystals and lipid nanoparticles, promising results have been obtained.

PARASITE AND HOST GENETIC DIVERSITY IN TRANSPLACENTAL TRANSMISSION OF *TRYPANOSOMA CRUZI*

NATALIA JUIZ, SILVIA LONGHI AND ALEJANDRO G. SCHIJMAN

LABMECH- INGEBI-CONICET, Buenos Aires, Argentina

Congenital Chagas diseases has striking impact in public health, being partially responsible of the emergent urbanization of Chagas disease, not only in endemic countries but also in non-endemic continents due to migration movements.

The occurrence of this type of transmission in a variable percentage from 2 to 10% of descendants of infected pregnant women depends on the geographic área and is a product of the complex interaction of parasitic factors, such as the parasitic maternal load and the parasite genotype as well as genetic factors of the mammalian host.

The placenta is the key barrier the parasite must invade to reach the faetus so it is crucial to investigate the nature of placental factors to understand the mechanism

of transmission. In this context, we have started to study the existence of association between genetic host patterns and the likelihood of congenital transmission. We have explored associations between SNP polymorphism in placentary genes and congenital infection and have initiated transcriptomic analyses in murine model and human placentas.

Analysis of SNPs in placentary genes. A retrospective study was carried out using human DNA obtained from 217 blood samples from 101 congenitally infected and 116 non-infected children born to Chagasic mothers. We have analyzed the sequences of eleven SNPs located in 4 loci that encode placentary enzymes, described top lay a role in congenital transmission, namely: rs2014683 and rs1048988 in *ALPP gene coding for placental alkaline phosphatase*, rs11244787 and rs1871054 in *ADAM12*, rs243866, rs243865, rs17859821, rs243864 and rs2285053 de *MMP2* and rs3918242 and rs2234681 in *MMP9, all three genes with metalloprotease activities. SNP identification was achieved after developing Real Time PCR systems followed by* "High Resolution Melting analysis" for discrimination of allelic copies and by sequencing in a microsatellite variant.

An association was observed between SNPs in gene MMP2 and ADAM 12. Logistic regression analysis under dominant and recessive models revealed that for SNPs rs243866 and rs17859821 of gene MMP2, one copy of the mutant allele, "A" in both cases, was necessary to increase the risk of congenital infection. In the case of SNP rs2285053, both copies of the "T" variant are necessary. With respect to gene ADAM12, the frequency of SNPs rs11244787 and rs1871054 differed between infected and non-infected groups by comparing genotypic and allelic frequencies as well as after regression analysis under the dominant model. In the case of SNP rs1871054, a protective effect of the allele "T" was also observed under the recessive model. Following recent studies that proposed gene to gene interactions as involved in the pathogenesis of diseases, we carried out a multifactorial dimensional analysis to identify the existence of differential SNP-SNP interactions between infected and non-infected groups. Genotyping of five sites rs11244787, rs1871054, rs243866, rs17859821 and rs243864 would be good predictors of the susceptibility of congenital infection.

Transcriptomic studies in placental genes. We performed functional genomics by microarray analysis in C57Bl/6J mice comparing placentas from uninfected animals and from animals infected with two *T. cruzi* strains: K98, a clone of the non-lethal myotropic CA-I strain (TcI), and VD (TcVI), isolated from a congenitally infected patient. Analysis of networks by GeneMANIA of differentially expressed genes showed that "Secretory Granule" was a pathway down-regulated in both infected groups, whereas "Innate Immune Response" and "Response to Interferon-gamma" pathways were up-regulated in VD infection but not in K98. Applying another approach, the GSEA algorithm that detects small changes in predetermined gene sets, we found that metabolic processes, transcription and macromolecular transport were down-regulated in infected placentas environment and some pathways related to cascade signaling had opposite regulation: over-represented in VD and down-regulated in K98 group. We also have found a stronger placental tropism of the VD strain, by detection and quantification of parasite satellite DNA and 18s RNA, indicative of living parasites in the tissue sample.

Transcriptomic analysis by means of RNAseq are currently undergone to compare differentially expressed genes in human placentas from pregnant women with and without detectable parasitic burden measured by Real Time PCR.

Our study is the first one to describe genetic characteristics, such as SNPs in placental expressed genes and the genetic response of placental environment to *T. cruzi* infection and suggests the development of a strong immune response, parasite genotype-dependent, to the detriment of cellular metabolism.

IMPACT OF PHARMACOKINETIC IN THE CLINICAL EFFICACY OF ANTHELMINTICS

LUIS I. ALVAREZ

Centro de Investigaciones Veterinarias de Tandil (CIVETAN), FCV, UNCPBA-CICPBA-CONICET, Tandil, Argentina.

Pharmacokinetics describes how the body affects the movement of a drug after its administration, and determines the drug concentration at the biophase (the site of action). Since the higher the concentration achieved at the tissue where the parasite is located, the greater the amount of drug reaching the target parasite receptor, pharmacokinetic is a key factor in anthelmintic efficacy. Understanding the mechanisms involved in drug access to the target parasite, together with drug pharmacodynamics, will enhance overall comprehension of anthelmintic drug activity. Two main issues are crucial to the comprehension of the process of drug accumulation into nematodes: (i) the oral versus transcuticular entrance routes, and (ii) identification of the main drug transport mechanism (active transport versus passive diffusion) involved in the transfer process. Lipophilicity and concentration of the active drug, the physico-chemical features of the parasite-surrounding medium, the structure of parasite's external surface, are among the factors affecting the transfer (diffusion) and accumulation of the active drug into the target parasite(s). Additionally, the particular mode of action of each compound will affect the onset and the character-

istic of the anthelmintic effect. Altogether, these different factors will determine the final anthelmintic activity. The pharmacokinetic "barrier" may explain many therapeutic failures observed in parasite control in both human and veterinary medicine, which in some cases have contributed to exposure of target parasites to subtherapeutic drug concentrations. The characterisation of drug concentration profiles in tissues of parasite location and within target parasites, and its relationship with the mode of action of each particular molecule provides a basis for understanding the differences in efficacy observed for the different chemical families. The entry of a drug into the parasite by a transtegumental/cuticular diffusion process may mainly depend on the diffusion surface, the concentration gradient across the membrane, the pH/pK relationship and the lipophilicity of the molecule. The amount of drug reaching the target nematodes is influenced by the drug concentrations in the tissues where the parasite is located. The higher concentrations measured in the gastrointestinal content, accounted for a greater amount of drug being measured within adult target worms recovered from treated animals. Drug absorption across nematode cuticle is restricted by lipid barriers in the hypodermis and collagen matrix. The rate of penetration across the cuticle depends mainly on lipophilicity, and, in the case of acidic or basic drugs, on the ionized and unionized (lipid-permeable) fractions of the drug, which is determined by the relationship between drug pK and pH of the aqueous environment within the cuticle. Although the oral route cannot be discarded, there is clear evidence that transcuticular diffusion is the common route of access for different anthelmintics in nematodes. A different situation occurs in trematode parasites such as *Fasciola hepatica*, in which the accumulated data confirm that oral ingestion is a main route of drug entry into adult liver flukes *in vivo* exposed to flukicidal drugs. Consequently, there is a strong relationship between blood concentration and flukicidal activity. This mechanism of drug entry has been observed under *in vivo* conditions for different flukicidal drugs such as triclabendazole, albendazole and closantel. The pharmacokinetic-pharmacodynamic relationship of anthelmintic drugs will be discussed based on different *in vivo* examples including trematode and nematode parasites.

SAIC SYMPOSIUM VII

ROLE OF MOLECULAR CHAPERONES IN HEALTH AND DISEASE

RELEVANCE OF THE HSP90-IMMUNOPHILIN CHAPERONE SYSTEM IN THE REGULATION OF BASIC BIOLOGICAL PROCESSES IN HEALTH AND DISEASE

G.I. MAZAIRA [1], C. DANERI [2], M.F. CAMISAY [1], N.R. ZGAJNAR [2], S.A. DE LEO [1], F. FEDERICCI [1], A.A. CAUERHFF [1], M.LAGADARI [2], A.G. ERLEJMAN [1], M.D. GALIGNIANA[1,2]

[1] Departamento de Química Biológica-Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina. [2] IBYME-CONICET, Buenos Aires, Argentina.

Hsp90 is the major soluble protein of the cell. Most of the Hsp90 population is primarily cytoplasmic, and a small fraction is also nuclear and plays several structural and functional roles. In the cell, Hsp90 is a homodimer. Each protomer contains three flexibly linked regions-the N-terminal domain (or ATP-binding domain), the middle M-domain, and the C-terminal domain or dimerization domain. The latter shows a conserved MEEVD motif that serves as the docking site for Hsp90 co-chaperones via a tetratricopeptide repeat (TPR) clamp. Although in some studies is still under discussion what the real stoiquiometry of the interaction between Hsp90 dimers and TPR-domain co-chaperones is, there is a general consensus that in the cell it is likely that there is only one TPR protein bound per dimer of Hsp90. This early finding of our laboratory was subsequently validated by the regulatory action observed for several biological properties of Hsp90 client proteins due to the functional exchange of high molecular weight immunophilins such as FKBP51 and FKBP52 associated to Hsp90 via that TPR clamp. Here we will discuss the biological relevance of the Hsp90-immunophilin heterocomplex in the regulation of several biological models such as the steroid receptor function in health and disease, its involvement in cancer development and progression, the regulation of telomerase activity, the ability to promote the nuclear retention of transcription factors by nucleoskeleton arrangement, and its role in cell differentiation. In all of these basic biological situations, the properties shown by the Hsp90-immunophilin chaperone heterocomplex in the cell supports the existence of a single Hsp90-binding immunophilin bound per Hsp90 dimer, which can be dynamically exchanged by other TPR-domain proteins in a mutually exclusive fashion. In view of the number and relevance of signalling cascades and cellular events affected by this heterocomplex, the potential use of drugs with therapeutic purposes that may affect the organization and function of such protein arrangement is currently assayed in clinical trials.

THE STRESS PROTEIN FKBP51 SHAPES ANTIDEPRESSANT PHARMACOLOGY AND LINKS TO A NOVEL DRUGGABLE ROUTE TO AUTOPHAGY

GASSEN, N.C.[1]; STEPAN, J.[2]; BALSEVICH,G.[2]; HARTMANN,J.[2]; GENEWSKY, A.[2]; HAFNER, K.[1]; SCHMIDT, M.V.[2]; EDER, M.[2]; REIN, T.[1]

[1]Max Planck Institute of Psychiatry, Department of Translational Research in Psychiatry, Kraepelinstr. 10, Munich 80804, Germany; [2]Max Planck Institute of Psychiatry, Department of stress neurobiology and neurogenetics, Kraepelinstr. 10, Munich 80804, Germany

FK506 binding protein 51 (FKBP51) is both regulator and target of the stress receptors. Genetic evidence implicated FKBP51 in stress-related psychiatric diseases such as depression and furthermore in the responsiveness to antidepressants. Since the molecular underpinnings remained elusive we set out to decipher intracellular pathways regulated by both antidepressants and FKBP51. Interaction analyses led to several convergently regulated molecular pathways in cells, mice and human. These pathways include GSK3beta, Akt1-Autophagy, and DNA methyltransferase 1 (DNMT1). Our studies characterize FKBP51 as remarkably versatile stress protein and scaffolder of multiple protein complexes. In patients suffering from depression, markers of these pathways predict clinical treatment response. To test whether these pathways might be causally involved in antidepressant action we used small molecules to address novel autophagy pathway components downstream of FKBP51. Some of them mimicked the action of antidepressants on autophagy pathway activity. Based in voltage-sensitive dye imaging, mini-EPSCs, and in vivo electrophysiological recordings, these compounds, acting downstream of FKBP51, also changed synaptic function similarly to antidepressants. Moreover, we observed antidepressant-like behavioral effects in mice. These compounds thus provide a novel route to autophagy, reveal a particular form of neuronal autophagy, and identify novel compounds for autophagic therapy in psychiatric and other diseases.

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DIRECT TARGETING OF THE FKBP52 COCHAPERONE FOR THE TREATMENT OF CASTRATION RESISTANT PROSTATE CANCER

N. GUY[1], H. XIE[2], J. CHAUDHARY[3], A. CHERKASOV[4] AND M.B. COX[1*]

[1]Border Biomedical Research Center and Department of Biological Sciences, University of Texas at El Paso, 500 W. University Ave., El Paso TX 79968, USA; [2]College of Pharmacy and Health Sciences, Texas Southern University, Houston, TX, USA; [3]Center for Cancer Research and Therapeutic Development, Clark Atlanta University, Atlanta, GA, USA; [4]The Prostate Centre, Vancouver General Hospital, Vancouver, Canada

The FKBP52 cochaperone is a positive regulator of androgen (AR), glucocorticoid (GR) and progesterone receptor (PR) function and represents an attractive target for the treatment of castration resistant prostate cancer. Towards this end, we previously identified MJC13, which represents a first-in-class drug for targeting the regulation of AR by FKBP52 through binding a putative FKBP52 regulatory surface on AR. While the targeting of the FKBP52 regulatory surface on AR is a promising therapeutic strategy, we propose that the direct targeting of FKBP52 offers a number of advantages over MJC13 that would lead to a more potent and effective drug. Thus, we performed a high-throughput *in silico* screen of the ZINC database consisting of 20-million lead-like compounds. We identified GMC1 as the initial hit molecule with the most potent inhibition of FKBP52-mediated AR reporter expression. GMC1 effectively blocks AR, GR, and PR activity, blocks endogenous AR-mediated gene expression, and inhibits the proliferation of prostate cancer cell lines. As proof-of-concept we developed a soluble GMC1 co-solvent formulation and demonstrated that GMC1 prevents tumor growth and causes tumor recession in LNCaP and CW22Rv1 xenograft mouse models. These studies are at the forefront of an emerging concept to target novel AR co-regulators for the treatment of prostate cancer.

(1073) THE PEPTIDYLPROLYL-ISOMERASE ACTIVITY OF FKBP52 IS REQUIRED TO ENHANCE NF-kB BIOLOGICAL ACTION

S.A. DE LEO[1], M.F. CAMISAY[1], M. D. GALIGNIANA[1, 2], A.G. ERLEJMAN[1]

[1]departamento De Química Biológica / IQUIBICEN, FCEN, UBA, Buenos Aires, Argentina. [2]IBYME, Buenos Aires, Argentina

Nuclear Factor κB (NF-κB) is a transcription factor that regulates the expression of genes involved in inflammation, cell cycle and cell death. NF-κB aberrant activation is relevant in chronic inflammatory diseases and cancer promotion. Inactive NF-κB is primarily cytoplasmic, and translocates to the nucleus upon activation. High MW FK506-binding proteins (FKBPs) are Hsp90-binding proteins first related to steroid receptor action. A signature property for FKBPs is the peptidylprolyl isomerase (PPIase) activity. Previously, we reported that FKBP51 and FKBP52 modulate canonical NF-κB (p65/p50) biological actions in an antagonistic fashion. In this work, we studied the contribution of FKBP52-PPIase activity on NF-κB biological action at different steps of its activation cascade, i.e. transcriptional activation by gene reporter assay, p65 nuclear relocalization by indirect immunofluorescence, and p65 phosphorylation at Ser536 by Western blot. NF-κB nuclear translocation was favored by overexpression of FKBP52 in HEK293T cells treated for 1 h with TNFalpha, while a mutant lacking enzymatic activity (FKBP52 F130Y) showed a decreased nuclear translocation. Accordingly, FKBP52 favored p65 phosphorylation as demonstrated by Western blot with a specific antibody and a band shift after alkaline phosphatase treatment. FKBP52 showed a strong stimulating effect on PMA-induced NF-κB transcriptional activation. The relevance of the PPIase activity for this effect was indirectly evidenced by the lack of stimulation in cells treated with the inhibitor FK506 (tacrolimus), and confirmed by overexpression of two inactive PPIase mutants. In contrast to FKBP52, its homologous partner FKBP51 impaired p65 nuclear translocation and transcriptional activity, the PPIase activity and its association with Hsp90 via the TPR domain being not required. In summary, FKBP52 activates NF-κB signalling cascade at various steps, its PPIase activity playing a key role in this regulation.

SAIC-SAFE SYMPOSIUM VIII

ONCO-HEMATHOLOGY AND INFLAMMATION

IDENTIFYING THE MECHANISMS DRIVING INV(16) ACUTE MYELOID LEUKEMIA

LUCIO H. CASTILLA

Department of Molecular, Cell and Cancer Biology, University of Massachusetts Medical School, USA

The pathology and treatment of acute myeloid leukemia (AML) is largely defined by the mutation composition in their blast cells. The development of effective specific therapies in AML that target the function of driver oncoproteins is lacking. A fraction of AML cases have the chromosome 16(p16;q22) inversion, which encodes the leukemia fusion protein CBFβ-SMMHC. We have shown that this fusion protein is a founding event that creates a pre-leukemic myeloid progenitor cell, and drives leukemia development and maintenance in mouse models and patient-derived AML cells. Recently, we have developed a specific inhibitor of CBFβ-SMMHC function, AI-10-49, with excellent potential as a candidate for inv16 AML targeted therapy. This small molecule binds to the CBFβ portion of CBFβ-SMMHC with high specificity, inhibits its binding to RUNX1, and restores expression of RUNX1 target genes. AI-10-49 induces apoptosis of leukemia cells expressing CBFβ-SMMHC with high potency and specificity within 24 hours. AI-10-49 presents excellent pharmacokinetics and negligible toxicity in mice, and delays leukemia latency in a mouse model. Furthermore, this molecule shows selective activity in human inv16 AML blasts in vitro. Recent studies in my laboratory have combined pharmacologic and genomic (ATACseq, ChIPseq and RNA-seq) approaches in human AML cells complemented with genetic mouse models to understand the how AI-10-49 induces apoptosis in inv16 AML. These studies have identified key enhancers driven by RUNX1 and BRD4 that provide survival of leukemia cells, and that are repressed in the presence of AI-10-49. These studies will provide a rationale for developing effective combined targeted therapies for inv(16) AML.

ONCOGENIC ENHANCER REARRANGEMENTS IN AML

RUUD DELWEL

Department of Hematology, Erasmus University Medical Center, Rotterdam, The Netherlands.

Introduction: The magnitude of data obtained through cancer genomics has greatly improved our insights into gene mutations and the effects of the mutated products on the biology of the disease, but it has yet to fulfil the promise of generating effective new therapies. Recent advances in molecular cancer research revealed entirely novel roles for epigenetic dysregulation of gene expression in the pathogenesis of acute leukemia. Since chromatin regulators are frequently amenable to small molecule inhibition, mutated regulatory regions are attractive targets for treatment of malignancies that are refractory to chemotherapy. In our program, we focus on advancing epigenetic approaches for a better understanding of leukemia dysregulation with the ultimate goal of pursuing unexplored epigenetic therapeutic opportunities.

An enhancer rearrangement uncovered in AML with inv(3)/t(3;3) applying functional genomics and genome editing.

Acute myeloid leukemia (AML) with chromosomal rearrangements inv(3) or t(3;3) is characterized by overexpression of the proto-oncogene EVI1 and clinically by an extremely poor response to therapy. We recently uncovered the molecular basis of EVI1 deregulation of AML subtype with inv(3)/t(3;3). High-resolution mapping of chromosomal breakpoints by 3q-seq revealed a breakpoint-free segment of 18 kb size near GATA2 (3q21) relocating to the EVI1 locus (3q26) in all analyzed cases. 4C-seq was carried out to study the three-dimensional chromatin environment of the EVI1 promoter, which revealed a contact hotspot of 9 kb within this commonly rearranged segment. ChIP-Seq analysis for enhancer-associated p300-binding confirmed a p300 interacting region in the center of the 9kb chromatin segment that formed a complex with EVI1 promoter. Excision of the ectopic enhancer element in an inv(3) cell line model (MUTZ-3), using CRISPR/Cas9 genome editing, abrogated EVI1 transcription and led to a profound reduction in cell viability, higher rates of apoptosis, along with increased maturation of the AML cells toward monocyte/macrophage phenotype. Exactly the same effects were observed upon EVI1 knock-down in these cells. Thus, aberrant EVI1 expression was caused by the reallocation of an enhancer, which caused uncontrolled expression of the transforming EVI1 gene.

We found that the identified enhancer element was a constituent of the of the GATA2 regulatory domain located at 3q21. RNA-seq and qPCR analysis confirmed reduced and monoallelic GATA2 expression only from the remaining normal chromosome 3 allele in inv(3)/t(3;3) AMLs, compared to control AMLs. Thus a single oncogenic enhancer rearrangement causes concomitant EVI1 and GATA2 deregulation in inv(3)/t(3;3) AMLs. In 20% of the inv(3)/t(3;3) AML patients, mutations were found in the coding region of GATA2 in the expressed the allele that was expressed. Thus, in those cases, no wild type GATA2 was present in those cells, emphasizing that aberrant GATA2 expression and function plays an important role in leukemia onset, development and/or maintenance in inv(3)/t(3;3) AML.

EVI1 is activated by an oncogenic super enhancer in AML with inv(3)/t(3;3)

We discovered that the GATA2 enhancer which translocates to EVI1, turns into a so-called oncogenic super-enhancer or stretched enhancer (OSE) in AML with inv(3)/t(3;3). The newly derived oncogenic EVI1-OSE is hyper-sensitive to pharmacologic BET-inhibitors, whereas the non-rearranged enhancer near GATA2 is insensitive to this compound. The mechanism of action of OSEs, why they are hyper-responsive to BET-inhibitors and which other components are essential for their activity is not understood. Nevertheless the lack of understanding, are BET-inhibitors currently applied in Phase I/II clinical trials, in particular for patients with acute myeloid leukemia (AML) that are refractory to chemotherapy. These patients are not selected based on prior knowledge about their sensitivity to those drugs or whether OSE-activated disease genes play a role in leukemic transformation of these patients. Since, normal super enhancers (SEs) are also sensitive to BET-inhibitors, there are question marks about the specificity of these compounds. An important difference between SEs and OSEs is that SEs are precisely regulated and can be switched on or off during cellular development, whereas OSEs are constitutively active. We hypothesize that altered regulation of the OSEs is caused by critical regulators of transcription and involves specific domains in the mutated OSE.

Conclusion: Our studies show that altered enhancer function is crucial in leukemia development in at least a portion of human AMLs and that molecular understanding of defective transcriptional control may provide leads for hypothesis driven targeting of cancer.

IDENTIFYING MECHANISMS OF GLUCOCORTICOID RESISTANCE IN RELAPSED PEDIATRIC T-ALL

JUSTINE E. RODERICK, KAYLEIGH GALLAGHER, KATHERINE M. TANG, OLIVIA KUGLER-UMANA, JULIE ZHU, MICHAEL R. GREEN AND MICHELLE A KELLIHER

Department of Molecular, Cell and Cancer Biology, University of Massachusetts Medical School, Worcester MA 01605, USA

While great strides have been made in the improvement of outcome for newly diagnosed pediatric acute lymphoblastic leukemia (ALL) patients, prognosis for relapsed leukemia patients remains poor. The synthetic glucocorticoid (GC) dexamethasone is part of the standard treatment for pediatric ALL and patient response to glucocorticoid treatment has proved to be a reliable prognostic indicator[1–3]the largest multicenter trial of the Berlin-Frankfurt-M\u00fcnster (BFM. Identifying the biological pathways responsible for glucocorticoid resistance may reveal novel therapeutic targets to prevent and treat relapsed ALL. Although genomic analyses of relapsed patients and matched diagnosis-relapse patient pairs have begun to define the genomic landscape of relapsed disease [4–6]but the prognosis is dismal for the minority of patients who relapse after treatment. To explore the genetic basis of relapse, we performed genome-wide DNA copy number analyses on matched diagnosis and relapse samples from 61 pediatric patients with ALL. The diagnosis and relapse samples typically showed different patterns of genomic copy number abnormalities (CNAs, discerning "driver from passenger" genetic lesions remains challenging. To identify glucocorticoid resistance genes in an unbiased, high-throughput manner, we conducted a genome wide, survival based, shRNA screen in dexamethasone sensitive murine T-ALL cells. Our preliminary data identify several hundred genes capable of mediating GC resistance, including several known GC resistance genes *Nr3c1*, *Rcan1, Btg1* and *Mllt10*, thereby validating our experimental approach. Candidate genes identified in the screen including *EP300* (p300), *GATA3* and *IKZF1* are known leukemia suppressors in pediatric ALL and the EP300 paralog CREBBP and IKAROS have been linked to GC resistance[5]but the biological determinants of treatment failure remain poorly understood. Recent genome-wide profiling of structural DNA alterations in ALL have identified multiple submicroscopic somatic mutations targeting key cellular pathways, and have demonstrated substantial evolution in genetic alterations from diagnosis to relapse. However, DNA sequence mutations in ALL have not been analysed in detail. To identify novel mutations in relapsed ALL, we resequenced 300 genes in matched diagnosis and relapse samples from 23 patients with ALL. This identified 52 somatic non-synonymous mutations in 32 genes, many of which were novel, including the transcriptional coactivators CREBBP and NCOR1, the transcription factors ERG, SPI1, TCF4 and TCF7L2, components of the Ras signalling pathway, histone genes, genes involved in histone modification (CREBBP and CTCF, indicating that suppressor genes involved in human leukemia and GC resistance are identified in our mouse screen. Consistently, we found the expression of several screen hits significantly decreased and/or mutated in relapse patient samples. Novel dexamethasone resistance genes identified in the screen interfere with GC-induced transcription (*Stat3, Rfx3, Sox6, Ncor2*), promote pluripotency (*Esrrb, Sox2)* or stimulate cAMP signaling (*Adcy3, Adcy9, Gnas, Creb1)*. Silencing of these genes in multiple mouse T-ALL cell lines has no detectable effects on leukemic growth/survival *in vitro,* but confers resistance to dexamethasone treatment *in vitro* and *in vivo*. Moreover, we show that silencing of some candidate dexamethasone resistance genes accelerates leukemogenesis *in vivo,* demonstrating that leukemia suppressor genes were identified. Effect(s) of silencing or inhibiting these novel dexamethasone resistance genes/pathways in human T-ALL cell lines, primary patient samples and xenografts will be discussed. We predict that targeting these dexamethasone resistance pathways may re-sensitize relapse pediatric T-ALL cells to dexamethasone and/or contribute to more effective patient stratification to prevent relapse and induction failure.

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NEGATIVE REGULATION OF THE IMMUNE RESPONSE

CARLA ROTHLIN

HHMI Faculty Scholar, Associate Professor Immunobiology and Pharmacology,
Yale School of Medicine, New Haven, USA

The innate immune response of dendritic cells (DCs) and others entinel cells functions as both the first line of defense against pathogens and also as the initiating trigger for T-cell-mediated adaptive immunity. These fundamental activities not with standing, DC activation must be tightly regulated. While reduced DC function leads to increased susceptibility to infections, unrestrained, overactive DC responses can lead to allergy, autoimmunity,

chronic inflammatory disease, and other pathological conditions. We have found that the TAM receptor tyrosine kinases, TYRO3, AXL and MERTK, are potent negative regulators of the immune response in DCs. I will present new findings that's how that TAM signaling in DCs is triggered by cells of the adaptive response with which DCs interact with. I will also discuss the specificity of different TAM receptors in the regulation of the immune response.

SAIC-SAI-SAFE SYMPOSIUM IX

NEW MOLECULAR TARGETS FOR ONCOLOGICAL THERAPY

PERSONALISED MEDICINE IN MELANOMA PATIENTS

ROMINA GIROTTI

Instituto de Biología y Medicina Experimental, IBYME-CONICET, Argentina

BRAF is mutated in about 50% of human melanomas and treatment with BRAF or MEK inhibitors have resulted in increased progression-free and overall survival in melanoma patients. However, the majority of patients relapse after a relatively short period of disease control. Furthermore, after treatment with targeted therapy, most patients derive little benefit from immune checkpoint inhibitors. Resistance to targeted agents is driven by several mechanisms, so selecting second line therapies is challenging. Current advice includes the option to continue treatment beyond progression, but it is unclear how to select the patients that will benefit from this, so detecting disease progression early and elucidating the mechanisms of resistance to therapy will help optimise the clinical care of these patients. Treatment options are also needed for the ~50% of melanoma patients who are BRAF wild-type.

We developed two novel compounds that target mutant BRAF and wild-type CRAF. Our compounds inhibited the growth of melanoma cells that were resistant to BRAF selective inhibitors. ERK pathway reactivation is responsible for resistance to BRAF targeted therapies in ~60% of the patients and in ~25% of patients resistance is driven by acquisition of mutations in NRAS. We show that our compounds inhibited the growth of melanoma cells that were resistant to BRAF-selective inhibitors due to pathway reactivation mediated by different mechanisms. We show that the drugs were active against patient derived xenografts (PDXs) from patients with acquired or intrinsic resistance to BRAF-selective inhibitors and in whose tumors resistance was associated with ERK pathway reactivation. Further, our compounds are active in a PDX from a patient whose tumor developed acquired resistance to a combination of a BRAF-selective plus a MEK inhibitor and associated with acquisition of an NRAS mutation. Thus, our panRAF inhibitors can inhibit melanomas with different mechanisms of acquired or intrinsic resistance to BRAF-selective and BRAFselective/MEK inhibitor combinations, potentially providing first-line treatment for naïve patients and second-line treatments for a range of relapsed patients (Girotti et al, Cancer Cell, 2015).

Moreover, we used whole exome sequencing (WES) to provide insight into the mechanisms of resistance to BRAF inhibition and identify new therapeutic strategies for BRAF wild-type melanomas. We present the case of a patient that was wild-type for V600 BRAF, but carried HRAS and Rb1 mutations, allowing us to predict that the patient's tumour would be sensitive to the combination of a MEK inhibitor plus paclitaxel and we validated this therapy in a xenograft derived from the patient (PDX). Thus we show that genome analysis can be used to develop novel hypothesis-driven therapeutic strategies for patients and we show that these treatments can be validated in the patients' PDXs. Finally, we describe the use of circulating tumour DNA (ctDNA) as a predictive biomarker of response to therapy and as a powerful approach to reveal and then monitor mechanisms of resistance. In summary, we are implementing a powerful combination of techniques for personalised medicine to improve clinical management of BRAF wild-type and BRAF mutant melanoma patients (Girotti et al, Cancer Discovery, 2016).

VOLTAGE DEPENDENT ANION CHANNEL, A MITOCHONDRIAL ANTI-PROLIFERATIVE SWITCH

EDUARDO N. MALDONADO

Department of Drug Discovery & Biomedical Sciences and Hollings Cancer Center
Medical University of South Carolina, Charleston, SC 29425, USA

Otto Warburg in the early 20th century described a metabolic tumor phenotype characterized by enhanced glycolysis and suppression of mitochondrial metabolism even in the presence of physiological levels of oxygen. Warburg also erroneously postulated that permanent defective respiration originates cancer. Further inves-

tigations showed enhanced glycolysis and functional mitochondria in nearly all tumors and cancer cell lines. Differentiated cells produce about 95% of the total ATP by mitochondrial oxidative phosphorylation and the remaining 5% by aerobic glycolysis. By contrast in cancer cells, glycolysis contributes 20-90% of total ATP production. A highly glycolytic phenotype has been associated with a high rate of cell proliferation and considered an indicator of malignancy. The "glucose avidity" of tumors is the foundation for the positron emission tomography (PET) of the ^{18}fluorodeoxyglucose to diagnose primary tumors, recurrences and metastasis. The advantage of the pro-proliferative Warburg phenotype for dividing cells is that the incomplete breakdown of glucose by glycolysis, although energetically less efficient than oxidative phosphorylation, provides carbon backbones for biomass generation (lipids, proteins, and nucleic acids). Biosynthesis of macromolecules is also contributed by intermediaries generated in the mitochondrial matrix.

In cancer cells, the interdependent "glycolytic" and "mitochondrial" metabolic compartments are separated by the mitochondrial outer membrane (MOM). The MOM is a functional barrier containing the voltage-dependent anion channel (VDAC) that comprises 3 isoforms in humans, VDAC1, VDAC2 and VDAC3. Flux of metabolites entering mitochondria including respiratory substrates, ADP and Pi cross the MOM through only one channel, VDAC. Once inside the matrix, respiratory substrates fuel the Krebs cycle generating mostly NADH that enters the electron transport chain (ETC). The ETC transfers electrons to the final acceptor O_2, pumps protons to the intermembrane space, and generates reactive oxygen species (ROS). Protons in the intermembrane space create a negative potential in the mitochondrial matrix (mitochondrial membrane potential, $\Delta\Psi$) and a proton-motive force utilized by the ATP synthase to generate ATP.

VDAC opening operates as a "master key" that "seal-unseal" mitochondria to modulate mitochondrial metabolism, ROS formation and the intracellular flow of energy. Tumors are metabolically flexible and can switch the bioenergetics phenotype from glycolytic to oxidative and vice versa in response to different stimuli. A predominantly oxidative metabolism characteristic of differentiated cells leads to a high cytosolic ATP/ADP ratio that inhibits glycolysis. A low ATP/ADP ratio, essential to maintain enhanced glycolysis independently of other pro-glycolytic variables, requires a partial or complete suppression of mitochondrial metabolism. VDAC, initially considered an all-time open channel, is actually regulated by several factors including free tubulin. We showed that high free tubulin in cancer cells decreases mitochondrial $\Delta\Psi$ by limiting ingress of respiratory substrates and ATP. Dimeric α-β tubulin also decreases conductance of VDAC inserted in lipid bilayers. We also showed that VDAC knockdown decreases mitochondrial metabolism. Our findings led to the hypothesis that VDAC closing by free tubulin contributes to the suppression of mitochondrial metabolism in the Warburg effect.

The VDAC-tubulin interaction is a potential pharmacological target to increase mitochondrial metabolism, promote ROS formation and revert the Warburg effect. We found that erastin, a small molecule that kills cells engineered to harbor a RAS^{v12} mutation, antagonizes the inhibitory effect of tubulin on VDAC. We also identified lead "erastin-like" compounds in a cell-based high throughput screening of a chemical library of 50,000 small molecules. Both erastin and lead compounds follow a "metabolic double hit model" characterized by induced oxidative stress and decreased glycolysis (anti-Warburg). Blockage of the inhibitory effect of tubulin on VDAC increases mitochondrial metabolism and ROS formation and activates the stress kinase c-Jun N-terminal kinase (JNK) leading to mitochondrial dysfunction, bioenergetics failure and cell death. VDAC opening is not expected to kill every metabolically heterogeneous tumor cell. Cells that survive the initial ROS-dependent hit may eventually take the second hit, the reversal of the Warburg phenotype. VDAC-opening reverts the Warburg phenotype by switching cells to an oxidative metabolism as evidenced by the decrease in lactic acid release after erastin/erastin-like compounds. In summary, VDAC-tubulin antagonists are a novel group of drugs that target a molecular interaction with global effects on cancer cell bioenergetics and promote a two-hit effect, damage by oxidative stress and reversal of the pro-proliferative Warburg phenotype.

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REPROGRAMMING OF TUMOR CELLS WITH THE MIR 302/367 CLUSTER SUPPRESSES TRANSFORMATION PHENOTYPES

CHUL MIN YANG AND BERND GRONER

Georg Speyer Haus, Institute for Tumor Biology and Experimental Therapy, Frankfurt am Main, D-60596, Germany

Cellular transformation is initiated by the activation of oncogenes and a closely associated developmental reprogramming of the epigenetic landscape. Transcription factors, regulators of chromatin states and microRNAs influence cell fates in development and stabilize the phenotypes of normal, differentiated cells and of cancer cells. The miR-302/367 cluster, predominantly expressed in human embryonic stem cells (hESs), can promote the cellular reprogramming of human and mouse cells and contribute to the generation of iPSC. We have used the epigenetic reprogramming potential of the miR-302/367 cluster to "deprogram" tumor cells, that is, shift their gene

expression pattern towards an alternative program associated with more benign cellular phenotypes. Induction of the miR-302/367 cluster in extensively mutated U87MG glioblastoma cells drastically suppressed the expression of transformation related proteins, for example, the reprogramming factors OCT3/4, SOX2, KLF4 and c- MYC, and the transcription factors POU3F2, SALL2 and OLIG2, required for the maintenance of glioblastoma stem-like tumor propagating cells. It also diminished PI3K/AKT and STAT3 signaling, impeded colony formation in soft agar and cell migration and suppressed pro-inflammatory cytokine secretion. At the same time, the miR 302/367 cluster restored the expression of neuronal markers of differentiation. Most notably, miR-302/367 cluster expressing cells lose their ability to form tumors and to establish liver metastasis in nude mice. The induction of the miR-302/367 cluster in U87MG glioblastoma cells suppresses the expression of multiple transformation related genes, abolishes the tumor and metastasis formation potential of these cells and can potentially become a new approach for cancer therapy.

Reference

Expression of the miR-302/367 cluster in glioblastoma cells suppresses tumorigenic gene expression patterns and abolishes transformation related phenotypes. Chul Min Yang, Tomohiro Chiba, Boris Brill, Natalia Delis, Viktoria von Manstein, Vida Vafaizadeh, Thomas Oellerich and Bernd Groner. Intern. J. Cancer. 137, 2296-309 (2015)

SAIC SYMPOSIUM X

REPRODUCTIVE HEALTH

MATERNAL DIABETES: MECHANISMS INVOLVED IN INTRAUTERINE PROGRAMMING OF METABOLIC, CARDIAC AND REPRODUCTIVE ANOMALIES

ALICIA JAWERBAUM

Laboratorio de Reproducción y Metabolismo. CEFYBO-CONICET. Facultad de Medicina. Universidad de Buenos Aires.

Maternal diabetes increases the risks for embryo resorption and malformations, feto-placental impairments and perinatal morbidities. Besides, the offspring have increased risks of metabolic and cardiovascular diseases in their adult life, as a result of an adverse process of intrauterine programming that is still poorly understood. Animal models of diabetes and pregnancy are valuable tools to improve the understanding of the mechanisms of induction of these alterations. In our laboratory, using a model of mild pregestational diabetes, we found that diabetic rats mated with control males lead to offspring that have increased markers of a pro-oxidant/pro-inflammatory state in their hearts from the neonatal stage. In the offspring from thesediabetic rats, increases in circulating lipid concentrations are evident from day 21 of age and increased circulating glucose concentrations are evident from the fifth month of age. Moreover, if normoglycemic three month-old offspring of pregestational diabetic rats are mated with control males, the pregnant rats develop gestational diabetes (GDM). At term, GDM fetuses are overweight and GDM placentas show reduced peroxisome proliferator activated receptors (PPARs) and increased mechanistic target of rapamycin (mTOR) signaling. PPARs and mTOR are nutritional regulators respectively activated by unsaturated fatty acids and amino acids, and respectively involved in anti-inflammatory and growth pathways. We found that PPARs can be activated in fetuses and placentas by supplementation of the maternal diet with oils rich in unsaturated fatty acids. Moreover, we found that these diets also have benefits in the offspring, as shown by reduced oxidative/inflammatory markers and reduced lipid content and peroxidation in the heart of the offspring of pregestational diabetic animals. These effects were similar to those found with the maternal administration of mitochondrial antioxidants, highlighting the relevance of oxidative stress in the intrauterine programming of offspring diseases in maternal diabetes. Besides, diets enriched in PUFAs in the pregnancy of pregestational diabetic animals (F0 generation)regulate placental PPAR and mTOR signaling, reduce feto-placental pro-oxidant/pro-inflammatory markers and prevent fetal overgrowth in the offspring that develop GDM during their pregnancy (F1). Our results suggest that impaired PPAR pathways are involved in the intrauterine programming of alterations in the heart, lipid metabolism, placental signaling and fetal growth in the offspring of pregestational diabetic rats, and that maternal supplements with oils enriched in PPAR ligands, possibly reducing the pro-oxidant/pro-inflammatory intrauterine environment, can prevent these alterations.

THE IMPACT OF THE SERUM OF PATIENTS WITH ENDOMETRIOSIS ON RECONSTITUTED 3D-ENDOMETRIAL TISSUE: EVALUATION OF INFLAMMATORY CYTOKINES

CAROLINE BORGATO[1]; ALINE LORENZON-OJEA[1]; ELAINE CARDOSO[1]; TATIANA BONETTI[2]; EDUARDO L. A. DA MOTTA[3]; PAULO C. SERAFINI[3]; VANESSA FREITAS[1]; ALEXANDRE BORBELY[1,4]; MAURICIO S. ABRAO[5]; LIDIA H. J. MYUNG[5]; AND ESTELA BEVILACQUA[1]

[1]Instituto de Ciências Biomédicas, Universidadede São Paulo, SP; [2]Depto de Obstetrícia, Universidade Federal de São Paulo, SP; [3]Depto de Ginecologia, Universidade Federalde São Paulo, SP; Centro de Medicina Reprodutiva Huntington, São Paulo, SP; [4]Institutode Biologia e Ciências da Saúde, Universidade Federal de Alagoas, AL; [5]Faculdade de Medicina da Universidade de São Paulo, SP, Brasil.

Background - Endometriosis is a chronic inflammatory disease characterized by the presence and growth of ectopic endometrial tissue. It affects 10% to 15% of women at reproductive phase and is associated with severe inflammationand infertility. Altered profile of systemic immunological factors seems to be the primary factor associated with subfertility in these patients, regardless the severity of the endometriotic injury. Objectives: Using a three-dimensional (3D) culture of partially reconstituted endometrium, we explored the possibility of factors present in the plasma of endometriotic women affect the endometrial profile of cytokines determining changes that can impair the fertility. Material and Methods: Samples were collected after written informed consent was obtained (Research Ethics Committee in Human Beings, USP, no. 692457). Endometrial biopsies (n=9) and plasma (n=31) were collected from healthy women at the Huntington Reproductive Medicine, SP. Plasma from endometriotic patients (with estrogen/progestin hormonal therapy, n=10and untreated n=5) was obtained from this clinic and from the Medical School Hospital, University of São Paulo, SP. The biopsies were digested with collagenase II/DNAse I and filtered for retention of the endometrial glands.CD105magnetic microbeads (MACS)were used for positive selection of endothelial cells from filtered preparations. Adhered cells (fibroblastsand decidual cells) that passed through the column were ressuspended in DMEM / F12 medium.To construct the 3D environment, 0.1 x 10^6 stromal cells in supplemented 199 medium were added to a mixture of extracellular matrix components (fibronectin and, collagen V, I and III) and placed in 48-well plates. After 12 hours in culture (37°C with 5% CO_2), endothelial cells were added (0.1 x 10^6 cells) on the surface of the culture. After 24h, medium was replacedby one containing 20%serum from healthy or endometriotic women as follow (n = 3-8): i) Serum of healthy patients; ii) Serum of patients with endometriosis; iii)Serum of patients with endometriosis, which received hormonal therapy. The system remained in culture for additional 24 and 48 h. Cells were then homogenized for inflammatory cytokines evaluations through cytometric bead array, according to the manufacturer's instructions. Results and Conclusions: This study showed that the endometrium is not responsive to the serum of patients with endometriosis treated with hormonal therapy; expression levels of inflammatory cytokines did not show changes. The use of serum of women with endometriosis without treatment induced a significant increase of inflammatory cytokines (IFN-gamma, TNF-alpha, and IL-2) produced by the endometrium, suggesting that the absence of therapy in these women may be a key factor compromising the physiology of the endometrium andits reproductive function.In conclusion, this study showed an imbalance in cytokine expression in 3D-endometrial co-cultures treated with serum from endometriotic patients emphasizing the ability of the uterus to respond and contribute to a systemic inflammatory environment with cytokine production, even when distant from the endometriotic lesion. Immunological mediators as inflammatory cytokines may alter tightly regulated crucial gene expression, which may be an additional aspect for infertility in these women.

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SPHINGOLIPIDS AND LOW-INTENSITY LASERS: ¿POSSIBLE STRATEGIES FOR ONCOFERTILITY OR SCIENCE FICTION?

FERNANDA PARBORELL

Instituto de Biología y Medicina Experimental (IBYME)-CONICET, Buenos Aires, Argentina

Oncofertility is an emerging interdisciplinary field that involves the study and development of new preventive and protective measures to reduce the impact of cancer treatments on reproductive health. Due to the increased survivorship of children and reproductive age patients treated with cancer thanks to the advances in anti-tumoral therapies, it is essential to understand its effects on future life quality and seek new techniques focused on preserving fertility. For example, in Argentina, breast cancer represents the highest incidence cancer in women, with a rate of 71 cases per 100,000 women. However, mortality rates from breast cancer have declined steadily and significantly since 1996. It is worth mentioning that,at a reproductive level, in patients older than 20 years who have received

anti-tumoral treatmentsthe rate of amenorrhea is 80%, the rate of premature ovarian failure (POF) is 90% and only 5-10% of these patients achieve spontaneous pregnancies. POF is a multi-disorder characterized by the disappearance or dysfunction of the ovarian follicles in women under 40 years. These patients present amenorrhea, hypoestrogenism and high levels of gonadotropins. POF affects about 1-2% of women under 40 years old and 0.1% of women under 30 yearsold and its causes can be grouped into: genetic, immune, infectious and iatrogenic (chemotherapy, radiotherapy) causes. In particular, POF can be consequence of treatment with chemotherapeutic drugs and/or radiation, with alkylating agents and radiotherapy being the most damaging agents to the ovarian reserve, and thus lead to infertility. Currently, treatments for POF consist mainly of hormone replacement therapy and oral contraceptives but are not fully effective. In addition, there are various options to preserve fertility (GnRH agonists, cryopreservation of oocytes, embryos and ovarian tissue). In our laboratory, we propose two new strategies to protect the ovary and restore fertility in patients who are diagnosed with cancer and undergoing chemotherapy treatment: 1) Local administration of ceramide-1-phosphate sphingolipid (C1P) (pharmacological strategy) 2) Local application of low intensity laser (LBI) (photobiomodulation).We have observed that both C1P and LBI are able to preserve ovarian reserve in a POF model induced by cyclophosphamide in mice. Finally, given the increased survival of cancer patients of reproductive age, it is necessary to develop effective, safe and inexpensive strategies to protect the ovary and preserve fertility, without neglecting the social, ethical and legal aspects, especially now that Argentina has a national law that provides medical assistance to those patients facing this difficult situation.

MOLECULAR MECHANISMS ASSOCIATED WITH THE FERTILIZING CAPACITY OF MAMMALIAN SPERM

MARIANO G. BUFFONE

Instituto de Biología y Medicina Experimental-CONICET, Buenos Aires, Argentina.

Mammalian spermatozoa are not able to fertilize oocytes immediately after ejaculation; they must first undergo a complex process called capacitation in the female reproductive tract or *in vitro*. These changes include the development of hyperactivated motility and the ability to undergo acrosomal exocytosis (AE) in response to specific stimuli. AE is essential for fertilization. Mice and men that produce sperm lacking acrosomes are sterile. The occurrence of AE allows IZUMO1, a protein that is essential for sperm-egg fusion, to relocalize to the equatorial region of mouse sperm head. Not long ago, it was broadly accepted that sperm undergo AE upon interaction with the zona pellucida (ZP) of the egg, and many of the advances in our knowledge of this process were derived from in vitro studies using solubilized ZP. However, recent evidence acquired using transgenic mice that produce sperm carrying enhanced green fluorescent protein (EGFP) in the acrosome and Ds-Red2 red fluorescence in the mitochondria of the flagellar midpiece suggest that sperm binding to the ZP is not sufficient to induce AE. Real-time imaging of in vitro fertilization of cumulus-oocyte complexes (COCs) showed that most fertilizing sperm undergo AE before contacting the ZP. Therefore, the aim of this study was to determine physiological sites of AE by using double transgenic mouse sperm, which carried EGFP in the acrosome and DsRed2 fluorescence in mitochondria. Using live imaging of sperm during in vitro fertilization of cumulus-oocyte complexes, it was observed that most sperm did not undergo AE. Thus, the occurrence of AE within the female reproductive tract was evaluated in the physiological context where this process occurs. Most sperm in the lower segments of the oviduct were acrosome-intact; however, a significant number of sperm that reached the upper isthmus had undergone AE. In the ampulla, only 5% of the sperm were acrosome-intact. These results support our previous observations that most of mouse sperm do not initiate AE close to or on the ZP, and further demonstrate that a significant proportion of sperm initiate AE in the upper segments of the oviductal isthmus.

SAIC SYMPOSIUM XI

VIROLOGY AND DISEASE

HERPESVIRAL ONCOGENESIS AND EFFECTS ON B CELL REPERTOIRE

ETHEL CESARMAN

Weill Cornell Medicine, New York, USA

Since the discovery of Epstein-Barr virus (EBV) in Burkitt lymphoma over 50 years ago, only one other herpesvirus, namely Kaposi's sarcoma-associated herpesvirus/human herpesvirus-8 (KSHV/HHV-8), has been shown to be a direct cause of cancer in humans. EBV causes a number of B cell lymphomas, carcinomas, and rarely leiomyosarcomas. KSHV is the infectious cause of three human malignancies, Kaposi Sarcoma (KS), an endothelial tumor, as well as Primary Effusion Lymphoma (PEL) and the plasma cell variant of Multicentric Castleman Disease (MCD), two B-cell lymphoproliferative diseases. The frequency of lymphoid malignancies related to infection by one of these two herpesviruses is greatly increased in individuals with immunodeficiency, whether primary or acquired, for example, as a consequence of HIV infection and AIDS or in the case of therapeutic immunosuppression for organ transplantation.

Understanding the causal associations of EBV and KSHV with certain cancers has allowed more accurate diagnosis and classification. Our current understanding indicates that EBV and KSHV contribute to lymphomagenesis by affecting genomic stability and by subverting the cellular molecular signaling machinery and metabolism to avoid immune surveillance and enhance tumor cell growth and survival. A deeper understanding of specific mechanisms by which EBV and KSHV cause cancer has been acquired over the past years, in particular with respect to viral protein interactions with host cell pathways, and microRNA functions. Specific therapies based on knowledge of viral functions are beginning to be evaluated, mostly in preclinical models.

KSHV is able to induce inflammation and angiogenesis when infecting endothelial cells, explaining its unique ability to cause KS. However, traditionally it has been challenging to do sophisticated studies in the context of full viral infection to assess specific KSHV properties because this virus does not infect cells or replicate efficiently *in vitro*. Recent advances have allowed us to use BAC systems to mutate specific viral genes, produce recombinant virus with selection markers, and assess the consequences of infection of different cell types. Using this technology, we have examined the effects of infection of primary B cells. We have known for almost 20 years that in MCD, KSHV infection of B lymphocytes is almost exclusively restricted to lambda light chain (IgL) expressing cells, and kappa light chain (IgK) cells that are KSHV positive are almost never observed. This specific association of KSHV biology with IgL has been a longstanding conundrum in the field given that IgL and IgK lymphocytes should be physiologically indistinguishable. In order to explore early pathogenic events during KSHV infection of lymphocytes, we have developed a model using *de novo* KSHV infection and maintenance of primary naïve B lymphocytes from human tonsil. Although at early stages of infection, we observe equal infection of B cells bearing immunoglobulin lambda (IgL) and kappa (IgK) light chains, the IgK positive cells are lost over time in the infected cultures. Experiments in which IgK and IgL naïve cells were sorted and infected separately reveal that KSHV infection induces IgK B cells to become IgL positive via a IgL/IgK double-positive intermediate. Consistent with these results, we observe polyclonal IgL genomic rearrangements in KSHV-infected IgK B cells by PCR, lambda light chain expression by RT-PCR and expression of the cellular V(D)J-recombination proteins Recombinase Activating Genes 1 and 2 (RAG1 and RAG2). Taken together our data demonstrates that KSHV infection of mature B lymphocytes causes re-induction of V(D)J-recombination in mature B lymphocytes, a process called B cell receptor (BCR) revision. Aside from providing a new and intriguing explanation for IgL restriction in KSHV infection, the potential implications of these results are far-reaching. BCR revision is associated with the induction of autoimmunity and these results could represent the first demonstration of a mechanism by which a lymphotropic human virus directly induces an autoimmune state. Autoimmunity is one of the diagnostic criteria for multicentric Castleman's disease, a KSHV-associated lymphoproliferative disorder, and moreover, autoimmune inflammation resulting from KSHV-mediated BCR revision could represent a critical driver of KSHV-associated lymphoma.

ANTIVIRAL, ANTIANGIOGENIC AND ANTI-INFLAMMATORY ACTIVITIES OF STIGMASTANE DERIVATIVES, A NOVEL CLASS OF BROAD SPECTRUM ANTIVIRAL EFFECTORS

LAURA ALCHÉ

Department of Biological Chemistry, School of Exact and Natural Sciences, IQUIBICEN-UBA-CONICET, University of Buenos Aires, Argentina

Many viral infections are associated with the development of immunopathologies for which no vaccines and/or antiviral drugs are available yet. The treatment of these diseases usually includes corticosteroids which can result in reactivation of the virus, as it occurs in the case of Herpes simplex virus (HSV) infections. Particularly, HSV type 1 (HSV-1) triggers an ocular disease in humans named herpetic stromal keratitis (HSK) that can lead to vision impairment and blindness. HSK develops as a consequence of the arrival of inflammatory cells to the cornea in response to viral infection through the appearance of new vessels. While inflammatory cells are responsible for the elimination of HSV-1 from the eye, they cause an uncontrolled inflammatory response that culminates in the development of HSK.

We have demonstrated that a polyfunctionalized stigmastane derivative (22S,23S)-22,23-dihydroxystigmast-4-en-3-one (**1**) inhibits HSV-1 replication in both human corneal and conjunctival cell lines with no cytotoxicity, and reduces the signs of HSK in a mouse experimental model. On the other hand, compound **1** decreases IL-6 production in stimulated macrophages, a cytokine which is crucial for the development of neovascularization along HSK progression. Besides, RNA microarrays have revealed various overexpressed and repressed genes in compound **1** treated HSV-1 infected cells and activated macrophages, many of which are associated with innate responses and inflammatory processes. Thus, compound **1**, and others belonging to the same family of molecules, proved to combine antiviral and anti-inflammatory properties in the same chemical structure.

Since angiogenesis plays a critical role in initiating and promoting several diseases, such as HSK and cancer, we have studied the effect of compound **1** on capillary tube-like structures and on cell migration of human umbilical vein endothelial cells (HUVEC), as well as on VEGF expression, given that VEGF is a primary angiogenic factor operating in HSV-infected cornea and is considered a target to treat corneal neovascularization. Compound **1** significantly restrains the ability of HUVECs to form capillary tubes when added together with cells, although it does not cause any cytotoxic effect on the tubes already established, and it efficiently suppresses IL-6-stimulated HUVEC migration, in a concentration-dependent manner. In addition, compound **1** diminishes VEGF expression when induced by two different stimuli in macrophages.

In vivo, a significant decrease in the incidence and severity of corneal neovascularization during the development of HSK has been achieved by compound **1**, which explains the improvement of the signs of disease in the murine experimental model.

Additional benefits of compound **1** have been observed, since it exerts an antiangiogenic effect on the neovascular response induced by LMM3 cells in mice, by reducing the number of neovessels in a murine model of breast cancer. This novel effect of compound **1** is not shared with other compounds belonging to the same family of synthetic analogs with antiviral and anti-inflammatory properties, which lead us to conclude that the antiangiogenic effect of compound **1** is not a consequence of its anti-inflammatory activity.

In summary, the synthetic stigmastane designed as compound **1** would be a promising compound not only to cure an immunopathology of viral origin like HSK, but also to improve other diseases where angiogenesis is the major pathogenic factor, as in the case of solid tumors.

HIGH-THROUGHPUT SEQUENCING GENOMIC APPROACHES TO UNDERSTAND KSHV/ HHV-8 ONCOGENESIS IN CELL AND MOUSE MODELS OF KAPOSI'S SARCOMA

JULIAN NAIPAUER (1), DARIA SALYAKINA (2), MARTIN ABBA (4) LUCAS CAVALLIN (1), VYTAS DARGHIS-ROBINSON (1), SANTAS ROSARIO (1), ENRICO CAPOBIANCO (2), COURTNEY PREMER (3), JOSHUA HARE (3), PASCAL GOLDSCHMIDT-CLERMONT (1) AND ENRIQUE A. MESRI (1)

(1) Miami CFAR, Department and Graduate Program of Microbiology and Immunology; Viral Oncology Program, Sylvester Comprehensive Cancer Center (2) Center for Computational Science and (3) Interdisciplinary Stem Cell Institute; University of Miami Miller School of Medicine, Miami FL, USA. (4) CINIBA, Facultad de Ciencias Medicas, Universidad Nacional de La Plata, La Plata, Argentina.

Human viral oncogenesis is the consequence of the transforming activity of virally encoded oncogenes and non-coding RNAs in combination with host oncogenic alterations. In the case of Kaposi's sarcoma (KS) and its etiologic agent, KS herpesvirus (KSHV/ HHV-8), there is a lack of experimental systems to dissect viral and

host contributions to the KS malignant phenotype. Using proteomic RTK arrays we found that PDGFRA the major oncogenic driver in our model of KSHV-driven mouse tumorigenesis and a key therapeutic target. We present two high throughput studies derived from this model. First we use RNA sequencing of KSHV Bac36 transfected mouse endothelial cells (mECK36) and their derived KSHV+ve and KSHV-ve tumors as a unique model to dissect genetic mechanisms of KSHV dependent and independent sarcomagenesis in an unbiased hi-throughput fashion since the system allows for unique experimental comparisons in the same cell and KS-like tumor types: 1) KSHV+ve vs KSHV-ve mECK36 were used to study KSHV mediated effects "in vitro" 2) KSHV+ve mECK36 grown in vitro and in tumors were used to study "in vitro" vs "in vivo" variations of host and viral gene expression induced by micro-environmental cues as well as the occurrence of host mutations in the tumors 3) KSHV+ve mECK36 vs KSHV-ve mECK36 tumors were used to dissect the role of KSHV genes and non coding RNAs in tumorigenesis by comparing mECK36 tumors driven by KSHV vs mECK36 tumors driven by host mutations. We performed Illumina, stranded, RNA seq analysis of all KSHV stages of this cell and animal model. Analysis of the host and viral transcriptome was used to characterize mechanisms of KSHV dependent and independent sarcomagenesis as well as the contribution of host mutations. Most significant results of analysis indicate that KSHV-driven in vivo growth display tumorigenesis pathways occurring predominantly by activation of developmental pathways while KSHV-ve tumors, driven by PDGFRA D842V activating mutations, occur with a predominance of proliferative pathways. Mutational analysis of mECK36 cells and tumors revealed a surprising set of mutations in inflammation/immune response related genes, absent in mECK36 cells but present in all mECK36 tumors in the same location. This indicates that these mutations should be the consequence of "in vivo" clonal selection of few mutated mECK36 cells of the population. This result suggests that in the context of in vivo tumorigenesis both these mutations and the virus may determine tumor growth. Our second model is a defined model of in vitro to in vivo tumorigenesis whereby KSHV is able to induce tumorigenesis in mouse mesenchymal stem cells (MSCs) only when infected MSCs are exposed to culture media reproducing tumor angiogenesis microenvironment conditions. Our results defines two useful cell and animal models to uncovered novel specific aspects of the interplay between host oncogenic alterations, virus-induced as well as environmentally induced transcriptional effects in the context of KSHV sarcomagenesis.

(341) NEUTROPHIL-COXSACKIEVIRUS INTERACTION

LEONARDO RIVADENEYRA[1], DENISE KVIATCOVSKY[2], SILVIA DE LA BARRERA[2], RICARDO MARTÍN GOMEZ[3], MIRTA SCHATTNER[1]

[1]Laboratorio de Trombosis Experimental e [2]Inmunología de Enfermedades Respiratorias, Instituto de Medicina Experimenta, CONICET-Academia Nacional de Medicina. Buenos Aires, Argentina. [3]Laboratorio de Virus Animales, Instituto de Biotecnología y Biología Molecular - UNLP-CONICET, La Plata, Argentina

Coxsackieviruses B (CVBs) belong to the genus Enterovirus within the Picornaviridae family. After infection by the oral route and before viremia, CVBs replicate in lymphoid tissues, such as the tonsils and the Peyer's patches. This early interaction with immune cells has been poorly studied. In this work, we focused in the CVB-neutrophil (Neu) interaction in order to clarify the role of these inflammatory cells in the CVB infection.

For this aim, Neu were incubated with CVB and one day later viral RNA and infectious virus were searched in cells and supernatants. RT-PCR and flow cytometry (FC) studies, showed the presence of both, viral RNA and antigen in Neu-infected cell pellets. Infectivity assays confirmed the presence of infectious viral particles in the supernatants. To determine whether this Neu-CVB interaction triggered cell activation we first analyzed expression of CD11b. FC analysis showed increased levels of this adhesion cell receptor that correlated with and augmented Neu adhesion to extracellular matrix proteins such as fibrinogen and fibronectin (acid phosphatase activity).

Observation of nuclear morphological changes by fluorescence microscopy and FC showed that apoptosis was significantly lower in infected cells compared to control samples. This increase of cell survival might be due to the release of proinflammatory cytokines by Neu-infected cells. In this regard, increased levels of IL-6, IL-1α and TNF-α production were detected by ELISA in CVB-Neu supernatants. Moreover, these supernatants also showed increased chemoattractant (Boyden chamber technique) and myeloperoxidase activity (ELISA). The interaction of CVB-Neu induced low levels of neutrophil extracellular traps (NETs) that were significantly potentiated in the presence of TNF-α. Our results indicate that interaction of Neu-CVB results in Neu activation and increased cell survival. This activation may play an important role in the subsequent pathogenesis of CVB infection.

SAIC SYMPOSIUM XII

ENVIRONMENTAL HEALTH

LOSING OUR MINDS: THE ONGOING CHEMICALS' ATTACK ON OUR CHILDREN'S BRAINS

MARICEL V. MAFFINI[1] AND THOMAS G. NELTNER[2]

[1]Independent Consultant, Maryland, US, [2] Environmental Defense Fund, Washington, DC, USA

According to the WHO, non-communicable diseases underlie almost two-thirds of all global deaths, and its incidence has increased over the past 40 years, in part due to environmental chemical exposures. Developmental disabilities affect millions of people and have a great impact on their lives, their families and the societies where they live. The prevalence of disorders such as autism, attention deficit hyperactivity disorder as well as subclinical decrements in brain function cannot be explained solely as genetic diseases. Exposures to environmental chemicals, especially during prenatal and early postnatal life, are one likely explanation for some of the decrements. The current chemical risk assessment approach is typically based on the toxicity caused by a single chemical on a variety of organs without acknowledging additional exposures to other chemicals also affecting the same organ or system. We analyzed data from the US Food and Drug Administration toxicology database, high-throughput screening ToxCast and Tox21 programs, and data obtained through Freedom of Information Act and available in the public domain. We identified more than 300 chemicals allowed in food, among them were 44 food ingredients, 109 food contact substances and 86 pesticides. These chemicals could be present in any diet in any combination that may have potential harmful effects on the developing brain. Each individual chemical may or may not be harmful if it were the only one present, but we know next to nothing about their cumulative biological effects on the brain. An expanded cumulative risk assessment approach is needed, and it should focus on health outcomes, like developmental disabilities, arising from the accumulation of effects of multiple chemicals on the brain. We must move beyond treating chemical exposures as isolated incidents and look at their cumulative biological effects on organs and their role in the onset of chronic diseases. The time has come to overhaul chemical risk assessment.

PESTICIDES AND FERTILITY: THE EFFECTS OF A BRIEF POSTNATAL EXPOSURE ON UTERINE DEVELOPMENT AND FEMALE FERTILITY

JORGELINA VARAYOUD, MARÍA MERCEDES MILESI, PAOLA INÉS INGARAMO, MARLISE GUERRERO SCHIMPF, JORGE GUILLERMO RAMOS, MARÍA PAULA GASTIAZORO, VIRGINIA LORENZ, MÓNICA MUÑOZ-DE-TORO, ENRIQUE H. LUQUE

Instituto de Salud y Ambiente del Litoral (ISAL), Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral - Consejo Nacional de Investigaciones Científicas y Técnicas, Santa Fe, Argentina.

The developmental programming hypothesis suggests that abnormal stimuli that occur during critical periods of development can permanently reprogram normal physiological responses and, consequently, give rise to reproductive health effects later in life. Early life exposures to chemicals in general, and pesticides in particular, have been associated with reproductive pathologies such as infertility and gynecologic tumors. Our research focuses on the effects of pesticides exposure on uterine development and their lasting consequences manifested later in life. The pesticides that we evaluated are the insecticide endosulfan and the herbicide glyphosate. In Argentina, glyphosate-based herbicides are the most commonly used, and although endosulfan has been banned in 2013, large quantities of this chemical continue to contaminate the environment because of its high persistence and lipophilicity. Using a rat model of early postnatal exposure we observed that low doses of endosulfan and low doses of a glyphosate-based herbicide disrupt the expression of genes that regulate uterine development and differentiation during the pre-pubertal period. In addition, we studied long-term effects on: 1) reproductive performance, 2) implantation and post-implantation processes, and 3) epigenetic modifications of endocrine-dependent genes. The results showed that both pesticides affected female fertility but in different ways. Low doses of endosulfan decreased the number of implantation sites. In the case of the glyphosate-based herbicide, there was an increased number of resorption sites. To address the effects of postnatal pesticide exposure on the pregnant uterus at the molecular level, we evaluated the endometrial proliferation and the expression of implantation and decidualization-associated genes. Both pesticides impaired endometrial proliferation and altered the expression of endocrine-regulated gene pathways. In addition, we found modifications on DNA methylation status of uterine genes,

showing evidence of epigenetic regulation of altered gene expression due to a postnatal pesticide exposure. Based on the evidence presented here and previously published data, we conclude that some pesticides are likely to diminish fertility in a laboratory animal model. More studies are needed to identify whether these or other pesticides may contribute to the decline in human fertility observed in the past decades.

EXPOSURE TO AIR PARTICULATE MATTER: MECHANISMS UNDERLYING LUNG AND CARDIOVASCULAR EFFECTS

PABLO EVELSON

Universidad de Buenos Aires. CONICET. Instituto de Bioquímica y Medicina Molecular (IBIMOL). Facultad de Farmacia y Bioquímica. Ciudad de Buenos Aires, Argentina.

The World Health Organization reports that in 2014 3.7 million deaths were recorded as a result of air pollution exposure. This mortality has been pointed out by several epidemiological studies, which have shown a positive correlation between decreased air quality levels and adverse health effects. Air pollution is a complex heterogeous mix whose complexity is increased due to the variation in its components, between places and over time. The most important pollutants in ambient air which are of concern regarding health effects, include sulfur dioxide (SO_2), nitrogen oxides (NOx), carbon monoxide (CO), volatile organic compounds (VOCs), and particulate matter (PM). Of the different air pollutants, it is accepted that PM is the major concern from a health perspective. Epidemiological studies have shown that the exposure to PM, at levels experienced by populations throughout the world, contributes to pulmonary and cardiac disease through multiple mechanistic pathways that are complex and interdependent. Experimental evidence suggests a series of events that are triggered by pollution-induced pulmonary inflammatory reactions and oxidative stress with an associated risk of vascular dysfunction, altered cardiac function, and obstructive pulmonary diseases. Pulmonary inflammation is the first event observed after PM exposure. A critical component of the inflammatory response to particles in the lung is the release of cytokines from activated macrophages and lung epithelial cells, resulting in neutrophil recruitment. This response may be caused by the deposition of PM into the alveolar space in the lung, inducing the release of cytokines from alveolar macrophages. The release of proinflammatory mediators from PM-exposed macrophages is a key event that causes cytokine release from lung epithelial cells, thus amplifying the inflammatory response. The activation of inflammatory cells leads to the generation of reactive oxygen and nitrogen species. It is understood that the oxidative stress caused by the activation of the inflammatory system, plays an important role in the deleterious effects of PM in multicellular organisms. Another proposed mechanism that may lead to oxidative damage is the direct generation of ROS at the surface of the particles. This is supported by the concept that the particle surface offers a unique physicochemical interface to catalyze reactions resulting in oxidant production. The interaction of PM with membrane components was recognized by the presence of free radicals and oxidants on the particle surface. Moreover, PM can also contain a large number of soluble metals that have the ability of redox cycling. The involvement of transition metals, such as Fe, Va, Cr, Mn, Co, Ni and Cu, which are able to catalyze Fenton-type reactions and generate hydroxyl radicals, has been proposed. Once the lung inflammatory response is initiated, it develops into a systemic oxidative stress and inflammatory response, characterized by alterations in circulating factors and cells associated with inflammation and oxidative damage. It has been proposed that the release of proinflammatory and oxidative mediators can alter heart O_2 metabolism and cardiovascular function. Given that mitochondria play an essential role in cellular O_2 and energetic metabolism, several authors suggested that mitochondrial dysfunction is a key feature in the development of cardiac alterations during the exposure to air pollution PM. Taking all this into account, we evaluated cardiac O_2 metabolism and contractile function, focused on mitochondrial function, in a mice model of acute exposure to Residual Oil Fly Ash (ROFA), a well-known PM surrogate. Our results indicate that PM exposure decreases heart O_2 metabolism, probably due to a mitochondrial dysfunction. Regarding cardiac function, we observed the myocardium fails to properly sustain contractile work when work output is increased in mice exposed to PM. Interestingly, pretreatment with Infliximab, a chimeric monoclonal antibody that blocks TNF-α biological activity recovered the positive correlation between cardiac contractile state and O_2 consumption. These findings support the notion that systemic inflammation is a key pathway in the alterations in cardiac function observed after PM exposure. A better understanding of the mechanisms underlying PM induced health problems would allow a more targeted approach to face the toxic effects of PM, and could possibly provide different ways to decrease individual sensitivity to PM.

(279) HEXACHLOROBENZENE INDUCES HYPERPLASIA AND ALTERS MOUSE MAMMARY BRANCHING MORPHOGENESIS THROUGH ARYL HYDROCARBON RECEPTOR

NOELIA MIRET[1], EVA RICO-LEO[2], CAROLINA PONTILLO[1], ELSA ZOTTA[3], PEDRO FERNÁNDEZ SALGUERO[2], ANDREA RANDI[1]

[1]Universidad De Buenos Aires, Facultad de Medicina, Departamento de Bioquímica Humana, Laboratorio de Efectos Biológicos de Contaminantes Ambientales, Argentina. [2]Universidad de Extremadura, Facultad de Ciencias, Departamento de Bioquímica y Biología Molecular y Genética, Laboratorio de Biología Molecular del Cáncer, Badajoz, España. [3]Universidad de Buenos Aires, Facultad de Medicina, Departamento de Ciencias Fisiológicas, Sección Patología, Laboratorio de Fisiopatogenia, Buenos Aires, Argentina.

Hexachlorobenzene (HCB) is an environmental pollutant that weakly binds to the aryl hydrocarbon receptor (AhR), being able to trigger AhR-dependent or -independent effects. Previous results showed a dose dependent effect of HCB in mouse mammary epithelial cells (NMuMG): 0.05 μM induced cell migration and transforminggrowth factor-β1 (TGF-β1) signaling, whereas 5 μM reduced cell migration, promoted cell cycle arrest, and stimulated AhR pathway. Our objective was to evaluate the HCB action in mammary gland from +/+AhR and -/-AhR C57BL/6N mice *in vitro* and *in vivo*. Because perturbations in mammary gland during puberty may enhance risk for later adverse effects, 4 weeks-old female mice were exposed to HCB (3 mg/kg body weight) for 21 days. Whole mount and immunohistochemistry results showed HCB to increase ductal hyperplasia (75% $p<0.05$), cell proliferation (PCNA levels, 100% $p<0.01$), nuclear estrogen receptor levels (80% $p<0.05$), branch density (15% $p<0.05$), and the number of terminal ends buds (18% $p<0.05$) in +/+AhR mammary gland. Interestingly, -/-AhR mice showed an increase in ductal hyperplasia (68% $p<0.05$) and growth (40% $p<0.05$) in absence of HCB treatment, thus revealing the importance of AhR in mammary development. However, HCB produced no changes in -/-AhR mouse mammary gland. Because interactions between epithelial cells and the stroma impacts in mammary and cancer development, immortalized mouse mammary fibroblasts (FGM) +/+AhR and -/-AhR were exposed to HCB (0.05 and 5 μM). 5 μM HCB enhanced α-smooth muscle actin expression (immunofluorescence, 508% $p<0.001$) and decreased TGF-β receptor II mRNA levels (RT-qPCR, 55% $p<0.05$) in FGM AhR+/+, resembling the phenotype of transformed cells. Accordingly, their conditioned medium was able to increase NMuMG cell motility (scratch motility assay, 84% $p<0.05$). These results show that environmental HCBconcentrations alter mammary branching morphogenesis, likely leading topreneoplastic lesions or enhanced malignancy.

SAIC SYMPOSIUM XIII

METABOLISM AND NUTRITION

MODULATING BIOENERGETIC METABOLISM FOR CANCER THERAPY

MARCELA S. VILLAVERDE

Unidad de Transferencia Genetica. Inst. de Oncología Ángel H. Roffo, Buenos Aires, Argentina

Cancer cells increase glucose uptake, even in the presence of adequate oxygen levels. This phenomenon is known as the Warburg effect and suggests a dependency on glycolysis, especially in rapidly growing tumors. Thus, it becomes cancer cell metabolism an attractive area of clinical and pre-clinical therapy developments. Metformin (MET) is a biguanide, clinically known as an oral well tolerated anti-diabetic drug. Numerous recent studies show that MET decreases cancer cell viability and tumor growth in different xenograft models. Furthermore, retrospective epidemiological studies revealed a decrease in the incidence of cancer in diabetic patients treated with MET. Apparently, MET modulates cell metabolism at different cell levels since MET increases glycolysis, inhibits respiratory chain complex I and ultimately inhibits mTOR leading to growth arrest and apoptosis. However, the molecular mechanisms underlying MET antitumor effects remains unclear. On the other hand, 2-deoxyglucose (2DG) is a reversible inhibitor of hexokinase, the first and rate-limiting enzyme of glycolysis. The inhibition of glycolysis decreases the production of glycolytic intermediates, which are the precursors of nucleic acids and phospholipids. In addition, depletion of glucose-6-phosphate also decreases pentose phosphate pathway (PPP) and consequently the antioxidant defenses of cancer cells. At present, different studies explored the combination of 2DG with chemotherapy as sensitizer. Here, we will describe the potential antitumor effect and the mechanism involve in the effectiveness of modulating bioenergetic pathways by MET in combination with 2DG or 6-aminonicotinamide (6AN, PPP inhibitor) on feline mammary carcinoma and melanoma cell lines as a preclinical approach of both veterinary and human disease.

(530) INHIBITION OF CYCLIN-DEPENDENT KINASE 4 (CDK4) ACTIVITY IN ADIPOCYTES ENHANCES THEIR THERMOGENIC PROGRAM

ANDREA PORTALES, IGNACIO MIGUEL, ANDRÉS GIOVAMBATTISTA

Instituto Multidisciplinario de Biología Celular (IMBICE-CONICET), Universidad Nacional de La Plata, Argentina

Beige adipocytes are thermogenically competent cells that develop in white adipose tissue (WAT) depots in response to b-adrenergic receptors stimulation. Two general mechanisms are accepted for beige adipocytes generation: *de novo* adipogenesis from beige precursor cells, and transdifferentiation from white adipocytes. In previous studies we found that inhibition of cyclin-dependent kinase 4 (CDK4) activity in stromal vascular fraction cells (SVF) from WAT led to an increase in beige adipocyte markers. Here we wanted to assess if the inhibition of CDK4 activity is involved in white to beige adipocyte transdifferentiation. To this aim, SVF cells from the epididimal depot of C57BL/6J mice were isolated (n=4), cultured to confluence and differentiated with an adipogenic cocktail. On day 8 post-differentiation, cells were treated or not with an inhibitor of CDK4 (Palbociclib (PAL) 1μM; CTR) for 48hs. In additional experiments, after the inhibition period, CTR and PAL cells were treated with Forskolin (FSK) 10μM for 4hs in order to activate the thermogenic program (CTR-F and PAL-F). Finally, on day 10 cells were processed for total RNA extraction and RT-qPCR quantification of thermogenic (*Ucp1*, *Prdm16*), beige (*Cd137*) and general adipogenic (adiponectin) markers. Results showed that inhibition of CDK4 in adipocytes resulted in a significant increase in *Ucp1* and *Prdm16* mRNA levels in the PAL group compared to the CTR ($p<0,05$), although *Cd137* mRNA levels remained the same. Interestingly, adiponectin was expressed at lower levels in the PAL group ($p<0,05$ vs CTR). As expected, upon FSK stimulation both, CTR-F and PAL-F groups, responded with a very large induction of *Ucp1* expression ($p<0,05$ CTR-F vs CTR, PAL-F vs PAL) while no induction of *Prdm16* was found. Our results allow us to conclude that inhibition of CDK4 would be involved in upregulation of thermogenic markers in adipocytes, suggesting that CDK4 may control the basal thermogenic state of adipocytes. PICT-2013-0930.

(751) ACTIVATION OF NFKB SIGNALING PATHWAY IN RENAL CORTEX OF FRUCTOSE-FED RATS: EFFECTS OF DIETARY (–)-EPICATECHIN

PAULA DENISE PRINCE[1], EZEQUIEL HID[1], JORGE E TOBLLI[2], CÉSAR G FRAGA[1,3], MÓNICA GALLEANO[1]

(1) Cátedra de Fisicoquímica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina. (2) Laboratorio de Medicina Experimental, Hospital Alemán, Argentina. (3) Department of Nutrition, University of California, Davis, EE. UU.

High fructose consumption (HFC) has been associated to deleterious metabolic conditions. In the kidney, high fructose causes alterations that contribute to the development of chronic kidney disease. Among them, inflammation is a key player of renal damage and loss of function. We evaluated the capacity of the flavanol (-)-epicatechin (EC) in attenuating the NFkB dependant inflammation induced by HFC in the kidney cortex of rats. Male Sprague-Dawley rats were fed standard diet and water (C), standard diet and fructose 10% (p/v) in the drinking water (F) and standard diet with EC (20 mg/kg BW) and the fructose solution (FE) for 8 w. No changes were observed in the expression levels of TLR-4 among the experimental groups. However, the NFkB activation signaling pathway resulted significantly increased in F group respect to C and FE groups, in terms of higher levels of activating phosphorylation in IKKα/β and p65, higher levels of phosphorylation of IkBα in Ser32 and a higher nuclear p65/cytosolic p65 ratio, an indicator of NFkB translocation to the nucleus (+99%*, +41%*; +113%* and +37%* F vs. C). In parallel with these results, F group showed a higher superoxide anion production (+107%* F vs. C) associated with an increased expression of NOX2 and NOX4 subunits, which were not observed in C and FE groups. EC supplementation also attenuated the increased expression of the inflammatory molecules iNOS, TNFα and IL-6 in kidney cortex induced by HFC. Although TLR-4 expression was not altered, a possible activation of this receptor by LPS may be possible, given that in HFC models a higher intestinal permeability is observed. Other TLR-4 independent mechanisms could be responsible for NFkB activation e.g. reticulum stress-induced IKK phosphorylation, triggered by metabolic surplus. This work supports the anti-inflammatory effect of EC, describing its modulation on NFkB signaling pathway in a context of chronic inflammation in rat kidney. *$p < 0.05$

(845) METABOLIC SYNDROME AND NEUTROPHIL RETENTION IN THE LUNG: A MOUSE MODEL

MARIA CECILIA DELLA VEDOVA, FLORENCIA MARTINA SOLER GARCIA, LUCIA BEATRIZ FUENTES, LUCAS DAMIAN SANTILLA, SANDRA ESTHER GOMEZ MEJIBA, NIDIA NOEMÍ GÓMEZ, DARÍO CEFERINO RAMÍREZ

Laboratory of Experimental and Translational Medicine, Laboratory of Molecular Biology, Laboratory of Experimental Therapeutics, Laboratory of Morpho-Physiology--IMIBIO-SL-CONICET-UNSL, San Luis, Argentina

The metabolic syndrome (MS) is a deadly metabolic abnormality-associated to obesity. The pulmonary microvasculature is a sink of circulating neutrophils; as well as is highly sensitive to small changes in the systemic oxidative/inflammatory profile, as occur in obesity. Previously, we have characterized a MS-mouse model in which animals were fed for 16 weeks a 22% p/p chicken-fat rich diet and 10 % fructose in the drinking water. These animals show several features of MS including obesity, central obesity, insulin resistance, hypertension, dyslipidemia and streatohepatitis. Using this model we envisioned to test whether MS predispose to retention/activation of neutrophils and how it affects whole-body insulin resistance (IR). To accomplish this goal we studied MS and control mice (B6 mice fed for 16 weeks with low-fat diet/tap water). MS animals had more IR than control mice. The MS mice had also higher concentration of inflammatory mediators than control mice. The lung tissue of MS mice was lighter and expressed more inflammation mediators (TNF-α; IL-6 and inducible nitric oxide) than the control lung. The lung of MS mice had more neutrophils (NIMP-14^+ cells), myeloperoxidase (MPO) activity and chlorotyrosine than control mice. ICAM-1 expression in MS mice's lung tissue was higher than control mice. In relation to control mice, intratracheal instillation (it) 2.5 ug lipopolysaccharide (LPS)/mouse to MS mice caused more retention/activation of neutrophils, ICAM-1 expression, MPO activity, chlorotyrosine, circulating inflammatory mediators and worse IR. These effects where damped by it of 5 nmol of 5,5-dimetil-1-pirrolina *N*-oxido (DMPO)/mouse. Our data suggest that retention/activation of neutrophils in the lung may be a potential therapeutic target to reduce IR and other complications of obesity. Supported by PROICO 2-3214 & PICT-2014-3369 (toDCR), PROICO 10-0414 (ToSEGM) and PIP2015-2017-112215-0100603CO (To DCR, SEA & SEGM).

(567) EFFECTS OF HEME OXYGENASE INDUCTION ON OXIDATIVE STRESS MARKERS IN THE LIVER OF INSULIN RESISTANT RATS

MORENA WISZNIEWSKI, CAROLINA VECINO, JUAN SALVADOR CALANNI, SILVIA SANCHEZ PUCH, CORA BEATRIZ CYMERYNG, ESTEBAN MARTÍN REPETTO

Laboratorio de Endocrinología Molecular (LEM). Centro de Estudios Farmacológicos y Botánicos –CEFYBO/CONICET. Facultad de Medicina, Universidad de Buenos Aires, Argentina

Insulin resistance (IR) is a key factor involved in the pathogenesis of non alcoholic fatty liver disease along with obesity and type 2 diabetes. As heme oxygenase-1 (HO-1) has been recognized as an antioxidant enzyme playing a role in cellular defense mechanisms the aim of this study was to examine the effects of pharmacological manipulation of HO-1 on cytoprotective systems in the liver of IR rats. Male Wistar rats were randomly distributed in different groups: control (C), sucrose-rich diet (SRD, 30% sucrose in the drinking water over 12 weeks). Hemin treatment (15 mg/kg/48h, ip) was initiated after 10 weeks of diet (H and SRD+H groups) and continued for 2 weeks. Insulin sensibility was evaluated with an insulin tolerance test. Serum samples were obtained for glucose and triglyceride (TG) determination as well as liver tissue for the analysis of superoxide dismutase (SOD) and catalase (CAT) activities. Results indicate that administration of SRD (SRD and SRD+H) correlates with a decrease in insulin sensitivity. No differences in glycaemia or body weight were observed due to pharmacological or dietary treatment vs C. TG levels were increased only in SRD group vs C ($p<0.05$). HO-1 induction was determined by western blotting in liver tissues of animals under SRD and/or H treatment. In addition, we detected an increase in the activities of SOD and CAT in samples obtained from SRD-treated rats, an effect that was prevented by H treatment: 1) SOD activity (U/mg protein) mean±SEM, (n=3/group), C 5.3±0.4, H 6.7±0.9, SRD 20.3±2.9 $p<0.001$ vs C, SRD+H 9.0±1.2, $p<0.01$ vs SRD. 2) CAT activity (mM H_2O_2/min/mg protein) mean±SEM, (n=4/group): C 1.48±0.02, H 1.03±0.09, SRD 3.73±0.72 $p<0.01$vs C, SRD+H 1.78±0.39, $p<0.05$ vs SRD. In summary our results suggest that long term administration of SRD to rats generates oxidative stress in the liver leading to an increase in the activity of antioxidant enzymes. We also suggest that HO-1 induction by H could attenuate these effects.

(281) ADAPTIVE RESPONSES OF CYTOSOLIC SUPEROXIDE DISMUTASE AND CATALASE IN RAT BRAIN AFTER ACUTE IRON AND COPPER OVERLOADS

JUAN MANUEL ACOSTA[1], ROSARIO NATALIA MUSACCO SEBIO[1], CHRISTIAN MARTÍN SAPORITO MAGRIÑÁ[1], MAURICIO CASTRO PARODI[2], ALICIA DAMIANO[2,3], JULIÁN FUDA[4], HORACIO TORTI[4], NIDIA FERRAROTTI[5], ALBERTO BOVERIS[1,6], MARISA GABRIELA REPETTO[1,6]

Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, (1) Cátedra de Química General e Inorgánica y (2) Cátedra de Biología Celular y Molecular. (3) Consejo Nacional de Investigaciones Científicas y Técnica, Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO-CONICET), (4) Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, (4) Cátedra de Física, (5) Departamento de Bioquímica Clínica. (6) Consejo Nacional de Investigaciones Científicas y Técnica, Instituto de Bioquímica y Medicina Molecular (IBIMOL, UBA-CONICET), Buenos Aires, Argentina

The time course of the genomic response that involves the expression of SOD1, catalase and Nrf2 in rat brain was evaluated in the period of 0-24 h after iron (Fe) and copper (Cu) ions loads. The molecule that seems most likely to be the signal for the genomic transcription is a soluble phospholipid hydroperoxide (ROOH), where R indicates a 4-6 carbon chain, that immediately increases its steady state concentration along with the increased rates of lipid peroxidation. The hypothesis is that the activation of Nrf2-ARE signaling pathway, one of the physiologic mechanisms in cellular defense against oxidative stress that controls the expression of genes whose proteins products, is involved in detoxification and elimination of reactive oxidants and by enhancing cellular antioxidant content. The aim of the present study was to determine the characteristics of the adaptive response in rat brain after Fe and Cu ions overloads. The activities and expressions of brain cytosolic superoxide dismutase (SOD1), and catalase were determined as well as the levels of the regulatory transcription factor Nrf2 in the cytosol and nucleus. The ratio of reduced glutathione/oxidized glutathione (GSH/GSSG) was determined as a reflection of the –SH/-SS- ratio of regulatory control proteins of neuronal metabolism and function. Rats (220 ± 8 g) were given single doses of 13.5 mg $FeSO_4$/100 g or of 2.8 mg $CuSO_4$/100 g and the brain response to these overdoses was followed. SOD1, catalase, glutathione transferase and Nrf2 increased their activities/expressions in an adaptive response to oxidative stress: after 24 h of Fe and Cu administration, expression was increased: SOD1, 2.5 and 1.8 times; catalase 2 and 2.5 times ($p<0.01$). Nrf2 expression increased 1.5 times after 6 h ($p<0.01$). Conclusions: Nrf2-ARE signaling pathway, associated to GSH homeostasis, regulates brain antioxidant enzymes and ROOH seems to be the likely signaling molecule for the adaptive response after Fe and Cu loads.

(342) MATERNAL FRUCTOSE RICH DIET INTAKE THROUGHOUT GESTATION INHIBITS THE ADIPOGENIC POTENTIAL OF WHITE ADIPOSE TISSUE PRECURSOR CELLS FROM THE MALE OFFSPRING

ANA ALZAMENDI[1], GUILLERMINA ZUBIRÍA[1], ANDREA PORTALES[1], EDUARDO SPINEDI[2], ANDRÉS GIOVAMBATTISTA[1]

1. Unidad de Neuroendocrinología. Instituto Multidisciplinario de Biología Celular (CONICET La Plata-CICPBA-UNLP). La Plata. (1900). 2. Centro de Endocrinología Experimental y Aplicada (CONICET La Plata-UNLP). La Plata, Argentina.

Previous studies from our group indicate that fructose rich diet (FRD) consumption during gestation induces a decrease in adipose precursor cells (APCs) from retroperitoneal adipose tissue (RPAT) thus reducing local adipocyte number and mass in the adult male progeny. These changes favor adipocyte hypertrophy and a distorted adipokine secretion pattern. We now evaluated the impact of FRD intake by the gestating dams on the adipogenic capacity of stromal vascular fraction (SVF) cells from RPAT, of their adult male progeny. On pregnancy day 1, dams were provided with either tap water alone (control) or containing fructose (10% w/v in drinking water; FRD) and fed *ad libitum* with chow up to delivery. Lactating dams and their pups (between 21 and 60 of age) received water and chow *ad libitum*. C and F animals will come from control and FRD dams, respectively. On experimental day (age 60 days) RPAT pads were dissected and SVF cells were isolated. The mRNA expression levels of adipogenic potential markers were assessed by qPCR in RPAT SVF cells and pads. PPARγ expression was quantified in differentiating cells and markers of terminal differentiation were also evaluated. Our data indicate that local F SVF cells express lower and higher levels ($p<0.05$) of CD34 and Pref-1, respectively. Immunofluorescence for PPARγ labeled cells (on day 4 post-differentiation) was lower in F than C animals ($p<0.05$). On day 10 post-differentiation, a lower number of differentiating F cells was noticed ($p<0.05$ vs

C). Increased Ob and LPL gene expression was found in RPAT pads from F rats ($p<0.05$). These results indicate that pre-natal nutritional intervention induces in adult male progeny a decrease in the adipogenic potential of RPAT APCs, favoring an unhealthy pad mass expansion (hypertrophic). A dysfunctional RPAT strongly contributes to develop multiple endocrine-metabolic disorders. PICT-2013-0930/CICPBA/FPREDM.

SAI SYMPOSIUM I

IMMUNITY AGAINST TUMORS

CONCOMITANT TUMOR RESISTANCE: A PUTATIVE MECHANISM TO CONTROL METASTATIC GROWTH.

RAÚL RUGGIERO, PHD.
IMEX-CONICET, Academia Nacional de Medicina, Buenos Aires, Argentina.

TUMOR INFILTRATING DYSFUNCTIONAL CD8+ T CELLS: A POTENTIAL IMMUNOMODULATORY ROLE.

CAROLINA MONTES, PHD.
CIBICI-CONICET, Facultad de Ciencias Químicas,
Universidad Nacional de Córdoba, Argentina.

UNWELCOME COMPLEMENT: IMMUNOSUPPRESSIVE FUNCTIONS OF COMPLEMENT IN TUMORS AND METASTASIS-TARGETED ORGANS.

MACIEJ MARKIEWSKI, MD, PHD.
Department of Immunotherapeutics and Biotechnology, Texas Tech University Health Science Center
School of Pharmacy, Abilene, Texas, USA.

(242) LOW PH IMPAIRS COMPLEMENT-DEPENDENT CYTOTOXICITY AGAINST IGG-COATED TARGET CELLS

EZEQUIEL DANTAS, FERNANDO ERRA DIAZ, PEHUÉN PEREYRA GERBER, ANTONELA MERLOTTI, AUGUSTO VARESE, MATÍAS OSTROWSKY, JUAN SABATTÉ, JORGE GEFFNER
CONICET, Consejo Nacional de Investigaciones Científicas y Técnicas y UBA, Universidad de Buenos Aires.

Extracellular acidosis is a hallmark of inflammatory conditions and solid tumors. However, few studies have addressed the influence of extracellular pH on the immune response. Here, we analyzed whether low pH could modulate complement-dependent cytotoxicity (CDC) against IgG-coated cells. Using human serum as a complement source, we found that extracellular pH values of 6.0 and 5.5 inhibit CDC against either the B cell line Raji coated with the chimeric anti-CD20 mAb rituximab (% inhibition = 48 ± 8 and 92 ± 7, respectively, n=6, $p<0.01$ vs controls) or PBMCs coated with the humanized anti-CD52 mAb alemtuzumab (% inhibition = 43 ± 6 and 88 ± 5, respectively, n=5, $p<0.01$ vs controls). Interestingly, low pH also impaired CDC assessed in the more physiologic milieu of whole blood (% inhibition at pH 6.0 and 5.5 = 49 ± 7 and 94 ± 7, respectively, n=5, $p<0.01$ vs controls). Suppression of CDC by low pH was shown to be a reversible phenomenon associated to the inhibition of both, the classical and alternative pathways of complement activation, which resulted in a reduced generation of C3a and C3b. This suggests that the major functions of the complement system triggered by IgG antibodies would be impaired by low pH. Local acidosis is a common feature of inflammatory reactions associated to infectious, allergic, vascular, autoimmune, and cancer diseases. Our observations suggest that in all these conditions, extracellular pH might exert important immunomodulatory effects by inhibiting the ability of IgG antibodies to activate the complement system.

(414) INTRINSIC ROLE OF GALECTIN-1 IN THE CONTROL OF THE FUNCTIONAL PROPERTIES OF IMMUNE CELLS IN A PROSTATE CANCER CONTEXT

ENRIQUE SEBASTIÁN CORAPI[1], GUSTAVO EZEQUIEL CARRIZO[1], LAURA GIRIBALDI[2], FELIPE MARTIN JAWORSKI[1], GABRIEL ADRIÁN RABINOVICH[2], DANIEL COMPAGNO[1], DIEGO JOSÉ LADERACH[1].

[1]Laboratorio de Glicooncología molecular y funcional, IQUIBICEN-CONICET, Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina. [2]Laboratorio de Inmunopatología, IBYME-CONICET, Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina.

The identification of effective new therapies for prostate cancer (PCa) requires a better understanding of the multiple molecular interactions between tumour cells and their associated stroma.In this context, Galectin-1 (Gal-1) plays a major role in determining the properties of the prostatic carcinoma microenvironment. The aim of this study is to elucídate whether Gal-1, in additionto promote tumor neoangiogenesis and immune regulations such as induction of apoptosis on activated T cells, inefficient antigen presentation and expansion of regulatory T cells, plays an additional role as an intrinsic regulator of CD8+ T cell functional properties. To address this concern, we used an in vitro T cell polyclonal activation model combining different cell types (purified by cell sorting) in a prostate tumor microenvironment. Thus, by combination of Gal-1 deficient (Lgals1-/-) and wild type (WT) cells, we were able to assess how the endogenous Gal-1 of each cellular compartment impacts on the CD8+ T cell properties (proliferation and citotoxicity). The absence of Gal-1 in antigen presenting cells (APCs) did not significantly modify the proliferative properties of CD8+T cells. Conversely, the absence of Gal-1 in CD4+ T cells induced a 1.21 fold increase in the proliferation of CD8+T cells. However, the most significant difference in the proliferation was obtained by absence of intrinsic Gal-1 in the CD8+ T cells themselves (2.12). Taking into account Gal-1 controls T cell proliferation, we further evaluated whether Gal-1 is relevant in controlling effector function. Our results-demonstrated that upon activation, Lgals1-/- T cells have increased ability to degranulate (evaluatedas % (1.87fold) and the content of granules (2.48 fold increase) ($p < 0.05$, t test Student). Altogether, these results place Gal-1 as a potent intrinsic molecular mechanism that down-regulates the functional properties of CD8+ T cells in PCa.

(539) TUMOR-INDUCED IL-18 PROMOTES PD-L1 EXPRESSION ON HUMAN NK CELLS

JESSICA MARIEL SIERRA, XIMENA LUCÍA RAFFO IRAOLAGOITIA, SOL YANEL NUÑEZ, ANDREA ZIBLAT, NICOLÁS IGNACIO TORRES, FLORENCIA SECCHIARI, CAROLINA INÉS DOMAICA, NORBERTO WALTER ZWIRNER, MERCEDES BEATRIZ FUERTES

Laboratorio de Fisiopatología de la Inmunidad Innata. Instituto de Biología y Medicina Experimental (IBYME-CONICET).

Natural killer (NK) cells are important mediators in the elimination of tumor and virus-infected cells, however, novel reports show a regulatory role for NK cells in different models of autoimmunity and viral infections. We have shown that NK cells from tumor bearing mice express the inhibitory molecule PD-L1 and are able to control $CD8^+$ T cell priming to tumor antigens in vivo. Moreover, in human NK cells, direct tumor recognition through NKG2D induced PD-L1 up-regulation, which was further enhanced in the presence of peripheral blood mononuclear cells (PBMCs). Therefore, the objective of this work was to elucidate the mechanisms involved in PBMC-mediated induction of PD-L1 expression on human NK cells after tumor recognition. To this end, PBMCs were cultured with K562 tumor cells and the conditioned medium (CM) obtained was used to stimulate NK cells. The CM was able to induce PD-L1 expression on NK cells ($CD3^-CD56^+$) as assessed by flow cytometry, suggesting the involvement of soluble factors. Aiming to identify these factors, PBMCs or isolated NK cells were stimulated with different doses of NK cell-activating recombinant cytokines (IL-12, IL-15 or IL-18) or cultured with K562 cells in the absence or in the presence of blocking antibodies to IL-12, IL-15 and/or IL-18. We found that IL-12 was not able to modulate PD-L1 expression. Although PD-L1 expression was induced by IL-15 on NK cells (within PBMCs, $p<0.01$ or isolated, $p<0.01$), IL-15 blockade during the co-culture of PBMCs with K562 cells did not modulate PD-L1 expression, suggesting that it is not involved in tumor-induced PD-L1 up-regulation. Finally, we found that PD-L1 expression on NK cells within PBMCs was induced by IL-18 ($p<0.0001$); moreover, IL-18 blockade in co-cultures of PBMCs with K562 cells abrogated tumor-induced PD-L1 up-regulation ($p<0.05$). Our results demonstrate that IL-18 produced by PBMCs after tumor recognition is able to up-regulate PD-L1 expression on human NK cells.

(848) DEFICIENCY IN THE IL-17RA/IL-17 PATHWAY AFFECTS PRIMARY AND SECONDARY ANTITUMOR RESPONSES PROMOTING TUMOR GROWTH

CONSTANZA RODRÍGUEZ, JIMENA TOSELLO BOARI, CINTIA ARAUJO FURLAN, FERNANDO PABLO CANALE, CRISTIAN GABRIEL BECCARIA, ADRIANA GRUPPI, CAROLINA LUCÍA MONTES, EVA VIRGINIA ACOSTA RODRÍGUEZ

Centro de Investigaciones en Bioquímica Clínica e Inmunología. CIBICI-CONICET. Departamento de Bioquímica Clínica. Facultad de Ciencias Químicas. Universidad Nacional de Córdoba.

The role of IL-17 cytokines in cancer remains controversial as both anti- and pro-tumoral effects have been described. We and others demonstrated that IL-17 family plays a central role for the induction of NK and CD8+ T cell (CTL) responses. As these subsets are critical for host resistance to cancer, we evaluated the role of IL-17/IL-17R in modulating anti-tumor immunity and tumor progression. To this end, B6 (WT), IL-17RA KO (RKO) and IL-17A/F double KO (DKO) mice were injected with tumor cell lines that displayed progressor (B16-OVA, B16-SIY and MCA101-OVA) and regressor (MC57-SIY) growth patterns. Tumor progression and immune responses were studied at different days (d) post-injection (pi). Upon injection of B16 cells, RKO and DKO mice showed increased tumor volume and weight in comparison to WT mice ($p<0.05$, d21pi). In contrast, tumors induced by MCA101 cells had similar volumes in DKO and WT mice. Although RKO and DKO rejected MC57 tumors as efficient as WT mice, they presented higher tumor volumes between d3-8pi ($p<0.05$). Initial studies showed that MC57-bearing DKO mice presented at d12pi reduced numbers of SIY-specific CTL in draining-lymph nodes in comparison to WT controls ($p<0.05$). In addition, SIY-specific CTL from DKO mice displayed decreased frequency of cells with memory phenotype (CD62L+CD127+). As the memory CTL response developed in WT mice upon MC57-SIY cell injection is critical to protect hosts against challenge with B16-SIY tumors, we evaluated whether the SIY-specific CTL elicited in immunized RKO and DKO mice were also efficient against challenge. Of note, while all immunized WT never develop tumor or were tumor-free at d20pi of B16-SIY cells, 100% of the immunized DKO and RKO mice developed tumors and only 50% were tumor-free at d20 post-challenge. Altogether, our results indicate that the IL-17/IL-17RA pathway likely modulate primary and secondary CTL responses against tumors and may have a protective role during certain types of cancers.

SAI SYMPOSIUM II

IMMUNITY AGAINST INFECTIOUS AGENTS

IMMUNOMODULATORY PROPERTIES OF THE TREMATODE FASCIOLA HEPATICA: EFFECTS ON DENDRITIC CELLS AND MACROPHAGE FUNCTIONS.

LAURA CERVI, PHD.

CIBICI-CONICET. Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina.

HIV-MEDIATED INDUCTION OF HYPOXIA INDUCIBLE FACTOR-1 ALPHA ACTIVITY IN CD4+ T CELLS MODIFIES IMMUNOMETABOLIC PHENOTYPE AND DECREASES CELL SURVIVAL.

MATÍAS OSTROWSKI, PHD.

INBIRS-CONICET. Facultad de Medicina, Universidad de Buenos Aires, Argentina.

HANTAVIRUSES AND THEIR TARGETS, AND THEIR TARGETS´ TARGETS, AND THEIR TARGETS´ TARGETS´ TARGETS.

JONAS KLINGSTÖM, PHD.

Department of Medicine, Karolinska Institute, Stockholm, Sweden.

(580) MTOR INHIBITION IN TRYPANOSOMA CRUZI INFECTED MACROPHAGES PRODUCES INFLAMMATORY MEDIATORS THAT REGULATE ITS SURVIVAL.

JORGE DAVID ROJAS MÁRQUEZ, YAMILE ANA, CINTHIA STEMPIN, FABIO CERBAN

Departamento de Bioquímica Clínica. Centro de Investigaciones en Bioquímica Clínica e Inmunología – CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba.

We have previously shown that *Trypanosoma cruzi* infection in macrophages (Mo) activates mTOR and its inhibition decreased parasite replication. Besides, in rapamycin (Rap) pre-treated and infected Mo we observed reduced arginase activity and expression compared to control cells. Surprisingly, we also found reduced iNOS activity and expression. Therefore, the aim of this work was to determine alternative mechanisms involved in controlling parasite replication in Rap pre-treated and infected Mo. In this context, we evaluated ROS production. We did not found differences in ROS production after 24h post infection (pi) between Rap or DMSO pre-treated and infected Mo. Also, we evaluated whether indoleamine 2,3-dioxygenase (IDO) enzyme was involved. To do that, we inhibit IDO activity by using 1MT (1-methyl-tryptophan) and we study parasite replication by immunofluorescence (IF). We did not found differences compared to control cells without 1MT and then IDO activity is either not involved in decreasing parasite replication in Rap pre-treated and infected Mo. Consequently, to study possible mediators involved in parasite killing in BMDM from different KO mice (TLR2, TLR4, IFNa-R, Caspase-1, IL-6, TNFa-R and NLRP3), parasite load was evaluated 72h pi by IF. We observed a significant increase in parasite load in Rap pre-treated and infected BMDM from IL-6-KO, TNFa-R-KO and NLRP3-KO mice compared to WT pre-treated and infected control cells for each strain. However, parasite number stands out in BMDM from NLRP3 KO ($p<0.05$). Taking into account that NLRP3 is one of the main components of the inflammasome our current studies are focused on demonstrating its activation during infection and mTOR inhibition. We found that Rap pre-treated and infected WT BMDM showed a significant increase in NLRP3 expression at 6h pi ($p<0.05$). These results would indicate that NLRP3 activation may be one of the mechanisms involved in reducing parasite replication in Rap pre-treated and infected Mo.

(1054) SEMINAL VESICLE FLUID INCREASES THE EFFICACY OF INTRAVAGINAL HSV-2 VACCINATION

AUGUSTO VARESE, JOSÈ ODDI, FEDERICO REMES LENICOV, MELINA GONZÁLEZ PRINZ, ANTONELA MERLOTI, JORGE GEFFNER, ANA CEBALLOS

Instituto de Investigaciones Biomédicas en Retrovirus y SIDA (INBIRS), Universidad de Buenos Aires -CONICET, Buenos Aires, Argentina.

Semen induces biological actions on the female reproductive tissues directed to modulate the immune response against paternal antigens. However, the influence of seminal plasma on the immune response against sexually transmitted pathogens has not been yet evaluated. Our aim was analyze whether the seminal vesicle fluid (SVF), might compromise the induction of a protective memory response induced by HSV-2 vaccination. SVF was extracted post-mortem from 10-week-old BALB/c males. Female BALB/c mice were vaccinated IVAG with inactivated HSV-2 ($1x10^4$ pfu/15 ìl), without or with SVF (protein concentration 5 mg/ml). Thirty days later, mice were challenged IVAG wiht HSV-2 lethal dose. Mice were examined for clinical score and survival. Draining lymph nodes (DLN) and genital mucosa cells were recovered and phenotype and cytokines production was assessed by flow cytometry, ELISA, and qPCR. We observed that SVF-vaccinated mice showed 90% survival (control 10%; n=20; $p<0.001$), and minor disease progression (1.278±0.1 Mean±SEM n=20; $p<0.001$). These mice had increased IFN-ã (n=3 $p<0.05$), TNF-á (n=3 $p<0.001$), IL-17 (n=3 $p<0.05$), IL-6 (n=3 $p<0.05$) and lower IL-10 (n=3 $p<0.05$) production at the site of infection. Also, we found SVF-vaccinated mice showed a higher frequency of memory/effector like T cells ($CD44^{high}CD62L^{low}$) and central memory T cells ($CD44^{high}CD62L^{high}$) in both CD4+ (n=3 $p<0,001$) and CD8+ (n=3 $p<0,05$) T cell compartments. These changes were shown to be associated to a marked increase in the production of TNF-á and IFN-ã (Mean±SEM n=3 $p<0,001$ and $p<0,0005$, respectively). We observe that SVF significantly increased the expression of CD86 (MFI 5982 vs 3808, n=3) in vaginal DCs 48 hs post vaccination and the frequency and the total number of DCs in DLN (n= 3, $p<0,05$). In contrast with the notion that semen acts as an immunosuppressive agent, our results suggest that SVF might induce an adjuvant effect on the female immune response against sexually transmitted pathogens.

(2008) CONTRIBUTION OF INFLAMMASOMES TO THE CONTROL OF THE RESPIRATORY INFECTION BY BRUCELLA SPP.

ANDREA GISELLE FERNANDEZ[1], MARÍA SOLEDAD HIELPOS[1], JULIANA FALIVENE[1], MARIANA C. FERRERO[1], SERGIO COSTA OLIVEIRA[2], PABLO C. BALDI[1]

[1]IDEHU (CONICET-UBA), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires. [2]Departamento de Bioquímica e Inmunología, Universidad Federal de Minas Gerais, Belo Horizonte, Brasil.

Inhalation of infected aerosols is one of the most frequent routes for acquiring *Brucella* spp. infection. The role of IL-1β and inflammasome activation in the initial control of this infection has not been studied. To determine whether pulmonary IL-1β contributes to the control of airborne *B. abortus* infection, IL-1 receptor *knock-out* mice (IL-1R KO) and wild type (WT) controls (C57BL/6 mice) were infected by the intra-tracheal route, and lung CFU were measured at 2 and 7 days p.i. While no significant difference was found at 2 days p.i., CFU counts were significantly higher in IL-1R KO mice at 7 days p.i. as compared to WT (mean, 3.15×10^7 vs. 1.12×10^6 CFU; $p<0.01$). Pulmonary levels of KC (neutrophils chemoattractant) were significantly lower in IL-1R KO mice at 7 days p.i. (88.3 vs. 146.2 pg/ml, $p<0.05$). To determine whether inflammasomes contribute to the control of airborne *B. abortus* infection, KO mice for caspase-1 (Casp-1), NRLP3 or AIM2 were infected by the intra-tracheal route, and lung CFU and cytokines were measured at different p.i. times. CFU counts were higher in Casp-1, NLRP3 and AIM2 KO mice (8.01×10^6, 1.18×10^7, and 5.43×10^6 CFU/ml, respectively) as compared to WT (0.81×10^6 CFU/ml), although differences only reached statistical significance for NLRP3 ($p<0.01$). In addition, at 2 days p.i. levels of IL-1β were lower in Casp-1 KO mice than in WT (213 vs. 465 pg/ml, $p<0.01$), and the same was true for KC (96 vs. 157 pg/ml, $p<0.01$), IL-12 (73 vs. 295 pg/ml, $p<0.05$) and TNF-β (130 vs. 260 pg/ml, $p<0.001$). To determine the role of different lung cells in these responses, alveolar macrophages and pneumocytes were obtained from WT mice and were infected in vitro in the presence or absence of a Casp-1 inhibitor (Z-WEHD-FMK). For both cell types, IL-1β levels were significantly lower in the presence of the inhibitor. These results show that inflammasomes play an important role in the initial control of *Brucella* infection acquired through the airways.

(54) B. ABORTUS MODULATES OSTEOBLAST FUNCTION THROUGH THE INDUCTION OF AUTOPHAGY

AYELÉN IVANA PESCE VIGLIETTI, PAULA CONSTANZA ARRIOLA BENITEZ, GUILLERMO HERNÁN GIAMBARTOLOMEI, MARÍA VICTORIA DELPINO

Instituto de Inmunología, Genética y Metabolismo (CONICET-UBA), Buenos Aires, Argentina

Osteoarticular brucellosis is the most common localization of human active disease. Osteoblasts are specialized mesenchyme-derived cells involved in bone formation and are considered as professional mineralizing cells. We demonstrated that *B. abortus* infection modified osteoblast metabolism by the inhibition of the deposition of organic and mineral matrix, leading to bone loss. *B. abortus* replicative vacuole involved autophagy pathway activation and on the other hand, autophagy has been involved in osteoblast metabolism. Then experiments were conducted to determine if *B. abortus* modulates osteoblast function through the activation of autophagy. To this end, *B. abortus* infected osteoblasts cells (MC3T3 E1 cell line) were lysed to determine LC3II, beclin-1 and p62 expression by Western blotting. MMPs production was determined by gelatin zymography, collagen deposition by Sirius red staining and alizarin red S staining to determine calcium deposition. *B. abortus* infection increased the levels of LC3II ($p<0.01$) and beclin-1($p<0.01$) and inhibited p62 expression indicating autophagy pathway induction. In addition, when *B. abortus* infection experiments were performed in the presence of bafilomycin A1 or chloroquine we did not observe inhibition of deposition of mineral and organic matrix ($p>0.05$). Taking together our results indicated that *B. abortus* induced the activation of autophagy pathway in osteoblast cells and this activation is involved in the modulation of osteoblast function and bone formation.

SAI SYMPOSIUM III

MEMORY T CELLS

T CELL PRIMING AND MEMORY T CELL RESPONSE TO PERIPHERAL VIRUS INFECTION

SCOTT MUELLER, PHD.

Dept. Microbiology & Immunology, The University of Melbourne and The Peter Doherty Institute for Infection and Immunity, Melbourne, Australia.

T CELLS AS SENTINELS OF THE INTESTINAL MUCOSA.

PABLO ROMAGNOLI, PHD

Instituto Universitario de Ciencias Biomédicas de Córdoba (IUCBC), Córdoba, Argentina.

(861) ARYL HYDROCARBON RECEPTOR SIGNALING MODULATES CD8 + T CELL EFFECTOR AND MEMORY SUBPOPULATIONS DURING THE INFECTION WITH TRYPANOSOMA CRUZI.

CONSTANZA INSFRÁN, LAURA FERNANDA AMBROSIO, XIMENA VOLPINI, HORACIO MARCELO SERRA, CLAUDIA CRISTINA MOTRÁN

Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI)-CONICET-UNC, Córdoba, Argentina

The acquisition of memory T cells is defined by the generation and persistence of T cells that can provide long-lasting protection against pathogens. Signals given by dendritic cells (DC) as TCR engagement, costimulation and cytokines, co-participate in the induction of memory T cells. Thus, changes in any of the factors controlling the activation of T cells during antigen presentation by DC can regulate T effector and memory cell differentiation. Different observations suggest that AhR, is a ligand-activated transcription factor that plays important roles in several biological processes, modulates critical events in the activation of naive T cells that could modify the development of effector and memory T cells. To investigate the role of AhR activated by natural ligands generated during *T. cruzi* infection, B6 (WT) and B6 mice carrying a mutant AhR protein with reduced affinity for its ligands (AhRd) were infected with 100.000 *T. cruzi* trypomastigotes and different splenic subpopulations of CD8+ T cells were studied by FACS at day 10 and 170 postinfection (pi). AhRd mice showed significantly lower frecuency and number of CD8+ T cells specific for the inmunodominant epitope TSKB20 (ANYKFTLV) than WT mice at day 10 pi, (p<0,05). Interestingly, analysis of CD44 and CD127 expression that distinguish memory precursors effector cells (MPECs: $CD44^{hi}$ $CD127^{hi}$) from short lived effector cells (SLECs: $CD127^{lo}$ $CD44^{hi}$) in subpopulations of CD8+ and CD8+TSKB20+ T cells revealed that, at day 170 pi, the frequency and number of splenic MPECS was higher in AhRd than in WT mice, (p<0,01). The study of memory subpopulations, $CD44^{hi}$ $CD127^{hi}$ $CD62L^{hi}$ (central memory, CM) or $CD62L^{lo}$ (effector memory, EM) at day 170 pi showed that the frequency and number of splenic EM and CM CD8+TSKB20+ T cells were higher in AhRd than WT mice, (p<0,01). Thus, during *T. cruzi* infection AhR signaling restricts the differentiation of CD8+ memory T cells.

(92) ALTERED EXPRESSION OF LTCD4 AND LTCD8 NAIVE AND MEMORY SUBSETS IN HIV INFECTED CHILDREN.

ALEJANDRA URIOSTE, ESTEFANIA CAPECCE, MARCELA CANDI, JEANETTE BALBARYSKI, GRACIELA BARBONI, EDUARDO GADDI

División Inmunología, Hospital General de Niños Dr. Pedro de Elizalde, Ciudad Autónoma de Buenos Aires, Argentina

CD28 and CD95 expression over LTCD4 and CD8 cells, define naïve (N) and memory (M) subsets. CD28 is a costimulatory T-cell molecule and its specific loss is associated with immune senescence. CD95 antigen promotes apoptosis pathways, and is expressed also, over activated T cells. HIV infection is characterized by LTCD4 depletion, chronic immune activation and quantitative and phenotypic changes in T cells subsets. Our aim was to describe T cells subsets levels in a cohort of HIV-infected children. The group evaluated comprised 47 HIV-infected

children, (age: 2-14 years), vertical transmission was confirmed in all patients, they were under antiretroviral therapy. LTCD4 and CD8 N (CD28+CD95-), central memory (CM) (CD28+CD95+), effector memory (EM) (CD28-CD95+), and senescence LTCD8 CD57+ subsets, were studied by flow cytometry. Clinical and virological status of all patients was also evaluated. Control samples (Co) were obtained from 10 HIV-seronegative healthy children. LTCD4 N subset percentage levels were decreased significantly ($p<0.05$) in patients with mild or severe immunosuppression: Group A (LTCD4<25%, n: 20), in comparison with children with no evidence of immunosuppression: Group B (LTCD4 ≥ 25%, n: 27), and Co, (A: 33.5±16.6 vs B: 48.5±20.6 vs Co: 67.4±9.5). Similar results were obtained with LTCD8 N (A: 10.6±10.3 vs B: 23.4±17.3 vs Co: 52.8±11.2). A significant increase in LTCD4 CM between groups A, B and Co, was also recorded (A: 58.9±17.2 vs B: 47.4±20.5 vs Co: 31.0±7.5). LTCD8 CM showed increase between A and B groups against Co (A: 24.7±10.8 vs Co: 16.0±7.1, B: 36.3±19.1 vs Co: 16.0±7.1). LTCD8 EM were increased significantly between A, B and Co (A: 57.4±20.3 vs B: 33.8±18.1 vs Co: 6.9±3.1). A positive correlation between LTCD8 EM and LTCD8 CD57+ was observed (r: 0.516, p: 0.003). Differential involvement of N subset and senescence characteristic of cytotoxic cells would be associated with persistent viral replication and repeated immune activation.

(904) PATIENTS WITH IL-17R DEFICIENCY SHOW ALTERATIONS IN THE NK AND CD8+ T CELL COMPARTMENTS.

JIMENA TOSELLO BOARI[1], NICOLÁS NUÑEZ[2], MIGAUD MELANIE[3], MARÍA CECILIA RAMELLO[1], CAROLINA MONTES[1], ELIANE PIAGGIO[2], ANNE PUEL[3], EVA ACOSTA RODRIGUEZ[1]

[1]Departamento de Bioquímica Clínica. Facultad de Ciencias Químicas. Universidad Nacional de Córdoba. Centro de Investigaciones en Bioquímica Clínica e Inmunología. CIBICI-CONICET. Córdoba – Argentina. [2]INSTITUT CURIE Laboratoire de Transfert INSERM U932. Paris - FRANCE [3]Génétique Humaine des Maladies Infectieuses. INSERM UMR 1163. Université Paris Descartes-Sorbonne Paris Cité. Institut Imagine. Paris – FRANCE.

The IL-17 cytokine family plays pivotal roles in inflammatory diseases and in host defense against several pathogens in mice and humans. Previously, we and others have demonstrated that IL-17RA deficient mice show compromised maintenance and activation of the main cytotoxic populations (i.e. NK and CD8+ T cells (CTL)) during infections. Here, we aimed at evaluating if IL17RA deficiency affects functional cytotoxic response also in humans. To do this, we studied by flow cytometry the frequency and phenotype of NK and CTL present on peripheral blood from three patients presenting genetic mutations that prevents IL-17RA expression. Blood from healthy donors (HD) were studied for comparison. Of note, IL-17RA deficient (RA-/-) patients showed a significant lower frequency of NK cells than HD ($p=0.0004$). Moreover, CTL from these patients showed a conserved frequency but a distinctive phenotype in comparison to those from HD. Hence, total and CD45RA- CTL from RA-/- patients showed a consistently higher frequency of OX40+ ($p=0.0469$ and $p=0.0281$) and CD38+ ($p=0.0418$ and $p=0.0123$) cells and, only within CD45RA- CTL, an increased % of ICOS+ cells ($p=0.0014$). In contrast, other activation markers such as CD25 ($p=0.0061$ and $p<0.0001$) and CD69 ($p=0.008$ and $p=0.0024$) showed reduced expression while HLA-DR and 4-1BB showed no changes in expression on total and CD45- CTL from RA-/-patients. Interestingly, CTL with a phenotype of central memory (CD45RA-CCR7+), which display a high expression of IL-17RA in HD, were significantly diminished in RA-/- patients ($p=0.0070$). This reduction in the frequency of the memory subset correlated with a significant increase in the percentage of CTL with phenotype compatible with senescence (KLRG1+CD57+; $p=0.0377$) and (CD27-CD28-CD45RA-, $p=0.0205$). These results suggest that, as it has been described in mice, IL-17RA signaling may modulate the maintenance, activation and differentiation of human cytotoxic immune populations.

SAI SYMPOSIUM IV

PLASTICITY OF MYELOID CELLS

DIVERSE ROLES OF MACROPHAGES IN THE INITIATION AND THE RESOLUTION OF INFLAMMATION.

PROFESSOR DAVID M. MOSSER, PHD.

Cell Biology and Molecular Genetics, Maryland Pathogen Research Institute, Maryland, USA.

HUMAN INFLAMMATORY DENDRITIC CELLS: ONTOGENY AND FUNCTION.

ELODIE SEGURA, PHD.

"Dendritic cells and antigen presentation" lab, INSERM U932, Institut Curie, Paris, France.

(715) M2 MACROPHAGES INHIBIT IFN-G PRODUCTION OF NK CELLS THROUGH TGF-B AND NK CELL-MEDIATED CYTOTOXICITY THROUGH CELL-TO-CELL CONTACT

SOL YANEL NUÑEZ, ANDREA ZIBLAT, FLORENCIA SECCHIARI, NICOLÁS IGNACIO TORRES, JESSICA MARIEL SIERRA, ROMINA ELIZABETH ARAYA, XIMENA LUCÍA RAFFO, CAROLINA INÉS DOMAICA, MERCEDES BEATRIZ FUERTES, NORBERTO WALTER ZWIRNER

[1]Laboratorio de Fisiopatología de la Inmunidad Innata. Instituto de Biología y Medicina Experimental (IBYME-CONICET).

Macrophages are highly plastic cells that can modify their functional response according to the surrounding microenvironment, becoming pro-inflammatory (M1) or anti-inflammatory (M2) macrophages. The outcome of the crosstalk between M1 and NK cells is well established playing a critical role in the protection against infections and tumor growth. However, the effect of M2 on NK cells is less clear. We previously demonstrated that M2 macrophages inhibit NK cell degranulation (n=8, p<0,001) and cytotoxicity (n=6, p<0,0001) against tumor cells and also IFN-γ secretion upon stimulation with cytokines (assessed by flow cytometry and ELISA, n=6, p<0,001) compared to M1. Thus, the aim of this work was to investigate the underlying mechanisms involved in this inhibition. Accordingly, we used co-cultures of human NK cells with M1 or M2 *in vitro* polarized macrophages. Human monocytes were differentiated to unpolarized macrophages (M0) with M-CSF for 6 days and then exposed overnight to LPS and IFN-γ or IL-4 to obtain M1 and M2 respectively. Then isolated NK cells (resting or stimulated with IL-12, IL-15 and IL-18) were co-cultured overnight with M1 or M2 cells. Transwell experiments and NK cell culture with M1 or M2 conditioned media demonstrated that the inhibition of IFN-γ secretion was due to soluble factors while inhibition of degranulation required contact between the respective cell types. Blockade of the immunosuppressive cytokine TGF-β in M2 macrophage-conditioned media restored the IFN-g secretion by NK cells (n>6, p<0,001) but blockade of TGF-β during co-cultures had no effect on NK cell degranulation (n=4, p>0,05), indicating that TGF-β is involved in silencing IFN-γ secretion but not NK cell-mediated cytotoxicity. Therefore, we conclude that M2 negatively regulate NK cell IFN-γ production through secretion of TGF-β but negatively regulate NK cell-mediated cytotoxicity through the interaction between cognate receptor-ligands expressed by these cells.

(326) FEVER-RANGE HYPERTHERMIA IMPROVES THE ANTI-APOPTOTIC EFFECT INDUCED BY LOW PH ON HUMAN NEUTROPHILS PROMOTING A PROANGIOGENIC PROFILE

FERNANDO ERRA DÍAZ[1], EZEQUIEL DANTAS[1], CABRERA MAIA[2], JOSEFINA CASTRO MAZZA[1], CONSTANZA ARRIOLA BENÍTEZ[3], MARÍA VICTORIA DELPINO[3], NORBERTO SANJUAN[4], ANALÍA SILVINA TREVANI[5], JORGE GEFFNER[1]

[1]Instituto de Investigaciones Biomédicas en Retrovirus y SIDA (INBIRS), CONICET, Facultad de Medicina, Universidad de Buenos Aires, Argentina. [2]Instituto de Investigaciones Farmacológicas (ININFA), CONICET, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina. [3]Instituto de Inmunología, Genética y Metabolismo (INIGEM), CONICET, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina. [4]Instituto de Investigaciones en Microbiología y Parasitología Médica (IMPAM), CONICET, Facultad de Medicina, Universidad de Buenos Aires, Argentina. [5]Instituto de Medicina Experimental (IMEX), CONICET, Academia Nacional de Medicina, Buenos Aires, Argentina.

Neutrophils have the shortest lifespan among leukocytes and usually die via apoptosis. We previously reported that low pH, a hallmark of inflammatory processes and solid tumors, moderately delays neutrophil apoptosis. Here we analyzed whether fever-range hyperthermia (39.5°C) was able to modulate the anti-apoptotic effect induced by low pH (pH 6.0). Hyperthermia markedly increased neutrophil survival induced by low pH (anexinV/PI staining/flow cytometry): % apoptosis = 72±5, 45±6, 70±7, and 19±3, for neutrophils cultured for 18 h at pH 7.3/37ºC, pH 6.0/37°C, pH 7.3/39.5ºC, and pH 6.0/39.5°C, respectively (p<0.001 for pH 6.0/39.5ºC vs pH 7.3/37°C, n=9). Similar results were observed when apoptosis was evaluated by different methodologies. Analysis of the mechanisms underlying this anti-apoptotic response revealed that hyperthermia further decreases cytosolic pH induced by extracellular acidosis, evaluated with BCECF-AM/flow cytometry (p<0.01, pH 6.0/37°C vs pH 6.0/39.5°C). The fact that two Na+/H+ exchanger inhibitors, EIPA(10mM) and amiloride (1mM) reproduced the effects

induced by hyperthermia suggested that it prolongs neutrophil survival by inhibiting the Na+/H+ anti-porter. Interestingly, we found that the anti-apoptotic effect induced by low pH and hyperthermia is associated to the induction of a functional profile characterized by a low phagocytic activity evaluated by fluorescence microscopy using FITC-labeled *Candida albicans* (% inhibition 56 ± 8, n=5, pH 6.0/39.5°C vs pH 7.3/37°C, p<0.001), an impairment in ROS production evaluated by DHR oxidation and flow cytometry (% inhibition >90%, n=7, pH 6.0/39.5°C vs pH 7.3/37°C, p<0.001), and a higher ability to produce the angiogenic factors VEGF, IL-8 and the matrix metallopeptidase 9 (MMP-9) evaluated by ELISA and zymography (p<0.01, pH 6.0/39.5°C vs pH 7.3/37°C, n=6). These results suggest that acting together fever and local acidosis might drive the differentiation of neutrophils into a proangiogenic profile.

(781) BORDETELLA PERTUSSIS EFFECT ON MACROPHAGE PHENOTYPE DURING THE INFECTION.

HUGO ALBERTO VALDEZ[1], LUCIANA BALBOA[2], JUAN PABLO GORGOJO[1], HILARIO CAFIERO[1], MARÍA DEL CARMEN SASIAIN[2], MARÍA EUGENIA RODRIGUEZ[1]

[1]CINDEFI (UNLP CONICET La Plata), Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina. [2]Laboratorio de Inmunología de Enfermedades Respiratorias, Instituto de Medicina Experimental (IMEX)-CONICET, Academia Nacional de Medicina, Buenos Aires, Argentina.

After bacterial products recognition macrophages initiate an immune response for removal of the microbes. Proper polarization of macrophages is critical for bacterial clearance. During microbial infection, macrophages are polarized to classically or alternatively activated cells (M1 or M2, respectively) in response to microbial components and host immune mediators. Recently we have demonstrated that *B. pertussis* (Bp) has the ability to manipulate the host defense response eventually enabling its own survival and replication inside the macrophages. In the present study we examined the evolution of macrophage phenotype during the Bp intracellular survival and proliferation. To this end, primary peripheral blood mononuclear cells (PBMC) were differentiated into macrophages in the presence of GM-CSF; GM-CSF + INF-gamma/LPS; M-CSF + IL-4 or M-CSF + IL10 to obtain the phenotypes M0, M1, M2a or M2c, respectively. Intracellular Bp infection evolution was determined by FISH staining and confocal microscopy. Macrophage expression of CD40, CD80, CD206, CD209 and CD163 was determined at 3 and 48 hours after infection. Three hours post infection macrophages cultured under all conditions displayed an increase in CD40 and CD80 expression and a decrease in CD206, CD209 and CD163 expressions as compared with the uninfected control. Forty eight hours post infection cells in which Bp infection had developed showed a decrease in CD40 and CD80 expression and an increase of CD209 and CD163 in all macrophage phenotypes (M0, M1, M2a and M2c) as compared with the uninfected population of the respective phenotype. These results indicate that early after bacterial phagocytosis macrophages develop an M1 like phenotype. However, as the intracellular infection progresses infected cells of any macrophage phenotype turned into an M2 like type, indicating the extraordinary ability of this pathogen to induce a permissive environment for its survival within human macrophages.

(494) PDL1 SIGNALING INHIBITS MACROPHAGE'S SUSCEPTIBILITY TO M. TUBERCULOSIS-SPECIFIC CD8+ T CELL INDUCED APOPTOSIS

GUADALUPE VERÓNICA SUÁREZ, MARÍA BELÉN VECCHIONE, FLORENCIA QUIROGA

Universidad de Buenos Aires. Consejo Nacional de Investigaciones Científicas y Técnicas. Instituto de Investigaciones Biomédicas en Retrovirus y Sida (INBIRS). Facultad de Medicina. Buenos Aires, Argentina.

CD8+ T cells contribute to the optimal control of Tuberculosis infection by inducing apoptosis on infected macrophages. M. tuberculosis (Mtb) infection is associated to alternative activation of macrophages. We aimed to study the effect of macrophage activation on Mtb-specific CD8+ T cell induced apoptosis. Monocyte Derived Macrophages (MDMs) from healthy donors were activated by IFNγ or IL4 addition during 48 h and loaded with heat-killed Mtb or CEF peptide pool. Macrophages' phenotype was determined by HLA-DR, CD86, DC-SIGN and CD14 staining and flow cytometry. Then, MDMs were co-cultured with antigen-stimulated, autologous CD8+ T cells. Specific killing was assessed by 7-AAD staining. Data was analyzed by ANOVA followed by Bonferroni posttest. IFNγ activation of MDMs tended to decrease CD8-induced Mtb-specific apoptosis but it remained sig-

nificant at a ratio of 1:1 ($p<0.01$). Contrary, IL4 activation completely abrogated Mtb-loaded MDMs apoptosis. Thus, IL4 significantly reduced Mtb-specific killing compared to non-activated MDMs ($p<0.05$). IFNγ production by CD8+ cells determined by ELISPOT was not affected by MDMs activation with IFNγ or IL4 ($p=0.49$ by Friedman test). HLA-ABC, CD80 and CD86 expression were increased by IFNγ or Mtb ($p<0.05$) but were unaffected by IL4 activation, suggesting that the observed differences in killing were not due to differences in antigen presentation or positive co-stimulation by macrophages. Interestingly, PDL1, but not PDL2 expression was acutely increased by IFNγ or Mtb ($p<0.001$) and to a lesser extent, by IL4 ($p<0.05$). Also, PDL1, but not PDL2-blockade improved CD8+ T cell-mediated cytotoxicity to IFNγ but not IL4-activated MDMs. Our results indicate that the increment of PDL1 by IFNγ activation inhibits macrophages susceptibility to CD8+ T cell induced apoptosis.

SAI SYMPOSIUM V

PHYSIOPATHOLOGY AND REGULATORY MECHANISMS OF THE IMMUNE RESPONSE

MECHANISMS OF ROBUST TRANSPLANTATION TOLERANCE.

MARÍA LUISA ALEGRE, MD, PHD

Gwen Knapp Center for Lupus and Immunology Research, University of Chicago, Chicago, Illinois, USA.

METABOLIC PATHWAYS SHAPING IMMUNE CELL FUNCTION

LUCIANA BEROD, PHD.

Institute of Infection Immunology, Twincore, Centre for Experimental and Clinical Infection, Research, Hannover School of Medicine, Hannover,Germany.

(484) IMPACT OF THE MACROPHAGE ACTIVATION STATE ON THE ACCUMULATION OF LIPID BODIES INDUCED BY THE TUBERCULOUS PLEURISY MILIEU

MELANIE GENOULA[1], DENISE KVIATCOVSKY[1], AYELEN MILILLO[2], BELÉN IMPERIALE[1], EDUARDO MORAÑA[3], PABLO GONZÁLEZ-MONTANER[3], DULCE MATA-ESPINOZA[4], ERIKA GONZÁLEZ-DOMÍNGUEZ[5], CARMEN SÁNCHEZ-TORRES[5], ROGELIO HERNÁNDEZ-PANDO[4], PAULA BARRIONUEVO[2], MARÍA DEL CARMEN SASIAIN[1], LUCIANA BALBOA[1]

[1]Laboratorio de Inmunología de Enfermedades Respiratorias, Instituto de Medicina Experimental (IMEX)-CONICET, Academia Nacional de Medicina, Buenos Aires, Argentina. [2]Laboratorio de Fisiología de los Procesos Inflamatorios, Instituto de Medicina Experimental (IMEX)-CONICET, Academia Nacional de Medicina, Buenos Aires, Argentina. [3]Instituto Prof. Dr. Raúl Vaccarezza, Hospital de Infecciosas Dr. F.J. Muñiz, Buenos Aires, Argentina. [4]Departamento de Patología Experimental, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico. [5]Departamento de Biomedicina Molecular, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Mexico City, Mexico.

During intracellular bacterial infection, the eukaryotic cells show metabolic adaptations that help them to eliminate the pathogen and conversely, the pathogen tries to profit from host metabolites. The ability of *Mycobacterium tuberculosis* (*Mtb*) to persist relies on its numerous immune evasion strategies such as the dysregulation of the lipid metabolism that can lead to foamy macrophage (FM) differentiation. So far, the specific host factors leading to FM induction are unknown. We aimed to characterize whether different profiles of macrophage (M0, M1, M2a, and M2c) differ in their propensity to accumulate lipid bodies (LB) upon exposure to tuberculous pleural effusions (TB-PE). For that, LB accumulation was evaluated by oil red staining, cytokines by ELISA, pSTAT-3 and ACAT by western blot, cholesterol by enzymatic assays, and bacillary loads by colony-forming units assay. TB-PE induced a FM phenotype in M0, M2c and M1 but not in M2a (n=8). After depleting IL-10, IL-4, IFN-g, IL-1b, IL-6, or TNF-a from TB-PE, only IL-10 depletion prevented FM differentiation (n=8). TB-PE increased the levels of

intracellular cholesterol, while IL-10 depletion reduced it. Besides, PE-TB or IL-10 addition induced CD36 expression, which mediates lipids uptake, and also pSTAT-3 levels, which governs the M2c program (n=5); moreover, pSTAT-3 inhibition prevented LB accumulation (n=4). Interestingly, the expression of ACAT, the enzyme that synthesizes cholesteryl esters, was induced by TB-PE in any macrophage profile except for M2a. Finally, TB-PE promoted the intracellular growth of *Mtb* in macrophages in an IL-10 dependent manner but not in M2a (n=10). Therefore, macrophage profiles differ in their propensity to accumulate LB, process that requires the activation of the IL-10/STAT-3 axis and favours *Mtb* replication. M2a was refractory to FM differentiation lacking ACAT expression. These results contribute to our understanding of the host metabolic alterations driven by *Mtb*.

(624) CORNEAL INJURY IN ONE EYE DISRUPTS MUCOSAL IMMUNE TOLERANCE OF THE FELLOW OCULAR SURFACE BY INDUCING SUBSTANCE P-MEDIATED NEUROGENIC INFLAMMATION.

MAURICIO GUZMÁN, IRENE KEITELMAN, FLORENCIA SABBIONE, ANALÍA TREVANI[1], MIRTA GIORDANO, JEREMÍAS GALLETTI

[1]Laboratorio de Inmunología Oncológica. Instituto de Medicina Experimental. Academia Nacional de Medicina/CONICET. Buenos Aires. Argentina. [2]Universidad de Buenos Aires. Facultad de Medicina. Departamento de Microbiología e Inmunología. Buenos Aires. Argentina.

In the clinic, it is commonly accepted that both eyes are functionally independent, but we unexpectedly observed immune tolerance perturbations in the opposite eye after unilateral manipulation in mice. Since there is no ocular lymphatic cross drainage that could account for this contralateral change, we studied the effect of a unilateral corneal lesion on conjunctival mucosal tolerance of the fellow eye. Using a delayed-type hypersensitivity (DTH) assay after s.c. immunization with ovalbumin (OVA)+adjuvant in Balb/c mice, we observed observed reduced responses in OVA-instilled mice compared with control mice ($p<0.05$). However, OVA-instilled mice in the same or in the opposite eye to the corneal burn developed full DTH responses. Consistently, mice injected with allogeneic B16 tumor cells in the subconjunctival space of the eye opposite to the corneal burn had a higher rejection rate than uninjured mice (89% vs 56%, $p<0.05$). In mice with a unilateral corneal burn, we observed an increase in the fraction of activated (CD69+ and CD25+) and interferon ã-secreting T cells and in the number of dendritic cells (CD11c+ MHC II+) in the opposite eye-draining lymph node that peaked 48-72 h after the injury ($p<0.05$). We then explored the contribution of neurogenic inflammation by using capsaicin (TRPV1 receptor agonist) and aprepitant (substance P receptor antagonist). Remarkably, unilateral ocular instillation of capsaicin also abrogated contralateral tolerance to OVA ($p<0.05$), and corneal burn-induced disruption of contralateral tolerance to OVA was prevented by aprepitant instillation ($p<0.05$). In summary, our results show that ocular mucosal tolerance was disrupted in the fellow eye after a unilateral corneal burn, and that this effect appears to be mediated by substance P-induced neurogenic inflammation. These findings could have major implications in the understanding and management of contralateral disease after single eye interventions.

(699) PARTICIPATION OF TYPE I-II IFNS IN THE REGULATION OF THE CNS IMMUNE SURVEILLANCE AFTER SYSTEMIC INFLAMMATION.

JAVIER MARÍA PERALTA RAMOS, CLAUDIO BUSSI, DANIELA SOLEDAD ARROYO, PABLO IRIBARREN

Center of Investigation in Clinical Biochemistry and Immunology (CIBICI-CONICET), Department of Clinical Biochemistry, National University of Córdoba.

Brain-resident microglia (Mi) and peripheral recruited leukocytes, play essential roles in shaping the immune response in the central nervous system (CNS). These cells activate and migrate in response to chemokines produced during active immune responses and may contribute to the progression of neuroinflammation. Recent findings have revealed distinct roles for type I (α and β)-II (γ) interferons (IFNs) in the recruitment of immune cells to the CNS and highlighted the importance of this process for brain protection/repair. In this study, we assessed the participation of type I-II IFNs in the innate immune response displayed by tissue-resident microglia and

recruited inflammatory leukocytes, to better understand the contribution of these cytokines in the establishment and development of a neuroinflammatory process induced by systemic TLR4 stimulation. We characterized the molecular and cellular players involved in neuroinflammation induced by i.p. administration of lipopolysaccharide (LPS - 1.6 mg/kg) to IFN-$\gamma^{-/-}$ and IFNAR$^{-/-}$ C57BL/6 mice, using flow cytometry combined with confocal microscopy. Following stimulation with LPS, we didn´t find any variation of CD11b^{+}CD45^{lo} microglial cells; however, we noticed a decrease of CD11b^{+}CD45^{hi} (Ly6C^{hi}/CD11c^{+}) myeloid recruited leukocytes in both KO mice strains compared to their WT-treated counterparts ($p<0.05$). Unexpectedly, no significant changes were observed neither in the absolute number of MHC-II^{+} cells nor in the MFI of Mi and peripheral leukocytes. Interestingly, we found an increase of CD11b^{+}CD45^{hi}Ly6C^{+}Ly6G^{+} neutrophils from LPS primed IFNAR$^{-/-}$ mice in comparison with their IFN-$\gamma^{-/-}$ littermates ($p<0.05$). Thereby, IFNs could prove to be important players in the regulation of leukocyte recruitment to the CNS by controlling the innate immune response in neuroinflammation. Furthermore, these findings highlight the ability of a systemic TLR4-mediated challenge to signal to the CNS and alter brain´s primary immunity.

(331) RELEVANCE OF THE BLASTOCYST CONDITIONED MEDIA ON IMMUNOTOLERANCE: FOCUS ON THE CONTROL OF THE INFLAMMATORY RESPONSE.

ESTEBAN GRASSO, ELIZABETH SOCZEWSKI, DAIANA VOTA, LAURA FERNÁNDEZ, LUCILA GALLINO, CLAUDIA PÉREZ LEIRÓS, ROSANNA RAMHORST

Immunopharmacology Laboratory, IQUIBICEN, University of Buenos Aires, CONICET, Argentina.

The decidualization of human endometrial cells involves changes in their secretome increasing the production of immunomodulatory mediators. It is associated with a sterile inflammatory response that should be later controlled to a tolerogenic microenvironment by maternal and bastocyst-derived factors. Here we focus on the production of immunomodulators during the decidualization process and we explored whether human Blastocyst Conditioned Media (BCM) could control the initial inflammatory response. As an in vitro model we used the Human endometrial stromal cell line (HESC) decidualized or not with medroxiprogesterone (10-6M)+dbcAMP (2,5 10-3M) during 8 days. We observed an increase in the production of IDO (indoloamine-2,3 di-oxigenase), CXCL8, CXCL12 and IL-1beta production ($p<0.05$ Studen T test) after the decidualization. Since IL-1beta can act as a 'double edge' mediator in early pregnancy, it is necessary for implantation but higher level display deleterious effects, we evaluated BCM effect on IL-1 production. BCM derived from human competent blastocyst reduced IL-1 intracellular production in decidualized cells, compared to BCM from blastocyst morphologically impaired. This effect was accompanied by decreased expression of ATF6 and PERK, two sensors of reticular stress, and TXNIP, a kinase/RNase associated with inflammasome activation by BCM and it was more pronounced with BCM derived from human competent blastocyst ($p<0.05$ Student t test). Finally, in an in vitro implantation model based on co-culture of blastocyst-like spheroids from trophoblast cells (BLS, from Swan-71 cell line) on decidualized-HESC cells, BLS were able to invade HESC decidualized monolayer and the BCM obtained from developmentally impaired blastocysts decreased their invasion index ($p<0.05$). In conclusion, the BCM might contribute to the cross-talk with decidualized cells controlling the inflammatory response and allowing blastocyst invasion accordingly with the blastocyst quality.

SAI SYMPOSIUM VI

IMMUNOTHERAPY

ANTI-STX2 ANTIBODIES FOR THE TREATMENT OF UREMIC HEMOLYTIC SYNDROME.

MARINA PALERMO, PHD.

IMEX-CONICET, Academia Nacional de Medicina, Buenos Aires, Argentina.

TOWARDS PERSONALIZED IMMUNOTHERAPY OF SOLID TUMORS

STEPHEN SCHOENBERGER, PHD.

Division of Developmental Immunology, La Jolla Institute for Allergy and Immunology, La Jolla, California, USA

(533) SENESCENCE AND IMMUNOTHERAPY IN BREAST CANCER MEDIATED BY STAT3 BLOCKADE

MARA DE MARTINO[1], MARÍA F. MERCOGLIANO[1], MERCEDES TKACH[2], LEANDRO VENTURUTTI[1], CECILIA J. PROIETTI[1], PATRICIA V. ELIZALDE[1], ROXANA SCHILLACI[1]

[1]Instituto de Biología y Medicina Experimental, CONICET, Buenos Ares, Argentina [2]Institut Curie, France.

Stat3 is constitutively active in 60% of breast cancer (BC) where it promotes tumor progression and immune evasion. We described in murine BC models that Stat3 inhibition leads to a senescence program and that immunization of mice with Stat3-blocked BC cells induces an antitumoral immune response that involves $CD4^+$ Th cells and NK cells. Here, we studied the mechanism of senescence induced by Stat3 inactivation and the use of the supernatant (SN) from Stat3-blocked cells to formulate an effective immunotherapy (IT). Knockdown of Stat3 with siRNA induced senescence in triple negative (4T1, MDA-MB-231 and MDA-MB-468 cells) and ErbB2 positive (C4HD, JIMT-1 and KPL-4 cells) BC models, determined by SA-â-gal staining. Also, we observed an increase in trimethylation of histone H3 at lysine 9 and in cell cycle inhibitors expression ($p16^{INK4a}$ (p16) or $p21^{CIP1}$). Simultaneous transfection with siRNAs targeting Stat3 and p16 or $p21^{CIP1}$ reverted the senescent phenotype. Interestingly, Stat3 inhibition in vivo induced senescence and an increased p16 expression in 4T1 tumor. Then, we embedded the SN of C4HD or 4T1 cells transfected with Control siRNA (SN-Control), Stat3 siRNA (SN-Stat3) or the combination of Stat3 and p16 siRNAs (SN-Stat3+p16) in a slow delivery depot as an adjuvant of a cellular IT. Prophylactic IT before C4HD tumor challenge with SN-Stat3 and SN-Stat3+p16 decreased tumor growth (72%, $p<0.05$ and 51%, $p<0.05$ respectively vs. SN-Control). Therapeutic IT after 4T1 tumor implantation with SN-Stat3 and SN-Stat3+p16 decreased tumor growth (51%, $p<0.001$ and 41%, $p<0.01$ respectively vs. SN-Control) and pulmonary metastasis (70%, $p<0.05$ and 50%, ns. respectively vs. SN-Control). In both IT protocols the result was associated with greater cytotoxic activity of NK cells and an increase in the number of memory $CD4^+$ T cells vs. SN-Control. These results suggest that Stat3 blockade drives a senescence program in BC cells and the SN-Stat3 is an effective adjuvant for IT.

(1066) EVALUATION OF HETEROLOGOUS PRIME-BOOST IMMUNIZATION STRATEGY AGAINST BORDETELLA PERTUSSIS USING A NOVEL OUTER MEMBRANE VESICLE BASED VACCINE AND COMMERCIAL ACELLULAR VACCINE

GRISELDA MORENO[2], EUGENIA ZURITA[1], EMILIA GAILLARD[1], DAVID SABATER[1], MARTÍN RUMBO[2], DANIELA HOZBOR[1]

[1]Laboratorio VacSal -IBBM FCE UNLP CONICET y [2]IIFP-FCE UNLP CONICET, La Plata, Argentina.

Pertussis remains an important health problem in many countries even in those with high vaccination coverage. From 1950s-1990s this disease was controlled by use of whole cell pertussis (wP) vaccines. Later in 2000s these vaccines were replaced in developed countries by acellular pertussis (aP) vaccines (purified components of *Bordetella pertussis* (Bp) absorbed to alum). The new aP vaccines, although safer, are not as effective as the wP vaccine and this has been attributed to: 1) escape form protective immunity, 2) waning immunity or 3) a failure of the aP vaccine to induced protective cellular immune responses. Under this context, we have developed a new vaccine candidate based on outer membrane vesicles (OMVs) derived from *Bp* which is capable of inducing a more robust immune response than commercial aP vaccines with a Th2/Th1/Th17 cellular profile. Here we evaluated the immunogenicity of a heterologous prime-boost regimen using OMV-base and aP vaccines. For comparison purposes homologous vaccination regimens with OMV based vaccine and aP were also performed. For all cases, the induced humoral and cell-mediated immune responses were evaluated. A robust total IgG antibodies with a high IgG2a/IgG1 ratio were detected in sera of mice primed with OMV based vaccine, suggesting that OMVs skewed the immune response to a Th1 profile. Spleen

cells from immunized mice were isolated and stimulated *in vitro* with B. pertussis antigens. Interestingly the obtained results showed that the priming with OMV vaccine induce a strong Th1/Th17 response with high values of INF-ϒ (2100 ± 350 pg/ml p<0,05), which was maintained after aP boost. In contrast, IL-5 secretion was mainly produced by spleen cells from mice primed with aP, which results in a Th2 response. The immunological characterization in the murine model of these vaccination schedules led us to propose that OMVs are highly reliable primer candidates to be considered in future as an alternative strategy against pertussis.

(2015) EFFECTS OF DIFFERENT DRUGS IN THE RELEASE AND PROTEIC EXPRESSION OF TUMOR CELLS DERIVED EXOSOMES TO BE USED AS ACELLULAR ANTIGENS.

FEDERICO COCOZZA[1], FLORENCIA MENAY[1], RODRIGO TSACALIAN[1], ALEJANDRINA VENDRELL[1], PURA SAMPEDRO[2], CLAUDIA WALDNER[1], CLAUDIA MONGINI[1]

[1]Centro de Estudios Farmacológicos y Botánicos (CEFYBO), CONICET-UBA. [2]Universidad de Morón.

Exosomes are 40-100 nm nanovesicles released by most of cells. Tumor cells derived exosomes (Tex), used as a vaccine, elicit a specific cytotoxic response against tumor cells, with a greater immunogenicity than lysated tumoral cells. The utilization of exosomes as an easily obtainable and stable defined source of antigens is a novel technique for treating cancer. However, the amount of exosomes purified from culture cells is limited. In recent studies it was observed that cells respond to different stressors stimuli (hypoxia, acidosis, radiation, cytotoxic drugs, oxidative stress and heat shock) by releasing microvesicles. The purpose of this work was to use different stressors to enhance the exosome production, to be used as acellular immunogens for the development of an antitumor vaccine. Tex released from tumor cells in a culture of the murine T-cell lymphoma, either growing in normal or stressed conditions, were purified by differential centrifugation and ultracentrifugation. Their concentration was measured by Bradford; the purity and also the expression of exosomes protein markers, such as Hsp's, Alix and TSG-101 and tumor antigens were determined by flow cytometry and Dot Blot. It was found that cyclophosphamide cellular stress enhances the exosome production (61 ± 16) % and that these exosomes express more Hsp90 in their membrane compared with exosomes of the same cells growing without stressors. Likewise, the immune response generated by the inoculation of exosomes in normal mice was study, evaluating the humoral response by Dot Blot, as well as the survival post-challenge with tumor cells. Tex isolated from tumor cells grown with 3mM cyclophosphamide or without stress induced an immune response with a high and similar titer of antibodies in serum, previous to the inoculation with the tumor cells and this induction was reflected in the percentage of the tumor rejection in mice, without differences within the groups.

(605) IL-2/ANTI-IL-2 COMPLEX TREATMENT INDUCES REGULATORY T CELLS AND AMELIORATES EXPERIMENTAL FOOD ALLERGY.

PAOLA LORENA SMALDINI[1], FERNANDO TREJO[1], JOSÉ COHEN[2], ELIANE PIAGGIO[3,4], GUILLERMO DOCENA[1].

[1]Instituto de Estudios Inmunológicos y Fisiopatológicos IIFP, Facultad de Cs. Exactas, UNLP, La Plata, Bs. As., Argentina. [2]Laboratoire de Biologie et Thérapeutique des Pathologies Immunitaires, Centre National de la Recherche Scientifique UMR 7087, Hôpital Pitié-Salpêtrière, 756651 Paris, France. [3]Institut Curie, PSL Research University, INSERM U932, F-75005, Paris, France. [4]Centre d'Investigation Clinique Biothérapie CICBT 1428, Institut Curie, Paris, F-75005 France.

Cow's milk allergy (CMA), mediated by an aberrant immunological reaction to cow's milk proteins (CMP), is one of the most prevalent food allergies in infants and young children worldwide. Oral and sublingual immunotherapies show promise as potential disease-modifying therapies, although no therapy has yet been approved. It has recently been demonstrated that the IL-2/ anti-IL-2 complex selectively expands regulatory T cells (Tregs). We aimed to combine sublingual immunotherapy (SLIT) using low doses of the allergen with systemic administration of IL-2/anti-IL2 to improve the safety and efficacy of the sublingual therapy Balb/c mice were sensitized with CMP and cholera toxin and then treated with PBS (Sens) or CMP (CMPdes) (sublingual) w/wo intraperitoneal injections of IL-2/anti-IL-2 (C or CMPdes/C). Mice were orally challenged with CMP and the immune response was in vitro (serum IgE, IL-5 and IFN-ã secretion by splenocytes, lamina propria Tregs, IL-10 and TGF-â) and in vivo (clinical score and cutaneous tests) evaluated. We found a lower medium clinical score in treated mice after the oral challenge with CMP, as compared with sensitized mice, with a reduction in skin mast cell reactivity in treated mice,

mainly in CMPdes/C group. Immunological changes included decreased specific IgE (2,1±0,3OD Sens vs 1,3±0.1CMPdes; 1,4±0,9C; 1,1±0,8CMPdes/C), decreased levels of Th2 cytokines and induction of IL-10 and Tregs in lamina propria of duodenum (14,6±1,2% Sens vs 28,0±0,6% CMPdes; 35,2±3,1% C; 42,5±1,8% CMPdes/C) and of sublingual mucosa of treated mice (p<0,05). The frequency of Tregs in submaxilar and sublingual mucosa was higher in CMPdes/C than CMPdes(p<0,05). However, CMPdes showed an increase of lamina propria tolerogenic dendritic cells CD11c+CD11b-CD8á+ (p<0,05). We demonstrated in a murine model of CMA that SLIT down-modulated the mucosal and systemic allergic immune response in sensitized mice and that the IL-2/anti IL-2 complex improved the sublingual immunotherapy.

SAFE SYMPOSIUM I

THE MEDICAL PRACTICE IN THE MULTIRESISTANT ERA

THE LABORATORY ON THE DIAGNOSTIC AND FOLLOW UP OF RESISTANT BACTERIAL PATHOGENS WITH PUBLIC HEALTH IMPACT.

FEDERICO NICOLA

Laboratorio de Bacteriología, Micología y Parasitología del Centro de Educación Médica e Investigaciones Clínicas "Dr. Norberto Quirno" (CEMIC), CABA, Argentina

Infectious diseases are one of the most important events of morbidity and mortality in inpatients.

Microorganisms that develop antimicrobial resistance are sometimes referred to as "superbugs". New resistance mechanisms are emerging and spreading globally, (KPC, OXA carbapenemases, colistin-resistant, vancomycin-resistant, MRSA, ESBL) threatening our ability to treat common infectious diseases, resulting in prolonged illness, disability, and death. The Microbiology Laboratory has a significant role in the prevention and control of these infections and is a key element of any infection control program and is the first alert for detection of new antibiotic resistance mechanisms, outbreaks of food borne infection and intra -hospitality infections. The work of the Microbiology Laboratory covers microbial isolation and identification, determination of phenotypic and genotypic antimicrobial susceptibility patterns, epidemiological surveillance and outbreak detection, education, and quickly report of quality assured results. There are described three revolutions areas in Microbiology Laboratory in the last three decades: automatization, molecular diagnosis, and proteomic and genomics methods. These news methodologies allowed reducing the TAT, increasing the quality of the results and improve the management of infections patients.

MULTIRRESISTANT STAPHYLOCOCCUS AUREUS INFECTIONS IN PEDIATRIC PATIENTS

EDUARDO LÓPEZ.

Hospital de Niños Ricardo Gutiérrez, CABA, Argentina.

SEVERE INFECTIONS BY MULTIRESISTANT BACTERIA IN INMUNOCOMPROMISED PATIENTS

NATALIA GARCIA ALLENDE

Servicio de Infectología, Hospital Alemán, CABA, Argentina.

Bacteria resistant to multiple antibiotics have become a public health problem. They are associated with an increase in direct and indirect costs. Mortality rates range from 8 to 53%, due to the difficulty in the initial empirical coverage. There was a report of an increase in mortality of 7.6% per hour because of inadequate treatment. The knowledge of local epidemiology and the possibility of performing rapid tests to search multiresistant bacteria are very important to allow adjustment of antibiotic therapy in the first 24 hours.

In immunocompromised hosts, this is even more important, due to the absence of an immune system that can't cooperate with the control of infection. Different cohorts show superiority, in terms of clinical efficacy, of the combined treatment over monotherapy. Furthermore, a significant decrease in mortality is reported when meropenem, administered by prolonged infusion and at maximum dose, is included as a part of the treatment.

Acquired resistance to beta-lactams is expressed in genetic platforms associated with resistance to other families of antibiotics (such as sulfonamides, fluoroquinolones, aminoglycosides). Recently a resistance plasmid mechanism to polymyxin has been documented. This

event has direct impact on the forecast, as their presence is associated with a 4-fold increase in mortality.

It is extremely important to reduce the spread of these "superbacterias" with the establishment of surveillance institutional policies of multiresistant microorganisms, isolation contact measures and the establishment of a program of rational antibiotic use.

SIMPOSIO I AACYTAL

II SEMINAR ON ALTERNATIVE METHODS

REGULATED ALTERNATIVE METHODS, A PRIORITY FOR THE REGION

THE ROAD THAT ALLOWS BRAZIL THE CURRENT SITUATION ON ALTERNATIVE METHODS

OCTAVIO PRESGRAVE

Department of Pharmacology and Toxicology, National Institute of Quality Control in Health (INCQS), Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro, RJ, Brazil

Brazil has recently approved legislation for regulating experimental animal use. On the other hand, it is a requirement that the safety of many products on the market is controlled on the basis of animal testing. Some groups at official laboratories, universities and industries are studying alternative methods, but there is no approved mechanism for funding collaborative studies, nor is there an institution responsible for coordinating these studies. These shortcomings obstruct the development of these assays in Brazil. The creation of a Brazilian Centre for the Validation of Alternative Methods (BraCVAM) would facilitate the development and validation of tests in all the institutions working on alternative methods in Brazil, and could also offer support to other Latin American countries.

MÉTODOS ALTERNATIVOS EN ARGENTINA, ESTRATEGIAS PARA SU DESARROLLO

MARCELO ASPREA

Hospital Pediátrico Juan P. Garrahan, CABA, Argentina

Experimentar con animales de laboratorio es un tema polémico, que lleva a discusiones acaloradas y apasionadas, sus implicancias no se reducen al ámbito científico, suele extenderse a legisladores, estudiantes, industrias, opinión pública y medios de comunicación, resultando difícil permanecer indiferente ante ciertos protocolos experimentales, entonces: ¿hasta qué punto es lícito, o científica y éticamente aceptable llevar a cabo experimentos con animales? ¿En qué medida el análisis de la relación sufrimiento del animal-beneficio humano, justifica ciertos experimentos?

Con la mirada puesta en las nuevas tecnologías de Métodos Alternativos al uso del Animal de Laboratorio y con el deseo de realizar nuevos aportes, hemos lanzado una Red Regional, para integrarnos al resto de la comunidad (www.facebook.com/alternativasenred)

Desde AACyTAL (Asociación Argentina de Ciencia y Tecnología en Animales de Laboratorio), junto a AUCYTAL (Asociación Uruguaya de Ciencia y Tecnología de Animales de Laboratorio), ASOCHICAL (Asociación Chilena en Ciencias de Animales de Laboratorio) y ACCBAL (Asociación Colombiana en Ciencias y Bienestar del Animal de Laboratorio), convocamos a que se unan a ella.

La realidad nos muestra que en la región es muy poco lo realizado hasta el momento en tecnología de métodos alternativos, debido a falencias en Legislación y Regulación sobre ensayos en animales de laboratorio

La ausencia gubernamental en la promoción de esta tecnología, nos priva de una herramienta muy importante, teniendo en cuenta factores económicos que favorecerían el alcance de objetivos

El desconocimiento del usuario a estas nuevas tendencias y la dificultad de abandonar formas tradicionales en sus tareas habituales es otra de las debilidades

Consideramos contar con suficientes fortalezas para cumplir nuestra misión y una de ellas es el apoyo de las Asociaciones en Ciencias de Animales de Laboratorio de la Región, lo que facilitará la recolección de información necesaria para construir una red entre áreas de investigación biomédica, educación y desarrollo de productos biológicos, para generar una base de datos confiables

Como oportunidad para ello hemos abierto este Portal en Internet que permitirá difundir el tema y como amenaza queda la irregularidad en las Legislaciones regionales

Participación en eventos para la difusión de los métodos alternativos desarrollados en instituciones y otras estrategias que previamente se vienen desarrollando se unen a nuestro proyecto

En Mayo del 2012 convocamos desde AACyTAL a referentes, investigadores y usuarios de animales de

laboratorio a nivel nacional a realizar 3 Workshops para la elaboración de un Proyecto de Ley, el cual recientemente ha sido presentado a la Legislación y que en su capítulo 16 contempla el uso de MA

Sumando todas las herramientas anteriores se presentó en Abril 2015 al SNB / MinCyT un proyecto nacional para organizar la Red Nacional de Métodos Alternativos (RAMA), dicha solicitud derivó a que en Junio del 2016 hayamos sido indicados como referentes del tema para trabajar junto al RECyT (Reunión de especialistas en Ciencia y Tecnología Plataforma Mercosur) para fomentar el desarrollo de los Métodos Alternativos en Argentina

Dentro de los desarrollos de MA en Argentina, podemos mencionar:

Tecnología IgY: producción y uso de anticuerpos de yema de huevo, donde el uso de anticuerpos de aves presenta varias ventajas: el costo de producción es relativamente más bajo que en mamíferos y se pueden producir en grandes cantidades, permitiendo su aplicación en el desarrollo de estrategias de inmunoprofilaxis e inmunoterapia. Algunas de estas aplicaciones son la prevención y tratamiento de diarreas humanas y animales, caries, xenotransplantes, síndrome urémico hemolítico, fibrosis cística, elaboración de antivenenos, etc. no presentando reacciones cruzadas con los factores reumatoideos o los anticuerpos humanos anti-ratón, ni activan el sistema de complemento mamífero

Monitoreo Endócrino No-Invasivo en Orina y Heces de Mamíferos, la extracción de muestras de sangre constituye, en sí mismo, un procedimiento que puede modificar los niveles hormonales. Una alternativa eficiente es el monitoreo no-invasivo de metabolitos de hormonas esteroideas excretadas en diversas matrices como materia fecal, orina, saliva, pelos , donde se pueden estudiar aspectos tan diversos como los ciclos reproductivos, variaciones estacionales en las concentraciones hormonales, diferencias sexuales y de comportamiento asociadas a hormonas, asociación entre posiciones jerárquicas, función tiroidea, efectos de tóxicos ambientales sobre la función endocrina, estrés y concentraciones hormonales con efectos sobre la reproducción, así como efectos de las actividades humanas sobre el bienestar animal

Galleria mellonella para sustituir a los modelos vertebrados en investigaciones científicas, en los últimos años, ha aumentado el uso de larvas de insectos en experimentos científicos para sustituir la demanda de pequeños mamíferos. En particular, *Galleria mellonella*, la “polilla grande de la cera” ha mostrado ser un modelo animal apto para reproducir algunas infecciones con un comportamiento patológico e inmunológico muy parecido al que se puede observar en los mamíferos. Este modelo reduce significativamente todo tipo de costos, incluyendo: instalaciones edilicias, alimentación, espacio para manipulación, controles de higiene y de homogeneidad genética, entre otros

Receptores Cys-loop en el organismo, modelo *Caenorhabditis elegans*, los receptores pentaméricos de la familia Cys-loop intervienen en procesos tales como transmisión neuromuscular, aprendizaje, cognición e incluyen al receptor nicotínico (AChR), de serotonina, de GABA y de glicina. El nematodo de vida libre *Caenorhabditis elegans* es un buen modelo para el estudio de comunicación neuronal y patologías humanas asociadas porque la transmisión sináptica y en particular los receptores Cys-loop se conservan con los de vertebrados. Mediante el análisis de las propiedades electrofisiológicas en cepas mutantes nulas para diferentes subunidades nicotínicas se descifra la composición de este receptor y se determina el rol funcional de cada una de sus subunidades. Se han generado y caracterizado cepas transgénicas conteniendo mutaciones que imitan las encontradas en síndromes miasténicos congénitos humanos. Se ha demostrado que es posible reproducir los cambios funcionales observados en los pacientes, por lo que *C. elegans* es un modelo válido para estos desórdenes musculares

Drosophila como modelo para identificar genes involucrados en neurodegeneración, en la última década Drosophila ha cobrado impulso como sistema modelo para desentrañar las bases moleculares de la neurodegeneración, el cáncer, la inmunidad innata y el envejecimiento. En ese contexto se han desarrollado distintos modelos que recrean diversos aspectos de la enfermedad de Alzheimer, Parkinson y Hungtinton, entre los cuales se basan en la sobre-expresión de variantes silvestres o mutadas de genes causales de enfermedad en el hombre. Se llevó a cabo un rastrillaje genético por desregulación de la expresión de genes al azar basado en un ensayo automatizado de actividad locomotora en dos estadios de la vida de la mosca *Drosophila melanogaster*. Como resultado de este esfuerzo se ha identificado varios loci que afectan específicamente este comportamiento en moscas de mediana edad, y uno de ellos, enabled, recrea tres de las características propias de enfermedades neurodegenerativas: la aparición tardía de síntomas, la progresión y la vulnerabilidad específica de ciertas poblaciones

Preservación de tejidos y simulación, el entrenamiento de personal médico quirúrgico tradicionalmente implicó la utilización de un número considerable de animales, a través de nuevos métodos alternativos se ha obtenido inmejorables beneficios en la adquisición de capacidades en la curva de aprendizaje y consecuente reducción del uso de animales para tales prácticas, mereciendo un capítulo aparte la Simulación, que consiste en un proceso de diseñar un modelo de un sistema real y llevar a término experiencias con él,

Nos encontramos, por tanto, en un punto de inflexión. La Ciencia y la Sociedad está avanzando respecto a hace unos años, pero aún quedan muchos puntos por cubrir y muchos obstáculos por superar. Por ello, es importante que no cesen los esfuerzos por parte de la comunidad

científica para que apoyen los métodos alternativos, para que se siga invirtiendo en su desarrollo e implementación en todos los ámbitos, ya que de este modo todos nos beneficiaremos: los humanos porque desarrollaremos modelos más fiables (Por ej: se está investigando sobre la metabolización de fármacos en cultivos de hepatocitos humanos y se están obteniendo resultados más fiables que en otras especies, cosa, por otra parte, lógica y esperable) y los animales no humanos, porque se evitaran sufrimientos innecesarios.

Pero hasta que la sociedad logre este noble fin... ¿Cuántos errores vamos a cometer por la prescripción de fármacos a humanos? ¿Cuántos animales deberán sufrir en silencio en los laboratorios? De nosotros depende

STRATEGIES TO REDUCE AND REPLACE IN VIVO EVALUATION OF OCULAR IRRITATION

SUSANA GORZALCZANY

Pharmacology Chair, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires
Junín 956 Piso 5, Ciudad Autónoma de Buenos Aires
sgorza@ffyb.uba.ar

The eyes are constantly being exposed to different substances. The exposure can be incidental, accidental, or intentional, with cosmetics, household products or drugs. For that reason, the evaluation of the potential irritation that, those products could be induced in eyes is essential to give evidence that their use is safe for human health. In this sense, in forties, Draize test sprang as the only assay to analyze the effect of different substances on or around the eyes. Rabbits are often preferred for their large eyes with well-described anatomy and physiology, ease of handling and availability. The eyes of rabbits are generally more susceptible to irritating substances than the eyes of humans but many exceptions do exist. However this procedure has been criticized because of the potential injuries on the eyes of the rabbits and for the subjectivity in the scoring system to measure the magnitude of the lesion produced by the consumer products.

The 3 Rs (replacement, refinement and reduction of the use of animals) derive from the humanitarian intent of minimizing unneccessary discomfort of the laboratory animals. Alternatives to the Draize eye test have been proposed. A refinement alternative aims to lessen animal distress, a reduction alternative decreases the number of animals used in testing and the replacement alternatives intend to eventually do away with whole-animal testing.

Different systems were used to evaluate the effect of substances in isolated environments devoid of hormonal, immune or neural influence, although the elimination of other biological factors does not allow the method to mimic interactions occurring in the whole organism, particularly with a specialized organ such as eye. Since the early 1980s, many *in vitro* methods have been developed, including isolated organ methods, organotypic models, reconstituted human tissue models, cell-based cytotoxicity methods, and cell function-based assays.

Although no single in vitro test has emerged as being completely acceptable for full replacement, various strategies are being used in order to contribute toward refining and reducing animal experiments.

NANOMED SYMPOSIUM
A GLIMPSE INTO THE LANDSCAPE OF NANOMEDICINES IN LATINO AMERICA

CHRONIC DISEASES, THERAPY, DIAGNOSTIC AND THERANOSTICS BY USING NANOPLATFORMS

MARCELO KOGAN

Department of Pharmacology and Toxicology. Faculty of Pharmacy. Universidad de Chile. Center for Advanced Study

The advent of nanotechnology has radically changed the way we diagnose, image and treat diseases, with novel nanoplatforms capable clinically important functions, including detecting cancer at its earliest stages and location, as well as delivering anticancer therapeutics specifically to tumor cells. The nanotechnology approach to cancer has focused on three main avenues: early detection; imaging for diagnostics or assessment of targeted delivery. Also multifunctional therapeutics are of interest, whereby nanoplattforms are loaded with multiple functional moieties capable of selective targeting, imaging and delivery of specific drugs to malignant cells (1,2,3). In relation with this is possible to mention the so called

theranostics which consist in the diagnostic and treatment of pathologies in a unique procedure (4) .

In the talk will be discussed the potential use of different nanomaterials multifunctionalized with different biomolecules for cancer theranostics, diagnostic *in vitro* for the ultrasensible detection of biomarkers and nanoplattorms for drug delivery. The state of the art of clinical applications of nanomaterials in diagnostic and treatment will be commented.

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WITH THE FOCUS IN MACROPHAGES: INHALABLE AND ANTI-ATHEROGENIC NANOMEDICINES.

EDER ROMERO

Nanomedicine ResearchProgram, Universidad Nacional de Quilmes, Buenos Aires, Argentina

The targeted delivery is one of the most popular promises of the nanomedicine. A promise however, that suffers from a number of limitations: inpharmacodynamics, those imposed by the restricted accessibility to the target site and the dependence of the convective extravasation; in security, because of the addition of exogenous ligands is frequently reactogenic-; in stability, because the higher the construct complexity, the more labile to physical chemical, enzymatic damagesand shear stress during storage or administration. Targeted nanoparticles are difficult to be scaled up since the addition of chemical bonds results in lengthy and expensive procedures. In a technical field where the product is the process, the artificial decoration of nanoparticles results in products difficult to be reproduced at high scale and also of challenging full structural characterization.

In this scenario, the development of new colloidal nanoparticles offering new functionalities such as a high endurance to casual manipulation and storage conditions, plus targeted delivery, with no aid of expensive additives or chemical derivatizations, is of utmost industrial relevance. To get these achievements however, the classical phospholipids employed to prepare nanoliposomes, (the massively accepted by the pharmaceutical industry nanoparticles), has to be replaced by a new type of building block, quite phylogenetically distant from phospholipids extracted from animals, plants, fungi or bacteria: the archaeolipids.

In this presentation I will address the potentialities of two of our most recent engineered nanoparticles prepared on the bases of archaeolipids (archaeosomes) at our Nanomedicine Research Program-2: inhalable pH sensitive archaeosomes for targeted delivery of anti-inflammatories to alveolar macrophages and long circulating savage archaeosomes for targeted delivery of bisphosphonates to macrophages of atheromatous plaque.

INNOVATIVE APPROACHES TO DECORATE THE SURFACE OF POLYMERIC NANOCAPSULES.

ADRIANA POHLMANN

Department of Organic Chemistry, Institute of Chemistry, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil

Biodegradable nanocarriers have been studied as a promising alternative to therapeutics contributing to expand the applications of nanotechnology. The control of size distribution, by using self-assembly methods of preparation, affects the drug biodistribution and release. Some advantages of the nanoparticulate systems are related to the drug targeting reducing side effects and increasing therapeutic index. The presentation addresses the aspects of the synthesis of lipid-core nanocapsules, an original type of carrier useful to encapsulate poorly water-soluble drugs, as well as their surface functionalization using an innovative approach based on an organometallic complex. Examples of physico-chemical characterization and biological applications of surface-functionalized lipid-core nanocapsules are discussed: i) LDL(-) recognition and ii) Mucopolysaccharidosis type I. In summary, this presentation shows that self-assembled nanoparticles are promising devices for drug delivery and targeting. (CNPq, CAPES, FAPERGS).

NANOMEDICINES FOR THE TREATMENT OF INFLAMMATORY BOWEL DISEASES.

HIGA L, JEREZ H, ROMERO E, MORILLA MJ.

Nanomedicine ResearchProgram, Universidad Nacional de Quilmes, Buenos Aires, Argentina

Inflammatory bowel diseases such as Crohn's disease and ulcerative colitis, are chronic relapsing disorders of the gastrointestinal tract, characterized bychronic inflammation and epithelial injuryinduced by the uncontrolled activation of the mucosal immune system. Dendritic cells and macrophages are key cells in the inflamed mucosa, which produce large amounts of pro-inflammatory cytokines. The current treatment is symptomatic, but the frequent oral intake of anti-inflammatory and immunosuppressant drugs or the systemic administration of biological agents such as the anti-TNF antibody infliximabis poorly effective and cause serious adverse effects.

More efficacious and safer therapies could rely on developing macrophages-targeted nanoparticles capable of specifically delivering high doses of immunosuppressantor anti-inflammatory drugs with minimal exposure of healthy. To that aim, we developedarchaeolipid nanoparticles made of a core of solid lipid and a shell of total polar archaeolipids (TPA) extracted from the halophilic archaebacterial *Halorubrumtebenquichense.* TPA are a mixture of saturated isoprenoid chains linked via ether bonds to the glycerol carbons at the *sn*2,3 position. In contrast to conventional phospholipids, TPA are hydrolytic, oxidative and enzymatic attack resistant. Besides, TPA are ligands for the macrophages scavenger receptors class A. Archaeolipid nanoparticles would combine high resistance under gastric tract with extensive uptake by macrophages.

Overall, our studies showed that ultra-small, highly negatively charge mucopenetratingarchaeolipid nanoparticles loaded with dexamethasone, but no nanoparticles lacking TPA, resulted highly stable under gastrointestinal conditions, were highly up taken by macrophages and reduced the secretion of pro-inflammatory cytokines from macrophages stimulated with lipopolysaccharide. We consider that archaeolipid nanoparticlescould improve the current therapies of inflammatory bowel diseases.

DESIGN AND DEVELOPMENT OF NEW NANOTECHNOLOGICAL PLATFORMS FOR PHARMACOTHERAPY

SANTIAGO PALMA

Pharmaceutical Technology Research and Development Unit (UNITEFA) (CONICET); Pharmacy Department, Faculty of Chemical Sciences, NationalUniversity of Cordoba (UNC), Córdoba, Argentina.

The use of active surface compounds (surfactants) in pharmaceutical technology has been widely explored. It is well known that surfactants at determined concentrations (minimal aggregation concentration) begin to form aggregates as consequence of the increment of interactions between molecules. As concentration is raised, the interactions between adjacent structures are increased leading to the coalescence of the system in larger structures usually denominated liquid crystals. The formation of such structures can be evidenced through noticeable changes in viscosity, conductivity, birefringence and X-ray diffraction patterns. The different liquid crystal systems formed from surfactant-solvent interactions are defined as lyotropic liquid crystals (LLC).

From several years ago we have been studying a group of polar lipids consisting of alkyl vitamin C derivatives (ASCn). Their amphiphilic nature allows these compounds to form aggregates, mainly lamellar mesophases. The performed studies allowed us to evaluate the potential utility of this new liquid crystal system, which have evidenced very interesting properties as pharmaceutical carrier. In this lecture, we described the general properties of these systems and the results concerning to their potential useful for different applications.

The nanostructured systems derived from the self-assembly properties of ASCn showed appropriated characteristics as pharmaceutical platform for drug delivery, especially through administration routes where permeation enhancement is necessary.

DESIGN OF SILICON OXIDE NANOPARTICLES. STUDIES OF THE INTERACTION WITH CELL SYSTEMS AND DRUG TRANSPORT.

MARTIN DESIMONE

IQUIMEFA-CONICET, University of Buenos Aires, CABA, Argentina.

The application of silica nanoparticles in the biomedical field experienced a great development in recent years. The driving forces for these and future developments are the possibility to design nanoparticles with homogeneous size and structure amenable to specific grafting. Indeed, it is possible to tune the characteristics of the silica nanoparticles to meet the requirements of each specific cell and desired application. Moreover, the effect of silica particle surface functionalization on antibiotic sorption was first studied, enlightening the role of electrostatic and hydrophobic interactions. Finally, core–shell silica particles were prepared allowing for the dual delivery of gentamicin and rifamycin.

TWO IN ONE: MULTIFUNCTIONAL NANOPARTICLES FOR THE TREATMENT OF BREAST CANCER.

MARÍA INÉS DIAZ BESSONE[1], LORENA SIMÓN GRACIA[2], PABLO SCODELLER[2], GALO SOLER ILLIA[1], TAMBET TEESALU[2], MARINA SIMIAN[1]

[1]Nanosystems Institute -University of San Martin, Argentina, [2]Laboratory of Cancer Biology, University of Tartu, Estonia

Seventy five percent of breast tumors express estrogen receptors. Tamoxifen, a selective estrogen receptor modulator, is the most widely used therapy for these patients. However, about one third of treated patients eventually developstamoxifen resistance and cancer reappears. We previously showed that fibronectin, when bound to cell surface β1-integrins,induces tamoxifen resistance in breast cancer cells. Moreover, we found that tamoxifen leads to an increase in breast cancer stem cells that are positive for β1-integrin. Nanoparticles (NPs) provide new features and functions that aredifferent from those present in the individual components. In particular, small size, high surface area/volume ratio and multifunctionality, make NPs attractive as carriers for drugs and diagnostics. The aim of this project was to investigate the effectiveness of multifunctional tamoxifen –loaded NPs,compared to free tamoxifen, for the treatment of breast cancer. To do so we to designed iRGD-coated and tamoxifen –loaded polimersomes and tested their efficacy on MCF-7 breast cancer cells in vitro and in vivo. Our results show that at equivalent concentrations, tamoxifen has a greater effect on cell viability when carried in NPs ($p<.001$). iRGD-coated tamoxifen NPs reduce stem cell growth ($p<.01$), contrary to what is observed with free tamoxifen. Cell uptake of NPs is increased when they are coated with iRGD, as well as in vivo tumor homing. Preliminary results show increased survival of mice carrying MCF-7 tumors when treated with iRGD-coated tamoxifen NPs, compared to free tamoxifen controls. Our results suggest that iRGD-coated tamoxifen NPs could be a rationale alterative for the treatment of estrogen receptor positive breast cancer, leading to increased disease free survival.

LIPOSOMES FOR TOPICAL TREATMENT OF CUTANEOUS LEISHMANIASIS.

DOLORES C. CARRER, MA. FLORENCIA PERALTA

Instituto de Investigación Médica Mercedes y Martín Ferreyra, INIMEC-CONICET-UNC, Córdoba, Argentina

Cutaneous Leishmaniasis is an endemic disease that affects millions of people worldwide. It is one of the five most widespread orphan diseases in Latin America in particular. In Argentina, it has traditionally been found mainly in the tropical/subtropical regions in the North-East of the country. This is however changing, with the vector and the disease being found, in the last few years, also in regions further South in the country and reaching the province of Córdoba. The reasons for the spreading of the disease are multiple and not straightforward to control. I will discuss the available methods for treatment and the new treatments being developed, in particular topical treatments. I will briefly present our own efforts in the field.

NANOTECHNOLOGYCAL TOOLS EMPLOYED TO IMPROVE THE OPHTHALMOLOGIC THERAPY.

DANIELA QUINTEROS

Pharmacy Department, Faculty of Chemical Sciences, National University of Cordoba (UNC), Córdoba, Argentina

Acetazolamide (AZM) is a carbonic anhydrase inhibitor, mainly used to reduce intraocular pressure in the treatment or long-term management of glaucoma. However, the potential of topical treatment is limited, due to its low permeability in ocular epithelium. An alternative to overcome this limitation is the incorporation of AZM in nanopar-

ticulate systems, such as polymeric nanocapsules (NC). In this way, this work aimed to prepare, characterize in vitro and in vivo AZM-loaded NC using ethylcellulose (EC) and Eudragit® RS100 (EUD) as encapsulating polymers. These NCs showed very high encapsulation efficiency, they were physically stable and their size was around 220-110 nm for NCEUD and NCEC, respectively. Due to the chemical characteristics of the polymers, NCEUD possesses positive surface charges, whereas for NCEC, the zeta potential was negative. In vitro release studies showed a characteristic release pattern corresponding to a classical behavior observed for devices based on a reservoir, where the properties of the polymer membrane modulate drug release. In both cases, the AZM release was practically independent regarding its concentration. Ex vivo assays, where the permeation through isolated cornea was studied, evidenced that the amount of AZM permeated from EC and EUD nanoparticles was quite higher than in the case of AZM solution. We hypothesized that nanoparticles may work as a carrier facilitating drug penetration across the cornea. Besides, the very small size and the mucoadhesive properties of NCs, particularly NCEC, could favor a close contact with the cornea surface facilitating its penetration. In vivo studies, related to the hypotensive effect of NCs in normotensive rabbits, showed that NCEC formulation was the most efficient, since an increased amount of permeated drug was observed, along with a greater IOP decrease and longer duration of the effect. This novel formulation could be a promising alternative for a more efficient treatment of glaucoma.

INDOCYANINE GREEN WITHIN POLYMERIC MICELLES AS POTENTIAL IMAGE AGENTS TO MAP SENTINEL LYMPH NODES

NICOLE LECOT[A], MARCELO FERNÁNDEZ LOMONACO[A], PABLO CABRAL[A] AND ROMINA GLISONI[B]

[a]Laboratorio Radiofarmacia, Centro de Investigaciones Nucleares, Facultad de Ciencias, UdelaR, Montevideo, Uruguay.
[b]NANOBIOTEC UBA-CONICET, Department of Pharmaceutical Technology, Faculty of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina.

The image-guided surgery (IGS) has been widely used in clinic to map sentinel lymph nodes (SLN) of primary tumors from breast, skin, colorectal, lung and other sites. IGS require a near infrared (NIR) fluorescent contrast agent to locate the position of SLN. A sentinel lymph node biopsy (SLNB), is a procedure in which SLN is identified, removed, and examined to determine whether cancer cells are presented. A positive SLNB result indicates that cancer is present in SLN and possibly may be present in other regional lymph nodes and organs. NIR indocyanine green (ICG) is a FDA-approved water soluble fluorophore, used for various diagnostic purposes for example IGS/SLNB. ICG tends to the self-aggregation in aqueous solution to form dimers, oligomers and polymers, depending the concentration used. As a result of this, the fluorescence quenching of ICG often occurs, reducing the fluorescence efficacy. On the other hand, due to the strong protein-binding and the fast uptake to the liver, ICG displayed only a half-life of 3-4 minutes when injected intravenously. The aim of this work was to develop a novel micellar formulation containing ICG with improved physicochemical properties such as: *(i)* an optimal fluorescence, *(ii)* a better stability in aqueous medium and *(iii)* a longer half-life in circulation, using polymeric micelles (PMs), made of pristine poly(ethylene oxide)-*b*-poly(propylene oxide) block copolymers (PEO-PPO) and their glucosylated derivatives, and finally to evaluate these nanocarriers loaded with ICG intradermally, in an *in vivo* assay against the commercially available free ICG in solution.

3D IN VITRO TISSUE MODELS IN NANOMEDICINE RESEARCH

PRISCILA SCHILRREFF, MORILLA MARIA JOSE, EDER ROMERO

Nanomedicine Research Program, C3R, Universidad Nacional de Quilmes, Buenos Aires, Argentina

With the increasing numbers of new nanomedicines there is a need to investigate their possible health adverse effects in humans.Nanomedicines can potentially be administered through a number of routes, including oral, dermal, pulmonar and ocular. Extensive*in vitro* and *in vivo*research is needed before nanomedicines will be suitable for medicinal use. For that purpose, millions of animals are used. However, 95 percent of new drugs fail because they do not work in humans or are unsafe, despite previously appearing safe in preclinical animal tests. According to US FDA, adverse drug reactions cause over 100.000 deaths annually. Besides, *being*costly and poor predictive of human toxicity due to inter-species differences, animal tests can *be cruel* and painful. Therefore,

the replacement of animals with *in vitro* human cell-based models is required. In this context, 3D *in vitro*models provides a cellular microenvironment that preserves cell-cell interactions, function and tissue architectureand could be a useful tool for *predicting*the effects of *nanomedicines* in humans.

A number of celular 3D models have been developed including skin, liver, cardiac, pulmonar, corneal and ephithelial intestinal tissues. This presentation will focus on technicalspecifications for the development of intestinal, normal and disease skin models usedfor the evaluation of toxicity, penetration and efficiency of new nanomedicines.

DENDRITIC THERMORESPONSIVE NANOGELS AS VERSATILE PLATFORMS FOR BIOMEDICAL APPLICATIONS

MARIA MOLINA[1,2], MARCELO CALDERÓN[2]

1:Universidad Nacional de Rio Cuarto. Rio cuarto, Argentina, 2:Institut für Chemie und Biochemie, Freie Universität Berlin. Berlin, Germany

Nanogels are nanosizedcrosslinked networks composed of hydrophilic or amphiphilic polymer chains. They are developed as carriers to transport small molecules such as drugs, dyes, or biomacromolecules. Thermoresponsive polymers undergo a phase transition at a certain temperature in aqueous media. As a consequence,they can change their aggregation state, exhibit conformational change and undergo shrinking, swelling, or micellization upon a thermal trigger. The combination of nanogel properties and thermoresponsiveness represents a promising approach for the development of smart nanocarrier systems, which reveals high loading capacity, improves drug stability, and thus can be used for stimuli-controlled release in drug delivery. Herein, the engineering of thermoresponsive dendritic nanogels using different thermoresponsive polymers and synthetic methodologies for biomedical applications is presented.

NANOMATERIALS AND CELLS: INTERACTIONS AND EFFECTS.

ALICIA LORENTI

Instituto de Ciencia y Tecnología Dr. Cesar Milstein, Fundación Pablo Cassara, CABA, Argentina

Tissue engineering is a field of Regenerative Medicine that combines cells, biomaterials, and suitable biochemical and physicochemical factors in order to obtain substitutes to improve or replace biological damaged tissues. The interactions between cells, extracelular matrix, and their microenvironment play key roles in controlling the cell fate (differentiation/dedifferentiation, proliferation, adhesión, spreading, migration, apoptosis).

Progress in the development of nanotechnology has stimulated the applications of nanomaterials for regenerative medicine/tissue engineering. However, thinking that a cell is a particle composed by nano compartments (cell membranes and nuclear, surface proteins, genetic material, cytoskeleton), the interactions between nanomaterials and cells should be taken in consideration when considered any tissue engineering development, even when these interactions are not well understood yet. Nanomaterials are not a simple miniaturization of macroscopic counterparts; they exhibit distinctive physical, chemical, optical, and mechanical properties.

The understanding of nanomaterial-cell interactions will facilitate improved biomaterial design for a range of biomedical and biotechnological applications. In this sense, the effects of the shapes, kind of surfaces, and chemical functionality of nanomaterials on cellular processes need critical evaluation, in order to understand the true nature of biological effects. Moreover, the intracellular safety concern of the nanomaterials as a result of its cellular uptake, its intracellular fate and degradation, and its influence on toxicity remains to be clarified in detail.

NANOTECHNOLOGICAL COLLAGEN SCAFFOLDS FOR DERMAL REGENERATION

HELENA PARDO[1], LUCIANA PEREIRA[1], ANALÍA CASTRO[1], ÁLVARO W. MOMBRÚ[1], RICARDO FACCIO[1], NATALIA ODDONE[2], JUAN C. BENECH[2], CRISTINA TOURIÑO[3], JUAN PABLO VILLANUEVA[1], PATRICIA ZIMET [1], PABLO MIRANDA[1], MARIANO ROMERO[1], INÉS ALVAREZ[4], HÉCTOR PÉREZ CAMPO[4]

[1]- Centro NanoMat, DETEMA, Facultad de Química, Universidad de la República, Montevideo, Uruguay; 2- Laboratorio de Nanobiología y Señalización Celular, Instituto de Investigaciones Biológicas Clemente Estable, Ministerio de Educación y Cultura, Montevideo, Uruguay; 3-Departamento Básico de Medicina, Hospital de Clínicas, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay; 4- Instituto Nacional de Donación y Trasplante, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay.

The treatment of hard-to-heal acute and chronic wounds represents a critical and expensive issue for medicine. In this regard, engineering skin substitutes arise as an alternative treatment suitable for dermal

regeneration, as they resemble structural and functional characteristics of the native extracellular matrix. Therefore, the main aim of this work is to develop a type I collagen scaffold which incorporates L-ascorbic acid (AA) loaded chitosan nanoparticles (CSNp) in order to improve the wound healing of the skin. The AA promotes the natural tissue collagen synthesis.

In order to develop a porous scaffold, type I collagen was obtained by chemical degradation of bovine tendon. The primary crosslinking was performed by the addition of native bovine chondroitin sulphate, afterwards the scaffolds were frozen and irradiated with gamma rays. Then, a medical grade silicone layer was adhered to the scaffolds, resulting in a bilayer material. Finally, the AA-CSNp were incorporated by inmmersion. The final product was characterized physically and chemically through: Scanning electron microscopy, atomic force microscopy and mechanical properties test by texturometry. The biologically cytotoxicity was assessed in vitro and in vivo.

The AA-CSNp were prepared by the ionic gelation technique and characterized using transmission electron microscopy and dynamic light scattering. Cell viability studies have been carried out in order to study de citotoxicity of the CSNp. Through this technique, were obtained monodisperse and spheric AA-CSNp with an average size of 109 nm, a 20 mV Z potential and 21 % of encapsulation efficiency.

15kGy gamma irradiated before lyophilisation collagen scaffolds has shown the best mechanical properties compared with other doses of gamma rays. This radiation also renders them sterile. The collagen scaffolds obtained had not shown cytotoxicity during in vitro studies. From in vivo results it has been observed an improvement in the skin healing.

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SAIC AWARDS

IRENE FARYNA RAVEGLIA: ONCOLOGY AWARD

IN VIVO HEMIN PRE-CONDITIONING TARGETS THE VASCULAR AND IMMUNOLOGICAL COMPARTMENTS AND RESTRAINS PROSTATE TUMOR DEVELOPMENT

FELIPE MARTÍN JAWORSKI (1a,1b), LUCAS DANIEL GENTILINI (1b), GERALDINE GUERON (1a), ROBERTO PABLO MEISS (2), EMILIANO GERMÁN ORTIZ (1a), PAULA MERCEDES BERGUER (3), ASIF AHMED (4), NORA NAVONE (5), GABRIEL ADRIÁN RABINOVICH (6), DANIEL COMPAGNO (1b), DIEGO LADERACH (1b), ELBA SUSANA VÁZQUEZ (1a)

(1a) Laboratorio de Inflamación y Cáncer, IQUIBICEN/Dpto. Química Biológica, CONICET/FCEN-UBA. (1b) Laboratorio de Glico-Oncología Molecular y Funcional, IQUIBICEN/Dpto. Química Biológica, CONICET/FCEN-UBA, (2) Departamento de Patología, Instituto de Estudios Oncológicos, Academia Nacional de Medicina. (3) Fundación Instituto Leloir-CONICET, (4) Aston Medical Research Institute, Aston Medical School, University of Aston, Birmingham, UK, (5) Department of Genitourinary Medical Oncology and the David H. Koch Center for Applied Research of Genitourinary Cancers, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, (6) Dpto. Química Biológica, FCEN-UBA / Laboratorio de Inmunopatología, IBYME-CONICET

Pre-conditioning strategies constitute a relatively unexplored and exciting opportunity to shape tumor fate. In this study we used hemin, an inducer of Heme Oxygenase-1 (HO-1), in an in vivo pre-conditioning model to assess prostate cancer (PCa) development. The stroma of immunocompetent C57BL/6 mice (n=5) was conditioned by subcutaneous administration of hemin prior to TRAMP-C1 (murine PCa cell line TC1) tumor challenge. Pre-conditioning resulted in increased tumor latency and decreased initial growth rate ($P<0.05$). Histological analysis of the tumors revealed impaired vascularization. Hemin-treated HUVEC exhibited decreased tubulogenesis in vitro only in the presence of TC1-derived conditioned media ($P<0.001$). Regarding the crosstalk between tumor and endothelial cells, TC1 cell motility and adhesion to the endothelium were significantly impaired in the presence of hemin pre-treated HUVEC conditioned media ($P<0.05$). An in vivo Matrigel plug assay confirmed that s.c. hemin pre-conditioning hinders tumor neo-vascularization in C57BL/6 mice (n=5; $P<0.01$). Furthermore, we also analyzed the effect of hemin treatment on the immune compartment. Hemin boosted CD8+ T-cell proliferation and degranulation in vitro, and antigen-specific cytotoxicity in vivo ($P<0.01$). Interestingly, a significant systemic increase in the frequency of CD8+ T lymphocytes was observed in hemin pre-conditioned tumor-bearing mice ($P<0.05$). Tumors from hemin-conditioned mice also showed reduced expression of Galectin-1 (Gal-1; $P<0.01$), key modulator of tumor angiogenesis and immunity, evidencing a clear and persistent remodeling of the microenvironment. Finally, data obtained at the mRNA and protein levels revealed a subset of PCa patients and PCa patient-derived xenografts, respectively, with mild HO-1 and low Gal-1 expression. Taken altogether, these data showcase a novel function of an already human-used drug as a novel means of boosting the endogenous anti-tumor response.

PROGNOSTIC IMPACT OF MINIMAL DISSEMINATION DETECTION IN NON-METASTATIC RETINOBLASTOMA WITH HIGH-RISK PATHOLOGY FEATURES

ANA VANESA TORBIDONI (1), VIVIANA E LAURENT (1), CLAUDIA V SAMPOR (1), ROSARIO ASCHERO (1), DANIELA OTTAVIANI (1), VALERIA VAZQUEZ(1), MARIANO R GABRI (2), MARÍA T GARCÍA DE DÁVILA (1), MARCO A RAMIREZ ORTIZ (3), CRISTINA ALONSO (1), JORGE ROSSI (1), DANIEL F ALONSO (2), GUILLERMO L CHANTADA (1)

1 Hospital de Pediatría S.A.M.I.C. "Prof. Dr. Juan P. Garrahan". Buenos Aires, Argentina, 2 Laboratorio de Oncología Molecular, Universidad Nacional de Quilmes. Bernal, Buenos Aires, Argentina, 3 Hospital Infantil de México Federico Gomez, Ciudad de México, D.F., México.

Introduction: Metastatic relapse may occur in children with retinoblastoma and high-risk pathology features (HRPF) and it is a major cause of mortality worldwide. Detection of minimal dissemination (MD) may be a tool for risk estimation. We previously reported the use of CRX mRNA for MD determination in metastatic retinoblastoma

but no data in non-metastatic children with retinoblastoma and HRPF are available. Objectives: To evaluate whether MD is detectable in children with non-metastatic retinoblastoma who have HRPF and to assess the prognostic impact of MD on disease-free survival (DFS). Material and methods: It was a prospective study carry on from 05-2007 to 10-2013. We studied 84 patients with non-metastatic retinoblastoma and HRPF (isolated massive choroidal invasion in 14, post laminar optic nerve invasion (PLONI) in 51, 12 with scleral invasion without PLONI and 7 with tumor at the resection margin of the optic nerve) were evaluated at the time of primary or secondary enucleation. CRX mRNA was evaluated by RT-qPCR in bone marrow (BM) and cerebrospinal fluid (CSF) at diagnosis and during follow-up. In 14 cases, GD2 synthase was used instead of CRX for CSF evaluation. The main outcome measure was the metastatic relapse. Results: MD was detected in 9 cases (BM=7, CSF=2). MD was significantly associated with tumor extension beyond the resection margin of the optic nerve (p=0.0005) and scleral invasion (p=0.002). MD occurred in 18.6% of the group E eyes with glaucoma and in 8/80 and 1/16 of initially and secondarily enucleated children, respectively. Children with MD had a significantly lower 3-year DFS (0.78 (95% CI= 0.37-0.94) versus 0.98 (95% CI= 0.93-1) p=0.004). Conclusions: We identified a very high-risk population of children with retinoblastoma and HRPF who have MD in whom DFS is significantly lower despite intensive adjuvant therapy. Children with group E retinoblastoma and glaucoma have a significantly higher risk of MD at diagnosis.

ID4 ACTS AS A TUMOR SUPPRESSOR IN ER+ BREAST TUMORS

DANIELA LUCÍA NASIF (1), EMANUEL CAMPOY (1,2), GUILLERMO URRUTIA (1), SERGIO LAURITO (1,3), MARÍA ROQUÉ MORENO (1,3), MARÍA TERESITA BRANHAM (1)

(1)IHEM, Universidad Nacional de Cuyo, CONICET, Mendoza, Argentina, (2) Facultad de Ciencias Médicas, Mendoza, Argentina , (3) Facultad de Ciencias Exactas y Naturales, Mendoza, Argentina

Inhibitor of differentiation proteins 1, 2, 3 and 4 (ID1–4), are dominant negative regulators of the basic helix-loop helix (bHLH) family of transcription factors. In human tumors, an increased expression of ID proteins has been associated with reversion to an embryonic-like state, loss of differentiation, high rates of proliferation, migration and neo-angiogenesis. In breast cancer there are controversial findings regarding the role of ID4 during tumorigenesis. For instance, ID4 silencing by promoter hypermethylation is a frequent event in ER+ (estrogen receptor) breast tumors and is associated with an increased risk of lymph node metastasis. However, in ER- breast tumors ID4 increased expression has been associated with the ability of cancer cells to exhibit anchorage-independent growth. Our group has previously shown that ID4 promoter's unmethylation is associated with the aggressive Triple Negative Breast cancer subtype. It seems then, that ID4 has a dual role in breast cancer. Here, we hypothesize that ID4 acts as a tumor suppressor in ER+ breast tumors. To test our hypothesis we performed data mining analyses from the TCGA database and cell culture experiments. In silico analyses, in a cohort of 872 invasive ductal breast carcinomas, reveal that ID4 is downregulated in ER+ breast tumors (p<0,001) due to promoter methylation. To test the effect of ectopic ID4 expression, we performed ID4 transient transfection on MCF-7 and T47D breast cancer cell lines. Both are ER+, present ID4 promoter methylation and do not express ID4. Ectopic ID4 expression on MCF-7 and T47D cells lead to decreased proliferation and increased apoptosis. Cell cycle analysis indicated that ID4 transfected cells accumulated in G1 phase. ID4 overexpression also reduced migration rate respect to cells treated with an empty vector. The results presented suggest that ID4 acts as a tumor suppressor in ER+ tumors by leading to reduced proliferation and migration in breast cancer cell lines

DR. CARLOS LANTOS – FUNDACIÓN HONORIO BIGAND: ENDOCRINOLOGY AWARD

SEX DIFFERENCES IN THE DEVELOPMENT OF PROLACTINOMA IN MICE OVEREXPRESSING HUMAN CHORIONIC GONADOTROPIN (HCGβ+): ROLE OF PITUITARY TGFβ1

ERIKA YANIL FARAONI; MARÍA ANDREA CAMILLETTI; ALEJANDRA ABELEDO; LAURA RATNER; SUSANA RULLI; GRACIELA DÍAS-TORGA

IBYME-CONICET, Buenos Aires, Argentina

TGFβ1 is an inhibitor of lactotroph proliferation and prolactin secretion, and the reduced TGFβ1 activity found in prolactinomas has been proposed to be involved in tumor development. hCGβ+ females, but not males, develop prolactinomas. In a previous work we found that hCGβ+ female pituitaries present decreased active TGFβ1 levels,

TGFβ1 biological activity and TGFβ1 receptors expression compared to their WT counterpart. This weaker TGFβ1 system was proposed to be involved in the development of prolactinomas in this group. The aim of the present work was to complete the previous work analyzing other components of the pituitary TGFβ1 system. As dopamine increases pituitary TGFβ1 expression and activity, acting through the dopamine D2 receptor (Drd2) expressed in lactotrophs, we also evaluated pituitary Drd2 expression, and hypothalamic tyrosine hydroxylase (TH) expression. Levels of LTBP1, Smad4 and Smad7, measured by qRT-PCR, were found increased in male pituitaries compared to females, without differences among genotypes, but were found decreased in tumoral hCGβ+ female pituitaries compared to the WT siblings. The lower expression of TGFβ1 system found in hCGβ+ female pituitaries was accompanied by a lower dopaminergic tone in this group, reflected by decreased hypothalamic TH and increased pituitary Drd2 expression. The high levels of progesterone present in hCGβ+ females could be involved in the decreased expression of hypothalamic TH found in this group. We did not find disturbances, neither in the pituitary TGFβ1 system, nor in hypothalamic TH in hCGβ+ males. We conclude that decreased TGFβ1 expression and activity found in hCGβ+ female pituitaries are undoubtedly involved in the development of prolactinoma in this group. Meanwhile, the stronger TGFβ1 system found in male pituitaries could be protecting them from excessive lactotroph proliferation and prolactinoma development, even in the presence of high levels of hCG.

HYPERMETHYLATION OF HOXA10 BY NEONATAL ENDOSULFAN EXPOSURE: AN EPIGENETIC MECHANISM FOR IMPAIRED EMBRYO IMPLANTATION

MARÍA MERCEDES MILESI; MARLISE LUCIANA GUERRERO SCHIMPF; ENRIQUE HUGO LUQUE; JORGELINA VARAYOUD

Instituto de Salud y Ambiente del Litoral (ISAL), Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral – Consejo Nacional de Investigaciones Científicas y Técnicas.

The homeobox gene, Hoxa10, is crucial for uterine development during embryogenesis and for embryo implantation at adulthood. In previous work we demonstrated that neonatal exposure to endosulfan alters uterine Hoxa10 expression in prepubertal rats, and decreases the number of implanted embryos at adulthood. This work investigates the effects of neonatal endosulfan exposure on Hoxa10 uterine expression and DNA methylation status during the pre-implantation period. Newborn female rats were treated by s.c. injections every 48 h, from postnatal day 1 (PND1) to PND7, with corn oil (vehicle, Control), 6 µg endosulfan/kg (Endo6, reference dose EPA) or 600 µg endosulfan/kg (Endo600, no observed effect level, EPA). On PND90 females were pregnant and on gestational day 5 (pre-implantation period) uterine samples were collected. The expression of Hoxa10 was determined at protein and mRNA levels by immunohistochemistry and real time RT-PCR, respectively. mRNA relative expression of the DNA methyltransferases (DNMT) 3a and 3b was also evaluated. Upon Hoxa10 gene we searched for CpG islands and restriction sites for the BstUI enzyme, to evaluate the methylation status of its regulatory regions by Methylation-Sensitive Restriction Enzymes-PCR technique (MSRE-PCR). Predicted binding sites for transcription factors were also investigated. Both doses of endosulfan decreased the expression of Hoxa10 mRNA, while only Endo600 down-regulated Hoxa10 at protein level. Endo6 and Endo600 groups showed increased DNA methylation levels in regulatory regions of Hoxa10 gene, that are potentially regulated by critical transcription factors associated with the implantation process. An up-regulation of DNMT3a and DNMT3b was detected in endosulfan-treated rats. Neonatal exposure to endosulfan decreases uterine Hoxa10 expression during the pre-implantation period, via hypermethylation of regulatory regions of the gene. These alterations could account for the endosulfan-induced implantation failures.

TESTOSTERONE FAVORS A HIGHER RECRUITMENT OF NEUTROPHILS WITH REDUCED EFFICIENCY IN KILLING BACTERIA

MARÍA VICTORIA SCALERAND; CAROLINA LEIMGRUBER; NAHUEL PEINETTI; JUAN PABLO NICOLA[2]; CRISTINA ALICIA MALDONADO; AMADO QUINTAR

Centro de Microscopía Electrónica, INICSA-CONICET. Facultad de Cs. Médicas, Universidad Nacional de Córdoba, Departamento de Bioquímica Clínica, CIBICI-CONICET. Facultad de Cs. Químicas, Universidad Nacional de Córdoba, Argentina

Although androgens have been suggested to exert modulatory functions on adaptive immunity, there is scarce evidence about their role on the innate immune/inflammatory response. Considering that neutrophils are es-

sential effector cells against bacterial pathogens, the aim of this work was to evaluate the effects of testosterone on neutrophils infiltrating the prostate gland. Adult male Wistar rats were first orchiectomized and immediately replaced with testosterone at physiological level (T) or vehicle (OX), and then subjected to acute prostatitis by intra-prostatic inoculation of E. coli (for 5 days, T+BP and OX+BP groups) or LPS (for 24 hs, T+LPS and OX+LPS groups). T+BP animals showed a higher neutrophil infiltration compared to OX+BP, with intense E. coli immunostaining, correlating with the presence of phagocytosed bacteria in active neutrophils by electron microscopy. In LPS-induced prostatitis, testosterone treatment also promoted a higher neutrophil recruitment (Gr+) cells per gland by flow cytometry, which was correlated to an increased mRNA expression of CXCL1 and CXCL2), with the cells having a lower myeloperoxidase (MPO) activity. Interestingly, sorted Gr+ infiltrating neutrophils showed a higher mRNA expression of IL10 and IL6 in T+LPS by qPCR. Finally, testosterone also increased thioglycollate-induced neutrophil recruitment in the peritoneum, with the cells exhibiting a reduced bactericidal ability when coincubating ex vivo with E. coli. These findings reveal a intriguing role for testosterone on the early inflammatory response in the prostate, with neutrophils being a main target. Testosterone increases local chemokine expression, leading to a higher recruitment of neutrophils to the site of infection. However, testosterone favors an IL10high MPOlow phenotype, with reduced efficiency in killing bacteria. This immunomodulatory effect of testosterone represents a novel factor to consider in alternative approches for inflammatory diseases.

INITIAL EXPERIENCE OF MOLECULAR STUDIES IN DIFFERENTIATED THYROID CANCER

NORMA NOEMÍ TOLABA[1]; PAOLA BAZZONI[2]; MARCELO MONTEROS ALVI[1,2,3]; CECILIA HERRERA[3]; LAURA SANCHEZ[3]; GILDA RICHTER[3]; LEOPOLDO VAN CAUWLAERT[4]; MARCELO NALLAR[4]; VALERIA CERIONI[5]; MACARENA GALINDEZ[5]; CHRISTIAN MARTÍN MOYA[1]

[1]Sector de Biología Molecular, [2]Sector de Anatomía Patológica, [3]Sector de Diagnóstico por Imagen, Programa de Diagnóstico y Tratamiento. [4]Programa de Cirugía. [5]Programa de Endocrinología. Hospital de Endocrinología y Metabolismo, Dr. Arturo Oñativia. Salta, Argentina

Differentiated thyroid cancer is the most common endocrine neoplasia. The Fine Needle Aspiration (FNA) of thyroid nodules, followed by cytological analysis is the standard preoperative diagnostic procedure, however the indeterminate FNA cytopathology is 10-30%. Molecular studies of indeterminate FNAs reduce the rate of diagnostic surgery and may define the extension of the surgery.

Aims: To evaluate the sensitivity and diagnostic accuracy of molecular studies of thyroid FNAs. To correlate these results with cytological and histological studies of the same patients. To confirm the utility of molecular studies to define diagnostic cytopathology.

The 7 most frequent genetic alterations in differentiated thyroid cancer were studied: mutations in BRAF, K/H/N-RAS genes and RET/PTC 1 and 3, and PAX8/PPARG gene fusions. The diagnosis was made by High Resolution Melting (HRM) for exons with most frequent mutations. Also, commercial kits were used to validate HRM results. Gene fusions were diagnosed by RT-nested PCR.

To date, were analyzed prospectively 80 thyroid FNA samples belong to the gray areas of Bethesda by molecular techniques. Of these, 29 underwent surgery and histological confirmation could be obtained. Of 80 FNAs, 17 were positive for a mutation in 1 of the 7 genes analyzed. Of the 29 patients operated, 13 were positive for 1 of these mutations. Histological analysis confirmed the presence of neoplasia in all positive samples and carcinoma in 11 of 13 molecular positive (PPV 84.6%). Of the 14 samples without mutations, only 2 were papillary carcinoma, the rest patients had benign histology (NPV 87.5%). So far, based on the sample analyzed we have a sensitivity, specificity and diagnostic accuracy above 84%.

The results confirm the high sensitivity and specificity of molecular studies. This methodology is already used in our hospital to improve the preoperative diagnostic accuracy, contributes to risk stratification and helps selecting the appropriate surgery.

AMERICAN SOCIETY OF MICROBIOLOGY AWARD

INFECTOLOGY AND PARASITOLOGY

CESTODE PARASITES SECRETE EXTRACELLULAR VESICLES CARRYING ANTIGENIC PROTEINS AND MICRORNAS

MARÍA EUGENIA ANCAROLA[1], ANTONIO MARCILLA[2,3], NATALIA MACCHIAROLI[1], MATÍAS PÉREZ[1], SEBASTIÁN ASURMENDI[4], CAROLINA PONCINI[1], MARA ROSENZVIT[1], MARCELA CUCHER[1]

1 Instituto de Investigaciones en Microbiología y Parasitología Médica (IMPAM, UBA-CONICET), Facultad de Medicina, Universidad de Buenos Aires (UBA). 2Área de Parasitología, Departamento de Farmacia y Tecnología Farmacéutica y Parasitología, Universitat de València, Burjassot, Valencia, Spain. 3 Joint Research Unit on Endocrinology, Nutrition and Clinical Dietetics, Health Research Institute-La Fe, Universitat De València, 46026 Valencia, Spain. 4 Instituto de Biotecnología, CICVYA-INTA, Buenos Aires, Argentina.

Cestode parasites are platyhelminths passively transmitted between the hosts involved in their life cycles and can infect almost all vertebrate species. Some of the zoonoses they cause are among the most severe neglected tropical diseases in humans prioritized by the World Health Organization. Lately, several studies described the secretion of extracellular vesicles (EV) as a path of intercellular communication in many organisms and also as a new mechanism of inter-species cross-talk in the host-parasite interplay. The term EV groups varying types of membranous structures which mainly differ in their biogenesis, morphology and protein content. EV can also carry lipids and nucleic acids, including DNA, mRNAs and small RNAs. It has been shown that nematode and trematode parasites secrete EV, which can be internalized by host cells. These EV contain proteins and small RNAs, among which microRNAs were identified. Here, we aimed to determine whether cestode parasites secrete EV and characterize their content. For this, we chose the larval stages of the model cestodes Taenia crassiceps and Mesocestoides corti. First, we demonstrated the in vitro secretion of membranous structures compatible with EV by transmission electron microscopy. Then, we characterized their protein content by LC-MS/MS. As a result we identified expected eukaryotic EV markers and also, among others, proteins tested for immunodiagnosis of cestode infection as well as host immunoglobulins. Finally, we proved by capillary electrophoresis that cestode EV carry small RNAs and then microRNAs were detected by RT-(q)PCR. This is the first report of EV as well as microRNAs secretion in cestode parasites and could represent a new cross-species communication mechanism with the host. We also provide evidence on a new route used by cestode parasites for the secretion of formerly studied proteins. These results provide relevant information for the improvement or development of new diagnosis methods of cestodiases.

CHLAMYDIA TRACHOMATIS NEITHER EXERTS DELETERIOUS EFFECTS NOR FIRMLY ATTACHES TO IN VITRO CAPACITATED SPERMATOZOA

Jenniffer Puerta Suárez[1, 3], Leonardo Rodolfo Sánchez[1], Héctor Alex Saka[1], Rosa Molina[2], Andrea Tissera[2], Virginia Elena Rivero[1], Walter David Cardona Maya[3], **Rubén Darío Motrich**[1]

(1) CIBICI-CONICET. Facultad de Ciencias Químicas. Universidad Nacional de Córdoba. Argentina. (2) LAR, Laboratorio de Andrología y Reproducción, Córdoba, Argentina. (3) Grupo Reproducción, Facultad de Medicina, Universidad de Antioquia, Medellín, Colombia.

Chlamydia trachomatis is the most prevalent sexually transmitted bacterial infection among sexually active young adults. Yearly, approximately 100 million new cases are diagnosed worldwide. Moreover, up to 90% infections in women and 50% in men are asymptomatic favoring bacterial spread. However, whether Chlamydia trachomatis has detrimental effects on sperm quality and male fertility is still a controversial issue. In the present study, we analyzed the effects of Chlamydia spp. on in vitro capacitated human and mouse spermatozoa. By in vitro and in vivo assays, we also analyzed the ability of Chalmydia spp. to firmly interact/attach to spermatozoa. Human and mouse sperm were obtained from healthy donors and cauda epididimys from C57BL/6 mice, respectively. Highly motile in vitro capacitated human or mouse spermatozoa were exposed, at different times, to increasing concentrations of elementary bodies of C. trachomatis (serovar E or LGV) or C. muridarum, respectively. Then, several sperm quality parameters were analyzed. In addition, confocal microscopy and in vitro and in vivo approaches were performed to analyze whether Chlamydia spp. firmly attach to spermatozoa. In vitro capacitated human or murine sperm exposed to increasing bacterial concentrations or soluble factors from C. trachomatis or

C. muridarum, respectively, did not show differences in the levels of sperm motility and viability, apoptosis, mitochondrial membrane potential, DNA fragmentation, ROS production and lipid peroxidation, when compared with control sperm ($p>0.05$). Moreover, Chlamydia spp. did not firmly attach to either human or mouse spermatozoa.

In conclusion, our results demonstrate that C. trachomatis does not directly exert deleterious effects on in vitro capacitated spermatozoa. Also, we provide evidence indicating that Chlamydia spp. does not firmly attach to spermatozoa shedding light on an old open question with significant implications for assisted reproduction.

MICRORNAS IN TAENIA CRASSICEPS AND TAENIA SOLIUM: CHARACTERIZATION AND FUTURE APPLICATIONS

MATÍAS GASTÓN PÉREZ, MARCELA CUCHER, NATALIA MACCHIAROLI, MARA ROSENZVIT

Instituto de Investigaciones en Microbiología y Parasitología Médica (IMPAM, UBA-CONICET), Facultad de Medicina, Universidad de Buenos Aires (UBA).

The cestode parasite *Taenia solium* is the etiological agent of neurocisticercosis, one of the 17 neglected tropical diseases prioritized by the WHO. Taenia crassiceps, another cestode from the same genus, is used as a laboratory model of *T. solium*. microRNAs (miRNAs) are small non-coding RNAs considered master regulators of gene expression with key roles in diverse cellular processes. At present there is no evidence of miRNAs from these parasites. High-throughput characterization of small RNAs (sRNAs) in *T. Crassiceps* larval stage (cisticercus) is presented. RNA was purified from cysticerci (3 biological replicates), sRNA library was constructed and sequenced with HiSeq 2500. The mirDeep2 software was used for miRNA identification. The *T. Solium* genome was used for bioinformatics analyses, since it is the closest genome available, allowing also to identify *T. solium* miRNAs at the genome level. Experimental validation of selected sequences was performed by Northern blotting. The results obtained showed that miRNAs were the most abundant category of sRNAs accounting for 83% of mapped reads. A final high confidence set of 42 miRNAs: 38 conserved and 4 novel, was obtained. Northern blot results showed bands compatible with miRNA biogenesis, allowing validating identified miRNAs. Expression analysis showed that few miRNAs accounted for most miRNA expression. Previous results of our group (Cucher et al, 2015; Macchiaroli et al, 2015, Basika et al, 2016) showed that these set of miRNAs is conserved and also highly expressed in other cestodes including *Echinococcus spp*, suggesting important roles in cestode biology. We are currently predicting genes regulated by the highly expressed miRNAs in cestodes, some of which are absent or divergent in the mammal hosts. This is the first report of miRNAs in *T.crassiceps* and *T. solium*. Highly expressed parasite miRNAs absent or divergent in the hosts were indentified and could be candidates for drug and diagnosis targeting.

RUBÉN CHERNY AWARD

HEME OXYGENASE -1 (HO-1) IN THE FOREFRONT OF A MULTI-MOLECULAR NETWORK THAT GOVERNS CELL-CELL CONTACTS AND FILOPODIA-INDUCED ZIPPERING IN PROSTATE CANCER

ALEJANDRA PÁEZ (1), CARLA PALLAVICINI (2), FEDERICO SCHUSTER (1), PIA VALACCO (1), JIMENA GIUDICE (3),EMILIANO ORTIZ (1),NICOLAS ANSELMINO (1),ESTEFANIA LABANCA (4),MARIA BINAGHI (1),MARCELO SALIERNO (1), MARCELO MARTÍ (1), JAVIER COTIGNOLA (1), ANNA WOLOSZYNSKA-READ (5), LUCIANA BRUNO (2), VALERIA LEVI (1), NORA NAVONE (4), ELBA VÁZQUEZ (1), GERALDINE GUERON (1).

(1) Department of Biological Chemistry / IQUIBICEN-CONICET, FCEN-UBA, Buenos Aires, Argentina (2) Department of Physics, CONICET, FCEN-UBA, Buenos Aires, Argentina (3) Department of Cell Biology and Physiology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States (4) Department of Genitourinary Medical Oncology, The University of Texas, M.D. Anderson Cancer Center, Houston, TX, United States (5) Pharmacology and Therapeutics Department, Roswell Park Cancer Institute, Buffalo, NY, United States.

Prostate Cancer (PCa) cells display abnormal expression of cytoskeletal proteins resulting in an augmented capacity to resist chemotherapy and colonize distant organs. We have previously shown that heme-oxygenase 1 (HO-1) is implicated in cell morphology regulation in PCa. Here, through a multi “omics” approach we define the HO-1 interactome in PCa, identifying HO-1 molecular partners associated with the integrity of the cellular cytoskeleton.

The bioinformatics screening for these cytoskeletal-related partners reveal that they are highly misregulated in prostate adenocarcinoma compared to normal prostate tissue. Under HO-1 induction, PCa cells present reduced frequency in migration events, trajectory and cell velocity and, a significant higher proportion of filopodia-like protrusions among neighboring cells. Moreover forced-expression of HO-1 was also capable of altering cell protrusions in transwell co-culture systems of PCa cells with MC3T3 cells (pre-osteoblastic cell line). Accordingly, these effects were reversed under siHO. Transcriptomics profiling evidenced significant modulation of key markers related to cell adhesion and cell-cell communication under HO-1 induction. The integration from our omics-based research provides a four molecular pathway foundation (ANXA2/HMGA1/POU3F1; NFRSF13/GSN; TMOD3/RAI14/VWF; PLAT/PLAU) behind HO-1 regulation of tumor cytoskeletal cell compartments. The complementary proteomics and transcriptomics approaches presented here promise to move us closer to unravel the molecular framework underpinning HO-1 involvement in the modulation of cytoskeleton pathways, pushing towards a less aggressive phenotype, showcasing its relevance as a key homeostatic factor against the aggressive disease.

MULTILAYERED VALIDATION OF ABCC4/MRP4 AS A THERAPEUTIC TARGET FOR PANCREATIC CANCER

CAROZZO ALEJANDRO (1), GÓMEZ NATALIA (1), MARÍA MAY (2), MARTÍN ABBA (3), AGUSTÍN YANEFF (1), JULIÁN ITURBE (4), NORA MOHR (5), FEDERICO MONCZOR (1), NATALIA FERNANDEZ (1), SHAYO CARINA (2), CARLOS DAVIO (1)

(1) Instituto de Investigaciones Farmacológicas (ININFA-UBA-CONICET), Facultad de Farmacia y Bioquímica, UBA, Buenos Aires, Argentina. (2) Instituto de Biología y Medicina Experimental (IBYME-CONICET), Buenos Aires, Argentina. (3) Centro de Investigaciones Inmunológicas Básicas y Aplicadas, Facultad de Ciencias Médicas, Universidad Nacional de La Plata. (4) Grupo Oncológico Cooperativo del Sur (GOCS), Unidad Oncológica de Neuquén, Neuquén, Argentina. (5) Sanatorio Boratti & Universidad Nacional de Misiones (UNAM), Misiones, Argentina.

Pancreatic ductal adenocarcinoma (PDAC) is one of the most severe types of cancer, and because of its early development of resistance to standard therapeutic agents, and its late diagnosis, it results imperative to identify and validate new and key therapeutic targets. A lot of evidence implicates disturbances in cAMP cascade with PDAC, suggesting the oncogenic potential of this signaling pathway in this setting. In addition to the known classical mechanisms of regulation of cAMP, it was recently described its extrusion to the extracellular compartment mediated by MRP4. The aim of the present work was to validate MRP4 as a therapeutic target and characterize the role of cAMP extrusion in PDAC progression. The analysis of MRP4 expression profiles in human PDAC samples indicated higher levels of expression in tumor cells (normal tissue vs primary tumor; $p<0,01$). We also determined an inverse relationship between MRP4 expression and the probability of survival of patients, establishing a clear association between the staining intensity of MRP4 and Ki67. This suggests the existence of a subset of cells within the tumor that are actively proliferating and express higher levels of MRP4. In vitro assays in PDAC cell lines (PANC-1, BxPC3 and HPAF-II) demonstrated a positive correlation between MRP4 expression, cell indifferentiation and cAMP extrusion. Pharmacological inhibition of MRP4, as well as its specific knockdown by shRNA, led to a significant decrease of cell migration and proliferation ($p<0,01$), the latter by regulating cell cycle progression in G1/S. Both effects were reversed by inhibition of the cAMP-dependant Epac/Rap1 cascade or by adding cAMP acting through a still unknown receptor. Furthermore, silencing MRP4 strongly reduced tumor growth and incidence in nude and NSG animal models. Altogether, these results validate MRP4 as a new therapeutic target for PDAC and demonstrate the oncogenic potential of cAMPextrusion.

ARE HIPPOCAMPAL NMDAR SUBUNITS RAISE AFTER LEARNING TASKS INVOLVED IN MEMORY TRACE?

MAGALI CECILIA CERCATO (1), EDGAR KORNISIUK (1), CECILIA ALEJANDRA VÁZQUEZ (1), NATALIA COLETTIS (1), MARINA SNITCOFSKY (1), DIANA ALICIA JERUSALINSKY (1,3), M. VERONICA BAEZ (1,2).

(1) Laboratorio de Neuroplasticidad y Neurotoxinas, Instituto de Biología Celular y Neurociencia, CONICET, UBA. Paraguay 2155 3th floor, CABA, Argentina. (2) Universidad de Buenos Aires. Facultad de Medicina. Departamento de Histología, 1UA de Biología Celular, Histología, Embriología y Genética. Buenos Aires, Argentina. (3) Universidad de Buenos Aires. CBC. Buenos Aires, Argentina.

NMDA receptors (NMDAR) play a critical role in synaptic plasticity, memory encoding and storage. These receptors are heterotetramers composed by two obligatory GluN1 subunits and two regulatory subunits: GluN2 (A-D) or GluN3 (A-B), being GluN2A and GluN2B the major regulatory subunits in central areas related to cognitive functions. It was already shown that there is an increase on hippocampal GluN1 and GluN2A 70' after 5' exploration of a new environment (open field, OF) leading to habituation of 1,2 and 3 month old Wistar rats. We hypothesized that this NMDAR subunits increase could be related to memory tracing; hence, we investigated if those changes would take place following other learning tasks like an object recognition task (OR). Along 3 consecutive days rats were left to explore an OF for 10', to habituate to it. In the 4th day, rats were exposed to 2 identical novel objects (A-A') for 5' in that familiar OF (training session: Tr); there was no significant difference in exploration time spent at each object. In the 5th day, each rat was left into the arena for 5' with either a familiar and a novel object (A-B), or with two familiar objects (A-A') (test session: Te) and time spent at exploring each object was recorded. Rats spent significantly longer time exploring the novel object than the familiar one. Immediately or 70' after Tr or Te rats were euthanized and hippocampal extracts were analyzed by western blot. There was a significant increase only in hippocampal GluN1 and GluN2A only 70' post-Tr though not after Te, nor at the hippocampus neither at CPF. These results suggest that changes in hippocampal NMDAR subunits could be related to early consolidation of new spatial memories, being involved in memory trace.

DNA REPLICATION IS REQUIRED FOR THE TRANSCRIPTIONAL SWITCH DURING MOUSE EMBRYONIC STEM CELL DIFFERENTIATION

ARIEL WAISMAN (1,2), CAMILA VÁZQUEZ ECHEGARAY (1,2), CLAUDIA SOLARI (1,2), SOLEDAD COSENTINO (1,2), MARÍA VICTORIA COSENTINO (1,2), MARCOS FRANCIA (1,2), LINO BARAÑAO (1,2), SANTIAGO MIRIUKA (3), ALI BRIVANLOU (4), ALEJANDRA GUBERMAN (1,2).

(1) Laboratorio de Regulación Génica en Células Madre, Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina. (2) Instituto de Química Biológica (IQUIBICEN), UBA-CONICET, Buenos Aires, Argentina. (3) Laboratorio de Investigación Aplicada a las Neurociencias (LIAN), Fundación para la Lucha contra las Enfermedades Neurológicas de la Infancia (FLENI), Buenos Aires, Argentina. (4) Laboratory of Stem Cell Biology & Molecular Embryology, Rockefeller University, New York City, United States.

A central question in developmental biology is how cells adopt different fates during differentiation. Mouse embryonic stem cells (mESCs) provide a good in vitro model to study this, since their differentiation recapitulates early embryonic development. Here, we aimed to gain insight on how transcriptional programs are switched during differentiation, with the hypothesis that the epigenetic transformation underlying gene expression changes is coupled to processes that normally reorganize the structure of chromatin, such as DNA replication. We have previously shown that inhibition of DNA replication when synchronized cultures of mESCs are set to differentiate to epiblast-like cells (EpiLCs) severely abrogates the transcriptional switch (TS) associated with this cell transition. However, inhibition of DNA synthesis is known to activate the DNA damage response (DDR), raising the possibility that failure to differentiate was connected to this process and not to replication itself. In this work, we evaluated the role of DDR in the TS repression upon DNA replication inhibition. We show that inhibition of DNA synthesis with mechanistically unrelated drugs activates DDR, as judged by Chk1 phosphorylation, p53 stabilization and upregulation of the p53 transcriptional target Mdm2. To comprehensively dissect the role of DDR, we used the CRISPR/Cas9 system to generate a mESC knockout line for p53 (p53 KO). After validation of several clonal lines by DNA sequencing and Western blotting, we studied the effect of replication inhibition in synchronized cultures of p53 KO cells differentiating to EpiLCs. Although we observed a partial rescue in the TS to EpiLCs, KO cells never reached the wild type control levels. We further inhibited DDR upstream of p53, targeting ATR and Chk1 proteins, and observed that TS was still inhibited even in the absence of an active DDR. Our results indicate that DNA replication is a critical process in the TS that takes place during cell differentiation.

HOMING AND THERAPEUTIC POTENTIAL OF MESENCHYMAL STROMAL CELLS AS VEHICLES OF ANTIFIBROTIC GENES IN ADVANCED LIVER FIBROSIS: KEY ROLE OF HEPATIC MACROPHAGES.

ESTEBAN JUAN FIORE (1), BAYO JUAN (1), MARIANA MALVICINI (1), ESTANISLAO PEIXOTO (1), CATALINA ATORRASAGASTI (1), ALEJANDRINA REAL (1), MARCELO RODRIGUEZ (1), SOFÍA GOMEZ BUSTILLO (1), MARIANA GARCÍA (1), JORGE AQUINO (2), GUILLERMO MAZZOLINI (1).

1 Gene Therapy Laboratory, Instituto de Investigaciones en Medicina Traslacional (IIMT) CONICET-Universidad Austral, Pilar, Buenos Aires, Argentina. 2 Developmental Biology & Regenerative Medicine Laboratory, Instituto de Investigaciones en Medicina Traslacional (IIMT) CONICET-Universidad Austral, Pilar, Buenos Aires, Argentina. Área temática:Medicina Regenerativa y Terapia Celular

Background: Hepatic macrophages (hMø) have a pivotal role in liver fibrogenesis. Mesenchymal stromal cells (MSCs) are actively recruited to injury sites, show immunomodulatory properties and can be a powerful tool as therapeutic gene carriers. We previously showed anti-fibrotic effects of in vivo application of MSCs engineered to exogenously express insulin growth factor like-I (IGFI MSCs). We aimed to characterize the main cytokines produced by the fibrotic liver involved in MSCs recruitment. We also analyzed the influence exerted by MSCs on hMø and if it could drive liver fibrosis resolution. Metodology: Experimental liver fibrosis was induced in BALB/c mice by 8 weeks administration of thioacetamide. For in vivo tracking of administrated MSC we used Xenogen InVivo Imaging System. Depletion of hMø was performed using clodronate. Results: MSCs in vivo and in vitro migration was higher to cirrhotic livers in comparison with healthy livers. Also, MSCs displayed a high migration to CM derived from liver of cirrhotic patient or cirrhotic mice or a hepatic stellate cell line (LX2). Analysis of cytokines expression by protein array of CM derived from patient and LX2 cells showed high levels of GRO, MCP-1 and IL-8. Incubation of MSCs with antibody against IL-8/GRO receptors resulted in a 50% reduction of their migration capacity toward LX2 CM. hMø isolated from IGFI-MSCs treated fibrotic livers showed reduced expression levels of pro-inflammatory and pro-fibrogenic genes and an up-regulation in pro-regenerative genes vs. control conditions. Similarly, hMø from cirrhotic patients showed a similar shift after incubation with CM from IGFI-MSCs. Factors secreted by MSCs preconditioned hMø reduced the activation status of hepatic stellate cells. Finally, hMø depletion abrogated the therapeutic effect and the pro-regenerative stimuli of IGF1 MSC therapy. Conclusions: Our data provide new early mechanisms which are required for MSCs homing and IGFI-MSCs liver fibrosis amelioration.

YOUNG INVESTIGATOR AWARD - FUNDACIÓN HONORIO BIGAND

NOVEL ROLES OF THE β-GALACTOSIDE-BINDING PROTEIN GALECTIN-1 IN HEPATOCELLULAR CARCINOMA DISSEMINATION

MARÍA FERNANDA TRONCOSO

Tumor Glycobiology Laboratory Institute of Biological Chemistry and Biophysics "Dr. A. Paladini" (IQUIFIB, UBA-CONICET) Department of Biological Chemistry, School of Pharmacy and Biochemistry University of Buenos Aires

Hepatocellular carcinoma (HCC) is the third cause of cancer-related deaths annually.

Although HCC treatment has improved during the past decade, its incidence still matches mortality. As metastasis is the most common cause of death among patients with this disease, it is important to explore the mechanisms underlying the spread of HCC cells for the development of new therapeutic agents. Galectin-1 (Gal-1) belongs to a family of lactose-binding lectins characterized by their affinity for β-galactoside moieties. Gal-1 is a multifunctional protein involved in different aspects of tumorigenesis. In human HCC tissues Gal-1 is up-regulated, and this overexpression correlates with HCC cell migration, tumor invasiveness, metastasis, and shortened patient survival. However, the role of Gal-1 in the molecular mechanisms leading to HCC dissemination remained uncertain. Further results obtained in our laboratory provided evidences of the involvement of Gal-1 in HCC cell adhesion, polarization, and epithelial-mesenchymal transition. Moreover, our findings revealed that Gal-1 overexpression, partly induced by transforming growth factor β1 (TGF-β1), promotes HCC cell proliferation, resistance to TGF-β1-induced growth inhibition and glycan-dependent adhesion to liver sinusoidal endothelial cells. Therefore, the novel contribution of Gal-1 to tumor hepatocyte dissemination highlights this glycan-binding protein as an interesting therapeutic target to restrain HCC progression.

RETINOID X RECEPTORS ON SURVIVAL AND MODULATION OF INFLAMMATORY RESPONSE IN A MOUSE MODEL OF RETINITIS PIGMENTOSA

OLGA LORENA GERMAN

Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB), CONICET, Argentina

Retina neurodegenerative diseases, which have no effective treatments, share as a final common step the death of photoreceptors (PhR). Degeneration or altered functionality of retinal pigmented epithelium (RPE) cells may also be involved. Inflammation has a role in these pathologies as well, involving cell types having immunomodulatory capacity such as Müller glial cells and RPE cells. Therapeutic strategies aim to reducing neuronal death or decreasing the effect of inflammation in the initiation and/or progression of these diseases. Retinoid X receptors (RXR) have a unique role in modulation and integration of multiple cell functions. Their agonists have shown beneficial clinical effects in animal models of chronic inflammatory diseases. Since little is known on the roles of RXR in the retina, we investigated whether these receptors might prevent PhR death and control inflammation. Using in vitro models of retinal degeneration induced by oxidative damage, we demonstrated that RXR activation promotes PhR survival and protect RPE cells from apoptosis. Therefore, we turned to a mouse model of Retinitis Pigmentosa, the rd mouse, to analyze the roles of these receptors using primary neuro-glial culture and performing in vivo experiments. We investigate whether the pattern expression of RXR is altered in association with the PhR degeneration, and whether different RXR agonists could modify this pattern of expression and promote PhR survival and an anti-inflammatory response in retina cells with immunomodulatory capacity. To have a global view of the impact of RXR activation in modulating the immune response in these conditions, we also would like to understand how these compounds affect the antiviral drug-response in infected cells. To this purpose we use RPE cells infected with Herpes Simplex-1 virus. Moreover, we are interested in identifying the specific RXR isoform and dimers implicated in the above mentioned effects, so that a pharmaceutical intervention could be developed successfully.

CHEMOTACTIC PATHWAYS INVOLVED IN MESENCHYMAL STROMAL CELL RECRUITMENT TOWARDS HEPATOCELLULAR CARCINOMA AND LIVER FIBROSIS. ROL FOR AUTOCRINE MOTILITY FACTOR, IL-8, GRO AND MCP-1.

MARIANA G. GARCIA

Laboratorio de Terapia Génica, Instituto de Investigaciones en Medicina Traslacional, CONICET-Universidad Austral, Pilar, Buenos Aires, Argentina

Hepatocellular carcinoma (HCC) is the 2nd cause of cancer-related death worldwide and the majority of patients are diagnosed at advanced stages. New therapies are needed and those focused on the delivery therapeutic genes by mesenchymal stromal cells (MSC) are gaining interest. Our aim was to investigate the chemotactic pathways involved in MSC recruitment towards HCC. We have demonstrated that soluble factors present in the conditioned media (CM) derived from HCC tumors induced in vitro and in vivo MSC chemotaxis towards the tumor. Autocrine Motility Factor (AMF), a cytokine released by HCC cells, has been previously described to stimulate tumor cell motility. In vitro chemotactic assays demonstrated that MSCs migrated to recombinant AMF (rAMF) and AMF blockage with a specific antibody reduced their migration toward HCC CM. Moreover, MSCs-primed with rAMF showed increased in vitro and in vivo migration towards HCC. Recently, we have demonstrated that HCC CM shared the presence of GRO, MCP-1 and IL-8, being the latter with the highest concentration. Blocking and knockdown experiments of MCP-1, IL-8, CXCR1 and CXCR2 reduced >20 % MSC migration. Simultaneous blockage of AMF, CXCR1 and CXCR2 resulted in >60% inhibition of MSC migration to HCC. Stimulation of MSCs with HCC CM (sMSC) resulted in increased in vitro migration and differential expression of ~500 genes, being 46 genes related with cell migration and invasion. Factors produced by sMSC were able to increase fibroblasts, mononuclear and endothelial cells chemotaxis in comparison to factors produced by unstimulated MSCs. We also demonstrated that injection of MSCs, primed with AMF or with HCC CM, in tumor bearing- mice did not modify tumor growth. We conclude that AMF, IL-8, GRO and MCP-1 play a critical role in MSC recruitment to HCC, and stimulation of MSC with rAMF or HCC CM increased MSC homing to HCC, thus becoming a promising strategy to improve their therapeutic efficacy.

IDENTIFICATION OF BIOMARKERS FOR CANCER DEVELOPMENT AND PROGRESSION.

JAVIER COTIGNOLA

IQUIBICEN – CONICET / Departamento de Química Biológica – FCEN, UBA, CABA, Argentina

All types of cancer are caused by the existence of genetic abnormalities within the cancerous cells. These abnormalities consist of the cumulative acquisition of genetic changes that make cells to bypass the cell cycle checkpoints that regulate the normal cell proliferation and physiology. These DNA anomalies are usually translated into mutations or altered expression of RNAs and proteins. In Oncology, it is extremely important that tumors are diagnosed during the early stages and to administer the most appropriate treatment because every time treatments fail, tumors become more aggressive and resistant to therapy, jeopardizing patients´ life. Up to date, there are only few validated biomarkers that are strong predictors of tumor development and progression; for example, the hormonal receptors in breast cancers. However, the availability of development/ progression predictors is non-existing for most types of cancer, and there is an urgent need to discover such biomarkers. The main aim of the projects is to identify genetic abnormalities (mutations, polymorphisms, gene expression patterns) that allow a better characterization of tumors in order to improve the diagnosis and to develop tailored treatments. We previously reported that polymorphisms in GSTP1, GSTT1 and GSTM1 were associated with the risk of disease relapse and shorter relapse-free survival in prostate cancer and childhood acute lymphoblastic leukemia. Currently, we are seeking mutations and gene expression profiles that help to stratify patients into: a) good/bad responders to therapy, b) low/high risk of disease progression, c) low/high risk of developing severe acute therapy-related toxicity. The identification and inclusion of these molecular biomarkers into the clinic will help to improve the diagnosis and prognosis of prostate cancer and acute lymphoblastic leukemia which, in turn, will increase survival and quality of life of the patients.

OCT4 EXPRESSION MEDIATES PARTIAL CARDIOMYOCYTE TRANS-DIFFERENTIATION OF MESENCHYMAL STEM CELLS: POTENTIAL ROLE IN THE CROSS-TALK WITH THE MICROENVIRONMENT AND REGENERATION CAPACITY

GUSTAVO YANNARELLI

Medicina Regenerativa: Cardiología (Instituto de Medicina Traslacional, Trasplante y Bioingeniería (IMeTTyB), Universidad Favaloro, CONICET, CABA, Argentina

Cell therapy using mesenchymal stem cells (MSCs) has shown their capacity to facilitate both myocardial repair and angiogenesis in models of cardiac injury. Mechanisms mediating tissue regeneration remain unclear and are likely multifactorial. Current opinion favors invoking paracrine actions of MSCs to explain hemodynamic improvement. While trans-differentiation of donor cells into cardiomyocytes is no longer considered a likely possibility, it is unclear whether or not partial cardiomyocyte differentiation enhances the release of soluble growth factors and cytokines in mediating tissue regeneration. Consequently, MSCs interaction with the cardiac microenvironment may be important to engender cardiac repair. OCT4 is a well-known transcription factor that regulates the self-renewal and pluripotency of embryonic stem cells. We recently showed that umbilical cord-derived MSCs have a higher OCT4 expression and an enhanced differentiation potential compared with bone marrow derived-MSCs. Moreover, we found a link between partial cardiomyocyte reprogramming and paracrine effects, as the improvement in cardiac function was significantly higher only after intramyocardial injection of UC-MSCs vs BM-MSCs in a mice model of acute myocardial infarction. Whether OCT4 is involved in this process or mediates MSC multipotency, however, has not been demonstrated. Thus, we investigated the role of the pluripotency factor OCT4 in partial cardiomyocyte reprogramming of MSCs by using our established co-culture system with rat embryonic cardiomyocytes. We found that MSCs must first gain OCT4 (de-diferentiate) before being able to trans-differentiate into cardiomyocytes, a mechanism that resembles the reprogramming process. Moreover, the specific silencing of OCT4 negated not only partial cardiomyocyte reprogramming but also MSCs differentiation into other lineages demonstrating that this factor is essential for the multipotent capacity of MSCs. Our findings suggest new mechanisms that may mediate MSC plasticity and their crosstalk with the microenvironment.

SAI AWARD

DR. LEONARDO SATZ AWARD

DEVELOPMENT OF INNATE T CELLS IN THE THYMUS UNDER INFECTIOUS / INFLAMMATORY SYSTEMIC CONDITIONS

NATALIA BÁEZ, CONSTANZA SAVID FRONTERA, FABIO CERBÁN, MARÍA CECILIA RODRÍGUEZ GALÁN, L

[1]Dpto de Bioquímica Clínica, Fac. de Cs. Químicas, UNC. CIBICI-CONICET, Córdoba, Argentina

Our previous work demonstrated that during the acute stage of certain infections (*Trypanosoma cruzi* or *Candida albicans*) with a strong Th1 component, the number of $CD8^+CD44^{hi}$ T cells in the thymus increased. These cells are named "innate T cells" and belong to a different lineage from conventional SP CD8 thymocytes that give rise in the thymus. They express the transcription factor EOMES, produce high levels of IFNg and differentiate in the presence of IL4 and IL15. This effect is mediated by the inflammatory process since we obtained similar data when we induced systemic expression of IL12 and IL18 by hydrodynamic injection of their cDNAs. The aim of our work is to determinate the origin and function of these cells. Our flow cytometry data demonstrated that these cells express the killing receptor NKG2D and have high cytotoxic activity measured by CD107a expression assay ($p<0,05$). Moreover, when mice are adoptively transferred with thymocytes from OT-I *T. cruzi*-infected or IL12 + IL18-treated mice previous to *T. cruzi* infection, a protective effect can be observed since the overall survival is increased and parasitemia is decreased compared to non-transferred control mice ($p<0,05$). When we injected $CD45.1^+$ control thymocytes directly into the thymuses of *T. cruzi*-infected $CD45.2^+$ mice, we observed that $CD45.1^+$ cells up-regulated CD44 and EOMES expression compared to $CD45.1^+$ cells injected in non-infected $CD45.2^+$ recipient mice ($p<0,05$). Moreover, when we co-cultured $CD45.1^+$ control thymocytes whit $CD45.2^+$ *T. cruzi*-infected thymocytes we observed up-regulation of CD44 and EOMES in a IL4 and IL15-dependent manner ($p<0,05$). Our results indicate that under systemic infectious/inflammatory processes, innate $CD8^+$ T cells are generated in the thymus by local IL4 and IL15 production. The presence of non-conventional $CD8^+$ T cells suggests a deviation in the normal thymic ontogeny that may have implication in the output and the repertoire of T cells in secondary immune organs.

GALECTIN-1 (GAL1) AND COMPLEX N-GLYCAN COORDINATELY REGULATE SPONTANEOUS DEVELOPMENT OF AUTOIMMUNITY

VERÓNICA MARTÍNEZ ALLO[1], VANESA HAUK[2], NICOLÁS PINTO[1], ROSA MORALES[1], JUAN CARLOS STUPIRSKI[1], SABRINA GATTO[1], ANGEL DELADOEY[3], PRISCILA MARCAIDA[4], VIRGINIA DURIGAN[4], ANASTASIA SECCO[4], MARTA MAMANI[4], ALICIA DOS SANTOS[3], ANTONIO CATALÁN PELLET[4], DIEGO CROCI[5], CLAUDIA PÉREZ LEIROS[2], GABRIEL RABINOVICH[1, 2], MARTA TOSCANO[1].

[1]Instituto de Biología y Medicina Experimental, CONICET. [2]Depto de Qca. Biológica, FCEyN. UBA. [3]Servicio de Patología, Hospital Rivadavia, [4]Servicio de Reumatología, Hospital Rivadavia. [5]Instituto de Histologia y Embriología de Mendoza, CONICET, Argentina

Galectin-1 (Gal1), an endogenous glycan-binding protein, plays a critical role in immune cell homeostasis. Here, we studied the relevance of endogenous Gal1 in the development of spontaneous autoimmunity. We found that aged Gal1-deficient mice (*Lgals1*$^{-/-}$; ~1-2 y) had increased titers of autoantibodies in circulation and more pronounced signs of inflammation and tissue modification in salivary glands (SG) compared to age-matched WT mice. Moreover, *Lgals1*$^{-/-}$ SG showed increased number of infiltrating cells, with a higher frequency of $CD8^+$ T cells. As Gal1 binds to N-acetyllactosamine residues in complex N-glycans, we analyzed signs of inflammation in β1,6N-acetylglucosaminyltransferase V-null (*Mgat5*$^{-/-}$) mice. Similar to *Lgals1*$^{-/-}$ mice, aged *Mgat5*$^{-/-}$ SG showed increased number of ducts/mm^2 and infiltrating cells, with higher proportion of $CD8^+$, $CD4^+$ and $B220^+$ cells, than WT SG. Seeking for possible mechanisms underlyig this effect, we studied migration and activation factors in $CD8^+$ T cells. We found increased expression of CXCL9 and CXCL10 in *Lgals1*$^{-/-}$ SG and higher percentage of $CD8^+CXCR3^+$ and $CD8^+PD1^+$cells in draining lymph nodes (DLN) and SG when compared to WT mice ($P<0.05$). Moreover, *Lgals1*$^{-/-}$ DLN had lower frequency of $CD11c^+$ dendritic cells (DCs), and these cells exhibited altered functionality. When co-cultured with $CD4^+$ cells, *Lgals1*$^{-/-}$ DCs induced higher IFN-g and lower IL-10 production ($P<0.05$), ulti-

mately leading to enhanced CD8+ T cell proliferation *in vitro,* compared to WT DCs. Furthermore, *Lgals1*$^{-/-}$ SG showed lower expression of PD-L1, supporting heightened CD8+ T-cell responses. Finally, we found downregulated Gal1 expression in NOD mice developing spontaneous sialadenitis and administration of rGal1 attenuated SG infiltration (P<0.05). Thus, Gal1 and complex *N*-glycans play critical roles in the control of salivary glands homeostasis, modulation of CD8+T-cell responses and development of autoimmune disease.

ROLE OF PROTEIN S AND TYRO3 RECEPTOR TYROSINE KINASE IN THE PATHOGENESIS OF MULTIPLE SCLEROSIS AND THE TYPE-2 PROTECTIVE ENVIRONMENT.

JUAN MANUEL ORTIZ WILCZYÑSKI[1,2], CINTHIA MARIEL OLEXEN[1,2], ANDREA EMILSE ERRASTI[2], MIRTA SCHATTNER[1], CARLA V. ROTHLIN[3], JORGE CORREALE[4], ANTONIO E. CARRERA SILVA[1].

[1]Instituto de Medicina Experimental, ANM-CONICET. Buenos Aires, Argentina. [2]Instituto de Farmacología, Facultad de Medicina, Universidad de Buenos Aires. Buenos Aires, Argentina. [3]Departamento de Inmunobiología, Universidad de Yale, New Haven, Estados Unidos. [4]Departamento de Neurología, Instituto de Investigaciones Neurológicas Raúl Carrea, FLENI, Buenos Aires, Argentina.

Multiple sclerosis (MS) is a chronic inflammatory and autoimmune disorder causing central nervous system demyelination and axonal injury by infiltration of autoreactive Th1/Th17 cells. The inflammatory environment is the main driver of damage and loss of neurologic function. Interestingly, helminth-infected MS patients showed lower number of relapses, lesion activity and minimal changes in disability scores compared with uninfected individuals with MS. Parasite-driven protection was associated with a reduction of pro-inflammatory cytokines via SOCS3, induction of Tregs and IL-10. TYRO3, AXL and MERTK (TAM) tyrosine kinase receptors and its ligand Protein S (PROS1) are critical regulators of the immune response and we have recently demonstrated that Th2 environment enhances PROS1 and TYRO3 expression. We hypothesize that the engagement of PROS1-TYRO3 axis will be enhanced during helminth infection negatively regulating an associated inflammatory response Th1/Th17. We evaluated TAM receptors and PROS1 expression in peripheral blood monocytes, dendritic and T cells compartment of patients with diagnosed MS (N=18), helminth-infected patients (HP, N=8) and healthy controls (HC, N=13-20). We found a significant increased level of PROS1 and MERTK in blood CD4 T cells of MS patients compared to HC. However, after in-vitro TCR activation, CD4 T cells from MS induced lower levels of PROS1 (100.3 ± 5.6) vs HC (120.7± 2.6) or HP (137.8 ± 6.0) measured as mean fluorescence intensity. Furthermore, PROS1 levels in CD4 + /IFNg + and CD4 + /IL13 + was higher in HP > HC > MS. Interesting, CD1c dendritic cells showed higher TYRO3 expression in HP (10.4 ± 0.9) vs HC (6.5 ± 0.3) and MS (4.9 ± 0.3) measured as fold increase referred to isotype. Our results suggest that the enhanced PROS1/TYRO3 axis in HP could be favoring a more efficient engagement of this anti-inflammatory pathway and could contribute to explain the parasite-driven protection observed in helminth-infected MS.

PROTUMORAL PROPERTIES OF NEUTROPHIL EXTRACELLULAR TRAPS (NETS) IN CHRONIC LYMPHOCYTIC LEUKEMIA.

ENRIQUE PODAZA[1,2], DENISE RISNIK[1,2], FLORENCIA SABBIONE[1,2], ANA COLADO[1,2], ESTEBAN ELÍAS[1,2], MARIA BELEN ALMEJÚN[1,2], RAIMUNDO FERNANDO BEZARES[4], HORACIO FERNÁNDEZ GRECCO[3], MERCEDES BORGE[1,2], ANALIA TREVANI[1,2], MIRTA GIORDANO[1,2].

[1]Laboratorio de Inmunología Oncológica- Instituto de Medicina Experimental (IMEX)-CONICET-ANM. [2]Laboratorio de Inmunidad Innata -Instituto de Medicina Experimental (IMEX) -CONICET-ANM . [3]Sanatorio "Dr. Julio Mendez". [4]Hospital General de Agudos " Teodoro Alvarez"

We previously reported that PMN from CLL patients are prone to release extracellular traps (NETs) in response to PMA. In the present work we extended these findings using ionomycin (1µg/ml) or TNF-α (10 ng/ml) plus LPS (100 ng/ml) to induce NETosis. We found higher concentrations of DNA and increased elastase activity in supernatants from CLL-PMN compared to those from healthy donors (HD) PMN (n=6, p<0.05 for ionomycin, n=5, p<0.05 for TNF-α+LPS). These differences in NETs formation were corroborated by fluorescent microscopy using propidium iodide and anti-elastase Ab. Our previous findings showed that plasma from CLL patients prime neutrophils to form NETs. Given that levels of IL-8, a cytokine involved in NET induction, are higher in CLL plasma, we supplemented HD plasma with IL-8 (0.15 ng/ml, the average concentration in our CLL patient cohort) and preincubated

HD PMN before triggering NETosis. IL-8 supplemented HD plasma promoted a higher response to PMA (n=5, p<0.05). Moreover, CLL plasma induced the activation of HD neutrophils as assessed by cell size increase and upregulated the expression of the IL-8 receptor, CXCR2 (n=6, p<0.05) without modifying CXCR1 levels. Given that both, stimulating and deleterious effects of NETs have been reported depending on the experimental model, we determined if NETs could modify the survival of leukemic B cells from CLL patients. Using NETs prepared with ionomycin or TNF-α+LPS we found protection from spontaneous apoptosis (n=10, p<0.05) and upregulation of the activation markers CD80, CD86 and CD69 (n=10, p<0.05). Of note, NETs were unable to delay apopotosis of B cells purified from HD blood. Our study provides new insights into the immune dysregulation in CLL and suggests that the chronic inflammatory environment typical of CLL probably underlies this inappropriate neutrophil priming.

SAFE AWARDS

BEST PRESENTATION AWARD

FLAVONOIDS ISOLATED FROM DALEA ELEGANS INHIBIT MELANOGENESIS IN MOUSE B16 MELANOMA CELLS

MARÍA DANIELA SANTI, MARIANA PERALTA, JOSÉ LUIS CABRERA, MARÍA GABRIELA ORTEGA

Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, IMBIV-CONICET, Argentina

Tyrosinase inhibitor compounds have importance in the treatment of hyperpigmentation diseases and are used as whiteners agents in cosmetics. Several currently marketed whiteners have adverse effects; for example Kojic acid (KA) is genotoxic, hepatocarcinogenic and produces dermatitis. For this reason is important the researching for new inhibitors of tyrosinase. Previously we have reported an important inhibitory activity on mushroom tyrosinase by a prenylated flavanone (8PP) and a chalcone (Triangularin) isolated from roots and aerial parts of Dalea elegans Gillies ex Hook. & Arn. In order to investigate this condition in cell line, we evaluate the melanogenesis inhibition of these compounds on mouse B16 melanoma cells through the tyrosinase intracelular inhibition and the extracelular melanin inhibition by spectrophotometrically measuring of the adduct formation between 3-methyl-2-benzothiazolinone and dopaquinona. The cytotoxicity assay was performed by MTT methodology. The maximum non-cytotoxic concentration (MNCC) for 8PP, Triangularin and KA were of 10 µM, 100 µM and 5000 µM, respectively and according with these results we evaluated the melanogenesis inhibition. The results demonstrated that these compounds have the ability to penetrate the membrane of B16 cells and inhibit the tyrosinase intracelular at non-cytotoxic concentrations. Comparing the inhibition % of each compound with reference inhibitor KA, 8PP and Triangularin would be two hundred-fold and five-fold more active than KA, respectively. In addition, it has been observed that these compounds decreased extracellular melanin. The 50 % of inhibition for 8PP and Triangularin were 1 and 25 µM, respectively. For KA, the 50 % of inhibition was 2000 µM. So, 8PP and Triangularin would be two thousand and eighty-fold more active than KA, respectively. It would be needed PCR and Western blot studies to establish the mechanisms by which these compounds act on melanin biosynthesis.

DEVELOPMENT OF A DRUG DELIVERY SYSTEM (DDS) BASED ON POLYMERIC NANOPARTICLES: THE POSSIBILITY OF AN ORAL ADMINISTRATION ROUTE FOR INTERFERON ALPHA

CAMILA CÁNEPA[1], CAROLINA A. BERINI[1], MAGDALENA GHERARDI[1], MARIANELA LEWICKI[2], GABRIELA B. ACOSTA[3], ALEJANDRO SOSNIK[4], MIRNA M. BIGLIONE[1], JULIETA C. IMPERIALE[3],

(1) Institute of Biomedical Research on Retroviruses and AIDS (INBIRS), UBA-CONICET, Buenos Aires, Argentina (2) Instituto de Investigaciones en Microbiología y Parasitología Médica, CONICET, Buenos Aires, Argentina (3) Institute of Pharmacological Research (ININFA), National Scientific and Technical Research Council, School of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina (4) Laboratory of Pharmaceutical Nanomaterials Science, Technion, Haifa, Israel

Interferon alpha (IFNa) is a protein drug used to treat oncological diseases and viral infections. Owing to its sensitivity to enzymatic degradation and limited absorption in the gastrointestinal tract, pegilated IFNa is administered via parenteral route once weekly which is associated with pain, allergic reactions and poor patient compliance. To

overcome these problems, the design of a suitable drug delivery system (DDS) able to protect the drug in order to administer it orally would lead not only to greater acceptance and adherence to the treatment but also to a better quality of life for patients. In this context, we prepared IFNa2b loaded chitosan nanoparticles (IFN CS NPs) by ionotropic gelation method. Infrared spectra supported the formation of CS NPs. The amount of CS that formed NPs, colorimetrically determined, was 95.5%. Size, determined by dynamic light scattering (DLS), showed a bimodal distribution; the mean sizes were 381.7 ± 35.2 nm and 50.17± 6.96 for blank CS NPs, 353.0 ± 31.2 nm and 42.49 ± 23.75 for IFNa-loaded ones. The polidispersity index was 0.472 ± 0.030 and 0.407 ± 0.010, while the zeta potential (Z-Pot) 31.4 ± 4.6 mV and 31.8 ± 1.7 mV, respectively. The Z-Pot value suggests not only a net positive surface charge but also physical stability of the DDS as was confirmed at 4 and 25°C for 30 days by DLS results. The encapsulation efficiency was 99.5%. Transmission electron microscopy confirmed the size obtained by DLS results. The antiviral activity of encapsulated IFN determined in Vesicular Stomatitis Virus (VSV) infected MDBK cells, was comparable to commercial IFN. Preliminary pharmacokinetic studies in Balb/C mice showed absorption of IFNa2b after oral administration of IFN loaded CS NPs in opposition to different studies in which the drug was not detected in plasma following administration of free drug. These promising CS NPs show great potential for application in oral delivery of IFNa2b allowing an enhancement of patient compliance.

SELF-GELLING ELASTIN AND SILK-ELASTIN RECOMBINAMERS FOR TIMOLOL OPHTHALMIC ADMINISTRATION IN THE TRATAMIENT OF THE GLAUCOMA

Daniela Alejandra Quinteros[1] Paula Lucía Grisanzio[1] Alicia Fernandez-Colino[2] José María Bermudez[3], Daniel Alberto Allemandi[1] Santiago Daniel Palma[2] José Carlos Rodríguez-Cabello[1], Francisco Javier Arias[2],
1UNITEFA-CONICET, Faculty of Chemical Sciences, National Univ. of Cordoba, Argentina 2 BIOFORGE Research Group, University of Valladolid, CIBER-BBN, Spain, 3 Facultad de Ingeniería, Instituto de Investigaciones para la Industria Química (INIQUI-CONICET), Universidad Nacional de Salta, Argentina

The development of topical ophthalmic formulations for the treatment of eye diseases such as glaucoma, present a challenge, since most drugs are hardly absorbed, having bioavailabilities from 1-10%. An alternative to increase the residence time of formulations is the use of bioadhesive systems. Taking this into account, here we developed an elastin-like and a silk-elastin like recombinamers, named respectively as ELR and SELR, for their incorporation in an ophthalmic formulation against glaucoma. Our hypothesis is that due to the thermo-sensitive behavior of these materials, the formulation could be administered topically as drops, and once sensing the temperature of the eye, suffer a shift to a gel system, which could help to prolong the permanence of the formulation in the eye, and therefore, could enhance the therapeutic effect.The thermosensitive gels each system Timolol (T) was charged and the release of T and erosion of ELR and SELR were evaluated for 8 hs. In vivo studies were performed on New Zealand rabbits. Each formulation was placed into the conjuntival fornix and the intraocular pressure (IOP), Irritation and Adhesion were measure.Both recombinamers display clearly differences on the release kinetics. It was determined using the model Korsmeyer, The results indicated the percent release at 8 hours was 80.39% and 40.04% of T for ELR and SELR, respectively. The rate constant, incorporating structural and geometric characteristics of the system was higher for ELR indicating a faster rate of drug release. In both cases the release mechanism T responded to an anomalous diffusion. This behavior can be inferred that the same erosion/dissolution of the matrix predominates in drug release, which coincides with erosion studies indicate greater resistance and mechanical stability of the gels SELR. *In vivo* tests evidenced that formulations containing the recombinamers further decreased the IOP when compared with the control solution formulation. Furthermore, SELR-formulation was found to be more effective that its counterparts ELR-formulation, which agrees with the enhanced mechanical properties provided by the presence of the silk moieties. These results evidenced that the self-gelling ELR and SELR have great potential for its use as components of ophthalmic pharmaceutical formulations.

EVALUATION OF SCHEDULE-DEPENDENT EFFECTS OF CHEMOTHERAPY IN VITRO AND IN ONE IN VIVO RETINOBLASTOMA MODEL

URSULA ANDREA WINTER[1,5], GUILLEM PASCO-PASCUAL [2], AGUSTINA MENA [1,3], SOLEDAD NEGROTTO [1,3], MARIONA SUÑOL [4], GUILLERMO CHANTADA [1,5], ANGEL MONTERO CARCABOSO[2], PAULA SCHAIQUEVICH [1,5],

#(1) Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) #(2) Fundación Sant Joan de Deu, Barcelona, España #(3) Instituto de Medicina Experimental. Academia Nacional de Medicina #(4) Hospital Sant Joan de Deu, Barcelona, España #(5) Hospital de Pediatria Prof. Dr. JP Garrahan, CABA, Argentina

Current treatment of retinoblastoma involves using the maximum dose of chemotherapy that induces tumor control and is tolerated by patients. The effect of metronomic chemotherapy treatment has not been assessed for retinoblastoma and may aid to decrease the incidence of adverse events by using lower doses. Our aim was to evaluate the cytotoxic and antiangiogenic effect of chemotherapy used in the clinics using different in vitro and in vivo models. Two patient-derived retinoblastoma cell types (007 and 008) and two human vascular endothelial cell types (HUVEC and EPC) were exposed to increasing concentrations of melphalan or topotecan in a conventional (single dose) or metronomic (7-day exposure) treatment scheme. The concentration of chemotherapy causing a 50% decrease in cell proliferation (IC50) was determined by MTT. The effect of treatments on endothelial cells was assessed by the ability of tube formation using matrigel assay. We also evaluated the in vivo response to conventional and metronomic topotecan in a retinoblastoma xenograft model. We compared the vascular density of tumors after treatments using CD31.

Melphalan and topotecan were cytotoxic to both retinoblastoma and endothelial cells after the two treatments schemes. Whereas the IC50 for melphalan and topotecan significantly decreased after metronomic compared to conventional treatment in endothelial and 008 cells ($p<0.05$), no change in the sensitivity to both agents was evident for 007 ($p>0.05$). Metronomic topotecan or melphalan significantly inhibited in vitro tube formation in HUVEC and EPC compared to vehicle treated cells ($p<0.05$). In mice, continuous topotecan lead to significantly lower tumor volumes compared to conventional ($p<0.05$). CD31 expression was lower after metronomic compared to conventional treatment ($p<0.05$). We propose that metronomic chemotherapy may be a valid option for retinoblastoma treatment based on the observed cytotoxic and antiangiogenic effect.

INCREASED INFLAMMATORY CELL PROFILE TOGETHER WITH MERTK UP REGULATION AND T CELL-DERIVED PROTEIN S REDUCTION IN IBD PATIENTS

LICINA TESSONE[1], PAULA CHAVERO[2], SILVIA PERÉS WINGEYER[3], GABRIELA DE LARRAÑAGA[3], ALICIA SAMBUELLI[2], CARLA V. ROTHLIN[4], EUGENIO A. CARRERA SILVA[5], ANDREA E. ERRASTI[1],

1- Instituto de Farmacología, Facultad de Medicina, Universidad de Buenos Aires. Buenos Aires, Argentina. 2-Hospital de Gastroenterología "Dr. Carlos Bonorino Udaondo", Buenos Aires, Argentina. 3-Hospital de Infecciosas"F. J. Muñiz", Buenos Aires, Argentina. 4-Departamento de Inmunobiología, Universidad de Yale, New Haven, Estados Unidos. 5-Instituto de Medicina Experimental, ANM-CONICET. Buenos Aires, Argentina.

Background: Crohn's disease (CD) and Ulcerative Colitis (UC) are Inflammatory Bowel Diseases (IBD) characterized by chronic inflammation and tissue damage. Protein S (PROS1), an agonist of TYRO3, AXL and MERTK (TAM) receptors, is expressed upon T cell activation reducing dendritic cell activation. Genetic ablation of Axl and Mertk increases the inflammatory signature and loss of tissue repair macrophage phenotype in a mouse model of IBD. Purpose: Our goal was to characterize the immune compartments, TAM receptors and PROS1 expression in monocyte/macrophage and lymphocytes of IBD patients. Results: Blood mononuclear cells from 33 IBD patients (16 CD/17 UC) classified as active disease (AD) or in remission by CDAI and Mayo scores, and 35 healthy controls were analyzed by FACS. Increased % of $CD14^{high}CD11b^{high}CD11c^{+}$ monocytes were observed in active IBD (21.1 ± 2.2 N=20) vs remission (9.6 ± 1.0 N=13) and controls (8.7 ± 0.6 N=19) and positively correlated with the CDAI ($r=0.67$ $p<0.01$) or Mayo scores ($r=0.82$ $p<0.001$). Interestingly, we observed an increased % of $CD11b^{low}CD64^{+}CD206^{-}$ cells in active IBD (48.2 ± 9.7 N=9) vs remission (13.3 ± 4.2 N=12) and controls (7.2 ± 1.2 N=14). Although AXL is highly expressed in circulating monocytes, no differences between IBD vs controls were observed. However, we do detect a significant up regulation of MERTK comparing IBD vs controls ($p<0.01$). TYRO3 was differentially expressed in monocytes of only CD vs controls. In-vitro TCR stimulation with a-CD3/CD28 leads to a significant lower level of PROS1 in CD4 T cells of active CD. Conclusions: Our results show a clear inflammatory cell profile with increased level of circulating monocytes and $CD64^{+}CD206^{-}$ cells in active IBD. Interestingly, MERTK that is associated with tolerogenic responses was consistently up regulated in monocytes of active IBD. Moreover, CD4 T cells showed reduced levels of PROS1 after activation reflecting a more inflammatory memory profile in IBD patients.

SUBLINGUAL ADMINISTRATION OF TACROLIMUS IN PEDIATRIC LIVER TRANSPLANT PATIENTS

NATALIA RIVA[1], PAULA SCHAIQUEVICH[2], MARIA EUGENIA GALVAN[3], PAULO CACERES-GUIDO[1], MARCELO DIP[4], MARIA DANIELA BORGNIA[5], DIANA VIALE[5], NIEVES LICCIARDONE [6], OSCAR IMVENTARZA[4], DANIEL BUAMSCHA[3].

(1) Unidad de Farmacocinética Clínica, Hospital de Pediatría Prof. Dr. JP Garrahan (2) Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) (3) Unidad de Cuidados Intensivos, Hospital de Pediatría Prof. Dr. JP Garrahan (4) Trasplante Hepático, Hospital de Pediatría Prof. Dr. JP Garrahan (5) Virología, Hospital de Pediatría Prof. Dr. JP Garrahan (6) Laboratorio, Hospital de Pediatría Prof. Dr. JP Garrahan, CABA, Argentina

Tacrolimus (FK) has led the market during the last ten years, as the calcineurin inhibitor of choice for prevention and treatment of rejection in solid organ transplantation. FK products include capsules and intravenous formulations. However, young children have difficulties in swallowing the capsules immediately post transplantation. Moreover, intravenous FK is very toxic. Therefore sublingual (SL) administration is an alternative to achieve therapeutic levels and avoid early graft rejection.

Objective: to study safety and efficacy of SL FK administration in pediatric patients who could not swallow FK capsules due to age, mechanical ventilation and/or sedoanalgesia, during their stay in the Intensive Care Unit post-transplantation.

Methods: pediatric patients with biliary atresia transplanted in 2014-2015 were studied. Trough FK levels, adverse events, clinical parameters and drug-drug interactions were recorded. Wilcoxon matched pairs test was used. Efficacy was evaluated by the occurrence of acute rejection (AR).

Results: 22 patients were included, with a median (range) follow-up and age of 2 days(6-68) and 0.9 years (0.6-6.3), respectively. Three AR and 3 adverse events (nephrotoxicity, hypomagnesemia and neurotoxicity) occurred during the study period. The median (range) daily dose and trough FK levels was 0.11mg/kg(0.02-0.31) and 6.4ng/ml(2.0-23.2), respectively. During concomitant administration of clarithromycin, a significant increase was observed in dose normalized FK trough levels ($p<0.05$).

Conclusion: Safety and efficacy parameters of SL-FK administration were studied in pediatric liver transplant patients who had difficulties in swallowing the capsules, under mechanical ventilation and/or sedoanalgesia. According to FK blood levels achieved, the SL route was effective. FK-clarithromycin interaction may affect safety of SL-FK administration. This study emphasized the role of therapeutic monitoring to maintain FK blood levels within the therapeutic range.

CD207+/CD1A+ CIRCULATING CELLS ARE PRESENT IN PATIENTS WITH ACTIV LANGERHANS CELL HISTIOCYTOSIS

WANDA NOWAK[1], LICINA TESSONE[1], IVANA G ESTECHO[1], ARNALDO A ARMESTO[1], GRACIELA ELENA[2], EUGENIO A CARRERA SILVA[3], DIEGO A ROSSO[1,2], ANDREA E ERRASTI[1],

1- Instituto de Farmacología, Facultad de Medicina, Universidad de Buenos Aires. Buenos Aires, Argentina. 2- Hospital "Pedro de Elizalde", Buenos Aires, Argentina. 3- Instituto de Medicina Experimental, ANM-CONICET. Buenos Aires, Argentina.

Background: Langerhans Cells Histiocytosis (LCH) is a disorder characterized by an abnormal accumulation of $CD207^{+}CD1a^{+}$ myeloid cells in almost any tissue. The precursors of these pathogenic cells were not yet clearly defined in vivo, however, it has been shown in vitro that monocytes and $CD1c^{+}$ dendritic cells can achieve high levels of CD207 and CD1a when exposed to TGFb and TSLP. We hypothesized that precursor cells expressing CD207 and/or CD1a should be circulating in active multisystem LCH patients. Methods: Pediatric patients with confirmed diagnosis of LCH were stratified as unisystem or multisystem; unifocal or mutifocal; with active disease (AD) or non-active disease (NAD). CD1a and CD207 expression was analyzed in blood mononuclear cells of LCH children and compared with same compartment of cells from healthy adults and umbilical cord blood by FACS analysis. Plasma TSLP and TGFb were analyzed by ELISA. **Results:** The myeloid compartment showed a significant increase of CD11b fraction including $CD11b^{high}$ plus $CD11b^{+}$ (36.4 ± 3.7, N=11) in AD vs NAD patients (18.9 ± 1.7, N=12) and healthy adults (24.9 ± 1.3, N=12). We have also identified the presence of high percentage of circulating $CD11b^{high}CD11c^{+}CD207^{+}$ cells (39 ± 9.4, N=12) in AD vs NAD (3.1 ± 0.5, N=11), healthy adults (0.6 ± 0.4, N=9) and umbilical cord blood (2.1 ± 0.5, N=5). Moreover, circulating $CD11c^{high}CD207^{+}CD1a^{+}$ cells (19 ± 7.4, N=12) are present in active multisystem patients compared with NAD (2.1 ± 0.4, N=12), healthy adults (0.7 ± 0.3, N=8) and umbilical cord blood (2.8 ± 1.1, N=4). TSLP and TGFb levels were significantly increased in active LCH compared to non-active patients. **Conclusions:** Circulating monocytes expressing CD207 as well as DCs expressing CD207/CD1a were found in multisystem active disease. In concordance with this result we observed increased plasma levels of TSLP and TGFb in active LCH patients suggesting that these cytokines could be key drivers of pathogenic LC in vivo.

BENEFICIAL PROPERTIES OF METHYL GALLATE ON EXPERIMENTAL COLITIS

ANGELES RODRIGUEZ BASSO, MARÍA LAURA ANZOISE, GRACIELA LÓPEZ ORDIERES, ANDREA CARRANZA, SUSANA GORZALCZANY

Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Argentina.

Chronic inflammation of the gastrointestinal tract is observed in inflammatory bowel disease (IBD). This condition encompasses two major conditions, known as Crohn's disease and ulcerative colitis. To date, no complete response has been achieved with conventional therapies, therefore, the development of new therapies is an important goal in the IBD therapy. Methyl gallate (MG) is a gallotannin that is widely distributed in herbal medicines, food plants and mushrooms. Previous studies reported that this bioactive phenolic compound presents antioxidant, anti-inflammatory, antimicrobial and anti-tumor activities. The aim of this study was to test the activity of MG on an experimental colitis model. Doses of MG (100 and 300 mg/Kg, vo) and mesalazine (100 mg/kg, vo), reference drug, were tested in colitis model, inducing by intracolonic instillation of a 2 mL of 4% (v/v) acetic acid solution. MG induced a significant reduction in the colon weight/length ratio, expressed in mg/2cm (control: 155.1±9.4, colitis: 545.2±36.3, MG 100 mg/Kg: 528.5±67.0, MG 300 mg/Kg: 374.0±34.7, mesalazine: 329.3±36.9), macroscopic lesion score (control: 0.14±0.08, colitis: 3.4±0.2, MG 100 mg/Kg: 2.5±0.6, MG 300 mg/Kg: 2.2±0.5, mesalazine : 1.8±0.2), GSSG/GSH ratio (control: 0.04±0.02, colitis: 0.70±0.2, MG 100 mg/Kg: 0.05±0.02, MG 300 mg/Kg: 0.06±0.02, mesalazine: 0.09±0.02), showing a similar pattern in TBARs levels. Na+K+ATPase activity were recovered in treated groups (control: 827.2±59.6, colitis: 311.6±54.8, MG 100 mg/Kg: 642.2±175.0, MG 300 mg/Kg: 809.7±100.6, mesalazine: 525.3±81.7). Futhermore, MG reduced the overexpression of COX2, IL-6, TNF and the severity of microscopic tissue damage induced by acetic acid intracolonic. The recovery of number of globet cells and mucin production in treated groups was also observed. Therefore, this study could demonstrate that methyl gallate possesses beneficial properties in a preclinical model of inflammation bowel disease.

PHARMACOKINETIC/PHARMACODYNAMIC ANALYSIS OF A THERAPEUTIC REGIMEN OF MARBOFLOXACIN IN GOATS WITH MASTITIS BY MONTE CARLO SIMULATION

AUGUSTO MATÍAS LORENZUTTI[1], JOSÉ JULIO DE LUCAS BURNEO[2], MANUEL IGNACIO SAN ANDRÉS LARREA[2], MARÍA DEL PILAR ZARAZAGA[1], MARTÍN ALEJANDRO HIMELFARB[1], NICOLÁS JAVIER LITTERIO[1],

1 Universidad Católica de Córdoba, Argentina. 2 Universidad Complutense de Madrid, Spain

Goat mastitis generates economic losses and threatens public health. Coagulase-negative staphylococci (CNS) and *S. aureus* are principal pathogens. Marbofloxacin (MFX) is a fluoroquinolone approved for veterinary use indicated for mastitis. The objectives of this study were to evaluate the pharmacokinetics and milk bioavailability of MFX by IM route in lactating goats, determine MIC and MPC from regional pathogens make a PK/PD analysis by Monte Carlo simulation, and correlate the PK/PD results with clinical response and milk culture. Seven goats with mastitis by CNS were included. MFX was administered by IM route at 10mg/kg/24hx5days. Milk production, pH and culture were performed once a day. Serum and milk samples were quantified by microbiological method, and a non-compartmental model was used. Milk bioavailability of MFX was evaluated by AUC24milk/serum ratio. MICs and MPCs were obtained from regional strains of CNS (*n*=106) and *S. aureus* (*n*=8) isolated from caprine mastitis in Córdoba, Argentina. 10000-subjects Monte Carlo simulation was carried out. PK/PD endpoints were AUC/MIC>40, 50 and 60, C_{max}/MIC>10, AUC/MPC>13.5 and Cmax/MPC>1.2. Probability of target attainment (PTA) and cummulative frequency of response (CFR) were calculated. AUCmilk/serum ratios were >1. During treatment, milk production increased and pH decreased in infected udders, consistent with lower values of AUC in serum and milk. No pharmacokinetic differences in milk between healthy and infected udders were observed. All animals completed the study with negative cultures. The proposed dose regimen of MFX presented a PTA>90% only for MICs of 0.2-0.4µg/ml for all PK/PD endpoints. CFR was >90% for all endpoints. For AUC/MPC endpoint a PTA>90% were achieved only for MPCs of 0.8-1.6µg/ml. CFR was <25% in all cases. For Cmax/MPC endpoint, a PTA>90% was observed with MPCs of 0.8-3.2µg/ml, with a CFR<75%. The results indicate that the proposed dose regimen of MFX in goat mastitis has good efficacy, but may promote the emergence of resistance.

INCREASED EXPRESSION OF TYRO3 AND PROTEIN S IN CIRCULATING CD11B+ CELLS OF PATIENTS WITH ACTIVE LANGERHANS CELL HISTIOCYTOSIS

LICINA TESSONE[1], WANDA NOWAK[1], IVANA G. ESTECHO[1], ARNALDO R. ARMESTO[1], GRACIELA ELENA[2], CARLA V. ROTHLIN [4], EUGENIO A. CARRERA SILVA[3], DIEGO A. ROSSO[1,2], ANDREA E. ERRASTI[1]

1- Instituto de Farmacología, Facultad de Medicina, Universidad de Buenos Aires. Buenos Aires, Argentina. 2- Hospital "Pedro de Elizalde", Buenos Aires, Argentina. 3-Instituto de Medicina Experimental, ANM-CONICET. Buenos Aires, Argentina. 4-Departamento de Inmunobiología, Universidad de Yale, New Haven, USA.

Background: TYRO3, AXL and MERTK (TAM) tyrosine kinase receptors and its cognate agonist Protein S (PROS1) have been identified as negative regulators of the immune response as well as non-classical proto-oncogenes, aberrantly expressed in multiple haematological and epithelial malignancies. Langerhans Cell (LC) Histiocytosis (LCH) is a disorder characterized by an abnormal accumulation of CD207+CD1a+ myeloid cells in almost any tissue. The etiology of this disease is still under scientific discussion and it is not clear if LCH results from malignant transformation orunbalanced immune response that leads to the proliferation of pathogenic LC-like cells. Our aim is to explore the role of the TAM axis in the pathogenesis of pediatric LCH. Methods: We analyzed the expression of TAM receptors and PROS1 in peripheral blood mononuclear cells of pediatric patients with confirmed diagnosis of LCH stratified as unisystem or multisystem with active disease (AD) or non-active disease (NAD) and adult controls. The expression levels of PROS1 and TAM receptors were determined by flow citometry and expressed as fold increase of mean fluorescence intensity (MFI) compared to the isotype control. Results: Circulating total $CD11b^+$ fraction was significantly expanded in AD (36.4 ± 3.7% N=11) vs NAD (18.9 ± 1.7% N=12) and adult controls (24.9 ± 1.3% N=12). Interestingly, this fraction that is consider the main source of inflammatory myeloid cells, showed higher levels of PROS1 in AD (14.2-fold N=4) compared with NAD (6-fold N=5) and adult controls (6.7-fold N=6). TYRO3 was also up regulated in circulating CD11b+ cells in AD (10.6-fold N=8) compared with NAD (5-fold N=9) and adult controls (4.5-fold N=6). Conclusion: Our results show that higher levels of TYRO3 and PROS1 are associated with active and multisystem LCH suggesting that this axis could be involved in the expansion of precursor and pathological LC-like cells.

ENHANCEMENT OF THERMAL NOCICEPTION AND ASTROCYTE REACTIVITY IN SOMATOSENSORIAL CORTEX INDUCED BY AMPHETAMINE INVOLVES CENTRAL AT1 RECEPTOR ACTIVATION

VICTORIA BELÉN OCCHIEPPO[1], OSVALDO MARTIN BASMADJIAN[1], NATALIA ANDREA MARCHESE[1], MARIELA FERNANDA PÉREZ[1], CLAUDIA BREGONZIO[1]

(1) Instituto de Farmacología Experimental Córdoba (IFEC-CONICET) Departamento de Farmacología. Facultad de Ciencias Químicas Universidad Nacional de Córdoba, Córdoba, Argentina.

The use of psychostimulants, such as amphetamine (Amph), is associated with inflammatory processes over glia and vasculature. Brain Angiotensin II (Ang II), through AT_1-receptors (AT_1-R), modulates dopaminergic neurotransmission and plays a crucial role in inflammatory responses in brain vasculature and glia. Studies from our laboratory showed the involvement of AT_1-R on astrocyte reactivity and neuronal survival in the pre-limbic cortex after repeated exposure to Amph. Our aim for the present work was to extend the role of AT_1-R in alterations induced by repeated exposure to Amph. Astrocyte reactivity, neuronal survival and brain microvascular network were analyzed at the somatosensory cortex. The thermal nociception was evaluated as a physiological outcome of this brain area. Male Wistar rats (250-320g), at standard laboratory conditions, were administered with AT_1-R antagonist Candesartan/vehicle (3 mg/kg p.o., day 1-5) and Amph/saline (2.5 mg/kg i.p., day 6-10). On day 17, animals were sacrificed and the brains processed for immunohistochemistry against Von Willebrand factor and glial fibrillary acidic protein (G-FAP), and Nissl staining. Thermal nociception was evaluated using hot plate test on day 17 in another group of animals. Data were analyzed with two-way ANOVA followed by Bonferroni test. Our results indicate that Amph exposure induces an increase in: occupied area by vessels and their tortuosity, astrocyte reactivity and neuronal apoptosis. Moreover, Amph exposure decreased the paw lick threshold behavior. Pretreatment with candesartan prevented the described alterations induced by psychostimulant. The Amph-induced structural changes at somatosensorial cortex, involving astrocytes, vasculature and neurons, implies AT_1-R activation. The decreased thermal nociception and the structural changes could be considered as extended neuroadaptative responses to Amph.

INHIBITORY EFFECT OF MELATONIN ON MAST CELL ACTIVATION

MARÍA DEL MAR CÚNEO YANZÓN, MARÍA LAURA MARIANI, ALICIA BEATRIZ PENISSI,
Instituto de Histología y Embriología (IHEM-CCT Mendoza-CONICET), Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Argentina.

Melatonin is a chronobiotic hormone widely distributed in the body. It has a variety of extrapineal non chronobiotic functions such as neuromodulator, antiproliferative, antioxidant, immunomodulatory and oncostatic actions. Immunomodulatory effects are exerted on different immune cells. Mast cells have been involved in the pathogenesis of a number of immune and inflammatory disorders including contact dermatitis, allergic rhinitis, asthma, cancer, multiple sclerosis, rheumatoid arthritis, ulcerative colitis and peptic ulcer. However, the effect of melatonin on mast cell activation remains unknown. The aim of this study was to determine whether melatonin inhibits mast cell activation induced by mast cell secretagogues that act by different molecular mechanisms of action. Peritoneal mast cells from adult male rats were removed and then purified and activated with compound 48/80, calcium ionophore A23187, neurotensin or a phospholipase A_2 activator peptide (PLA_2AP). Morphological studies by light microscopy and confocal fluorescence microscopy were performed. The percentage of serotonin release was determined by HPLC as a marker of degranulation. Melatonin inhibited mast cell activation induced by the calcium ionophore A23187 (10 µg/ml A23187: 16.35±1.6% versus 20 µg/ml melatonin: 2.8±0.21%, $P<0.001$) and did not alter mast cell activation induced by compound 48/80, neurotensin or PLA_2AP. Granule morphological changes induced by the calcium ionophore A23187 were also inhibited by melatonin at non-cytotoxic doses, suggesting an interaction of the hormone with calcium-binding proteins, among other mechanisms. Melatonin is an attractive molecule, which could be useful for prevention and/or treatment of mast cell-mediated disorders.

PREMIO JOVEN INVESTIGADOR / YOUNG INVESTIGATOR AWARD

DESARROLLO DE ESTRATEGIAS FARMACÉUTICAS PARA LA REDUCCIÓN DE LA TOXICIDAD LEUCOCITARIA, INCREMENTO DE LA ACTIVIDAD ANTIBIOFILM Y MEJORA DE LAS PROPIEDADES FISICOQUÍMICAS DE CLORANFENICOL/ DEVELOPMENT OF PHARMACEUTICAL STRATEGIES FOR THE REDUCTION OF LEUCOCYTIC TOXICITY, INCREMENT OF ANTIBIOFILM AND IMPROVEMENT OF PHYSICOCHEMICAL PROPERTIES OF CHLORANPHENICOL

MEJORA DE LAS PROPIEDADES FISICOQUÍMICAS DE CLORANFENICOL MEDIANTE COMPLEJACIÓN CON CICLODEXTRINAS Y AMINOÁCIDOS/ IMPROVEMENT OF PHYSICOCHEMICAL PROPERTIES OF CHLORAMPHENICOL BY CYCLODEXTRINE AND AMINOACID COMPLEXATION

ARIANA ZOPPI

Las infecciones causadas por patógenos multirresistentes a antimicrobianos requiere la introducción de ingredientes farmacéuticos activos (IFA) nuevos para su tratamiento. Entre 1980 y 1984, la Food and Drug Administration aprobó 20 antimicrobianos (ATM) nuevos, sin embargo entre 2005 y 2009 sólo tres fueron aprobados. Esta disminución en la aprobación de nuevos ATM refleja la caída brusca de la productividad en el sector de desarrollo de ATM de la industria farmacéutica. También, la creciente resistencia en las bacterias exige que los nuevos agentes presenten diferentes mecanismos de acción, lo que aumenta aún más el desafío. De acuerdo a lo expuesto se torna más factible y promisoria la aplicación de la síntesis supramolecular para un mejor aprovechamiento de los ATM de uso aprobado como es el caso de CP. El desarrollo de sistemas supramoleculares es un campo muy activo, con retos pendientes que extienden la actividad tradicional dedicada a la preparación de nuevos IFA, hacia la obtención de materiales con características determinadas, que se puedan seleccionar en lo posible sobre la base de mejorar los inconvenientes que presenten ATM de importante relevancia terapéutica, tales como solubilidad, estabilidad, permeabilidad y toxicidad. La formación de complejos ofrece la oportunidad de modificar la composición de un ATM y sus propiedades fisicoquímicas, biofarmacéuticas, microbiológicas, toxicológicas y farmacotécnicas, sin alterar los enlaces covalentes preexistentes en este, lo que implica una mejora indiscutida en la relación costo-beneficio de la obtención de nuevos medicamentos ya que se ven acorta-

dos ciertos plazos de obtención, especialmente aquellos relacionados con el descubrimiento y la toxicología. CP presenta baja solubilidad en soluciones acuosas por lo cual la formación de complejos multicomponentes con ciclodextrinas utilizando compuestos con actividad antioxidante como tercer componente (cisteína, glicina y N-acetilcisteína) resultó una estrategia útil para sortear este inconveniente.

ESTRATEGIAS FARMACÉUTICAS PARA PREVENIR EL ESTRÉS OXIDATIVO INDUCIDO POR CLORANFENICOL EN LEUCOCITOS/PHARMACEUTICAL STRATEGIES TO PREVENT OXIDATIVE STRESS INDUCED BY CHLORAMPHENICOL IN LEUCOCYTES

VIRGINIA AIASSA

Es ampliamente conocido que CP está relacionado con la producción de anemia aplásica en personas sensibles o en casos de sobredosis. La depresión de la médula ósea (MO) es el efecto adverso más serio del cloranfenicol. Existen dos tipos de depresión de la MO: una que no depende de la dosis administrada ni del tiempo de uso (anemia aplásica irreversible) y otro dependiente de la dosis y de las concentraciones plasmáticas que revierte espontáneamente al suspender la medicación. Estos efectos secundarios graves e incluso fatales limitan el uso de este fármaco.

La toxicidad de diversos fármacos está relacionada con un aumento de la producción de especies reactivas de oxígeno (ROS) con la consecuente producción de estrés oxidativo. Por lo tanto, el desarrollo de ensayos de toxicidad para evaluar alteraciones metabólicas previas a la anemia aplásica podría ser útil para reducir este grave riesgo. Estudios previos han demostrado que las ROS y la producción de nitrito, junto con la alteración de las enzimas antioxidantes, pueden explicar la leucotoxicidad de CP. De hecho, se sabe que las células contienen algunos sistemas antioxidantes para protegerse de la lesión inducida por el aumento de ROS intracelular. En consecuencia, resultó interesante determinar el efecto de los antioxidantes glicina, cisteína y N-acetilcisteína (utilizados como tercer componente en los sistemas supramoleculares) sobre el estrés oxidativo causado por CP en leucocitos humanos. Nuestros resultados confirmaron la producción de estrés oxidativo en leucocitos inducido por CP, mientras que, cuando se ensayaron los sistemas supramoleculares multicomponentes, la producción de ROS fue significativamente menor incluso que en las muestras no tratadas indicando de este modo las ventajas de estas formulaciones de múltiples componentes que contienen glicina, cisteína o N-acetilcisteína en la reducción de los efectos nocivos de CP.

AUMENTO DE LA ACTIVIDAD ANTIBIOFILM DEL SISTEMA MULTICOMPONENTE CLORANFENICOL: β-CICLODEXTRINA: N-ACETILCISTEÍNA/ INCREASE OF ANTIBIOFILM ACTIVITY OF THE MULTICOMPONENT SYSTEM CHLORANPHENICOL: BCYCLODEXTRINE: N-ACETYLCISTEINE

DIAMELA M. ROCCA

Un biofilm es una comunidad microbiana caracterizada por células unidas irreversiblemente a un sustrato biótico o abiótico, embebidas en una matriz polimérica extracelular producida por ellas mismas y que exhiben un fenotipo alterado con respecto al índice de crecimiento y trascripción de genes. Los biofilms son formas de vida adaptadas para sobrevivir en medios hostiles, por lo tanto su resistencia a agentes antimicrobianos y a las defensas del huésped es entre cien a mil veces superior a la de su contraparte planctónica (células en estado libre). Dado que los biofilms están involucrados en más del 80% de las infecciones bacterianas, existe una creciente necesidad de prevenir su formación ya que aumentan aún más la posibilidad de resistencia a los agentes antimicrobianos. La erradicación de biofilms preformados requiere de estrategias especiales, dado que la matriz reduce la interacción del antimicrobiano con la bacteria, y consecuentemente, aumenta el número de fallas terapéuticas. N-acetilcisteína es una droga no antibiótica que presenta acción dispersiva sobre la matriz del biofilm, lo cual hace a las bacterias que lo forman más susceptibles a los agentes antimicrobianos y además, posee un excelente perfil de seguridad siendo ampliamente utilizada en la práctica médica por vía inhalatoria, oral e intravenosa por lo que resulta un tercer componente apropiado para ser utilizado en una formulación farmacéutica de CP.

En nuestro trabajo, la formación del complejo multicomponente cloranfenicol:β-ciclodextrina: N-acetilcisteína mostró tener un efecto significativo en la reducción de la actividad metabólica y biomasa de biofilms de *Staphylococcus aureus* y *Staphylococcus* coagulasa negativo, incluso resultó más efectivo que CP a una concentración igual a 10 veces su concentración inhibitoria mínima (CIM).

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RESÚMENES DE LAS COMUNICACIONES

PRESENTACION DE POSTERS SAI I / SAI POSTERS PRESENTATION I

INMUNOLOGÍA TUMORAL/ TUMOR IMMUNOLOGY

001 (892) **CD39 DELINEATES CELL EXHAUSTION IN MOUSE AND HUMAN TUMOR-ASSOCIATED CD8+ T CELLS: A POSSIBLE IMMUNOMODULATORY ROLE OF A "DYSFUNCTIONAL" CELL SUBSET**

Fernando Pablo Canale[1], María Cecilia Ramello[1], Nicolás Núñez[2], Cintia Liliana Araujo Furlan[1], Melisa Gorosito Serrán[1], Jimena Tosello Boari[1], Sabrina Noemí Bossio[1], Andrés Del Castillo[3], Marta Ledesma[3], Christine Sedlik[2,4], Eliane Piaggio[2,4], Adriana Gruppi[1], Eva Virginia Acosta Rodríguez[1], Carolina Lucía Montes[1].

[1]Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI-CONICET), Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Haya de la Torre y Medina Allende, Ciudad Universitaria, Córdoba, Argentina. [2]Institut Curie, PSL Research University, INSERM U932, F-75005, Paris, France. [3]Hospital Rawson, Av. Gdor Amadeo Sabattini 2025, Polo Sanitario, Córdoba, Argentina. [4]Centre d'Investigation Clinique Biothérapie CICBT 1428, Institut Curie, Paris, F-75005 France.

Tumor-infiltrating CD8+ T lymphocytes (CD8+ TILs) are crucial to eliminate tumors through cytotoxicity and cytokine production. Cancer cells blunt this process developing a microenvironment that induces dysfunctional and regulatory T cells. Here, we aimed to study by flow cytometry the expression of the immunomodulatory ecto-enzyme CD39 on CD8+ T cells in the context of anti-tumor response. Studying B16F10-OVA and other mouse cancer models, we defined three subsets: CD39-, CD39int and CD39high CD8+ T cells, being the latter predominant in tumors but absent in lymphoid organs ($p \leq 0.0001$). Of note, the frequency of CD39high CD8+ TILs increased with tumor growth ($p \leq 0.0001$). CD39high CD8+ TILs exhibited an exhausted phenotype with lower production of TNF ($p \leq 0.001$) and IL-2 ($p \leq 0.01$) than CD39-/CD39int CD8+ TILs, and higher expression of inhibitory receptors (PD-1, Tim-3, LAG-3, TIGIT and 2B4) ($p \leq 0.0001$ for all). Murine exhausted CD8+ T cells displayed high ability to hydrolyze extracellular ATP in vitro, indicating that CD39 is enzymatically active on these cells. Moreover, co-culture experiments showed that exhausted CD8+ TILs are able to reduce IFNã production by conventional CD8+ T cells ($p \leq 0.05$). Interestingly, in samples from 29 breast cancer and 4 melanoma patients we observed that CD39+ CD8+ T cells were present in tumors and increased in invaded/metastatic lymph nodes compared to non-invaded lymph nodes ($p \leq 0.001$), while absent in peripheral blood. These cells exhibited impaired production of IFNã ($p \leq 0.001$), TNF ($p \leq 0.01$) and IL-2 ($p \leq 0.05$ in lymph nodes) when compared to CD39- CD8+ T cells, and higher expression of PD-1 ($p \leq 0.01$), TIGIT ($p \leq 0.01$) and BTLA ($p \leq 0.05$). These findings suggest that beside the loss of effector functions, the tumor environment also drives the acquisition of regulatory molecules on CD8+ T cells. CD39 may emerge not only as a marker of CD8+ T cell dysfunction but also as a possible target for treatments aimed to restore anti-tumor immunity.

002 (412) I**NNATE CD8+ T CELLS: AN UNEXPLORED LINE OF DEFENSE OF THE IMMUNE SYSTEM IN CANCER?**

Constanza Savid-Frontera[1], Natalia Baez[1], María Cecilia Rodriguez-Galán[1].

[1]Dpto de Bioquímica Clínica, Fac. de Cs. Químicas, UNC. CIBICI-CONICET.

Innate CD8$^+$ T cells give rise in the thymus apart from the conventional T CD8$^+$ development pathway. One particular marker that distinguishes CD8$^+$ innate from conventional thymocytes is high expression of CD44 similar to memory T cells in the innate subset. It has been recently demonstrated that these cells could play an important role during neoplasic processes due to their capacity to produce large amounts of interferon-gamma after interleukin 12 (IL-12) and IL-18 stimulation and their high cytotoxic activity mediated mainly by the NKG2D receptor. In our group we developed an experimental model where we induce systemic and transitory expression of IL-12 and IL-18 by hydrodynamic injection of their cDNAs. We observed that the levels of both cytokines are significantly higher in 12+18 group than control group, injected with the empty plasmid, up to 7-10 days post-treatment ($p<0,05$). Interestingly we found in lymph nodes and spleen, a large number of CD8$^+$ CD44^{hi} T cells after IL-12 and IL-18 systemic expression ($p<0,05$). Moreover, these cells also express high levels of EOMES, a transcription factor characteristic of innate CD8$^+$ T cells compared to CD8$^+$ CD44^{lo} T cells ($p<0,05$). In vitro antitumor assays demonstrated that splenocytes from 12+18 mice, enriched in CD8$^+$ CD44^{hi} EOMES$^+$ NKG2D$^+$ T cells, kill more efficiently YAC-1 tumor cells that express ligands for NKG2D than splenocytes from control mice ($p<0,05$). In vivo experiments demonstrated that IL-12+IL-18 cDNA treatment significantly attenuate tumor growth in a B16 murine melanoma model measured by the size and the weight of the tumors ($p<0,05$). Interestingly, in the absence of CD8$^+$ T cells (CD8KO mice), the size of the tumors is larger than in control mice after IL-12+IL-18 systemic expression. All together, our data indicates that systemic expression of IL-12 and IL-18 selectively expands a population of CD8$^+$ T cells with an innate phenotype with a high antitumoral capacity.

003 (944) **STUDY OF THE EXPRESSION OF CD39 ON CD4 CONVENTIONAL T CELLS FROM TUMOR-BEARING MICE AND BREAST CANCER PATIENTS**

Sabrina Noemi Bossio[1], María Cecilia Ramello[1], Fernando Pablo Canale[1], Nicolás Gabriel Núñez[2], Eliane Piaggio[2,3], Adriana Gruppi[1], Eva Virginia Acosta Rodríguez[1], Carolina Lucía Montes[1].

[1]Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI-CONICET), Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Haya de la Torre y Medina Allende, Ciudad Universitaria, Córdoba, Argentina. [2]Institut Curie, PSL Research University, INSERM U932, F-75005, Paris, France. [3]Centre d'Investigation Clinique Biothérapie CICBT 1428, Institut Curie, Paris, F-75005 France.

CD39 is an ecto-enzyme capable of hydrolyzing extracellular ATP to AMP, which can be further hydrolyzed into adenosine by CD73. CD39 is expressed by different cell populations such as B cells and Foxp3+ regulatory CD4+ T cells (Tregs), among others. We aimed to study the expression of CD39 on conventional Foxp3- CD4+ T cells (Tconv) from tumor-bearing mice and patients with breast cancer. C57BL/6 mice were injected with MCA cancer cells. Tumors, spleens and draining lymph nodes (dLN) were extracted on day 17. We observed higher frequency

of CD39-expressing Tconv in tumor respect to spleen ($p \leq 0.001$) and dLN ($p \leq 0.001$). In addition, CD39 expression was higher in tumor-infiltrating Tconv respect to Tconv from spleen and dLN and comparable to the expression on Tregs. We detected in tumors that 57,7±5,9% and 59,5±3,0% of CD39+ Tconv express CD73 and PD-1 respectively, while they do not express TIGIT. PD-1 expression in CD39+ Tconv was significantly higher in tumors compared to spleens and dLN ($p \leq 0.001$ in both). We observed that around 60% of tumor-infiltraiting CD39+ Tconv exhibited effector memory phenotype. Analysis of CD39 expression on T cells from tumor and metastasic (Met) or non-metastasic (NonMet) LN from cancer patients, revealed that in tumor 13,7±5,7% of Tconv expressed CD39. The frequency of CD39-expressing Tconv was higher in MetLN respect to Non-MetLN and absent in peripheral blood. CD39+ Tconv in tumors and MetLN exhibited higher frequency of PD-1+, TIGIT+ and BTLA+ cells than CD39- Tconv ($p \leq 0.05$). The higher expression of inhibitory receptors is associated with functional impairment, in fact, within CD39+ Tconv we detected decreased frequency of TNF and IFNγ-producing cells compared to CD39- Tconv, in both, tumors and MetLN ($p \leq 0.05$). Together these results suggest that tumor microenvironment drives the acquisition of immunoregulatory molecules on conventional $CD4^+$ T cells which may impact in tumor progression.

004 (910) **MILK FERMENTED BY LACTOBACILLUS CASEI CRL431 IMPROVES HOST IMMUNE RESPONSE IN 4T1 BREAST CANCER MODEL AND ITS METASTASIS**

Virginia Emilce Méndez Utz[1], Féliz Fernando Aragón[1], Gabriela Perdigón[1,2], Alejandra de Moreno de LeBlanc[1].
[1]Centro de Referencia para Lactobacilos (CERELA)- CONICET [2]Cátedra de Inmunología, Facultad de Bioquímica, Química y Farmacia. UNT.

Metastasis (MTS) from breast cancer is associated to high mortality rate. Despite the host immune response, tumor cells can stimulate the production of cytokines and growth factors to favour their growth and MTS. Previously, we reported that administration of milk fermented by *Lactobacillus casei* CRL431 (PFM) to mice bearing breast cancer diminished tumor growth, angiogenesis and lung MTS, and decreased the macrophages in mammary glands. The aim of this study was to evaluate the effect of PFM administration on immune cells in tumor and lungs, and to analyse systemic production of cytokines in a MTS model (without tumor presence).Mice were injected subcutaneously with 4T1 cells to induce primary tumors. When the tumors reached a volume of $1cm^3$, mice were randomly divided into 2 groups according they received PFM or milk. They were sacrificed after 30days and tumors were removed to isolate immune cells. For the study of MTS, tumors from other 10 mice (without special feeding) were surgically removed and mice were divided into 2 groups (PFM or milk). Mice were sacrificed after 70days; blood was collected for cytokine assays and lungs were removed to isolate immune cells. Immune cells from tumors and lung were analysed by flow cytometry. Like previous results, mice that received PFM decreased tumor growth. F4/80+cells showed decreased percentages in tumors from mice given PFM compared to milk group. PFM group showed the highest percentage of CD4/CD8 double-positive cells. In the MTS model, lungs from PFM group showed significant decreases in the percentage of F4/80+ cells compared to milk group. The analysis of cytokines in serum revealed that proinflammatory cytokines IL17, IL6 y TNFá were significantly diminished in PFM group compared to milk group. In conclusion, our results show that beneficial effects of PFM in mice bearing breast tumor and MTS was associated to changes on the host immune response, with diminution of macrophages and the pro-inflammatory environment.

005 (376) **CLUSTERIN PRESENT ON HUMAN BREAST CANCER BEARS DC-SIGN BINDING FUCOSYLATED GLYCANS**

Antonela Merlotti[1], Álvaro López Malizia[1], Sol Carregal[1], Augusto Varese[1], Ana Ceballos[1], Christine Sedlik[2,3], Jorge Geffner[1], Sebastián Amigorena[2,3], Eliane Piaggio[2,3], Juan Sabatté[1].
[1]Universidad de Buenos Aires. Consejo Nacional de Investigaciones Científicas y Técnicas. Instituto de Investigaciones Biomédicas en Retrovirus y Sida (INBIRS). Facultad de Medicina. Buenos Aires, Argentina. [2]Institut Curie, PSL Research University, INSERM U932, F-75005, Paris, France. [3]Centre d'Investigation Clinique Biothérapie CICBT 1428, Institut Curie, Paris, F-75005 France.

Clusterin (CLU) is a ubiquitous glycoprotein with extracellular chaperone activity. We have previously shown that clusterin isolated from human seminal plasma bears a set of glycans rich in fucose with high affinity for DC-SIGN present on dendritic cells (DCs); it targets misfolded proteins to DC-SIGN promoting their endocytosis and degradation; and that semen clusterin treated DCs promotes expansion of CD4+CD25+FOXP3+ T-cells. On the other hand, numerous studies have shown that glycosylation changes are associated with the development of cancer and CLU overexpression has been reported in different tumors types. Furthermore, DC-SIGN ligands impose different functional profiles on DCs depending on their glycosylation pattern. Hence, we hypothesize that tumor-CLU has a similar glycosylation pattern and properties of seminal CLU. The presence and properties of CLU were analyzed on human luminal breast cancer samples, and non-invaded breast tissue was used as control. The concentration of total CLU showed no significant differences between tumor and "healthy" tissues (n=20, p=0.3890). The presence of fucose motifs on clusterin was analyzed by ELISA using the fucose-binding lectin Lotus tetragonolobus. The presence of fucose motifs was higher on tumor-CLU than on healthy-tissue CLU (n=14 p=0.004). Moreover, tumor-CLU bound to DC-SIGN when analyzed by western-blot, while CLU from healthy tissue did not (n=5). Finally, we observed that CLU strongly co-localized with Lewis X type fucose motifs on the breast cancer cell line MCF7 and on primary breast cancer cells (n=3). These results showed that breast tumor-CLU bears fucosylated glycans and binds to DC-SIGN. Ongoing experiments will confirm whether tumor-CLU also has chaperone activity and promotes expansion of CD4+CD25+FOXP3+ T-cells, acting as a new mediator of tumor immune-evasion.

006 (430) **LURBINECTEDIN ENHANCE THE PRODUCTION OF IL-1a BY MONOCYTES AND MACROPHAGES FROM HEALTHY DONORS AND CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS**

Denise Risnik[1], Enrique Podaza[1], Ana Colado[1], Maria Belen Almejun[1], Esteban Elias[1], Raimundo Fernando Bezares[2], Horacio Fernández Grecco[3], Maria Cabrejo[3], Mercedes Borge[1], Carlos M. Galmarini[4], Romina Gamberale[1], Mirta Giordano[1].
[1]Laboratorio de Inmunología Oncológica, IMEX-CONICET-ANM, Buenos Aires, Argentina [2]Hospital General de Agudos Dr. Teodoro Álvarez, Buenos Aires, Argentina [3]Sanatorio Municipal Dr. Julio Méndez, Buenos Aires, Argentina [4]Cell Biology Department, PharmaMar SAU, Madrid, Spain.

Chronic lymphocytic leukemia (CLL), the most frequent leukemia in adults, remains an incurable disease. Novel treatments should affect both, neoplastic B cells and the protective microenvironment. Lurbinectedin (Lur) is a synthetic alkaloid currently in phase II/III clinical trials for solid and hematological tumors. We previously reported that monocytes are particular sensitive to Lur. In this work, we study the effect of Lur on IL-1β production by monocytes and macrophages (Mac) from healthy donors (HD) and CLL patients. We found that incubation of HD monocytes with sub-apoptotic doses of Lur (1-3 nM) for 24 h significantly increased the release of IL-1β measured by ELISA (n=9, $p<0.01$). Moreover, Lur enhanced the production of IL-1β induced by LPS or urate monosodium crystals (n=3, $p<0.05$). By contrast, Lur had no effect on IL-1β release by Mac differentiated in vitro from monocytes. Given that the inflammasome is constitutively activated in monocytes but not in Mac, we added ATP to Mac to activate the inflammasome. We found that ATP induced the release of IL-1β only in Mac cultured with Lur for 24 h (n= 6, $p<0.05$). Comparable results were obtained with nurse-like cells, a particular type of CLL-associated Mac differentiated from monocytes in the presence of leukemic B cells. Since the release of ATP is a hallmark of immunogenic cell death, we asked if Lur could induce cell death leading to IL-1β

secretion by Mac. To that aim, we incubated the human stroma cell line, HS5 with Lur (20 nM) for 6 h. After washing out the drug, dying cells were added to Mac treated or not with subapoptotic doses of Lur. The amount of IL-1β was assessed 24 h later. We found that Mac exposed to Lur in the presence of dying HS5 cells secreted higher levels of IL-1β compared to control Mac with or without HS5 cells (n=9, p<0.05). Our results suggest that, unlike other chemotherapeutic drugs, Lur induces the synthesis of pro-IL-1β by monocytes and Mac without activating the inflammasome.

007 (454) **EXPRESSION OF LIGANDS OF THE ACTIVATING RECEPTOR NKG2D IN PRE-MALIGNANT (MYELODYSPLASTIC SYNDROME) AND MALIGNANT (LEUKEMIA) BLASTS AND LYMPHOID CELLS**

Florencia Secchiari[1], Sol Yanel Nuñez[1], Nicolás Ignacio Torres[1], Jessica Mariel Sierra[1], Andrea Ziblat[1], Mercedes Beatriz Fuertes[1], Carolina Ines Domaica[1], Norberto Walter Zwirner[1].
[1]Laboratorio de Fisiopatología de la Inmunidad Innata. Instituto de Biología y Medicina Experimental (IBYME-CONICET).

Different tumors express a diverse array of ligands of the NK cell activating receptor NKG2D. These molecules comprise MICA, MICB and 6 members of the ULBP family (ULBP1 to 6) and are generically known as NKG2D ligands (NKG2DLs). However, the pattern and relevance of the differential and/or combined expression of these NKG2DLs remains unknown in many cases. Nonetheless, as these molecules may constitute attractive targets for immunotherapy and potential prognostic and/or therapeutic biomarkers, a thorough characterization of the expression pattern of NKG2DLs or "NKG2DL*oma*" may reveal interesting information associated with disease status, progression, response to therapy and other clinical parameters. Therefore, the aim of this study was to initiate the characterization of the NKG2DL*oma* expressed by peripheral blood mononuclear cells (PBMCs) from patients with leukemia (with focus on acute myelogenous leukemia) and myelodysplastic syndrome (MDS), as well as from healthy donors using multicolor flow cytometry. For leukemia patients (n=6), variable degrees of expression of MICA, MICB, ULBP2,5,6, ULBP3 and/or ULBP4 were observed on blasts and, unexpectedly, on lymphoid cells; while for MDS samples (n=5) only MICB and/or ULBP4 expression was observed on blasts (and in a few cases also on lymphoid cells). Conversely, very low levels of NKG2DLs were observed on lymphoid cells from healthy donors (n=6), while higher expression of MICA, ULBP2,5,6 and ULBP3 were expressed by lymphoid cells from leukemia samples (p<0.05 for all of them). Our results suggest that a more restricted array of NKG2DLs is expressed during pre-malignancy stages on blasts; while such array of NKG2DLs broadens as the disease progresses towards malignancy and also extends from blasts to lymphoid cells.

008 (820) **IMPACT OF ADJUVANT FORMULATION IN CANCER IMMUNOTHERAPY**

Gustavo Ezequiel Carrizo[1], Enrique Corapi[1], Daniel Compagno[1], Diego Laderach[1].
[1]Laboratorio de Glico-Oncología Molecular y Funcional, IQUIBICEN-CONICET, Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires.

Cancer is one of the major health problems worldwide. The lack of effective treatments has led to explore new alternatives. In recent years the modulation of immune system emerged as a tool with great potential. However, our knowledge in this field remains incipient. Actually, most of the current immunotherapy protocols for cancer have been inspired from responses against pathogens, in this way, different types and concentrations of adjuvants are used to increase immune effector functions; however concomitantly regulatory T cells (Treg) burst is generated. The main goal of this study was to evaluate different adjuvant formulations comparing their potential to induce anti-tumor immunity, aiming to increase effector over regulatory T cells. Results from our laboratory propose Galectin-1 as a tumor associated antigen since it is highly expressed on tumor cells. Preliminary immunizations against a Gal-1-derived peptide and challenge with tumor (B16-F10) cells in adult male C57BL/6 mice demonstrated the adjuvant relevance. The mix of 25uM CpG with 900mM Poly-U-PEI was the only condition that caused a delayed tumor emergence. To evaluate the impact of adjuvant on immune system, we quantify total lymph node cells, total CD4+ and CD8+ T cells and Tregs obtained at different doses of the mix. Our results show a concomitantly burst of effector and Treg populations at higher doses, while at lower doses we obtained a moderate lymphocyte expansion without Tregs burst (p<0.05, ANOVA). Importantly, low doses of adjuvant mix were required to obtain anti-Gal-1 specific cytotoxicity (p<0.05, t-test). These results challenge current concepts of cancer immunotherapy that associate high immune expansion with greater effector function, in contrast, our results showing a better effector function is consistently associated with moderate T cell expansion and no suppressive microenvironment.

009 (939) **SENESCENT T CELLS FROM BREAST CANCER PATIENTS ARE ARRESTED IN THE CELL CYCLE BUT SHOW POLYFUNCTIONAL EFFECTOR PHENOTYPE**

María Cecilia Ramello[1], Fernando Pablo Canale[1], Sabrina Noemí Bossio[1], Nicolás Gonzalo Núñez[2], Andrés Del Castillo[3], Marta Ledesma[3], Eliane Piaggio[2; 4], Adriana Gruppi[1], Eva Virginia Acosta Rodríguez[1], Carolina Lucía Montes[1].
[1]Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI-CONICET), Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Haya de la Torre y Medina Allende, Ciudad Universitaria, Córdoba, Argentina. [2]Institut Curie, PSL Research University, INSERM U932, F-75005, Paris, France. [3]Hospital Rawson, Av. Gdor Amadeo Sabattini 2025, Polo Sanitario, Córdoba, Argentina. [4]Centre d'Investigation Clinique Biothérapie CICBT 1428, Institut Curie, Paris, F-75005 France.

Tumor-induced dysfunction of T cells in patients with cancer may contribute to immune escape. Exhaustion and senescence of T cells have been described as dysfunctional states induced in cancer patients. KLRG-1 and CD57 have been considered as senescent markers in aged T cells. Herein, we aim to study the phenotypic and functional characteristics of senescent T cells from breast cancer patients. We observed that the frequency of KLRG-1+CD57+ T cells (CD8+ and CD4+) were significantly increased in peripheral blood of cancer patients compared to healthy donors (p=0.019 and 0.025, respectively). We confirmed that KLRG-1+CD57+ T cells showed a senescent phenotype since they were CD27-CD28-, gH2AX+ and exhibited the highest activity of b-galactosidase. CD57+ T cells (CD8+ and CD4+) exhibited higher frequency of cell-cycle arrested cells than CD57- T cells (p=0.005 and 0.001, respectively). Interestingly, these populations exhibited co-expression of inhibitory receptors such as 2B4, BTLA and CD160 but not PD-1. Moreover, we found that KLRG-1+CD57+CD8+ T cells and KLRG-1+CD57- or KLRG-1-CD57+ CD4+ T cells infiltrate tumors and metastatic-draining lymph nodes from breast cancer patients. Senescent T cells exhibited higher ability to produce IFNg and TNF and increased capacity to degranulate compared to non-senescent T cells in all tissues studied (p<0.05). KLRG-1+CD57+ peripheral CD4+ and CD8+ T cells expressed higher levels of granzyme B and perforin than non-senescent T cells (p<0.05). Senescent T cells from cancer patients and aged-matched healthy donors exhibited a polyfunctional effector phenotype, however we found that in peripheral blood of patients, KLRG-1+CD57+CD8+ T cells were higher TGFb-producers compared to donors (p=0.008). Moreover, there was a similar trend, although not statistically significant, in KLRG-1+CD57+CD4+ T cells. Thus, our data suggest that senescent CD8+ and CD4+ T cells are not completely dysfunctional cells in cancer patients.

010 (1060) **EFFECT OF BLS IN REGULATORY T CELLS AND ITS IMPACT ON ANTITUMORAL IMMUNITY**

Ana Farias[1], Andrés Hugo Rossi[1], Luciana Berod[2], Fernando Alberto Goldbaum[1], Tim Sparwasser[2], Paula Mercedes Berguer[1].

[1]Instituto Fundación Leloir, IIBBA-CONICET, Buenos Aires, Argentina [2]Institute for Infection Immunology, TWINCORE, Centre for Experimental and Clinical Infection Research, Hannover, Germany.

Melanoma patients harbor increased numbers of Foxp3(+) regulatory T cells (Treg), impeding the onset of antitumoral response. Treg depletion has been adopted as a promising therapeutic approach however the precise mechanism and receptors involved remains unclear. Brucella spp. lumazine synthase (BLS) is a decameric protein with highly immunogenic properties. We have previously demonstrated that BLS induces a protective antitumoral response in mice with B16 melanoma. We inoculated mice sc with BLS and 20 or 35 days later induced tumor development by sc injecting B16 cells. Treatment significantly inhibited tumor growth and 50% of mice failed to develop a tumor. Furthermore, the protective effect by BLS was abolished in TLR4 deficient mice suggesting the importance in signaling through this receptor. In a first attempt to decipher the mechanism involved, we analyzed the effect of BLS in Treg generation from naïve T cells *in vitro* under Treg differentiation conditions. BLS decreased the percentage of $Foxp3^+$ Treg, with a marked reduction in the expression of FoxP3. Moreover, the effect of BLS was partially abolished in $MyD88^{-/-}$ mice suggesting that signaling through alternate receptors may be involved. We next evaluated the effect of BLS in Treg *in vivo*. Our results showed that after 20 days post BLS treatment there is a higher number of $CD4^+$ cells and $CD4^+Foxp3^+$ cells in the draining lymph node. However, after 35 days only recruitment of $CD4^+FoxP3^-$ cells was observed.Thus decrease in Tregs in the tumor microenvironment by FoxP3 downregulation may explainhow BLS mechanistically regulates antitumoral responses.

011 (2050) **KLF6 SUBCELLULAR DISTRIBUTION AS A MARKER OF TUMOR AGGRESSIVENESS IN HUMAN COLON ADENOCARCINOMA**

Verónica Grupe[1], María Eugenia Sabatino[2], Vanesa Cordero[3], María Elisa Cabalier[3], Andrea Lucca[1], Enriqueta Cortiñas[1], Laura Monteverdi[1], Gerardo Gatti[1], José Luis Bocco[2].
[1]Fundación para el Progreso de la Medicina, Córdoba, Argentina [2]CIBICI-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina [3]Hospital Nacional de Clínicas, Facultad de Ciencias Médicas, Córdoba, Argentina.

Colorectal cancer (CRC) is the second most frequent cancer type in our country, representing the 12% of total cancer deaths. However, it is one of the most preventable tumors due to its slow progression and early detection increases complete remission chances up to 90%. Unfortunately, useful molecular biomarkers for CRC early detection are not available. Krüppel-like factor 6 (KLF6) is a tumor suppressor related with several types of cancers though its function in CRC is not deeply known. Our aim was to investigate whether KLF6 expression could be associated with CRC pathogenesis. We establish the frequency of KLF6 subcellular expression in 37 human CRC by immunohistochemistry and we found that 83% of the cases showed a generalized KLF6 cytoplasmic expression. However, nuclear localization revealed a more gradual pattern, even showing null expression. We correlated these data with CRC pathology grades and we found a positive correlation between KLF6 nuclear expression and tumor stage ($r= 0.36$) and a negative correlation with tissue differentiation ($r= -0.33$). In addition, null nuclear expression correlated with low proliferation index ($p<0.05$). In summary, increased KLF6 nuclear localization in CCR could be related with a more advanced and an aggressive tumor behavior, suggesting that KLF6 subcellular expression might be a plausible marker for prognosis of CCR.

012 (644) **LOSS OF IRF8 EXPRESSION CORRELATES WITH THE METASTATIC PHENOTYPE IN BREAST CANCER**

Gerardo Gatti[1], Emiliano Roselli[2], Paula Araya[2], María Elisa Caballier[3], Vanesa Cordero[3], Cecilia Di Tada[1], Veronica Grupe[1], Mariana Maccioni[2].
[1]Fundación para el Progreso de la Medicina, Córdoba, Argentina. [2]CIBICI-CONICET, Facultad de Ciencias Químicas. Universidad Nacional de Córdoba, Córdoba, Argentina. [3]Hospital Nacional de Clínicas, Facultad de Medicina, Universidad Nacional de Córdoba, Córdoba, Argentina.

The transcription factor interferon regulatory factor-8 (IRF-8) is crucial for myeloid cell development and immune response and also acts as a tumor suppressor gene. Tumors silence the expression of IRF8, and disruption of its function leads to acquiring resistance to apoptosis, decreasing the activation of antitumor immune response and increasing the incidence of metastasis, however there is little information about IRF8 expression in breast cancer. The main goal of this work was to evaluate whether loss of IRF8 expression in breast cancer is an important factor in disease progression and metastasis. IRF8 expression was assessed by immunohistochemistry in normal breast tissue, breast ductal carcinoma and sentinel nodes samples. To evaluate whether silencing of IRF8 expression correlates with epigenetic changes, we performed Metylation-specific PCR. IRF8 is expressed in normal breast tissue and breast tumor samples of different tumor histological grades, although with less intensity in tumors with histological grade III. In contrast, expression of IRF8 was not detected in metastatic foci in sentinel nodes. Furthermore, expression of IRF8 was not evidenced in human tumor cell lines MCF7 and MDA231. DNA methylation assays showed that the promoter region of IRF8 was methylated in both cell lines, suggesting a mechanism responsible for the silencing of IRF8 in breast cancer samples. In conclusion, our results indicate that the expression of IRF8 correlates inversely with the metastatic phenotype in breast cancer, and that transcriptional silencing of IRF8 in breast tumor cells correlates with epigenetic changes. These results suggest that IRF8 may have clinical value as potential prognostic marker in breast cancer.

013 (645) **GALECTIN-1 EXPRESSION BY 4T1 MAMMARY TUMORS CONTROLS DEVELOPMENT AND ACTIVATION OF B CELLS**

Nicolás Sarbia[1], Verónica Martinez Allo[1], Florencia Moses[1], Sabrina Gatto[1], Rosa M. Morales[1], Montana N. Manselle Cocco[1], Tomás D´Alotto-Moreno[1], Gabriel A. Rabinovich[1,2], Mariana Salatino[1], Marta A. Toscano[1].
[1]Laboratorio de Inmunopatología, Instituto de Biología y Medicina Experimental, CONICET. [2]Departamento de Química Biológica, FCEN, UBA

Galectin-1 (Gal1) is an endogenous lectin capable of silencing immune responses and blunting anti-tumor immunity. Since Gal1 has been involved in shaping the B cell compartment and the role of these cells in cancer is not fully understood, we sought to understand the role of tumor-derived Gal1 on B cell development and activation. For this, we used the 4T1 murine breast cancer model, which recapitulates human metastatic disease and expresses high amounts of Gal1. As expected, targeted disruption of Gal1 expression in 4T1 cells (shRNA, 4T1 KD) reduced tumor growth and the number of lung metastases ($p<0.05$) in BALB/c mice, compared to wild-type 4T1 cells (4T1 WT). At day 28 post-injection, mice were sacrificed and splenocytes were isolated in order to analyze the different B cell subsets. We found that growth of 4T1 WT tumors was accompanied by an increased frequency of splenic transitional 1 (T1) B cells as compared to tumor-free mice. Downregulation of Gal1 in KD tumors prevented T1 accumulation during B cell development ($p<0.05$). In line with this evidence, we found a decreased frequency of splenic follicular B cells (Fo) in 4T1 WT tumor-bearing mice ($p<0.05$). In addition, within draining lymph nodes Gal1 downregulation induced a reduction in the percentage of activated B cells ($p<0.05$). Our findings suggest that breast tumor-derived Gal1 may affect B cell development and activation, promoting an accumulation of immature B cells. These results may have implications in tumor progression, immune-escape and metastasis.

014 (673) **THE BTK INHIBITOR IBRUTINIB IMPAIRS THE IMMUNE RESPONSE AGAINST MYCOBACTERIUM**

TUBERCULOSIS OF MONOCYTE-DERIVED MACROPHAGES FROM CLL PATIENTS AND HEALTHY DONORS

Ana Colado[1], Denise Kviatcovsky[1], Melanie Genoula[1], María Belén Almejún[1], Enrique Podaza[1], Denise Risnik[1], Esteban Elias[1], Céline Cougoule[3], Isabelle Maridonneau-Parini[3], Fernando R. Bezares[2], Mirta Giordano[1], María del Carmen Sasiain[1], Romina Gamberale[1], Luciana Balboa[1], Mercedes Borge[1].

[1]Instituto de Medicina Experimental (IMEX)-CONICET--ANM. CABA, Argentina. [2]Hospital de Agudos T. Álvarez. CABA, Argentina. [3]CNRS, Institut de Pharmacologie et de Biologie Structurale (IPBS), Département of Tuberculosis and Infection Biology, Toulouse, France.

Ibrutinib is an oral irreversible inhibitor of the Bruton Tyrosine Kinase (Btk) recently approved for CLL treatment. We previously reported that ibrutinib impairs M1 polarization in macrophages from healthydonors (HD). In general, alterations on M1 functions can impact on the resistance against intracellular pathogens, such as *M. tuberculosis* (Mtb), increasing the occurrence of infectious diseases. Therefore, we aimed to explore the effects of ibrutinib on Mtb-response by macrophages from HD and CLL patients. Macrophages were differentiated by culturing monocytes with M-CSF for 7 days. TNFα, IL-8 and IL-10 secretion were measured by ELISA after macrophage or monocyte stimulation with irradiated Mtb for 24 h in the presence or absence of different doses of ibrutinib (0.3-3 µM). Phagocytosis ofMtb-FITC by macrophages was evaluated by flow cytometry. Bacillary loads were determined by colony-forming units assays. Migration in response to CCL5 was measured in matrigel chambers. We found that ibrutinib significantly reduced the release of TNF-α, IL-10 and IL-8 by Mtb-stimulated macrophages from HD, being the production of TNF-α severely affected even at low concentrations of the drug, while IL-10 and IL-8 secretion was only affected at 3 µM (n=7, $p<0.05$). Additionally,ibrutinib enhanced macrophage migration in Matrigel (n=3, $p<0.05$). Although ibrutinib did not affect Mtb phagocytosis (n=10), preliminary results showed a slight increase in the bacillary loads recovered from macrophages at day 3 and 6 post-infection. Finally, we observed that 0.3 µM of ibrutinib also affected Mtb-induced TNF-α production in macrophages (n=6, $p<0.05$) and in freshly isolated monocytes from CLL patients (n=6, $p<0.05$). Ibrutinib concentration in plasma from CLL-treated patients were found to be between 0.25 and 1 µM. Thus, our results show that clinically relevant doses of ibrutinib can impair the in vitro immune response of monocyte-derived macrophages against Mtb.

015 (697) **PHENOTYPIC CHARACTERIZATION OF NK CELLS IN HUMAN RENAL CELL CARCINOMA**

Andrea Ziblat[1], Nicolás Ignacio Torres[1], Ximena Lucía Iraolagoitia[1], Raúl Germán Spallanzani[1], Sol Yanel Nuñez[1], Florencia Secchiari[1], Jessica Mariel Sierra[1], Romina Elizabeth Araya[1], Fernando Pablo Secin[2], Agustín Rovegno[2], Carolina Inés Domaica[1], Mercedes Beatriz Fuertes[1], Norberto Walter Zwirner[1].

[1]Laboratorio de Fisiopatología de la Inmunidad Innata, IBYME-CONICET. [2]CEMIC

Renal cell carcinoma (RCC) is among the 10 most frequent cancers in the western world. Surgery is the main treatment as kidney cancers are resistant to radiation and chemotherapy. Natural killer (NK) cells play a key role in tumor immune surveillance through a cytotoxic activity and the secretion of pro-inflammatory cytokines that promote an adaptive antitumor response. Several activating and inhibitory receptors regulate NK cells effector functions. In RCC patients it was observed a positive correlation between the percentage of tumor infiltrating NK cells (TINKs) and a better prognosis. Moreover, the frequency of TINKs and of NK cells in peripheral blood mononuclear cells (PBNKs) inversely correlated with tumor grade, suggesting the existence of a tumor immunosuppressive effect on NK cells. However, the phenotype of NK cells in RCC remains unexplored. Therefore, the aim of this work was to phenotypically characterize TINKs and PBNKs of RCC patients. Flow cytometry analysis revealed a higher percentage of positive cells and/or expression of CD25, CD69, ILT2 ($p<0.01$), DNAM-1, CD45 ($p<0.001$) and CD48 ($p<0.0001$), and a lower percentage of positive cells or expression of CD62L, CD56 ($p<0.05$) and NKG2D ($p<0.001$) in PBNKs of RCC patients in comparison with healthy donors. Also, we detected a higher percentage of positive cells and/or expression of CD56 ($p<0.05$), CD69 ($p<0.0001$) and PD-1 ($p<0.01$), and a lower percentage of positive cells and/or expression of CCR7, CD57, NKG2D ($p<0.05$), 2B4, NKp46 ($p<0.01$), CD16, DNAM-1, NKp80 ($p<0.001$), CD62L and NKp30 ($p<0.0001$) on TINKs compared to PBNKs of RCC patients. Therefore, our results suggest that tumor microenvironment induces TINKs with an altered phenotype that may lead to NK cells with less functional capacity. Furthermore, PBNKs phenotype of RCC patients may compromise the immune surveillance against circulating cancer cells. Besides, CD48 and CD45 expression in PBNKs may constitute diagnostic biomarkers in RCC patients.

016 (766) **CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) AND TUMOR MICROENVIRONMENT: THE IMPACT OF THE NOVEL THERAPEUTIC AGENT BCL-2 INHIBITOR ABT-199**

Esteban Elías[1], Ana Colado[1], Enrique Podaza[1], Denise Risnik[1], María Belén Almejún[1], Mercedes Borge[1,2], Raimundo Fernando Bezares[3], Mirta Giordano[1,2], Romina Gamberale[1,2].

[1]Laboratorio de Inmunología Oncológica, Instituto de Medicina Experimental (IMEX)-CONICET-Academia Nacional de Medicina (ANM) [2]Cátedra de Inmunología, Facultad de Medicina, Universidad de Buenos Aires. [3]Sección Hematología, Hospital Álvarez, Ciudad de Buenos Aires.

Leukemic B cells from CLL patients survive and proliferate within lymphoid tissues in contact with activated T cells and myeloid cells and receiving signals through the BCR. Effective therapy should target both, leukemic cells and the protective microenvironment. Combination of anti-CD20 MoAbs and novel agents such as BCR-associated kinase inhibitors and/or specific BCL2 inhibitor ABT-199 appears as an attractive strategy. The aim of this study was to evaluate whether ABT-199 was able to impair T cell activation and phagocytosis of anti-CD20 coated CLL cells by macrophages. Peripheral blood mononuclear cells (PBMC) from CLL patients were cultured with vehicle (DMSO) or ABT-199 and cell survival was evaluated by flow cytometry using Annexin V-FITC at 24hs. PBMC cultured on plate bound anti-CD3 induced the expected upregulation of the activation markers CD25 and CD69 on T cells and also the activation of CLL cells. The phagocytosis was evaluated by flow cytometry with macrophages and CFSE-labeled CLL cells coated with Rituximab. We found that CLL cells were very sensitive to ABT-199 (mean±SEM for CLL cell survival: 85±5, 59±4, 30±5 and 16±4 for DMSO, ABT-199 0.01, 0.1 and 1µM, n=5, $p<0.01$), while T cells were less sensitive to the drug (n=5, $p<0.05$). The presence of ABT-199 did not modify CD25 and CD69 upregulation induced by anti-CD3 stimulation (n=5). Interestingly, leukemic cell activation significantly reduced its sensitivity to the drug (mean±SEM for CLL cell survival: 89±2, 86±2, 81±4 and 76±3 for DMSO, ABT-199 0.01, 0.1 and 1µM, n=4, $p<0.05$). Finally, we found that ABT-199 did not affect the phagocytosis of rituximab-coated CLL cells (n=4). Our results showed that ABT-199 does not affect T cell activation in CLL patients or the phagocytosis of CLL cells by macrophages. The resistance observed in vitro when CLL cells where activated suggests that tissue resident cells from the supportive microenvironment may not be targeted by the drug.

017 (970) **NO NEURONAL CHOLINERGIC SYSTEM INFLUENCE IN THE PROGRESSION OF U251MG HUMAN GLIOBLASTOMA CELL LINE AND ITS IMPACT ON THE IMMUNE RESPONSE**

Luciana Moverer[1], María Soledad Gori[1], Mariela Moreno[2], Antonela Asad[2], Julieta Alcain[1], Walter Scordo[3], Mónica Vermeulen[1], Marianela Candolfi[2], Gabriela Salamone[1].

[1]Instituto de Medicina Experimental (IMEX)-CONICET Academia Nacional de Medicina. [2]INBIOMED (UBA-CONICET)

[3]*Servicio de Medicina Transfusional, Hospital Italiano de Buenos Aires.*

Glioblastoma multiforme (GBM) is the deadliest and most common type of human primary brain tumor. This tumor is defined by the hallmark features of uncontrolled cellular proliferation, diffuse infiltration, propensity for necrosis, robust angiogenesis, intense resistance to apoptosis and genomic instability. Acetylcholine (ACh) is a neurotransmitter which can also modulates cell survival, proliferation and differentiation in neuronal and non-neuronal cells such as immune cells, which has been referred to as a "non-neuronal cholinergic system". The aim of this work is to elucidate the relevance of the non-neuronal cholinergic system in the interaction immune-GBM cells. Methods: Human U251 GBM cells were cocultured with human dendritic cells (DC) obtained from healthy adult volunteer. Mononuclear cells were isolated from buffy coats of healthy adult nonsmoker volunteer and CD14+ cells were then isolated by positive selection and then were cultured with GM-CSF and IL-4. The cells were cultered in the presence of cholinergic agonists (carbachol, muscarine and nicotine) and antagonists (atropine and mecamelamine) and evaluated DC maturation. Results: We found that coculture of dendritic cells with U251 cells in the presence of carbachol and nicotine treatment increased the release of IL-8 (P<0,01) as well as the expression of CD86 and HLA-DR, we did not find difference in the production of IL-10 citokine. These effects were completely blocked when the co-culture was treated with selective antagonists for mAChR and nAChR, Atropine and Mecamelamina receptors respectively. Conclusions: The cholinergic system stimulates the cytokines production in U251 and DC cells and modulates the activation and maturation of DC. Our findings suggest that the no neuronal cholinergic system could emerge as a therapeutic target for the immunotherapy of GBM.

018 (592) **INDOLEAMINE 2,3-DIOXYGENASE, TUMOUR INFILTRATING LYMPHOCYTES, AND FOXP3 EXPRESSION ARE DIFFERENTIALLY ASSOCIATED TO BREAST CANCER MALIGNANT PARAMETERS**

Marina Teresita Isla Larrain[1], Martín Enrique Rabassa[1], Alberto Barbera[1], Aldo Cretón[1], Amada Segal-Eiras[1], María Virginia Croce[1].

[1]*Centro de Investigaciones Inmunológicas Básicas y Aplicadas, Facultad de Ciencias Médicas, Universidad Nacional de La Plata.*

Breast cancer development and dissemination are influenced by intrinsic molecular heterogeneity as well as by the balance between effector and suppressive immune responses. One of the mechanisms of tumor evasion involves Indolamine-2,3-dioxygenase (IDO), which catalyzes the degradation of tryptophan to kynurenine. It has been observed that IDO restricts host immune surveillance and is related to immune cells in the tumor microenvironment. Objective: To study the immunomodulatory response in relation to molecular and histopathological breast cancer characteristics. Materials and Methods: Cell lines and 100 samples from breast cancer patients; normal breast tissue samples were used as controls. The clinical and histopathologic features were obtained from medical records. Expression of estrogen (ER) and progesterone receptors (RP), Her2-neu, IDO, Foxp3, CD45RO and CK 5/6 was determined by IHC. Intratumoral (II) and extratumoral tumor infiltrating lymphocytes (EI) were analyzed in tumor sections. Univariate statistical analysis was performed by Kendall correlation and multivariate analysis by principal component. Results: By IHC, samples expressed ER in 81% of cases; PR, 71% and Her2-neu, 9%. IDO was positive in 61% of samples; FOXP3, in 54%; CK5/6 was positive in 18%. IHC results were validated by RT-PCR and Western blot in tissue samples and cell lines. II were found in 69% of samples, while 36% presented EI. CD45RO+ TILs were found in 78% of samples. ER expression was negatively correlated with the expression of IDO and detection of II. Moreover, a negative correlation between the EI and Foxp3 expression in tumor cells was found. A positive correlation between the stage of disease and the expression of IDO, CK 5/6, as well as the presence of EI and nuclear grade was also found. Conclusions: IDO and TILs were associated to highly malignant breast cancer characteristics; in contrast, Foxp3 expression was related to less aggressive parameters.

ENFERMEDADES INFECCIOSAS I/ INFECTIOUS DISEASES I

019 (582) **ASSOCIATION OF THE IL-17A RS2275913 SINGLE NUCLEOTIDE POLYMORPHISM WITH TUBERCULOSIS SEVERITY IN ARGENTINA**

Agustín Rolandelli[1,2], Rodrigo Hernández Del Pino[1,3], Joaquín Pellegrini[1,2], Nancy Tateosian[1,2], Nicolás Amiano[1,2], Paula Morelli[1,2], Florencia Castello[1,2], Nicolás Casco[4], Alberto Levi[4], Marisa Gutierrez[5], Domingo Palmero[4], Verónica Garcia[1,2].

[1]*Departamento de Química Biológica. Facultad de Ciencias Exactas y Naturales. UBA, Intendente Güiraldes 2160, Pabellón II, 4°piso, Ciudad Universitaria (C1428EGA), Buenos Aires, Argentina.* [2]*Instituto de Química Biológica, Facultad de Ciencias Exactas y Naturales (IQUIBICEN), UBA (Universidad de Buenos Aires)-CONICET, Intendente Güiraldes 2160, Pabellón II, 4°piso, Ciudad Universitaria (C1428EGA), Buenos Aires, Argentina.* [3]*Centro de Investigaciones y Transferencia del Noroeste de Buenos Aires (CIT NOBA), CONICET. Newbery 261, Junín (6000), Buenos Aires, Argentina.* [4]*División Tisioneumonología Hospital F.J. Muñiz, Uspallata 2272, (C1282AEN) Buenos Aires, Argentina.* [5]*Sección Bacteriología de la Tuberculosis, Hospital General de Agudos "Dr. E. Tornu", Combatientes de Malvinas 3002, (C1427ARN) Buenos Aires, Argentina.*

Mycobacterium tuberculosis (Mtb) causes nearly 10 millions of new tuberculosis disease cases annually. However, most individuals exposed to *Mtb* do not develop tuberculosis, suggesting the influence of a human genetic component. Previously, we investigated the association of the rs2275913 SNP (G->A) from IL-17A and tuberculosis in Argentina, and found an association between the AA genotype and tuberculosis resistance. In this work, we evaluated the functional relevance of this SNP during the immune response of the host against Mtb and analyzed its impact on clinical parameters of the disease severity. Thus, peripheral blood mononuclear cells (PBMCs) from GG, GA and AA healthy donors (HD) and tuberculosis patients (TB) were obtained and stimulated for five days with a *Mtb*lysate (*Mtb*-Ag), and IL-17A and IFN-gamma production were measured by ELISA and Flow Cytometry. Our findings indicated that, within the HD population, AA *Mtb*-Ag stimulated cells produced the highest amounts of IL-17A ($p<0.05$) and IFN-gamma ($p<0.05$), supporting the genetic evidence we have previously found. In contrast, within the TB population, AA *Mtb*-Ag stimulated cells produced the highest amounts of IL-17A ($p<0.05$) but the lowest levels of IFN-gamma ($p<0.05$). Furthermore, AA patients showed the lowest proliferation index in *Mtb*-Ag stimulated PBMCs ($p<0.05$) and the lowest SLAM expression on CD3+ T cells ($P<0.05$), two immunological parameters that suggest a relationship with tuberculosis severity. In fact, we found an association between the AA genotype and clinical parameters of disease severity, such as severe radiological lesions ($p<0.05$) and higher AFB in sputum smear ($p<0.05$). Overall, our present results evidenced that the rs2275913 SNP could be a biomarker of resistance to tuberculosis and disease severity in the Argentinean population.

020 (586) **HUMAN B CELLS ARE SUSCEPTIBLE TO ANDES VIRUS INFECTION AND UP-REGULATE CD61 -THE HANTAVIRUS ENTRY RECEPTOR- UPON ACTIVATION**

Marina Garcia[1], Carles Solà Riera[2], Sabrina Bassi[3], Ayelen Igleasis[3], Kimia Maleki[2], Shawon Gupta[2], Veronica Landoni[4], Carla Bellomo[3], Maria del Carmen Sasiain[1], Valeria P. Martinez[3], Jonas Klingström[2], Pablo Schierloh[1].

[1]*Laboratorio de Inmunología de Enfermedades Respiratorias, Instituto de Medicina Experimental (IMEX)-CONICET-Academia Nacional de Medicina (ANM), Ciudad Autónoma de Buenos Aires (CABA), Argentina.* [2]*Center for Infectious*

Medicine, Department of Medicine Huddinge, Karolinska Institutet, Stockholm, Sweden. [3]Laboratorio Nacional de Referencia para Hantavirus, Servicio de Biología Molecular, Instituto Nacional de Enfermedades Infecciosas, ANLIS -"Dr. Carlos G. Malbrán", CABA, Argentina. [4]Laboratorio de Fisiología de procesos Inflamatorios, IMEX-CONICET-ANM, CABA, Argentina.

Hantaviruses produce 2 zoonotic life-threatening diseases: Hemorrhagic fever with renal syndrome and Hantavirus pulmonary syndrome (HPS). In a previous study, we observed a massive polyclonal activation of B cells in HPS patients which led us to wonder if these cells may constitute an alternative target for Andes virus (ANDV) infection. To test this hypothesis, purified normal B cells were exposed to ANDV for 24 to 72hs, demonstrating for the first time that these cells are susceptible to infection. Importantly, in our hands, the *in vitro* infection success was strongly donor-dependent (45%, n=10) which is consistent with the low basal expression level of CD61 among peripheral B cells (n=10,% of $CD61^+$ B cells Mean ($IC_{95\%}$) = 6 (4-7.8)). The clear co-expression pattern of CD41 with CD61 ($p<0.001$) strongly suggests the presence of aIIbâ3 integrin complex on this small B cell subset. The basal CD61 expression seemed not to be related with the BCR Ig-class expression ($p>0.05$ for IgD^+, IgA^+, and IgG^+ B cells) but was associated to B cell activation state ($p<0.05$ for $CD69^+$ and $CD25^+$ B cells). Next, the modulation of CD61 expression under different culture conditions was analyzed. We observed that overnight PHA-treatment of PBMC strongly induced CD61 on a subset of activated ($CD69^+$) B cells (n=6, $p<0.05$). Interestingly, depletion of $CD2^+$ cells before PHA stimulation strongly reduced CD61 up-regulation on B cells, suggesting that such phenomenon requires the presence of bystander T cells (n=4, $p<0.05$). Consistently, B-cell specific stimulation with PWM lectin failed to induce CD61 expression on activated ($CD69^+$) B cells. Finally, the increased binding to fibronectin of PHA-pretreated B cells demonstrates the functionality of CD61/CD41 receptor complex ($p<0.05$). These results indicate that human B cell may constitute an alternative cell target for ANDV. Moreover, by mean of CD61 regulation, as yet unknown factors may enhance the susceptibility to infection in this cell type.

021 (684) **B. PERTUSSIS SUPPRESSES PROINFLAMMATORY CITOQUINES-MEDIATED NETS-INDUCTION RELEASED BY EPITHELIAL CELLS DURING INFECTION**

Juan Pablo Gorgojo[1], Yanina Lamberti[1], Juan Marcos Oviedo[1], María Eugenia Rodriguez[1].
[1]CINDEFI (UNLP CONICET La Plata), Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina.

Recent studies demonstrated that *B. pertussis* (Bp) has the ability to inhibit neutrophil extracellular traps (NETs) formation in response to the proinflamatory stimulus of IL-8. However, this cytoquine is a weak inducer of NETs. Moreover, in our hands, IL-8 alone does not induce significant NETosis even at supraphysiological concentrations (1000 ng/ml). Since, Bp might be exposed to a more complex, usually stronger NETs-inducer stimulus in vivo, we here investigated the interaction of Bp with epithelial cells and its relevance in NETs production. We used the 16HBE14o- epithelial cell line derived from human bronchial epithelium, which retains differentiated epithelial morphology features and functions. We found that 16HBE14o- epithelial cells secrete IL-8, IL-6 and TNF-á in response to Bp infection, as determined by ELISA. By mean of DNA labeling with propidium iodide, antibodies against NET-associated proteins, and fluorescence microscopy we observed that conditioned medium, obtained after incubation of bacteria with 16HBE14o- cells, induced NETs release. Our results show that the NETs-inducer stimulus associated to bacterial infection of epithelial cells can be inhibited by Bp. This effect proved dependent on CyaA-mediated ROS inhibition activity as determined by blocking antibodies against this toxin. These results show that Bp is able to control this essential neutrophil bactericidal mechanism even in a complex proinflammatory environment. Together with previous results these data highlight the role of CyaA in Bp evasion of the immune response against this bacterium.

022 (788) **DIFFERENTIAL ACTIVITY OF TWO SAGS OF THE EGC OPERON ON PHAGOCYTIC CELLS**

Sofia Noli Truant[1], Sarratea María Belén[1], M. Julieta Fernández Lynch[1], Antonoglou M. Belén[1], De Marzi Mauricio C[1,2], Malchiodi Emilio L.[1], Fernández Marisa M.[1]
[1]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Inmunología-IDEHU (UBA-CONICET), Buenos Aires, Argentina. [2]Universidad Nacional de Lujan, INEDES, Lujan, Buenos Aires, Argentina.

Bacterial superantigens (SAgs) are enterotoxins that bind to the MHC-II molecules and the TCR, activating as much as 20% of T cells and promoting a cytokine storm which enhances endotoxic shock and produces immunosuppression, hindering the immune response against bacterial infection. The egc operon reported in autochthonous *S. aureus* strains includes natural variants of the SAgs genes *seg*, *sei*, *sem*, *sen* and *seo*. Here, we characterize the complementary activity of SEO and SEM on phagocytic cells in the absence of T cell. SEO and SEM were cloned in pET26 vector and expressed in *E coli*. To test biological activity, SAgs were exposed to different cell cultures to evaluate proliferation and viability by using [methyl-^{3}H] thymidine and MTT. Macrophage activation was characterized measuring arginine activity in cell lysates and by nitrite determination in culture medium. SEO interaction with MCH-II DR1 was evaluated by Western Blot and their affinity constants were estimated by surface plasmon resonance. We have found:1-PBMCs activation was determined for SEO at 0.001ìg/ìl and SEM at 0,01 ìg/ìl; 2-SAgs cytotoxic activity on THP-1 was triggered by SEO at 10 ìg/ìl, and SEM at 100 ìg/ìl; 3-Both SAgs are toxic to macrophages-differentiated THP-1 at 100 ìg/ìl at 24/48 h, while SEM showed cytotoxicity at 0.1 ìg/ìl on Raw cells at 24/48 h, though SEO only showed effect after 48 h; 4-Exposure of macrophages-differentiated THP-1 to SEM, but no SEO (neither SEI or SEG) showed up to 7-fold nitrite increase; 5-Only SEO increased nitrite up to 1.6 fold in culture medium of RAW cells; 6-SEO reduced arginase activity in cell lysates; 7-SEO-DR1 specific interaction was determined. SEO and SEM exhibited SAg activity on different phagocytic cells showing a complementary cytotoxic activity, favoring tendency to a very soft M1 response that is triggered at different stages for each SAg. The singularity of this differential response may be a key for bacteria's survival in the host.

023 (863) **YERSINIA ENTEROCOLITICA ORAL INFECTION INDUCES EXPANSION OF CELLS WITH PHENOTYPE OF MYELOID-DERIVED SUPPRESSOR CELLS INTESTINAL MUCOSA AND SPLEEN**

Marianela Leporati[1], Silvia Di Genaro[1,2], Javier Eliçabe[1,2].
[1]Laboratory of Immunopathology, IMIBIO-SL, CONICET, San Luis, Argentina. [2]Division of Immunology, School of Chemistry, Biochemistry and Pharmacy, National University of San Luis, Argentina.

Background: Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immature and immunesupressive myeloid cells. These cells are characterized by coexpression of CD11b and Gr-1, and are divided into two major subsets, granulocytic ($CD11b^+Ly6G^+Ly6C^{low}$) and monocytic ($CD11b^+Ly6G^-Ly6C^{hi}$) MDSCs. *Yersinia enterocolitica* (Ye) are Gram-negative bacteria that cause food-borne acute or chronic gastrointestinal and systemic diseases. The role of MDSCs in Ye infection has not been determined. Objective: The purpose was to elucidate whether oral Ye infection induces the expansion of MDSCs and to define the role of these cells in the infection. Methods: First, C57BL/6 mice were orally infected with Ye WAP-314 serotype O:8. On days 5 and 10 post-infection (p.i), cell infiltration in mesenteric lymph nodes (MLN), Peyer´s patches (PP) and spleen was analyzed. Moreover, total and specific IgA responses in feces, and IL-6 levels in sera were measured. Results: We observed that Ye-infected mice pre-

sented a dramatic increases in the frequencies as well as in the absolute numbers of $CD11b^{+}GR\text{-}1^{+}$ cells in PP, MLN and spleen on days 5 and 10 p.i ($p<0.05$ compared with uninfected mice). In PP and spleen, $CD11b^{+}Ly6G^{+}Ly6C^{low}$ subset was expanded while in MLN both $CD11b^{+}Ly6G^{-}Ly6C^{hi}$ and $CD11b^{+}Ly6G^{+}Ly6C^{low}$ subsets were detected. Additionally, the MDSC expansion in infected mice did not require TNFR1 signaling. Moreover, IL-6 levels in sera from infected mice were significantly increased on day 5 p.i ($p<0.05$). Interestingly, the increases of MDSCs frequency and IL-6 levels were accompanied with elevated total IgA levels and Ye-specific IgA response in intestinal mucosa from infected mice ($p<0.05$). Conclusion: We conclude that oral Ye infection induced the expansion of granulocytic MDSCs in spleen and intestinal mucosa. Production of IL-6 may be implicated in the development and regulation of MDSC. Granulocytic MDSCs may promote anti-Ye IgA response during Ye infection.

024 (890) **RNASEQ ANALYSIS REVEALED THAT FOXP3+ REGULATORY T CELLS ACQUIRE TH1-LIKE AND TISSUE REPAIR PROGRAMS DURING EXPERIMENTAL TRYPANOSOMA CRUZI INFECTION**

Cintia Araujo Furlan[1], Véronique Adoue[2], Joanna Fourquet[2], Jimena Tosello Boari[1], Constanza Rodriguez[1], Fernando Canale[1], Facundo Fiocca[1], Cristian Beccaria[1], Adriana Gruppi[1], Carolina Montes[1], Olivier Joffre[2], Eva Acosta Rodriguez[1].

[1]CIBICI-CONICET, Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, UNC, Córdoba, Argentina. [2]Centre de Physiopathologie de Toulouse Purpan, Toulouse, France.

$Foxp3^{+}$ $CD4^{+}$ T cells (Tregs) present a dual role during infections as they limit immunopathology but also restrain immunity to the pathogen. Recently, a new role in repairing tissue injury was also ascribed to Tregs. During *T. cruzi* (Tc) infection, Tregs response has been poorly characterized. Previously, we determined that Tregs become activated during Tc infection, upregulating Foxp3, CD25, GITR and CTLA-4. Moreover, the frequency of Tregs that produce IL-10, TGF-beta and, interestingly, IFN-gamma, was increased by the infection. Here, our aim was to examine the emergence of specialized Tregs populations during Tc infection. For this, Tregs transcriptome was analyzed by RNAseq. Briefly, Tregs were purified from the spleen of non-infected (NI) or 22-day-infected (INF) Foxp3-GFP mice, RNA was isolated and cDNA libraries were sequenced. Bioinformatics analysis revealed that 5175 genes were differentially expressed in INF Tregs compared to NI Tregs. In agreement with our previous results, RNAseq further confirmed the activated status of INF Tregs as these cells showed increased amounts of transcripts for suppressive and activation markers. Remarkably, Tbx21 and Areg (coding for amphiregulin) appear among the most upregulated genes in INF Tregs. A more exhaustive analysis showed that not only Tbx21 but also other genes related to Th1 responses (e.g., Ifng, Cxcr3, Stat1, Il12rb2 and Ifngr1) were also significantly upregulated, suggesting the acquisition of a Th1-like profile by INF Tregs. Of note, in addition to Areg, INF Tregs upregulated other genes associated with tissue repair properties of muscle and lung Tregs, including Il18r1, Il18rap, Neb, Ccr2, Il10, Gzmb, Itgae and Havcr2. As previously reported for spleen Tregs, INF Tregs showed no changes in the expression of St2 (coding for IL-33 receptor) compared to NI Tregs. These results suggest that during Tc infection, Tregs acquire a program specialized in controlling Th1 immune responses and promoting tissue repair.

025 (923) **INFLUENCE OF OUTER MEMBRANE VESICLES OF BORDETELLA PERTUSSIS ON BACTERIAL INTERACTION WITH MACROPHAGES**

Bruno Blancá[1], Jimena Alvarez Hayes[1], María Eugenia Rodriguez[1].

[1]CINDEFI (UNLP CONICET La Plata), Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina.

Outer membrane vesicles (OMVs) are naturally produced by Gram negative bacteria. These vesicles contain adhesins, toxins, lipoproteins, LPS, among other bacterial factors, and interact with host cells acting as virulence factor delivery vehicles. OMVs interact with the host immune effectors influencing the outcome of the infection in different ways. *Bordetella pertussis,* the causative agent of whopping cough, is able to produce OMVs not only during *in vitro* growth but also during infection inside host. In the present study we isolated OMVs from a late log phase culture of *B. pertussis*. We recovered them from the culture supernatant by sterile filtration followed by an ultracentrifugation step. The purity and morphology of OMVs were analyzed by transmission electron microscopy. We next studied the interaction of *B. pertussis* OMVs with macrophages, a kind of cells in which we previously showed that this pathogen is able to develop intracellular infections. Human monocytes were obtained from fresh buffy coats and differentiated into macrophages *in vitro*. Fluorescence microscopy studies showed that OMVs are associated with macrophages after 4 h of incubation. The interaction of the immune cell with vesicles prior bacterial infection led to a significant decrease in bacteria uptake (12 bacteria/cell) as compared with control macrophages not treated with OMVs prior infection (33 bacteria/cell). At later time points confocal microcopy studies showed no difference in the intracellular bacterial trafficking as determined by the use of specific markers and confocal microscopy. However, a higher intracellular infection rate was observed in OMVs treated macrophages. As seen for other Gram negative pathogens, these results suggest that *Bordetella Pertussis* OMVs are relevant actors in the infection process and the pathogenesis of this bacterium.

026 (2044) **PLANKTONIC AND BIOFILM LIFESTYLES: SETUP AND PRELIMINARY RESULTS OF A METHODOLOGY TO STUDY THE INTERACTIONS OF MICROORGANISMS IN A BOVINE MASTITIS IN VITRO MODEL**

Luciana Paola Bohl[1,2], Walter Ezequiel Lerda[2], Agustín Conesa[1,2], Paula Isaac[1,2], María Laura Breser[1,2], Jaione Valle[4], Nori Tolosa de Talamoni[3], Carina Porporatto[1,2].

[1]Centro de Investigaciones y Transferencia de Villa María (CIT-VM), CONICET -Universidad Nacional de Villa María. Córdoba, Argentina. [2]Instituto Académico Pedagógico de Ciencias Básicas y Aplicadas. Universidad Nacional de Villa María. Córdoba, Argentina. [3]INICSA-CONICET-UNC. Córdoba, Argentina. [4]Idab-Universidad Pública de Navarra-CSIC-Gobierno de Navarra. Pamplona, España.

Staphylococcus aureus (*SA*) is the main pathogen isolated into intramammary infections from dairy cattle. *SA* has the ability to grow as biofilm, showing a reduction to antimicrobial susceptibility and to the recognition by the immune system. However, the response of epithelial cells to planktonic and biofilm cultures has not been characterized. The aim of this work was to study the immune response of bovine mammary epithelial cells to *SA* grown in planktonic and biofilms form. The study was performed using a cell line of bovine mammary epithelial (MAC-T) infected with different form of *SA* V329 grown. To determinate the interaction of biofilm grown, bacteria was cultures in a specific device consisting of a lid with 96 pegs. The CFU of planktonic and biofilm grown was normalized to co-cultures assays. MAC-T cells infected with planktonic and biofilms *SA* was evaluated to 2, 4, 6 and 24 h. Co-cultures interaction was evaluated by the internalization of *SA* (CFU) (2 h), pro-apoptotic (Bax) and anti-apoptotic (Bcl-2) mRNA expression (4 h) and IL-1β and IL-6 production in the supernatants by ELISA (2, 4, 6 and 24 h). Bacteria from planktonic cultures shown higher internalization than bacteria from biofilms cultures ($p<0.05$). On the another hand, both co-cultures show upregulation of Bcl-2 and downregulation of Bax, but the effect of planktonic bacterial on Bcl-2 was higher than the bacteria grown in biofilm ($p<0.05$). Although both *SA* co-cultured conditions showing a significant increase of IL-6 and IL-1β ($p<0.05$) (2 and 4 h), we did not find differences between the levels of pro-inflammatory cytokines between bacterial forms of growth. These results shown a usefulness method to evaluate the response to epithelial cells after planktonic and biofilm co-cultivation and provide important information about

differentiates in the immune response. More studies are needed to understand. the role of different forms of bacterial growth and its relation with immune response.

027 (2067) **DEVELOPMENT OF A NOVEL METHOD TO CHARACTERIZE AND FUNCTIONALLY ANALYSE EXTRACELLULAR VESICLES ISOLATED FROM THE PLASMA OF HIV-1-POSITIVE PATIENTS**

Andrea Morales[1], Pehuen Pereyra Gerber[1], Gabriel Duette[1], Julia Rubione[1], Matías Ostrowski[1].
[1]Instituto de Investigaciones Biomédicas en Retrovirus y Sida (INBIRS).

Extracellular Vesicles (EVs) encompass a heterogeneous group of structures surrounded by a lipid bilayer, which are produced and secreted to the extracellular space by almost every cell type. Consequently, EVs are present in several biological fluids, including plasma. Given the capacity of EVs to transfer molecules from a donor to a target cell, they are considered to mediate a novel and particular type of intercellular communication, with involvement in a number of physiological and pathological processes. The main hypothesis of our team is that EVs present in the plasma of patients chronically infected with HIV-1 promote immune activation and inflammation, thus contributing to the pathogenesis of the immune disorders observed in these patients. A major limitation to address this question is obtaining large amounts of highly pure plasma-derived preparations of EVs. Here, we developed a method that combines the ability of Size-Exclusion Chromatography (SEC) to separate vesicles from soluble proteins from plasma followed by a high-molecular cut-off ultrafiltration device to concentrate purified EVs. Finally, we set up a bead-based immunocapture assay to phenotypically characterize purified EVs using a panel of antibodies against vesicle-enriched proteins by flow cytometry. Overall, this method is fast, reproducible, quantitative and inexpensive, allowing to overcome many of the actual technical limitations in the field. Moreover, even if our main interest is to analyze EVs from HIV-1 chronically-infected individuals, this method could also be applied to the analysis of EVs in other pathologies, such as cancer.

028 (588) **ELEVATED LEVELS OF PLASMA LBP AND SCD14 IN PUUMALA VIRUS-INFECTED PATIENTS SUGGEST A POTENTIAL GASTROINTESTINAL INVOLVEMENT DURING HEMORRHAGIC FEVER WITH RENAL SYNDROME**

Kimia Maleki [1], Clas Ahlm [2], Jonas Klingström[1]
[1]Center for Infectious Medicine, Department of Medicine Huddinge, Karolinska Institutet, Karolinska University Hospital, SE-141 86 Stockholm, Sweden. [2]Department of Clinical Microbiology, Division of Infectious Diseases, Umeå University, SE- 901 87, Umeå, Sweden.

Hantaviruses cause two severe human diseases; hemorrhagic fever with renal syndrome (HFRS) in Eurasia and hantavirus pulmonary syndrome (HPS) in the Americas, with case-fatality rates of up to 10% and 40%, respectively. As of today, no specific treatment or FDA-approved vaccine is available. Hantavirus infection of humans is characterized by vascular leakage and a strong inflammatory response displayed by high levels of pro-inflammatory cytokines and vigorous NK cell and CD8 T cell responses. The mechanistic background to what is driving these responses is however unknown. Microbial translocation (MT), referring to the leakage of bacterial products from the gastrointestinal tract into the systemic circulation, has been suggested as a driver of immune activation and inflammation during certain viral and inflammatory diseases. As HFRS-patients often present with various gastrointestinal symptoms, we aimed to investigate if MT could be a driver of inflammation during HFRS. Plasma levels of two surrogate markers of MT; LPS-binding protein (LBP) and sCD14, were measured in 31 patients during acute and convalescent Puumala virus-caused HFRS, as well as in 26 age- and sex-matched healthy controls, using ELISA. Furthermore, the levels of pro-inflammatory cytokines were measured using a custom 12-plex immunoassay. Increased plasma levels of LBP and sCD14 were observed during the acute phase, suggesting the potential occurrence of MT. A positive correlation was found between levels of LBP and fever in patients. Further, elevated plasma levels of TNF, IL-6, IL-10, IL 15 and IL-18 were detected. Our preliminary data confirm a strong inflammatory response during the acute phase of HFRS and suggest a potential gastrointestinal involvement during the disease.

029 (654) **INQUILINUS LIMOSUS INVADE AND PERSIST INSIDE BRONQUIAL EPITHELIAL CELLS**

Yanina Andrea Lamberti[1], Pablo Mieres[1], María Eugenia Rodriguez[1].
[1]CINDEFI-CONICET-UNLP

Inquilinus limosus is an emerging multi-resistant opportunistic pathogen documented mainly in Cystic Fibrosis patients. Infection with I. limosus is sometimes accompanied with exacerbation or respiratory decline. However, the pathogenic potential and the impact on respiratory function are still unclear. This study examined the interactions of Inquilinus limosus DSM16000 with lung epithelial cells and its ability to induce a proinflammatory response, adhere and invade bronquial human epithelial cells 16HBE14o-. ELISA studies indicated the I. limosus, triggered a pro-inflammatory response as soon as 4,5 hs post-infection, with a significant increase of interleukin (IL)-6 and IL-8 in culture supernatant as compared with uninfected 16HBE14o- cells ($p<0.05$). Immunofluorescence confocal studies further indicated that I. limosus is not only capable to adhere but also to invade epithelial cells. About 30% of I. limosus associated with 16HBE14o- cells were found intracellularly in LAMP2 negative compartments suggesting that I. limosus avoids the trafficking to lysosomal compartments. Meropenem, an antibiotic frequently used to treat the infection, was ineffective to eliminate intracellular bacteria at concentrations of 800ig/ml, a concentration 8 fold above the minimum bactericidal concentration (MBC). Overall, this study suggests a potential prospect on the impact of I. limosus on cystic fibrosis lung infections though its capacity to induce a proinflammatory response and survive intracellularly in respiratory epithelial cells possibly contributing to the pathogenesis and the antibiotic treatment failure.

030 (716) **CATHEPSIN L3 PROMOTES IFN-γ RESPONSE BY CD8 T CELLS DURING FASCIOLA HEPATICA INFECTION**

Daiana Pamela Celias[1], Leonardo Micael Silvane[1], Ileana Corvo[2], José Tort[3], Cristina Motrán[1], Laura Cervi[1].
[1]Departamento de Bioquímica Clínica Facultad de Ciencias Químicas. Universidad Nacional de Córdoba. CIBICI-CONICET. Córdoba, Argentina. [2]Laboratorio de I+D de Moléculas Bioactivas, Centro Universitario de Paysandú, Universidad de la República, Uruguay. [3]Facultad de Medicina. Universidad de la República, Uruguay.

During its migration through host tissue, the helminth parasite *F. hepatica*, secretes a number of distinct cathepsin L cysteine peptidases. Among them, cathepsin L3 (CL3) is highly expressed in the juvenile larvae, which has the ability to degrade collagen. However, the effect of CL3 on the modulation of immune response is still unknown. The aim of this work was to study the ability of CL3 to induce an immune response during *F. hepatica* infection in mice. A role of dendritic cells (DC) in this modulation was also investigated. C57BL/6 mice (B6) were orally infected with 12 *F. hepatica* metacercariae per animal. After 4 and 17 days post infection peritoneal cells (PECs) and splenocytes were removed, respectively. These cells were cultured in presence or absence of CL3 or total parasite lysates (TE) for 48h. Cytokines production was detected by ELISA or FACS analysis. Moreover, DC were differentiated from B6 bone marrow with GM-CSF and cultured for 18 h with CL3 (produced in *Hansenula polymorpha*) or LPS, and injected in B6. After 7 days lymph nodes were obtained and stimulated with CL3. Both PECs and splenocytes from infected animals restimulated with CL3, secrete significantly higher levels of IFN-γ and IL-17 than those observed in uninfected mice ($p<0,05$). We found that IFN-γ-producing cells in spleen were mostly CD8 T cells, and in a lesser extent CD4 T cells, while IL-17-producing cells were not CD4 T cells. Being DC potent antigen presenting cells, we stimulate these cells with CL3, injected them in mice and 7 days later we evaluated the response of lymph node cells (LNC)

to CL3. CL3-treated DC were able to induce a specific IFN-γ CD8 T cells response by LNC from injected animals after CL3 stimulation. Our data show that during *F. hepatica* infection, CL3 was able to trigger a specific IFN-γ response by CD8 T cells, being DC probably involved in this effect. Accordingly, CL3 appears as an interesting target for vaccines development against *F. hepatica.*

031 (740) **DEVELOPMENT OF A PLASMONIC BIOSENSOR FOR THE DETECTION OF STAPHYLOCOCCAL ENTEROTOXINS**

María Belén Sarratea[1], Sofía Noli Truant[1], Maria Julieta Fernández Lynch[1], María Belén Antonoglou[1], Romina Mitarotonda[2], Pablo Nicolás Romasanta[1], Mauricio De Marzi[1,2], Emilio Luis Malchiodi[1], Marisa Mariel Fernández[1].
[1]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Inmunología-IDEHU (UBA-CONICET), Buenos Aires, Argentina. [2]Universidad Nacional de Lujan, INEDES, Lujan, Buenos Aires, Argentina.

Staphylococcal enterotoxins are one of the most important causative agents of food poisoning. These molecules function as both gastrointestinal toxins and superantigens (SAgs) which can simultaneously bind MHC-II and T cell receptor leading to a non-specific polyclonal T cell activation and massive proinflammatory cytokine release. Common symptoms include vomiting and diarrhea; however, in more severe cases, systemic dissemination may result in toxic shock syndrome and can be lethal in a few hours. Only small amounts of these heat-stable toxins are needed to cause the disease. Therefore, it is highly important to detect low concentrations of SAgs in biological samples. We have previously reported a surface plasmon resonance (SPR) method based on a double antibody sandwich approach that can detect pM levels of the SAg SEG. In the present work, we aimed to assess the influence of the amount of immobilized antibody and the use of alternatives enhancing reagents in order to improve the limit of detection. Three different quantities of primary antibody were covalently coupled on the independent surfaces of CM5 sensor chip: 3500, 6800 and 11000 RU. In concordance with our previous assays, 1.10^{-12} M of SEG was detected in the surface with 6800 RU. We also used SAgs natural ligand, TCR Vb8.2, as a secondary reagent to increase the signal. However, it did not prove to be useful probably due to the occlusion of the binding site during the capture. We also studied the potential use of antibody-coated nanoparticles (NPs) as an alternative amplification strategy. NPs were coated with the antibody after treatment with glutamine, dopamine or alone. For the same amount of Sag tested, NP gave a higher response level than antibodies alone. Overall, SPR biosensors offer the capability for continuous real-time monitoring and high sensitivity that can be befitting for the detection of enterotoxins in food industries, laboratories and regulatory agencies.

032 (747) **DETECTION OF GENITAL INFECTIONS AND ANALYSIS OF CYTOKINE EXPRESSION IN INFERTILE PATIENTS OF HOSPITAL JB ITURRASPE SANTA FE, ARGENTINA**

Romina Cecilia Russi[1], Carla Bernasconi[2], Evelin Cáceres[2], German Henrich[2], María Ines García[1], Carolina Veaute[1].
[1]Laboratorio de Inmunología Báscia, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina. [2]Laboratorio Central, Hospital JB Iturraspe, Santa Fe, Argentina.

Sexually Transmitted Infections (STI) are a major global cause of illness and infertility. Among them, *Chlamydia trachomatis (Ct), Mycoplasma hominis (Mh)* and *Ureaplasma urealyticum (Uu),* have a significant impact on human reproduction. Our aims were to assess the prevalence of STIs in infertile patients and the local cytokine profile produced by genital infections. Assessment of *Ct, Mh* and *Uu* infections was performed in 97 patients attending at the JB Iturraspe Hospital (Santa Fe, Argentina), from March 2015 to June 2016. *Ct* diagnosis was performed by PCR and detection of *Mh* and *Uu* by culture. The results showed that 27% of the population studied had a genital infection: *Ct* (5%), *Uu* (10%), *Mh* (9%) and 3% had mixed infections. Cytokine expression was evaluated in 70 patients: 27 infertile with genital infections (IGI), 26 infertile without genital infections (IWGI), and 14 fertile without genital infections (FWGI). Cytokine mRNA was analyzed in cervico-vaginal lavage and seminal plasma, by quantitative PCR using the ΔΔCt comparative method, with β-actin as housekeeping gene. The cytokine analysis showed a significant increase of IFNγ ($p<0.0001$), TNFα ($p<0.01$) and IL10 ($p<0.0001$) expression in IWGI patients *vs.* FWGI patients. In the IGI group, the *Mh* infection was associated with a significant decrease in expression of IFNγ ($p<0.01$), IL10 ($p<0.05$) and IL17A ($p<0.01$), compared to IWGI patients. Infection with *Uu* was associated with a decrease in the TNFα expression ($p<0.05$) and an increase in the TGFβ1 expression ($p<0.01$). In summary, infertile patients showed a high prevalence of STIs, with an altered cytokine expression profile. Moreover, IWGI patients present altered cytokine expression profile respect to FWGI patients. Therefore, we suggest that an alteration of immunological factors in the genital tract could contribute to infertility. Consequently, it is interesting to investigate the immunological mechanisms involved in the development of infertility.

033 (822) **SIGNALING LYMPHOCYTIC ACTIVATION MOLECULE (SLAM) AS A REGULATOR OF MACROPHAGES FUNCTIONS**

Angela María Barbero[1], Josefina Celano[1], Martin Andres Estermann[1], Rodrigo Emanuel Hernández Del Pino[1,2], Luciana Balboa[3], Paula Barrionuevo[4], Verónica Edith García[2,5], Virginia Pasquinelli[1].
[1]Centro de Investigaciones y Transferencias del Noroeste de la Provincia de Buenos Aires (CITNOBA), Universidad Nacional del Noroeste de la Provincia de Buenos Aires - Consejo Nacional de Investigaciones Científicas y Técnicas (UNNOBA-CONICET). Centro de Investigaciones Básicas y Aplicadas (CIBA, UNNOBA), Argentina [2]Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina [3]IMEX-CONICET, Academia Nacional de Medicina, Buenos Aires, Argentina [4]Instituto de Estudios de la Inmunidad Humoral (CONICET), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires (UBA), Buenos Aires, Argentina. [5]Instituto de Química Biológica, Facultad de Ciencias Exactas y Naturales (IQUIBICEN), Universidad de Buenos Aires- Consejo Nacional de Investigaciones Científicas y Técnicas (UBA-CONICET), Argentina.

Tuberculosis ranks alongside HIV as a leading cause of death worldwide affecting one third of world's population. *Mycobacterium tuberculosis* (*Mtb*) has developed many strategies to evade immune response and survive within host's macrophages (MΦ). SLAM is a self-ligand and a costimulatory molecule that promotes Th1 response against *Mtb*. SLAM can also act as microbial sensor that regulates Gram- bacterial phagosome functions in MΦ. Here, we study SLAM role against *Mtb* on MΦ activation and functions. Human monocyte derived MΦ were obtained from healthy donors after Ficoll-Percoll gradient. After adherence for 2h, the cells were culture in complete media for 16-18h before stimulation with sonicated *Mtb*. SLAM, CD86 and CD163 expression was studied by flow cytometry and bacterial uptake by using rhodamine stained *Mtb*. In some experiments MΦ were also stimulated with IFN-g or agonistic antibody áSLAM. Production of VEGF was measured by ELISA in THP1 cells differentiated with PMA and stimulated with *Mtb*. Our results show that *Mtb* induces SLAM expression in monocytes and MΦ. Interestingly, preliminary results indicate that these MΦ present high CD86 and low CD163 expression (M2 marker) suggesting that SLAM promotes M1 activation during *Mtb* infection. Moreover, the great majority of phagocytic MΦ (62.5%) were SLAM+ as seen by rhodamine *Mtb* uptake. However we found a reduction of phagocytosis when macrophages were treated with a-SLAM. This could be due to a negative role of SLAM during phagocytosis or to a blocking effect of the interaction SLAM-Mtb. More studies are required to elucidate this function. IFN-g stimulation significantly increased SLAM expression on *Mtb*-stimulated MΦ and the number of SLAM+ rhodamine+ cells. Moreover; a role of SLAM on angiogenesis was seen as the levels of VEGF were significantly decreased after áSLAM treatment on THP1 cells. Taken together, these results demonstrate the role of SLAM as a regulator of MΦ functions in response to *Mtb* infection.

034 (855) **INFLUENCE OF HEPATITIS C COINFECTION ON IMMUNE ACTIVATION IN HIV VIRAL SUPPRESSED SUBJECTS**

María Noel Badano[1], Natalia Aloisi[1], Marcelo Corti[2], Luis Viola[3], María Marta E de Bracco[1], Patricia Bare[1].
[1]Academia Nacional de Medicina, IMEX CONICET [2]Fundación de la hemofilia [3]Centro Integral de Gastroenterología.

Despite effective antiretroviral therapy (ART) achieves Human Immunodeficiency Virus (HIV) viral suppression, a high immune activation levels may persist in HIV infected patients. We investigated the presence of Hepatitis C Virus (HCV) as a possible factor contributing to the immune activation in HIV suppressed patients coinfected with HCV. Patients were grouped according to HCV viral load and to the non-invasive markers of liver fibrosis (APRI, FORNS and FIB-4) as follows: patients that cleared HCV and display low liver indexes (G1), patients with detectable HCV viral load and low liver indexes (G2) and patients with detectable HCV viral load and high liver indexes (G3). IL-10 and IL-2 plasma levels and CD4/CD8 ratios were analyzed as markers of immune activation and liver fibrosis in HIV/HCV patients. Healthy blood donors were used as controls (C). Statistical analysis was performed with Kruskal-Wallis test and Dunn's post-test to compare differences between groups. Similar IL-10 and IL-2 levels were found in G1, G2 and control groups. However, G3 displayed significantly higher IL-10 (G1: 5.1; G2: 6.3; G3: 11.3, C: 6.4 pg/ml; $p<0.0001$) and IL-2 (G1: 6.3; G2: 6.6; G3: 14.2 pg/ml; C: 6.7; $p<0.02$) amounts compared to all groups. No significant differences were found between G1 and G2 in CD4/CD8 ratios. On the other hand, G3 displayed significantly lower CD4/CD8 ratios compared to G1 and G2 (G1: 0.8; G2: 1.1; G3: 0.5; $p<0.01$). These results suggest that the mere presence of HCV would not contribute to the immune activation in HIV/HCV patients. However, HCV mediated liver pathology seems to be involved in the chronic immune activation present in coinfected subjects despite successful HIV suppression by ART. Since inflammatory cytokine production and immune activation play a key role in the immunopathology of HIV/HCV coinfection and liver fibrosis, HCV treatment and monitoring of hepatic damage must be considered priority in this group of patients.

035 (828) **BORDETELLA PARAPERTUSSIS, INFECTIVE PHENOTYPE CHARACTERIZATION AND ITS RELEVANCE IN VACCINE DEVELOPMENT**

Juan Marcos Oviedo[1], Jimena Alvarez Hayes[1], Yanina Lamberti[1], Kristin Surmann[2], Frank Schmidt[2], Uwe Volker[2], Juan Martín Laborde[3], Miguel Ayala[3], Fabricio Maschi[3], María Eugenia Rodriguez[1].
[1]CINDEFI (UNLP CONICET La Plata), Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina. [2]Interfaculty Institute for Genetics and Functional Genomics, University Medicine Greifswald, Friedrich-Ludwig-Jahn-Str. 15a, 17475 Greifswald, Germany [3]Cátedra de Animales de Laboratorio, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, Buenos Aires, Argentina.

Bordetella parapertussis (Bpp) is one of the causes of whooping cough whose incidence has been rising in the last decade. Opsonic antibodies are necessary to eliminate this pathogen. The only opsonins induced by current acellular pertussis (aP) are those directed against pertactin. aP is composed of three antigens common to both species FIM, FHA and Prn. According to previous studies aP don't induce proper immunity against Bpp. Partially because Prn isn't exposed on the surface of Bpp due to the shielding properties of the O antigen of this species. Iron starvation is a critical stress a bacteria has to overcome during infection and induces deep changes in bacterial phenotype also at antigen level. In this study we used shotgun proteomics to study this bacterium and the phenotype induced under iron starvation. We found that Fim expressed by Bpp is different from that expressed by Bp and there is not cross reactivity between them. On the other hand, under iron starvation FhaC, a protein necessary for FHA exportation is downregulated determining a decrease in surface-bound FHA as confirmed by western blot. According to these results under physiological conditions Bpp should be more effective in avoiding aP induced immunity than expected. However, we also found that the lack of iron induced a significant decrease of wbmM expression which eventually introduced changes in the O-antigen molecule as confirmed by electrophoresis analysis. This change seemed to induce variations in the shielding property of the O antigen since antibodies directed against Prn were found able to attach to iron starved Bpp more efficiently as determined by ELISA. Accordingly, *in vivo* studies showed that aP induced significantly higher protection against the iron starved Bpp than that reported for the iron replete phenotype. Since the infective phenotype is iron starved our results suggest that might be easier than expected to improve the protective capacity of current whopping cough vaccines.

036 (914) **COMPLEX MECHANISMS REGULATE T CELL ANERGY DURING THE ACUTE PHASE OF TRYPANOSOMA CRUZI INFECTION**

Yamile Ana[1], David Rojas Marquez[1], Fabio Cerban[1], Stempin Cinthia[1].
[1]CIBICI-CONICET. Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba.

We have previously shown that decreased T cell proliferation during acute phase of Trypanosoma cruzi infection is related to increase of inhibitory receptor and gene related to anergy in lymphocytes (GRAIL) expression, reduced IL2 production and expression of its regulator Otubain 1 (Otub-1). The aim of this study was to evaluate if GRAIL expression during infection is regulated by IL-2. BALB/c mice were infected i.p. with 500 trypomastigotes of Tulahuen strain and CD4+T cells were purified from spleen of uninfected or infected mice at 21 days post infection (d.p.i). First, we measured whether impaired proliferative response could be reversed by adding IL-2. CD4+T cells from control and 21 d.p.i animals were stimulated with anti-CD3/CD28 in the presence or absence of IL-2 for 3 days and then cell proliferation was assessed by CFSE. We found that CD4+T cells from control as well as 21 d.p.i animals had an increase in proliferation when treated with IL-2 together with the TCR stimulatory ligands. In addition, we also evaluated GRAIL and Otub-1 expression as well as mTOR activation in CD4+T cells with or without IL-2. In agreement with the increase in CD4+T cell proliferation, we found a slight rise in the phosphorylation of 4EBP1 (mTOR) and Otub-1 expression and a reduction in GRAIL expression. Besides we observed by FACS an increase in GRAIL expression in naïve and memory CD4+T cell population ($p<0.05$ vs control). Furthermore, we tested if GRAIL expression could be induced directly and by different parasite strains. To perform this, CD4+T cells purified from BALB/c mice were incubated with Tulahuen or Y strain and 24h later GRAIL expression was assess by intracellular FACS staining. We have observed that both stains are able to induce GRAIL expression in vitro. These results may indicate that although GRAIL expression could be induced directly by different parasite strains its regulation may implicate a complex mechanism involving Otub-1, mTOR activation and IL-2.

037 (971) **MONOCYTIC MYELOID SUPPRESSOR CELLS ARE INVOLVED IN THE EXACERBATED INFLAMMATION DURING ACUTE TRYPANOSOMA CRUZI INFECTION IN TLR4 KNOCKOUT MICE**

Patricia Verónica Gonzalez[1], Augusto Paroli[1], Sabrina Rizzo[1], Roxana Cano[1], Susana Gea[1].
[1]Dpto Bioquímica Clínica CIBICI CONICET Facultad de Ciencias Químicas Universidad Nacional de Córdoba.

Background: Myeloid-derived suppressor cells (MDSCs) are key players in the resolution of hepatic inflammation during acute *T. cruzi* infection. There are two subsets: granulocytic (G-MDSCs: $CD11b^{+}LY6G^{+}LY6C^{low}$) and monocytic (M-MDSCs: $CD11b^{+}LY6G^{-}LY6C^{high}$). MDSCs may suppress immune response through re-

active oxygen species (ROS) and nitric oxide (NO) production. Recognition by toll-like receptors (TLRs) is critical for defense against *T. cruzi* and for triggering hepatic pathology. Previously, we reported that TLR4 deficiency produces an increase in hepatic M-MDSCs and ROS-producing M-MDSCs associated with high levels of GPT in plasma. Here, we evaluated plasma cytokine levels, hepatic intracellular IL10+, NO+, CCR5+ or CXCR2+ G- or M-MDSCs in TLR2 and TLR4 deficient and WT mice. Methods**:** Male C57BL/6 WT, TLR2-/- and TLR4-/- mice were i.p. infected with 1000 trypomastigotes (Tulahuén strain). MDSCs phenotype, intracellular IL10, CCR5 and CXCR2 expression, and NO detection were analyzed in G- or M-MDSCs from intrahepatic leukocytes by flow cytometry at 19 days post infection. Plasma cytokine levels (TNF-á, IL6, IL12, IFN-ã and IL10) were quantified by ELISA. Results: During infection, TLR4KO mice presented a higher number of NO producing hepatic M-MDSCs and lower number of NO producing G-MDSCs than WT and TLR2KO. TLR4KO mice showed a strong increase of inflammatory cytokines vs. other groups while IL10 remained similar to uninfected control. Likewise, intracellular IL10 expression did not change in any MDSCs population. The infection induced an increase in CCR5 expressing hepatic M-MDSCs from TLR4KO and WT mice while the number of CXCR2 expressing G-MDSCs was similar between both groups. Conclusion: The absence of TLR4 during acute infection triggered an increase in NO producing hepatic M-MDSCs as well as in plasma inflammatory cytokines without changes in IL10 levels, suggesting that the lack of TLR4 would be involved in the exacerbated inflammation of the liver.

PRESENTACIÓN DE POSTERS SAIC I / SAIC POSTER PRESENTATION I

CARDIOVASCULAR Y APARATO RESPIRATORIO/ CARDIOVASCULAR AND RESPIRATORY SYSTEM

038 (886) **EARLY HEART MITOCHONDRIAL DYSFUNCTION IN DIABETES INDUCED BY STREPTOZOTOCIN**

Ivana Agustina Rukavina Mikusic[1], Silvina Sonia Bombicino[1], Laura Beatriz Valdez[1].
[1]University of Buenos Aires, Institute of Biochemistry and Molecular Medicine (IBIMOL; UBA-CONICET), School of Pharmacy and Biochemistry, Physical Chemistry Division, Buenos Aires, Argentina.

Ventricular dysfunction in the absence of hypertension or coronary arterial disease is a complication of Diabetes Mellitus (DM) that could be associated to mitochondrial dysfunction. Results from our laboratory have shown a mitochondrial dysfunction in rat heart, 28 days after Streptozotocin (STZ)-injection, without alterations in cardiac performance in resting conditions but with a cardiac compromise against a work overload.

The aim is to study early events in heart of diabetic rats concerning mitochondrial function and nitric oxide and hydrogen peroxide metabolisms, using an experimental model of type 1 DM. Diabetes was induced by a single dose of STZ (60 mg/kg, ip.) in male Wistar rats. Glycemia values after 3 days of injection were 282±6 mg/dl. At 7, 10 or 15 days after STZ-injection, animals were sacrificed. Results were compared with ones obtained at 28 days after STZ administration. At 10, 15 and 28 days, the state 3 respiration sustained by malate-glutamate (308±14 ng-atO/min.mg protein) or by succinate (262±32 ng-atO/min.mg protein) were reduced by 20-22% or by 15-16%, respectively. Because of resting mitochondrial respiration rates were not modified in diabetic rats respect to control animals (malate-glutamate and succinate: 36±2 and 95±12 ng-atO/min.mg protein) respiratory controls were declined. Moreover, respiratory complexes activities (I-III, II-III and IV) were significantly reduced (20-22%) at 15 and 28 days, but slightly declined (10%) at 10 days post-STZ administration. After 7 days of injection, no difference in oxygen uptake and complexes activities were detected. Thus, an incipient heart mitochondrial dysfunction was observed after 10 days of STZ administration, a process that could be triggered in response to 7 days of a sustained hyperglycemia. More studies are going to be performed at 10 days post-STZ injection, in order to study the role of nitric oxide and hydrogen peroxide as molecules involved in mitochondrial-cytosol signalling

039 (37) **AMPK ACTIVATION IS HARMFUL FOR POSTISCHEMIC RECOVERY OF ISOLATED RAT ATRIA SUBJECTED TO SIMULATED ISCHEMIA-REPERFUSION (IS-RS) WHEN PALMITATE IS AVAILABLE AS ENERGY SUBSTRATE**.

Romina Hermann[1], Victoria Mestre Cordero[1], Federico Reznik[1], María de las Mercedes Fernández Pazos[1], Débora Vélez[1], Enrique Savino[1], María Gabriela Marina Prendes[1], Alicia Varela[1].
[1]Cátedra de Fisiología, Facultad de Farmacia y Bioquímica, Uba. IQUIMEFA-CONICET.

AMPK is a cellular “fuel gauge” enzyme, that regulates cellular fatty acid and glucose metabolism. The effects of AMPK in myocardial ischemia and reperfusion, when fatty acid levels are elevated, as observed in patients suffering acute myocardial infarction, are less well established.

We explored the effects of the pharmacological inhibitor, Compound C (CC;10 µM), in isolated rat left atria subjected to 75min Is-75min Rs, on inotropic response to isoproterenol (ISO) 2 **µM (contractile reserve: CR), tissue ATP content,** mitochondrial ATP synthesis and pyruvate dehydrogenase (PDH) activity after Is-Rs.

SD rats weighing 220–270 g were used. Atria were incubated in Krebs–Ringer containing glucose 10 mM (G) or G and 1,2 mM palmitate-3% BSA (P), 95% O_2–5% CO_2, pH 7,4. For Is, the incubation medium contained 10 mM 2-deoxy-D-glucose, 95% N_2–5% CO_2, pH 6,8. ANOVA, n=6.

Results showed a significant increase in AMPK activity during Is, remaining activated during Rs. CC prevented AMPK activation. CR was attenuated by Is-Rs, P increased this attenuation. CC increased the attenuation of CR in the presence of G as sole exogenous substrate, but reversed the harmful effect of P (Systolic force%: Rs:91±9; Rs+CC:67±7*; Rs+P:66±8*; Rs+CC+P:92±5;*p<0,05). This results were accompanied by similar changes in tissue ATP content (Rs:547±23; Rs+P:249±48*; Rs+CC:322±60*; Rs+CC+P:501±88 pmol/mg prot;*p<0,05). The rate of ATP synthesis by isolated mitochondria showed no significant differences between groups (Rs:1,0±0,1; Rs+CC:0,9±0,1; Rs+P:1,3±0,1; Rs+CC+P:1,3±0,4 nmol/min/mg mit prot). PDH activity was reduced by P or by CC in the presence of G as sole exogenous substrate (Rs:2,4±0,5; Rs+P:1,0±0,2*; Rs+CC:1,1±0,2*; Rs+CC+P:1,9±0,1 mU/mg mit prot;*p<0,05).

These results suggest that endogenous AMPK activation is harmful for postischemic recovery when P is available as energy substrate, possibly because of inhibition of PDH through Randle cycle effect and thus reduction of energy efficiency.

040 (97) **ANALYSIS OF BIOMARKERS OF INFLAMMATION AND OXIDATIVE STRESS IN CHAGAS DISEASE**

María Belén Martí[1], Susana Lioi[1], Gabriela Gerrard[1], Romina Diviani[1], María José Ceruti[1], Juan Beloscar[2], Mabel D Arrigo[1].
[1]Area Clinical Analytical Chemistry Fbioyf UNR 2 Cardiology Career. FCM. UNR.

Introduction: The pathophysiologic factors that control the formation and perpetuation of heart inflammation in chagasic patients were not yet fully clarified. Chronic inflammatory processes induce oxidative/nitrosative stress and lipid peroxidation. Objectives: Descriptive study of biomarkers of oxidative stress: enzymatic activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), lipid peroxidation (MDA / TBARS) and tumor necrosis factor alpha (TNFalpha) as a marker of inflammation. Materials and Methods: Samples of serum / blood were analyzed of chagasic individuals without cardiomyopathy (ECsinMCC: 45), chagasic with MCC (MCC: 48) and controls (CN: 65) underwent cardiovascular examination, electrocardiogram, chest radiography and supplementary examinations. All gave their consent. SOD, CAT, GPx and MDA / TBARS were analyzed by spectrophotometric methods (Kits Ransel Labs); TNFalpha by ELISA (BD). RESULTS: SOD (USOD/gHb): MCC 3600±750,

ECsinMCC 2710±190, CN 890±310; MDA / TBARS (nmol/ml): MCC 4.34±1.52, ECsinMCC 3.39±1.20, CN 2.33±0.60; CAT (K/gHb): MCC 316±68, ECsinMCC332±41, CN 185±28; GPx (U/gHb): MCC 98±17, ECsinMCC 102±20, CN 61±11; TNFalpha (pg / ml): MCC 33.3±7.2, ECsinMCC 26.1±6.8, CN 7.7±2.4. For the statistical study analysis of variance was performed and Kruskal Wallis. Level of significance was set at $p < 0.05$. The results show for SOD, MDA / TBARS and TNFalpha significant differences ($p < 0.01$) between MCC, ECsinMCC and CN. DISCUSSION: Increased activity of biomarkers of oxidative stress and inflammation in MCC is observed.The persistence of the parasite, would maintain an active and cronic inflammatoryprocess, that depending on the immunogenic or immunological characteristicsinduced by environment, would predominate a cellular response with specific-cytokine production. The TNF-alpha selectively would induce Mn-SOD. Further research should be done with more patients to clarify these pathophysiological aspects in the MCC

041 (136) **THIOREDOXIN-1 IS INVOLVED IN CARDIOPROTECTION CONFERRED BY ISCHEMIC POSTCONDITIONING**

Virginia Perez[1], Verónica D´Annunzio[1], Tamara Mazo[1], Timoteo Marchini[2], Lourdes Caceres[2], Pablo Evelson[2], Ricardo J. Gelpi[1].

[1]Institute of Cardiovascular Physiopathology, Department of Pathology, Faculty of Medicine, University of Buenos Aires, Argentina. [2]Institute of Biochemistry and Molecular Medicine (IBIMOL UBA-CONICET), School of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina.

Thioredoxin-1 (Trx1) maintains the cellular redox status and decreases the infarct size in ischemia/reperfusion injury (I/R). However, it is not fully understood the role of Trx1 in ischemic postconditioning (PostC). The aim was to study if Trx1 is involved in the PostC cardioprotection mechanism.

Wild type mice hearts (Wt), transgenic mice hearts overexpressing Trx1, and a dominant negative (DN-Trx1) mutant (C32S/C35S) of Trx1 were used. The mice hearts were subjected to 30 min of I and 120 of R (Langendorff technique) (I/R group). In the PostC group, after I, a protocol of 6 cycles of R/I (15 sec and 10 sec each), was performed. The assessment of the infarct size was performed using TTC. Reduced (GSH) and oxidized (GSSG) glutathione levels were measured by HPLC and Trx, pAkt and pGSK3beta expression were evaluated by western blot. Data are expressed as mean ± SEM and $p<0.05$ was considered statistically significant. n= 6 each goup.

The infarct size in the Wt-PostC group decreased in comparison to the Wt-I/R group (54.6±2.4 vs 40.0±2.1%, $p<0.05$), but this protection was abolished in DN-Trx1-PostC (47.7±1.1%). The Trx1-I/R and PostC reduced infarct size at the same magnitude (35.9±2.1 and 38.4±1.3%, respectively, $p<0.05$ vs. Wt-I/R). After I/R, the Trx1 expression decreased in Wt (0.32±0.09 AUDO). However, the PostC preserved the Trx1 expression (0.65±0.08 AUDO, $p<0.05$ vs. Wt-I/R), meanwhile DN-Trx1-PostC did not preserved Trx1 levels. In Wt-PostC, I/R-Trx1 and PostC-Trx1 increased Akt and GSK3beta phosphorylation compared to Wt-I/R, without changes in DN-Trx1 groups. In conclusion, Trx1 plays a key role in the PostC protection mechanism, since when this protein is inactive the cardioprotective mechanism was abolished. Given that the cardioprotection confer by both, Trx1 overexpression and PostC, is through the activation of Akt/GSK3beta cell survival pathway, no synergic effect was evidenced between both protection mechanisms.

042 (163) **DECREASE OF PLASMA LIPIDS WITHOUT IMPAIRMENT OF BLOOD FLUIDITY BY LIGARIA CUNEIFOLIA (LC) PROTOANTHOCYANIDINE ENRICHED FRACTION IN WISTAR RATS FED WITH HIGH FAT DIET (HFD)**

Natasha Gerschcovsky[1], Sebastian Galliano[1], Gloria García[1], Alicia Dominighini[1], José Gonzálvez[1], Leda Urli[1], Diego Crosetti[1], Juan Monti[2], Flavia Lambertucci[2], María Teresa Ronco[2], Marcelo Wagner[3], Cristina Ester Carnovale[2], Alejandra Nora Luquita[1].

[1]Cátedra de Biofísica. Facultad de Ciencias Médicas. CIURN. UNR. [2]Cátedra de Fisiología,FCByF-UNR, IFISE-CONICET. [3]Cátedra de Farmacobotánica. Fac. de Farmacia y Bioq.-UBA.

We have previously demonstrated that intraperitoneal (i.p.) injection of *Lc* crude extract produces a plasma cholesterol (Cho) decrease and blood viscosity increase in rats. Proanthocyanidine (P*Lc*) was purified to analyze the effect on Cho, Triglycerides and blood fluidity in adult male Wistar rats (aged 70 days, n=16) fed with standard chow diet added with 40% bovine meat juice during 28 days (HFD). Rats were administered with either physiological solution (controls C, n=8) or P*Lc* 3mg /100g body weight (treated T; n=8) i.p. each 24hr during 7 days. On day 8 they were anesthetized i.p. with Ketamine/Xylazine (100mg/kg/3mg/kg) to obtain blood samples by cardiac puncture. Plasma assays: Cho (enzymatic method with cholesterol oxidase esterase), Triglycerides, HDL-Cho, and LDL-Cho. Blood assays: total blood viscosity (TBV) and plasma viscosity (PV) by rotational viscosimeter. Standardized relative BV (SBV) to a 45% hematocrit [(BV/PV)45/Hto]; rigidity index (RI), the inverse of erythrocyte deformability (ED) by filtration method. Results: (mean ± SD). Plasma Cho (mg%): C:145.25±8.53 , T: 62.88±3.23**; ChoHDL: C:25.00±1.35; T:29.5±2.29 (n.s.); ChoLDL: C:28.86±2.41; T:21.37±2.19*; Triglycerides: C:360.71±27.48, T:71.38±11.04**; SBV to a 45% hematocrit: C:5.09±0.45, T:5.41±0.49(n.s.); RI: C:8.37±1.45, T:9.61±1.43(n.s.); Mean Corpuscular Volume (µm3): C:72.02±5.74; T:65.26±4.65(n.s.) (*$p<0.05$; **$p<0.001$vs C, Student's *t* Test for unpaired data). P*Lc* treatment decreases Cho, ChoLDL and Triglycerides without alteration of blood viscosity neither in RI nor in MCV in rats fed with a hig fat diet, thus showinga lipid-lowering effect.

043 (138) **GENETIC DELETION OF GALECTIN 3 REDUCED THE INFARCT SIZE AND PRESERVED MYOCARDIAL CONTRACTILITY IN AN EXPERIMENTAL MODEL OF ISCHEMIA AND REPERFUSION IN MICE**

Nadia Laura Martinez Naya[1,2], Pablo Cassaglia[1], Maria Eugenia Aruanno[1], Celina Morales[1,2], Germán E. González[1,2].

[1]Instituto de Fisiopatologia Cardiovascular, Departamento de Patologia, Facultad de Medicina, Universidad de Buenos Aires. [2]Instituto de Bioquimica y Medicina Molecular

It has been previously shown that genetic deletion of Galectin 3 (Gal-3) reduces inflammation, oxidative stress and unfavorably modifies the ventricular remodeling after permanent coronary artery ligature in mice. Despite its active involvement in the inflammatory response, the role of Gal-3 in a model of myocardial ischemia and reperfusion has not been studied. Therefore, we hypothesize that genetic deletion of Gal-3 reduces myocardialinfarct size and improves ventricular function after 2 hours of reperfusion in mice.

Male C57 (n=6) and Gal-3KO (n=7) mice were subjected to 30 minutes of regional myocardial ischemia followed by 2 hours of reperfusion. Meanwhile, heart rate (HR, BPM), LV systolic pressure (LVSP, mmHg), LV $+dP/dt_{max}$ (mmHg/sec) and LV end diastolic pressure (EDP, mmHg) were continuously monitored by cardiac catheterization. Once the protocol was finished, hearts were perfused with Evans blue to determine the area at risk. Then the infarct size was measured by using the triphenyltetrazolium chloride technique.

The area at risk was similar between groups (C57: 38±4% vs Gal-3KO: 36±4%; p=NS) while the infarct size was significantly reduced in Gal-3KO mice (C57: 71±4% vs Gal3KO: 52±5%; $p<0,001$). Thirty minutes of myocardial ischemia diminished the contractility by 36±10% in C57 mice and by 16±10% in Gal-3KO mice (P=NS between groups). At this time, every other ventricular function parameter was preserved.

At the end of the follow up, myocardial contractility in C57 mice was still reduced ($p< 0.05$ vs C57 baseline) but it was preserved in Gal-3KO mice (p=NS vs Gal-3KO baseline). Thus, we observed a significant difference in this parameter between groups (99 ± 13% of Gal-3KO baseline vs 61 ± 5% of C57 baseline; $p<0,05$).

In conclusion, the lack of Gal-3 reduced the infarct size and improved myocardial contractility after two hours of reperfusion in an experimental model of myocardial ischemia and reperfusion in mice.

044 (185) **P66SCH AND ITS ROLE IN MITOCHONDRIAL HOMEOSTASIS THROUGHOUT THE AGING PROCESS**

Hernan Perez[1], Maria Eugenia Elguero[1], Nerina Mariel Villalba[1], Yael Alippe[1], Juan Jose Poderoso[1], Maria Cecilia Carreras[1]

[1]Laboratory of Oxygen Metabolism, INIGEM, University Hospital, University of Buenos Aires

In accordance with the free radicals dependent aging theory, it has been widely accepted that this process is triggered and sustained by the deleterious accumulative effect of oxidative damage caused by mitocondrial respiration generated ROS. P66shc has been proposed as a longevity genetic determinant that regulates both apoptosis and metabolism through ROS generation.

In the present work we assessed p66shc function on mitochondrial metabolism and physiology throughout the aging process. With this in mind, and by means of a murine model consisting of p66shc (p66shc-/-) KO mice, we assessed oxidative metabolism in brain mitochondria of 3-24-month-old mice. The p66shc-/- mice present a longer life expectancy (30%), show diminished weight, evidenced by a 25% body weight reduction, and exhibit changes in mitochondrial function and physiology while aging, such as increased mitochondrial DNA content, not significantly decreased activity in the respiratory chain complexes, elevated ATP levels (P<0,05), higher NAD+/NADH ratio (P<0,05), and a reduction in H2O2 production rate concomitant with a decrease in MnSod activity (P<0,05). In aged KO mice we also observed an increment in RNS by means of Western Blot and flow cytometry essays (P<0,05).

As previously reported, an increase in NO levels, triggered mitochondrial biogenesis modulating mfn2. WB and qPCR results showed a coordinated regulation of protein expression related to the mitochondrial fusion/fission and biogenesis such as Drp1, Opa1, Mfn2 and PGC1a during aging in KO mice. Changes in mitochondrial dynamics were evidenced by transmission electron microscopy imaging. We therefore conclude that the metabolic rate reduction in p66shc-/- mice improves mitochondrial homeostasis coordinating mitochondrial biogenesis as well as the fusion/fission balance. This in turn, corresponds with a delay in the aging process.

045 (492) **ROSUVASTATIN (R): CHANGES IN MITOCHONDRIAL PERMEABILITY TRANSITION PORE (MPTP)**

Debora Elisabet Velez[1], Victoria Evangelina Mestre Cordero[1], Juliana Andrea Perego[1], Romina Hermann[1], Enrique Alberto Savino[1], Alicia Varela[1], Maria Gabriela Marina Prendes[1].

[1]Cátedra de Fisiología. Departamento de Ciencias Biológicas. Facultad de Farmacia y Bioquímica. Universidad de Buenos Aires. IQUIMEFA-CONICET

Previously in our laboratory we found that acute administration of R in hearts subjected to ischemia-reperfusion (I-RP) has direct cardioprotective effects, apparently linked with PI3K/Akt pathway. The goal of the present work was to determine the relationship between this route, MPTP opening , mitochondrial morphology and lactic acid production in Langendorff perfused hearts from female Wistar rats (250-300g body weight) feeding ad libitum. R (3uM) and wortmannin (W), PI3K/Akt inhibitor (100nM), were added 10 and 15 min before global I respectively. The hearts were subjected to 25 min of I and 60 min of RP. Tissue samples were taken at the end of stabilization and ischemia to determinate lactic acid content, electronic microscopies were performed at the same time for assess mitochondrial damage. Activation of PI3K/Akt pathway was studied by western blot. Mitochondrias were isolated at the end of RP to evaluate MPTP opening using cyclosporine A as MPTP opening inhibitor in order to indicate that changes in Abs were due to MPTP opening. The R delayed the opening, while W annulled the differences between the experimental groups (results expressed as% drop in initial 200uM and 300uM Abs with $CaCl_2$ respectively) C: 2.50±0.34; R: 0.75±0.15*; W: 2.55±0.22; R+W:2.43±0.28/C: 2.25±0.30; R: 1.24±0.20*; W: 2.50±0.23; R+W: 2.43±0.30 (*p<0.05 against% Abs drop in response to all lower concentrations of $CaCl_2$ in the same group). Electronic microscopy showed greater mitochondrial conservation in the group treated with R. Lactic acid content were meaningfully lower in those hearts treated with R (umoles/g dw): C: 146.28±6.60*; R: 56.68±6.77; W: 65.31±5.22; R+W: 61.35±4.10 (*p<0.05 against all groups), showing a possible link between the lower production of fixed acids in the tissue and a delay in the opening of MPTP. On account of bigger relation between Akt-P/Akt-T in R group, these events could be link with the activation of PI3K/Akt pathway.

046 (188) **INCREASED CARDIAC SYMPATHETIC TONE INDUCES DEPRESSIVE BEHAVIOR IN TRANSGENIC MICE OVEREXPRESSING CARDIAC GS-ALPHA**

Méndez Diodati Nahuel[1], Bruno Buchholz[1], Jazmín Kelly[1], Christian Höcht[2], Julieta S. Del Mauro[2], Stephen F. Vatner[3], Ricardo J. Gelpi[1]

[1]Instituto de Fisiopatología Cardiovascular, Departamento de Patología, Facultad de Medicina, Universidad de Buenos Aires. [2]Departmento de Farmacología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires. [3]Department of Cell Biology & Molecular Medicine, New Jersey Medical School, Rutgers University.

Cardiac specific transgenic (TG) overexpression of Gsa protein in mice increases sympathetic drive and cardiac function, which is generally associated with enhanced cardiac activity. The goal of this investigation was to determine if increasing sympathetic tone in this model affected behavior. We subjected TG mice and their wild type (WT) littermates to a novel, open field environment for 2 hours, a forced swimming test, i.e., Porsolt test, for 6 min, a tail suspension test for 6 min, a light/dark box test for 10 min, and a rotarod test. Gsa transgenic mice displayed marked hypoactivity, which was characterized by significant reduction in locomotion, jumps, turns and vertical activity in the open field, but no behavioral changes were observed in the home cage. Latency time and swimming time were significantly less in the Porsolt test in TG mice and immobility time in the tail suspension test was higher in the same group. These behavioral changes were reversed by treatment with propranolol. The anxiety test in the light/dark box and motor coordination in the rotarod test were not significantly altered in TG mice. Western blotting confirmed that Gsa protein was markedly increased in the heart of TG mice compared to WT, but not in areas of the brain likely involved in the regulation of behavior, such as the amygdala, the olfactory bulb, the hippocampus the midbrain and the striatum. TG mice with cardiac specific overexpression of Gsa exhibit enhanced cardiac function, but show blunted behavioral responses to stress, indicative of depression, as confirmed by the Porsolt and tail-suspension test, providing a link between increased sympathetic regulation of the heart and behavior. The novel features of this investigation is the finding of depressive disorders as a result from overexpression of a cardiac protein without primary alterations in the brain, or from enhanced cardiac sympathetic tone.

047 (903) **INTRAMYOCARDIAL TREATMENT WITH HIGH DOSES OF HIGH MOBILITY GROUP BOX-1 (HMGB1) PROTEIN IMPROVES CARDIAC FUNCTION IN SHEEP WITH MYOCARDIAL INFARCTION**

María del Rosario Bauzá[1], Paola Locatelli[1], Carlos sebastián Gimenez[1], Andrea De Lorenzi[1], Sandra Wray[1], Luis Alberto Cuniberti[1], Alberto Jose Crottogini[1], Fernanda Daniela Olea[1]

[1]IMETTYB - Universidad Favaloro- CONICET Hospital universitario Fundación Favaloro

Introduction: The effect of the pro-inflammatory HMGB1 protein on experimental infarct size is controversial. A possible reason is the lack of knowledge about the therapeutic doses. In a previous study on sheep with acute myocardial infarction (AMI) comparing 1 ug and 10 ug injected intramyocardially, we have shown that only the 10 ug dose induces angio-arteriogenesis and overexpression of genes involved in cell differentiation. We now aimed at evaluating the effect of this dose on left ventricular function in the ovine AMI model.

Methods: Four hours after coronary ligation, sheep were randomized to receive intramyocardial HMGB1 10 ug or PBS (placebo) in the peri-infarct zone. Echocardiographic assessment of percent wall thickening of the peri-infarct septum (%SWT) and the peri-infarct antero-lateral wall (%AWTh), and percent ejection fraction (%EF) was performed at baseline, 3 and 30 days post-AMI. Statistics: two-way ANOVA-Bonferroni; data expressed as X±SE; significance: p<0.05.

Results: As expected, at 3 days after AMI, all 3 indexes decreased significantly with regard to baseline in both groups. At 30 days, in the placebo group%SWTh decreased from 42.2±3.2% at baseline to 7.4±5.7% (p<0.005),%AWTh from 41.6±5.9% to 14.6±5.7% (p<0.005) and%EF from 68.5±5.6% to 51.8±6.3% at 30 days (p<0.01). In contrast, in the HMGB1 group,%SWTh was 34.3±6.8% at baseline and 30.9±6.8% at 30 days (p=NS),%AWTh was 42.6±3.6% at baseline and 27.3±5.1% at 30 days (p=NS), and%EF 66.8±3.2% at baseline and 54.4±5.4% at 30 days (p=NS).

Conclusion: Intramyocardial high dose HMGB1 preserves the assessed regional and global indexes of cardiac function in a sheep model of AMI at 30 days after treatment.

048 (295) **ASSOCIATION OF ANGIOTENSIN II TYPE 1 RECEPTOR A1166C GENE POLYMORPHISM AND ESSENTIAL HYPERTENSION IN A POPULATION FROM SAN LUIS**

María Milagros Correa[1,2], María Elena Arce[1], Gladys María Ciuffo[1,2], Lucia Beatriz Fuentes[1,2].
[1]Universidad Nacional de San Luis. [2]IMIBIO-SL-CONICET

Hypertension is associated commonly with stroke and cardiovascular disease, leading to significant morbidity and mortality. Epidemiological studies reported that environmental factors could contribute to the risk of essential hypertension (EH), and suggested that is a complex disease resulting the combined influence of environmental factors and genetic determinants. The rennin angiotensin system (RAS) may play a crucial rolein the regulation of blood pressure and pathogenesis of EH. Genetic polymorphismsin the RAS genes, such as angiotensinogen, angiotensin II type 1 receptor (AT_1) and angiotensin converting enzyme have been investigated as potential genetic factors involved in EH. The aim was to analyses the association between EH and the main genetic polymorphisms $A^{1166}C$ of AT_1 in hypertensive and normotensive individuals from San Luis (Argentina). 112 patients were selected from general population of our community during 2015. Ethics committee approval the study and informed consent was obtained. Hypertensive subjects 57 (49% women), age 54.5±8.6 years, systolic and diastolic blood pressure 152.7±17.8/88.9±11.1 mmHg respectively. Normotensive individuals 55 (69% women), age 40.8±15.6 years, systolic and diastolic blood pressure 116.8±11.3/69.8±10.1 mmHg. The $A^{1166}C$ polymorphism was analyzed by PCR-RFLP and biochemical parameters were determined. Frequency genotypes in hypertensive/control: AA 43.9/58.2%, AC 43.9/32.7% and CC 12.3/9.1%, frequency alleles A^{1166}0.65/0.75 and C^{1166} 0.35/0.25. Chi square analysis shows genotypic CC was statistically significant in hypertensive women >38 years (p<0.03), systolic and diastolic blood pressure (156.2±22/94.0±9 mmHg), body mass index (35.3±4.2) and biochemical risk parameters were increased. We suggest a correlation between $A^{1166}C$ polymorphism and risk of cardiovascular disease in hypertension women. Further studies in a larger population are needed to clarify the genetic association between candidate genes and EH.

049 (1065) **VALPROATE DECREASES TRAN GENERATIONALLY BLOOD PRESSURE BY AFFECTING TRH PROMOTER DNA METHYLATION AND GENE EXPRESSION IN SHR**

Maria Silvina Landa[(1,2)], Mariano Luis Schuman[(2)], Ludmila Peres Diaz[(2)], Maia Aisicovich[(2)], Mariela Gironacci[(3)], Silvia Ines García[(2)], Carlos J Pirola.
(1) Department of Molecular Genetics and Biology of Complex Diseases, Institute of Medical Research A Lanari-IDIM, University of Buenos Aires-National Scientific and Technical Research Council (CONICET), Ciudad Autónoma de Buenos Aires, Argentina (2) Laboratory of Molecular Cardiology, Institute of Medical Research A Lanari-IDIM, University of Buenos Aires-National Scientific and Technical Research Council (CONICET), Ciudad Autónoma de Buenos Aires, Argentina. (3) Departamento de Química Biológica, IQUIFIB-CONICET, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina

In the central nervous system, TRH acts as neurotransmitter involved in cardiovascular regulation. We demonstrated that the overexpression of diencephalic TRH (dTRH) in SHR rats could be reverted by antisense treatment normalizing the blood pressure (BP). Valpoate (VPA), an inhibitor of histone deacetylases, can modulate gene expression through epigenetic alterations such as DNA methylation. Here, to study the role of HDAC inhibition in the regulation of the TRH expression and its effect on the pathogenesis of hypertension, we treated seven weeks old male SHR and WKY with VPA. Blood pressure was recorded weekly; following 10 weeks of treatment rats were euthanized. BP and dTRH expression were increased in SHR vs WKY. VPA attenuated the higher blood pressure seen in untreated SHR, without effect in WKY strain. Changes in blood pressures were paired with alterations in the dTRH mRNA expression. Indeed we found a significant 62% reduction in the abundance of dTRH mRNA of the SHR+VPA group compared to SHR control group. Decreased TRHmRNA induced by HDAC inhibition was confirm “in vitro” by neuron primary culture using TSA. We performed methylation specific PCR and demonstrated a significant increase of the DNA methylated level in SHR+VPA group compared to SHR control and a significant negative correlation between methylation status and dTRH mRNA expression. Another group of male and female SHR and WKY were treated with VPA as described. After 2 weeks of the treatment interruption, rats were mated. Offspring born from VPA treated parents did not receive VPA ever. We observed that changes in BP, TRH expression and methyaltion status were reproduced in offspring showing a transgenerational inheritance. Thus, these results suggest that TRH modulation by epigenetics mechanism may affect BP and could be inherited by the next generation in SHR rats.

050 (302) **EFFECT OF CIGARETTE SMOKE ON HUMAN RESPIRATORY CULTURE EPITHELIUM: EXPRESSION AND ACTIVITY OF CFTR, OXIDATIVE STRESS AND INFLAMMATORY RESPONSE**

Angel Gabriel Valdivieso[2], Andrea Vanesa Dugour[1], Mariangeles Clauzure[2], Juan Manuel Figueroa[1], Tomas Santa Coloma[2].
[1]Centro de Biología Respiratoria (CEBIR)-Fundación Pablo Cassara. [2]Instituto de Investigaciones Biomédicas (BIO-MED, CONICET). Universidad Católica Argentina (UCA).

The chronic obstructive pulmonary disease (COPD) is directly related to cigarette consumption. Recent evidences suggest a possible relationship between the CFTR Cl^- channel and COPD (CFTR mutations cause cystic fibrosis (CF)). Both disorders share clinical and functional similarities, in particular oxidative stress and inflammation. In CF cells, a CFTR failure is also associated with a mitochondrial complex I (mCxI) dysfunction. Here we initiated studies on the mechanism involved in the inflammatory response in COPD, and the possible role of CFTR. Calu-3 cells (human lung epithelium) were treated with a concentrated extract of smoke (CSE) for 24 h and the CFTR activity (using SPQ) and expression (qRT-PCR), the production of reactive oxygen species (ROS) (DCFH-DA for total ROS and MitoSOX™ for mitochondrial ROS), the mCxI activity (measuring mitochondrial NADH-cytochrome c reductase activity) and the release of IL-8 (by Elisa) were measured. CSE treatment significantly decreased the CFTR expression and activity. This was accompanied by a significant decrease of the mCxI activity and a significant increase in ROS levels, in concordance with the increased release of IL-8. The addition of NAC together with CSE prevented the increased ROS and IL-8 levels, but did not prevented the decreased CFTR

expression nor the mCx-I dysfunction. Also, treatment with the NF-kB inhibitor IKK-2 abolished the increased IL-8 secretion. The results suggest that CSE effects on IL-8 are mediated through a ROS-induced activation of NF-kB. CSE is also able to cause changes in CFTR, mCx-I and ROS similar to those observed in CF cells. The understanding of the mechanisms involved in these effects would be of interest for the development of new therapeutic strategies. Acknowledgements: Supported by grants PICT 2007-00628 and PICT 2012-1278 from ANPCYT to TASC; PIP 11220080102551 2009-2011 and PIP 11220110100685 2012-2014 to TASC (CONICET); UCA to TASC; and Fundación Cassará to FJM and DAV.

051 (419) **25-HYDROXYVITAMIN D IS RELATED TO MARKERS OF VULNERABLE PLAQUE IN ACUTE MYOCARDIAL INFARCTION**

Nahuel Fernandez Machulsky[1,2], Juan Gagliardi[3], Magalí Barchuk[1,2], Diego Gonzalez[1,2], Laura Schreier[1,2], Bibiana Fabre[1,2], Gabriela Berg[1,2,4]

[1]Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Departamento de Bioquímica Clínica. Cátedra de Bioquímica Clínica I. Buenos Aires, Argentina. [2]Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Instituto de Fisiopatología y Bioquímica Clínica (INFIBIOC). Buenos Aires, Argentina. [3]Hospital General Dr. Cosme Argerich. Division Cardiologia. Unidad de Hemodinamia. Buenos Aires, Argentina [4]Universidad de Buenos Aires. CONICET. Facultad de Farmacia y Bioquímica. Buenos Aires, Argentina.

Vitamin D is a fat soluble vitamin mainly involved in the calcium and bone metabolism; recently its deficiency has been related to cardiovascular disease (CVD). In cardiac tissue, 1,25 (OH)2 vitamin D suppress metalloproteinases (MMP) expression directly associated with vulnerable plaque, although no data was reported in acute myocardial infarction (AMI) patients. Otherwise, leptin, produced by adipose tissue, is associated with CVD, however there are no reports about the association between leptin and vitamin D in AMI patients. Our aim was to investigate whether there is a relationship between MMPs activity, leptin and vitamin D in patients with AMI. Methods and results: we studied 66 consecutive male patients (62±1.6 years) with AMI from Argerich Hospital, Buenos Aires, referred for primary angioplasty. Half of the studied population was recruited in spring/summer seasons. The protocol was approved by the Ethics Committee of the Argerich Hospital. Blood samples were obtained at admission and 24hs after the cardiac protocol. Leptin (RIA) and vitamin D (chemiluminescence) determinations were performed in serum; MMP-2 and -9 activity was determined in plasma by gelatinolytic zymography. Vitamin D was higher in patients hospitalized in spring/summer (p=0.007). MMP-2 activity was significantly higher in patients with deficient/insufficient concentration of vitamin D at admission (p=0.02) and 24hs later (p=0.03). In a linear regression model, vitamin D explained 24% of the variance of MMP-2 activity (F=2.839 p=0.04). At admission, vitamin D correlated with serum leptin (r=-0.302 p=0.033) and in a linear regression model, explained 39.5% of the variation of serum leptin (F=4.432 p=0.003). Both models independently of the season. Conclusions: In the studied population, vitamin D was related to MMP-2 and leptin which are involved in the pathogenesis of coronary artery disease and AMI, holding a possible mechanism for the effect of vitamin D on CVD.

052 (457) **OXIDATIVE STRESS MARKERS AND TRANSPORTERS EXPRESSION IN THE HEART OF FRUCTOSE-FED RATS TREATED WITH THE CARDIOTOXIC ANTINEOPLASIC DOXORUBICIN**

Natalia Soledad Ogonowski[1], Natalia Lucía Rukavina Mikusic[2], Nicolás Kouyoumdzian[2], Andrea Fellet[1], Inés Rosón[2], Marcelo Choi [2], Ana Balaszuk[1], Stella Maris Celuch[3].

[1]Cátedra de Fisiología (FFyB, UBA). [2]Instituto de Investigaciones Cardiológicas (ININCA, UBA-CONICET). [3]Instituto de Investigaciones Farmacológicas (ININFA, UBA-CONICET)

Doxorubicin (DOX) clinical use as chemotherapeutic agent is limited due to the development of cardiomyopathies and heart failure. Our interest is to study whether the cardiotoxicity of DOX could be increased in conditions with cardiovascular risk factors. In this sense, we reported that in a model of metabolic syndrome caused by fructose-feeding (FRU) in rats, a single dose of DOX decreased the ejection and shortening fractions in the left ventricle, suggesting greater cardiotoxicity than in control (C) animals. Both FRU and DOX produce oxidative stress. Moreover, DOX changes the expression of the plasma membrane transporter P-GP which extrudes DOX from cells. Also, a possible mechanism of its cardiotoxicity is the depletion of carnitine which is uptaken by OCTNs transporters. The aim of this study was to analyze the effects of DOX on oxidative stress markers and expression of P-GP and OCTNs in cardiac tissue of C and FRU rats.

Male Sprague-Dawley rats receiving either tap water or water with 10% fructose during 8 weeks were treated with DOX (6 mg/kg, ip, md) or vehicle (ISS) (n=4/group). Three days after injection, rats were sacrificed and left ventricles were excised to measure oxidative stress markers and perform western blot for P-GP and OCTN1/2/3.

Both FRU and DOX enhanced TBARS production with a significant increase in the FRU+DOX group compared to C (1.71±0.27 vs 0.97±0.11 nmol/mg prot; p<0.05). There were not changes in superoxide dismutase and catalase activities. The expression of P-GP in FRU was 55% lower than in C (p<0.01). DOX showed a tendency to increase P-GP expression, although FRU+DOX values remained 35% lower than C (p<0.01). Similar results were obtained with OCTNs. It is suggested that a minor efflux of DOX due to reduced expression of P-GP and a possible depletion of carnitine related to decrease in OCTNs expression could contribute to the greater cardiotoxicity of DOX in FRU rats. Supported by CONICET (PIP 112-201201-00425).

053 (518) **THE ROLE OF THIOREDOXIN 1 IN THE ISCHEMIC POSTCONDITIONING ON DYSLIPIDEMIC MICE**

Tamara Mazo[(1)], Virginia Perez[(1)], Anabella Gómez[(1)], Magali Barchuk[(2)], Gabriela Berg[(2)], Verónica D´Annunzio[(1)], Ricardo J Gelpi[(1)].

(1)Department of Pathology. Institute of cardiovascular physiopathology. Faculty of Medicine. University of Buenos Aires, Argentina. (2) Department of Clinical Biochemistry, Laboratory of Lipids and Atherosclerosis, Faculty of Pharmacy and Biochemistry, INFIBIOC-University of Buenos Aires, Argentina.

It is known the damaged by reactive oxygen species due toischemia/reperfusion (I/R) is exacerbating in dyslipidemia condition and thereforeincreased infarct size. Regarding to cardioprotection mechanism, it has beenshown that dyslipidemia could modify the infarct size reduction conferred byIschemic postconditioning (IP). However, it has not been showed whether thelack of protection if PI could be related with thioredoxin 1 system (Trx1).Therefore the aim was to evaluate if IP exerts cardioprotective effect ondyslipidemic mice and determine if these lack of reduction of infarct size arerelated to changes in Trx1 expression. C57 / BL6 males mice were used, fed withcontrol diet or high-fat diet (HFD) during 12 weeks. We measured clinical biochemistry parameters and thehearts were subjected to 30 min of I and 120min R (Langendorff) (Group I/R, n=7), or an IP protocol (n=7, 6 Cycles of R/I, 10 sec each). We assessed ventricular function, infarct size and Trx 1 expression (western blot, n = 5 per group). In HFD mice did total cholesterol, LDL and HDL increased compared to control mice, but triglycerides did not change. The behavior of ventricular function was similar in all the groups. In control mice IP decreased infarct size compared to I/ R gorup (I/R: 55.2±2.6 vs.PI:40.2 ±1.6,p =0.05), however the cardioprotection was abolished in DAG mice (I/R: 67.0±4.0vs.PI:61.0±4.2) .In basal conditions, the Trx1 expression was higher in HFD mice compared with control group (control: 0.79±0.14 vs.DAG: 1.98 ± 0.51p <0.05). In conclusion, our data suggest that the cardioprotection afforded by PI is abolished in mice HFD, at least in part by inactivation of Trx1, since the antioxidant expression did not change.

054 (603) **REMOTE ISCHEMIC PRECONDITIONING ACTIVATES ADENOSINE A1 RECEPTOR AND ATTENUATES MITOCHONDRIAL DAMAGE DURING MYOCARDIAL REPERFUSION**

Diamela Paez[1], Martin Donato[1, 2], Mariana Garces[2], Timoteo Marchini[2], María Ailín Goyeneche[1], Pablo Evelson[2], Ricardo J Gelpi[1,]

[1]Instituto de Fisiopatología Cardiovascular, Facultad de Medicina, Universidad de Buenos Aires [2]Instituto de Bioquímica y Medicina Molecular (IBIMOL), CONICET

Introduction: Remote ischemic preconditioning (rIPC) reduces infarct size through the activation of pre-ischemic muscarinic pathway. However, the mechanism activated during reperfusión remains unclear.

Objective The first aim was to evaluate whether A_1 adenosine receptor is part of the rIPC cardioprotective mechanism during early reperfusion. A second objective was to evaluate the effect of rIPC on ischemia/reperfusion mitochondrial damage.

Methods: Isolated rat hearts were subjected to 30 minutes of global ischemia and 60 minutes of reperfusión (I/R, n=9). In a second (n=10) group, before the isolation of the heart, a rIPC protocol (three cycles of left femoral artery ischemia/reperfusion) was performed, followed by I/R protocol. In a third group (n=5), the above protocol was repeated but, during the first 5 min of reperfusion, an adenosine A1 receptor blocker was administered (DPCPX). We evaluated infarct size using triphenyl tetrazolium chloride staining and oxidative damage to macromolecules by TBARS and carbonylated proteins. Additionally, we measured mitochondrial respiration and H_20_2 production rate in freshly isolated mitochondria.

Results: rIPC significantly decreased infarct size (50,33±2,74 vs 31,29±2,52%, p< 0,05 Vs I/R) and this effect was abolished by DPCPX administration (55,74±6,41%). rIPC significantly decreased carbonylated proteins without any modification in TBARS content. Finally, 30 min of global ischemia followed by 60 min of reperfusion induced an impairment of mitochondrial respiration and decreased H_2O_2 production rate, which was attenuated in the rIPC group.

Conclusions: rIPC reduces infarct size by activation of adenosine A_1 receptors at reperfusion, and attenuates oxidative stress, preserving mitochondrial function.

055 (680) **THYROID DESEASE: CARDIOVASCULAR NITRIC OXIDE TO HYPOVOLEMIA**

Natalia Ogonowski[1], Gisselle Piro[1], Déborah Pessah[1], Noelia Arreche[1], Bernardita Puchulu[1], Ana M Balaszczuk[1], Andrea Fellet[1]

[1]Department of Physiology, School of Pharmacy and Biochemistry, IQUIMEFA-CONICET,Universidad de Buenos Aires, Buenos Aires, Argentina

We previously demonstrated that activation of nitric oxide (NO) pathway is involved in the restoration of vascular volume and blood pressure following bleeding. Cardiovascular function is influenced by the autonomic nervous system and numerous endocrine hormones in which thyroid hormones have relevance. Additionally, a functional relation involving thyroid hormones, endothelial cells and NO has been extensively described in the past several years. This study aimed to investigate whether NO participates in the cardiovascular function and haemodynamic adaptation to acute haemorrhage in animals with thyroid disorders. Sprague-Dawley rats aged 2 months old treated with T3 (hyper, 20 µg/100 g body weight) or 0.02% methimazole (hypo, w/v) during 28 days were pre-treated with NG nitro-l-arginine methyl ester (L-NAME) and submitted to 20% blood loss. Heart function was evaluated by echocardiography. Measurements of arterial blood pressure, heart rate, nitric oxide synthase activity and protein levels were performed. Hypothyroid animals had decreased fractional shortening and ejection fraction and increased left ventricle internal diameter. However, hyperthyroid rats had decreased ventricle diameter and no changes in cardiac contractility. Haemorrhage elicited a hypotension of similar magnitude within 10 min. Then, this parameter was stabilized at about 30–40 min and maintained until finalized, 120 min. L-NAME rats showed that the immediate hypotension would be independent of nitric oxide. Nitric oxide synthase inhibition blunted the changes of heart rate induced by blood loss. Animals with thyroid disorder had lower atrial enzyme activity associated with a decreased enzyme isoform in hypothyroid group. In ventricle, thyroid abnormalities were associated with higher enzyme activity, which was not correlated with changes in protein levels. Haemorrhage induced an increased heart nitric oxide production. We concluded that thyroid disorders were associated with hypertrophic remodelling which impacted differently on cardiac function and its adaptation to a hypovolemia. Hypovolemia triggered a nitric oxide synthase activation modulating the heart function to maintain haemodynamic homeostasis. This involvement depends on a specific enzyme isoform, cardiac chamber and thyroid state.

056 (686) **ANALYSIS OF ANTICOAGULANT AND ANTIPLATELET CAPACITY OF NITINOL-POLYPYRROLE DEVICES DOPED WITH DRUGS**

María Ivone Valle[1], Daniel O Flamini[2], María Julia Sandoval[1], Silvana Beatriz Saidman[2], Virginia Massheimer[1]

[1]Instituto de Ciencias Biológicas y Biomédicas del Sur (INBIOSUR)- Universidad Nacional del Sur (UNS)-Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)-Departamento de Biología, Bioquímica y Farmacia-Cátedra de Bioquímica Clínica II- Bahía Blanca- Argentina. [2]Instituto de Ingeniería Electroquímica y Corrosión (INIEC)- Universidad Nacional del Sur (UNS)- Departamento de Ingeniería Química- Bahía Blanca - Argentina.

Hemostasis plays an active role in atherosclerotic disease and arterial stenosis. Nitinol (NiTi) is used in several biomedical applications such as stents. Despite its biocompatibility, elasticity and corrosion resistance, some Ni^{2+} and Ti^{2+} ions may be released, resulting in local and systemic unwanted effects. The use of polypyrrole (PPy) coating, protects from ion release and, allows doping with drugs designed such as heparin (Hep) and sodium salicylate (NaSa). This work evaluates the effect on blood coagulation and platelet aggregation (PA) of NiTi-PPy devices (slices of $1cm^2$) doped with Hep (0.2; 0.3; 0.4 g/L), NaSa (0.1 and 0.5 M) or the combination of NaSa plus Hep (0.5M + 0.2 g/L). The slices were incubated 35 min with human plasma, and immediately after aliquots were taken for coagulation tests. Platelet poor plasma (PPP) was used for thrombin time (TT) and fibrinogen (F) measurements, and platelet rich plasma (PRP) for PA assays. TT was prolonged by Hep device. The anticoagulant effect was proportional to Hep concentration (25 - >120 sec; 0.2 to 0.4 g/L). F content was unchanged compared to PPP (316±16.5 vs 322±35.7 mg/dL). The 0.1 M NaSa slice inhibited PA (42% IPA). TT time was enlarged-(34.8±9.9 sec.), F content decreased (15% vs PPP) and IAP was higher (90% IAP, p<0.05) using 0.5 M NaSa. Both drugs doping showed an anticoagulant additive effect (TT: 60±20.5 sec.) with significant F diminution (49% vs PPP). Scanning electron microscopy showed that slices that contained 0.5 M NaSa or NaSa plus Hep, exhibited a complex microtubular structure. The anticoagulant effect of NaSa slices could be due to the hiding of F in these three-dimensional structures. Platelet deposition was rule out since platelet counting in the remaining PRP was not changed respect to basal count. The results suggest that, these devices are able to produce an *in situ* beneficial regulation of hemostasis, and could represent a potential design to prevent restenosis of blood vessels.

057 (778) **THE EXPOSURE TO AIR POLLUTION PARTICULATE MATTER AGGRAVATES EXPERIMENTAL MYOCARDIAL INFARCTION IN MICE BY POTENTIATING CYTOKINE SECRETION FROM ALVEOLAR MACROPHAGES**

Timoteo Marchini[1,2], Dennis Wolf[2], Nathaly Anto Michel[2], Maximilian Mauler[2], Natalia Magnani[1], Deborah Tasat[3], Silvia Alvarez[1], Ingo Hilgendorf[2], Andreas Zirlik[2], Pablo Evelson[1].

[1]Universidad de Buenos Aires. CONICET. Instituto de Bioquímica y Medicina Molecular (IBIMOL). Facultad de

Farmacia y Bioquímica. Buenos Aires, Argentina. [2]University of Freiburg. University Heart Center. Cardiology and Angiology I. Freiburg, Germany. [3]Universidad Nacional de General San Martín. Escuela de Ciencia y Tecnología. Buenos Aires, Argentina.

Environmental particulate matter (PM) exposure is associated with increased cardiovascular morbidity and mortality rates, mainly due to myocardial infarction (MI) and its complications. We hypothesize that, following PM inhalation, alveolar macrophages orchestrate a local inflammatory response within the lung, which afterwards leads to systemic inflammation that affects disease progression. In the present work, we aimed to describe the mechanisms and consequences of PM exposure in an experimental model of MI. C57BL/6J mice were exposed to a PM surrogate (Residual Oil Fly Ash, ROFA) by intranasal installation, prior and after the permanent ligation of the left anterior descending coronary artery. Histological analysis of the myocardium at 7 days after MI showed increased infarct area and enhanced inflammatory cell recruitment in ROFA-exposed mice. Time-course evaluation of cell populations in infarcted tissue by flow cytometry revealed increased numbers of Ly6C^{high} monocytes at day 3 after MI, as well as increased macrophages at day 7. Mechanistically, the ROFA exposure increased plasma TNF-α levels, induced the activation and expression of adhesion molecules in myeloid and endothelial cells, respectively, and enhanced leukocyte recruitment in models of sterile peritonitis and intravital microscopy. We identified alveolar macrophages as the primary source of elevated cytokine production after PM exposure. Accordingly, *in vivo* depletion of alveolar macrophages by an intranasal instillation of clodronate liposomes inhibited local and systemic ROFA-mediated cytokine secretion, as well as inflammatory cell recruitment in infarcted tissue. Taken together, our data demonstrate that the exposure to environmental PM worsens MI healing in mice. These findings provide a novel link between air pollution and inflammatory pathways, and emphasize the importance of environmental factors in cardiovascular disease.

058 (817) **THE CARDIAC HYPERTROPHY OF THE OBESE AGOUTI MICE IS NOT DUE TO HYPERTENSION: ROLE OF THE CARDIAC TRH SYSTEM**

Ludmila Peres Diaz[1], Maia Aisicovich[1], Mariano Schuman[1], Jorge E Toblli[2], Maria Silvina Landa[1], Silvia Ines García[1].
[1]Laboratory of Molecular Cardiology, Scientific Research Institute "A. Lanari", Buenos Aires University; IDIM-CONICET. Argentina. [2]Hospital Aleman, Buenos Aires, Argentina.

Cardiac TRH (cTRH) is overexpressed in SHR left ventricle. Specific TRH-iRNA treatment induced downregulation of LV-TRH production, preventing cardiac hypertrophy and fibrosis demonstrating that TRH is involved in hypertrophy associated with hypertrophic and fibrotic processes. We confirmed TRH hypertrophy and fibrotic effects in myocytes and fibroblasts. We showed that cTRH participates in the AII-induced hypertrophy mice model. On the other hand, we described the TRH-leptin interaction in the nervous system where leptin induces TRH gene suggesting that this interaction could function also in the heart. Hyperleptinemic obese models present hypertension and hypertrophy, primarily attributable to high pressure. In contrast, we hypothetized that in obese agouti mice (AG) cardiac alterations could be provoked by high leptin levels which could interact with leptin receptors thereby inducing a cTRH increase. Indeed, in AG obese adult males n=10 we confirmed obese phenotype through the increase ($p<0.01$) of body weight, blood pressure, leptin levels and cardiac hypertrophy index in the AG vs lean C57. As thought AG shows 3-fold TRH expression increase (PCR) confirmed by IHC suggesting that heart alterations could be induced by cTRH. Indeed AG hearts showed higher ($p<0.01$) BNP, BMHC and col III expression confirmed by Masson. Then, we treated an AG group (n=12) with the diuretic hydrochlorotiazide (AG+HC) from 2 to 18w.Treatment was effective as AG+HC presented similar pressure vs lean C57 but lower than AG obese ($p<0.01$) without effect on body weight.Although normal blood pressure AG+HC presented higher hypertrophy index similar to AG mice with higher cTRH expression accompanied by an increase ($p<0.01$) in hypertrophy markers and fibrosis (BNP, BMHC, Col III) pointing out that in obesity, cardiac hypertrophy is independent of hypertension, opening the possibility that high leptin evokes cTRH induction responsible for AG obesity-induced cardiac alterations.

059 (826) **HEXACHLOROBENCENE TREATMENT INDUCES DIFFERENT RESPONSES TO ISCHEMIA/REPERFUSION INJURY IN MALE RAT HEARTS**

Patricia Bonazzola[1], Giselle Romero Caimi[2], María Inés Rosón[1], Susana Gorzanzky[3], Laura Alvarez[2], Rocío Castilla[1].
[1]Instituto de investigaciones Cardiológicas Prof. Dr. Alberto C. Taquini, Facultad de Medicina, UBA-CONICET. [2]Laboratorio de Efectos Biológicos de Contaminantes Ambientales, Dpto de Bioquímica Humana, Facultad de Medicina, UBA. [3]Cátedra de Farmacología, Facultad de Farmacia y Bioquímica, UBA.

We have previously demonstrated that the bio-accumulated organ chlorinade pesticide hexachlorobenzene (HCB) induced hypertension in female rats. This work focuses on HCB subchronic *in vivo* and acute *in vitro* effect on male rat heart function. For *in vivo* studies, male Wistar rats were treated 3 times a week with HCB (500 mg/kg b.w.) by garbage intubation for 45 days, and hearts were then extracted and either used for histological analysis or for mechanical and energy assays. To this end the hearts were arterially perfused at 37 °C by Langendorff method, electrically stimulated at 3 Hz, exposed to 25 min ischemia followed by 45 min. reperfusion while simultaneous mechanical and heat measurements were done. For *in vitro* studies hearts from control rats were treated with HCB (5 µM) for 30 min. before ischemic insult.

Results: *in vivo* studies, HCB increased systolic blood pressure in male rats (142 ± 7 vs. 117 ± 2, $p<0.01$). Histological studies showed no alterations in muscular thickness of septum or the edge free of left ventricle but subpericardial inflammatory lymphocytic infiltrated and subendocardial foci of fibrosis are observed. Hearts from HCB-treated rats showed a decrease in resting pressure during reperfusion (58.5 ± 4.5 vs. 89.2 ± 6.3 mmHg, $p<0.05$ at 45 min of reperfusion) and an improvement of developed pressure (P) expressed as percentage of preischemic value during post ischemic recovery (46.2 ± 6.0 vs. 18.8 ± 2.3%, $p<0.001$ at 45 min of reperfusion). Total heat production (Ht) revealed no differences from control hearts, so that contractile economy expressed as percentage of preischemic value (P/Ht%) was higher than control during reperfusion (67.8 ± 7.2 vs. 35.0 ± 5.1%, $p<0.05$ at 45 min reperfusion).

In vitro pre-treatment with HCB rendered a decrease of 25% in preischemic values of P but no significant changes in postischemic P, Ht or P/Ht were observed.

Conclusion: HCB protects male rat hearts from ischemic insult. Such protection is a long term event probably as a response to pesticide-induced hypertension.

NEUROCIENCIAS I/ NEUROSCIENCES I

060 (109) **BRAIN SITE OF ACTION OF TRACE ELEMENTS (I): ROLE OF SE IN THE ACCUMBENS NUCLEUS OF THE RAT**

Silvia G. Ratti[1], Anabella A. Orozco[1], Edgardo O. Alvarez Toro[1].
[1]Laboratorio de Neuropsicofarmacología Experimental, Facultad de Ciencias Médicas, UNCuyo, IMBECU-CONICET

Previously in our laboratory, evidence was presented showing that after systemic administration of Te in non-toxic doses to pregnant mother and its litter rats, several behavioural parameters related to motivated and lateralized exploration in the offspring were affected. Administration of systemic Se simultaneously with Te, blocking of Te effects was found. Since this evidence suggested a central site of action in the brain, the purpose of the present work was to study the possible neural structures involved in the behavioural effects of trace elements. For this purpose,

adult male rats were implanted with microinjection cannulae into the accumbens nuclei (left and right), and48h later, they were injected with 1µl of saline or Na_2O_3Se(0.17 ng/L) into the left (ACC_L; n=10), right (ACC_R; n=15)or both neural structures (n=11) at 12:00h for three days. At four day, all animals were tested individually in a general motor activity detector (OVM) and in a Double Lateral Hole-Board Labyrinth (DHBL) to register general, motivated exploratory activity, and lateralized exploration induced by novelty. Results shown that in the OVM, stimulation of the ACC_R significantly inhibited head-dipping (5±1 Counts/5min Vs. 14.5±1 Counts/5min; Se Vs. Control,p <0.01) with no effect on rearingor focalized exploration. In the DHBL, general parameters of displacement and non-exploratory activity were not affected by treatments. However, lateralized exploration was inhibited when Se was injected into both ACC. In conclusion, results suggest that the ACC might be one possible site of action of trace elements, and evident lateralization was found in the ACC_R with on especific motivated exploration.

061 (110) **SE IS ABLE TO COUNTERACT THE INHIBITORY EFFECTS OF TE ON SELF-DEFENSE AND SOCIAL BEHAVIOURAL ACTIVITY IN THE RAT**

Silvia G. Ratti[1], Anabella A. Orozco[1], Carla N. Agüero[1], Edgardo O. Alvarez Toro[1].
[1]Laboratorio de Neuropsicofarmacología, Facultad de Ciencias Médicas, UNCuyo, IMBECU-CONICET.

Previously in our laboratory, evidence was presented showing that after systemic administration of Te in non-toxic doses to pregnant mother and its litter rats, several behavioural parameters related to motivated and lateralized exploration in the offspring were affected. Administration of systemic Se simultaneously with Te, blocked the inhibitory effect of Te on lateralized exploration, recovering the spontaneous left-biased exploration of animals in a double lateral hole-board labyrinth. In the present complementary work, the possible effect of Se on the inhibitory action that Te has on social and defensive behavioural activity, two important processes to the animal was studied. Four experimental groups were formed. Animals that received water (Control, n=10);animals that received ZnTe (0.03µg/L; n=10); animals that received Na2O3Se(0.268µg/L; n=10), and animals that received Na2O3Se +ZnTe (n=10). Treatments started with the pregnant mother and were continued along delivery, lactation and prepuberal stage of litter rats. At 30 day of age of the offspring, animals were tested individually in an intruder-resident(SIT), and a forced swimming test (FST) to measure social interaction and defense behaviour. Results in the SIT shown that, animals receiving ZnTe, latency to confront the intruder was significantly increased compared to control (131±36.5 Counts/3min Vs 13±3 Counts/3min, p<0.01). Animals receiving Se+Te showed a latency not different from Control and significantly lower than the ZnTe group (10±3 C/3min Vs 131±36.6C/3min; [Se+Te] Vs [Te], p<0.01. In the FST, ZnTe group showed a significant decrease in active swimming behaviour compared to Control; diminished activity that was blocked and restored to normal values in the (Se+Te) group. In conclusion, results show that social interaction and defensive behaviour, affected by the ZnTe treatment is restored to normal values by Se treatment.

062 (141) **DESIGN OF A LENTIVIRAL VECTOR AS A THERAPEUTIC STRATEGY AGAINST ALZHEIMER´S DISEASE**

Maria Jimena Abrey Recalde[1], Paula Gonzalez Hermida[1], Veronica Baez[2], Diana Jerusalinsky[2], Cecilia Ariana Frecha[1].
[1]Laboratorio de Vectores Virales y Terapia Genica, Instituto de Ciencias Básicas y Medicina Experimental, Instituto Universitario Hospital Italiano de Buenos Aires (ICBME-IUHIBA). [2]Laboratorio de Neuroplasticidad y Neurotoxinas, Instituto de Biología Celular Y Neurociencias Prof E De Roberts, Facultad de Medicina, Universidad de Buenos Aires.

Alzheimer's disease (AD) is a chronic neurodegenerative disorder characterized by a progressive loss of cognitive functions. One of the hallmarks is the formation of amyloid plaques, composed mainly by Aß peptide oligomers (AβOs). Neprilysin (NEP) is the most important endopeptidase in charge of the degradation and clearance of Aß in the brain. Therefore, strategies aiming to increase NEP levels should contribute to decrease the amount of Aβ, AβOs and their deleterious effects on neurons, and could have a therapeutic effect. We are developing a lentiviral vector (LV), to deliver human NEP exclusively in hippocampal neurons. We will study its performance in vitro and in an AD transgenic rat model.

A construct containing the complete cDNA of NEP downstream of the hippocampal-specific promoter human synapsin (SYN-1) was developed. NEP cDNA was obtained from pBOB-NEP plasmid and was cloned under the SYN-1 promoter to obtain SYN-NEP plasmid. The correct cloning was checked by BamHI/KpnI digestion followed by 1% agarose gel electrophoresis. We obtained two bands of 3392pb and7644pb, which coincided with the molecular weight of NEP insert and plasmid backbone, respectively. The insert was also verified by sequencing. To test NEP expression by the plasmid, 293T cells were transfected with pSYN-NEP. pSYN-RFP plasmid expressing the reporter red fluorescent protein (RFP) and pBOB-NEP were used as transfection and positive controls, respectively. After 48 hours NEP expression was evaluated by western blot (WB) and RFP expression by fluorescence microscopy. Transfection efficiency was 80% and the WB showed a85kDa band only in those lanes corresponding to transfection with pBOB-NEP andSYN-NEP. In conclusion, NEP was successfully cloned downstream of SYN-1 promoter and is expressed correctly under a ubiquitous promoter. We are currently using this construction to produce LV for neuron-restricted expression of NEP.

063 (152) **ANGIOTENSIN II AT1 RECEPTORS MEDIATE NEURONAL SENSITIZATION AND THE SUSTAINED BLOOD PRESSOR RESPONSE INDUCED BY A SINGLE INJECTION OF AMPHETAMINE**

Natalia Andrea Marchese[1], Maria Constanza Paz[1], Ximena Caeiro[2], Florencia Maria Dadam[2], Gustavo Baiardi[3], Mariela Fernanda Perez[1], Claudia Bregonzio[1].
[1]Instituto de Farmacología Experimental Córdoba (IFEC-CONICET) Departamento de Farmacología. Facultad de Ciencias Químicas Universidad Nacional de Córdoba, Córdoba, Argentina. [2]Instituto de Investigación Médica Mercedes y Martín Ferreyra (INIMEC-CONICET), Universidad Nacional de Córdoba, Córdoba, Argentina. [3]Laboratorio de Neurofarmacología, (IIBYT-CONICET) Universidad Nacional de Córdoba Facultad de Ciencias Químicas, Universidad Católica de Córdoba, Córdoba, Argentina.

A single exposure to amphetamine induces neurochemical sensitization in striatal areas. The neuropeptide angiotensin II, through AT_1 receptors (AT_1-R) activation, is involved in these responses. However, amphetamine-induced alterations can be extended to extra-striatal areas involved in blood pressure control and their physiological outcomes. Our aim for the present study was to analyze the possible role for AT_1-R in these events using a two-injection protocol and to further characterize the proposed AT_1-R antagonism protocol.

Central effect of orally administered AT_1-R blocker (Candesartan, 3mg/kg p.o. × 5 days) was analyzed recording spontaneus activity of neurons within locus coeruleus. In another group of animals, pretreated with AT_1-R blocker or vehicle, sensitization was achieved by a single administration of amphetamine (5mg/kg i.p.- day 6) followed by a 3week period off drug. After receiving an amphetamine challenge (0.5mg/kg i.p.),we evaluated: 1) the sensitized c-Fos expression in locus coeruleus (LC),nucleus of the solitary tract (NTS), caudal ventrolateral medulla (A1) and central amygdala (CeAmy); and 2) the blood pressor response. AT_1-Rblockade decreased LC neurons' spontaneous firing rate. Moreover, sensitizedc-Fos immunoreactivity was found in LC and NTS; and both responses were blunted by the AT_1-R blocker pretreatment. Meanwhile, no differences were found neither in CeAmy nor A1. Sensitized pressor response was observed as sustained changes in mean arterial pressure and was effectively prevented by AT_1-R blockade.

Our results support the important role for brain AT_1-R in amphetamine-inducedsensitization in extra-striatal areas and over its related cardiovascular output.

064 (153) **A SPECIFIC GLUCOCORTICOID RECEPTOR (GR) ANTAGONIST (CORT113176) PREVENTS INFLAMMATION AND NEURODEGENERATION IN THE WOBBLER MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS**

Maria Meyer[1], Maria Claudia Gonzalez Deniselle[1, 2], Analia Lima[1], Hazel Hunt[4], Joseph Belanoff[4], Alejandro Federico De Nicola[1, 3].

[1]Instituto de Biología y Medicina Experimental-CONICET, [2]Departamento de Fisiología, Facultad de Medicina, UBA ,[3]Departamento de Bioquímica, Facultad de Medicina, UBA, [4]CORCEPT Therapeutics, Menlo Park, CA, USA.

Wobbler (WR) mice show motoneuron degeneration, astrogliosis and microgliosis of the spinal cord. Additionally, increased plasma and brain corticosterone and focal adrenocortical hypertrophy suggest a role of hyperadrenocorticism in the WRdisease. In the present work we evaluated if antagonizing the GR with CORT113176prevents development of spinal cord abnormalities. CORT113176 shows high affinity towards GR without binding to progestin, androgen or estrogen receptors. Five month old genotyped WR mice received s.c. vehicle or 30 mg / kg / day for 4 days of CORT113176 dissolved in sesame oil. The mice were used the 4th day, 2 hours after the last dose of CORT113176. Antagonistnaïve WR showed several abnormalities of the spinal cord, such as vacuolated motoneurons, increased glial fibrillary acidic protein (GFAP) + astrocytes, decrease glutaminesynthetase (GS) + cells, increased number of IBA1+ microglia and decreased number of cells + for the calcium-binding protein B (S100B). Treatment of WR with CORT113176 normalized these altered parameters. Furthermore untreated WR expressed high levels of mRNA for CD11b (microglia marker) ($p < 0.01$ vs.control mice) and increased mRNA of proinflammatory markers TNa($p < 0.001$ vs. control) and iNOS ($p < 0.001$ vs.control). These markers were normalized in WR receiving CORT113176 (WRvs. WR CORT113176: CD11b: $p<0.05$; TNFa:$p<0.01$; iNOS: $p<0.001$). The TLR4 mRNA increased in the spinal cord of WR without treatment ($p < 0.01$ vs. control) and decreased with CORT113176 ($p<0.01$ vs. WR without treatment). In conclusion, the GR antagonist CORT113176 proved a powerful tool to block proinflammatory mediators, development of astrogliosis and microgliosis, thus holding back spinal cord neuropathology of WR mice.

065 (169) **PROTECTIVE ROLE OF LIPOIC ACID IN THE OXIDATIVE DAMAGE OF BRAIN STRUCTURES IN A GLAUCOMA RAT MODEL**

Claudia Gabriela Reides[1,2], Agustina Peverini[1], Ailen Gala Hvozda Arana[1], Natasha Stephanie Janezic[1], Romina Mayra Lasagni Vitar[1,2], Fabian Lerner[1], Sandra Maria Ferreira[1,2], Susana Francisca Llesuy[1,2].

[1]Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Cátedra de Química General. [2]IBIMOL, UBA-CONICET.

Evidence of oxidative process was found in glaucoma brain so the use of an antioxidant therapy may hold a promise for treatment. The purpose was to evaluate the possible protective role of lipoic acid (LA) in the oxidative damage of geniculate nucleus (GN) and visual cortex (VC) in an experimental glaucoma model. Wistar rats (3 months) weredivided in four groups (n=20): glaucoma in which rats were operated under a microscope by cauterized two of the episcleral veins (G), glaucoma treated with LA 100 mg/kg i.p. (LG), control which received a sham procedure (C) and control treated with lipoic acid 100 mg/kgi.p. (LC). Seven days after surgery rats were euthanized, brains were removed and GN and VC were separated. The following markers were evaluated:thioredoxin reductase (TRxR), glutathione reductase (GR) and superoxidedismutase (SOD) activities, protein oxidation (PO), damage to lipids (TBARS) and glutathione (GSH).

Comparing LA treated glaucoma to glaucoma: TRxR increased 52% in GN (G 7.6±0.4 nmol/min.mg protein *p<0.01*) and 26% in VC (G 9.9±1.0 nmol/min.mg protein *p<0.05*), GR increased 82% in GN (G 8.2±1.2nmol/min.mg protein *p<0.01*) and 300%in VC (G:5.1±1.3 nmol/min.mg protein *p<0.001*),SOD increased 23% in GN (G 18.0±1.1 U/mg protein *p<0.05*) and 80% in VC (G 6.0±0.4 U/mgprotein *p<0.001*), PO diminished 52%in GN (G 22.9±2.3 nmol/mg protein *p<0.05*)and 58% in VC (G 9.34±1.30 nmol/mg protein *p<0.05*),TBARS diminished 25% in GN (G 4.8±0.4 nmol/mg protein *p<0.05*) and 36% in VC (G 5.3±0.6 nmol/mg protein *p<0.05*), GSH increased 42% (G 0.20±0.04µmol/g *p<0.05*) in GN and 73% (G 0.41±0.03µmol/g *p<0.01*) in VC.

The increase in GSH and in the activities of antioxidant enzymes could have been a consequence of the protective role of LA in oxidative processes in glaucoma. Furthermore, the protective effect against lipid and protein damage and the improvement inGSH recycling support that LA could be used as a novel therapy for reducing oxidative damage in glaucoma.

066 (189) **KISSPEPTIN PARTICIPATION IN DELETERIOUS GHRELIN EFFECTS ON SPERMATOGENESIS**

María Belén Poretti[1, 2], Santiago Bianconi[1,2], Giulia Maestri[2], Ana Carolina Martini[1], Laura Vincenti[1], Marta Fiol de Cuneo[1], Helgi Schiöth[2], Valeria Paola Carlini[1, 2].

[1]Instituto de Fisiología, Instituto de Investigaciones en Ciencias de la Salud (INICSA, UNC-CONICET), Facultad de Ciencias Médicas, CONICET and Universidad Nacional de Córdoba, Argentina. [2]Uppsala University, Dept. of Neuroscience, Section of Pharmacology, Uppsala, Sweden.

Physiological mechanisms that control energy balance are reciprocally related to those that control reproductive function. In previous work we have shown that chronic intrahypothalamic ghrelin(Ghr) administration (42 days), an orexigenic peptide of 28 amino acids, decreases epididymal sperm concentration and motility in mice, these results correlate with a reduction in plasma testosterone concentration.

In this study, we investigated the involvement of kisspeptin (Kiss-1) system and its receptor (GPR54) in deleterious effects on spermatogenesis after chronic Ghr central administration. Albino Swiss adult male mice were implanted with osmotic pumps (Alzet) Model 1007D (0.5 l / hour-7 days) ormodel 2006 (0.15 l / hour-42 days) on hypothalamus and infused with sterile cerebrospinal fluid (CSF-control) or different Ghr doses (0.3 or 3.0 nmol /ul). Animals were sacrificed and the hypothalamus dissected to assess the expression of genes encoding gonadotropin releasing hormone (GnRH), Ghr receptor (GHRS), Kiss-1 and GPR54 by real time PCR.

Results show a significant decrease of relative Kiss-1 expression ($F = 10.25$, $p \leq 0.05$) and its receptor GPR54 ($F = 11.34$, $p \leq 0.05$) in animals treated per 7 days with Ghr 3,0 nmol/ul, while peptide administration for 42 days only decreases GPR54 expression ($F = 9.03$; $p \leq 0.05$). No significant differences were found in GHRS or GnRH expression ($p > 0.05$).

This paper provides new evidence about possible mediators involved in Ghr deleterious effect on male reproductive system, indicating that this peptide induces a negative modulation at central level on the expression of Kiss-1 and / or its receptor.

067 (400) **EFFECTS OF EARLY LIFE STRESS ON NEUROGENIC BRAIN REGIONS: ACTIVITY OF LINE-1 RETROTRANSPOSONS DECREASES IN HIPPOCAMPAL NEURONS IN RATS SUBJECTED TO NEONATAL-MATERNAL SEPARATION.**

Mariana Salcedo[1], Andrés J Orqueda[1].

[1]Instituto de Ciencias Básicas y Medicina Experimental, Instituto Universitario del Hospital Italiano.

Long Interspersed Nuclear Elements-1 (LINE-1) retrotransposons are repetitive elements that encode an RNA binding protein (ORF1p)and an endonuclease with reverse transcriptase activity (ORF2p), that control LINE-1mobilization via target prime reverse transcription. Despite most human LINE-1insertions occur during early embryonic development, somatic retrotransposition also takes

place in the brain. Thus, by inserting into new genomic locations, LINE-1activity is a potential source of genotypic variation among neurons. In humans, the consequences of somatic mosaicism are most apparent in disease, including many neuropsychiatric disorders (*e.g.*Rett syndrome and schyzophrenia) and cancer, which show increased LINE-1activity. Thus, the possibility that LINE-1-driven somatic mosaicism alters functional properties of the brain arises. On the other hand, the formation of neuronal networks is also affected by adverse early life experiences. Indeed, it is known that *in vivo* neonatal maternal separation (NMS) attenuates the capacity of adult hippocampal neuronal precursor cells to differentiate in to neurons. However, the precise mechanism of this process is still poorly understood. In this study we examined how NMS impacts on theactivity of LINE-1 in adult hippocampal neuronal precursor cells. We found thatrelative ORF2 DNA content is significantly reduced in hippocampal tissue of adultrats subjected to NMS, suggesting a reduction of new LINE-1 insertions. RelativeORF2 DNA content was not affected in the cerebellum, a non-neurogenic brain region. In addition, behavioral tests were used to evaluate cognitive functions: preliminary results of episodic-like memory and anxiety tests show no significant difference. Taken together, these findings show that retrotransposition of LINE-1 in rats is negatively affected by early life stress of pups, and suggest that altered hippocampal neurogenesis by NMS is associated to a decreased LINE-1 activity.

068 (191) **ACUTE GHRELIN EFFECTS ON MEMORY RETENTION IN AN OLFACTORY BULBECTOMY MODEL: A POSSIBLE MECHANISM OF ACTION**

Maria Belen Poretti[1,2], Santiago Bianconi[1,2], Guilia Maestri[2], Paula Rodriguez[1], Susana Rubiales de Barioglio[3], Helgi Schiöth[2], Valeria Paola Carlini[1,2].

[1]Instituto de Fisiología, Instituto de Investigaciones en Ciencias de la Salud (INICSA, UNC-CONICET), Facultad de Ciencias Médicas, CONICET and Universidad Nacional de Córdoba, Argentina. [2]Uppsala University, Dept. of Neuroscience, Section of Pharmacology, Uppsala, Sweden. [3]Departamento de Farmacología, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina.

Ghrelin (Ghr) is an orexigenic peptide that is being investigated for its potential role in development of anxiety-like behavior and modulation of depressive-like symptoms induced by bilateral olfactory bulbectomy (OB) in rodents. Olfactory bulbectomy is a useful animal model to study of depression and Ghr could be an alternative therapeutic tool in depression therapy. In present work, we studied the ability of Ghr to reverse amnesic effects induced by OB in mice, using the object recognition test (ORT) and possible Ghr effects on genes implicated in memory modulation into hippocampus. Adult male Albino Swiss mice were divided in sham and OB, and immediately after training in ORT were infused with saline (S) orGhr 0.03 nmol/µl (doses that reversed depressive-like behavior induced by OB in tail suspension test) in the hippocampus. Animals were tested in ORT, sacrificed and hippocampus was dissected in order to study the mRNA expression of genes related to memory using real time PCR.

The OB animals treated with S (OB-S) presented impair on memory retention compared to sham($p<0.05$), but acute Ghr 0.03 nmol/µl infusion produced an increase on this parameter in OB animals ($p \leq 0.05$). In addition, OB induced low expression of Calcium/calmodulin-dependent protein kinaseII isoform2 (CamKII iso2) ($F= 9.12$, $p<0.05$) and Nmda1 N-methyl-D-aspartate receptor (NMDA1 ($F= 8.96$, $p<0.05$), which were reverted by acute Ghr 0.3 nmol/ul administration($p \leq 0.05$). Results show that Ghr 0.3 nmol/µl reverts memory impairment induced by OB in mice and provide information that this effect could be mediate at least in part by CamKII iso2 and NMDA1.

069 (195) **COMPARTMENTALIZATION AND SEXUAL DIMORPHISM IN THE STRIATUM OF THE SOUTH AMERICAN PLAINS VIZCACHA, LAGOSTOMUS MAXIMUS**

Alejandro Raúl Schmidt[1,2], Santiago Elias Charif[1,2], Pablo Ignacio Felipe Inserra[1,2], Alfredo Daniel Vitullo[1,2], Veronica Berta Dorfman[1,2].

[1]Centro de Estudios Biomédicos, Biotecnológicos, Ambientales y Diagnóstico (CEBBAD), Universidad Maimónides, [2]CONICET.

Some brain nuclei as striatum show sexual dimorphism in a variety of mammals from rodents to humans. To appropriately respond to environmental stimuli, sensory information is integrated into the reward circuit, being the striatum the critical structure to combine information. Striatum collects inputs from cortex, thalamus and midbrain. The South American plains vizcacha is a caviomorph rodent native from the Pampean region of Argentina. It is a close evolutionary relative to chinchilla with peculiar reproductive characteristics as an activereproductive axis with ovulation at mid-gestation and massive poly-ovulation upto 800 oocytes per estrous cycle. In order to characterize the striatum of the vizcacha and to determine striatal sexual dimorphism, we used adult non-pregnant female and male vizcachas to evaluate striatum histology byKlüver-Barrera staining and tyrosine hydroxylase (TH) and parvalbumin (PV) localization by immunohistochemistry to study matrix and striosome compartments. A bigger level of compartmentalization of the striatum was observed in the vizcacha related to other rodents like mouse. In addition, caudate and putamen nuclei resulted separated by the internal capsule similarly to the striatum of chinchilla. PV and TH distribution showed a complementary pattern of expression that defines the striatum histology with striosomal and cellular distribution for PV and matrix localization for TH. Sexual dimorphism was determined by TH immunoreactive area in caudate, with a significant increase in males than in females ($p<0.02$). PV did not show significant immunoreactive area differences between sexes. In addition, in the same platethan striatum, both sexes showed PV immunoreactive granular cells in the layer IV of the parietotemporal cortex. The observed striatum compartmentalization suggests an adaptation of the vizcacha to improve its motor skills and facilitate the reproductive success.

070 (197) **CHANGES IN FREE RADICAL PRODUCTION IN BRAIN CORTEX SYNAPTOSOMES DURING AGING**

Paulina Lombardi[1], Analia Karadayian[1], Federico Orgambide[1], Juanita Bustamante[2], Silvia Lores Arnaiz[1].

[1]Instituto de Bioquímica y Medicina Molecular (IBIMOL)-Universidad de Buenos Aires (UBA), CONICET, Fisicoquímica, Facultad de Farmacia y Bioquímica, Junín 956 (C1113AAD), Buenos Aires, Argentina. [2]Centro de Altos Estudios en Ciencias Humanas y de la Salud (CAECIHS), Universidad Abierta Interamericana, Av. Montes de Oca 745 (1270AAH), Buenos Aires, Argentina.

During aging, changes in brain cortex mitochondrial bioenergetics and in active oxygen species generation have been extensively described. Previous results from our laboratory showed impairment of mitochondrial respiratory chain in aged mice. The susceptibility of brain cortex synaptic mitochondria to age-dependent oxidative damage was studied in 3 and 17 months old mice. Synaptosomal fractions were isolated by Ficoll gradient procedures. Superoxide anion levels and hydrogenperoxide (H_2O_2) production were assayed. Also, cardiolipinoxidation, uncoupling protein (UCP-2) expression and enzyme activities of monoamino oxidase (MAO) and acetylcholinesterase were determined.

At17 months of age, superoxide levels were 19% lower than in young animals, whileH_2O_2 production increased by 32%. H_2O_2production would be a consequence of both MAO activity and mitochondrial respiratory chain (derived from superoxide). In the presence of the MAO inhibitor deprenyl, H_2O_2 production was not affected, suggesting that the increase in H_2O_2 could be due to MAOactivity. This enzyme activity was 62% increased in synaptosomes from 17-monthsold mice. Intact cardiolipin content was 42% lower in synaptosomes from oldmice, reflecting an increase in cardiolipin oxidation with age. UCP-2expression was 61% increased in synaptosomes from 17-months old mice compared with young animals. An increase of 57% in acetylcholinesterase activity was observed in synaptosomes from old mice.

The results of this study suggest that oxidative damage to mitochondria in nerve terminals from brain cortex would be caused by an increase in H_2O_2 generation with age and would not be a consequence of superoxide generation at the mitochondrial respiratory chain. Our results suggest that the increment in H_2O_2 levels would be due to MAO increase, while low levels of superoxide would be maintained by the uncoupling effect of UCP-2 in 17 months-old mice.

071 (201) **DIFFERENT PATTERNS OF ACTIVATION OF FOS-SEROTONIN NEURONS IN THE DORSAL RAPHE NUCLEUS IN AN ANIMAL MODEL OF DEPRESSION**

Antonella Pollano[1], Marta Magdalena Suárez[1], Verónica Trujillo[1].
[1]Universidad Nacional de Córdoba. Facultad de Ciencias Exactas Físicas y Naturales. Cátedra de Fisiología Animal.

Dysregulations in the brain serotonergic system and exposure to stressors through life have been implicated in the development of major depressive disorder. Maternal separation (MS)may sensitize specific neurocircuits to subsequent stressors.

The dorsal raphe nucleus (DRN) is the main brain source of serotonin, neurotransmitter implicated in the pathophysiology of depression. Administration of tianeptine activates its reuptake, opposite to the action of other antidepressants. Also, tianeptine has been shown to be clinically effective, and has fewer side effects compared with most antidepressants.

We assessed the number of immunoreactive Fos-serotonin cells in the DRN in a model of depression employing maternal separated and chronically stressed adult rats, treated with the antidepressant tianeptine. We hypothesized that the interplay between early and late-stress would result in a fewer number of neurons immunoreactive for Fos-serotonin in the DRN, and that tianeptine could correct some of the alterations induced by our model.

Wistar derived male rats were separated from their mother for 4.5 hr during the first 3 weeks of life. From day postnatal 50, were exposed to an unpredictable chronic stress (UCS) paradigm during 24 days and were daily treated with tianeptine (10 mg/kg i.p.) or vehicle. The number ofneurons double labeled with Fos and serotonin were quantified in the DRN at three rostrocaudal levels.

We found a significant interaction between MS and UCSin the number of Fos-serotonin cells at -8,04 mm bregma. UCS decreased the number of Fos-serotonin cells on DRN compared with controls and maternal separated-chronic stressed rats. Tianeptine administration had no main effect inthe number of Fos-serotonin cells on DRN. The combination of the two stressors produced a similar phenotype than control rats, indicating that maternal separation could trigger an adaptive response in rats subjected to chronic stress.

072 (203) **EXTRACELLULAR GAL-3 AND OLIGODENDROGLIAL DIFFERENTIATION**

Laura Thomas[1], Leandro Nazareno Marziali[1], Gabriel Adrian Rabinovich [2], Juana Maria Pasquini[1], Laura Andrea Pasquini[1].
[1]Department of Biological Chemistry, School of Pharmacy and Biochemistry, Institute of Chemistry and Biological Physicochemistry (IQUIFIB), School of Pharmacy and Biochemistry, University of Buenos Aires and National Research Council (CONICET), Argentina. [2]Laboratory of Immunopathology, Institute of Biology and Experimental Medicine (IBYME; CONICET), C1428 Buenos Aires, Argentina and Department of Biological Chemistry, School of Exact and Natural Sciences, University of Buenos Aires, C1428 Buenos Aires, Argentina.

Gal-3, a chimeric protein structurally composed of unusual tandem repeats of proline and short glycine-rich segments fused onto a carbohydrate recognition domain, possesses multifaceted roles in physiological processes including the regulation of innate and adaptive immune responses (Rabinovich et al, 2012). Previous results have demonstrated that recombinantGal-3 (rGal-3) treatment accelerates oligodendrocyte (OLG) differentiation in adose- and carbohydrate-dependent manner, which is in accordance with the N-glycosylationprofile observed in immature versus differentiated OLG (Pasquini et al, 2011). The aim of our work is to elucidate the mechanism involved in Gal-3-induced OLG differentiation by means of rGal-3 obtained and purified optimizing the standard protocol for recombinant protein production. rGal-3 was obtained from the *Escherichia coli BL21 star*/*pET28b+-Gal-3* system. Inducer concentration and period of induction were improved to increase the overall yield. rGal-3 release from bacteria was optimized through changes in ultrasonication time and acoustic power. rGal-3 was purified by affinity chromatography with agarose-lactose and bacterial LPS was removed by affinity chromatography with polymyxinb. Protein biological activity was controlled in every step by hemagglutination and finally by the evaluation of the dose-dependent viability of treated OLG. The evaluation of migration *in vitro* showed cells treated with Gal-3 to migrate significantly less than control cells. Also, *ex vivo* mice brain slices were used in order to study the effect of Gal-3 on myelination, dysmyelination induced byLPC and remyelination after LPC, both in wild type and *LGALS-3*$^{-/-}$. Results indicate that Gal-3 is a key factor to achieve correct myelination, and that the rGal-3 obtained is biologically active to induce correct oligodendroglial maturation.

073 (202) **CHRONIC UNPREDICTABLE STRESS IMPAIRS HIPPOCAMPAL DEPENDENT MEMORY: CONSISTENT DATA FROM FEAR CONDITIONING AND BARNES MAZE**

Antonella Pollano[1], Marta Magdalena Suárez[1], Julieta Aguggia[1].
[1]Universidad Nacional de Córdoba. Facultad de Ciencias Exactas Físicas y Naturales. Cátedra de Fisiología Animal.

It has been widely reported that chronic stress affects the morphology of the hippocampus and causes a decline in spatial hippocampal dependent memory. In previous studies from our laboratory we exposed Wister derived male rats to a chronic unpredictable stress paradigm (five types of stressor at different times of day) from postnatal day 50 to 74. In the stressed animals we found a reduction of26% and 23% in the volume from areas CA1 and CA3, respectively, and spatial memory impairments in a fear conditioning test compared with controls. Since both the stress protocol and the fear conditioning task have aversive components, we asked whether the less freezing response exhibited by stressed animals in the fear conditioned test occurred because they were habituated to aversive stimuli or because in fact they had impaired memory associated with the reduction of the hippocampal volume.

We submitted a group of rats to the same chronic unpredictable stress paradigm during 24 days and the next day conducted the Barnes maze test, which measures spatial memory performance but lacks of electric shocks or any other aversive stimulus, and consist in find an escape hole in a circular platform employing visual cues.

According our data, chronically stressed animals committed significantly more errors finding the escape hole compared with controls ($p < 0.05$). Therefore, our previous results obtained with the fear conditioning test are strengthened by Barnes maze. We conclude that contextual memory is compromised in animals exposed to chronic unpredictable stress, despite the test used.

074 (210) **A CHRONIC CUPRIZONE-INDUCED DEMYELINATION MODEL FOR THE DEVELOPMENT OF THERAPIES IN THE PROGRESSIVE STAGE OF MULTIPLE SCLEROSIS**

Victoria Sofia Berenice Wies Mancini[1], Juana Maria Pasquini[1], Jorge Daniel Correale[2], Laura Andra Pasquini[1].
[1]Department of Biological Chemistry, School of Pharmacy and Biochemistry, Institute of Chemistry and Biological Physicochemistry (IQUIFIB), School of Pharmacy and Biochemistry, University of Buenos Aires and National Research Council (CONICET), Argentina. [2]Institute for Neurological research Dr. Raúl Carrea, FLENI.

Multiple Sclerosis (MS) is one of the most common causes of progressive disability affecting young people. Most patients

initially present a relapsing-remitting course which, after 10 to 15-yearevolution, becomes progressive in up to 50% of untreated patients, with clinical symptoms slowly but steadily deteriorating. In about 15% MS patients, disease progression is relentless from disease onset. A better understanding of relapsing-remitting MS disease mechanisms has led to the development of different disease-modifying therapies, reducing both severity and frequency of new relapses by modulating or suppressing the immune system. In contrast, therapeutic options available for progressive disease are comparatively disappointing and remain a challenge. A 0.2% cuprizone (CPZ) diet administered to 5 to 6-week mice is known to induce demyelination in the corpus callosum (acutemodel), while CPZ withdrawal triggers a spontaneous remyelination process. When this diet is maintained for 12 weeks (chronic model), remyelination fails, leading to progressive disability. The present work evaluates the use of the chronic model in the development of therapies targeting the progressive stages of MS. Our results show severe myelin damage in the corpus callosum (MBP, Olig 2 and Redoil staining), accompanied by the activation of astrocytes (GFAP), neural precursors (Nestin), and microglial cells (Iba1). Axons from chronic CPZmice were more sparsely distributed, indicating neuronal loss. Unlike features observed in the acute model, our results in the chronic model show demyelination to reach the spinal cord, as evidenced by immunohistochemical(MBP and RIP) and electron microscopy analyses of myelin. These findings were concomitant with astroglial (GFAP) and microglial (Griffonia and ED1) activation. These findings hint at the possible use of the chronic CPZ model to develop therapeutic agents enabling remyelination and preventing neurodegeneration.

075 (246) **THE PREFRONTAL LOBE ON MAGNETIC RESONANCE IMAGING OF BOTH SEXES OF IN TWO AGE RANGES: SIMILARITIES AND DIFFERENCES OF ABSOLUTE AND RELATIVE UNDIMENSIONAL VALUES.**

Alicia Beatriz Merlo[1], Alfonso Miguel Albanese [1], Jorge Horacio Miño[1], Eduardo Francisco Albanese[1].
[1]Facultad de Medicina Universidad del Salvador. USAL.

In previous SAIC meetings we show that surfaces measures on parasagittal magnetic resonance imaging (PMRI) of prefrontal lobe (PFL) decrease with advancing of age.

Objective: To obtain, with advancing of age, absolute and relative lenght values of PFL.

Material and method: In PMRI of PFL (38 female and 38 male subjects) without psychiatric or neurological disease, using reliable anatomic landmarks, was drawn in each hemisphere an anteroposterior line that intercepts the edges of the brain. The length of the corresponding segment on PFL and the rest was measured. The percentage corresponding to the PFL to the sum of both segment was calculated.

The data were processed by sex and age groups (41-60 and 61-84 years). For statistical significance ANOVA was used.

Results: Lengths in cm(mean ± SE) of the PFL from female and male between 41-60 years were 3.30± 0.06 y 3.62 ± 0.12 ($p < 0.01$) for the right hemisphere and 3.20 ± 0.04and 3.35 ± 0.05 ($p < 0.05$) for the left and between 61-84 yearsrespectively 3.02±0.06 y 2.98±0.11 and 2.89±0.06 y 2.90±0.10.

The% (mean ±SE) provided by the PFL by female and male between 41-60 yearswere 22.40±0.35 y 22.38 ±0.47 for theright hemisphere and 20.51±0.15 y 20.40 ±0.20 for the left and between 61-84years respectively 19.18±0.31y 19.10 ±0.16 and 19.51±0.31 y 19.50±.0.28. In both sexes the% of PFL are lower ($p < 0.05$) in the range 61-84 years. Gender differences are not statistically significant.

Conclusion: While the segment on the PFL of each hemisphere of the male group in the range of 41-60 years has a significantly greater length than the female, in the range of 61-84 years decreases to values that do not differ significantly between the sexes, indicating greater fall in males . The percentage contribution of the prefrontal segment in both age ranges shows to be independent of sex and falls significantly in the range of 61-84 year.

076 (288) **NEURONS OF THE RAT CERVICAL SPINAL CORD EXPRESS VIMENTIN AND NEUROFILAMENT AFTER INTRAPARENCHYMAL INJECTION OF KAINIC ACID**

Fabian Nishida[1], Maria Susana Sisti[1, 4], Carolina Natalia Zanuzzi[1, 2, 3], Claudio Gustavo Barbeito[1, 2, 3], Enrique Leo Portiansky[1, 3].
[1]Image Analysis Laboratory, School of Veterinary Sciences, National University of La Plata (UNLP), Buenos Aires, Argentina. [2]Department of Histology and Embryology, School of Veterinary Sciences, National University of La Plata, Buenos Aires, Argentina. [3]National Council of Scientific and Technical Research (CONICET). Argentina. [4]Research fellowship. National Agency for Science and Technology Promotion. Ministry of Science and Technology, Argentina.

Intermediate filaments are the major components of the cytoskeleton, together with microtubules and microfilaments. Their expression can be altered in neurodegenerative diseases and cancer and, therefore, they can be used as biological markers. Rats injected with Kainic acid (KA) show behavioral changes and histopathological degenerative alterations of their spinal cords. In the present study we evaluated whether vimentin (VIM) and neurofilament (NF)expression are modified in a KA-induced neurodegenerative rat model. Animals were injected with KA at the C5 segment of the cervical spinal cord and euthanized at 1, 3 and 7 post injection (pi) days. Immunohistochemistry and double immunofluorescence were carried out for quantification of NeuN, VIM and NF positive cells and to determine colocalization of enolase (NSE)-VIM and NSE-NF. Cell counting of NeuN positive perikarya showed a significant loss of neurons at the injected site(ipsilateral) when compared with those of sham and non-operated animals. The contralateral side remained unchanged in all groups. When the VIM/NeuN positive neurons ratio was calculated in sham animals, a significant reduction was observed at day 7 pi whereas that of NF/NeuN was reduced from day 3 pi. KA-injected rats showed a constant ratio for both markers through the experimental days. Colocalization analysis confirmed a high index of VIM-NSE and NF-NSE in both experimental groups at day 1 pi. This index decreased in sham animals by day 3 pi whereas that of KA-injected rats remained high throughout the experiment. These results suggest that neurons initiate an unconventional intermediate filaments expression, which may respond to both the neuronal damage induced by the mechanical injury and the excitotoxic effect of the KA. The VIM and NF expression here described may function as a relevant molecular tool to reestablish the synaptic connections lost as a result of the neurodegenerative process.

077 (310) **EXPRESSION OF CLOCK GENES AND XENOBIOTIC METABOLIZING GENES IN A MURINE MODEL OF CHRONIC JET LAG AND TUMOR DEVELOPMENT**

María Belén Cerliani[1], Fernanda Román[2], Ignacio Aiello[2], Malena Mul Fedele[2], Silvina Richard[1], Diego Andrés Golombek[2], Juan José Chiesa[2], Natalia Paladino[2].
[1]Lab. de Citogenética y Mutagénesis, Instituto Multidisciplinario de Biología Celular (CIC-CONICET-UNLP), La Plata, Buenos Aires, Argentina. [2]Lab. de Cronobiología, Universidad Nacional de Quilmes, Bernal, Buenos Aires, Argentina.

The circadian clock synchronizes physiological and behavioral rhythms with the daily light-dark cycle (LD). Previous studies in animal models showed that chronic advances in the LD cycle (chronic jet lag, CJL) accelerate tumor growth. Moreover, the World Health Organization established that shift-work is a cancer risk factor. Xenobiotic metabolizing genes (XMG) are responsible for activating and/or detoxify endo or exogenous carcinogenic compounds. These enzymes are under circadian clock regulation. The aim was to analyze mRNA expression of Per1 and Bmal1 clock genes, and XMG Nat1, Cyp1a1 and Gstt1, in the suprachiasmatic nuclei (SCN, the central clock), and in liver and tumor tissues, using a murine model of CJL. C57BL/6 male mice were housed under the following conditions: 1)healthy animals in LD 12:12; 2) healthy animals under CJL (6 h advances of the LD cycle every 2 days);3) animals inoculated with B16 cell line (melanoma), under LD 12:12; 4) animals inoculated with B16 cell

line under CJL. Samples were taken at two time points(CT8 and CT20), from 3-6 animals/group. mRNA was quantified by qPCR, following the $2^{-\Delta\Delta Ct}$ method. Results showed that CJL alter daily (LD) Bmal1expression both in SCN and liver of healthy animals. This effect was also seen in animals with tumors, for Bmal1, Per1, Gstt1 and Cyp1a1 in liver tissue, and forBmal1 in SCN. With respect to mRNA expression in tumor tissue, groups showed no differences, except for Per1 that showed significant low levels under CJL. On the other hand, tumor development was able to disturb Cyp1a1 circadian expression, in the liver of animals under LD 12:12; however Bmal1 displayed an expression pattern similar to that seen in healthy animals. Data suggest that both central and peripheral clocks can be disturbed by CJL, affecting the expression of clock-controlled genes. Tumor seems not to be able to alter the expression of clock genes, but could alter hepatic expression of XMG by other signaling pathways.

078 (380) **ROLE OF NEUROSTEROIDS, STEROID RECEPTORS AND THE GABAERGIC PATHWAY ON THE EXACERBATED NEUROGENESIS OF THE GABA B RECEPTOR KO MOUSE**

Laura Inés Garay[1,2], Noelia Di Giorgio[1], Paula Gonzalez-Giqueaux[1], María Claudia González Deniselle[1,3], Analía Lima[1], Bernhard Bettler[4], Victoria Lux-Lantos[1], Alejandro De Nicola[1,2].

[1]Instituto de Biología y Medicina Experimental. [2]Dto de Bioquímica Humana, Facultad de Medicina, UBA. [3]Dto de Fisiología, Facultad de Medicina, UBA. [4]Universidad de Basilea, Suiza.

Adult hippocampal neurogenesis is regulated through the interaction of neural progenitor cells with diverse signals in the dentate gyrus. These signals include, among others, neurotrophins, neurotransmitters, and steroids. γ-Aminobutyricacid (GABA) signaling through GABAA receptors positively regulates the entire process of neurogenesis while GABAB receptor exerts an inhibitory role. Here, we analyzed the influence of GABA and the steroidogenic pathway on the exacerbated neurogenesis observed in the GABAB1 receptor knockout mice (GABABKO). GABAB KO showed a 2.2 fold increase in the number of double cortin (DCX)+cells vs. WT ($p<0.001$). Aberrant expression of young neurons may contribute to network hyperexcitability since a higher expression of early genes c-fos andc-jun were observed in CA1, CA3 and the hilus of the hippocampus of GABAB KO($p<0.05$). Lack of GABAB receptor increased 1.5 fold the number of GABA+interneurons in the stratum lacunosum moleculare ($p<0.05$), and the number ofGFAP+ astrocytes and glutamine sintetase (GS)+ cells ($p<0.05$). To evaluate if neurosteroidogenesis was also affected in GABAB KO, we determined the expression of steroidogenic proteins (STAR, PBR), enzymes (3βHSD, 5α-reductase, aromatase) and steroid receptors (AR, ER, MR). None of these were modified in GABA KO vs.WT in the analyzed tissues. Since adult neurogenesis is enhanced by estrogens, we analyzed if these steroids were involved in the aberrant neurogenesis observed in GABAB KO. Anastrazole, an aromatase enzyme inhibitor, was administered daily (1mg/kg) for fifteen days. No changes were observed inDCX, GFAP or GS hyperexpression after anastrazole treatment. In conclusion, aberrant neurogenesis and gliosis in this model may be more related to abnormal GABA signaling (high GABA, absent GABAB receptor) than the neurosteroidogenic pathway. These results may contribute to understand molecular mechanisms associated to aberrant neurogenesis in human diseases such as epilepsy.

METABOLISMO Y NUTRICIÓN SESION I/ METABOLISM AND NUTRITION I

079 (772) **TRIGGERING FACTORS IN PORPHYRIA CUTANEA TARDA ARGENTINEAN YOUNG WOMEN**.

Viviana Alicia Melito[1], Pablo Winitzky[1], Alcira Batlle[1], Maria Victoria Rossetti[1], Victoria Estela Parera[1].

[1]Centro de Investigaciones sobre Porfirinas y Porfirias- CIPYP-CONICET-UBA. Hospital de Clinicas Jose de San Martin.

Porphyria Cutanea Tarda (PCT) is a rare metabolic disease produced by reduced activity of Uroporphyrinogen Decarboxylase. Accumulation of highly carboxylated porphyrins in liver and plasma, mainly excreted by urine, is responsible of cutaneous damage: skin photosensitivity and fragility, blistering on exposed areas, hyperpigmentation and hyperthricosis. There are two principal types: Type I or A-PCT (acquired, 75-80%) and Type II or H-PCT (hereditary). It is a pharmacogenetic disease triggered by hepatotoxic drugs and hepatothropic virus. Men/women relationship is 4:1 in overt PCT. In this work the precipitating agents in young women were analyzed. We studied 80 women up to 40 years old without HBV, HCV or HIV infection. PCT diagnosis was made by fluorometric measure of plasma porphyrin index (PPI), total urinary porphyrins (TUP) by ion exchange chromatography and porphyrin profile by thin layer chromatography (CRO). Mean age of PCT manifestation was significantly lower (29.0±6.4, $p<0.05$) than in total PCT (49.5±9.3). In 60% of patients (G_1) the only triggering factor was hormonal treatment ($p<0.05$), being significant different respect to G_2 (alcohol 10%, corticoids 5%, anxiolytic drugs 5% among others); hormones plus other risk factors were identified in 10% of these patients. No significant differences were found between G_1 and G_2 in PPI, TUP and CRO at the time of diagnosis, not either the mean age of first symptoms. G_1 and G_2 represent 20% of PCT women, and 35% of them are H-PCT. The main triggering factors in our female and male populations are hormones (35%) and ethanol (55%) respectively. In the analyzed group the prevalence of hormonal treatment was 60%. On the contrary, there are not young PCT males (except in childhood PCT or PCT/HIV). Hormones are potent porphyrinogenic agent, mainly via CYP 450 as a suicide metabolite. In young women undergoing hormone treatment, the presence of cutaneous manifestation is an important signal of a possible PCT.

080 (91) **COMPARISON OF EFFECT ON CALCIUM RETENTION BETWEEN WHITE BREAD AND WHITE BREAD WITH INCORPORATION OF FUNCTIONAL INGREDIENTS AS GARLIC AND RESISTANT STARCH IN AN EXPERIMENTAL MODEL IN RATS**

Adriana Ruth Weisstaub[1], Jimena Correa[2], Victoria Salinas[2], Patricio Parra[1], Luis Dyner[1], Laura De La Casa[1], Angela Zuleta[1].

[1]Facultad de Farmacia y Bioquímica. Universidad de Buenos Aires. CABA, Argentina. [2]CIDCA, Facultad de Ciencias Exacta, Universidad Nacional de La Plata.

White Bread is a widely consumed food by the population and suitable for the introduction of functional ingredients as a strategy to overcome deficiencies such as calcium. Resistant starch and garlic, due to its content of fructooligosaccharides, arrive intact into the colon where are fermented by bifidobacteria and lactobacilli, producing end products such as short-chain fatty acids, increasing mineral bioavailability. The aim of this study was to evaluate the effect of three different diets intake on calcium bioavailability during 60 days, in a model rat. A total of 24 male Wistar rats recently weaned (8/group) were fed with a control diet prepared according American Institute of Nutrition Diet (C), and two semisynthetic diet prepared with bread formulated with wheat flour (WB) and bread formulated with wheat flour and adding garlic (3%), resistant starch (20%) and calcium citrate (1.1%) (GRB). At the end of the study rats were anesthetized and changes in total skeleton bone mineral density (BMDT), femur (BMDf) and tibia (BMDt) were determined (mg/cm^2). After sacrifice of rats, the cecum from each animal was excised, split open, and the pH of the cecal content was measured. The results showed that GRB had a higher BMDT than WB and C (269±10 vs 238±9 vs 238±9; $p<0.0001$). Nevertheless, GRB produced higher values of BMDf (mg/cm2) and BMDt than WB but lower than C (249±9vs232±10vs258±9; $p<0.0001$) and (226±11vs213±16vs232±4; $p<0.05$). The cecal content of GRB was acidified significantly greater extent than the cecal content of WB and C showing a lower cecal pH (6.78± 0.06vs vs7.15±0.29vs7.42±025; *$p< 0.0001$*). Bread containing resistant starch, garlic and calcium citrate, showed a prebiotic effect increasing calcium bioavailability and deposition in bones, comparing

with white bread. The observed beneficial health effects allow us to consider the design of breads healthier than those made only with wheat flour. Financed by UBACyT N° 20020130200028BA.

081 (171) **EFFECTS OF HEME OXYGENASE INDUCTION IN ADRENOCORTICAL FUNCTION IN A RAT MODEL OF IN INSULIN RESISTANCE**

Carolina Verónica Vecino[1,2], Esteban Martín Repetto[1,2], Silvia Sanchez Puch[2], Morena Wiszniewski[1,2], Juan Salvador Calanni[1,2], Cora B. Cymeryng[1,2].
[1]Departamento de Bioquímica Humana. Facultad de Cs. Médicas. Universidad de Buenos Aires. [2]CEFYBO-CONICET

Previous studies from our laboratory have shown morphological and functional changes in the adrenal gland upon the establishment of insulin resistance (IR) in rats by the administration of a sucrose rich diet (SRD). Our results indicated that in these animals, oxidative stress is generated within the adrenal cortex. Given the cytoprotective function associated with the activity of heme oxygenase 1 (HO-1) we hypothesize that HO-1 induction by hemin may mitigate these effects. Wistar adult male rats were randomly distributed into four groups (n=4): Control (C), Hemin (H), SRD and SRD + Hemin (DRS + H). Both SRD groups received 30% sucrose in the drinking water for 12 weeks. H and SRD + H rats were treated with hemin (15 mg/ kg ip/48 h) during the last two weeks of treatment and all animals were sacrificed 48 h after the last dose of hemin was administered. The insulin tolerance test (ITT) and the ACTH stimulation test were used to evaluate peripheral insulin sensitivity (0.75 IU insulin/ kg ip) and the adrenal response capacity, respectively. Plasma corticosterone levels were determined by RIA, and both HO-1 and catalase expression levels were assessed by western blotting.

Our results showed that, compared to controls, rats from DRS and DRS + H groups exhibited a significant decrease in insulin sensitivity ($p<0.005$). Blood triglyceride levels were only increased in the DRS group ($p <0.005$) while no differences in body weight and glycemia were observed between groups. At adrenal level, HO-1 induction by hemin was confirmed ($p <0.005$) and catalase expression levels were increased in the DRS group ($P <0.005$).

Hemin treatment prevented the decrease in basal corticosteronemia observed in rats from the SRD group ($p<0.05$) but not their diminished response to ACTH.

Our results indicate that administration of a SRD triggers a redox imbalance at adrenocortical level and suggest that HO-1 induction could prevent changes in basal corticosterone production in IR rats.

082 (178) **EFFECT OF HIGH- DIETARY LIPID CONCENTRATION FROM DIFFERENT SOURCES ON FATTY ACID PROFILES AND TBARS**

Paula Daniela Perris[1], Inés Fernandez[1], María Cecilia Mambrin[1], Nora Slobodianik[1], María Susana Feliu[1].
[1]Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica, Cátedra de Nutrición.

Diet lipid profile is important to prevent chronic diseases. Objetive: analyze the effect of high- dietary lipid concentration from different sources, on serum and liver fatty acid profiles and oxidative stress parameter. Weanling Wistar rats were fed during 40 days diet containing 42Kcal of lipids% provided by: butter(B);Olive oil(O); high oleic acid sunflower oil(HO) and sunflower oil(S). Control group(C) received normocaloric diet according to AIN´93. Serum and liver fatty acids profile were determined by gas chromatography. Liver lipid peroxidation was determined by TBARS (µg malondialdehyde-MA/g of tissue). Statistical analysis: Dunnett/ Kruskal-Wallis(*p<0.01). Results:%Area±SD PALMITIC SERUM B:20.63±2.54*▲ O:18.23±1.09 HO:12.96±2.99 S:15.83±1.48 C:16.08±2.15 LIVER B:22,53±1,53*▲O:16,88±0,57*▼ HO:12,09±0,66 *▼ S:14,55±0,98*▼ C:19.14±1.19, OLEIC SERUM B:20.37±2.23*▲ O:21.03±2.41*▲ HO:26.96±3.71*▲ S:11.46±3.86 C:11.29±2.27 LIVER B: 22,73±5,06*▲ O:23,86±2,31*▲ HO:39,03±7,45*▲ S:14,77±2,26 C:11,11±1,25 LINOLEIC SERUM B:9.79±1.12*▼ O:14,59±1,09 HO:7.41±1.89*▼S:30,87±2.58*▲ C:18.50±3.31 LIVER B:6,31±1,46*▼ O:15,63±3,52 HO:5,89±1,36*▼ S:25,33±4,05*▲ C:17,15±3,18 LINOLENIC SERUM B:0.44±0.14*▼ O:0.29±0.05*▼ HO:0.32±0.13*▼ S:0.28±0.05*▼ C:0.81±0.22 LIVER B:0,23±0,06*▼ O:0,29±0,11*▼ HO:0,14±0,03*▼ S:0,19±0,05*▼C:0,69±0,15. TBARS (MEDIA±DS) LIVER B:0,07±0,02 O:0,05±0,01 HO:0,06±0,02 S:0,05±0,02 C:0,06±0,02. B, O y HO groups showed higher serum and liver oleic acid levels with decrease of essential fatty acids levels compared to C. This fact would exacerbate the route of the ω9 family. S group presented high level of linoleic acid and low levels of linolenic in serum and liver. All groups didn´t show an increase in lipid peroxidation, this suggest that antioxidants provided by the diet might be playing a protective role. The changes in serum and liver fatty acid profile levels were in response to differences in the sources of dietary lipids.UBACyT 20020150100011BA.

083 (179) **ASSOCIATION BETWEEN LOW LEVELS OF HIGH DENSITY LIPOPROTEIN CHOLESTEROL (HDL-C) AND CHOLESTERYLESTER TRANSFER PROTEIN (CETP) IN PRE-SCHOOL CHILDREN (PSC) AND THEIR MOTHERS**

Maximiliano E. Martin[1], Karen Oestreicher[1], Eliana E Botta[1], Claudia Molinari[1], Walter Tetzlaff[1], Laura E. Boero[1], Fernando Brites[1], Valeria Hirschler[1].
[1]Laboratorio de Lipidos y Aterosclerosis, FFyB, UBA. Buenos Aires, Argentina.

Obesity has been associated with high prevalence of low HDL-Ceven at very early ages. In plasma, HDL is bound to CETP, which is responsible for the exchange of triglycerides (TG) and cholesterylesters between HDL and apolipoprotein B-containing lipoproteins. As far as we know, there are no studies involving the association between HDL-Cand CETP in PSC and their mothers. The aim was to determine the association between these two parameters in PSC and their mothers. A cross-sectional study was performed in November 2015 in a kindergarten from the Buenos Aires suburbs. Body mass index (BMI), blood pressure, lipids and CETP activity were obtained from 56 PSC and their mothers. HDL-C was determined by a standardized method and CETP activity by a radiometric assay. HDL-C levels<45 mg/dl for PSC and <50 mg/dl for their mothers were considered abnormal.Univariate correlations were performed to determine the associationsofage, BMI, lipids and CETP. Multiple linear regression analyses were performed to examine the associations of HDL-C, as dependent variable, with age, gender, BMI, TG, and CETP, as independent ones. The mean age was 5±1 years for PSC and 33±7 years for their mothers. The prevalence of overweight/obesity was 36% in PSC and 78% in their mothers. The prevalence of low HDL-C was 37% in PSC and 71% in their mothers.HDL-C was significantly associated with age (r;p<) (0.23;0.05), gender (0.23; 0.04) and CETP (-0.62;0.01) for PSCand with BMI (-0.46;0.01), and CETP (-0.49;0.01) for their mothers.Multiple linear regression analyses showed that HDL-C was significantly and inversely associated with CETP activityboth in PSC ($b=-0.16$; $R^2=0.41$) and their mothers ($b=-0.13$; $R^2=0.53$),adjusted by age, gender (only for PSC), BMI and TG. This study shows that HDL-C levels were inversely associated with CETP activity, suggesting that the activity of this protein could be at least partially responsible for the decrease in HDL-C levels observed in mothers and in their PSC.

084 (221) **IRON STATUS AND CHRONIC DISEASES RISK IN A GROUP OF HEALTHY ADULTS MALES FROM ARGENTINA**

Ana Lia Felipoff[1], Romina Airaldi[2], M. Luján Donadío[2], Vanesa Sebastiano[2], María C Maselli[3], Marcela Pandolfo[3], Silvana J Fleischman[1], Jorge A Rey[4], Alejandra Vellicce[4], Marta M Lardo[3)], Silvia H Langini[1].
[1]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica. Departamento de Sanidad, Nutrición, Bromatología y Toxicología. Cátedra de Nutrición. Buenos Aires, Argentina. [2]Universidad de Buenos Aires, Facultad de Medicina. Escuela de Nutrición. Buenos Aires, Argentina. [3] Universidad de Buenos Aires, Facultad de Farmacia y

Bioquímica. INFIBIOC. Buenos Aires, Argentina. [4] Universidad de Buenos Aires. Hospital de Clínicas "José de San Martín". Servicio de Hemoterapia. Buenos Aires, Argentina

Excess body Fe has been related with alterations in glucose homeostasis.To study relationship between Fe status and increased risk of prediabetes, 135 male blood donors, attending, Hospital de Clínicas José de San Martín, Universidad de Buenos Aires (2012-2014) were enrolled. Total Fe intake (FeI), including Fe from mandatory wheat flour fortification (FeIf), was estimated (ARGENFOODS and USDA National Nutrient Database on Standard Reference). Serum ferritin (SF) (IMMULITE Ferritin, DPC); transferrin saturation (TS) (%) (serum Fe/total iron binding capacity x 100) (IRON2 and Tina-quant Transferrin, Cobas) and HbA1c (COBAS, Roche) were determined in blood samples negative for infectious diseases and C-reactive protein (PCR-latex, Wiener lab). The characteristics of the studied population (mean±SD) were: age (y): 34.9±10.4 (older than 40y: 30%); body mass index (BMI): 27.1±3.9 Kg/m^2, overweight (OW) 44% and obesity (Ob) 20% ; FeI and FeIf (mg/d) (range): 21.9 ± 9.2 (7.6-58) and 10.4±6.8 (1.5-45) respectively; SF (ng/mL): 230±205 (4.9-1403); TS(%): 29.7±10.6 (6-77.7) and HbA1c (%): 5.3±0.5 (4.3-7.7) (ref. range: 4.5 to 5.9%). To determine interaction between HbA1c and FeI, SF and/or TS, data were divided into FeI quartiles (Q) (mg Fe/d) (mean±DS): 12.5±1.8; 17.6±1.7; 23.4±1.4 and 34.4±8.2, respectively (RDA: 8 mg Fe/d, FNB, 2001). Between FeI Q, HbAc1(%) values were not different (mean± SD):5.4±0.8, 5.2±0.3, 5.2±0.2, 5.3±0.3 (p=0.8878); and no subject showed criteria of Fe overload (TS and SF higher than 50% and 300 ng/mL, respectively). However, 7 subjects from different FeIQ, showed HbA1c values compatible with increased risk for prediabetes (5.7%-6.4%). As FeI was higher than Fe RDA in 157, 220, 292 and 430%, Q1 to Q4, and the FeIf accounts for 50% of FeI, increased risk of chronic diseases with age may be possible in people healthy and unaware of any family history of Fe overload. *Universidad de Buenos Aires, Programación Científica 2016, UBACyT 20720150100004BA.*

085 (248) **TIME AND DOSE PROFILE OF CLINICAL SYMPTOMS, MITOCHONDRIAL DYSFUNCTION AND OXIDATIVE DAMAGE BY COPPER TOXICITY IN RAT LIVER AND BRAIN**

Juan Manuel Acosta[1], Rosario Natalia Musacco Sebio[1], Christian Martín Saporito Magriñá[1], María Victoria Tuttolomondo[2], Martín Desimone[2,3], Alberto Boveris[1,3], Marisa Gabriela Repetto[1,3],

[1]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Química General e inorgánica. Junín 956 (C1113AAD), Buenos Aires, Argentina. [2]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Química Analítica Instrumental. Junín 956 (C1113AAD), Buenos Aires, Argentina. [3]Consejo Nacional de investigaciones Científicas y Técnicas, Instituto de Bioquímica y Medicina Molecular (IBIMOL-UBA-CONICET)

The epidemic increase in obesity causing insulin resistance and type 2 diabetes has become a worldwide problem. To better understand the mechanisms that lead to metabolic disorders, it is crucial to develop a better underlying knowledge of the molecular events that regulate adipocyte differentiation. Many positive modulators of this process have been identified, such as the CCAAT/enhancer binding protein (C/EBPβ) and peptide hormones like insulin.

The insulin receptor (IR) is transmembrane tyrosine kinase receptor, encoded by a single gene composed of 22 exons. Due to alternative splicing of exon 11, the gene gives rise to two protein isoforms that differ by a 12-amino-acid insertion: the IR lacking exon 11 (IR-A) and the IR containing exon 11 (IR-B). We hypothesize that IR plays an important role in regulating adipocyte differentiation process.

To address this issue, we have generated two IR KO clones from the 3T3-L1 cell line, using the CRIPSR/Cas9 system, and tested it as an adipocyte differentiation model. We verified accurate genome editing through sequencing and IR protein lacking through WB. These clones were unable to differentiate under standard differentiation protocols.

In order to recover the differentiation capacity in these KO clones, we re-expressed IR-A or IR-B through retroviral infection. Correct isoform expression was determined at mRNA level through RT-PCR. Preliminary results show that they recapitulated some of the molecular events typical of adipocyte differentiation. This was evaluated at the level of early adipogenic markers such as C/EBP and in late adipocyte-specific genes, such as the gene encoding aP2, a lipid-binding protein.

Taken together, these results suggest that IR could be modulating important steps of adipocyte differentiation. Further studies are necessary to elucidate the targets of IR action.

086 (265) **BONE CELLULAR ACTIONS OF GENISTEIN (GEN) INVOLVES ENDOTHELIAL CELLS ACTIVATION VIA NITRIC OXIDE PATHWAY**

Sabrina Cepeda[1], Carla Crescitelli[1], María Belén Rauscemberger[1], Marisa Sandoval[1], Virginia Massheimer[1].

[1]Instituto de Ciencias Biológicas y Biomédicas del Sur (INBIOSUR), Universidad Nacional del Sur (UNS), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Departamento de Biología, Bioquímica y Farmacia, Cátedra de Bioquímica Clínica II, Bahía Blanca, Argentina.

Previously we showed that, Gen enhances endothelial cells (EC) proliferation and nitric oxide (NO) synthesis, through a mechanism of action that involves the estrogen receptor. In this work, we studied the effect of Gen on bone-vascular axis and, its relationship with osteoblastic (OB) differentiation. To that end, primary cultures of murine (Wistar rats) endothelial cell (EC) or calvarial preosteoblast cell (OB) were used. OB monolayers were incubated (24 h) with culture medium obtained from EC (conditioned medium C). Thus, OB proliferation was measured (MTT assay and cell counting). Medium C stimulated OB proliferation (13%, $p<0.01$), mitogenic action that was enhanced (1-5 fold above control, $p<0.01$) when EC were exposed to different concentrations of Gen (10nM-5uM). Similar results were observed at different time intervals of Gen treatment. Medium C obtained from EC cultures pre-incubated with 10 uM L-NAME (nitric oxide synthase inhibitor), diminished OB proliferation. When OB were directly exposed to sodium nitroprusside, an exogenous nitric oxide (NO) donor, stimulation of OB proliferation (48% above control, $p<0.01$) was detected. Direct treatment of OB with Gen or estrone, a natural estrogen ER agonist, did not modified cell growth. The effect of Gen on OB differentiation was studied using two markers: alkaline phosphatase activity (AP) and extracellular calcium deposition (HCl leaching of calcium assay). Both markers were enhanced after 12 days of culture (0.42±0.1 vs 0.25±0.08 IU/mg prot. AP activity, $p<0.05$; 144±43 vs 80±19 ugCa/mg prot., $p<0.001$, Gen vs. C). Similar results were obtained after 15 days of culture. Using red Alizarin staining, an increase in the number and size of calcification nodules was also observed. These results suggest that Gen promotes bone cells growth and differentiation through its action at endothelial level. Nitric oxide is a potential biochemical messenger involved in this close link between bone and vascular systems.

087 (303) **COMPARISON OF REDOX STATUS IN LIVER AND BRAIN IN AN EXPERIMENTAL MODEL OF GLUTAMATE MEDIATED EXCITOTOXICITY**

Fabiana Lairion[1,2], Ailen Hvozda Arana[1], Valeria Calabro[2], Claudia Reides[1], Nathalie Weichsler[1], Susana Llesuy[1,2], Sandra Ferreira[1,2].

[1]Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Cátedra Química General e Inorgánica. [2] IBIMOL, UBA-CONICET.

Increased levels of glutamate, main excitatory neurotransmitter in the central nervous system, have been associated with various neurological disorders, such as epilepsy, cerebral ischemia, Parkinson and glaucoma. The aim of this study was to assess changes in the redox status in the liver and the brain of rats subject to glutamate mediated excitotoxicity.

The experimental model consisted of one group (n=3) injected ip with 1g glutamate/kg weight (GHD), another group (n=3) injected ip with 0.5 g glutamate/kg weight (GLD) and control group (n=3) injected ip with saline solution (CG). All groups received glutamate or saline solution at days 1, 5 and 9. The following markers were evaluated in liver and in brain homogenates: lipid oxidation (TBARS), catalase (CAT) levels, and the activities of superoxide dismutase (SOD) and glutathione transferase (GT). The results in brain showed that both doses of glutamate produced a significant increase of SOD (31% GLD *p<0.001*; 27% GHD *p<0.001*) and a significant increase of CAT levels (28% GLD *p<0.05*; 94% GHD *p<0.05*). An increase of 36% in GT activity was only observed in the highest dose of glutamate (*p<0.05*).

The results in liver showed a significant increase in SOD (45% GLD *p<0.01*; 73% GHD *p<0.001*), with significant differences between both doses (19% *p<0.05).* However, no changes in glutathione transferase neither in catalase, were found in liver, even at the highest dose.

No significant differences were observed in TBARS levels in both organs.

In this excitotoxicity model, the brain is more vulnerable than liver to oxidative stress at both glutamate doses; an up-regulation of antioxidant enzymes activities may be a consequence of an increase in oxidative process.

088 (365) **EFFECTS OF METFORMIN ON METABOLIC AND CARDIOVASCULAR DISORDERS ON A MODEL OF INSULIN RESISTANCE IN RATS**

Hyun Jin Lee[1], Silvana María Cantú[1], Nahuel Mariano Sánchez Eluchans[1], Adriana Susana Donoso[1], Horacio Angel Peredo[1], Ana María Puyó[1].

[1]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Anatomía e Histología.

Metformin (Mf) is used as therapy for type 2 diabetes and metabolic syndrome (MS). A high-fat (HF) diet produces insulin resistance in the rat, which is related to metabolic and hemodynamic alterations that resemble human MS There is evidence of the pathogenic potential of adipose tissue which could explain some of the risk factors of MS. We analyze the effect of Mf on metabolic and hemodynamic parameters; adiposity index; fatty liver; vascular remodeling of abdominal aorta (AA) in male Sprague-Dawley rats. Four groups were studied during 12 weeks (n=6 each): Control (C), standard diet (SD) and tap water to drink; HF diet (HF), 50% (w/w) bovine fat added to SD; C + Mf (CMf), 500 mg/Kg/day Mf in drinking water; and HF + Mf (HFMf). The adiposity index was calculated as: mesenteric vascular fat bed (MVB) weight/body weight (BW) x 100. Morphometry of liver and AA samples were performed by image analysis. HF diet increased BW gain (g, 264±7 vs. 229±13, p<0.05); glycemia and triglyceridemia (mg/dl, 144±4 vs. 124±3; 166±21 vs. 86±9, both p<0.05); insulinemia (ng/dl, 3.9±0.7 vs. 1.1±0.2, p<0.05); systolic BP (mmHg, 150±2 vs. 123±3, p<0.001); MVB weight/BW ratio (1.72±0.1 vs. 0.8±0.1, p<0.001); fat liver index (%, 87±4 vs. 0.8±0.3, p<0.001); and AA wall thickness (WT)/lumen diameter (LD) ratio (µm/mm, 6.4±0.4 vs. 4.8±0.3 p<0.05). Mf decreased BW gain (g, 198±19 vs. HF, p<0.05); glycemia, triglyceridemia (mg/dl, 110±11 vs. HF; 65±13 vs. HF, both p<0.05); insulinemia (ng/dl, 1.3±0.1 vs. HF, p<0.05); systolic BP (mmHg, 138±1 vs. HF, p<0.05); MVB weight/BW ratio (1.38±0.1 vs. HF, p<0.05); fat liver index (%, 72±4 vs. HF, p<0.05); and AA WT/LD ratio (5.4±0.1 vs. HF, p<0.05). In conclusion, our findings show that Mf treatment ameliorates metabolic and cardiovascular dysfunctions in a rat model of MS. We have demonstrated by liver biopsy that Mf prevents fatty liver development and avoids vascular remodelling, peripheral resistance and PAS elevation.

089 (434) **DIETARY SOY PROTEIN IMPROVES ADIPOSE TISSUE DYSFUNCTION BY MODULATING PARAMETERS ASSOCIATED WITH OXIDATIVE STRESS IN RATS FED A SUCROSE-RICH DIET**

Paola Guadalupe Illesca[1], Silvina Mónica Álvarez[2,3], Dante Alejandro Selenscig[1], María del Rosario Ferreira[1,3], María Sofía Giménez[2,3], Yolanda B. Lombardo[1,3], María Eugenia G D'Alessandro[1,3],

[1]Departamento de Ciencias Biológicas. Facultad de Bioquímica y Ciencias Biológicas. Universidad Nacional del Litoral, Ciudad Universitaria, El Pozo cc 242, 3000, Santa Fe, Argentina. [2]Laboratorio de Bioquímica Molecular. Facultad de Química, Bioquímica y Farmacia. Universidad de San Luis, Avenida Ejercito de los Andes 950, 5700, San Luis, Argentina. [3]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Santa Fe, Argentina.

Numerous studies link dietary soy protein with beneficial effects on different metabolic disorders (dyslipidemia, diabetes, atherosclerosis). One of the potential mechanisms mediating these effects might be the reduction of oxidative stress. The adipose tissue (AT) is especially susceptible to the damage of oxidative stress. The aim of this study was to evaluate the possible beneficial effect of dietary intake of soy protein isolated (SPI) on AT dysfunction - antioxidant defenses and reactive oxygen substances (ROS) production- in a rat model that mimics several aspects of the human Metabolic Syndrome. Male Wistar rats were fed a sucrose rich diet (SRD) (62.5% sucrose) for 4 months, after were divided into two subgroups, one continued with SRD until month 8, while the other received SRD in which casein was replaced by SPI for additional 4 months. A reference group consumed a control diet all the time. In AT were determined: i) the activities of antioxidant enzymes (SOD, CAT, GPx), GR and gene expression of Mn-SOD and GPx; ii) ROS levels, xanthine oxidase (XO) activity and gene expression of NAD(P)H oxidase; iii) the expression of Nrf-2 transcription factor. Besides, plasma levels of uric acid, protein carbonyl groups, TBARS and the inflammatory cytokine TNF-α were also determined. Compared with the SRD-fed rats, SRD-S normalizes the activities of SOD and GR, improves/normalizes gene expression of SOD and GPx respectively without changes in the expression of Nrf2. In addition, both XO activity was normalized and ROS levels were improved. The SPI decreased the TNF-α and restored plasma levels of uric acid, protein carbonyl and TBARS. The present study show a beneficial effect of SPI upon dysfunctional adipose tissue in dyslipemic and insulin-resistant rat model, suggesting that soy protein can be a complementary nutrient to ameliorate alterations associated with Metabolic Syndrome.

090 (395) **PREVENTIVE EFFECT OF A PROBIOTIC ADMINISTRATION ON THE METABOLIC ALTERATIONS CAUSED BY A FRUCTOSE RICH DIET INTAKE**

María Guillermina Zubiría[1], Sabrina Eliana Gambaro[1], Paula Carasi[2], Martín Rumbo[3], María de los Angeles Serradell[2], Andrés Giovambattista[1].

[1]Instituto Multidisciplinario de Biología Celular (IMBICE) CICPBA-CONICET-UNLP. [2]Laboratorio de Microbiología, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, UNLP. [3]Instituto de Estudios Inmunológicos y Fisiopatológicos (IIFP), Facultad de Ciencias Exactas, UNLP-CONICET.

There is large evidence that gut microbiota modulates metabolic disorders associated with obesity. We evaluated the preventive effect of a probiotic Lactobacillus administration on the metabolic alterations caused by a fructose rich diet (FRD). Four different Swiss mice groups were studied (n=10), CTR: mice were given tap water, FRD: mice were given 20% w/v fructose in the drinking water, and two similar groups that received by gavage 10^8 CFU of *Lactobacillus kefiri* CIDCA 8348 every 48 hs (CTR-Lk or FRD-Lk, respectively). Caloric intake and body weight were recorded every 48 hs. After 6 weeks, mice were euthanized and plasma samples were collected. Epididymal adipose tissue (EAT) was dissected, weighted and processed for qPCR and cellular analysis. Stromal vascular fraction (SVF) cells were isolated from EAT and processed for gene expression analysis. FRD and FRD-Lk showed an increased in caloric intake (P<0.05 *vs.* CTR and CTR-Lk), but only the FRD mice showed an increase in weight gain and EAT mass (P<0.05 *vs.* CTR, CTR-Lk and FRD-Lk). Plasmatic triglycerides levels were higher in FRD mice (P<0.05 vs CTR and

CTR-Lk) and revert to control levels in FRD-Lk group (P<0.05), while blood glucose did not display any difference between groups. The mRNA expression of functional adipose tissue genes (Ob, LPL, Adiponectin, GPR43) and pro-inflammatory genes (INFγ and TNFα) in EAT were assessed. All these genes were higher in FRD mice, but were reverted with the lactobacillus administration (FRD-Lk, P<0.05). For the SVF from FRD mice, where the immune cells are in high proportion, the expression levels of INFγ and TNFα were higher (P<0.05 vs CTR and CTR-Lk), while for FRD-Lk the expression values were similar to CTR mice. With this preliminary results we can state that the administration of *L. kefiri* prevents the deleterious effects of a FRD, impacting the weight gain and the functional profile and pro-inflammatory state of the EAT.

091 (538) **TRIGLYCERIDE RICH LIPOPROTEINS IN INSULIN-RESISTANCE: COORDINATED ACTION OF ADIPONECTIN AND LIPOPROTEIN LIPASE ON LARGE VLDL AND REMNANT LIPOPROTEINS**

Diego Lucero[1, 2, 3], Verónica Miksztowicz[1, 2, 3], Valeria Zago[1, 2, 3], Graciela I López López[1, 2], Katsuyuki Nakajima[4], Gabriela Berg[1, 2, 3], Laura Schreier[1, 2],

[1]Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Departamento de Bioquímica Clínica. Laboratorio de Lípidos y Aterosclerosis. [2]Universidad de Buenos Aires. Instituto de Fisiopatología y Bioquímica Clínica (INFIBIOC). [3]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) [4]Graduate School of Health Sciences, Gunma University, Gunma, Japan.

Insulin-resistance (IR) is characterized by increased circulating triglyceride rich lipoproteins (TRL), result of increased production of altered VLDL and accumulation of remnant lipoproteins (RLP), of great atherogenicity. Reduced Adiponectin (ADP) and lipoprotein lipase (LPL) activity are related to TRL metabolism. It is not clear how these factors influence TRL levels in IR. Aim: to evaluate the role of ADP and LPL on circulating TRL in IR. Methods: We studied 30 patients with metabolic syndrome (MetS) (ATPIII) and 15 healthy controls. In fasting serum we measured lipid profile, glucose, insulin levels and calculated HOMA-IR. ADP was measured by ELISA. TRL was isolated by ultracentrifugation (d<1,006 g/L), the sum of all TRL components was considered as its total mass and TRL sub-fractions were analyzed by size exclusion HPLC. RLP cholesterol (RLP-c) and triglycerides (RLP-TG) were measured by immunoaffinity separation using a commercial kit. LPL activity was assessed in post-heparin plasma obtained 10 min after injection of 60 U/kg heparin. Results: As expected, MetS patients showed higher plasma triglycerides and reduced HDL-c and ADP levels (p<0.05). MetS showed increased TRL mass (p<0.01), large VLDL% (p=0.01), RLP-c (29.8±12.9 *vs.* 13.9±11.2 mg/dl; p=0.02) and RLP-TG (58.4±29.7 *vs.* 12.5±9.9 mg/dl; p=0.003). Whereas, LPL activity was reduced in MetS (p=0.01). After adjusting by HOMA-IR, ADP was negatively associated with TRL mass (β= -0.42, p=0.01), large VLDL (β= -0.35, p=0.03), and positively associated to LPL activity (β= -0.35; p=0.02). Interestingly, ADP negatively correlated with RLP-c (β= -0.55, p=0.03) and RLP-TG (β= -0.50, p=0.03), independently of IR. LPL activity negatively correlated with RLP-TG (r= -0.56, p=0.05). Conclusion: In MetS, ADP reduction induces increase in TRL by secretion of large VLDL particles. Also, the coordinated reduction of ADP and LPL activity favors the accumulation of RLPs, enhancing the atherogenic risk of MetS.

092 (536) **MITOCHONDRIAL RESPIRATORY CHAIN ALTERATIONS DUE TO PORPHYRINOGENIC AGENTS IN A MOUSE MODEL OF ACUTE INTERMITTENT PORPHYRIA**

Johanna Romina Zuccoli[1], Silvina Fernanda Ruspini[1], Alcira Maria del Camen Batlle[1], Ana Maria Buzaleh[1,2].

[1]Centro de Investigaciones sobre porfirinas y porfirias (Cipyp), Hospital de Clinicas, CONICET-UBA. [2]Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires.

The mitochondria play a vital role in energy metabolism, reactive oxygen species generation and cell death. Heme is one of the most biologically diverse molecules in nature; its deficiency by a reduced synthesis or an accelerated catabolism would trigger severe cell damage. Previously we demonstrated that porphyrinogenic agents affected several brain metabolisms included respiratory mitochondrial chain in encephalon *CF1* mice. The aim was to study the effects of volatile anaesthetics and other xenobiotics on the activity of I to IV complexes of respiratory chain in a mouse model of Acute Intermittent Porphyria. For this purpose we use male knockout mice that have 50% reduced the activity of the enzyme Porphobilinogen deaminase, treated with Isoflurane (2 ml/kg), Sevoflurane (1,5ml/kg), ethanol (30%), allylisopropylacetamide (AIA, 350 mg/kg) and Veronal (167 mg/kg). The activities of I-III, II-III, II and IV complexes were measured in mitochondria of encephalon. Veronal increased the activity of II-III complexes (370%; p<0.01) and II complex (218%; p<0.01). Ethanol decreased I-III and II complexes activities (48%; p<0.01), moreover complex IV activity was induced (86%; p<0.01). Sevoflurane increased only the activity of complex II (218%, p<0.05) and AIA augmented complexes II-III activities (218%; p<0.01). Results reinforce our previous reports and support the hypothesis that there would be more than one factor to explain the pathogenesis of acute attacks. The alterations observed in the activities of the respiratory complexes in this genetic model of PAI could be the result of a deficiency of the donor reduction equivalents, NADH and $FADH_2$, as a consequence of Krebs cycle alteration, caused by the decrease of succinyl-CoA, substrate of 5-Aminolevulinic acid synthetase, the heme regulatory enzyme.

093 (627) **METABOLIC EFFECTS OF CONSUMING CHOLESTEROL RICH-DIETS AND THERMALLY OXIDIZED VEGETABLE OIL, IN GROWING RATS**

Estefania Alsina[1], Cecilia Ramos[1], Valeria Zago [3], Silvia Giacomino[2], Iana Belen Perez Alcaraz[1], Elisa Vanesa Macri[1], Silvia Friedman[1].

[1]Department of Biochemistry. Faculty of Dentistry.University of Buenos Aires. [2]Department of Bromatology. Faculty of Pharmacy and Biochemistry.University of Buenos Aires. [3]Laboratory of Lipids and Lipoproteins, Department of Clinical Biochemistry, Faculty of Pharmacy and Biochemistry, INFIBIOC, University of Buenos Aires.

Conventional sunflower oil is the most widely used in the gastronomic sector. However, the consumption of oils subjected to repeat frying may affect lipid and energy metabolism with potential health risks. The effects of consuming thermally oxidized vegetable oil and cholesterol (chol) rich-diet on body growth, body fat and serum lipid lipoprotein profiles in growing rats were investigated.

At weaning, male Wistar rats (n=24; 21±1d) were randomly assigned to 1 of 4 groups according to the lipid source supplemented to a commercial diet (13% w/w). Rats were fed ad libitum for 8 wk. SFO received sunflower oil; AT, an atherogenic diet (saturated fat plus chol); SFOx, SFO oxidized frying oil at 180 °C for 40 hours and SFOx-chol (SFOx plus chol). Anthropometry (body weight and length (Wt and L) and food consumption were weekly recorded. At T=8wk: anthropometry,% body fat distribution (epididymal (EF) and perirenal (RF)),% total body fat (%TF, DXA), serum (mg%) total chol (T-chol), triglyceride (TG) and nonHDL-chol were assessed. Atherogenic index (AI) expressed as T-chol/HDL-chol was analyzed.

Results (mean±SD, ANOVA-SNK, p<0.05). There were no significant differences in energy intake among groups; however, final Wts and Ls of SFOx and SFOx-chol were lower than SFO and AT (p<0.001).%EF and%RF were significantly decreased in AT, SFOx and SFOx-chol as compared to SFO (p<0.01), despite SFOx-chol and SFO rats showed similar% TF (p>0.05). Trygliceridemia remained similar among groups (p> 0.05). Serum T-Chol and nonHDL-Chol were significantly increased in AT and SFOx-chol groups (p<0.001). Although these differences were not detected in AI (SFO;1.37±0.10=SFOx;1.42±1.37< AT;3.38±0.73=SFOx-chol; 3.18±0.30; p< 0.001).

Conclusion: Feeding rats with diets containing thermally oxidized vegetable oils impaired body growth and appeared to have

less power on body fat, body fat distribution or serum lipids than the presence of hypercholesterolemia. UBACyT: 20020130100506BA.

094 (614) **PARADOXICAL INCREASE OF ADIPONECTIN IN CHRONIC KIDNEY DISEASE: EVALUATION OF ITS EXPRESSION IN ADIPOSE TISSUE**

Leonardo Cacciagiu[1], Veronica Miksztowicz[1], Fernando Chiquilito[2], Magali Barchuk[1], Elsa Zotta[3], Ana Gonzalez[1], Gabriela Berg[1], Laura Schreier[1],

[1]Laboratorio de Lípidos y Lipoproteínas, Departamento de Bioquímica Clínica, Facultad de Farmacia y Bioquímica - INFIBIOC, UBA. [2]IFIBIO Houssay Conicet. [3]IFIBIO Houssay Conicet, Cátedra de Fisiopatología, Facultad de Farmacia y Bioquímica, UBA.

Chronic kidney disease (CKD) at stage 5 is associated with a marked increase in cardiovascular mortality, even in hemodialysis. The presence of insulin-resistance (IR) in CKD is a common metabolic condition. On the other hand adiponectin (ADPN) is an anti-inflammatory and insulin-sensitizing cytokine, which decreases in IR conditions. Paradoxically ADPN is elevated in CKD, probably due to an increased synthesis or a decreased clearance. Aim: To elucidate the cause of paradoxical increase circulating ADPN in rats with CKD. Materials and Methods: A model of renal failure was implemented by 5/6 nephrectomy in male Sprague Dawley rats, which were divided into two groups: control group (C,n = 10) and group nephrectomy 5/6 (NX,n = 10) . NX group underwent ablation of renal mass by cutting the two left renal poles and the total removal of the right kidney. C rats were undergone to sham operations without ablation of renal tissue. Animals were sacrificed at 8 weeks. Lipid profile and ADPN (ELISA with specific antibodies) were measured in serum. Adipose tissue was extracted and measured ADPN expression by western blotting using polyclonal rabbit primary specific antibody. Results: NX had lower creatinine clearance validating the CKD model (p <0.01). Regarding lipid profile NX had higher triglycerides levels: 161±65 *vs* 74±40 mg/dL and low levels of HDL-chol: 13±8 *vs* 38±7 mg/dL; p <0.001. ADPN was higher in NX: 16.2±1.6 *vs* C: 13.7±1,8 ug/mL; p <0.05. ADPN adipose tissue expression showed no differences between groups (p = 0.65). Conclusions: in CKD, increased circulating ADPN does not respond to an increase in its expression, suggesting that it may be due to a decrease in its clearance through the kidney. The unfavorable lipid profile indicates that, although ADPN is high, it would not be functioning

095 (739) **THE IMPACT OF THE PRENATAL STRESS IN THE DEVELOPMENT OF OBESITY**

Yamila R Juárez[1], Alejandro Emiliano Mercado[1], Maria Rosa Gonzalez Murano[1], Ana Maria Genaro[1], Adriana Laura Burgeño[1].

[1]Instituto de Investigaciones Biomédicas (BIOMED UCA-CONICET).

According to the '*fetal programming hypothesis*', prenatal exposure to suboptimal intrauterine conditions could predispose the individual to chronic disease at adult age. Over the past decades, obesity has increased its worldwide prevalence. Therefore identifying the factors that predispose its development is essential. In the present work, we studied the effect of prenatal stress (PS) on the development of obesity. For this purpose pregnant C57BL/6J female mice were stressed during the last week of pregnancy for 2 h daily (from 10 AM to 12) by placing them in a plastic restraining tube. Non-exposed control pregnant females were left undisturbed during all the gestation period (NPS). At 4th week of age, both PS and NPS offspring were fed with one of the following diets: High Fat diet (HFD, 4800kcal/kg) or a standard diet (SD, 3000kcal/kg). After 12 weeks of diet, PS/SD males showed no differences in body weight compared to NPS/SD. Instead, PS/HFD males gained more body weight than the NPS/HFD (p <0.05). Within the group of PS males we observed that those fed with HFD had more body weight than SD (p <0.001). After 16 weeks of diet we observed a difference in body weight between PS/HFD vs PS/SD females (p<0.01). Unlike males, PS/HFD vs NPS/HFD females did not show a difference in their body weight. When we performed a glucose tolerance test, we observed that all males (PS + NPS) fed with HFD showed a higher area under the curve than those who were fed with SD (p<0.00001). While females showed no significant difference. We conclude that the PS predisposes the development of obesity in male mice, but this only happens under the intake of a HFD. Furthermore, these results suggest that exists a sexual dimorphism response to the development of obesity after prenatal stress.

096 (717) **PROTEIN MALNUTRITION DURING THE CRITICAL DEVELOPMENTAL STAGE INDUCES HEPATIC INJURY AND AN INCREMENT OF INFLAMMATION AND OXIDATIVE STRESS MARKERS IN ADULT OFFSPRING FEMALE WISTAR RATS**

Sabrina Edith Campisano[1], Stella Maris Echarte[2], Rocío Abalo[1], Matías Preisegger[3], Ricardo Dewey[3], Andrea Chisari[2].

[1]Departamento de Química, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata. [2]Instituto de Investigaciones Biológicas, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata. 3Instituto de Investigaciones Biotecnológicas – Instituto Tecnológico Chascomús (IIB-INTECH).

Individuals who suffer malnutrition during gestation, lactation and childhood have an elevated predisposition to develop metabolic diseases in adulthood, a phenomenon called fetal programming. We evaluated the effects of protein malnutrition on liver damage and expression levels of inflammation and oxidative stress markers in adult offspring female rats subjected to different malnutrition schemes. Pregnant rats were fed with a control diet (CD) or a low protein diet (LPD). Female offspring rats received a LPD during gestation, lactation and until they were 120 days of age (MM group), a late LPD that began after 60 days (CM), or a LPD administrated during gestation, lactation and up to 60 days, followed by a CD (MC). The control group received only a CD. On day 120 the liver was dissected out. The ultrastructure was analyzed by TEM and the expression levels of TGF-β, TNF-α and GST-pi 1 were determined by RT-qPCR. Hepatocytes from MM livers showed a large number of lipid vacuoles and the cellular matrix was lost. The level of liver injury was lower in the hepatocytes from MC group, which showed mitochondrial swelling and vacuolation. Hepatocytes in CM group showed the lowest injury and the cytoplasmic matrix was more intact than that in MC group. We found high expression levels of TGF-β and GST-pi 1 in the malnourished rats: TGF-β (MM: 10.68±0.94; p<0.01, CM: 2.7±0.23; p<0.01, MC: 6.59±0.11; p<0.01), GST-pi 1 (MM: 5.88±0.04; p<0.01, CM: 1.85±0.04; p<0.01, MC: 3.08±0.06; p<0.01). MM group showed the highest expression of both (p<0.01), and the CM group the lowest (p<0.01). The level expression of TNF-α was higher in MM (6.61±0.59; p<0.01) and MC rats (2.04±0.48; p<0.05). MM group showed the highest expression (p<0.01). These results suggest that protein malnutrition during the development predisposes to the occurrence of fatty liver and the increment of inflammation and oxidative stress markers in adulthood, in accordance with the fetal programming phenomenon.

097 (762) **ENDOTHELIAL DYSFUNCTION IN INSULIN-RESISTANCE: EFFECT OF SUPPLEMENTATION WITH OLEIC ACID FROM HIGH OLEIC SUNFLOWER OIL**

Carolina Olano[1,2], Diego Lucero[1,2,6], Susana Gorzalczany[3], Celina Morales[4], Michelle Bursztyn[1], Silvia Friedman[5], Vanesa Macri[5], Laura Schreier[1,2], Valeria Zago[1,2,6].

[1]Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Departamento de Bioquímica Clínica. Laboratorio de Lípidos y Aterosclerosis. [2]Universidad de Buenos Aires. Instituto de Fisiopatología y Bioquímica Clínica (INFIBIOC). [3]Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Cátedra de Farmacología. [4]Universidad de Buenos Aires. Facultad de Medicina. Instituto de Fisiopatología Cardiovascular (INFICA). [5]Universidad de Buenos Aires. Facultad de Odontología. Cátedra de Bioquímica General y Bucal. [6]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

In insulin-resistance (IR), VLDL is partly responsible of the associated endothelial dysfunction. Consumption of monounsaturated fatty acid rich oils, such as high oleic sunflower oil (HOSO), has been one of the advised strategies with beneficial effects on plasma lipid profile and possibly on VLDL and endothelium in the IR context. Aim: to evaluate in IR rat model, the effect of HOSO supplementation on endothelial function and morphology, and to assess the role of isolated VLDL on endothelium. Methods: Male Wistar rats (180-200g) were fed with standard diet (Control, n=5), standard diet+sucrose 30% (DRS, n=5), standard diet+HOSO 15mg% (HOSO, n=5) and standard diet+HOSO 15mg%+sucrose 30% (HOSO-SRD, n=5) for 12 weeks. Thoracic aorta rings were removed, suspended in an organ chamber, and contracted with noradrenaline. Dosis-response relaxation curves, by cumulative acetylcoline (10-9-10-5M), were evaluated and the percentage of relaxation (%R) was calculated. An aorta fragment from each group was taken for histological analysis. In parallel, VLDL ability to inhibit vasorelaxation was evaluated with healthy aorta rings by the same dosis-response curves, in the presence of isolated VLDL (d=1.006 g/ml) from each group. Results: Aorta rings from DRS and HOSO-DRS showed decreased endothelium-dependent relaxation compared to control (67±2, 69±5 vs 82±11%R, $p<0.05$), without differences between them (p=0.531). Histological analysis revealed that HOSO-DRS showed greater focal loss of endothelial continuity solution with presence of suggestive deposits of lipid material, in comparison to DRS ($p<0.05$). Moreover, VLDL from DRS and HOSO-DRS showed lower%R than VLDL from controls (69±7, 65±4 vs 85±6; $p<0.01$). Conclusion: Supplementation with oleic acid from HOSO, not only did not improve endothelial function and VLDL impact in IR, but also impaired the artery intima status. Thus, the proposal of HOSO supplementation in diet should be revised.

MEDICINA REGENERATIVA Y TERAPIA CELULAR/ REGENERATIVE MEDICINE AND CELULAR THERAPY

098 (493) **DIFFERENTIAL EXPRESSION OF MEMBERS OF THE E2F TRANSCRIPTION FACTORS FAMILY IN HUMAN PLURIPOTENT STEM CELLS AND IN ITS DIFFERENTIATED NEURAL PROGENY**

María Soledad Rodríguez Varela[1], Verónica Alejandra Furmento[1], Guillermo Videla Richardson[1], Nicolás Alexis Dimopoulos[1], Santiago Gabriel Miriuka[1], Gustavo Emilio Sevlever[1], María Elida Scassa[1], Leonardo Romorini[1]

[1]Laboratorio de Investigación Aplicada a Neurociencias (LIAN)-CONICET, FLENI.

Embryonic or induced human pluripotent stem cells (hESCs and hiPSCs) have an unusual cell cycle structure which consists of a short G1 phase and absence of the G1/S checkpoint. E2F transcription factors are critical for the temporal expression of oscillating cell cycle genes. In somatic cells, both activator (E2F1, E2F2, E2F3a) and repressor E2Fs (E2F3b, E2F4, E2F5) show a periodic expression profile. Activator E2Fs present higher expression levels in the S phase while repressor E2Fs in the G2/M phase. In humanpluripotent stem cells (hPSCs), the role of RB/E2F complexes still remains uncertain and their expression profiles during cell cycle progression have *not been fully studied yet*. Thus, the aim of this work was to explore if E2Fs mRNAs are constitutively or periodically expressed in hPSCs. We found by BrdU-7AAD flow cytometry analysis that hPSCs have a S phase cell population of 43,5±12%, whereas their differentiated neural progeny, Nestin$^+$/ Neurofilament light chain$^+$/ Doublecortin$^+$ progenitors (NP), one of 9,2±0,7% (similar to somatic cells, fibroblasts). At the same time, we observed by RT-qPCR analysis that hPSCs express higher levels of E2F1, E2F3, E2F4 and E2F5 transcripts than fibroblasts. In contrast, E2F2 and E2F3b mRNA expression levels did not significantly differ from somatic cells in hESCs, whereas hiPSCs show higher expression levels of both transcripts. NP exhibit similar expression profiles than the hPSCs from which they derive, except for E2F2 mRNA, which is highly expressed in NP. Finally, to determine if E2Fs are periodically or constitutively expressed we synchronized the cells in G1/S with PD0332991, specific inhibitor of CDK4/6 (30h 5 µM for hPSCs and 24h 1 µM for NP), and in G2/M with Nocodazole (24h 100 ng/ml for hPSCs and 54h 200 ng/ml for NP). RT-qPCR analysis of synchronized cells revealed significant differences in the expression levels of E2Fs transcripts suggesting that they are expressed in a cell cycle phase-specific manner.

099 (113) **MESENCHYMAL STEM CELL THERAPY FACILITATES ORGAN PRESERVATION FOR LUNG TRANSPLANTATION**

Natalia Pacienza[1], Diego Santa Cruz[1], Oscar Robledo[2], Gaston Lemus[2], Alejandro Bertolotti[3], Martin Marcos[2], Gustavo Yannarelli[1]

[1]Laboratorio de Regulación Génica y Células Madre, Instituto de Medicina Traslacional, Trasplante y Bioingeniería (IMeTTyB), Universidad Favaloro-CONICET, Solís 453, (1078) CABA, Buenos Aires, Argentina. (2) Departamento de Cirugía. Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, Calle 60 y 118, La Plata (1900), Buenos Aires, Argentina. (3) Instituto de Cardiología y Cirugía Cardiovascular, Fundación Favaloro. Av. Belgrano 1746, (1039) CABA, Buenos Aires, Argentina.

Lung transplantation is a lifesaving therapy for patients suffering from end-stage lung diseases. As only 15% of lungs from multi-organ donors are deemed usable for transplantation, the waiting list mortality can be as high as 30%. Today, the emphasis of lung preservation is tending to facilitate organ recovery and regeneration prior to implantation using different technologies. Here, we propose to increase organ procurement by using a mesenchymal stem cell (MSCs) therapeutic strategy to better preserve lungs from non-heart-beating donors which are currently discarded. We developed an ex-vivo lung perfusion rat model that mimics the different stages through which donor organs must pass before implantation. The therapeutic schema was as follow: cardiac arrest, hot ischemia (2h at RT), cold ischemia (1.5h at 4°C), alveolar recruitment, and lung reperfusion with ventilation (Steen solution, 1h at RT). Human umbilical cord MSCs were infused via the pulmonary artery after 1h of hot ischemia (1x106 cells or PBS). Physiologic data (pressure-volume curves, lung compliance) were acquired right after cardiac arrest and during reperfusion. Pulmonary edema, redox profile and neutrophil infiltration were determined from lung biopsies (Basal, PBS and MSCs). MSCs therapy significantly reduced oxidative damage by controlling ROS production (Protein carbonyl: Basal, 2.2±0.5; PBS, 12.6±1.5; MSCs, 8.8±0.7 µmol/mg, $p<0.05$). Consequently, catalase and superoxide dismutase enzyme activities remained at baseline levels in MSCs-treated lungs. Although lung edema did not change among groups, MSCs infusion decreased neutrophil infiltration by 40% compared with PBS ($p<0.01$). Interestingly, lung compliance dropped a 38% in the MSCs group, while the PBS group showed a stronger reduction (65%, $p<0.05$). In conclusion, these results demonstrate that MSCs infusion prevents lung injury through an anti-inflammatory mechanism and promote MSCs therapy as a novel tool in organ preservation.

100 (112) **CHROMATOGRAPHICALLY ISOLATED EXOSOMES FROM MESENCHYMAL STEM CELLS (MSCS) OVER-EXPRESSING HEME OXYGENASE-1 FOR TISSUE REGENERATION**

Diego Mario Santa Cruz[1], Natalia Alejandra Pacienza[1], Dong-ki Kim[2], Ricardo Malvicini[1], Darwin Prockop[2], Gustavo Gabriel Yannarelli[1]

[1]Laboratorio de Regulación Génica y Células Madre, Instituto de Medicina Traslacional, Trasplante y Bioingeniería (IMeTTyB), Universidad Favaloro-CONICET, Solís 453, C1078AAI Buenos Aires, Argentina. (2) Institute for Regenerative Medicine at Scott & White, College of Medicine, Texas A&M Health Science Center, Temple, TX 76502.

Regenerative properties of mesenchymal stem cells (MSCs) are largely mediated by paracrine mechanisms, including the

secretion of extracellular vesicles (EVs). Exosomes are EVs of endosomal origin that may constitute a safe and effective cell-free therapy. We developed a scalable chromatographic procedure for production of exosomes derived from human MSCs over-expressing heme oxygenase-1 (HO-1) that might have anti-inflammatory/immunosuppressive potential. MSCs were activated with hemin, an HO-1 inducer, for 24 h and subsequently cultured in a chemically defined protein-free media (CDPF) during 48 h to collect the exosomes. Hemin treatment up-regulated HO-1 mRNA expression by 54±14-fold compared with control MSCs ($p<0.01$). The MSCs did not expand, but there was little evidence of cell death in CDPF. In addition, culture in CDPF medium increased the mRNA expression for the inflammation-modulating protein TSG-6 by 74±6-fold and 68±6-fold in control and hemin-treated MSCs, respectively. There were no significant changes in the mRNA expression of the pro-inflammatory cytokines IL-1b and IL-8. CDPF conditioned media was subjected to ion exchange chromatography (IEX). The protein eluted with 0.5 M NaCl was recovered as a single broad peak that contained CD63, a marker for exosomes. Quantification of CD63 by ELISA showed that the column provided a 150-fold concentration with a recovery of up to 80% of the exosomes. Assay of the peak fractions with a nanoparticle tracking system demonstrated a similar vesicle size for control and hemin-treated MSCs (86.9±1.3 and 86.1±0.9 nm, p=ns). This result infers the presence of exosomes in the absence of other types of EVs. Only exosomes derived from activated MSCs contained HO-1. In conclusion, we provide a scalable protocol to isolate exosomes from MSCs fully compatible with clinical applications by using CDPF media and IEX. Moreover, we pre-activated MSCs with hemin to produce a designer exosome suitable for regenerative therapies.

101 (131) **ACELLULAR URETHRAL SCAFFOLD FROM SEX REASSIGMENT PATIENTS: DEVELOPMENT AND CRYOPRESERVATION EFFECTS**

Nicolas Fraunhoffer[1], Javier Belinky[2], Horacio Rey[2], Analia Meilerman Abuelafia[1], Lourdes Rey[1], Yago Blumengarten[1], Nir Schvarzman[1], Marina Paci[1], Sergio Ferraris[3], Marcela Barrios[1].
[1]Carrera de Medicina, Universidad Maimónides. [2]División Urología, Hospital G. Durand. [3]CIDME, Universidad Maimónides.

Urethralreconstruction for both congenital and acquired causes remains challenge for urological surgeons. A wide variety of tissues have been used in urethral repair. However, all of these substitutes have limitations compared to the urethral tissue (UT). In this context, acellular scaffolds from human urethras would be the best alternative, although the access to this tissue is limited. The objectives of this study were to develop a decellularization method for UT and analyze UT cryopreservation effects on the acellular scaffold. 4 urethral samples from male patients without any urological pathology were used. Samples were obtained from Hospital Durand. Two decellularization protocols in 2 periods (3 or 7 days) were analyzed: sodium deoxyxholate 1% (PR1) and Triton X-100 1% (PR2). Additionally, two freezing media with DMSO 0.7M (PRA) and 1.5M (PRB) were evaluated. Decellularization and structural integrity were assessed by histological analysis, actin WB, DNA levels and scanning electron microscopy (SEM). Extracellular matrix (EM) proteins (collagen I and IV, laminin, fibronectin and elastin) and VEGF were studied by IHC and dot blot. PR1 and PR2 applied for 3 days, maintained high cellular components, while PR2 applied for 7 days showed total decellularization, undetectable DNA and actin levels with high structural integrity. EM proteins and VEGF were higher in PR2 than PR1. Comparing the two freezing protocol, PRA presented better integrity and protein levels than PRB, in combination with both decellularization protocols. However, PRA/PR2 showed the highest levels of EM proteins and VEGF, even better than PR2 without freezing cycle. These results show that PR2 applied for 7 days is the best decellularization protocol. Furthermore, our results suggest that a freezing cycle, previously to decellularization promotes the UT integrity. Therefore, these results suggest that urethral scaffold from sex reassignment patients represents a feasible tissue for urethral repair.

102 (216) **AMNIOTIC MEMBRANE CONDITIONED MEDIUM PROMOTES CELL DEATH AND INHIBITS PROLIFERATION OF HEPATOCARCINOMA HEPG2 CELLS**

Rodrigo Nicolas Riedel[1], Antonio Perez Perez[2], Bernardo Markin[3], Mariana Jaime[3], Ornella Parolini[4], Victor Sanchez-Margalet[2], Cecilia Varone[1], Julieta Maymó[1].
[1]Depto. de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires- IQUIBICEN-CONICET, Buenos Aires, Argentina. [2]Depto. de Bioquímica Médica y Biología Molecular, Universidad de Sevilla, Sevilla, España. [3]Hospital Nacional Alejandro Posadas, Buenos Aires, Argentina. [4]Centro di Ricerca E. Menni- Fondazione Poliambulanza- Istituto Ospedaliero, Brescia, Italia.

The placenta and fetal membranes have recently been proposed as an important stem cells source for regenerative medicine. Stem cells derived from amniotic membrane offer considerable advantages over other stem cells because of the ease of collection, their low immunogenicity and inability to form tumors after transplantation. Moreover, minimal ethical and legal barriers are associated with their use. Epithelial amniotic cells isolated from the amnion express embryonic stem cells markers and have the ability to differentiate towards all three germ layers. Not only are amnion-derived stem cells applicable in regenerative medicine, but also have antitumoral properties. Hepatic failure is one of the major causes of morbidity and mortality and despite the development in therapies, hepatocarcinoma rates are high worldwide. A few studies have demonstrated the antittumoral effects of the amniotic membrane and their cells but little is known about the molecular and cellular mechanisms involved. The aim of this work was to study some aspects of cell death and proliferation induced by amniotic membrane conditioned medium (AM-CM) in hepatocarcinoma cells. We have analyzed the expression of pro-apoptotic proteins (p53, Caspase-3, PARP-1) by Western blot, in HepG2 cells treated with AM-CM. We have also analyzed cell proliferation by tritiated-thymidine (H^3-T) incorporation assay and cell viability by MTT assay. We found a significant increment in p53 expression, Caspase-3 fragmentation and cleaved PARP-1 -measured by western blot-, after 24, 48 and 72 h of treatment with AM-CM in hepatocarcinoma cells. We have also observed that AM-CM significant decreased cell proliferation and viability, up to 7-fold compared with control, measured by H^3-T incorporation and MTT assay, respectively. Finally, we have observed by immunofluorescence a diminished Ki-67 expression in HepG2 cells treated with AM-CM. Our results begin positioning amnion-derived stem cells as emerging candidates in anticancer therapy.

103 (241) **DIFFERENTIAL GENE EXPRESSION ASSOCIATED TO MIGRATORY, SECRETORY AND MORPHOGENESIS PROCESSES, AND VEFG-MEDIATED SIGNALING IN OSTEOSARCOMA CELL LINES THAT DIFFER IN THEIR METASTATIC ABILITY**

Luciana Mariel Gutierrez[1], Mariana Andrea Amoros[1], Juan Bayo[3], Guillermo Mazzolini[3], Eugenie S. Kleinerman[4], Mariana Garcia[3], Alejandro Correa Dominguez[2], Marcela Fabiana Bolontrade[1].
[1]Laboratorio de Células Madre, IBYME – CONICET. [2]Laboratorio de Biología Básica de Células Tronco (Labcet), Instituto Carlos Chagas - FIOCRUZ Curitiba. [3]Laboratorio de Terapia Génica, Universidad Austral – CONICET. [4]The University of Texas MD Anderson Cancer Center, Division of Pediatrics and Department of Cancer Biology, Houston.

Despite combination of chemo- and radiotherapy, survival rate for osteosarcoma (OS) remains 60-70%. OS presents major therapeutic challenges at lung metastasis stage with a survival rate of 15-30% at diagnosis time. We used a metastatic OS model that includes a subline (LM7) with enhanced lung metastatic ability. Proteomic analysis identified differentially expressed proteins

both in the secretory and cellular compartments of the metastatic and parental (SAOS2) cell lines. We identified an increased gene expression associated to VEGF signaling, morphogenesis and tissue remodeling and migratory ability in the metastatic subline. A functional profile evaluation revealed no significant differences between both cell types secretome´s ability to induce angiogenesis in vitro, suggesting that a differential role for VEGF could be associated to non-angiogenic related mechanisms. LM7 showed increased in vitro migratory behavior; moreover, mesenchymal stem cells (MSC) displayed enhanced migration towards the parental secretome (1200±98 vs 820±158 cells/field, SAOS2 vs LM7 respectively, p<0.001); this suggests that the differential protein expression associated to endosome trafficking and microtubule processes, together with the presence of individual chemotactic proteins in SAOS2 secretome could be associated to a parental secretome specialized in cell recruiting, particularly in our model, of MSC towards the primary tumor. MSC compose a heterogeneous population with the ability to migrate and home to tumors. The identification of the cellular and secretory proteome associated to OS cells with differential lung metastatic abilities would allow identifying mechanisms associated to tumor progression, including the relevance of recruiting MSC. Understanding the interaction between tumor cells and tumor-recruited cells such as MSC in terms of tumor progression and metastasis will permit the development of new therapeutic strategies for OS treatment.

104 (259) **CRISPR-ON SYSTEM FOR THE ACTIVATION OF ENDOGENOUS HUMAN INS, NGN3, NKX6.1 AND PAX4 PANCREATIC β CELL GENES**

Carla Alejandra Giménez[1], Marcelo Ielpi[1], Luis Grosembacher[2], Sung Ho Hyon[2], Federico Pereyra Bonnet[1],
[1]Basic Science and Experimental Medicine Institute, University Institute of the Italian Hospital of Buenos Aires (HIBA), Buenos Aires, Argentina. [2]Endocrinology and Nuclear Medicine Service, HIBA, Buenos Aires, Argentina.

The CRISPR-on system consists of the inactive DNA nuclease Cas9 (dCas9) fused to activation domains and co-expressed single guide RNAs (sgRNAs) that are designed to hybridize a target sequence. Combined, these elements can generate a DNA complex that recognizes a target locus and activates a specific gene. We sought to determine whether the CRISPR-on system fused with transcriptional activators (dCas9-VP160) could activate gene expression of pancreatic β cell genes (NGN3, NKX6.1, PAX4, INS) on different cell lines (HEK293T cells and human fibroblast). Therefore, five sgRNAs were designed to target proximal promoter for activation of each gene using CRISPR Design Tool (Feng Zhang Lab, MIT). The sgRNAs target sequences were cloned on the sgRNA expression plasmid (Addgene #47108). For all cell types, the dCas9-VP160 plasmids (Addgene #48226) were transfected at a mass ratio of 1:1 to either individual sgRNA expression plasmids or a mixture of equal amounts of the different sgRNAs plasmids. Control groups cells were untransfected. Our results revealed that the CRISPR-on system activated endogenous human INS, and β cell transcription factors such as NGN3, NKX6.1 and PAX4. We observed a synergistic effect on gene activation by RT-qPCR at day 4 when multiple sgRNAs were used on HEK293T cells. Also, we co-transfected all sgRNAs into skin fibroblasts. We observed gene activation with the CRISPR-on system via RT-PCR (NKX6.1, NGN3 and PAX4) and no activation in the non-transfected control cells. However, gene induction was weak on those primary culture cells. In conclusion, we could apply the CRISPR-on system to active pancreatic genes on human cells. In the future, the CRISPR-on system could be tested to improve reprogramming strategies to pancreatic linage.

105 (385) **ROSCOVITINE, A SMALL PURINE-LIKE CDK INHIBITOR, TRIGGERS APOPTOSIS IN HUMAN EMBRYONIC STEM CELLS**

Verónica Alejandra Furmento[1], Carolina Paola García[1], Guillermo Agustín Videla Richardson[1], Leonardo Romorini[1], Santiago Gabriel Miriuka[1], Gustavo Emilio Sevlever[1], María Elida Scassa[1],
[1]Fundación para la Lucha contra Enfermedades Neurológicas de la Infancia

Human embryonic stem cells (hESCs) possess the unique ability of self-renewal while retaining their undifferentiated state. Protein kinase complexes formed by the association of cyclins and their catalytic subunits, the cyclin dependent kinases (CDKs), represent key molecules in regulating cell cycle. In hESCs, elevated cyclin activity combined with lack of endogenous CDK inhibitors results in augmented CDK1 and CDK2 activity and consequently, diminished G1 and G2 phases. Acute inhibition of CDK1 or CDK2 in proliferating somatic cells generally induces a reversible arrest of the cell cycle without significant cell death. However, in other cellular contexts, CDK inhibitors can promote cell differentiation or apoptosis. Herein, we used Roscovitine (ROSC), a smallpurine-like CDK inhibitor, to examine the role of CDK1 and CDK2 inhibition in WA09 hESC line. Flow cytometric analysis of DNA content revealed that ROSC treatment (20 μM) increases the percentage of cells in G1 phase and declines cyclin E mRNA levels (N=3, p<0.05).XTT/PMS vital dye, Trypan blue and flow cytometry assays showed that ROSC exposure reduces hESCs viability in a dose-dependent manner (N=3, p<0.05). By Western blot (WB) analysis we determined that the loss in cell viability was accompanied by apoptotic features such as caspase-9 and caspase-3 activation and PARP-1 cleavage. Moreover, the presence of ROSC led to a decrease in the anti-apoptotic factor Mcl-*1* at both mRNA (N=4, p<0.05) and protein levels. Immunofluorescence assays indicated that ROSC induces p53 nuclear accumulation while WB assays showed that p53 undergoes site-specific phosphorylation at *Ser46.* ROSC treatment also downregulated MDM2 mRNA levels (N=6, p<0.05) and increased mRNA expression levels of p21 (N=6, p<0.01) and p53AIP1, a downstream target of p-p53 (Ser46). As a whole, we found that, similar to what occurs in many types of cancer cells, in rapidly proliferating hESCs ROSC triggers apoptosis and activates p53 signaling.

106 (306) **NEURAL CREST-DERIVED CELLS IN THE LIVER DURING DEVELOPMENT AND FIBROGENESIS**

Romina Sierra[1], A. Furlán[2], I. Adameyko[3], P. Ernfors[2], Jorge Benjamín Aquino[1].
[1]IIMT Universidad Austral-CONICET, Argentina. [2]Medical Biochemistry and Biophysics, Karolinska Institutet, Sweden. [3]Physiology and Pharmacology, Karolinska Institutet, Sweden.

Cirrhosis results from repeated cycles of liver damage and scar formation. Little is known regarding the contribution of neural crest-derived cells (NCDCs) to the liver in health and disease. Previous in vitro findings likely suggest that some hepatocyte-like cells (HLCs) might be of this origin. Objective: The aim of this work was to analyze the contribution of NCDCs to the liver during development and liver fibrogenesis. Methodology: Wnt1-Cre, PLP-CreERT(2), Sox10-CreERT(2) mice were mated with Rosa26-loxP-Tomato or Rosa26-loxP-YFP and the co-expression of the reporter gene with glial, hepatocyte and hepatic stellate cell markers was analyzed. Two models of liver fibrogenesis were generated: 1) chronic applications of thioacetamide, and 2) bile duct ligation. Results: Data from in vivo studies with Wnt1-Cre:Rosa26-Tomato mice suggest a contribution of NCDCs with HLCs in the adult. For studies during embryonic stages, PLP-CreERT(2):Rosa26-YFP and Sox10-CreERT(2):Rosa26-YFP mice were used. Pregnant females were tamoxifen-injected at embryonic day 15.5 (E15.5). Numerous YFP^+ cells were found at E18.5 and some of them co-expressed cytokeratin 18 and alpha-fetoprotein. None of them were $desmin^+$. Interestingly, NCD-HLCs were anatomically distributed following a similar pattern in all strains and stages analyzed. Finally, liver fibrogenesis was found to induce a gliosis-like process and it was associated with a significant increased in numbers of HLCs. Conclusions: During normal development some HLCs could be NCD. Numbers of glial cells and HLCs increased during liver fibrogenesis, which may have implications on fibrogenesis control and on liver regeneration.

107 (472) **A NOVEL NATURALLY OCCURRING SPLICE VARIANT OF THE HUMAN TGF-β TYPE II RECEPTOR (TβRII) ENCODES A TRUNCATED SOLUBLE MOLECULE**

Marcela Soledad Bertolio[1], Tania Melina Rodríguez[1], Alejandra Carrea[1], jorge Velasco Zamora[2], Marcelo Javier Perone[3], Ricardo Alfredo Dewey[1].

[1]Instituto de Investigaciones Biotecnológicas-Instituto Tecnológico de Chascomús, Consejo Nacional de Investigaciones Científicas y Técnicas-Universidad Nacional de San Martín, Chascomús, Argentina. [2]Centro de Enfermedades Reumáticas/Fundación Articular, Quilmes, Argentina. [3]Instituto de Investigación en Biomedicina de Buenos Aires, Consejo Nacional de Investigaciones Científicas y Técnicas, Partner Institute of the Max Planck Society, Buenos Aires, Argentina.

Here we describe for the first time the presence in human cells of a new splicing variant of TGF-β type II receptor. Compared to the known TβRII-A variant mRNA sequence, the new molecule lacks the last 63 nucleotides of exon II and the first 86 nucleotides of exon III. This deletion of 149 nucleotides causes a frameshift with the appearance of an early stop codon rendering a truncated protein of 80 amino acids lacking the transmembrane domain. Here we show that this new splicing variant, named by us TβRII-Soluble endogenous (TβRII-Se), is transcribed by most cell types analyzed but absent/highly downregulated in solid tumor-derived cell lines (A549, HT1080, CaCo-2, SK-Mel). Moreover, overexpression of TβRII-Se by means of lentiviral vectors in A549 cells, increased the sensitive to TGF-β growth inhibition, as determined by MTT proliferation assay. To allow increased expression in mammalian cells, we codon optimized the TβRII-Se cDNA, and fused it in frame with the human IgG1 Fc domain to ease protein purification, and to enhance *in vivo* half life. In addition, we generated a specific monoclonal antibody directed against TβRII-Se which allowed us the intra and extracellular detection of the novel secreted protein by means of Western blot and flow cytometry. Moreover, we identified the interactome of the TβRII-Se fusion protein by screening, in duplicates, the Huprot v3.0™ Human Proteome array (CDI), containing more than 15,000 genes covering around 75% of the proteome. This assay indicated that TβRII-Se fusion protein is able to bind to a panel of 150 proteins. Gene ontology analysis using the PANTHER gene list analysis, indicated that, at the molecular function, the novel protein interacts with binding proteins (56%). Of them, 59% are nucleic acid binding, and 33% protein binding proteins, being 54% of the latter, receptor binding proteins. Members of the TGF-β pathway are known targets for diseases such as cancer, fibrosis, osteoarthritis, and Type II diabetes

108 (435) **STRONTIUM RELEASE SYSTEM BASED ON ALGINATE FOR BONE TISSUE ENGINEERING: GELATION TIME EFFECT**

María Luz Torres[1,2], Ana María Cortizo[1], Tamara Gisela Oberti[2], Juan Manuel Fernández[1].

[1]Laboratorio de Investigación en Osteopatías y Metabolismo Mineral (LIOMM), Fac. de Cs. Exactas, UNLP, 47 y 115, La Plata, Argentina. [2]Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA), Dpto. de Química, Fac. Cs. Exactas, UNLP, CCT- La Plata, CONICET CC 16, Suc. 4. La Plata, Bs. As., Argentina.

Alginate (Alg) is a natural, water-soluble, polysaccharide obtained from brown algae. Due to many attractive features such as good biocompatibility as well as ease of hydrogel formation with divalent cations, it has been widely used in biomedical application. Strontium ranelate (SR) is an orally administered antiosteoporotic agent that includes 2 strontium ions and an organic moiety (ranelic acid). Sr+2 can partially substitute Ca+2 in hydroxyapatite, and thus be incorporated into bone. SR has been shown to effectively reduce the risk of vertebral and femoral bone fractures in women with osteoporosis. In vitro, strontium increases osteoblast-mediated bone formation, while decreasing osteoclastic bone resorption. We study the release kinetics of Strontium in Alg hydrogels (HG). The HG were prepared with 2% Alg (Aldrich) and 0.05, 0.1, 0.25 and 0.5 mM of Sr+2, the times of gelation were 1, 12 and 24hs. The release profile of Sr+2 from the HG was determined by incubating samples with 1mL of Dulbecco's Modified Eagle Medium (DMEM) at 3°C for different periods of time. At appropriate times, the supernatant was removed and replaced by fresh media. The time-dependent release of the Sr+2 was followed by monitoring the amount of Sr+2 present in the supernatant medium, using a flame spectrophotometer. The assay was performed in quadruplicate and results were expressed as the fractional release ($Mt/M\infty$) versus time of release (t). Besides, swelling of all HG in water was studied. Our results show that Sr+2 concentrations released to medium depend of time gelation and the initial amount of strontium in the HG. In order to analyze the kinetics of Sr+2 releases, we used Fick's second law. These results suggested that the mechanism of Sr+2 releases occurs by an Anomalous (non-Fickian) transport. As for the swelling of HG, a large percentage of these in some cases the 10,000% in 24hs are observed. In conclusion, we have obtained a delivery system Sr+2 with high swelling capacity.

109 (581) **LARGE SCALE MESENCHYMAL STEM CELLS PRODUCTION FROM PLURIPOTENT STEM CELLS ON MICROCARRIERS**

Olivier Blond[1], Lucia Moro[1], Alessandra Norris[1], Alejandro La Greca[1], Leonardo Romorini[1], Darío Fernandez Espinosa[1], María Elida Scassa[1], Gustavo Emilio Sevlever[1], Carlos Luzzani[1], Santiago Gabriel Miriuka[1].

[1]Laboratorio de Biología del Desarrollo Celular, LIAN-Unidad Asociada al CONICET, Fundación FLENI, Ruta 9, Km53, Belen de Escobar, Argentina

Mesenchymal stem cells (MSC) are strong candidates for regenerative therapies, because of their involvement in stemness for tissue repair and their low immunogenicity. Among their main applications are exosomal preparations or direct cell injections. Since MSC expansion has some issues, due to their limited number of divisions, it is more convenient to derive and amplify MSC from pluripotent stem cells (PSC). In a previous work done in our laboratory, we have adapted this protocol of differentiation for clinical purposes, using platelet lysate instead of animal serum, making it more affordable and biocompatible. We now demonstrate that it is possible to scale up this protocol by growing cells in 3D, on microcarriers. Once the most suitable microcarrier was found, the most challenging aspects were to adapt the agitation settings and to obtain individualised cells. The best microcarrier and coating combination was cytodex 3 coated with or without geltrex. The most efficient agitation setting (discontinuous shaking at less than 100 rpm) was the one dedicated to MSC, not the one meant to grow undifferentiated PSC. Furthermore, the dissociation step was optimised by combining strong enzyme digestion with an agitation at 80 rpm and a Rho kinase inhibitor (Y27632) to relax actin-mediated aggregate compaction. After thirty to forty days of differentiation, the MSC phenotype was analysed by different methods. The combination of cell surface markers, including CD90, CD73 and CD105 and quantitative RT-PCR allowed us to conclude that these cells are indeed MSC.We have now proved that mesenchymal stem cells can be produced from PSC with this differentiation protocol in 3D, on cytodex 3 microcarriers. As the phenotype was confirmed with specific markers, our next objectives will be to confirm that MSC are fully functional by immunomodulation experiments and multilineage differentiation assays.

110 (436) **COMMERCIAL AND EXTRACTED SODIUM ALGINATE ANALYSIS AFTER PURIFICATION FOR BONE TISSUE ENGINEERING APPLICATION**

María Luz Torres[1, 2], Juan Manuel Fernández[1], Fernando Gaspar Dellatorre[3], Ana María Cortizo[1], Tamara Gisela Oberti[2].

[1]Laboratorio de Investigación en Osteopatías y Metabolismo Mineral (LIOMM), Fac. de Cs. Exactas, UNLP, 47 y 115, La Plata, Argentina. [2]Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA), Dpto. de Química, Fac. Cs. Exactas, UNLP, CCT- La Plata, CONICET CC 16, Suc. 4. La Plata, Bs. As., Argentina. [3]CENPAT – CONICET, Puerto Madryn, Chubut.

Alginate is a natural, water-soluble, polysaccharide obtained from brown algae consisting of β-D-mannuronate(M) and α-L-guluronate(G) residues (1→4). Due to many attractive features such as good biocompatibility, low toxicity as well as ease of hydrogel formation with divalent cations, it has been widely used in a variety of biomedical application, such as medical delivery and tissue engineering. These applications required highly purified materials. However the alginate extracted from brown seaweed and commercial alginate contain a large number of impurities, such as proteins and polyphenols, which might lead to an intense host immune reaction and reduce the biocompatibility of alginate. The brown algae, *Undaria pinnatifida,* native from Japan, China and Korea is a powerful invasive species which was introduced worldwide in different coasts, including the one of Puerto Madryn (Argentina). Sodium alginate can be obtained from this seaweed using and extraction process based on successive treatments with acid and alkaline solutions.In this study, we compare *U. pinnatifida*'s sodium alginate extracted from stem tissue with the commercial salt (Sigma Aldrich) and its derivates after a purification method based on chloroform/butanol. The results showed that the purification procedure is necessary and effective to reduce protein content. Total protein measurement was performed using the method of Bradford. Neither de raw material nor the purified one showed significant amount of polyphenols after a determination using a spectrofluorimeter. Assay realized with an Ostwald viscosimeter showed that intrinsic viscosities of the purified samples were lower compared with the raw material. Samples characterization was perform by FTIR and TGA analysis. In conclusion, purified sodium alginate materials present a reduction of contamination by using a simple method that might generate a better biocompatibility than the unpurified ones (extracted and commercial).

111 (634) **POLYLACTIC ACID SHEETS SEEDED WITH GENETICALLY MODIFIED OVINE DIAPHRAGMATIC MYOBLASTS FOR MYOCARDIAL REGENERATION. PRELIMINARY RESULTS**

Carlos Sebastián Giménez[1], Daniela Fernanda Olea[1], Paola Locatelli[1], Maria del Rosario Bauzá[1], Ricardo Dewey[2], Florencia Montini Ballarin[3], Gustavo Abraham[3], Alejandro Orlowski[4], Andrea De Lorenzi[5], Luis Cuniberti[1], Alberto Crottogini[1],

[1]IMETTYB-Universidad Favaloro-CONICET. [2]IIB- INTECH-UNSAM-CONICET. [3]INTEMA-UNMDP-CONICET. [4]CIC-UNLP-CONICET. [5]Hospital Universitario Fundación Favaloro.

We tested the hypothesis that polylactic acid (PLA) sheets seeded with diaphragmatic myoblasts (DM) overexpressing connexin-43 (Cx43) would decrease infarct size and improve left ventricular (LV) function in sheep with chronic myocardial infarction (MI). Forty minutes after coronary artery ligation, PLA sheets without DM (PLA, control group, n=4) or PLA sheets with DM overexpressing Cx43 (PLA-DM-Cx43 group, n=4) were sutured covering the infarcted zone and 1 cm of the adjacent peri-infarct zone. Baseline echocardiograms confirmed that all animals displayed normal LV function prior to infarction. Cardiac magnetic resonance with gadolinium enhancement was performed at 3 and 45 days after MI to determine infarct size and LV function early and late after treatment, respectively. Blinded data analysis revealed that infarct size expressed as ml of infarcted tissue decreased in the PLA-DM-Cx43 group from 8.0±1.83 ml at day 3 to 6.25 ±2.2 ml at day 45 ($p<0.01$, X±SD, ANOVA-Bonferroni) but did not change in the PLA group (9.4±3.15 ml at day 3 vs. 8.67± 3.79 ml at day 45, p=NS). As regards LV function, LV% ejection fraction increased in the PLA-DM-Cx43 group from 24.8±5.9% at day 3 to 41.73±7.53% at day 45 ($p<0.01$) but remained unchanged in the PLA group (35.5±12.9% at day 3 vs. 41.4±4.3 at day 45, p=NS). Conclusion. Our preliminary results show that the application of PLA scaffolds seeded with DM overexpressing Cx43 on the surface of the infarct and its adjacent peri-infarct zone reduce infarct size and preserves LV function in this large mammalian model of MI.

112 (488) **MESENCHYMAL STROMAL CELLS ARE MODULATED BY A THYROID HORMONE-RESPONSIVE TUMOR MICROENVIRONMENT**

Mariana Andrea Amorós[1], María Florencia Cayrol[2], Luciana Mariel Gutiérrez[1], Leandro Cerchietti[5], Alejandro Correa Dominguez[4], Carlos Luzzani[3], Santiago Miriuka[3], Graciela Cremaschi[2], Marcela Fabiana Bolontrade[1].

[1]Laboratorio de Células Madre, IBYME - CONICET, Buenos Aires. [2]Laboratorio de neuroimmunología y Oncología Molecular, BIOMED - UCA - CONICET, Buenos Aires. [3]FLENI-CONICET, Buenos Aires. [4]Laboratorio de Biologia Basica de Celulas Tronco (Labcet), Instituto Carlos Chagas - FIOCRUZ Curitiba. [5]Weill Cornell Medical College, New York, NY.

Mesenchymal Stromal Cells (MSC) are recruited and home in the tumor stroma. Functional properties such as immunomodulation, migration and secretion of paracrine factors turn MSC as modulators of the tumor environment. Identifying the niche components that modulate the cross talk between tumor cells and MSC is key to understand progression mechanisms. Tumor environmental factors may affect MSC functions, influencing their tumor modulation ability with an impact in tumor progression. In this regard thyroid hormones (TH) affect tumor growth. In order to study the contribution of MSC to tumor development we used our previously established model for T-cell lymphoblastic leukemia with MSCs as recruited tumor stromal cell components, evaluating the modulation exerted by TH on MSC in a tumor environment context. We demonstrated that TH-modulated tumor cells (canonical pathway) produced a secretome that induced lower migration rates and higher adhesiveness, together with an increased ability to induce angiogenesis *in vitro* and a higher proliferation rate on MSC. When tumor cells were modulated by TH via surface receptors (non-canonical pathway) the resultant secretome induced higher migration rates (252,6±35,49 vs 168,9±9,22 No. of cells/field, non-canonical vs canonical respectively, $p<0.01$), lower adhesiveness, lower ability to induce angiogenesis (6,88±2,05 vs 30±3,99 No. of branches/field, non-canonical vs canonical respectively, $p<0.001$), and lower proliferation rates on MSC, suggesting a differential mechanism of action. TH also directly affects MSC inducing a differential proteomic profile on the secretome produced by TH-stimulated MSC. Our data suggest that tumor recruited MSC articulate the effect of TH on tumor cells, being modulated and simultaneously modifying the tumor environment. By discerning the mechanisms that govern MSC/tumor cells cross talk we could contribute to elucidate underlying mechanisms involved in tumor stroma transformation and progression.

113 (691) **TUNNELING NANOTUBES: AN EMERGING INTERCELLULAR ROUTE FOR THE EXCHANGE OF COMPONENTS BETWEEN NEIGHBORING CELLS CHARACTERIZED IN MESENCHYMAL STEM CELLS**.

Nerina Villalba[2], Viviana Sanchez[1], Luciano Fiore[1], Carlos Luzzani[5], Santiago Miriuka[5], Ricardo Gelpi[3], Alberto Boveris[4], Alicia Brusco[1], Juan José Poderoso[2].

[1]IBCN (UBA-CONICET). Facultad de Medicina UBA. [2]INIGEM (UBA-CONICET). Hospital de Clínicas, UBA. [3]IBIMOL (UBA-CONICET), Facultad de Medicina, UBA. [4]IBIMOL (UBA-CONICET), Facultad de Farmacia y Bioquimica, UBA. [5]LIAN (CONICET) FLENI, Belén de Escobar.

Intercellular communication is one of the most important events in cell population behavior. In the last decade, tunneling nanotubes (TNTs) have emerged as a newly discovered form of long distance intercellular connection. TNTs transfer molecules and organelles such as calcium ions, prions, viral and bacterial pathogens, small lysosomes and mitochondria between neighboring cells. New findings suggest that the ability of mesenchymal stem cells (MSCs) to alter tissue microenvironment via the secretion of soluble factors or transfer of their own cellular components to neighboring cells may contribute significantly to tissue repair, although the underlying mechanisms remain poorly understood. In this work we present an extensive and detailed description of types, structure, components,

dynamics and functionality of TNTs bridging neighboring human Wharton Jelly MSCs (WJ-MSC) using phase contrast, fluorescence and electron microscopy, as well as time lapse images. Our results show that TNTs either extend between two neighboring cells or form a network connecting various neighboring cells. In both cases, they exhibited a long rectilinear extension whose length ranged between 100 and 700 mm. Two different types of TNTs wereidentified: one containing only actin filament cytoskeleton (Type I), and the other with both actin and tubulin filaments (Type II). *In vivo* time lapse imaging employing a mitochondrial marker allowed us to see mitochondria lining up and moving along TNTs at a velocity of 0.8 ± 0.2 mm/ min. As shown by ultrastructural analysis, Type I TNTs did not exceed 100-nm diameter and had no organelles inside, while Type II TNTs had 600-700nm diameter and contained polyribosomes, rough endoplasmic reticulum, vesicles, mitochondria and lipid drops. Caveoles were present on the cell surface of both types of TNTs. MSCs have many advantages for implementation in regenerative medicine and TNTs in this cell type may constitute a suitable route.

114 (600) **MYST4 IS REQUIRED FOR NEURAL DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS**

María Soledad Cosentino[1,2], Ariel Waisman[1,2], Camila Vazquez Echegaray[1,2], Claudia Solari[1,2], María Victoria Petrone[1,2], Marcos Gabriel Francia[1,2], Santiago Miriuka[3], Lino Barañao[1,2], Alejandra Guberman[1,2],
[1]Laboratorio de Regulación Génica en Células Madre, Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina. [2]Instituto de Química Biológica (IQUIBICEN), UBA-CONICET, Buenos Aires, Argentina. [3]Laboratorio de Investigación Aplicada a las Neurociencias (LIAN), Fundación para la Lucha contra las Enfermedades Neurológicas de la Infancia (FLENI), Buenos Aires, Argentina.

The transition between transcriptional programs associated with Stem Cell (SC) differentiation is related to changes in chromatin structure. In this work we studied Myst4, a transcriptional coactivator with histone acetyltransferase activity. It was reported that Myst4 gene has a regulatory element highly occupied by Embryonic SC (ESC) key transcription factors (TFs), but the relevance of this protein in ESC remains to be established. Additionally, this gene is important for the establishment and self-renewal of adult neural SC, and loss of only one allele in humans leads to intellectual disability. We have previously shown that Myst4 is expressed in mouse ESC (mESC) and is repressed during differentiation. Moreover, our previous results also suggest that its expression is regulated by pluripotency TFs. To study the role of Myst4 in the maintenance of ESC's properties, we used the CRISPR/Cas9 strategy to generate mESC *knock-out* cell lines. Most of the clones analyzed presented indel mutations. We selected a clone with a frameshift mutation that generated a premature stop codon in both alelles (M4 -/-), and confirmed the lack of Myst4 protein expression by Western blot. This clone displayed normal morphology and had no significant differences to the *wild type* (WT) control cells regarding pluripotency markers expression. Surprisingly, M4-/- mESC failed to differentiate to neural derivatives during a directed differentiation protocol, with most of the cells dying at day 12. Gene expression analysis during initial stages of neural differentiation showed that M4 -/- mESC had lower expression of neural progenitor and neuron markers than WT cells. These results suggest that Myst4 is required for the differentiation of mESC to neurons, and provides a platform to study the epigenetic mechanisms of normal development as well as human disease. We consider that understanding the processes involved in ESC chromatin structure regulation is critical to their future application.

115 (641) **ROLE OF INTEGRINS (α3, α5 AND α6) ALONG CARDIAC DIFFERENTIATION THROUGH SILENCING BY CRISPR-EFFECTOR SYSTEM**

Gabriel Neiman[1], Fernanda Mesquita[2], Ximena Garate[1], Adriana Bastos Carvalho[2], Antonio Campos de Carvalho[2], Alejandra Guberman[3], Gustavo Sevlever[1], Santiago Miriuka[1].
[1]Laboratorio de Biología del Desarrollo Celular, FLENI, Buenos Aires. [2]Laboratorio de Cardiologia Celular y Molecular, Universidad Federal de Rio de Janeiro, Brasil. [3]Laboratorio de Regulación de Expresión Génica en el Crecimiento, Supervivencia y Diferenciación celular, FCEyN, UBA, Buenos Aires.

Pluripotent stem cells (PSC) are a model to study embryological development and also have the potential to provide a supply of different cell types for tissue replacement and drug screening. Owing to this, increasing efficiency of differentiation protocols is of vital importance. The role of ECM in cell adhesion and signaling to cells through receptors such as integrins has received much attention. Previous publications showed that specific integrins and their ligands such as fibronectin, laminins are responsible of the cellular fate. Our goal is to find out what role integrins (α3, α5 and α6) are playing during cardiac differentiation. It will be achieved through the CRISPR-effector system where we can specifically and reversibly inhibit gene expression allowing us to understand the contribution of integrins to cardiac development. A new specific protocol was developed in our lab and it has 80% of efficiency. We performed the integrin expression profile on days d0, d3.5, d7.5 and d15 at both levels: mRNA and proteins. Through qPCR, we observed that α5 has its peak at d3.5 where increases 10 times compared to d0 and its main ligand (Fibronectin) has a d7.5 peak, 50 folds higher. Integrins α3 and α6, whose ligand is laminin, show an opposite behavior: α6 decrease an 80% at d3.5 while α3 increase 8 folds at d15. By citometry, we got a similar profile to mRNA expression and each mesodermal or cardiac population is correlated with specific population markers. All this data together proves there is a regulation along differentiation and they change significantly on different cell states: early mesoderm, late mesoderm or cardiomiocyte. These profiles were already done in hESC and hiPSC. We are under way to obtain CRISPR hPSC targeting these integrins, which will allow us to see how inhibition will affect the specific protocol, not only regarding the efficiency but also the morphology of the beating bodies and changes in the signaling pathways.

116 (670) **EFFECT OF ACUTE HYPOXIA IN HUMAN PLURIPOTENT STEM CELLS SURVIVAL**

Jonathan Vera[1], Maria Elida Scassa[1], Gustavo Emilio Sevlever[1], Santiago Gabriel Miriuka[1], Leonardo Romorini[1]
[1]Laboratorio de Investigación Aplicada a Neurociencias (LIAN)-CONICET, FLENI.

Human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) are self-renewing pluripotent stem cells (PSC) that can differentiate into a wide range of specialized cells. The first stages of embryogenesis evolve in a hypoxic environment. In this sense, it is well known that moderate hypoxia (5% O2) improves PSC self-renewal, pluripotency and cell survival. However, the effect of acute hypoxia (1% O2) in PSC has not been reported yet. The aim of this work was to explore the effect of acute hypoxia in PSC survival and cell death rate. To address this issue, hESCs (WA09/H9 line) and hiPSCs (FN2.1 line) were cultured under physical (1% O2) or chemical acute hypoxia conditions. The latter were generated with CoCl2 (250 μM) and dimethyl oxal glycine (DMOG) (100 μM and 1 mM) treatments, which stabilizes HIF-1α (key hypoxia inducible transcription factor) mimicking hypoxia. We observed an increase in the levels of HIF1-α target gen *bnip-3* concomitant with a decrease (measured by XTT/PMS vital dye assay) in cell viability at 24 hours post-hypoxia induction. Importantly, at 24 hours post-hypoxia induction we observed the appearance of cell ballooning and detachment, hallmarks of programmed cell death, a reduction on the percentage of surviving cells (Trypan blue dye-exclusion assay), an increment on the percentage of apoptotic nuclei (Hoechst staining of nuclear DNA), and an increase in late apoptosis or necrosis (flow cytometry analysis with PI staining) and apoptotic DNA fragmentation (DNA oligomers quantified by ELISA) rates. Moreover, immunofluorescence analysis revealed activation of the effector Caspase-3 as soon as 6 hours after hypoxia induction. Collectively, in this study we

demonstrated that acute physical (1% O2) and chemical hypoxia reduced human PSC survival and triggered apoptosis.

117 (750) **HYPOXIA MODULATES DECORIN EXPRESSION BY MICROVASCULAR ENDOTHELIAL CELLS**

Florencia Analia Funez[1], Claudia Amusquivar[1], Ana Clara Eiguren[1], Graciela Cristina Calabrese[1].
[1]Universidad de Buenos Aires- Facultad de Farmacia y Bioquímica- Cátedra de Biología Celular y Molecular. Junín 956 Primer Piso (CABA- C1113AAD, Argentina).

Decorin (DCN) is a stromal proteoglycan synthesized by stressed vascular endothelial cells. DCN effectively represses pro-angiogenic gene targets under normoxia and simultaneously promotes the expression of various anti angiogenic molecules. Moreover, DCN modulates the inflammatory response through Toll like Receptor family. The aim of the present work was to analyze the expression of DCN by endothelial cells under hypoxia conditions. Hypoxia was induced on murine heart microvascular cells (H5V line) after incubating with 250 mM cobalt chloride from 15 min to 24 hs. Lipopolysaccharide treatment (1.5 µg/ml) was performed as a positive control of endothelial cell injury. Inmunofluorescence, immunoblotting and reverse transcriptase PCR assays were carried out to study DCN expression. Besides, H5V cells were homogenized and fractionated by ultracentrifugation to analyze NFkB subcellular distribution by immunobloting assays. A significant decrease of DCN expression was determined after 24 hs of lipopolysaccaharide treatment ($p< 0.05$; control vs lipopolysaccharide), accompanied by an increase in NFkB nuclear translocation observed by immunofluorescence. On the other hand, an increase in DCN expression was detected by immunofluorescence from 15 to 60 min of hypoxia, with a homogeneous cellular distribution. However, 24 hs of hypoxia shows no difference of mRNA DCN expression compared with control cells. Our results show that (1) long-term lypopolysaccharide treatment activates microvascular endothelial cells accompany by a reduction in DCN expression; (2) short-term hypoxia induces an increase in the proteoglycan expression while long-term hypoxia produces its decrease and (3) no aberrant localization of the core protein of DCN was detected in injured endothelial cells. Taking into account our results, DCN could be considered as a new therapeutic molecule for the regeneration of tissue vasculature.

118 (841) **ERYTHROPOIETIN GENE TRANSGENESIS ALLOWS SELF-INDUCED IN VITRO GENERATION OF ERYTHROID CELLS FROM HUMAN HEMATOPOIETIC STEM/PROGENITOR CELLS (HSPC)**

Luisina Anabel Cappellino[1,2], Ricardo Kratje[1,2], Marina Etcheverrigaray[1,2], Claudio César Prieto[1,3].
[1]UNL, Laboratorio de Cultivos Celulares, FBCB. [2]CONICET. [3]UNL, Laboratorio de Desarrollo Biotecnológico, FBCB.

HSPCs are an adequate source of erythroid cells. However, culture conditions require utilization of costly growth factors, which turns erythroid production into a complicated process to be scaled-up for future medical applications. Our goal was to genetically modify HSPCs with human erythropoietin (hEPO) gene through lentiviral transgenesis in order for cells to secrete the hormone into the culture medium, and to analyze *ex vivo* erythroid differentiation (ED) in absence of exogenous hEPO (ehEPO). Preliminarily, we evaluated ED in CFU assays for non-modified cells (NMC) and hEPO-modified cells (EMC), both cultured with and without ehEPO. We further studied cell expansion and ED in suspension cultures of NMC in presence of ehEPO (NMC-E+) and of EMC in absence of ehEPO (EMC-E-). Immunophenotyping by flow cytometry and morphological analysis by May-Grünwald-Giemsa staining were carried out throughout culture time. Additionally, we assessed hEPO production and its isoforms profile. CFU assay showed higher erythroid colonies development for EMC, which correlated with higher hEPO concentrations in supernatants. This encouraged us to further investigate in suspension cultures, in which EMC-E- reached a maximum of 953-fold mean amplification (FMA) by day 15 (post-transduction) and NMC-E+ reached 369-FMA by day 11. ED was demonstrated in both conditions: CD34 stemness marker and CD45 leukocyte marker decreased during the first week, in correlation with appearance of early erythroblasts. From day 11, polychromatic and orthochromatic erythroblasts were detected. CD235a and CD71, expressed at early erythroid and early reticulocyte stages, increased. Secreted hEPO reached its maximum by day 7, and its isoforms profile was similar to that of ehEPO. Thus, it was possible to develop a culture system in which HSPCs were self-induced into ED without addition of ehEPO.

119 (956) **KIDNEY ACELLULAR MATRIX AND NEW STRATEGIES OF RECELLULARIZATION**

Diego Guerrieri[1], Francisco Sanchez[1], Geraldine Haenblein[1], Fernanda Toniolo[2], Julian Drago[1], Marcos Aranda[1], Pablo Uva[2], Domingo Casadei[2], Eduardo Chuluyan[1].
[1]CEFYBO-CONICET, Facultad de Medicina, UBA. [2]Instituto de Nefrología Nephrology.

Replacement therapy is the best treatment option against organ failure, however graft rejection and adverse effects of immunosuppression remain major clinicalcomplications. The aim of this work was to obtain kidney acellular matrix and develop new strategies of recellularization. Wistar rats were used, which were made nephrectomy, then the kidney was cannulated for renal artery, placed in a bioreactor at room temperature and connected to a pump with a constant flow of 0.5 ml/min for the passage of 1% SDS and distilled water (18 h). Macroscopic changes in the kidney during the decellularization can be displayed in our video: (https://youtu.be/NI2OU-PE15g). In histological sections an acellular matrix was observed with preserved renal architecture being positive for markers of collagen and laminin. For recellularization process we develop an *in vitro* and another *in vivo* protocol. In the first, $1{,}5.10^6$ human renal epithelial cells (HK-2) was administered by ureter. At 72 hours, with histological techniques viable cells mainly located in the cortical tubules were observed. For *in vivo* protocol, acellular scaffold were implanted into recipient rats in subcutaneousand intraperitoneal location for one or two weeks. Once recovered the scaffolds showed a large inflammatory infiltrate, along with some positive cells markers for transgenlin and podocin. Conclusion: These results show the feasibility of renal decellularization techniques for obtaining acellular scafolds, and the possibility of recellularize at least partially both in vitro and in vivo. However, the development of more complex recelullarization techniques is required to generate more complex and functional renal structures.

120 (961) **SERTOLI CELLS AS CARRIERS FOR GENE THERAPY VECTORS IN BRAIN AND CANCER**

Antonela Sofía Asad[1], Patricia Jacobo[1], Cristian Sobarzo[1], Cinthia Soledad Méndez[1], Flavia Zanetti[2], Mariela Alejandra Moreno Ayala[1], Camila Zucatto[1], María Verónica Baez[3], Diana Jerusalinsky[3], Adriana Seilicovich[1], María Susana Theas[1], Livia Lustig[1], Marianela Candolfi[1].
[1]Instituto de Investigaciones Biomédicas (INBIOMED, UBA-CONICET), Facultad de Medicina, Universidad de Buenos Aires. [2]Instituto Cesar Milstein (CONICET). [3]Laboratorio de Neuroplasticidad y Neurotoxinas. Instituto de Biología Celular y Neurociencia "Profesor E. De Robertis". Facultad de Medicina, UBA.

Since Sertoli cells are key regulators in testis immunoprivilege, they constitute good candidates for cell-mediated gene therapy. Viral vectors are excellent tools for the delivery of therapeutic transgenes in chronic diseases. However, these vectors could be immunogenic leading to transient expression *in vivo*. Our hypothesis was that Sertoli cells could be useful carriers for the delivery of immunogenic gene therapy vectors into the brain parenchyma and the tumor microenvironment. Sertoli cells were obtained by enzymatic digestion (90% purity) from prepuberal or adult mice testis and infected with adenoviral (Adv) vectors (MOI 15) encoding the green fluorescent protein (GFP) under the CMV promoter. Trans-

duction efficiency was assessed *in vitro* by fluorescent microscopy. Sertoli cells were harvested for *in vivo* injection 24 h after infection. Naïve mice received intracranial injections of Adv-infected Sertoli cells (60,000). Infected syngeneic Sertoli cells (35,000) were also injected in breast tumors growing in the flank of Balb/c mice or into glioblastomas growing in the brain of C57Bl/6 mice. In Sertoli cells from prepuberal mice, transgene expression was detected in 38% of cells 24 h post-infection. Transduction efficiency was higher when cells were obtained from adult mice (90%) in comparison with prepuberal mice (60%). GFP^+ Sertoli cells from prepuberal or adult mice were readily detected in the normal brain parenchyma 72 h after injection. However, brains injected with adult-derived Sertoli cells exhibited higher frequency of GFP^+ cells. Adult-derived GFP^+ Sertoli cells were also detected within the tumor microenvironment in breast and brain tumors. CONCLUSIONS: Our results show that Sertoli cells from adult animals exhibit more stable transgene expression than prepuberal animals, probably due to their low proliferation rate. Sertoli cells could be useful carriers for the delivery of therapeutic transgenes for chronic diseases.

121 (2052) **PRODUCTION, PURIFICATION AND IN VITRO ASSESSMENT OF SOLUBLE GLYCOSYLATED RHSCF FOR IMPROVING UMBILICAL CORD BLOOD CD34+ HSCS EX VIVO PROLIFERATION**

Antonela Fuselli[1], Marina Etcheverrigaray[1], Ricardo Kratje[1], Claudio Prieto[1].
[1]Laboratorio de Cultivos Celulares -Facultad de Bioquímica y Ciencias Biológicas - Universidad Nacional del Litoral - Santa Fe, Argentina.

Stem cell factor (SCF) is a growth factor capable of promoting proliferation, differentiation and survival of hematopoietic stem cells (HSCs). Different *in vivo* and *in vitro* experiments have established its role mediated by C-kit receptor in early stages of hematopoiesis, gametogenesis, melanocyte proliferation and mastocyte maturation and activation. SCF potential therapeutic applications include anemia treatment, HSCs mobilization from bone marrow to peripheral blood for recovery and transplant, and improvement of transduction efficiency on gene therapy. The aim of this work was to produce and purify glycosylated recombinant human SCF (rhSCF) for ex vivo expansion of HSCs. Two rhSCF-producing adherent cell lines were generated using different promoting sequences through lentiviral transgenesis (HEK ACTB SCF and HEK CMV SCF). Gradual antibiotic selection was performed in order to increase specific productivity. SDS PAGE followed by western blot analysis evidenced the presence of rhSCF in all cell lines culture supernatants. A C-terminal HisX6 tag was used to purify rhSCF from HEK CMV SCF cell line supernatant by Immobilized Metal-ion Affinity Chromatography (IMAC) obtaining 98% purity in one step. $CD34^+$ HSCs were isolated from umbilical cord blood samples by positive immunomagnetic selection and cell proliferation assays in presence of rhSCF were performed. HSCs culture reached ten-fold expansion when stimulated with purified rhSCF, and six-fold expansion when supplemented with Escherichia *coli*-derived rhSCF. These results demonstrated an improved proliferative capacity of glycosylated rhSCF compared to E. *coli*-derived rhSCF.

122 (2069) **OSTEOSYNTHESIS MAXILLOFACIAL CUSTOMIZED PLATES DESIGN FOR 3D PRINTING**

Enrique Frayssinet[1,2,3,4], Carlos Atienza[2], Miguel Angel Utrera[2], Álvaro Page[2], Jaime M Prat[2].
[1]National Council of Scientific and Technical Research ,CONICET, (Argentina). [2]Valencia Biomechanical Institute, Valencia, Spain. [3]National Institute of Material Technology, INTEMA, Argentina. [4]Project Research and Industrial Design Actions Center CIPADI, Argentina.

Abstract. Objectives: Functional geometrical optimization of maxilofacial customized implants through 3D printing. *Materials and Methods:* A comparison of different designs of customized maxillofacial plates with different grades of topological and functional optimization was carried out. The geometric simple of a human jaw was taken from a computed tomography of a symphysis frontal impact fracture, through a *MIMICS 16 y 3MATIC 8 of Materialize* software. The maxillofacial plates design combine medical especifications, topological and functional optimization criteria derived from finite element models (FEM) and its morphological adaptation to the patient. Boundary conditions and FEM evaluation criteria were taken from literature; a simulation of an occlusal obstruction by restricting movement in perpendicular plane to one of the molars. These forces were represented by simulating the active maxilofacial muscles during chewing. *Result:* maximum strains registered for different osteosynthesis plates models resulted below the material limits failure in every case. Maximum tension registered was observed in the proximal fracture area. Fragmentary displacement was below 0.05 mm in every case. Failure limits were considered according to current regulations. Osteosynthesis plates physical demonstrators were obtained printed in Titanium medical grade as well as from the mandibular model of the clinical case. *Conclusions:* Results suggest that with the current technology in 3D printing, osteosynthesis customized and optimized plates can be printed according to the medical especifications with a high level of morphological freedom.

ONCOLOGÍA I / ONCOLOGY I

123 (73) **TNF ALPHA INDUCES MULTIRESISTANCE TO HER2-TARGETED THERAPIES IN BREAST CANCER**

Sofia Bruni[1], Mara De Martino[1], Cecilia J. Proietti[1], Patricia V. Elizalde[1], Roxana Schillaci[1], María Florencia Mercogliano[1].
[1]Instituto de Biología y Medicina Experimental

HER2 positive (HER2+) is a breast cancer (BC) subtype characterized by HER2 overexpression/amplification and affects ~15% of BC patients, who receive trastuzumab (T), an anti-HER2 monoclonal antibody, but resistance events hamper its clinical benefits in 40-60% of the cases. We have demonstrated that TNFα overexpression turned T-sensitive cells and tumors into resistant ones, and this resistance mechanism was mediated by upregulation of mucin 4 (MUC4). Nowadays there are new anti-HER2 therapies such as the dual tyrosine kinase inhibitor lapatinib (L) and antibodies, like T-DM1 and pertuzumab (P). The aim of this work was to explore whether TNF and TNF-induced MUC4 expression play a role as a multiresistance factor to these new therapies.We performed cell proliferation assays by cell count and by 3H-thymidine incorporation using T-sensitive (C) and T-resistant BT-474 cells (T2), the latter engineered to overexpress TNF. The combination of T+P (10 µg/ml each) was more effective that T alone in C cells (–65.7% and -30.6% respectively, and, $P < 0.001$). T2 cells proliferation was slightly inhibited with T+P treatment (-24.1%, n.s). Dose-response curves showed that L inhibits C cells proliferation at 1 µM (-51.8%, $P<0.0001$ vs vehicle) and T-DM1 at 0.01 µg/ml (-40.7% vs IgG $P<0.05$), whereas T2 cells were resistant to both treatments at these concentrations. On the other hand, when MUC4 expression was abrogated, T2 cells were sensitized to T-DM1, showing that TNF-induced MUC4 expression is responsible for T-DM1 resistance in this cell line. These results suggest that overexpression of TNF in HER2+ BC confers resistance to T+P, T-DM1 and lapatinib. For T-DM1 treatment this resistance is mediated by TNF-induced MUC4 expression. We then propose to study MUC4 and TNF expression in tumor samples. Furthermore, resistant patients to HER2-targeted therapies with expression of TNF and MUC4, could be eligible for a combination with a TNF blocking treatment to overcome resistance.

124 (83) **TRANSITION METAL COMPLEXES WITH 6-METHOXYQUINOLINE AS ANTITUMOR AGENTS. BIOLOGICAL STUDIES ON TWO AND THREE-DIMENSIONAL CELL CULTURED MODELS**.

Juan Cadavid Vargas[1,2], Cristian Villa Pérez[1], María Carolina Ruiz[1,2], Ignacio Esteban León[1,2], Gloria Cristina Valencia[3], Delia Beatriz Soria[1], Ana Laura Di Virgilio[1,2], Susana Beatriz Etcheverry[1,2].

[1]CEQUINOR-CONICET-UNLP [2]Cátedra de Bioquímica Patológica, Facultad Ciencias Exactas, UNLP [3]Universidad Nacional de Colombia - Sede Medellín

Coordination complexes have been extensively studied as antitumor agents, offering a potential alternative for cancer treatment. Both, the ligand and the metal play an important role in the pharmacological properties of the complex. This study evaluates the effect of 6-methoxyquinoline complexes with copper, zinc and silver as antitumor agents in 2D (monolayer) and a 3D (multicellular spheroids) models derived from A549 and MG-63 cell lines. Ag, Cu and Zn complexes significantly reduced the cell viability (P<0,001) evaluated by the MTT assay in the 2D model (MG-63: IC50 40.6,50.7,63.8 µM, respectively, A549: IC50 26.8, 61.8, 245.8 µM, respectively). Moreover, reactive oxygen species (ROS) level was determined using dihydrorhodamine 123 as a fluorescent probe. Only the Cu complex induced ROS production (P<0,001). Preliminary studies on synergy of the complexes were performed using the Median-Drug Effect analysis for Cu and Zn complexes at fixed ratio concentrations (1:1, 1:3 and 1:5). The affected fraction was measured after 24h of incubation. It could be observed that Zn and Cu complexes presented a synergic effect at the ratios tested with a CI <1.On the other hand, spheroids were cultured by the liquid overlay technique and were treated with the complexes for 48h. The spheroids were treated with the Cu and Zn complexes. However, this model was more resistant and the concentrations tested were raised 2 to 3 times the IC50 to reach a significant viability reduction (P<0,01). Morphological changes were observed in the whole range of concentrations using phase contrast microscopy. Moreover, the spheroids treated with low doses were capable to re-attach on the surface. All together, these results suggest that the four complexes showed a differential effect on the cultured cells. Zn and Cu complexes showed the higher cytotoxic activity in both 2D and 3D models. These compounds are good potential candidates for alternative antitumor treatment alone or in combination.

125 (85) **PLAGL1 GENE FUNCTION DURING HEPATOMA CELL PROLIFERATION**

Ana Florencia Vega Benedetti[1], Cinthia Natalia Saucedo[1], Patrizia Zavattari[2], Roberta Vanni[2], Luis Antonio Parada[1].
[1]Institute of Experimental Pathology, UNSa-CONICET, Salta, Argentina [2]Biochemistry, Biology and Genetics Unit, Department of Biomedical Sciences, University of Cagliari, Cittadella Universitaria di Monserrato SP 8, Km 0.700, 09042 Monserrato, Cagliari, Italy

PLAGL1 gene encodes a homonymous Zinc-finger protein that regulates cell cycle arrest and apoptosis. Such regulation occurs through pathways that include p53 and PPARγ, which induce p21Cip1, a cell cycle regulator. To elucidate its role in tumor growth, we studied the transcript and protein levels of PLAGL1, p53, PPARγ and p21 in hepatoma proliferating cells. Hepatoma cell lines HepG2, Huh7, PLC and SkHep1, and normal fibroblasts (control) were cultured according to standard protocols for proliferation studies. Then, cell count, flow cytometry, Western blot and RT-qPCR analyses were performed at 48, 72 and 96hs. The transcript level was quantified by the 2-ΔΔCt method, using PPIA as a reference gene. Protein level was quantified by the ImageJ program, using Actin as a loading control. Statistic analyses were performed by ANOVA. We determined that fibroblasts have lower proliferation rates than cancer cell lines. In general, the PLAGL1 mRNA level was significantly higher in fibroblasts than in tumor cell lines, which exhibited distinct patterns of transcription and expression. In fibroblasts, the PLAGL1 transcript and expression levels decreased significantly after 48hs post release from serum starvation, and then gradually increased until 96hs, while p53 and p21 followed this PLAGL1 pattern. In contrast, the tumor cell lines showed uniform low transcription and expression levels of PLAGL1 during the experimental period, except for SkHep1 cells. Despite of this, the transcript and expression levels of p53 and p21 presented different dynamics among tumor cell lines. Regarding PPARγ, our experiments demonstrated that its transcriptional level was significantly low during proliferation in normal and and tumor cell lines, except only for Huh7.Our results show that there is not a straight forward functional relationship between PLAGL1 and p53, PPARγ and p21 in the cell-cycle control of hepatoma cells.

126 (121) **PROGNOSTIC SIGNIFICANCE OF TRAIL-R3 AND CCR-2 EXPRESSION IN TUMOR EPITHELIAL CELLS OF PATIENTS WITH EARLY BREAST CANCER**

María Belén Giorello[1], Francisco Raúl Borzone[1]Leandro Marcelo Martinez[1], Ayelén Matas[1], Vivian Labovsky[1], Norma Alejandra Chasseing[1], Kevin Mauro Davies[2], Hernán García-Rivello[2], Alejandra Wernicke[2], María de Luján Calcagno[3].
[1]Instituto de Biología y Medicina Experimental, Laboratorio de Inmunohematología - Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina. [2]Departamento de Anatomía Patológica, Hospital Italiano, Buenos Aires, Argentina. [3]Departamento de Bioestadística, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina.

Despite improvements in technology and early diagnosis, many patients still succumb to the disease if the primary breast tumor metastasizes to secondary organs. Thereby, detection of new prognostic and predictive factors in breast cancer that could provide a target for new therapeutic treatments as well as to define prognosis is being sought worldwide. In previous work, we found that tumor epithelial cells (TEpCs) and spindle-shaped stromal cells (SCs), not associated with the vasculature, of early breast cancer patients (BCP) expressed OPG, TRAIL, RANKL, SDF-1, IL-6, M-CSF, CCL-2 and their receptors at significantly higher levels compared with non-neoplastic breast tissues. Therefore, our aim was to explore the clinicopathological significance of these ligands and receptors for TEpCs and SCs, as prognostic determinants of early BCP. We conducted immunohistochemical analysis of these proteins expression in TEpC and SCs of invasive ductal primary tumors with early BCP, and analyzed their association with clinicopathological characteristics, including local relapse, metastatic recurrence, disease-free survival (DFS), metastasis-free survival (MFS), and overall survival (OS). We found that elevated levels of TRAIL-R3 and CCR-2 (CCL-2-R) in TEpCs and OPG and CCL-2 in these SCs were associated with a higher risk of metastasis (p=0.032, p= 0.003, p= 0.038, and p= 0.049; respectively). Moreover, high expression of TRAIL-R3 and CCR-2 in TEpCs was associated with shorter DFS, MFS, and OS. Finally, high TRAIL-R3 expression in TEpCs was an independent prognostic factor for DFS and OS, and high CCR-2 expression in these cells was an independent prognostic factor for MFS. This study is the first todemonstrate that high levels of TRAIL-R3 and CCR-2 expression in TEpCs identified patients with early breast cancer with poor outcomes.

127 (137) **BIOPLAT2 AN OPEN-SOURCE SOFTWARE FOR ONCOGENOMICS DATA ANALYSIS**

Martin Carlos Abba[1,2], Diego Ariel Martinez[1], David Alejandro Nastasi[1], Juan Martín Lichowski[1], Hernán Chanfreau[1], Juan Manuel Jaime[1], Ezequiel Lacunza[2], Matias Daniel Butti[1].
[1]CAETI - Facultad de Tecnología Informática, Universidad Abierta Interamericana, CABA, Argentina. [2]CINIBA – Facultad de Ciencias Médicas, Universidad Naciona de La Plata, La Plata, Argentina

BioPlat2 is a user-friendly bioinformatic resource (http://www.cancergenomics.net), which provides a set of analytic tools for the discovery and in silico evaluation of novel prognostic and predictive cancer biomarkers based on integration and re-use of gene expression signature in the context of follow-up data. Here, we describe the main improvements implemented in the second version of the software. The new distinctive features include the capability to analyze the gene expression profiles in the context of follow-up among thousand of samples obtained from TCGA project (The Cancer Genome Atlas, NCI – NHGRI) in almost every human cancer disease. The desktop cross-platform client applicationis now supported by a dedicated Bioplat backend for the statistical

and computational analysis of very large databases. Furthermore, we have refurbished its graphical interface, added new visualization tools and up-graded the BioPlat data bases providing access tomore than 10.000 gene signatures. In conclusion, BioPlat2 facilitates the mining process of oncogenomics data obtained from publicly available geneexpression profiling repositories.

128 (208) **INTERACTION BETWEEN THE FIBROBLAST GROWTH FACTOR 2 (FGF2) AND THE PROGESTERONE RECEPTOR (PR) PATHWAYS IN BREAST CANCER**

Virginia Figueroa[1], Ana Sahores[1], Claudia Lanari[1], Caroline A. Lamb[1].
[1]Instituto de Biología y Medicina Experimental (IBYME)

About 75% of all breast cancers express hormone receptors and these patients are eligible to receive endocrine therapy. However, some patients are unresponsive or develop resistance to hormone therapy. Dysregulation of the FGF2/FGFR signaling pathway has been associated with different types of cancer. In humans, FGF2 consists of five protein isoforms with different molecular weights: 18 kDa (low molecular weight, LMW-FGF2) and 22, 22.5, 24 and 34 kDa (high molecular weight, HMW-FGF2). In murine and human breast cancer models, we demonstrated that tumor variants resistant to the antiprogestin mifepristone (MFP), express a higher PR isoform B/isoform A ratio compared to responsive tumors. We recently observed that hormone resistant variants also express higher levels of HMW-FGF2. Our aim is to investigate the interaction between the PR and FGF2/FGFR pathways in order to determine the mechanisms that drive breast cancer progression. We worked with the T47D human cell line that expresses high levels of PR, low levels of HMW-FGF2 and responds to MFP treatment. We infected T47D cells with plasmid constructions containing an empty vector, a sequence for the 18 or the 22 kDa FGF2 isoforms. We found that T47D-18 and -22 were unresponsive or increased cell proliferation ($p<0.05$) with MFP treatment, respectively, compared to control treatment. Moreover, we found that PR levels, particularly PRA, were significantly reduced in cell lines that overexpress FGF2 compared to the cell line expressing the empty vector. Our findings evidence a negative regulation of PR levels, especially PRA, by FGF2. These results suggest that FGF2 might induce endocrine resistance by altering the PR isoform ratio. Unravelling the mechanisms involved in endocrine resistance are essential for the development of new therapies to treat breast cancer patients.

129 (205) **PROTEOME-BASE BIOMARKERS IN PROSTATE CANCER: PROTEIN PROFILING USING MASS SPECTROMETRY AND A BIOINFORMATICS BASED APPROACH**

Emiliano Germán Ortiz[1], Alejandra Verónica Páez[1], Federico Schuster[1], Nicolás Anselmino[1], Sofía Lage Vickers[1], Javier Cotignola[1], Elba Vazquez[1], Geraldine Gueron[1].
[1]Laboratorio de Inflamación y Cáncer, IQUIBICEN/Dpto. Química Biológica, CONICET/FCEN-UBA

Proteomics represents an important tool for the identification of new molecular targets for prostate cancer (PCa) tailored therapy. Considering the heterogeneity of the prostate tumors, a single biomarker does not seem sufficient to predict the disease outcome. Towards this end, we undertook an in-depth mass spectrometry-based proteomics study to build the Heme-oxygenase 1 (HO-1) interactome in PCa. We have previously shown the anti-tumoral role of HO-1 in PCa. We propose HO-1 and its interactors reprogram PCa cells and modify the tumoral microenvironment, favoring a less aggressive phenotype. FLAG immunoprecipitation assays were performed using lysates from PC3 cells transfected with FLAG-tagged HO-1, and the isolated proteins were subjected to LC/ESI-MSMS analysis. To address the relevance of the HO-1 interactome in prostate carcinogenesis we evaluated multiple microarray datasets for across the Oncomine database. We grouped the interactome in 3 clusters according to the following expression profiles: Cluster A- genes that were over or under-expressed in^3 60% of datasets, Cluster B- genes that were over or under-expressed in approx. 50% of datasets, Cluster C- genes that were over or under-expressed in similar percentages of datasets. Cluster A rendered the following genes: NOA1, CBX3, RCC1, EEF2, ASPH, SQSTM1, HSPB1 and ANXA2. Results show that for prostate adenocarcinoma *vs.* normal prostate gland these genes lie within 20% of the most consistently high or low-expressed genes across this comparison. We selected cluster A for further analysis and assessed their expression profile across The Human Protein Atlas platform observing positive correlation for Cluster A genes compared to Oncomine. We also analyzed whole exome data for these genes (cBioportal) revealing amplification as the most frequent genetic alteration in the prostate cancer vs normal prostate comparison, ascertaining them as potential biomarker candidates for PCa.

130 (220) **BONE MARROW MESENCHYMAL STEM CELLS ORCHESTRA A PRO-INFLAMMATORY MICROENVIRONMENT CREATING A PRE-METASTATIC NICHE IN UNTREATED ADVANCED BREAST CANCER PATIENTS**

Francisco Raúl Borzone[1], Maria Belén Giorello[1], Vivian Labovsky[1], Valeria Beatriz Fernández Vallone[2], Hosoon Choi[3], Raúl Horacio Bordenave[4], Emilio Batagelj[5], Leonardo Feldman[6], Federico Dimase[7], Leandro Marcelo Martinez[1], Norma Alejandra Chasseing[1].
[1]Laboratorio de Inmunohematología, Instituto de Biología y Medicina Experimental (IBYME) - Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina. [2]Institut de Recherche Interdisciplinaire en Biologie Humaine et Moléculaire (IRIBHM), Faculty of Medicine, Université Libre de Bruxelles ULB, Belgium [3]Central Texas Veterans Research Foundation, Texas, USA [4]Departamento de Oncología, Hospital Iriarte, Quilmes, Buenos Aires, Argentina [5]Departamento de Oncología, Hospital Militar Central, Argentina [6]Departamento de Trasplante de Medula Ósea, Ciudad de Buenos Aires, Argentina [7]Departamento de Hematología, Hospital Militar Central, Ciudad de Buenos Aires, Argentina

Most of advanced breast cancer patients (BCP) develop osteolytic bone metastasis. Our previous studies demonstrated that bone marrow-mesenchymal stem cells (BM-MSC) of untreated BCP (invasive ductal carcinoma, stage III-B, without metabolic bone disease) have a decrease of self-renewal, proliferation, and osteogenic/ adipogenic differentiation capacities, as well as, a positive regulation of osteoclastogenic process versus healthy volunteers (HV). Also, we observed elevated levels of TBARS in BM-plasma from these patients, suggesting an increase in reactive oxygen species (ROS) production. It has been proposed that, the oxidative stress inhibits β-catenin signaling, and thus, increase RANKL/OPG ratio, as well as, CCL-2 expression in BM-stromal cells leading to bone resorption. Therefore, we hypothesized that the BM-MSC orchestra a pro-inflammatory microenvironment leading to future imbalance of bone metabolism, and thus favoring bone metastasis appearance in advanced-BCP. We evaluated total and mitochondrial ROS levels in BM-MSC from BCP and HV. Also, we quantified the levels of CCL-2, RANKL and OPG in conditioned medium (CM) of colony-forming unit fibroblast (CFU-F) primary cultures (1CFU-F=1MSC) from both groups. Finally, we investigated the membrane-RANKL expression in BM-MSC. Data showed higher levels of intracellular ROS in MSC from BCP vs HV ($p=0.02$). Moreover, CCL-2 levels in BCP were higher than HV-values ($p=0.0374$). In contrast, OPG levels were lower in BCP ($p=0.0482$). Although the RANKL levels in these CM were lower than detectable doses, membrane-RANKL expression/MSC was higher in BCP compared with HV. In conclusion, high levels of ROS, CCL-2 and membrane-RANKL in BM-MSC would increase bone resorption, leading to breast tumor cells invasion and proliferation. Taken together, the BM-MSC of advanced BCP creates an ideal environment ("pre-metastatic niche") for the metastatic development.

131 (214) **INHIBITION OF MITOCHONDRIAL FISSION INHIBITS REPLICATION OF GLIOBLASTOMA CELLS AND INCREASES MITOCHONDRIAL MASS BUT HAS NO EFFECT ON CELL DEATH**

Nerina Mariel Villalba[1], Ines Rebagliati[1], Juan Jose Poderoso[1], Guillermo Blanco[2], Maria Cecilia Carreras[1, 3].
[1]Instituto de Inmunología, Genética y Metabolismo (INIGEM-CONICET-UBA) [2]Instituto de Estudios de la Inmunidad Humoral (IDEHU-CONICET-UBA) [3]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Dpto. Bioquímica Clínica

During cell replication (CR), mitochondria must provide the energetic requirements of G1 and S phases, and further split between the daughter cells. Thus, blocking fission can have detrimental effects on tumour cells replication; while, a persistent mitochondrial fusion can lead to reactive oxygen species overproduction and initiation of apoptosis. We evaluated the effect of Mdivi1, a mitochondrial fission inhibitor, on U87 and HeLa cells over CR, mitochondrial mass (MM), and cell death by flow cytometry or fluorescence microscopy. CR was evaluated by DNA content with propidium iodide (PI), MM by staining mitochondrial cardiolipin with nonyl-acridine-orange (NAO), mitochondrial membrane potential with tetra-methyl-rhodamine-esther, and cell viability was determined by staining cells with PI. Mdivi1 at 25-100 µM induced fusion of the mitochondrial network of U87 cells at 24-72h. The round-shaped mitochondria of untreated cells were progressively lost and replaced with elongated thread-like shapes ($p<0.001$ for the difference of roundness index between Mdivi1-treated and basal cells). In cells treated with 100µM Mdivi1 donut-shaped mitochondria were also noted. Mdivi1 at 25-75 µM had no effect on cell viability at 48h in U87 or HeLa cells; however, the MM was significantly increased ($p<0.001$). At 72h, while U87 cells showed no change in viability, HeLa cells were all dead. DNA content analysis showed a progressive accumulation of U87 cells in S and G2/M phases with increasing Mdivi1 doses (5.6 and 18.4% for basal and 50µM Mdivi1 in G2 phase and 33.4% and 42.6% for basal and 50µM Mdivi1 in S phase, respectively). Cells arrested in S and G2 phase showed increased MM. We conclude that blocking of mitochondrial fission in U87 cells inhibits CR probably owing to impairment of the normal split of mitochondria to daughter cells. The differential susceptibility between U87 and HeLa cells to the increase in MM may be due to the different extent of OXPHOS and glycolysis usage.

132 (667) **INVOLVEMENT OF NOTCH SYSTEM AND PDGFB IN OVARIAN CANCER STEM CELLS SUB-POPULATION**

Maria Camila Pazos[1], Andrés Bechis[1], Marta Tesone[1,2], Ronald J. Buckanovich[3], Griselda Irusta[1].
[1]Instituto de Biología y Medicina Experimental (IByME - CONICET) [2]Departamento de Química Biológica (FCEN - UBA) [3]Division of Gynecologic Oncology - University of Michigan

Ovarian cancer remains one of the most lethal gynecologic cancers, in part due to a regenerative tumor cell sub-population that acquired stem cell properties. Notch and PDGF systems are involved in proliferation, apoptotic and angiogenic processes. To assess the rol of these systems in oncogenic stem cell properties, we incubated two epithelial ovarian cancer cell lines (SKOV3 and HEY1) in the presence of a gamma-secretasa inhibitor, DAPT (20uM), recombinant-PDGFB (10ng/ml), DAPT+rec-PDGFB and in the absence of stimulus (Control). We determined ovarian oncogenic stem cell markers by flow citometry (CD133, CD44 and ALDH activity), and the capability of these cell lines to form spheres. A xenograft model in female SCID mice was developed using SKOV3 cells. During 4 consecutive days, mice received 1.DMSO 20% (Control), 2.A gamma-secretase inhibitor (DBZ, 1mg/ml/d), 3.rec-PDGFB (25ug/ml/d) and 4.DBZ+PDGFB. Animals were sacrificed 2 days post-treatment. The in vitro studies showed a decrease of the stem cell population in both cell lines when incubated with DAPT. The incubation with DAPT, PDGFB, or both, significantly decreased the number of spheres formed by cells. In vivo treatment with DBZ or DBZ+PDGFB significantly decreased tumor weight and growth compared to the control group. No differences were found between Control and PDGFB treatments. Also, DBZ, PDGFB, or DBZ+PDGFB significantly decreased the tumor stem cell pool. Tumor proliferation (Ki67 levels) significantly decreased and cellular apoptosis (cleaved Caspase-3 staining) slightly increased with DBZ and significantly increased with DBZ+PDGFB co-treatment. Also endothelial area increased with DBZ and DBZ+PDGFB co-treatment. In conclusion we demonstrate that Notch system is involved in ovarian cancer stem cell properties and also ovarian tumor stem cell content. The administration of PDGFB cooperates with gamma secretase inhibitor action on tumors intensifying the inhibitor antitumor effect.

133 (228) **OVARIAN CANCER AND HIPPO PATHWAY: CHARACTERIZATION AND ESTROGEN MODULATION**

Nicolas Fraunhoffer[1], Analia Meilerman Abuelafia[1], Ines Stella[1], Silvia Galliano[2], Pablo Lopez Bergami[1], Alfredo Vitullo[1].
[1]CEBBAD, Universidad Maimónides [2]Servicio de Anatomía Patológica, Hospital Eva Perón de Merlo

Despite improvements in therapeutic strategies, prognosis for patients with ovarian cancer (OC) remains dismal. Hormonal therapy could be an option. Tamoxifen and selective estrogen receptor modulator, produce only a response of 10% in OC. A key player in hormonal therapy resistance may be the Hippo pathway (HP) bypassing the classical estrogen cascade. YAP is a downstream effector of HP binding to transcriptional co-factors TEAD. YAP is phosphorylated and restricted to the cytoplasm by MST1/2- and LATS1/2- kinases. To investigate HP involvement in OC, we first analyzed the expression of YAP, LATS1 and MST1/2 by immunochemistry in 14 OC (3 mucinous, 4 serous, 4 endometrioid and 3 clear cell). Samples were obtained from Hospital Eva Peron. We found that ovarian cancer presented different pattern of YAP expression. Endometrioid and clear cell carcinomas showed strong nuclear YAP expression in extracellular matrix cells. On the other hand, serous and mucinous carcinomas presented nuclear YAP signal mainly in cancer cells. LATS1 and MST1/2 showed low expression in all samples. In order to analyze the effect of 17β-estradiol (E2) on HP in OC, SKOV-3 cells were treated with 50 and 100 nM of E2. YAP, p-YAP (S127), LATS1 and MST1/2 expression was analyzed by western blot at 0, 1 and 4h of treatment for each E2 concentration. In addition, cellular localization of YAP and TEAD4 was studied by IF. E2 induced a reduction of p-YAP (S127) at 1h in a dose of 100 nM. LATS1 and MST1/2 levels were constant in all treatment conditions. Additionally, YAP nuclear staining was 80% higher in 100 nM at 4 h than control, while TEAD4 was 40% higher. These results show that YAP presents a differential pattern of expression depending on OC type, but not LATS1 and MST1/2 kinases which present low levels in all OC types. Furthermore, our results suggest that E2 acts on HP promoting nuclear translocation of YAP. Therefore, these results suggest that HP plays a role in ovarian tumorigenesis and hormonal therapy resistance.

134 (711) **VEMURAFENIB REVERTS HYPOXIA-INDUCED INCREASE IN CELL VIABILITY OF BRAFV600E MELANOMA CELLS**

Melina Gabriela Castro[1,2], Celia Noemi Perez[1,2], Ludmila Estefania Campos[1,2], Yamila Isabel Rodriguez[1,2], Dario Ramirez[1,2], Sergio Alvarez[1,2]
[1]Instituto Multidisciplinario de Investigaciones Biológicas (IMIBIO-CONICET) San Luis [2]Universidad Nacional de San Luis (UNSL)

Melanoma is the most aggressive form of skin cancer with a high mortality percentage. Half of melanoma patients display the BRAFV600E kinase mutation. Indeed, Food and Drug administration (FDA) and ANMAT have approved the use of Vemurafenib and Dabrafenib (BRAF inhibitors) in melanoma patients. Unfortunately, patients develop resistance after a short period of disease control, indicating that new targets are needed. In that regard, several evidences confirm that tumor microenvironment modulate prolif-

eration, migration and acquired resistance of cancer. Precisely, hypoxia is a remarkable feature of this picture that control cancer growth and progression. Thus, the aim of this study was to elucidate how hypoxia influences the viability of melanoma cells. We used two melanoma cell lines: SKMel2 (BRAFWT) and Lu1205 (BRAFV600E). Surprisingly, hypoxia exerts a differential effect according to the culture conditions: while decreases viability of melanoma cells cultured in the presence of fetal bovine serum (FBS), it partially protects of the reduced viability observed under serum deprivation conditions. To further investigate the effect in absence of serum, we used pharmacological inhibitors of different signaling pathways. Certainly, inhibition of NF-kB, PI3-K, MEK and hypoxia inducible factor 1 alpha (HIF1a) pathways do not have an effect on viability of SKMel2 melanoma cells in hypoxia. On the other hand, HIF1a is crucial for the hypoxia-induced protection of Lu1205 melanoma cell viability. Interestingly, inhibition of BRAF with vemurafenib also reverts the effect of hypoxia in Lu1205 melanoma cells. Altogether, these results suggest that viability of cancer cells in the hypoxic zones of the tumor is not reduced by the deficiency of growth factors, but may be diminished by inhibition of BRAF with vemurafenib.

135 (713) **SPHINGOSINE-1-PHOSPHATE INDUCES CYTOKINE EXPRESSION, MIGRATION AND MMPS SECRETION IN MELANOMA CELLS: ROLE OF FILAMIN-A**

Ludmila Estefanía Campos[1], Luciana Morellatto Ruggieri[1], Melina Gabriela Castro[1], Yamila Isabel Rodriguez[1], Sergio Eduardo Alvarez[1]

[1]Institución: Instituto Multidisciplinario de Investigaciones Biológicas (IMIBIO – SL) – Facultad de Química, Bioquímica y Farmacia. CONICET – Universidad Nacional de San Luis. San Luis, Argentina

Sphingosine-1-phosphate (S1P) is a bioactive sphingolipid linked to chronic inflammation and cancer. Once produced intracellularly by two sphingosine kinases SK1 and SK2, S1P might act as a) a second messenger or b) as autocrine/paracrine metabolite by acting through G protein-coupled receptors (GPCRs), named S1PR1-5. Most of S1P actions are mediated by its receptors, including migration, invasion and angiogenesis. Previously, we have shown that extracellular S1P induces NF-κB activation in melanoma cell lines that do not express Filamin-A (FLNa), an actin binding protein. Here we explored some biological actions triggered by extracellular S1P in two melanoma cell lines: M2 cells (FLNa-) and A7 cells (FLNa+). First, we analyzed mRNA expression of NF-κB-regulated cytokines and chemokines by qPCR. Our results demonstrate that S1P stimulates mRNA expression of IL-6, TNFα, CCL2 and CCL5 only in M2 cells. By using Boyden's chamber migration assays, we found that S1P induces migration in M2 cells but the effect is minor in A7 cells. Moreover, S1P protected from serum depletion-induced apoptosis in M2 cells. In concordance with these results, MTT experiments indicate that S1P preserve cell viability in M2 cells while its effect is less noticeable on A7 cells. In addition, gelatin degradation assays showed that S1P induces MMP9 secretion in M2 cells. Interestingly, a differential behavior is observed in A7 cells: S1P induced MMP2, but not MMP9, secretion, suggesting that FLNa may play a role in the regulation of MMPs release. In summary, our experiments indicate that FLNa expression modulates extracellular S1P induced-cytokine expression, MMP secretion, migration and proliferation in melanoma cells.

136 (476) **EFFECT OF RETINOIC ACID AND LAPATINIB COMBINED TREATMENT ON STEM/PROGENITOR CELLS GROWTH IN A TRIPLE NEGATIVE MAMMARY CANCER CELL LINE**

Agustina Taruselli[1], Damian Berardi[1], Alejandro Urtreger[1], Laura Todaro[1].

[1]Instituto de Oncología Angel H. Roffo

Cancer stem cells (CSC) have been described as resistant to chemotherapy and radiation and they are also considered as the metastasis seed. Recent studies have determined that HER2 receptor would be essential for the maintenance of the stem capacity of CSC, for that reason our objectives are: A) Assess the expression of HER2 receptorboth in 4T1 monolayers as well as in its derived CSC population and B) Analyze the modulation of growth potential under the treatment with retinoids (ATRA), Lapatinib (Lp, HER2 inhibitor) and their combination. By Western blot we could determine that HER2 is selectively expressed in the CSC population, mainly in its active form (phosphorylated). The effect of ATRA (1 uM), Lp (1 uM) and their combination on cell growth was evaluatedduring 96 hours. Lp caused a marked decrease in the proliferative capacity from 72 hours in 4T1 monolayers ($p<0.05$, evaluated by cell count). The same treatments were applied on CSC enriched mammospheres. The combination of ATRA with Lp strongly decreases the formation of these 3D structures, being them very small and irregular, with evident presence of numerous dead cells (diameter in um: Control 150±8; Lp120±22; ATRA 93±16; ATRA/Lp 72±19; $p<0.05$). Pretreated mammospheres were disaggregated and seeded at low density in order to determine the clonogenic capacity. While ATRA and Lp reduced this capacity, the combined treatment induced a significantly greater effect than either treatment alone (colony number: C 241±19; Lp 142±14; ATRA 202±6; ATRA/Lp 65±5; $p<0.05$). In conclusion, we have promoted an important decrease in the CSC population growth of a triple negative breast tumor, through the treatment with Lapatinib, together with retinoid's mediated cell differentiation. This result could allow a new therapeutic approach for a subset of tumors lacking treatment till nowadays.

137 (685) **"A SUCCESSFUL THERAPY COMBINING TUMOR MICROENVIRONMENT REMODELING AND ACTIVATION OF IMMUNE RESPONSE INHIBITS TUMOR GROWTH IN A MURINE MODEL OF HEPATOCELLULAR CARCINOMA ASSOCIATED WITH FIBROSIS"**

Marcelo Maximiliano Rodriguez[1], Juan Bayo[1], Esteban Fiore[1], Sofia Gomez Bustillo[1], Mariana Malvicini[1], Guillermo Mazzolini[1]

[1]Laboratorio de Terapia Génica, Instituto de Investigaciones en Medicina Traslacional (IIMT), Universidad Austral-CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas), Buenos Aires, Argentina

Currently, strategies for targeting the interaction between tumor microenvironment components that includes tumor cells, fibroblasts, endothelial, immune and cancer stem cells (CSCs) and the extracellular matrix (ECM), could delay tumor progression. In fact, immunotherapy to activates antitumor response and inhibits tumor growth is an attractive option. Liver fibrosis, a pre-neoplastic condition for hepatocellular carcinoma (HCC) is usually accompanied with the accumulation of ECM components including hyaluronan (HA). Our aims were to evaluate the effects of 4-methylumbelliferone (4Mu), an inhibitor of HA synthesis combined with gene therapy of interleukin 12 mediated by an adenovirus (AdIL-12) in a murine model of HCC associated with fibrosis induced by thioacetamide (TAA). C3H mice received Hepa129 cells intrahepatically (day 0) after 4 weeks of 200mg/kg TAA administration. On day 5 mice were distributed in groups (n=8/group): saline; 4Mu 200 mg/Kg, drinking water (day 5); AdIL-12 1x10e9 DICT50/ml, i.v (day 9) and 4Mu + AdIL-12. Antitumor efficacy and survival were studied. Liver and spleen samples were taken to determine prevalence and function of immune cells and the expression of CSCs markers. Combined treatment induced a significant decrease of HCC growth ($p<0.05$), and increased animal survival ($p<0.05$). The level of CD4+ and CD8+ cells in spleen and liver of treated mice were significant higher than untreated mice ($p<0.01$). Also, AdIL-12 and AdIL-12+4Mu therapies cause a stronger response mediated by cytotoxic T cells. Combined therapy decreases the hepatic mRNA levels of CSCs markers CD133, CD90, EpCAM and CD44 ($p<0.01$). In vitro studies showed that 4Mu reduces the expression of CSCs markers in Hepa and when cells are exposed to supernatants of splenocytes from treated mice, become more susceptible to apoptosis. Our results propose that this strategy achieves a successful antitumor immune response and reduces the CSCs markers in HCC associated with liver fibrosis.

138 (748) **RADIORESISTANCE ASSOCIATED MARKERS IN LUNG CANCER AND DESIGN OF THERAPEUTIC STRATEGIES BASED ON SIRNA**

Florencia Giannoni[1, 2], María Belén Cerda[1], Rodrigo Lloyd[1, 3], Agustín Giannoni[1], María Elena Iezzi[2, 4], Osvaldo Podhajcer[2, 4], Lucía Policastro[1, 2]

[1]Laboratorio de Nanomedicina, Comisión Nacional de Energía Atómica [2]CONICET [3]Instituto Nacional del Cancer [4]Fundación Instituto Leloir

Tumor radioresistance is one of the main causes that lead to the failure of radiation treatments. Silencing the expression of radioresistant-associated genes by small interference RNA (siRNA) is a promising strategy to optimize radiotherapy. On the other hand, for an optimal delivery of therapeutics siRNAs it is important to design effective nanovehicles. The aims of this work were to characterize the intrinsic radiosensitivity (IR) of human lung cancer cell lines to identify potential resistant-associated genes to be silencing by siRNAs molecules in a lung cancer model and also evaluate efficient strategies to delivery siRNA molecules. We have evaluated the expression of Ku80, a gene associated to DNA repair and the peroxirredoxin2 (Prx2) a critical enzyme of cellular antioxidant systems in A549, H292 and H1975 human lung cell lines. We have evaluated cellular radiosensitivity by survival curves. A549 was the most radioresistant cell line and H292 and H1975 have shown similar radiosensibility and significantly lower respect to A549. The expression of Ku80 was inducted by IR in all human lung cell lines, but Prx2 was not modulated after IR exposition. Silencing of Ku80 and also Prx2 in A549 by siRNAs have induced a significantly radiosensitization in A549 cells. We also have explored the encapsulation of plasmid containing a shRNA against the firefly luciferase reporter gene complexes with the cationic polymer poly(ethylene imine) (PEI) in liposomes. We encapsulated the complexes by the thin film hydration method. Nanovectors have shown a hydrodynamic diameter of 140-170nm and polydispersity index of 0.2. The stability of complexes was visualized in agarose gel. In future experiment we will evaluate the silencing potential of nanovehicles using a constitutive luciferase cell line, in order to obtain an optimized non-viral nanovehicle to delivery siRNAs against radioresitant-associated gene in lung cancer.

139 (721) **DETERMINATION OF BRAF ONCOGEN MUTATIONAL STATUS IN CUTANEOUS MELANOMA LIQUID BIOPSIES**

Mariana Aris[1], Eduardo Urigüen Zabalgogeazcoa[2], María Belén Sánchez[1], Michele Bianchini[1], Maite Iglesias Badiola[2], María Marcela Barrio[1], José Mordoh[1,3,4]

[1]Centro de Investigaciones Oncológicas-FUCA, CABA, Argentina [2]Universidad Francisco de Vitoria, Madrid, España [3]IIBBA-CONICET, Fundación Instituto Leloir, CABA, Argentina [4]Instituto Médico Especializado Alexander Fleming, CABA, Argentina

Cutaneous Melanoma is the most aggressive skin cancer, several targeted-therapy and immunotherapy strategies have been developed. Around 60% of melanoma tumors harbor mutations in the oncogene BRAF; most frequent mutations include BRAFV600E (75%) and BRAFV600K (20%). The study of liquid biopsies allows monitoring of patients periodically; however sensitive methods are required to detect low levels of circulating tumor DNA in blood. Thus our main goal was to establish a feasible method to determine BRAF mutational status in liquid biopsies. Calibrators with either BRAFV600E, BRAFV600K or BRAFV600 WT DNA were generated by their cloning in plasmids and confirmed by Sanger sequencing. Mutational status determination was set up by quantitative PCR. Specific primers were designed for detecting either BRAFV600E or BRAFV600K mutation, and a blocking-primer was used for the WT allele. Different cycling conditions were assayed for optimizing these reactions. Determination of BRAFV600 status in liquid biopsies was set up by spike-in of calibrators in plasma from healthy donors, isolation of circulating DNA with a column-based method, and qPCR. Parameters were established that allowed detection of up to 1 copy of BRAFV600E DNA variant in 10000 copies of BRAFV600 WT DNA (0.01%); a specific reaction was set up for BRAFV600K, obtaining the same sensitivity. When testing each reaction with the other template, specific cross reactivity in both cases was detected only at 1%. When analyzing BRAF mutational status in liquid biopsies, all mutated copies were detected in at least 0.1ng of mutated DNA per ml of plasma. We optimized a method for detecting the most frequent mutations in BRAF oncogene, BRAFV600E and BRAFV600K, in a highly sensitive, specific and processive way. This method can be implemented to improve the clinical follow up of melanoma patients in time, contributing to a more personalized medicine.

140 (792) **PHOTODYNAMIC THERAPY WITH A LIPOPHILIC ZN(II) PHTHALOCYANINE LEADS TO APOPTOTIC CELL DEATH THROUGH LYSOSOMAL PERMEABILIZATION, ER STRESS AND CASPASES ACTIVATION**

Nicolás Chiarante[1], Julieta Marino[1], María C. García Vior[2], Osvaldo Rey[3], Josefina Awruch[2], Leonor Roguin[1]

[1]IQUIFIB (UBA-CONICET) [2]Dpto de Química Orgánica, FFyB, UBA [3]INIGEM, CONICET

Phthalocyanines (Pcs) have been found to be useful photosensitizers for photodynamic therapy (PDT). In a previous work, we demonstrated the cytotoxic action of a lipophilic Zn(II) phthalocyanine (Pc9) encapsulated into poloxamine polymeric micelles (T1107) in CT26 cells derived from a murine colon carcinoma (IC50=11±1 nM). In order to elucidate the mechanism of phototoxic action, we explored both the subcellular localization of Pc9 as well as the possible induction of an apoptotic response. After staining Pc9-loaded cells with fluorescent dyes for specific organelles, Pc9 was detected in lysosomes and endoplasmic reticulum (ER), but not in mitochondria. A significant increase in the cytosolic levels of the lysosomal enzyme cathepsine D was observed after irradiation of Pc9-treated cells, suggesting the permeabilization of the lysosomal membrane. In addition, an enhancement of cell viability was obtained after incubating Pc9-exposed cells with lysosomal proteases inhibitors, such as Pepstatin (cathepsin D inhibitor), CA-074 Me (cathepsin B inhibitor) or aprotinin (serine proteases inhibitor). A time-dependent increment of the cytosolic amounts of calcium together with the regulation of the expression levels of ER proteins suggested the involvement of ER stress. Consistently, a lower cytotoxic effect was obtained when cells were pretreated with the calcium chelator BAPTA. An apoptotic response was then demonstrated by the increase of caspase-3, -8 and -9 activities, the decrease of anti-apoptotic Bcl-2 family protein levels, the cleavage of PARP and the visualization of apoptotic nuclei in cells stained with Hoechst 33258. Finally, PDT also provoked cell cycle arrest in the S and G2/M phases. In conclusion, our results showed that Pc9 behaves in vitro as a potent photosensitizer capable of inducing an apoptotic cell death through lysosomal permeabilization and ER stress, both contributing to the activation of a mitochondrial caspase cascade.

141 (773) **CANCER STEM CELLS INCREASE IN HUMAN BREAST CANCER 3D CULTURES WITH ACQUIRED RESISTANCE TO TRASTUZUMAB**

Cristina Elisa Rodriguez[1], Damian Emilio Berardi[1], Laura Todaro[1], Elisa Dora Bal de Kier Joffe[1], Gabriel Leon Fiszman[1]

[1]Area Investigación, Instituto de Oncología Angel H. Roffo, Facultad de Medicina, Universidad de Buenos Aires

HER2, overexpressed in 20% invasive breast tumors, correlates with low disease free survival. Trastuzumab (Tz) is a monoclonal antibody used to treat HER2+ tumors; however more than half of the patients are resistant or acquire resistance during treatment. Cancer stem cells (CSC) are associated with chemotherapy resistance. Tumor spheroids are a 3D culture model that mimics in vivo avascular tumors. Previously we showed that BT474 (HER2+) spheroids chronically treated with Tz developed resistance. Our aim was to analyze the resistance acquired in 3D and the impact of the CSC developed in these conditions. First, we analyzed the CSC subpopulation shaped in BT474 spheroids

by studying the CD44+CD24low cells by flow cytometry. We found that 15 days Tz treatment increased the CSC population by 1.5 fold vs controls ($p<0.05$). Moreover, by qPCR we observed an increase in the expression of the pluripotent genes Nanog, Sox2 and OCT4 (2.5, 2 and 2.3 fold higher than controls, $p<0.05$). Interestingly, this population showed a higher HER2 expression than controls ($p<0.05$). Unlike monolayers with homogeneous expression of HER2, in spheroids we found two HER2 populations, HER2high and HER2low (37% vs 63% respectively $p<0.05$). Interestingly, HER2high population increased to 50% after Tz treatment. In order to analyze the influence of 3D architecture in cells refractory to Tz, we used MCF7, a breast cancer cell line without HER2 amplification and unresponsive to Tz both in 2D and in 3D cultures. In MCF7 spheroids, by flow cytometry we detected low expression of HER2 in 82% of the cells. Though spheroids size did not change with Tz treatment, HER2+ cells were diminished by 20% and CD44+CD24low population was reduced by 30%. Furthermore, only 28% CSCs expressed HER2 and this population was reduced to 10% after Tz treatment. These results suggest that the hostile microenvironment in 3D has a key role in the acquisition of resistance to Tz, associated with an increase in CSCs.

142 (802) **PORPHYRIN PHOTOSENSITIZATION AND REACTIVE OXYGEN SPECIES SCAVENGERS PROTECTION IN 5- AMINOLEVULINIC ACID- BASED PHOTODYNAMIC THERAPY**

Haydée Fukuda[1], María Julieta Teijo[1], María del Carmen Martínez[2], María Victoria Rossetti[1], Alcira Batlle[1].
[1]Centro de Investigaciones sobre Porfirinas y Porfirias (CIPYP, CONICET_UBA). Hospital de Clínicas José de San Martín. Av Córdoba 2351. 1120 AAR, CABA [2]Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, UBA. Pab II, Ciudad Universitaria. 1428 EHA, CABA

Photosensitization of endogenously synthesized porphyrins after administration of 5- aminolevulinic acid (ALA), are used in Photodynamic Therapy of tumours and microorganisms infections. Irradiation with appropriate wavelength light triggers photochemical reactions and ROS production are induced, leading to cell death. Several ROS scavengers agents (ascorbate , trolox, mannitol, GSH, l-tryptophan) were assayed to their ability to modulate ALA-PDT performed on A549 human lung adenocarcinoma cells, by incubating 3h with 1mM ALA, followed by irradiation with or without 1 h pre-incubation with the modulators. The ratio between cell survival after ALA-PDT in the presence and in the absence of the scavenger agent (protection grade: PG) was determined, and the concentrations showing no cytotoxicity/ photoactivity and providing the highest PG were used. ALA-PDT alone induced a high percentage of apoptotic cell death (98.4±3.5%). Pre-incubation with modulators at their highest PG concentration significantly reduced apoptotic cells to 48.3±2.7% (ascorbate), 58.8±4.2% (trolox), 78.5±3.1% (GSH), 64.3±1.6% (mannitol), and 74.6±2.3% (l-tryptophan). ROS production in early cell death induction after ALA-PDT was tested by flow cytometry; ROS increased after ALA-PDT (H2-DCFDA + cells: control: 1.1±0.1%; 10 min-PDT: 69.3±5.6%; MVP positive cells: control: 0.65±0.35%; 10 min-PDT: 83.5±1.9%). Ascorbate prevented peroxide formation and singlet oxygen increased, whereas trolox limited peroxides generation, but did not affect significantly singlet oxygen production. These results suggest that undesired photodamage to normal tissue might be attenuated by antioxidant agents administration, showing strong correlation between porphyrins, ROS production, scavengers and cell death, reinforcing the photodynamic properties of endogenously synthesized porphyrins, which are of high relevance in the photosensitization of porphyric patients.

143 (799) **ROLE OF GLUCOCORTICOIDS IN MYELOID LEUKEMIA CELLS**

Micaela Silbermins[1], Adali Pecci[1,2], Luciana Rocha Viegas[1].
[1]Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE-UBA-CONICET) [2]Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, UBA

The leukemias are malignant diseases of hematopoietic cells in which the proper balance between proliferation, differentiation and apoptosis is no longer operative. Synthetic glucocorticoids like dexametasone (Dex) are frequently used in the treatment of hematopoietic diseases due to its pro-apoptotic properties. Previously we demonstrated that human U937 cells undergo significant cell death after Dex treatment, in correlation with the down-regulation of the anti-apoptotic isoform Bcl-XL, over-expressed in myeloid leukemia. On the other hand, in many clinical trials the differentiation inducer retinoic acid (RA) resulted not encouraging in most myeloid patients. In this sense, our hypothesis suggests that a combination of steroid hormones and RA could represent an alternative and promising therapy. The main goal of this project is to study the role of glucocorticoids in RA-induced human promyelocytic leukemia cell differentiation. Undifferentiated HL60 cells were treated with RA in the presence or absence of Dex over 72h. Our results showed that Dex markedly enhances a RA-induced cell differentiation response, observed as a potentiated expression of the cell surface marker CD11b by flow cytometry analysis (control: 1.54% RA: 28.6% RA+Dex: 38.1%). To gain functional insights into this differentiation process, the expression of hox genes, pscd4, meis2 and rarβ was monitored in RT-qPCR assays. Notably, upon 24h of combined treatment the up-regulation of hoxA3 expression was observed. Finally, the addition of Dex potentiates RA-induced expression of hoxA3 and pscd4 genes after 48h of treatment, and the expression of hoxA4 gene after 72h. Overall, our data reveals the existence of a synergistic effect of Dex and RA on HL60 cell differentiation. Further characterization of this molecular context could thus be instrumental in defining a general molecular mechanism that identifies attractive targets for therapeutic strategies in myeloid leukemia.

TRANSDUCCION DE SEÑALES/ SIGNALS TRANSDUCTION

144 (2062) **TRANSCRIPTIONAL AND POST-TRANSLATIONAL ERK 1/2 REGULATION OF MKP-3 SPLICE VARIANTS IN BREAST CANCER CELLS**

Silvana Iris Nudler[1], Ana Fernanda Castillo[1], Juan Manuel Cohen Sabban[1], María Mercedes Mori Sequeiros García[1], Cristina Paz[1]
INBIOMED (UBA-CONICET). Dpto. Bioquímica Humana, Facultad De Medicina, Universidad De Buenos Aires, Argentina

MKP-3 is a member of the family of mitogen-activated protein kinase phosphatase (MKPs) that specifically dephosphorylates ERK 1/2, acting as a key effector of the MAPK signal pathway. Variable expression of MKP-3 was detected in different cancer types. In human tissues and cell lines, two splice variants of MKP-3 are expressed: the full transcript or isoform L, and isoform S, where exon 2 is skipped. The function of MKP-3 and its isoforms in breast cancer is still not well known. The aim of this work was to analyze and to compare the role of P-ERK on mRNA and protein stability of both isoforms in the breast cancer cell line MDA-MB-231. Real time RT-PCR analysis showed that both isoforms, S and L, are induced by a mitogenic stimuli (fetal bovine serum, FBS) in a time and ERK-dependent manner. The maximal induction of L and S isoforms was detected after 3 h of stimulation (1.4 and 1.8-fold, for L and S respectively). In cells stimulated with FBS for 3 h, a MEK (MAPK kinase) inhibitor (PD 98059) reduced L and S mRNA levels in a time dependent manner, reaching a 10% of the initial value after 3 h of treatment. In actinomicyn D-treated cells, the decay of both isoforms was different. Indeed, mRNA of the L isoform showed higher stability as compared to the S isoform (half life: 60 vs. 38 min), while blockade of P-ERK signaling by PD 98059 reduced mRNA stability of both isoforms. Western blot analysis revealed the presence of L and S proteins in MDA-MB-231 cells, being L levels up-regulated by the MEK inhibitor. Together, our results show that P-ERK regulates the expression and stability of mRNAs of both isoforms of MKP-3 but only the stability of the L protein.

145 (143) **LEYDIG CELL STEROIDOGENESIS: CYCLOOXYGENASE-2 AND HEME OXYGENASE-1 CROSSTALK**

Trinidad Raices[1], M. Eugenia Matzkin[1], Marcos Besio Moreno[1], Mónica Frungieri[1], Omar P. Pignataro[1,2], Elba N. Pereyra[1].
[1]Instituto De Biología Y Medicina ExperimentaL (IBYME-CONICET). [2]Depto. de Q. Biológica-FCEN-UBA

Heme oxygenase (HO-1) is one of the factors involved in testicular steroidogenesis fine tuning and, as we have previously demonstrated, has an inhibitory effect on progesterone (P4) synthesis both in basal and stimulated (dibutyril cAMP) conditions. So as to continue with the characterization of this regulatory network, we aimed to study the involvement of cyclooxygenases (COXs) in steroidogenesis and its interaction with HO-1. Two different tumoral Leydig cell lines were used: MA-10 and R2C. They were treated with COX-1 (SC-560) and COX-2 (Meloxicam) selective inhibitors and hemin, an HO-1 inductor (5 - 20 μM). Protein levels were analysed by means of Western Blotting and RIAs were performed to determine P4 concentration. Subcellular localization of COX isoenzymes was assessed using confocal microscopy. Results: COX-1 and COX-2 protein levels were unaffected by the inhibitors SC-560 and Meloxicam. Besides, they are significantly higher in R2C than in MA-10. COX-1 inhibition did not affect P4 levels neither in MA-10 nor in R2C. On the contrary, COX-2 inhibition resulted in an increase in P4 levels in both cell lines. COX-1 and COX-2 are located in the cytoplasm and nuclear envelope. The analysis of the interaction between COX-2 and HO-1 denotes that in basal conditions in MA-10, 1 μM Meloxicam does not modulate the inhibitory effect of Hemin on P4 levels. Contrarily, in stimulated conditions in MA-10, 1 μM Meloxicam partially counteract 20 μM Hemin inhibitory effect. In R2C, when Hemin is added together with 1 μM Meloxicam, stimulation with this inhibitor is reverted almost to control values. Additionally, Meloxicam increases HO-1 protein levels beyond hemin stimulation in a concentration-dependent manner. In conclusion, the results suggest that COX-2 and HO-1 interact in the cross-regulation of testicular steroidogenesis and HO-1 protein levels.

146 (126) **INTRACELLULAR CHLORIDE ACTS AS A SECOND MESSENGER FOR CFTR MODULATING IL-1β EXPRESSION**

Mariángeles Clauzure[1], Ángel Gabriel Valdivieso[1], María Macarena Massip Copiz[1], Consuelo Mori[1], Andrea Vanesa Dugour[2], Juan Manuel Figueroa[2], Tomás Antonio Santa Coloma[1].
[1]Laboratorio de Biología Celular Y Molecular, Instituto de Investigaciones Biomédicas (Biomed). [2]Centro de Biología Respiratoria (CEBIR), Fundación Pablo Cassará

The CFTR gene is responsible for Cystic Fibrosis (CF). The CFTR protein is a cAMP-regulated chloride channel. Several cellular functions are altered in CF cells. However, it is not clear how the CFTR failure induces those alterations. By using differential display, we have found previously several CFTR-dependent genes, including c-Src, MUC1, MTND4 and CISD1. We also reported the existence of chloride-dependent genes, such as GLRX5 and RPS27. Here, using nigericin and tributyltin to clamp the pH and the intracellular chloride concentration [Cl-]i of IB3-1 epithelial cells, we show that IL-1β is also a Cl-dependent gene that can be modulated by changing the intracellular chloride concentration, in a biphasic way. The IL-1β secretion also showed a similar pattern of response to changes in [Cl-]i. The mechanism involves an IL-1β autocrine effect, since in the presence of the IL-1β receptor antagonist IL1RN and anti-IL-1β blocking antibody, Cl- effects disappeared. Similar effects were obtained with the JNK inhibitor, c-Src inhibitor and the IKK inhibitor, suggesting that JNK, c-Src and NF-kB are important mediators of the [Cl-]i signaling. In conclusion, the Cl- anion act as a second messenger for CFTR, modulating the expression and secretion of IL-1β, through an autocrine IL-1β loop that involves IKK, c-Src and JNK kinases. This work was supported by National Agency for the Promotion of Science and Technology (ANPCYT) [grant numbers PICT 2007-00628 and PICT 2012-1278] to TASC; National Scientific and Technical Research Council of Argentina (CONICET) [grant PIP 112200801025512009-2011 and PIP 11220110100685 2012-2014] to TASC; Pontifical Catholic University of Argentina (UCA) to TASC and a grant from Pablo Cassará Foundation (AVD and JMF); and research fellowships from CONICET (to MMMC, CM and MC).

147 (367) **MODULATION OF THE TRANSCRIPTIONAL ACTIVITY OF THE GLUCOCORTICOID RECEPTOR BY β2-ADRENERGIC LIGANDS. ROLE OF PKA AND ERK**

Gina Granja-Galeano[1], Natalia Cristina Fernandez[1], Carlos Daniel Zappia[1], Antonela Diaz-Nebreda[2], Carina Shayo[2], Carlos Davio[1], Carlos Fitzsimons[3], Federico Monczor[1].
[1]Instituto de Investigaciones Farmacológicas (Ininfa), FFYB, UBA, Conicet, Argentina. [2]Instituto de Biología y Medicina Experimental (IBYME), CONICET, Argentina. [3]Swammerdam Institute for Life Sciences, University of Amsterdam. The Netherlands

Glucocorticoid effects on memory consolidation after stressful events are mediated by binding and activation of the Glucocorticoid Receptor (GR) and its interaction with the noradrenergic arousal system. β2-adrenergic receptors (β2AR) are membrane receptors that signal independently through both the Gs-AC-cAMP-PKA and ERK pathways, while the GR is classically considered a ligand-activated transcription factor that modulates the transcriptional activation of responsive genes. Here we set to study the modulation of GR transcriptional activity by β2AR ligands that differentially activate PKA or ERK signaling. We show that, in HEK293T cells transfected with β2AR, isoproterenol and clenbuterol behave as full agonists, stimulating both cAMP and ERK pathways, whereas propranolol and carvedilol behave as biased ligands diminishing cAMP concentration and increasing ERK activity. Cotransfection of a luciferase reporter plasmid under the control of a promoter regulated by the GR-responsive promoter (TAT3-Luc) with plasmids coding for GR and β2AR, resulted in inhibition of dexamethasone-induced GR activity by 50% ($p<0.05$) by propranalol and carvedilol, while isoproterenol and clenbuterol slightly increased it by 20% ($p<0.05$). The inhibitory effect induced by propranolol and carvedilol was precluded by the ERK kinase inhibitor PD98059, while isoproterenol and clenbuterol's potentiating effect turned inhibitory in the presence of the PKA inhibitor KT5720 or a dominant negative PKA mutant. Importantly, dexamethasone-induced GR nuclear translocation was hampered by all four β2AR ligands to a similar extent. Our results suggest that ERK activation blocks GR nuclear translocation diminishing GR transcriptional activity. However, when PKA is simultaneously activated, GR transcriptional activity is restored, suggesting a cross-talk between cAMP and ERK pathways. This cross-talk provides new insights into the crossregulation of Glucocorticoid and adrenergic signals.

148 (652) **CONTROL OF PARATHYROID HORMONE RELATED PEPTIDE EXPRESSION BY CALCIOTROPIC HORMONES IN COLON CANCER CELLS**

María Julia Martín[1], Natalia Calvo[1], Claudia Gentili[1].
[1]INBIOSUR

Parathyroid hormone (PTH) and Calcitriol (1α,25(OH)2 Vitamin D3) are essential regulators of calcium homeostasis. Depending on cellular context, both hormones may also be related with apoptosis and/or cell proliferation and even with cell cycle regulation. Previously, we demonstrated that PTH (10-8 M) exerts pro-apoptotic and anti-proliferative effects in human colon cell line Caco-2 while others authors observed that the treatment with Calcitriol at doses above 10-10 M induces anti-proliferative effects in these cells. Moreover, previous studies showed that in prostate cancer cells, Calcitriol induces the anti-proliferative response through down-regulation of PTHrP expression. Recently we found that exogenous PTHrP promotes the survival, proliferation and migration of Caco-2 cells. Based on these findings, this study is aimed to evaluate if the response of Caco-2 cells to PTH or Calcitriol occurs through

the modulation of PTHrP expression. We found by qPCR assay that PTH treatment for 5 h increases PTHrP mRNA levels. However, at 24 h of PTH exposure, these levels were similar to those observed in control cells. Exposition to PTH for 4 days followed by proliferation assay revealed a significant difference in the number of live cells between Caco-2 cells overexpressing PTHrP respect to control cells suggesting that PTH exerts its anti-proliferative role through the control of PTHrP expression. Then we found by qPCR assay that Calcitriol at the times studied (5-48 h) down-regulates the expression of PTHrP. However, the number of live cells was similar when both, Caco-2 cells overexpressing PTHrP and control cells were exposed to Calcitriol, suggesting that PTHrP does not participate in the effect of this hormone on Caco-2 cells. These results expand our knowledge about the molecular mechanisms that mediate the action of calciotropic hormones on tumor intestinal cells.

149 (465) **CYCLIC AMP EXTRUSION ABROGATION INHIBITS PANC-1 CELL PROLIFERATION THROUGH ACTIVATION OF EPAC/RAP1 PATHWAY**

Agustín Yaneff[1], Nicolás Di Siervi[1], Alejandra Attorresi[2], Alejandro Carozzo[1], Carina Shayo[3], Natalia Gómez[1], Carlos Davio[1].

[1]Instituto de Investigaciones Farmacológicas, Facultad de Farmacia y Bioquímica, UBA, ININFA-CONICET-UBA. [2]INBIOBA MPSP - Instituto de Investigaciones en Biomedicina de Buenos Aires, CONICET. Instituto Partner de La Sociedad Max Planck [3]Instituto de Biología y Medicina Experimental-CONICET

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal cancer with a 5-year survival rate of 4%. This is caused by its high resistance to conventional therapies, its delayed diagnosis and high metastatic potential. Previously, we established a correlation between MRP4 expression and the level of differentiation, malignancy and cAMP extrusion capacity of PDAC cells, thus proposing it as a potential target for this disease. Our working hypothesis is that the antiproliferative effect of MRP4 inhibition is a consequence of the modulation of cAMP extrusion. This work concentrates in unraveling which cAMP pathways are leading this process. The temporal progression of intracellular cAMP (i-cAMP) induced response was performed using FRET assays in PANC-1 cells transfected with a plasmid encoding a cAMP molecular sensor (Epac-SH187). MRP4 inhibition using MK571 evoked a significant increase in i-cAMP within the first minutes of measurement after stimulation with 10 μM Isoproterenol ($p<0.01$). Furthermore, neither incubation with PKA inhibitor KT5720 nor transfection with PKA inhibitor peptide PKI were able to reverse the anti-proliferative effect observed with MRP4 inhibition. Instead, transfection of PANC-1 cells with a plasmid encoding a dominant negative mutant of EPAC (N-EPAC) or with a Rap1-GAP construction (which increases Rap1 inactive form) both annulled the anti-proliferative effect of MK571. Our work demonstrates that MRP4 inhibition produces an increase on cAMP response which in turns diminish cell growth rate through activation of the EPAC/Rap1 pathway.

150 (289) **NF-KB EFFECTS ON ESTRADIOL INDUCED PLACENTAL LEPTIN EXPRESSION**

Malena Schanton[1], Antonio Pérez-Pérez[2], Yésica Gambino[1], Bernardo Maskin[3], Victor Sánchez Margalet[2], Cecilia Varone[1].

[1]Departamento de Química Biológica, FCEN, UBA, IQUIBICEN, CONICET, Buenos Aires, Argentina. [2]Departamento de Bioquímica Médica y Biología Molecular, Universidad de Sevilla, Sevilla, España. [3]Hospital Nacional Profesor Alejandro Posadas, Buenos Aires, Argentina

Leptin is a key hormone in placental physiology. Previous results from our lab demonstrated that estradiol (E2) regulates leptin expression involving genomic and non-genomic effects. On the other way, considering that we have found, by in silico analysis, potential binding sites for NF-κB between -2850 and -2838bp, the leptin promoter region necessary to evidence E2 response, we decided to analyze the involvement of this transcription factor on E2 effects on leptin placental expression. BeWo cells cultured under standard conditions, as well as human placental explants were used. Western blot, qRT-PCR and transfection assays with reporter constructs and expression vectors were carried out. All procedures were approved by ethical review committee at the A Posadas National Hospital. Experiments carried out with E2 during 48 hours in presence of sulfasalazine, an Iκκ inhibitor, showed that the treatment with the drug reduce the E2 action over endogenous leptin expression, suggesting the involvement of NF-κB transcription factor in this regulatory effect. On the other hand, BeWo cells were transiently transfected with an expression vector expressing the NF-κB subunit p65 (Rel A). Surprisingly the expression of this protein also significantly decreased E2 effects on the transcriptional activity of leptin promoter reporter pL1951 vector. BeWo-Sh2 cells, expressing and shRNA against ERα protein, showed no effect to E2 treatment neither Rel A overexpression, suggesting that this factor might require ERα to exert its effects on E2 induced leptin expression. These results provide new evidence that leptin induction by estradiol is modulated by NF-κB transcription factor.

151 (522) **ROLE OF RSK IN THE CELL CYCLE PROGRESSION AND MIGRATION OF INTESTINAL TUMORAL CELLS INDUCED BY PARATHYROID HORMONE-RELATED PEPTIDE**

Natalia G. Calvo[1], Pedro Carriere[1], Claudia Gentili[1].

[1]INBIOSUR (UNS-CONICET)

Parathyroid Hormone-related Peptide (PTHrP) is implicated in several human cancers such as colon carcinoma. This disease is a complex multistep process that involving enhanced cell cycle progression and migration. In previous work we obtained evidence that in the human colorectal adenocarcinoma Caco-2 cells, exogenous PTHrP increases cell cycle progression via Erk1/2, p38 MAPK and PI3K. Recently we observed that the treatment with the peptide also increases cell migration and the phosphorylation of p90 ribosomal S6 kinase (RSK) (which is an enzyme that regulates cell motility and cell cycle) via Erk1/2 in these intestinal cells. However, if RSK is implicated in cell cycle progression and cell migration of Caco-2 cells induced by PTHrP is not known. The objective of this study was to further delineate the molecular mechanisms involved in Caco-2 cells response to PTHrP. To that end, Caco-2 cells were pre-incubated with an inhibitor of RSK, SL0101, and treated with PTHrP followed by wound-healing, transwell or western blot assays. PTHrP effects on cell migration and on the expression of focal adhesion kinase (FAK), a regulator of cell motility, were prevented by RSK inhibition. In addition, the inhibitor also reversed the up-regulation of cyclin D1 and CDK6 (two positive cell cycle regulators) and the down-regulation of p15 and p53 (two negative cell cycle regulators) induced by PTHrP. Finally, performing subcellular fractionation followed by Western blot analysis we found that the peptide induces the nuclear translocation of RSK, where many of its substrates are located. Taken together, these data suggest that RSK modulates the expression of cell cycle regulators and cell migration in Caco-2 cells treated with PTHrP. In conclusion, the results obtained in this work expand our knowledge on the signaling pathways that are involved on Caco-2 cells response to PTHrP. Strategies aimed at blocking PTHrP action in colon cancer may thus provide therapeutic benefits.

152 (348) **ROLE OF RETINOID X RECEPTORS ON SURVIVAL AND MODULATION OF INFLAMMATORY RESPONSE, IN RETINAL PIGMENT EPITHELIUM CELLS UPON DIFFERENT STRESSORS, SUCH AS H2O2 AND VIRAL INFECTION**

Victoria Belen Ayala-Peña[1, 2], Nieves Armiento[2], Luis Alberto Scolaro[2], Olga Lorena German[1].

[1]Instituto de Investigaciones Bioquímicas de Bahía Blanca-Depto de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur-Conicet. [2]Laboratorio de Virología, Depto. Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires -CONICET

Age-related macular degeneration (AMD) is the main pathology leading to blindness in adults and has currently no cure or effective treatment. Retinal pigment epithelial (RPE) cells have immunomodulatory properties and their degeneration contributes in AMD development. Oxidative stress is one of the events involved in the pathogenesis of this disease, and herpes simplex virus type 1 (HSV-1) infection is currently proposed as a probable risk factor. We previously demonstrated that RXR activation with HX630 protects RPE (D407) cells from H_2O_2-induced apoptosis. HX630 prevents p65NFκB nuclear translocation (which is also crucial for triggering inflammation) and increases PPARγ mRNA levels. LG100754 (a RXR homodimers antagonist and a RXR agonist that could activate RXR/PPARγ) also had a protective effect. In this work using a PPARγ specific agonist and their antagonist, we reproduced the protective effect of RXR activation against oxidative damage, determined by MTT assay and fluorescence microscopy. We observed by qRT-PCR that RXR activation decreased IL-6 (a pro-inflammatory cytokine) and increased IL-10 and TGFβ (anti-inflammatory cytokines) mRNA levels significantly. It is known that HSV-1 blocks the synthesis of RXRα in macrophage promoting the host antiviral response. In HSV-1 infected D407 cells, PPARγmRNA level but not RXRα was significantly decreased compared to mock infected cultures, even under LG100754 treatment. Using fluorescence microscopy, p65NFkB protein level was increased in the whole cell; however, the nuclear/total p65NFkB fluorescence proportion in infected cells was decreased compared to the mock infected cells, even under LG100754 treatment. Furthermore, the infection blocked the synthesis of TGFβ mRNA and the RXR agonist could not improve it. As a whole, our results suggest that RXR/PPARγ heterodimers would be a central signaling in survival and inflammatory response upon H_2O_2 in RPE cells, which could be regulated by HSV-1 infection.

153 (753) **IN C2C12 CELLS HETEROCHROMATIN PROTEIN 1 GAMMA INTERACTS WITH ACTIN TO REGULATE GENE EXPRESSION AND, IN THE CYTOPLASM, IS ASSOCIATED TO MICROFILAMENTS**

Nancy Lorena Charó[1], Natalia Maricel Galigniana[1], Graciela Piwien Pilipuk[1].
[1]Laboratorio De Arquitectura Nuclear, Instituto De Medicina Y Biología Experimental (IBYME), CONICET, Buenos Aires, Argentina

HP1 proteins belong to the chromodomain superfamily that in mammals correspond to HP1α,-β and -γ. HP1s, initially implicated in gene silencing, also participate in DNA repair, DNA replication, telomere stability and active transcription. We have previously shown that HP1γ, a protein so far exclusively nuclear, is also present in the cytoplasm of C2C12 myoblasts and myotubes. Importantly, myoblasts were unable to differentiate or gave rise to thin myotubes when the expression of HP1γ was interfered. Due to the filamentous pattern of staining of HP1γ observed in myoblasts, myotubes and myofibrils isolated from mice, we now explored its possible association with proteins of the cytoskeleton. In myoblasts, actin co-immunoprecipitates with HP1γ in both nuclear and cytosolic fractions. Immunoelectron microscopic analysis performed in samples of murine skeletal muscle shows high-density immunogold particles that correspond to HP1γ localized to the Z-disk and the I- and A-bands of the sarcomere. These results raise the possibility that cytoplasmic HP1γ may play a role in sarcomere organization, which remains to be further explored. As for the role of the HP1γ-actin complex in the nucleus, a great body of evidence shows the importance of nuclear actin in the control of gene expression. Using chromatin immunoprecipitation (ChIP) assays, we show that both proteins are present in the promoter and transcribed region of the house keeping gene GAPDH. Further, re-ChIP analysis shows that both proteins belong in a complex associated to those same regions of GAPDH, suggesting that HP1γ may function as a scaffold protein for actin to bind to chromatin to regulate transcription. In summary, HP1γ forms complexes with actin in both the cytoplasm and the nucleus, which may regulate sarcomere organization in the cytoplasm and participate in the control of gene expression in the nuclear compartment.

154 (957) **THE SPLICING VARIANTS OF MAP KINASE PHOSPHATASE-3 (MKP-3) EXHIBIT DIFFERENT PROPERTIES: IMPACT ON ERK-DEPENDENT EVENTS**

Juan Manuel Cohen Sabban[1], M Mercedes Mori Sequeiros Garcia[1], Alejandra B Gorostizaga[1], Silvana Nudler[1], Paula Maloberti[1], Cristina Paz[1].
[1]INBIOMED (UBA-CONICET). Dpto. Bioquímica Humana. Facultad de Medicina. Universidad de Buenos Aires. Argentina

MAP kinase phosphatase-3 (MKP-3) is an enzyme specific for ERK1/2 and induced exclusively by proliferative stimuli. Two MKP-3 transcripts are expressed in some human cells, a short form (S) and a long form (L), which are products of alternative splicing. The ratio L/S is variable among different tissues and also between normal and tumoral tissues. As MKP-3 S lacks sites that could be involved in the regulation of this enzyme, the aim of this work was to compare the properties of both isoforms. MKP-3 S and L were cloned for the expression of Flag-MKP-3 S or L under a constitutive promoter; then, HEK293 cells were transiently transfected with these vectors. The subcellular localization of each recombinant protein was analyzed by fluorescence microscopy, while P-ERK and flag-MKP-3 S and L levels were evaluated by Western blot using an anti P-ERK or anti-Flag antibody. The results show that MKP-3 S is accumulated equally in the nucleus and cytosol, while MKP-3 L is mainly localized in the cytosol, reflecting the lack of a nuclear export sequence in the S isoform. The levels of P-ERK reached after 15 min of stimulation with fetal bovine serum (FBS) were lower in cells transfected with either isoform, which indicates that these expression products are active. In addition, the isoforms presented differences in post-translational regulation, as similar levels of flag-MKP-3 L were detected after different times of FBS stimulation, whereas isoform S was accumulated over the same period (4.5-fold at 90 minutes, $P<0.01$, $n=3$). Our results show that L and S variants exhibit different behavior. Therefore, alterations in the L/S ratio can be expected to have a differential impact on cell biology, mainly ERK-dependent processes such as cell proliferation.

155 (833) **PROTECTIVE EFFECT OF 17B-ESTRADIOL AND TESTOSTERONE AGAINST APOPTOSIS INVOLVING FOXOS AND P53-RELATED GENES IN SKELETAL MUSCLE CELLS**

Lucía Pronsato[1], Anabela La Colla[1], Dario Lincor[1], Andrea Vasconsuelo[1], Lorena Milanesi[1].
[1]INBIOSUR-CONICET. Universidad Nacional Del Sur. Bahía Blanca

In aged skeletal muscle, a prominent apoptosis associated to a deficit of sex hormones is observed, contributing to the loss of muscle mass and strength, pathology known as sarcopenia. Previously we have demonstrated the protective effect, at morphological, physiological, biochemical and molecular level, of both 17β-estradiol (E2) and Testosterone (T) against H2O2-induced apoptosis in C2C12 skeletal muscle cell line. It has been established that the exposure of these cells to H2O2 represents a comparable phenotype to aged skeletal muscle, constituting a useful tool for the study of sarcopenia. Since genomic actions underlying the regulation of nuclear gene transcription are a classical mechanism of action of these hormones, we studied the transcriptional activity modulated by E2 and T in order to understand the molecular mechanisms involved in the antiapoptotic action of these steroids. We reported that the hormones protect skeletal myoblasts against apoptosis induced by H_2O_2 by modulating p53 and FoxO transcription factors and then, their target genes Bcl-2, Bim, Puma, PERP and MDM2, without affecting Noxa gene. Furthermore, ERK and JNK kinases have been demonstrated to be linked to FoxOs phosphorylation and thus its subcellular distribution and activation. The results presented in this work support the notion that E2 and T modulate the oxidative stress-induced apoptosis in skeletal muscle involving the transcription factors FoxO and p53. Altogether, these data expose some of the puzzle pieces of the intricate network that hormonal regulation of skeletal muscle apoptosis represents.

156 (559) **THE CFTR CHLORIDE CHANNEL REGULATES EGFR LIGANDS EXPRESSION**

María Macarena Massip Copiz[1], Mariángeles Clauzure[1], Consuelo Mori[1], Ángel Gabriel Valdivieso[1], Tomás Antonio Santa Coloma[1].

[1]LABORATORY OF CELLULAR AND MOLECULAR BIOLOGY, INSTITUTE FOR BIOMEDICAL RESEARCH (BIOMED), UCA-CONICET

Cystic fibrosis (CF) is a rare autosomal recessive disease, caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. CFTR is a cAMP-activated chloride channel, member of the superfamily of ABC (ATP Binding Cassette) transporter proteins. CFTR appears to function not only as a regulated chloride channel; it also acts as a signalling molecule regulating different genes. Previously, we found that several genes had altered expression due to the CFTR failure, such as SRC (a tyrosine-kinase which in turn regulated MUC1), MTND4 (a mitochondrial gene encoding a subunit of the mitochondrial Complex I), and CISD1 (a mitochondrial protein encoded in the nucleus with a yet ill-defined function). Hence, the aim of the present work was to determine if a failure in the CFTR activity (or expression) determines a differential regulation in the EGF receptor and its ligands. We use a cellular model consisting of Caco-2 cells (human colon carcinoma epithelial cells) expressing wt-CFTR that were previously selected and cloned after transfections with short hairpin RNA interference (shRNA) directed against different regions of CFTR (CaCo-2/pRS26) or with its control plasmid (CaCo-2/pRSctrl). The results obtained suggested that CFTR modulates significantly the expression of TGF-alpha, epiregulin and amphiregulin but not the EGFR expression. As we observed an important regulation of epiregulin ligand by CFTR, we continue to study the possible mechanisms involved in its modulation. In conclusion, CFTR channel activity failure or CFTR inhibition regulates the expression of different EGFR ligands possible involved in phenotype changes present in CF-like cells. Acknowledgements: grants from ANPCYT (PICT 2012-1278), CONICET (PIP 2012-0685), and UCA; and research fellowships from CONICET (MMMC, MC and CM).

157 (960) **CYCLOOXYGENASE-2 GENE EXPRESSION REGULATION. TRANSCRIPTIONAL AND MRNA STABILITY APPROACHES**

M. Victoria Medina[1], Daiana A. Sapochnik[1], Agata M. D'Agostino[2], Julian Naipauer[2], Enrique Mesri[2], Omar A. Coso[1].

[1]Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE-FCEN- UBA-CONICET). [2]University of Miami School of Medicine Miami, USA

Kaposi sarcoma (KS) is the most frequent AIDS-related cancer and arises when endothelial cells are transformed by the KSHV virus. Encoded within the KSHV genome, vGPCR is a consitutively active receptor that plays a key role in the oncogenesis induced by the virus. In order to understand mechanisms that impinge upon gene expression control in cells subject to proliferative stimuli as the ones triggered by the vGPCR oncogene, we have been studying Cyclooxygenase 2 (COX-2) gene expression as it is induced by tumor promoter factors and presents an important role in cancer progression. We intend to characterize the putative link between signal transduction pathways triggered by vGPCR and transcription factors and AUBPs that bind the COX-2 promoter and 3'-untranslated regions of its mRNAs, respectively. To achieve our goal we extracted mRNA from SVEC control cells and SVEC cells that express the vGPCR oncogene (SVEC vGPCR) and measured mRNA by Real Time PCR (qPCR). Using tranfections with reporter plasmids we measured promoter regulation and mRNA stability. We observed that SVEC vGPCR cells overexpress COX-2 mRNA respect to control cells. We transfected SVEC cells with plasmid containing serial deletions of the COX-2 promoter (1.2; 0.8; 0.65 and 0.4 kb long) followed by a luciferase open reading frame. We observed vGPCR dependent increments in luciferase expression which peaked with the 0.4 kb construct. On the other hand, we co-transfected SVEC cells with a plasmid which expresses luciferase fused to the 3' UTR of COX-2 mRNA together with different AUBPs expression vectors and observed a decrease in luciferase expression when coexpressing TTP. Our experiments show that vGPCR-dependent COX-2 overexpression is due to a dual effect upon its promoter region (mainly in the 0.4 kb proximal end) and upon elements in the 3'UTR region, where its mRNA may bind TTP and therefore negatively regulate the stability of COX-2 mRNA.

158 (646) **PIVOTAL ROLE OF PKC, SRC AND AKT IN COLON CANCER CELLS RESPONSE TO PTHRP: CONTROL OF MITOGENIC SIGNALING**

María Julia Martín[1], María Belén Novoa Díaz[2], Graciela Gigola[2], Martín Carriquiborde[3], Florencia Gentil[3], Claudia Gentili[1].

[1]INBIOSUR. [2]Depto. Biología, Bioquímica y Farmacia - Universidad Nacional del Sur [3]Facultad de Cs. Veterinarias - Universidad de La Plata

Parathyroid hormone related peptide (PTHrP) is present in the fetus, in most adult tissues and in various tumors. Its expression correlates with the severity of colon carcinoma. Previously we demonstrated that exogenous PTHrP (10-8 M) stimulates Caco-2 cells proliferation through MAPK and PI3K/Akt pathways. The aim of this study was to investigate the molecular mechanisms that modulate the activation of these signaling pathways induced by PTHrP in two tumor intestinal cell lines, Caco-2 and HCT116. We also evaluated if the hormone exerts proliferative effects in vivo. It is known that Src and several isoforms of PKC have a key role in the development and progression of multiple cancers. Western blot analysis revealed that after PTHrP treatment, PKC levels decreased and increased in Caco-2 cells and HCT116 cells, respectively. Also, Src is phosphorylated/activated by the hormone in Caco-2 cells as well as in HCT116 cells. Furthermore, using specifics inhibitors we observed that PKCδ, Src and Akt signaling pathways modulate the phosphorylation of ERK 1/2 induced by PTHrP in both tumor cell lines. Moreover, the expression of c-Myc oncoprotein induced by PTHrP was reverted when ERK 1/2 signaling pathway was inhibited. Finally, when Akt and PKC signaling pathways were blocked, we observed a reversion on Caco-2 cell proliferation induced by the hormone. On the other hand, xenograft tumor model studies revealed that when a low number of HCT116 cells (1x106) are inoculated into nude mice, the administration of PTHrP (40 ug/kg) promotes the formation and tumor growth. Also, immunohistochemical and western blot analysis of these tumors showed that PTHrP increased the expression of cyclin D1, a positive regulator of cell cycle progression, and c-Myc. Together, the results from in vitro and in vivo studies provide information about the molecular mechanisms that are modulated by exogenous PTHrP in tumor intestinal cells.

159 (2065) **MKP-1 MODULATES ENDOPLASMIC RETICULUM STRESS EVENTS TRIGGERED BY CISPLATIN IN RENAL TUBULE CELLS**

Luciana Andrea Cabrera Escobar[1], Andrea Beatriz Acquier[1,2], CF Mendez[1,2], Cristina Paz[1], Alejandra Beatriz Gorostizaga[1].

[1]INBIOMED, UBA-CONICET, Departamento de Bioquimica Humana, Facultad de Medicina, Universidad de Buenos Aires. Argentina. [2]Cátedra de Farmacologia, Facultad De Odontologia, Universidad de Buenos Aires. Argentina

A wide variety of agents induce endoplasmic reticulum stress (RE) in different cell types. RE produces a complex signal transduction pathway known as Unfolding Protein Response that includes activation of the MAP kinases (MAPKs) ERK1/2, JNK1/2 and p38 which have different effects on survival or apoptosis. Stimuli that promote the activation of MAPKs induce the expression of different members of the family of MAPK phosphatases (MKPs), which promote the inactivation of these kinases. MKP-1 is a well characterized member of this family induced by several stimuli and able to dephosphorylate members of the three MAPKs subgroups. Cisplatin (CPT), a known chemotherapeutic agent, is widely used as RE inductor. The aim of this work was to determine

if MAPK phosphatase 1 (MKP-1) modulates aspects related to RE in a human renal proximal tubule-derived cell line (HK-2) exposed to CPT. First, we analyzed MAPKs activation by CPT. Western blot analysis using specific antibodies against the phosphorylated forms of ERK1/2 showed that 50 mM CPT promotes ERK1/2 activation in a time-dependent manner. The increase was evident after 4 h of stimulation (6-fold) extending to 12 h. We also analyzed MKP-1 mRNA levels and we observed that CPT increases mRNA levels in a time- and concentration-dependent manner. In addition, we analyzed the expression of GRP78, a RE marker involved in cell survival by semiquantitative RT-PCR. We found that CPT increased GRP78 levels reaching a maximum at 6 h (3-fold). PD98059 (50 uM), an inhibitor of ERK1/2 activation, prevented the effect of CPT on GRP78 levels, while SB203580 and SP-600125 (JNK and p38 inhibitors, respectively) had no effect, thereby suggesting that GRP78 expression is modulated by ERK1/2. Moreover, in cells transfected with a construction for transient expression of flag-MKP-1 recombinant protein, MTT assay showed that transfection with MKP-1 modulates cell viability. Collectively, our data suggest that MKP-1 is induced by CPT and may contribute to turn off MAPKs-dependent events triggered by CPT.

160 (489) **ZEB1 IS REGULATED BY PKC-ALPHA IN BREAST CANCER CELL LINES**

Maria Candelaria Llorens[1], Cynthia Lopez Haber[2], Laura Barrio-Real[2], Maria Victoria Vaglienti[1], Marcelo Kazanietz[2], Ana Maria Cabanillas[1].

[1]Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional Córdoba. CIBICI-CONICET, Argentina. [2]Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, USA

Cancer progression can be activated by cytokine signals which regulate transcription factors such as ZEB1 or Snail. We intended to uncover the role of PKC signaling in ZEB1 regulation. PKC isoforms and EMT markers were determined by immunoblotting in 9 breast cancer cell lines. PKCα and ZEB1 had a significant positive correlation ($p \leq 0.05$). The interference of PKCa expression by 2 siRNAs significantly reduced ZEB1 expression at 72hs in MDA-MB-231 cells (siPKCα1,2: 0.64±0.06 and 0.67±0.06 vs siNonTarget siNT:1)($p \leq 0,05$) as well as in MDA-MB-453 and BT549 (72/96hs) and in MDA-MB-231 (96hs). EMT markers changed their expression at no time. However, ZEB1 mRNA (qPCR) did not differ from control in siPKCα/MDA-MB231 cells which suggests that PKCα could regulate ZEB1 expression by diminishing its protein stability. In addition, motility and invasive abilities (Matrigel®invasion assay) of MDA-MB-231 cells silenced by 6 siPKCα or 4 siZEB1 lowered significantly against siNT cells (siPKCα1,2,3,4,5,6: 40.3±3.5; 52.6±4.2; 53.4±2.6; 52.0±2.8; 42.7±2.4; 59.6±3.1 vs siNT: 83.1±2.9) ($p \leq 0.0001$) (siZEB1 1,2,3,4: 25.2±2.3; 31.0±2.49; 80.3±3.0; 39.7±2.7 vs siNT: 150.6±3.9) ($p \leq 0.0001$). We also tested the capacity of actin cytoskeleton to respond to a 10 minute stimulus of 10% Fetal Bovine Serum by Phalloidin-rhodamine stainining on siPKCa or siZEB1MDA-MB-231 cells. Control and siZEB1 cells showed ruffles and lamellipodia structures as a response to 10%FBS while siPKCα MDA-MB231 cells were unable to have a normal response. Results were expressed as mean ± SEM. The results suggest that PKCα could regulate ZEB1 expression in breast cancer cell lines by modifying its protein stability at short term. The poor ruffle formation only in siPKCa cells compared to a normal response in siZEB1 cells suggest that PKCα is able to regulate cell invasion by two mechanisms: ZEB1 dependent and ZEB1 independent. Conclusion: PKCα is novel regulator of ZEB1 and therefore the cell invasion in breast cancer.

161 (1083) **ALTERNATIVE RNA EXPRESSION OF B1-INTEGRIN IN THE MAMMARY GLAND**

Martín Emilio García Solá[1], Julián Naipauer[2], Edith Claudia Kordon[1], Omar Adrián Coso[1].

[1]Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE-FCEN-UBA-CONICET) [2]University Of Miami.

Integrins are cell surface receptors that play a critical role in normal and tumor tissue development. We have reported that the messenger RNA (mRNA) encoding the B1-integrin subunit (Itgb1) has an alternative cleavage and polyadenylation site that renders a mRNA isoform (Itgb1-S), 578 nucleotide shorter than the one already reported (Itgb1-L). In its 3'UTR, Itgb1-L contains regulatory sequences, which are absent in the Itgb1-S 3'UTR. We have previously shown that during mammary gland development, expression levels of each Itgb1 mRNA isoform are determined, at least in part, by the alternative use of these signals. The cell line HC11 allows to study, in culture, the behavior of mammary epithelium at different developmental stages. When confluent, apoptosis is induced in these cells by starvation. However, this effect is reversed by treatment with lactogenic hormones, which induce mammary differentiation, or by EGF that triggers cell survival pathways. We hypothesized that regulation of Itgb1 mRNA might be relevant for these effects. Therefore, first, we analyzed the consequences of treating starved HC11 cells with EGF on the different Itgb1 mRNA isoforms. To this goal, we performed a time course of EGF induction and tested by RTqPCR the expression levels of Itgb1 mRNA variants. Our results show an increase in the amount of total Itgb1 mRNA during the first 5 hours post-stimulation. Interestingly, during the time window between 2 and 4 hours, we observed a decrease of Itgb1-L mRNA levels together with an increase in the amount of Itgb1-S mRNA. This data suggest that in that time frame there is a change in the selection of the cleavage and polyadenylation site that privileges use of the alternative (weaker) proximal site generating a peak of expression for the shorter isoform. Currently, we are analyzing the signaling pathways triggered by EGF, which may lead to a change in the selection of the Itgb1 polyadenylation site in Itgb1 mRNA.

162 (620) **ANALYSIS OF PIN1 EXPRESSION AND FUNCTION DURING ZEBRAFISH DEVELOPMENT REVEALS A DYNAMIC REGULATION**

Solange Ibarra[1], Ezequiel Margarit[2], Marina Mione[3], Javier Girardini[1].

Instituto de Biología Molecular y Celular de Rosario.[2]Centro de Estudios Fotosintéticos y Bioquímicos. [3]Centre For Integrative Biology - CIBIO, University Of Trento.

The prolyl isomerase Pin1 plays a key role in the modulation of proline-directed phosphorylation signaling by inducing local conformational changes in phosphorylated protein substrates. Besides being involved in physiological processes, a large body of evidences has shown that Pin1 may affect pathological conditions. For example, clinical studies have shown that Pin1 is frequently overexpressed in different cancers and in some cases its overexpression correlates with clinical outcome. Also, its deregulation has been related to brain disorders, including Alzheimer's, Parkinson's and Huntington's disease. Despite several evidences on the effect of Pin1 on specific substrates or signaling pathways, there are still several unanswered questions regarding its biological role and, particularly, little is known about its regulation. Notably, the current evidences showing that Pin1 expression and activity may be regulated by different mechanisms were generated only on cultured cells, and there is almost no evidence of its relevance in vivo. Considering the dependence of Pin1 on the particular combination of protein substrates present in a specific cell type and on the activation of signaling pathways able to phosphorylate its binding sites, studies in animal models may provide novel insights, useful to understand proline-directed phosphorylation signaling and the consequences of Pin1 deregulation. Using Danio rerio (zebrafish) as a vertebrate model organism, we showed that pin1 expression is regulated during embryogenesis to achieve specific mRNA and protein distribution patterns. Moreover, we found that Pin1 overexpression affected specific regions of the developing embryos and we extended the study of Pin1 expression to the adult zebrafish brain. Our results suggest that specific mechanisms are operated in different cell types to regulate Pin1 function.

163 (701) **IDENTIFICATION AND CHARACTERIZATION OF THE SIGNALING PATHWAYS TRIGGERED BY THE MAS RECEPTOR**

Valeria Burghi[1], Diego Tomás Quiroga[1], Emiliana Echeverría[2], Natalia Cristina Fernández[2], Fernando Pablo Dominici[1].
[1]IQUIFIB, Cátedra de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires. [2]ININFA, Cátedra de Química Medicinal, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires.

The Mas receptor (MasR) is a class A Orphan G-protein-coupled receptor (GPCR). Although angiotensin-(1-7) [Ang-(1-7)] has been reported as its putative ligand, the intracellular signaling pathways activated by the MasR remain partially characterized preventing definitive knowledge of ligand/receptor pharmacology. In this study we examined MasR-dependent activation of canonical (G protein-mediated) and non-canonical (MAP kinase mediated) GPCRs' signaling pathways. With that aim, we used endogenously expressing human lung carcinoma A549 cells to evaluate Gq-coupled activity, as it was previously described by other authors, and we monitored changes in intracellular Ca2+ levels after Ang(1–7)-stimulation. Incubation with 100nM Ang(1-7) failed to increase Ca2+ levels either in endogenously expressing or MasR transfected A549 cells. On the other hand, transfection of HEK293T cells with MasR resulted in a significant decrease in basal cAMP levels ($p<0.05$) that depended on the amount of MasR protein expressed. Pretreatment of MasR expressing cells with pertussis toxin restored basal cAMP levels whereas no effect was observed in mock-transfected cells. Also, cAMP production after forskolin stimulation was lower in cells expressing MasR than in control cells. These results indicate a high level of constitutive receptor activity towards cAMP modulation. Activation of MasR with 100nM Ang(1–7) caused a rapid increase in ERK1/2 phosphorylation only in HEK293T cells expressing MasR. This modulation was dampened by cotransfection with G□-transducin, a widely used scavenger of Gβγ, indicating that Ang(1-7) stimulated activation of ERK1/2 involves G□□ action. Based on our data, we propose that MasR signaling involves both cAMP and ERK1/2 pathways. As the MasR plays a role in regulating cardiovascular and renal functions, comprehensive pharmacological characterization of MasR signaling is essential for developing clinical therapeutics targeting MasR function.

164 (837) **GROWTH HORMONE (GH) AFFECTS CELLULAR SIZE AND SIGNALING PATHWAYS RELATED TO TUMOROGENESIS PROCESSES IN THE LIVER OF MICE EXPOSED TO DIFFERENT ADMINISTRATION PATTERNS OF GH AT THERAPEUTIC DOSES**

Verónica Gabriela Piazza[1], Nadia Cicconi[1], Sofía Valquinta[1], Lorena Gonzalez[1], Daniel Turyn[1], Johanna Gabriela Miquet[1], Ana Isabel Sotelo[1].
[1]Universidad de Buenos Aires, Consejo Nacional de Investigaciones Científicas y Técnicas, Instituto de Química y Fisicoquímica Biológicas (IQUIFIB), Facultad de Farmacia y Bioquímica, Buenos Aires, Argentina

Prolonged exposure to growth hormone (GH) in mice is associated with hepatomegaly, due to hypertrophy and hyperplasia of hepatocytes; old GH-transgenic mice frequently develop liver tumors. Alterations in signaling pathways involved in cell growth and proliferation have been related to sustained exposure to GH in young adult transgenic mice. These changes could be a direct effect of prolonged GH action or secondary to the pathological context produced by hormone excess. Consequently, the aim of this work was to assess if lower levels of GH administered for a limited period would also induce any of these alterations. For that purpose, mice were treated with 6 µg/g of body weight of GH per day between 3- and 8-weeks of age, by two daily injections (intermittent treatment) or by osmotic pumps (continuous treatment). In comparison to controls, intermittent GH-treatment increased body size and induced a proportional enlargement of the liver. Continuous treatment provoked similar effects in males, despite differences did not reach statistical significance. Liver sections were analyzed in search of preneoplastic morphological alterations. Mice intermittently treated with GH exhibited a significantly lower number of hepatocytes per microscope field, denoting cell enlargement. Continuous treatment produced similar changes, only in males. Activation and protein content of STAT3, which is known to be involved in liver tumorogenesis, was evaluated by immunoblotting.In females, both kinds of GH treatment slightly increased basal phosphorylation of STAT3, while in males only continuous treatment produced a similar change. A small increment in STAT3 abundance was observed in males exposed to both administration patterns. Therefore, limited exposure to low levels of GH can be associated with hypertrophy of hepatocytes, although hepatomegaly is not evidenced, and sexually dimorphic alterations in the activation of STAT3 that depend on hormone administration pattern are also observed.

165 (309) **CAFFEIC ACID, WHICH PROTECTS RETINAL PIGMENT EPITHELIUM (RPE) CELLS FROM PREMATURE CELL SENESCENCE, MODULATES CREB ACTIVATION**

Pablo Sebastian Tate[1], Mariela C. Marazita[1], Melisa D. Marquioni Ramella[1], Tomas P. Bachor[1], Angela M. Suburo[1].
[1]IIMT Universidad Austral-CONICET, Argentina

Age-related macular degeneration (AMD) is a progressive disease which leads to irreversible loss of vision. We have previously reported that oxidative damage, a hallmark of AMD, promotes premature cellular senescence in RPE cells. Furthermore, these findings support a role for oxidative stress-induced senescence of RPE cells in the etiopathogenesis of AMD. Polyphenols, micronutrients present in the diet, may protect against degenerative diseases. Caffeic acid (CAF) is a polyphenol present in yerba mate in high concentrations. Since we have earlier shown that CAF prevents senescence induction in RPE cells, we now aimed to determine the signaling pathways involved in CAF-mediated protection. We hypothesized that CAF would modulate the activation of cAMP-response element binding protein (CREB), targeting prosurvival pathways. Methods. ARPE-19 cells, derived from human RPE, were incubated with or without caffeic acid (12.5 mg/ml) for 2 hours and then exposed to H_2O_2 (150 mM) for 90 minutes. Cells, collected at different time points following damage, were used to evaluate CREB activation and BCL-2 expression by Western blot, and to measure Reactive Oxygen Species (ROS) with the fluorescent probe 2′,7′-Dichlorofluorescein diacetate (DCFH-DA) and flow-cytometry. Results. Phosphorylation of CREB (CREB- S133) was increased ($p < 0.05$) at 4 and 24 h in CAF-treated H_2O_2-damaged samples compared to untreated damaged cells. Similarly, ROS levels significantly decreased ($p < 0.05$) at 24 h in CAF treated samples, whereas the BCL-2 pro-survival factor was enhanced ($p < 0.05$) at 4 and 24 h. Conclusions. CAF-mediated CREB activation might impact on downstream effectors, decreasing oxidative stress and promoting RPE survival, thus limiting the appearance of senescent phenomena in RPE cells.

166 (2058) **ANALYSIS OF TYPICAL AND ATYPICAL MAPK PHOSPHATASES (MKPS) EXPRESSION IN THE AGGRESSIVE PHENOTYPE OF BREAST CANCER CELLS**

Maria Mercedes Mori Sequeiros Garcia[1], Ulises D Orlando[1], Ana Fernanda Castillo[1], Juan M Cohen Sabban[1], Paula M Maloberti[1], Cristina Paz[1]
[1]INBIOMED (UBA-CONICET). Dpto. Bioquímica Humana. Facultad de Medicina. Universidad de Buenos Aires. Argentina

MKPs are a heterogeneous group of dual-specificity phosphatases (DUSP) that can dephosphorylate both phosphotyrosine and phosphoserine/ phosphothreonine residues. This group of enzymes includes typical MKPs (such as MKP-1, 2 and 3), which specifically dephosphorylate members of the MAPK family, and atypical MKPs (such as DUSP15, 18, 22), much less characterized than the first group. A large number of phosphatases are involved in tumor development, pointing to a central role of this group of enzymes in the regulation of proliferation and cell differentiation. The expression of acyl-CoA synthetase 4 (ACSL4), an enzyme that participates in arachidonic acid metabolism, has been associated

with more aggressive forms of several types of cancer. ACSL4 is part of the mechanism responsible for increased breast cancer cell proliferation, invasion and migration. Thus, the aim of this work was to analyze the expression of a typical, MKP-3, and an atypical MKP, DUSP18, in cells with different ACSL4 expression levels. The experimental model is based on the stable transfection of MCF-7 cells with ACSL4 using the tetracycline Tet-Off system (MCF-7 Tet-Off/ACSL4), in which doxycycline down-regulates ACSL4 expression. mRNA levels of MKP-3 and DUSP18 were evaluated by semiquantitative RT-PCR. The expression of MKP-3 and DUSP18 was increased 1.5 and 1.8-fold ($P<0.05$) in ACSL4 overexpressing MCF-7 cells. Doxycycline treatment reduced the increase in mRNA levels of both phosphatases to control values (MCF-7), confirming the participation of ACSL4 in this effect. The biological significance of the variation of phosphatase expression and the increase in tumor aggressiveness mediated by ACSL4 have not been determined yet. However, these studies may contribute to molecular and functional characterization of these phosphatases, mainly DUSP18.

167 (2075) **EFFECT OF LEPTIN ON THE APOPTOSIS OF TROPHOBLAST EXPLANTS TRIGGERED BY ACIDOSIS**

Antonio Pérez-Pérez[1], Ayelen Rayen Toro[2], Teresa Vilariño-García[1], Malena Schanton[2], Julieta L Maymó[2], Cecilia L Varone[2], Víctor Sánchez-Margalet[1]

[1]Departamento de Bioquímica Médica y Biología Molecular, Universidad de Sevilla, España.[2]Departamento de Química Biológica, Fcen, UBA, IQUIBICEN, CONICET, Buenos Aires, Argentina.

Decrease in gas exchange at the level of the placenta is a serious complication during pregnancy. Disturbance pH of the internal environment can alter fetal, maternal and placental functions. In particular, the decrease in pH can alter the fetal tissue homeostasis causing irreparable fetal death or to damage by metabolic acidosis. In this context we decided to study the effect of lowering the pH of the process of apoptosis in placental explants and leptin action under such conditions. Based on previous results of our research group and the central role of p53 in the regulation of apoptosis, we hypothesize that pH changes in the placental cells increase apoptosis by altering the expression of p53 as well as their target genes. In this context we investigated the posible antiapoptotic effect of leptin in trophoblast cells subjected to different pHs. Explants human placenta at term were incubated in DMEM F-12 at different pH (7.4, 7 and 6.8) in the presence or absence of 10 nM leptin. They were analyzed by Western blot and qRT-PCR p53 levels and its downstream effectors p21, BAX and BCL-2, as well as the cleaved fragment of PARP-1 and the active form of caspase-3. We found an increase in phosphorylation (Ser 46) p53 and p21 expression, PARP-1 and activation of caspase-3 in the explants incubated at pH 7 and 6.8. Conversely, these effects were attenuated in the presence of 10 nM leptin both pH 7 and pH 6.8. The BCL-2 / BAX ratio decreased to pH 7 and 6.8 compared with explants incubated at pH 7.4, an effect that is counteracted by treatment with leptin. These data demonstrate an anti-apoptotic effect of leptin potential involving the regulation of p53 axis explants cultured placental acidic pHs, suggesting that placental leptin has a protective effect on trophoblast cells.

168 (150) **MODULACION DE LA EXPRESION DE CISD1 POR LA ACTIVIDAD DEL CANAL CFTR**

Consuelo Mori[1], Mariángeles Clauzure[1], María Macarena Massip Copiz[1], Ángel Gabriel Valdivieso[1], Tomás Antonio Santa Coloma[1].

[1]Instituto De Investigaciones Biomédicas (BIOMED).

Cystic fibrosis (CF) is an autosomic recessive disease caused by mutations affecting the activity of CFTR chloride channel, which indirectly affect the expression of a net of genes. CDGSH iron sulfur domain 1 (CISD1; initially cloned in this laboratory as named successively CFTR-RG2, KLPX, ZCD1 and CISD1), also referred to as mitoNEET, is a protein localized on the outer membrane of mitochondria. Oriented toward the cytoplasm, the CDGSH domain of CISD1 contains a redox-active 2Fe-2S cluster, which is stabilized by pioglitazone. We have previously described that CISD1 gene expression was decreased in a CF cellular model and restored in the same cells ectopically expressing wt-CFTR (CFDE and CFDE/6RepCFTR cells). The initial results were further validated by using confocal FISH and real-time PCR, using CFDE and CFDE/6RepCFTR cells incubated or not with glibenclamide (50, 100 µM, 24h) or CFTR(inh)172 (2.5, 5 µM, 24h), another Cl- transport inhibitor (more potent and specific than glibenclamide). A similar differential expression was obtained using another CF cellular model: IB3-1(CF cells) and S9 cells (IB3-1 corrected cells), now using CFTR-stimulation (isoproterenol, db-cAMP, IBMX) instead of CFTR-inhibition. Now we used another cellular model, human colon adenocarcinoma T84 cells (known to express the CFTR), and by real-time PCR we determined that CISD1 gene expression was decreased in T84 cells treated with CFTR(inh)172 (10 µM, 24h), compared to control cells. These results confirmed that CISD1 expression is decreased in CF cells or in cells with impaired CFTR function. We are now studying the possible mechanisms involved in this regulation, which will be discussed. Acknowledgments: Grants from ANPCYT [PICT 2007-00628 and PICT 2012-1278], CONICET [PIP 11220080102551 2009-2011 and PIP 11220110100685 2012-2014] and UCA to TASC; and research fellowships from CONICET (to MMMC, CM and MC).

PRESENTACIÓN DE POSTERS SAFE I / SAFE POSTER PRESENTATION I

ENDOCRINOLOGÍA / /ENDOCRINOLOGY

169 (630) **ANTIPROLIFERATIVE EFFECTS OF OXYTOCIN AND DESMOPRESSIN ON CANINE MAMMARY CANCER CELLS**

Micaela Andrea Benavente[1], Carolina Paula Bianchi[1], Fernanda Imperiale[2], Marcelo Alfredo Aba[1].

[1]Laboratorio de Endocrinología, Centro de Investigación Veterinaria de Tandil (CIVETAN), CONICET, Facultad de Ciencias Veterinarias, U.N.C.P.B.A., Tandil, Buenos Aires, Argentina. [2]Laboratorio de Farmacología, Centro de Investigación Veterinaria de Tandil (CIVETAN), CONICET, Facultad de Ciencias Veterinarias, U.N.C.P.B.A., Tandil, Buenos Aires, Argentina.

Neoplasms of the mammary gland represent the most frequent tumor type in the female dog, and according to the histologic criteria, approximately 50% are malignant. In the most aggressive cases of mammary cancer, surgery is not enough to warrant a favorable outcome, and adjuvant therapies are needed to improve the patient's overall survival. Several *in vitro* studies performed on murine and human cancer cells have suggested that some peptide hormones can modúlate tumor growth. However, little is known about its effects on canine cancer. The aim of the present study was to evaluate the effects of Oxytocin (OT) and Desmopressin (DDAVP) on proliferation of the canine mammary cancer cell line CMT-U27. The cells were grown in 96-well plates, at two densities ($4x10^3$ and $8x10^3$ cells/well) in 200 µl of RPMI-1640 supplemented with 10% Fetal Bovine Serum. After overnight culture, the medium was removed and replaced with médium containing OT or DDAVP at five concentrations: 10, 50, 100, 500 and 1000 nM, or medium without drugs (controls). After 72 h of incubation, cell viability was determined using the MTT colorimetric dye reduction method. With $4x10^3$ cells/well, OT at 1000 nM resulted in a 25% of inhibition of cell viability($p<0.01$). Surprisingly, OT 50 nM resulted in a higher inhibitory effect (18%) than 500 nM (9%). At $8x10^3$ cells/well, OT showed a significant antiproliferative effect (almost 25%) with the highest concentration ($p<0.05$). Desmopressin at 1000 nM and $4x10^3$ cells/well reduced cell growth by 22% ($p<0.05$). At 50, 100, 500 nM the inhibitory effect was lower. With $8x10^3$cells/well, DDAVP at 100, 500 and 1000 nM exhibited a mild antiproliferative effect ($p>0.05$). In conclusion, OT and DDAVP can inhibit proliferation of CMT-U27 cells, by almost 20% at the highest concentrations.

Further studies are required to evaluate its potential as antitumor agents, alone or combined with conventional cytotoxic drugs, for the treatment of dogs with advanced mammary cancer.

170 (2042) **GROWTH HORMONE INDUCED EPIGENETIC CHANGES IN LIVER SEXUALLY DIMORPHIC GENE EXPRESSION**

Belen Brie[1], María Cecilia Ramirez[1], Catalina De Winne[1], Ana María Ornstein[1], Damasia Becu-Villalobos[1].[1]*Instituto de Biología y Medicina Experimental.*

Growth hormone (GH) secretion is sexually dimorphic in many species, such as rodents and humans. In the liver GH regulates gene expression in a sexual dimorphic manner. Previous results from our laboratory showed that central disruption of the Dopamine D2 Receptor (D2R) altered the growth axis, we thus inferred and searched for alterations of sexually dependent liver gene expression. To this end, we used transgenic mice with the D2R depleted from the nervous system (*neuroDrd2KO*). Furthermore, because neonatal sexual steroids are known to imprint the growth axis, we tested the effects of the neonatal administration of testosterone in females on genetic and epigenetic regulation of the liver.

We sustained that liver sexual dimorphism is mediated by epigenetic mechanisms, induced by the GH secretion pattern. Hypermethylation of promoter regions of genes generally results in their silencing. We measured promoter methylation levels using a methylation sensitive restriction enzyme, followed by specific qPCR.

We found that the mRNA expression of female predominant genes Alcohol Dehydrogenase 1 (Adh1) and Hepatocyte Nuclear Factor 6 (Hnf-6) are masculinized in the *neuroDrd2KO* mice, and so was the Adh1 protein. On the other hand, mRNA expression of the male predominant gene*Cyp7b1* was feminized (decreased) in both our mice models. Increased methylation of the promoter region of *Hnf-6* correlated with its decreased expression in neuroDrd2KO females, while increased expression of *Adh1* in neuroDrd2KO males did not correlate with methylation status of its promoter. Furthermore, methylation could not explain the changes in *Cyp7b1* in neuroDrd2KO mice. Lastly *Adh1 and Hnf6* mRNA in the testosterone treated females was masculinized and altered methylation of the promoter correlated only with *Hnf6* expression.

Our results demonstrate that the growth axis modulates the sexual dimorphic expression of several genes in the liver, in part through DNA methylation.

FARMACOLOGÍA / PHARMACOLOGY

171 (140) **TIME COURSE OF RENAL EXPRESSION AND URINARY EXCRETION OF CAVEOLIN-2 (CAV2) IN RATS WITH CISPLATIN INDUCED ACUTE KIDNEY INJURY**.

Romina Paula Bulacio[1], Adriana Mónica Torres[1].
[1]*Area Farmacología. Facultad de Ciencias Bioquímicas y Farmacéuticas. Universidad Nacional de Rosario. CONICET.*

Caveolins (Cav), main component of caveolae, are a family of integral membrane proteins that are involved in cell surface signaling, endocytosis and in repair processes. The role of Caveolin 2 (Cav2) in kidney disease is unclear. Cisplatin (Cis) is a chemotherapeutic agent that might cause acute kidney injury (AKI). The aim of this study was to evaluate the time course of Cav2 renal expression and urinary excretion in a Cis induced AKI model. Adult male Wistar rats were injected with 5 mg/kg i.p. of Cis and the studies were performed 2 (T2, n=4), 4 (T4, n=6), 7 (T7, n=6) and 14 days (T14, n=5) after treatment. Control rats (C, n=16) received the vehicle. Plasma samples and renal tissue were collected. Urea plasma levels (Ur_p) were determined spectrophotometrically. Cav2 in renal homogenate ($Cav2_H$), in renal apical membranes ($Cav2_{ap}$) and in urine samples ($Cav2_U$) were evaluated by immunoblotting. ANOVA/Newman-Keuls test, P<0,05: a vs C; b vs T2; c vs T4; d vs T7; e vs T14. Results: Ur_p(g/L): C=0.28±0.01, T2=0.49±0.08^{c,d}, T4=2.94±0.24^{a,b,e}, T7=3.82±0.91^{a,b,e}, T14=1.03±0.23^{c,d}; $Cav2_H$(%): C=100±4, T2=102±11, T4=102±6, T7=81±6, T14=85±5; $Cav2_{ap}$(%):C=100±2, T2=28±2^{a,d,e}, T4=23±1^{a,d,e}, T7=42±4^{a,b,c,e}, T14=76±6^{a,b,c,d}; $Cav2_U$(%): C=100±3, T2=96±10^{e}, T4=114±8^{e}, T7=86±13^{e}, T14=159±21^{a,b,c,d}. Ur_p was significantly altered on days 4 and 7 of treatment and on the day 14 tended to return to its basal value.$Cav2_H$ did not change through the experiments, but $Cav2_{ap}$ was found significantly decreased in all the times evaluated. $Cav2_U$ was only increased on the 14th day. The decrease in $Cav2_{ap}$ expression could be due to Cav2 internalization, probably together with different signalling molecules. So, Cav2 might have a role in the regulation of signal transduction after AKI. The increase observed in $Cav2_U$ only in the recovery phase of AKI (day14), let us to propose it as a urinary biomarker useful to monitor reversibility of renal injury after Cis induced AKI.

172 (307) **ROLE OF ALLOPREGNANOLONE IN THE MORPHOPHYSIOLOGY OF THE RAT OVARY**.

Antonella Rosario Ramona Cáceres Gimenez[1, 2, 3], Laura Tatiana Pelegrina[1, 2], Joana Antonela Asensio[1], Fernanda Parborell[4], Myriam Raquel Laconi[1, 2].
[1]*Laboratorio de fisiopatología ovárica y neurobiología. Instituto de medicina y biología experimental de Cuyo (IMBECU - CONICET)* [2]*INBIOMED - UM* [3]*Universidad Juan Agustín Maza* [4]*IBYME – CONICET*

Allopregnanolone (ALLO), a progesterone metabolite, is a neurosteroid synthesized *de novo* in the nervous system. ALLO icv reduces LH secretion, increases progesterone and prolactin serum levels,inhibits corpora lutea apoptosis, alters follicular steroidogenesis and impairs ovulation. It also modifies ovarian angiogenesis, which takes place cyclically in the ovary and is essential for follicular and luteal development. The aim of this study was to evaluate the effect of a pharmacological dose of ALLO, using a new administration model (intrabursa), on ovarian morphophysiology, vascular development and stability.

A dose/response curve of 5 points was performed. The results presented correspond to the pharmacological dose of 6 µM. Adult Sprague-Dawley rats were used. On the morning of proestrus, vehicle and ALLO were administered at 5 µl intrabursa. The animals were sacrificed during the morning of estrus, 24 h after treatment. To assess vascular density and stability, immunohistochemistry for the von Willebrand factor (present in endothelial cells) and α-actin (present in smooth muscle cells of blood vessels) was performed. For the ovarian structures morphometric analysis, histological sections were made. Oocyte count was performed by puncturing the ovarian ampulla. ALLO caused a significant increase of angiogenesis, measured as an elevation in the detection of the W factor ($p < 0.001$) and of the α-actin protein ($p < 0.05$). It also induced a significant increase in the number of atretic follicles ($p < 0.001$) and the formation of cystic follicular entities. In this model, the treatment did not alter ovulation unlike previous findings with the model icv. Also, asignificant increase in the diameter of the corpora lutea ($p < 0.05$) of treated animals was found. We can conclude that ALLO, at this concentration, alters follicular and luteal development as well as ovarian angiogenesis. ALLO is a molecule candidate to be used as a new therapeutic alternative for ovarian disease.

173 (532) **BISPHOSPHONATES REGULATE DIFFERENTIAL CA2+ INTAKE DEPENDING OF CELL TYPE AND ORIGIN: VERO, HT29 OR EGPE, CELL LINE FROM ECHINOCOCCUS GRANULOSUS.**

Mariana Ferrulli[1], Andrea Granada[1], Emilio Roldán[1], Alicia Graciela Fuchs[1].
[1]*Centro de Altos estudios en Ciencias Humanas y de la Salud (CAECIHS), Universidad Abierta Interamericana (UAI) Avenida Montes de Oca 745, CABA.*

The Ca^{2+} constitutes one of the most relevant cations to cell metabolism. Different channels are responsible for Ca^{2+} intake. We study antiparasitic effects of bisphosphonates (BPHs). Previously we described the antiproliferative effect of Ibandronate (IB) and Etidronate (EHDP) on EGPE cellline (Fuchs et al, 2014). Ca^{2+} accumulation and alkaline phosphatase inhibition was observed in cells treated with EHDP. Moreover, calcium entry in EGPE is

comparable to HT29 cells, a mucous cells line (Ferrulli et al,SAP 2015). The aim of this work is to study Ca^{2+} intake regulation by EDPH and IB on EGPE, HT29 and Vero cell lines using $CaCl_2$ and $CaGluc_2$, as permeant and non-permeant anions. Mat. and Meth.: HT29, Vero and EGPE (Echeverria et al, 2010) cells were cultured. Espectrofluorometry:(n=5 exp) suspension of EGPE (10^6),HT29 (10^5) and Vero (10^5) cells were incubated with Fluo-4AM according to the dye use instructions. Cells were distributed in a black multiplate in buffer (mM): 124, Na+; 1 K+, 125 Cl-. pH 7.2; 1.5 mM Cl_2Ca or $CaGluc_2$ were added to each wells after base fluorescence was recorded in Glomax (Promega). Controls were performed without calcium addition. To end, 90 µM of ionimicine was added. Calcium entry was calculated. Proteins were measured by Bradford in parallel cell sample. Statistics: [Ca^{2+}] is expressed in nM of Ca^{2+}/ cell/µg proteins. Results were evaluated by student T test (significantly p<0,05; one tail). On EGPE, EDPH decreased Ca^{2+} uptake with $CaCl_2$ and IB increased its entry with $CaGluc_2$. On Ht29 cells EDPH, the calcium increment was with both salts but IB decreased its entrance only with $CaCl_2$. On Vero, EDPH increased Ca^{2+} uptake only with $CaGluc_2$. Conclusions: The results show that although in EGPE cells the calcium entry is similar to HT29 cells the BPHS effects are different. On Vero and HT29 cellsEDPH may act on different channels, regarding the use of $CaCl_2$ or Gluc2Ca. The Cl^- channel seems to be involved in Ca^{2+} uptake mediated by BPHS.

174 (534) **GASTROPROTECTION OF ARISTOLOCHIA ARGENTINA: STRUCTURAL BASIS OF INTERACTION WITH CYCLOOXIGENASE COX2**

Jésica Daniela Paredes[1], Ángela Sosa[1], María Fusco[1], Alejandra Rotelli[1], Graciela Wendel[1], Carlos Aguilar[1], Lilian Pelzer[1], Alejandra Olivia Margarita Maria[1].
[1]Facultad de Química, Bioquímica y Farmacia- Universidad Nacional de San Luis.

Aristolochiaargentina Griseb. (family Aristolochiaceae) is popularly know as “charrúa”, “buche de pavo”, “charruga”, “mil hombres”, “patito”. The roots of this plant are used in folk medicine for the treatment of ulcers (Barboza et al., 2009). Previously, we have demonstrated that *A. argentina* prevented the gastric ulcer induced by several necrotizing agents (ethanol, HCl, NaOH). Allantoin was isolated from the roots of *A. argentina* (Priestap et al., 1972). This compound showed gastrointestinal antiulcer activity (de Sousa Falcão et al., 2008). Here we study the allantoin effect to probe the structural basis of possible interaction with cyclooxigenase COX_2 and the role ofprostaglandins in antiulcer activity of *A. argentina*. Gastric lesions were produced according to the method of Robert et al. (1979). The present study showed that the protective activity of *A. argentina*against ethanol-induced gastric mucosal lesions in Wistar rats was reduced significantly when indometacin (10 mg/kg), a classic inhibitor of prostaglandins synthesis, was given before *A.argentina*. (lesion area: 20.94±1.39 mm^2 vs. 7.5±2.98 mm^2, p<0.01). The docking of alantoina into the crystallographic structure of COX_2 was done using AUTODOCK4. The allantoin docking with COX_2 showed that it binds to the protein active site, a long and deep hydrophobic channel, between C8 and C11. Allantoin occupies a similar region as several NSAIDs in the crystal structure of these complexes with COX_2. The structure of the complex COX_2-allantoin revealed contacts within a 4 Å radius with Trp 387, Tyr 348, Ser 530, and Tyr385. The interaction of allantoin with COX_2 active site may indicate that has a role in the antiulcer mechanism. Therefore, it seems possible that allantoin may act as a natural inhibitor of COX_2. Present findings suggest the possible involvement of prostaglandins in the antiulcer effect of *A. argentina.*

175 (710) **MULTIDRUG RESISTANCE PROTEIN 4/ ATP BINDING CASSETTE TRANSPORTER 4 IS OVEREXPRESSED CLEAR CELL RENAL CELL CARCINOMA (CCRCC)**

Juan Pablo Melana Colavita[1] María May[2] Tania Stoyanoff[1] Natalia Gómez[3], Juan Santiago Todaro[1], María Victoria Aguirre[1], Francesco Mignolli[4], Carlos Davio [3], Juan Pablo Rodríguez[1].
[1]Laboratorio de Investigaciones Bioquímicas (LIBIM), IQUIBA-CONICET-UNNE. [2]Instituto de Biología y Medicina Experimental-CONICET. [3]Instituto de Investigaciones Farmacológicas, Facultad de Farmacia y Bioquímica, UBA, ININFA-CONICET-UBA. [4]Laboratorio de Fisiologia vegetal, Instituto de botánica del nordeste (IBONE, UNNE-CONICET).

Clear cell renal cell carcinoma (ccRCC) is the most common subtype of renal cell carcinoma, which is the most prevalent kidney cancer among adults, accounting for approximately 89% of all diagnosed cases. Presently, there is a paucity of effective therapies designed to target ccRCC effectuating long-term durable response in patients with advanced disease. In addition, there is a lack of molecular markers that can be remedially targeted, showing tumor-specific inhibition. Recently, it was demonstrated that besides playing a role in drug-resistant tumoral cell lines, multidrug resistance protein 4 (MRP4/ABCC4) regulates cell proliferation and differentiation through the endogenous MRP4/ABCC4 substrate, cAMP. Both features make it an attractive option to use it as a therapeutic target.

Up to now, it has not been reported that MRP4/ABCC4 is involved in the biology of renal cell carcinoma. Thus, the objective of this work was to determine the level of expression of this protein in renal carcinoma and its implication in cell proliferation.

Using quantitative PCR, we detected an average 30-fold increase in mRNA of MRP4/ABCC4 in tumors, compared to a distal portion of kidney taken as control (n=27). Western blott analysis confirmed the overexpression of this protein and its localization was studied by immunohistochemistry. We detected an aberrant expression of MRP4/ABCC4 in renal tumor cells, localized in concentrated foci. In order to determine whether the intracellular cAMP concentration interferes in cell proliferation, functional studies were developed using the cell line Caki-2. Treatment of these cells with Forskolin and MK571 resulted in significant inhibition of cell proliferation.In the best of our knowledge, this work first informs the overexpression of this protein in ccRCC. These results and further functional studies, in renal cell lines, may validate MRP4/ABCC4 as a possible therapeutic target in ccRCC.

176 (712) **ALBENDAZOLE RESISTANCE IN FASCIOLA HEPATICA: IN VITRO DIAGNOSTIC AND P-GLYCOPROTEIN GENE EXPRESSION**

Luis Ignacio Alvarez[1], Laura Ceballos[1], Laura Maté[1], Mariana Ballent[1], Laura Moreno[1], Rodrigo Sanabria[2], Carlos Lanusse[1].
[1]Centro de Investigaciones Veterinarias de Tandil (CIVETAN), Fac. Cs. Vet., UNCPBA-CICPBA-CONICET, Tandil, Argentina. [2]Instituto de Investigaciones Biotecnológicas-Instituto Tecnológico Chascomús (IIB-INTECH), CONICET-UNSAM, Chascomús, Argentina. Fac. Cs Vet., UNLP, La Plata, Argentina.

The main goals of the current work were to confirm the albendazole (ABZ) resistance status of a *Fasciola hepatica* isolate by the egg hatch (EHT) and the controlled test, and to compare the gene expression profiles of the efflux transporter, P-glycoprotein (P-gp), in the same specimens of *F.hepatica* recovered from ABZ treated or untreated animals. Metacercariae of a *F. hepatica*isolated previously classified as ABZ-resistant by the EHT were used to artificially infect eight (8) healthy sheep. Sixteen (16) weeks after infection, animals were randomly distributed either in an untreated control or in an ABZ-treated (7.5 mg/kg) group. The flukicidal efficacy of ABZ was assessed by the comparison of the number of flukes recovered from untreated and treated sheep at 7 days post-treatment. Additionally, adults *F. hepatica* specimens from both experimental groups were used to determine the P-gp gene expression profiles by real-time PCR, using GAPDH as a reference gene. No statistical differences were observed in the number of adult liver flukes between untreated (18.5 ± 3.6) and ABZ-treated group (18.2 ± 8.5, P<0.05), confirming the ABZ-resistant statuspreviously observed by the EHT. The expression level of P-gp in *F. hepatica* was 2.5- fold higher(P<0.05) in those recovered from ABZ-treated sheep compared to collected from untreated control group. Further studies involving more individuals

are needed to confirm if the higher P-gp expression observed in the ABZ-exposed *F. hepatica* could be a potential resistance mechanism. Furthermore, the observed results confirm that the EHT is a reliable method to determine the state of susceptibility/ resistance in *F. hepatica.*

177 (729) **HYPOXIC TUMORAL MICROENVIRONMENT AND STEAROYL-DESATURASE EXPRESSION ARE ASSOCIATED WITH CLEAR CELL RENAL CELL CARCINOMA SURVIVAL AND PROLIFERATION**

Tania Stoyanoff[1], Juan Pablo Rodríguez[1] Juan Santiago Todaro[1], Joaquín Diego Espada[2] , Juan Pablo Melana Colavita[1] Adriana Mónica Torres[3], María Victoria Aguirre[1].
[1]Laboratorio de Investigaciones Bioquímicas (LIBIM), Facultad de Medicina, Universidad Nacional del Nordeste (UNNE), IQUIBA-CONICET, Corrientes, Argentina. [2]Servicio de Urología Hospital "Juan R. Vidal", Corrientes, Argentina. [3]Área Farmacología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario (UNR), CONICET, Rosario, Argentina.

Adapting to hypoxic stress is crucial in tumour progression and in determining its malignancy. The transcription factor for hypoxic adaptation (HIF-1α) is pivotal in modulating tumorous hypoxic responses. The most common of renal carcinomas is the clear cell adenocarcinoma (ccRCC) There is great interest to know the molecular basis of ccRCC tumor biology that might contribute to a better understanding of its aggressive biological behaviour Thus, the aim of this study was to describe the relationship among the expression of HIF-1α, a key enzyme related to mono unsaturated fatty acid synthesis, stearoyl desaturase-1 (SCD-1) and the angiogenic pair VEGF/VEGF-R2 in early stages ofccRCC. Tissue samples were obtained from patients at the Urology Unit of the J.R. Vidal Hospital (Corrientes, Argentina) who underwent radical nephrectomy for renal cancer. Four experimental groups according to pathological stage and nuclear grade were organized: T1G1(n=6), T2G1(n=4), T1G2(n=7), and T2G2(n=7). The expression of HIF-1α, VEGF, VEGFR-2, Bcl-x_L and SCD-1 were evaluated by immunohistochemistry, Western blotting and/or RT-PCR. Apoptosis was assessed by the TUNEL in situ assay and tumor proliferation was determined by Ki-67 immunohistochemistry. Data revealed that HIF-1α, VEGF and VEGF-R2 were overexpressed in most samples. Bcl-x_L enhancement was concomitant with the increment of proliferative indexes. SCD-1 expression increased with the tumor size and nuclear grade. Particularly, data analysis reveals statistical positive correlations between SCD-1 and HIF-1 alfa (r^2=0.97; p=0.0018), as well as, between SCD-1 and Ki-67 (r^2=0.97; p=0.0018), suggesting a putative involvement of SCD-1 in tumor progression inearly stages of ccRCC. This study provides new information of tumoral biology of ccRCC regarding to hypoxic microenvironment, lipogenesis and tumor cell proliferation that might contribute to propose SCD-1 as a biomarker and/or an oncotherapeutic target for this complex carcinoma.

178 (1016) **EARLY PROTEIN MALNUTRITION ATTENUATES THE ANTIDEPRESSANT-LIKE EFFECT OF DMI IN THE FORCED-SWIM TEST IN ADULTS RATS MATERNALLY SEPARATED**

María Cecilia Gutiérrez, Lucía NasiMedeot, María Cecilia Perondi, Gabriel Cuadra, Analía Valdomero.
IFEC-CONICET, Departamento de Farmacología, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba Argentina.

Previous results demonstrate that perinatal protein malnutrition facilitates depressive-like behaviors in rats that have experienced maternal separation. Thus, undernutrition significantly increases the immobility time in the forced- swim test (FST). Considering the long-lasting alterations produced in the central monoaminergic systems, as a consequence of nutritional insult, we study the treatment effects of desipramine (DMI) - a highly selective reuptake inhibitor of norepinephrine- on the behavioral changes found in the FST. To this aim, different groups of adult control (C) and protein deprived rats (D) maternally separated daily for 180 min from PND 1 until PND 10 (MS-group) were treated with DMI (15 mg/kg/day, IP) or saline during 7 days. Total immobility time was measured for 5 min period in the FST and compared with rats that were not submitted to maternal separation (NMS). Although DMI treatment produced an antidepressant-like effect in both C (NMS and MS) and D (NMS and MS) groups, rats in D-MS group showed a significant higher immobility time in the forced-swim test. These preliminary results indicate that early nutritional insult alter the pharmacological reactivity to DMI, highlighting an incapacity to induce neuroadaptative changes incentral pathways involved in DMI mechanism of action. Indeed, they suggest the possibility of an altered reactivity to therapeutic treatments in adult subjects malnourished at early life.

FARMACOLOGÍA DEL DOLOR / /PHARMACOLOGY OF PAIN

179 (87) **SOLID LIPID NANOPARTICLES LOADED WITH ANTI-INFLAMMATORY ANTIBIOTICS OF PROLONGED RELEASE. LONG-TERM STUDY IN RATS WITH CHRONIC NOCICEPTION.**

Claudio Laurido[1], Teresa Pelissier[2-], José Luis Martínez[1], Carlos Valdés[1].
[1]University of Santiago of Chile, Faculty of Chemistry and Biology, Dept. of Biology, Santiago, Chile. [2]ICBM, University of Chile, Santiago, Chile.

Chronic pain is a sensory experience which involves the glial cells, whose participation is such that some authors have proposed to chronic pain as a gliopathy. Because of this, the drug target of possible treatments focuses on modulating nociceptive response affecting transduction into the central nervous system through affecting synapses in the dorsal horn of the spinal cord. Solid lipidnanoparticles (SLNP) were utilized and loaded with the antibiotics minocycline and ciprofloxacin. Both present significant anti-inflammatory properties. The anti-inflammatory effect of these SLNP wastested in a rat model of Freund's adjuvant monoarthritis. Sprague-Dawley male rats weighing 250-350 g. were used in the present work. The animals were obtained from the vivarium of the University of Chile, Faculty of Medicine and kept in a light/dark cycle 12/12 hours, with food and water ad libitum. All experiments were performed according to the regulations of the International Association for the Study of Pain and the Ethics Committee of the University of Santiago, Chile. Results showed when the monoarthritis was established, (14 days after the Freund's adjuvant injection) that intrathecally injecting a dose of 20 micrograms/rat of non encapsulated antibiotics (minocycline or ciprofloxacin) at day 15, and measuring the effect by using the Randall-Selitto test at days 16, 17, 19 and 21, there was an antinociception corresponding to an increase in vocalization threshold of 56.7% for minocycline and 71.8% for ciprofloxacin, both values compared to monoarthritic saline control. In the other hand, injecting SLNP with encapsulated antibiotics, there was an increase in the antinociceptive effect of 85.9% for minocycline and 74.7% for ciprofloxacin. The former SLNP were loaded only with 10 micrograms/rat, meaning half of the dose used for the non encapsulated ones. We can conclude that these antibiotics encapsulated in SLNP increased their pharmacological efficacy for a long period of time spanning to seven days. These results may have an impact on the Clinic, because with reducing the dosage, undesirable side effects may also be reduced or completely abolished. Acknowledgments: Funded by DICYT 021643LF, University of Santiago, Chile.

METABOLISMO / METABOLISM

180 (168) **HIGH FRUCTOSE-HIGH FAT DIETS MODIFY BODY COMPOSITION IN RATS, BUT NOT BODY MASS**

Marina Laura Wallinger[1], Laura Mercedes Linares [1], María Patricia Reyes[1], Verónica Zuccarella[1], Eleonora Pagano[2,3], Daniel Pedro Cardinali[1], Carlos Felipe Reyes Toso[1].

[1]Universidad de Buenos Aires. Facultad de Medicina. Departamento de Ciencias Fisiológicas. Unidad Académica II [2]BIOMED (UCA-CONICET) [3]Universidad Católica Argentina. Facultad de Ciencias Médicas.

Introduction: The diets of populations in industrialized nations have shifted to dramatically increased consumption of fructose and saturated fat. In animal models high fructose-high fat diets (known as Western diet –WD-) induce metabolic syndrome (MS), but the mechanisms linking peripheral metabolism and body composition is unclear.

Objetive: the present work was designed to evaluate body composition modification in WD fed rats

Methods: Animals were exposed from 35 days to 6 months of age to a standard diet–SD- (n=8) or WD (n=8). Every 6 weeks rats were fasted overnight and baseline blood samples (150–200 µL) were collected from the tail vein. Blood glucose, insulin and fasting triglyceride and cholesterol levels were assayed. Body mass was measured weekly and Systolic blood pressure (BP) was measured monthly by using a manometer and employing an inflatable tail-cuff pressure transducer connected to an amplifier and a data adquisition system. An average value from three BP readings (that differed by no more than 2 mmHg) was determined for each animal. Weight gainwas calculated by subtracting the final weight from the initial weight. After euthanasia, adiposity measures were obtained from abdominal (retroperitoneal and visceral) fat masses. Abdominal white adipose tissue (WAT) was expressed as a percentage of body weight. All data are shown as the mean ± SEM. Data were analyzed by two-way ANOVA tests adjusted by Bonferroni correction. Thesignificance level was set at $p \leq 0.05$.

Results: WD induced a metabolic syndrome. We did not find diet-induced obesity in WD-treated group. However, significant differences were detected in abdominal white adipose tissue ($p<0,05$), systolic blood pressure ($p<0,01$), fasting serum triglycerides levels ($p<0,05$), fasting serum cholesterol levels ($p<0,01$), insulin ($p<0,01$) and fasting total blood glucose ($p<0,05$) between SD- and WD-treated group.

Conclusions: WD induces MS and modified body composition in rats but not body mass.

NEUROCIENCIAS / NEUROSCIENCE

181 (312) **GHRELIN ENHANCES BDNF EXPRESSION IN HIPPOCAMPAL SLICES**

Mary Luz Perea Vega[1], Mauricio Martin[2], Susana Rubiales De Barioglo[1].

[1]Depto Farmacología Universidad Nacional de Córdoba - IFEC- Conicet. [2]Instituto de Investigaciones Médicas Mercedes y Martín Ferreyra (INIMEC-CONICET-UNC).

Ghrelin (Gr) is a peptide involved in the modulation of various biological processes. In previous works, we have shown that the stereotactic injection of Gr either in the ventricle or directly in the hippocampus (HP) of rats, improves memory retention in a dose dependent manner when the animals were challenged in different behavioral paradigms. Administration of Gr in the Hp also increases neuronal excitability facilitating the induction of long-term potentiation (LTP, a process underlying memory formation) and glutamate release. In addition, we have demonstrated that Gr increases the expression of NR2B subunit N-methyl-D-aspartate receptor (NMDARs). Activity regulated gene transcription is required in multiple biological processes, including memory formation. One of the most extensively studied genes that are regulated by neuronal activity is the brain derived neurotrophic factor (*Bdnf*), which plays important roles in synaptic plasticity. Accordingly, the increased expression of Bdnf has been associated with the activation of NMDARs after induction of LTP and LTD (long term depression) in hippocampal neurons. Thus, taking in mind the previously demonstrated effects of Gr on NMDARs, we examined if Gr could also influence the transcription of BDNF promoters Using total RNA preparations obtained from mouse acute hippocampal slices in control conditions or after NMDA stimulation, we have observed that Gr is able not only to increase the transcriptional activity of specific Bdnf promoters but also able to potentiate the transcriptional effect of NMDA. Our data suggest that the ability of Gr to enhance memory formation would be mediated by increased Bdnf transcription.

182 (384) **A NOVEL ANTICONVULSANT/ANTIDEPRESSANT Î±-HYDROXYAMIDE EXERTS ITS MECHANISM OF ACTION THROUGH VOLTAGE GATED SODIUM CHANNELS**.

Valentina Pastore[1], Cristina Wasowski[1], Josefina Higgs[1], Pedro Martin[2], Veronica Milesi[2], Mariel Marder[1].

[1]Universidad de Buenos Aires. Consejo Nacional de Investigaciones Científicas y Técnicas. Instituto de Química y Fisicoquímica Biológicas Prof. Dr. Alejandro C. Paladini (IQUIFIB). Facultad de Farmacia y Bioquímica. Buenos Aires, Argentina. [2]Universidad Nacional de La Plata. Consejo Nacional de Investigaciones Científicas y Técnicas. Instituto de Inmunología y Fisiopatología IIFP. Facultad de Ciencias Exactas. Argentina.

In patients with epilepsy, anxiety and depression are frequent psychiatric comorbidities but they often remain unrecognized and untreated. We have synthesized a novel α-hydroxyamide, N-propyl-2,2-diphenyl-2-hydroxyacetamide (**C1**), which is effective as anticonvulsant in the maximal electroshock test (ED_{50}: 2.5mg/kg). Taking into account that there are clinical anticonvulsants that are also used as mood stabilizers, i.e. lamotrigine, topiramate, phenytoin; we evaluated if this novel compound also has antidepressant activity in two experimental models: forced swimming (FST) and tail suspension (TST) tests. It is known that antidepressant drugs modify, in different ways, the activity of neurons, by increasing monoamine levels and by modulating ion channels. Sodium channels are molecular targets for antiepileptic drugs, which can also be mood stabilizers. So, in order to elucidate its mechanism of action, we made different *in vivo/in vitro* assays. Compound C1 demonstrated antidepressant activity (FST and TST) in Swiss male mice at 0.3-30 mg/kg i.p. (CICUAL EXP-FYB N° 0031682/2014). Its capacity to act via $GABA_A$ receptor ([^{3}H] flunitrazepam binding assay); serotonin 5-HT_{1A} receptor ([^{3}H] 8-OH-DPAT binding assay) and voltage gated sodium channels using veratrine, a voltage gated sodium channel agonist (*in vivo*) and in a HEK 293 cell line that expressed Nav 1.1 (electrophysiology) was also evaluated. As a result, we found that its effects are not likely related to 5-HT_{1A} or GABAergic pathways; but its anticonvulsant/antidepressant-like effects could be due toits voltage gated sodium channel blocking properties. We observed that the antidepressant-like effect induced by C1, at 1 mg/kg and 10 mg/kg, was reversed by the presence of veratrine and a 30% inhibition of the sodium current using patch clamp technique in HEK 293 cell line that expressed Nav 1.1. This combined anticonvulsant/antidepressant-like profile may represent a valuable tool for the treatment of these disorders.

183 (554) **THE ROLE OF SEROTONERGIC G-PROTEIN COUPLED RECEPTORS (SGPCRS) IN ECHINOCOCCUS GRANULOSUS AND OTHER CESTODES SUGGESTS FUTURE APPLICATIONS AS DRUG TARGETS OF NEGLECTED DISEASES**

Federico Camicia[1], Ana María Celentano[1,4], Nicolás Di Siervi[2], Hugo Vaca[1], Lucas Maldonado[1], Sergio Simonetta[3], Laura Kamenetzky[1], Carlos Davio[2], Mara Rosenzvit[1].

[1]Instituto de Investigaciones en Microbiología y Parasitología Médica, IMPAM-UBA-CONICET, Facultad de Medicina. [2]Instituto de Investigaciones Farmacológicas, Facultad de Farmacia y Bioquímica, UBA, ININFA-CONICET-UBA. [3]Centro de InnovacionTecnologica, Empresarial y Social (CITES), Sunchales. [4]Departamento de Microbiología, Parasitología e Inmunología, UBA, Facultad de Medicina.

Introduction: Cestode parasites are large multicellular organisms, many of them cause neglected zoonoses with major impact in local health and global economy. The adecuate nerve and

muscle function is essential for the parasitic way of life and is a target for cestocide drugs. Genomic and transcriptomic data of E. granulosus (Tsai et al., 2013) and experimental results showing the motor response to serotonin (5-HT) of the larval stage (Camicia et al., 2013) suggest the existence of sGPCRs in this parasite. Drugs that modulate human GPCRs have numerous examples in the pharmaceutical industry but drugs that could discriminate between parasite and human receptors have not been discovered yet.

Hypothesis: we propose the existence of sGPCRs with a major role in cestode movement.

Results: BLASTp search on Mesocestoidescorti, Taeniasolium, Echinococcua granulosus and E, multilocularis databases showed 4 groups of aminoacidic sequences with homology to 5HT1 human GPCR. Percentage identity, E-value and presence of transmembrane domains and of critical residues for sGPCR activation and 5HT binding allowed us to select 8 candidates, some of them were already cloned and sequenced. A similar tridimensional structure and position of the 5HT binding site between was confirmed between the human and E. granulosus sGPCRs. However interesting differences could be found. The addition of 5-HT to the larval stages of M. corti and Taeniacrassiceps stimulated the motility and increased the endogenous levels of cyclic AMP. The stimulatory effect of 5HT in parasite movement was abolished by the addition of gramine and sumatriptan, two known sGPCR modulators.

Conclusion: The present findings support our hypothesis about the importance of sGPCRs as a new target for development of new cestocidal drugs for neglected diseases.

184 (698) **INVOLVEMENT OF IL-10 AND TGF-Î² IN CHRONICALLY STRESSED MICE COGNITIVE DEFICIT**.

Alejandro David Moroni[1], María Emilia Di Rosso[2], Laura Daniela Alaniz[1], Ana María Genaro[2,3], María Laura Palumbo[1].
[1]CIT NOBA-UNNOBA-CONICET; J.Newbery 261, Junín, Bs. As. [2]BIOMED-UCA-CONICET; A.M. Justo 1600, CABA. [3]Depto. de Farmacología-Fac. Med.-UBA; Paraguay 2155, CABA.

In previous reports we found that chronic mild stress (CMS) exposure induces a decrease in learning and memory in female BALB/c mice. This cognitive deficit correlated with a decrease in $CD4^+CD25^+FOXP3^+$and an increase in $CD4^+CD25^-FOXP3^+$ regulatory T cells (Tregs) in CMS mice spleen. No differences in the $CD4^+CD25^+FOXP3^+$ and $CD4^+CD25^-FOXP3^+$ Tregswere found in lymphnodes in stressed mice. Tregs have an important role in maintaining self-tolerance through the inhibition of effector T cells. The main cytokines involved in this mechanism are IL-10 and TGF-β, both released by Treg cells. In this context, the aim of this work was to evaluate the levels of the cytokines IL-10 and TGF-β in CMS mice and its correlation with cognitive deficit. Here, we show that CMS mice presented a poor learning performance in the Y-maze (% spontaneous alternation = control: 63.52 ± 1.93; CMS: 50.46 ± 1.96; $p<0.01$). The evaluation of mRNA expression by qRT-PCR indicated a decrease in the mRNA levels of IL-10 and TGF-β in spleen (IL-10 = control: 1.01 ± 0.07; CMS: 0.35 ± 0.001; $p<0.001$ and TGF-β = control: 1.27 ± 0.24; CMS: 0.28 ± 0.15; $p<0.05$). However, no differences were observed in lymph nodes in CMS mice respect to control mice. Furthermore, we found a decrease in the levels of IL-10 in serum of CMS mice detected by ELISA (IL-10 (pg/ml) = control: 147.45 ± 9.05; CMS: 118.09 ± 3.87; $p<0.05$). Our findings indicate that chronic stress affects the immune system through a mechanism that involves Treg cells possibly by the release of IL-10 and TGF-β. This suggests Treg cells participation in the cognitive deficit observed in chronic stressed female mice. Knowledge of thesemechanisms could be useful to find new therapeutic targets for reversing the deleterious cognitive effects induced by chronic stress.

185 (990) **SOMATOSTATIN MODULATION OF ACETHYLCHOLINESTERASE ACTIVITY IN CEREBRAL CORTEX OF MICE SUBJECTED TO AN EPIGENETIC MODEL OF SCHIZOPHRENIA.**

María Graciela López Ordieres, María Laura Peralta, Alma Kemmling, Susana Gorzalczany, Andrea Induni.
Cátedra de Farmacologia.Facultad de Farmacia y Bioquímica. UBA.Junín 956 (1113) CABA.

Somatostatin (SST) is a peptide present in the CNS and peripheral tissues that interacts with five receptor subtypes (SSTRs). The biological effects of somatostatin were studied by the use of antagonists, such as somatostatin-cycle (cSSTA) or somatostatin analogs. The purpose of this study was to evaluate the somatostatin modulation of acethylcholinesterase activity in cerebral cortex ofmice subjected to an epigenetic model of schizophrenia. For this purpose, Swiss Albino mice were injected with a daily dose of methionine 3.1 mmol / kg for 15 days. Exploratory behavior is fundamental to the rodent nature and the open field task is currently used to test drugs. Present results showed that the number of crosses in the open field task for control animals is lower in the third period of exposure. Another test used was the Y maze in which the percentage of alternance,represented as (Ā± SE) was 10% ± 1.7 (n = 5) for control mice and 16% ± 0.8 (n = 5) for mice injected with methionine. Acetylcholinesterase activity was also determined, it was 1.67 ± 0.12 μmol.mg $prot^{-1}.h^{-1}$ (n = 5) and 1.15 ± 0.11 μmol.mg $prot^{-1}.h^{-1}$ (n = 5) assayed in cortical membranes from mice administered with methionine and saline (vehicle) respectively, a significant increase of 45% was recorded (according to Student *t* test, $P<0.05$). Then, acetylcholinesterase activity was 131 ± 11% (n = 4) in cortical membranes from mice injected with methionine, the addition of SST 10^{-6}M and SST previous cSSTA in equimolar concentrations reduced this enzyme activity in bothsituations at about 37%. Therefore, it may be concluded that somatostatin reduced the activity of acetylcholinesterase in cerebral cortex of mice subjected to this epigenetic model of schizophrenia and since its action was not blocked by the antagonist of somatostatin receptors, it is likely that thisinhibition was produced by a direct action on the enzyme.

186 (1028) I**NVOLVEMENT OF CB1 RECEPTOR IN MORPHINE WITHDRAWAL OF ADOLESCENT MICE PRENATALLY TREATED WITH A CANNABINOID AGONIST.**

Eliana Micaela Canero[1,2], Valeria Teresa Pedron[2], Andrés Pablo Varani[2], Amira Jazmin Aon[2], Delia Beatriz Soriano[3], Laura Romina Caltana[3], Herminia Alicia Brusco[3], Graciela Noemí Balerio[1,2].
[1]ININFA (UBA - CONICET). Junín 956 5°P (1113), C.A.B.A. [2]Cát. de Farmacología – (FFyB-UBA). Junín 956 5° P (1113), C.A.B.A. [3]IBCN (UBA - CONICET). Facultad de Medicina UBA. Paraguay 2155 3er piso. CABA 1121.

There is evidence that prenatal exposure to cannabinoids agonists induce long-term alterationsin the opioid system. In previous studies from our laboratory performed in adolescent CB1 KO mice prenatally treated with vehicle (VEH), we reported a decrease in the expression of naloxone (NAL) precipitated morphine (MOR) withdrawal syndrome compared to their wild-type (WT) littermates. In addition, WIN prenatal treatment induced an attenuation of MOR withdrawal syndrome in the WT,but not in the CB1 KO mice.

The aim of the present study was to evaluate the effect of the prenatal exposure to the cannabinoid agonist, WIN, in c-Fos expression of certain brain areas of MOR withdrawn CB1 KO and WT adolescent mice.

Pregnant female CB1 KO and WT mice received WIN (0.75 mg/kg, s.c.) or VEH once daily from 5th gestational day to parturition day. From the postnatal day 25 forward, mice were treated for 9 days with MOR (2 mg/kg, i.p.) or saline (SAL) twice daily. On the tenth day, the animals received NAL (6 mg/kg, i.p.) or SAL 60 min after the last injection in order to precipitate the withdrawal. Thirty minutes after NAL or SAL administration, mice were perfused with 4% paraformaldehyde solution.Brains were removed and coronal frozen sections were made at 30 μm on a freezing microtome to perform the c-Fos immunohistochemistry.

WT but not KO adolescent mice prenatally treated with VEH showed a decrease in c-Fosexpression during MOR withdrawal syndrome in Cg ($p<0.01$) and CA3 ($p<0.05$). On the contrary, no significant differences were observed in c-Fos expression of both WT and KO adolescent mice prenatally treated with WIN.

These results suggest that the behavioural alterations induced by prenatal exposure to the cannabinoid agonist WIN, observed in our previous studies, could be related to the changes observed in c-Fos expression during MOR withdrawal syndrome.

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187 (1079) **ADDRESSING THE MECHANISM OF PRIMING OF DOPAMINE RECEPTORS AND ITS EFFECT ON DRUG-INDUCED DYSKINESIA**

Gimena Gomez[1], Pedro Ballestero[1], Sara Sanz-Blasco[1], Maria Alejandra Bernardi[1], Oscar Gershanik[1], Irene Taravini[2], Juan Ferrario[1].

[1]Laboratorio de Parkinson Experimental. ININFA. UBA-CONICET. [2]Laboratorio de Neurobiología Experimental. FBRO-UNER.

Parkinson's disease (PD) is characterized by dopamine (DA) depletion in the striatum and the gold standard treatment is still the use of *3,4-dihydroxyphenyl-l-alanine* (L-DOPA), despite its prolonged use induces severe motor complications, known as L-DOPA-induced dyskinesia (LID). Not all dopaminergic agonists share the same capacity to induce dyskinesia, and D2 agonists are frequently used in clinical practice because its low dyskinetogenic profile. *Priming* is defined as the behavioral and molecular sensitization occurring after the first exposure to L-DOPA or full DA agonist. Priming is not necessarily associated with dyskinesia but once it has occurred, lower doses of L-DOPA or dopamine agonists are enough to induce dyskinesia. Priming has been extensively studied by pharmacological approaches but the molecular mechanism underlying is not well understood. The aim of this work is to understand the mechanisms of priming and to evaluate the effect of selective D1 or D2 receptor stimulation on subsequent DA agonist responses. This knowledge will contribute to elucidate the mechanisms underlying dyskinesia. To reach this goal, we developed a mice model of PD. C57 mice were injected with 6-OHDA and received dyskinetogenic doses of L-DOPA or saline for 3, 5 or 7 days to induce priming. We tested the effect of different days of priming to induce Quinpirole-induced dyskinesia. Using a 3 day priming protocol, a group of mice was tested with different doses of the D2 agonist Quinpirole or L-DOPA. We determined the score of dyskinesia and analyzed striatal molecular changes by immunohistochemistry, including TH (to determine dopaminergic loss), pERK, FosB and cFos (molecules related to LID). We compared dyskinetic potentials of L-DOPA or Quinpirole treatment, as well as their ability to induce expression of markers that have been already related to dyskinesia.

Our results represent the first step to explore the molecular mechanisms of priming and the differential induction of dyskinesia by selective or full dopaminergic agonist.

188 (1080) **THE FYN INHIBITOR SARACATINIB REDUCES LEVODOPA INDUCED DYSKINESIA IN PARKINSON'S DISEASE**

María Alejandra Bernardi[1], Sara Sanz Blasco[1], Gimena Gomez[1], Melina Bordone[1], Ana Damianich[3], Irene Taravini[2], Elena Avale[3], Oscar Gershanik[1], Juan Ferrario[1].

[1]Laboratorio de Parkinson Experimental. IININFA, UBA-CONICET. [2]Laboratorio de Neurobiología Experimental. FBRO-UNER. [3]Laboratorio de Terapeutica Experimental en Procesos Neurodegenerativos. INGEBI-CONICET.

Levodopa-induced dyskinesia (LID) is one of the major side effects of the treatment of Parkinson's disease (PD), a neurodegenerative condition caused by progressive degeneration of dopaminergic neurons in the SubstantiaNigra pars compacta.

The aim of this study was to pharmacologically manipulate the tyrosine kinase Fyn as a novel target to control LID. We investigated the role of Fyn in the development and maintenance of dyskinesia in PD and tested it as a potential pharmacological target to prevent LID. To reach this goal we induced LID in a mouse model of PD by dopaminergic denervation induced by 6-OHDA. We evaluated the degree of dopaminergic loss by observation of the spontaneous rotation, and cylinder test quantification. Mice that were correctly denervated were randomly assigned to receive either vehicle or the Fyn-inhibitor Saracatinib (AZD0530), which was given starting 8 days before L-DOPA or 7days after LID has been started. In both cases, Saracatinib and L-DOPA treatment were continued until the end of the protocol, which last for 25 days on which LID were quantified every 3 days. Finally, dopaminergic denervation was carefully determined postmortem, to ensure the same level of degeneration in all groups. At the experimental doses used in this procedure, we found that pre-administration of Saracatinib prevented by 30% the development of LID. But in the case that co-administration began one week after L-DOPA, when LID were fully developed, the treatment was not effective. This data strongly supports the idea that Fyn is involved in the development of LID in our model of PD, maybe avoiding the consolidation of some maladaptive process. However, when LID are already established, the inhibition of Fyn seems to have no effect. We propose to block Fyn as a novel pharmacological target to manage LID in PD patients, although further work is still necessary to improve Fyn inhibition, either by development of new drugs or by improving Saracatinib delivery.

189 (468) **IMPACT OF 5-HTTLPR POLYMORPHISM IN THE LETHALITY AND IMPULSIVENESS OF SUICIDAL BEHAVIOR IN PATIENTS WITH MAJOR DEPRESSION**

Angeles Romina Arena[1], Arnaldo Raúl Armesto[1], Soledad Puppo[1, 3], Leandro Grendas[1, 2], Federico Rebok[1, 2], Demián Rodante[1, 2], Gisela Lado[4], Alicia Portela[4], Patricia Vidjen[4], Andrea Emilse Errasti[1], Federico Manuel Daray[1, 2].

[1]Instituto de Farmacología, Facultad de Medicina, Universidad de Buenos Aires [2]Hospital Neuropsiquiátrico, "Braulio A. Moyano", Ciudad de Buenos Aires [3]Hospital de Clínicas "José de San Martín", Ciudad de Buenos Aires [4]Hospital Neuropsiquiátrico, "José Tiburcio Borda", Ciudad de Buenos Aires.

Suicidal behavior is a complication of psychiatric illness, most commonly observed in major depression. This behavior is comorbid with other disorders like violence, anxiety and aggressive impulsiveness. These disorders have been related to a dysfunction in the serotonergic pathway, which was also considered as a biological substrate of suicidal behavior. Due to twin and adoption studies there is evidence that suicide has a genetic background and, because of that, several genes related with serotonin neurotransmission were evaluated in order to find genetic markers. One of the most studied is a functional polymorphism in the promoter region of the serotonin transporter gene (5-HTTLPR) that is being composed of a 44bp insertion/deletion which determinate a long allele (L) or a short one (S). The alleles differ in the transcription of the SCL6A4 gene and have differences in the efficiency of reuptake. The current study was observational, descriptive, transversal and multi-centric.The project aims to evaluate the relationship between 5-HTTLPR genotypes and suicidal behavior in patients with major depression. Patients were interviewed by a psychiatrist belonging to our research group. A suicide attempt was defined as an intentional self-harm in which there is intent to die. Genomic DNA was obtained from blood samples of 86 patients using spin-columns, amplified with PCR and resolved by electrophoresis in agarose gel. The study was approved by the Research Ethics Committee and all patients enrolled gave written informed consent. One-way analysis of variance and a likelihood ratio test were performed as statistical analysis. Results suggest a positive association between SS genotype, impulsiveness $p<0.1$ and low lethality in the suicide attempt $p<0.1$. These results suggest a cluster of patients with a genetic substrate that express a particular type of suicidal behavior (impulsive and with low lethality).

190 (992) **BACLOFEN PRETREATMENT PREVENTS BEHAVIORAL AND MOLECULAR CHANGES INDUCED BY MORPHINE CONDITIONED PLACE PREFERENCE IN MALE, BUT NOT IN FEMALE MICE**

Valeria Teresa Pedrón[1], Eliana Micaela Canero[1,2], Amira Jazmín Aon[1], Andrés Pablo Varani[1a], Graciela Noemí Balerio[1,2].

[1]ININFA (UBA-CONICET) Junín 956 5°P (1113) C.A.B.A. [2]Cát de Farmacología (FFyB-UBA) Junín 956 5°P (1113) C.A.B.A.

It is known that sex differences exist with respect to various pharmacological responses of morphine (MOR). Previous studies from our laboratory showed sex differences in the behavioral, molecular and neurochemical responses to MOR withdrawal syndrome and they were related to an increased sensitivity to MOR effects in males. In addition, baclofen (BAC), a $GABA_B$ receptor agonist, was able to prevent the somatic expression and the molecular and neurochemical changes induced by MOR withdrawal syndrome in mice. On the contrary, little is known about BAC effects on the reinforcing properties on MOR in male and female mice. Taking this into account, the aims of the present study were: 1) to evaluate the effect of BAC pretreatment in the conditioned place preference (CPP) induced by MOR in prepubertal male and female mice; 2) to analyze the effect of BAC pretreatment in the expression of BDNF induced by the reinforcing effects of MOR (7 mg/kg, sc) in prepubertal male and female mice in order to rule out the influence of sexual hormones. The CPP paradigm consists in three phases: pre-conditioning, conditioning and post-conditioning. Brains were obtained by transcardial perfusion immediately after the post-conditioning session to perform BDNF immunohistochemistry. Our results showed that MOR administration was able to induce CPP in both male and female mice ($p<0.01$ and $p<0.001$, respectively). BAC pretreatment was able to prevent the reinforcing effect of MOR only in male mice ($p<0.01$). Moreover, these reinforcing effects of MOR were associated with a decrease in BDNF expression in Cg ($p<0.05$);NAcSh ($p<0.01$) and CA3 ($p<0.05$) only in male mice. BAC pretreatment was able to prevent these changes in BDNF expression. In conclusion, our results suggest that $GABA_B$ receptors could have a regulatory role in MOR reinforcing property and BDNF expression in male but not in female mice. UBACyT N° 20020120100244 y PIP N°: 00269.

191 (1020) **POSITIVE EFFECTS IN DOPAMINERGIC NIGROSTRIATAL SYSTEM BY PROGESTERONE IN HEMIPARKINSONIAN MODEL WITH 6-OHDA**.

Eliana Gaglio, Roberto Yunes , Ricardo Cabrera.*/ NBIOMED Universidad de Mendoza PROBIOL Universidad Nacional de Cuyo IMBECU CONICET.*

Parkinson's disease is a progressive disorder that involves domapinergicnigrostriatal neuronal death. One of the main proteins that take part of the neurodegeneration is alpha synuclein (alpha syn). In an animal model of hemiparkinsonism with a neurotoxin 6-HODA we previously observed thatprogesterone subcutaneous treatment delay the appearance of motor signs. The objectives of this work were to test the alpha syn expression in different groups of rats, to analyze how does it work under progesterone effects and evaluate possible genomic effects of this protein.

We worked with male rats Sprague Dawley strain, adults of 250 – 300gr, of 8 weeks postinjured with 6-OHDA in left striatum. Rats of different groups were evaluated by 2 behavioral tests: Raturn and Stepping, to verify its alteration in motor dysfunction. Rats were divided into 3 groups: 1) sham group (vehicle injured, n:8), 2) hemiparkinsonian group (HP) (6-HODA injected, n:8), 3) progesterone treated group (6-HODA , progesterone injected for 3 days a week after theneurotoxic injury, 4mg/day/kg of rat, n:8). Results were expressed as ± S.E.M by ANOVA I and Test of Student relying on the analysis. We observed a significant rise in alpha syn expression. We also found that progesterone treatment diminished significantly ($p< 0.05$) the expression of alpha syn with respect to HP group, as sham levels. With this results we proposed to find weather progesterone treatment induce any change in alpha syn expression due to genomic effects of alterations in nucleotide sequence. DNA was extracted from substance nigra and striatum of all groups of rats and made a sequencing analysis. Results showed no changes in nucleotide sequence of HP groups regarding progesterone treatment group. We concluded that a possible mechanism of action in overexpression observed of alpha syn and the delay in appearance of motor signs could be associated to a neuroprotective effect not genomic of progesterone or its metabolites.

SÍNDROME METABÓLICO / /METABOLIC SYNDROME

192 (752) **ANALYSIS OF THE INTERACTION BETWEEN HYPERGLYCEMIA AND OXIDATIVE STRESS ON IMMUNE CELLS OF PATIENTS WITH TYPE 2 DIABETES**

Esther Gerez[2], Martín Torraco Llanes[1], Natalia Laguarde[3], Alejandro Serra[4], Gustavo Frechtel[3, 5], Miriam Ruth Wald[1].
[1]BIOMED, UCA -CONICET [2]CIPYP, UBA-CONICET [3]Hospital Sirio Libanés [4]Cátedra de Farmacología, Facultad de Medicina, UCA [5]INIGEM, UBA-CONICET.

Diabetics are predisposed to infections, suggesting an association between diabetes and immunosuppression, being hyperglycemia the main factor involved. High glucose (HG) induces reactive oxygen species (ROS) and oxidative stress is involved in the pathophysiology. However, there are patients who recover normally from infections. Genetic conditioning could be a factor involved. In a previous work we observed that lymphocytes isolated from BALB/c mice were sensitive to the deleterious effect of hyperglycaemia, while C57 were resistant. Oxidative stress was implicated in this deleterious effect. In the present study we start to analyze the interaction between hyperglycemia and oxidative stress on immune cells in type 2 diabetic patients (D2). Antioxidant status was estimated by measuring glutathione content (GSH) and oxidative stress by measuring ROS generation in lymphocyte and monocytes isolated from blood samples (basal). The immune function was studied by proliferation assays. The influence of hyperglycaemiawas evaluated by preincubation of mononuclear cells in a HG-containing medium. 23 D2 and 11 clinically healthy individuals were evaluated. 14 D2 were metabolically decompensated with glycosylated hemoglobin (HbA1c) > 8.5 and 9 had HbA1c < 8.5. The age ranged from 65-77 years old, with 14 males and 9 females. From the 23 patients analyzed, only 9 (39%) presented lower basal GSH content (range10-18% of control) and an increment in ROS generation (range 29-122% of basal) after an incubation with HG. In parallel, these patients presented a decrease in lymphocyte proliferation after incubation with HG ($p<0.05$). There was no correlation between these parameters and the level of HbA1c in neither sex. These results encourage the need to consider the state of oxidative stress in orderto understand the predisposition of D2 patients to immunosuppression.

193 (964) **DIFFERENTIAL EFFECT BY SEX OF A HIGH FAT DIET IN C57/BL6J MICE BEHAVIOUR AND GLUCIDIC METABOLISM. INFLUENCE OF CHRONIC MILD STRESS**.

Andrés Prochnik[1], María Rosa González Murano[1], Miriam Ruth Wald[1], Ana María Genaro[1,2].
[1]BIOMED-UCA-CONICET [2]Primera Cátedra de Farmacología, Facultad de Medicina, UBA.

In the past decades obesity have turned into one of the most common diseases, spreading worldwide. In recent clinical studies obesity has been associated with impairment in cognitive function and pointed as a risk factor in the development of dementias, such as Alzheimer's disease. In the present study we analyze if a high fat diet (HFD)can lead to behaviour alterations and wether these are associated with metabolic changes. Furthermore, we investigated if stress aggravates these alterations. Male and female C57/BL6J mice were fed with a high fat diet (35% w/w) or a standard diet (SD, 5% w/w) during 6 months. Two months after starting the diet, mice under HFD were subdivided in two groups: one received chronical mild stress (CMS) and the other was left undisturbed. By the end of the treatment we measured body weight, performed behavior test relevant to anxiety, context and spacial memory and run a glucose tolerance test. Results

show that mice under HFD had a higher body weight (Male: N=9; p<0,05. Female: N=9; p<0,01); which reverted with CMS in both sex. Under HFD, males had glucose intolerance (p<0,05), which worsen with CMS (p<0,05) and exhibit a reduced discrimination ratio in spatial object recognition (SOR) (N=4; p<0,05) while CMS caused a worst performance in SOR and a anxious-like behaviour (N=9. p<0,01). We also found a negative correlation between glucose intolerance and SOR results (Correlation:-0,6257;p<0,05) and glucose intolerance and anxious-like behaviuor (Correlation:-0,4798;p<0,05). On the other hand, the treatments seem to have no effect on females neither in their metabolism nor their behaviour. Evidence shows that, in this model, both male and female have a body weight increase with a HFD. However, only male develop disorders associated with overweight, both metabolic and behavioural. Moreover, in males stress exposure has a paradoxical effect preventing weight gain but increasing the HFD deleterious effects on metabolism and behavior.

PRESENTACION DE POSTERS SAIC II / SAIC POSTER PRESENTATION II

NEUROCIENCIAS II / NEUROSCIENCES II

194 (490) **IRON OVERLOAD AND CHLORPROMAZINE EFFECT ON OXIDATIVE STRESS IN BRAIN**

Natacha Estefania Piloni[1,2], Susana Puntarulo[1,2].
[1]Instituto de Bioquímica y Medicina Molecular (IBIMOL)-CONICET. [2]Universidad de Buenos Aires (UBA).

Schizophrenia and other psychotic disorders are widely treated with chlorpromazine (CPZ), which is frequently associated with several adverse side effects like metabolic disorders due to changes in lipid and glucose metabolism. Oxidative stress triggered by Fe overload was recently reported to be followed by beneficial responses, that were described as an hormetic effect. Fe administration pattern is a critical factor to determine the type of alterations to the cellular oxidative metabolism observed. The hypothesis tested here was that the pattern of administration of Fe affects the response to CPZ in the rat brain. Male Sprague-Dawley rats (180 ± 10 g) were used. In the acute Fe overload, a single dose of 500 mg Fe-dextran /kg was intraperitoneally (ip) injected. In the subchronic Fe overload, 6 doses of 50 mg Fe-dextran /kg was ip injected every second day. In both protocols, control rats were sham-injected ip with saline solution. At 8 h after the single or the6th dose in the acute and subchronic Fe treatment, respectively, a single dose of 10 mg CPZ/kg was ip injected. At specific time points after CPZ treatment (1, 2 and 4 h), brain was removed. Lipid radical (LR$^{\bullet}$) generation rate, determined by Electron Paramagnetic Resonance (EPR), was increased at 1 and 2 h post administration of CPZ, and returned to control values at 4 h. Catalase (CAT) activity, assayed spectrophotometrically, was no affected by CPZ administration. After acute and subchronic Fe treatment, a single dose of CPZ did not lead to modifications in LR$^{\bullet}$ generation rate from 1 to 4 h post CPZ administration. However, after acute Fe overload, CAT activity was not modificated by CPZ administration, but after subchronic Fe treatment, a single dose of CPZ lead to an increase in CAT activity at 2 and 4 post administration. These results suggest that the hermetic effect is triggered by Fe overload by both administrations protocols, nevertheless it seems that the mechanisms are different.

195 (626) **BLUE LED LIGHT IRRADIATION AND A2E PERTURB MITOCHONDRIAL MORPHOLOGY AND FUNCTION IN RETINAL PIGMENT EPITHELIAL CELLS. NEW INSIGHTS INTO THE PATHOGENESIS OF AGE-RELATED MACULAR DEGENERATION**

Agustina Alaimo[1], Juan Marco Bujjamer[2], Guadalupe Garcia Liñares[3], Roxana Mayra Gorojod[1], Soledad Porte Alcon[1], Alicia Baldessari [3], Hernan Edgardo Grecco[2], Monica Lidia Kotler[1].
[1]Laboratorio de Disfunción Celular en Enfermedades Neurodegenerativas y Nanomedicina. Departamento de Química Biológica. Facultad de Ciencias Exactas y Naturales. Universidad de Buenos Aires. IQUIBICEN-CONICET. [2]Laboratorio de Electrónica Cuántica. Departamento de Física. Facultad de Ciencias Exactas y Naturales. Universidad de Buenos Aires. IFIBA-CONICET. [3]Laboratorio de Biocatálisis. Departamento de Química Orgánica. Facultad de Ciencias Exactas y Naturales. Universidad de Buenos Aires. UMYMFOR-CONICET.

Age-related macular degeneration (AMD) is a neurodegenerative disease of the elderly. AMD pathogenesis is characterized by retinal pigment epithelium (RPE) degeneration that progress with the light exposure-induced injury. RPE cells accumulate lipofuscin which contributes to their susceptibility to photo-oxidation. Blue light reaches deep into the eye causing cumulative retinal damage and increased AMD risk. Here, we studied the effect of blue light and A2E (major component of lipofuscin) on mitochondrial integrity in the RPE. Human ARPE-19cells were exposed to blue light (LED λ=445nm; 1.7mW/cm^2)for 1-30min and incubated for an additional 24h. The following parameters werestudied: mitochondrial metabolic activity (MTT assay), generation of ROS (DCFDAprobe) and superoxide ($O_2^{\bullet-}$) (MitoSOX probe) (fluorometry and fluorescence microscopy), mitochondrial mass and shape-changes quantification (Tom-20 ICC/ Mito-Morphology ImageJ Macro**).** Blue light significantly reduced RPE viability (1min: 89±6%, 5min:81±2%, 15min: 76±2% 30min: 52±5%) while increased intracellular ROS levels (1min:34±6%, 5min: 37±2%, 15min: 45±13%, 30min: 53±4%). An early increment in $O_2^{\bullet-}$ levels occurred after 1min (74%, p<0.01).Light-exposed cells showed a gradual mitochondrial mass reduction (e.g. 30min: 69%,p<0.05) suggesting a decreased biogenesis. Also, mitochondrial elongation and interconnectivity significantly diminished (e.g. 30min: 38%, p<0.001and 17%, p<0.05, respectively)and correlates with the appearance of small dots and donuts-like organelles instead of a tubular network. On the other hand, cells exposed to exhibited a dose-dependent decrease of cell viability(e.g. 10μM: 78±1%, p<0.01). Moreover, mitochondrial metabolic activity, $O_2^{\bullet-}$ generation and morphology indicated that A2E-laden cells were more susceptible to phototoxicity thanA2E-free cells. Our findings provide insights supporting the relevance of design mitochondrial quality-based therapies for AMD.

196 (629) **RETINOID X RECEPTORS ON SURVIVAL AND MODULATION OF INFLAMMATORY RESPONSE IN A MOUSE MODEL OF RETINITIS PIGMENTOSA.**

Olga Lorena German[1,2], Yanel Volonté[1,2], Andres Garelli[1,2], Victoria Belen Ayala-Peña[1,2], Nora Rotstein[1,2], Luis Politi[1,2].
[1]Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB)-CONICET. [2]Universidad Nacional del Sur (UNS), Dept de Biología, Bioquímica y Farmacia.

Retinal neurodegenerative diseases, which have no effective treatments, share as a final common step the death of photoreceptor cells (PhR). Inflammation has a role in these pathologies as well, involving cell types having immunomodulatory capacity such as Müller glial cells (MGC). Retinoid X receptors (RXR) have the capacity of modulate and integrate multiple cell functions; and their agonists have shown beneficial clinical effects in animal models of chronic inflammatory diseases. Since little is known about RXR participation in the retina, we assessed whether this receptors might prevent PhR death and/or inflammation.

Using a murine model of retinitis pigmentosa (rd mice), we analyze in vivo and in vitro the roles of RXR in retina degeneration. Our results using qRT-PCR show that the mRNA levels of the alpha isoform increased in the firsts postnatal days (PN) reaching the high levels at PN4 and then decreasing more in rd mice retina respect to their wt counterparts. This supports our previous data obtained by immunohistochemistry from retina slices. Noteworthy, RXR activation modulated the mRNA levels of all three RXR isoforms in mixed neuroglial cultures from rd retina. Moreover, it also delayed the onset of PhR apoptosis analyzed by TUNEL assay and decreased the protein expression of a glial marker for cell inflammatory response (GFAP) apparently without affecting its mRNA level. This last result led us to evaluate whether RXR could regulate anti-inflammatory response in the retina. Our preliminary

results suggest that RXR activation increased the transcription of the anti-inflammatory cytokine interleukin (IL)-10 in rd mixed neuroglial cultures. As a whole, the activation of RXR could promote survival of PhR either by direct action on them, or indirectly by modulating the inflammatory response of MGC.

197 (776) **PHARMACOLOGICAL EFFECTS OF TESTOSTERONE IN A MOUSE MODEL OF MOTONEURON DISEASE**

Agustina Belén Lara[1], Gisella Mariana Gargiulo Monachelli [1], María Meyer[1], Laura Inés Garay[1,4], Noelia Di Giorgio [2], Victoria Lux-Lantos[2], Michael Schumacher[5], Rachida Guennoun[5], Alejandro Federico De Nicola[1,4], Maria Claudia González Deniselle[1,3].

[1]Laboratorio de Bioquímica Neuroendócrina, [2]Laboratorio de Neuroendocrinología. Instituto de Biología y Medicina Experimental-CONICET [3]Dto de Ciencias Fisiológicas, [4]Dto de Bioquímica Humana. Facultad de Medicina, UBA [5]U 1195 Inserm, University Paris-Sud and University Paris Saclay.

Wobbler (WR) mouse, a useful model for human amyotrophic lateral sclerosis (ALS), shows motoneuron degeneration with increased levels of intramitochondrial nitric oxide synthase(NOS) and astrogliosis in the spinal cord. Some steroids such as progesterone favor mitochondrial function and neuroprotection, while others are deleterious. Here, we determined endogenous testosterone (T) levels in male WRs and controls by radioimmunoassay and gas chromatography/mass spectrometry (GC/MS) at symptomatic stage. We demonstrated a strong decrease of T levels in serum, spinal cord and brain ($p<0.05$) in male WRs vs male controls. Therefore, a silastic tube containing T crystals was implanted s.c.in WR mice at symptomatic stage for 2 months. We analyzed in cervical spinal cord from male controls, WRs and WRs+T: 1) the% of vacuolated neurons, 2) the number of glial fibrillary acidic protein (GFAP)+ astrocytes, 3) the number of neurons and glial cells+ for NADPHd/NOSreaction, and 4) immunoreactivity (IR) for mitofusin-2 (Mfn2) and dynamin-like protein (DLP1), essential proteins in mitochondrial biogenesis. T serum levels and seminal vesicles weight were higher in WRs+T vs WRs ($p<0.001$) and vs controls ($p<0.01$), while hypophysis mass, a parameter sensitive to aromatizable androgens, was not affected by T. T reduced the% of vacuolated neurons in ventral horn ($p<0.05$vs WR), without changing GFAP+ astrogliosis. Hyperactivity of NADPH-d/NOS was reduced in glial cells from WRs+T vs WRs ($p<0.01$), but not in neurons. The IR for Mfn-2 and DLP1 was increased in the ventral horn of WR mice ($p<0.001$ and $p<0.01$),while T treatment only decreased DLP1 IR ($p<0.05$). In summary, supraphysiological T levels in WRs lessened neuronal vacuolation, NADPH-d/NOS activity in glial cells and DLP 1 but not Mfn2 IR. These effects may depend more on binding of Tto the androgen receptor rather than on T aromatization. Future studies are needed to disclose the usefulness of T effects on WR mice.

198 (704) **EARLY GABAPENTIN TREATMENT DURING THE LATENCY PERIOD REDUCES EPILEPTOGENESIS IN AN ANIMAL MODEL OF TLE**

Alicia Raquel Rossi, Gerardo Ariel Rosciszewski, Alberto Javier Ramos.

Instituto de Biologia Celular y Neurociencias Prof. E. De Robertis (IBCN), Facultad de Medicina. UBA.

Temporal lobe epilepsy (TLE) affects adult population but retrospective studies have shown in most patients an event of febrile seizures with status epilepticus (SE) during childhood followed by a latency period (LP) until the epileptic seizures in the early adulthood. Using an animal model of TLE we have previously shown that latency period brains presents neurodegeneration, macrophages infiltration and reactive gliosis. A short 4-days Gabapentin (GBP) treatment following the SE reduced neurodegeneration and reactive gliosis in the TLE model (Rossi et al 2013). GBP reduces microglial activation but also is an antagonist of the TPS-1 receptor probably involved in spurious sinaptogenesis proposed as the epileptogenic process. The aim of this work was to ascertain if the early GBP treatment after SE is able to increase the seizures threshold after the LP thus preventing the epileptogenesis. Adult male Wistar rats were subjected to the lithium-pilocarpine model of TLE and behavioral changes were evaluated according to the Racine scale. 24 h later animals received intraperitoneally (ip) 400mg GBP during 4 days. By 21 days after the SE animals were exposed again to repeated sub-convulsive doses of pilocarpine and the development of seizures were recorded. Animals treated with the 4-day GBP paradigm showed a reduced SE development (34% vs. 72%), 33% of them reached lesser Racine scale levels showing a fast and better recovery.Our results show that early GBP treatment after SE not only reduced neurodegeneration, neuroinflammation and reactive gliosis, but also may have an effect preventing the establishing of epileptic seizures after the LP. These results open the possibility of developing new treatment strategies for febrile seizures during the childhood.

199 (853) **INHIBITION OF GABAARHO1 RECEPTOR FUNCTION BY HISTAMINE**

Andrea N Beltrán González[1], Paula Zubiry[1], Alejandro Olaviaga[1], Daniel J Calvo[1].

[1]Laboratorio de Neurobiología Celular y Molecular. INGEBI-CONICET.

Histamine, a local mediator of the immune response, can also act as neurotransmitter and neuromodulator. Histamine-releasing neurons, located in the tuberomammillary nucleus of the hypothalamus, project all over the brain and are involved in the control of arousal, sleep, cognition, learning, appetite and endocrine output. Histamine actions are commonly mediated by metabotropic receptors, but several evidences indicated that histamine can also modulate the function of different ligand gated ion channels. For example, histamine gates homomeric GABAA-beta2 and GABAA-beta3 receptors with a higher efficacy than GABA and is a positive allosteric modulator of heteromeric GABAA-alfa1beta2 and GABAA-alfa1beta2gamma2L receptors and an inverse agonist of strychnine sensitive glycine receptors. In retinal bipolar neurons, ambient concentrations of GABA elicit tonic currents mediated by GABAA-rho1 receptors. The retina receives histaminergic input from the brain via retinopetal axons. The role of histamine in the retina is still far from being entirely understood. In the present study we analyzed the effect of histamine on responses mediated by GABAA-rho1 receptors. Homomeric GABAA-rho1 receptors were expressed in Xenopus laevis oocytes and GABA-evoked chloride currents were recorded by two-electrode voltage-clamp. Histamine inhibited GABAA-rho1 receptor responses. Inhibition was dose-dependent, reversible, voltage independent and depended on GABA concentration. Pre-incubation or repeated applications of histamine gave similar results. This is the first report of a direct modulatory action of histamine on tonic GABAA receptors. The underlying mechanism of action is currently under study.

200 (775) **NEUROLOGICAL AND INFLAMMATORY DISORDERS PRODUCED BY CROTALUS DURISSUS TERRíFICUS VENOM IN A MURINE MODEL.**

Adriana Mónica Cangelosi[1], Alipio Vasconcelos Esteves Pinto[2], Virginia Laura Mariconda[1], Jorge Goldstein[2], María Luisa Brero[1], Patricia Andrea Geoghegan[1].

[1]Centro Nacional de Control de Calidad de Biológicos, ANLIS "Dr Carlos G. Malbrán" [2]Laboratorio de Neurofisiopatología, Dpto de Fisiología Fac. de Medicina, UBA.

The ofidic species *Crotalusdurissus terrificus* (*Cdt*), popularly known as rattlesnake, is distributed in northern and central areas ofArgentina, and is responsible for almost 3% of all snakebites in this country. These incidents may lead to physical and/or psychological sequelae or even death by paralysis of skeletal muscles and cardio-respiratory arrest as a consequence of a neurotoxic venom. As far as we know, CNS dysfunction following *Cdt* bites has never been studied. Thus, the aim of this study was to evaluate the effects of the *Cdt* venom on mice cortical motor and striatal neurovascular units, and secondly, to determine whether inflammatory mediators are involved. NIHmale mice were injected

intravenously with vehicle (control) or 1 LD_{50}(5.66 µg) of *Cdt* venom. Mice were intracardially perfused 3, 6, 20, 24 or 48hours after the above mentioned treatments and their brains were subjected to immunofluorescence by using an anti-GFAP antibody to identify reactive astrocytes, or anti-NeuN (Neuronal nuclei marker) antibody to determine early signs of neuro degeneration. Another group of mice were subjected to measure cytokine levels in the brain and in serum by a flow cytometer (BD Accuri C6). The immunofluorescence assay showed that the venom altered the neurovascular unit of the cortex and the striatum, which included a significant reactive astrocytes and neurodegenerative events at 6, 20, 24 y 48 hours after *Cdt* venom treatment in comparison to controls ($p<0.05$). The maximum serum levels of INF-γ,IL-6, IL-4, IL-17A and IL-10 were observed at 24 and 48 hours post-injection($p<0.05$), while no significant differences were observed in mice treated brains in comparison to controls. In conclusion, intravenous *Cdt* venom damages mice CNS and increases pro- and anti-inflammatory cytokine serum.

201 (925) **DIETARY N-3 FATTY ACID DEFICIENCY INDUCES DEPRESSION-RELATED BEHAVIOR MEDIATED BY VASOPRESSIN**

Santiago Bianconi[1, 2, 3], María Belén Poretti[1, 3], Giulia Maestri[3], María Emilia Santillán[2], María del Rosario Solís[2], Helgi B Schiöth[3], Michael Williams[3], Graciela Stutz[2], Valeria Paola Carlini[1,2,3].

[1]Instituto de Investigaciones en Ciencias de la Salud (CONICET/FCM-UNC), Córdoba, Argentina. [2]Cátedra de Fisiología Humana, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Córdoba, Argentina. [3]Department of Neuroscience, Functional Pharmacology, Uppsala University, Uppsala, Sweden.

Adverse fetal and postnatal conditions, such as nutritional imbalance, can predispose to psychiatric disorder development inthe adulthood. Omega-3 (n-3) polyunsaturated fatty acid (PUFA) offer can modify the central nervous system development and function. Thus, we propose to study the effect of long term exposition to different dietary levels of n-3PUFA, from gestation to adulthood, among depression-related parameters. Three groups of albino Swiss female mice (during gestation-lactation) and then their male offspring (during weaning-adulthood; n˜10per group) were fed with C (control, adequate in n-3 diet; 7% soya oil; n-3:0.57%; n-6/n-3: 5.7), D (deficient in n-3 diet; 7% sunflower oil; n-3: 0%;n-6/n-3: 0) or E (excessive in n-3 diet; 7% blend oil: cod liver 60% + soya40%; n-3: 1.25%; EPA 0.43% y DHA 0.32% n-6/n-3: 1.29). At postnatal day 63,open field test was performed in order to study locomotor activity. Then, animals were sacrificed to determine plasma corticosterone levels by ELISA and gene expression of vasopressin (AVP) and corticotropin releasing hormone (CRH) -in hypothalamus- and their receptors AVPr1b, CRHR1and CRHR2 -in the pituitary gland- by RT-qPCR. The D group showed lower locomotor activity (crossing number: D 415 ± 37 *vs* C 195 ± 25; $p<0.05$)and gene expression of AVP ($F(23,2)=4.96$;$p<0.05$ *vs* C y E) and its receptor ($F(31,2)=7.27$; $p<0.05$ *vs* Cy E). Furthermore, themessenger level of CRH and its receptors as well as plasma corticosterone concentration did not change significantly. Our results show that n-3 PUFA deficiency could selectively modify the hypothalamic-pituitary-adrenal axis function and induce depression-related behavior.

202 (840) **THE INFLAMMATORY CONTRIBUTION OF SHIGA TOXIN 2 (STX2) AND LIPOPOLYSACCHARIDE (LPS) FROM ENTEROHEMORRHAGIC ESCHERICHIA COLI (EHEC) IN THE BRAIN STRIATUM OF MICE.**

Alipio Pinto[1], Adriana Cangelosi[2], Patricia Geoghegan [2], Jorge Goldstein[1].

[1]Universidad de Buenos Aires, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Instituto de Fisiología y Biofísica "Houssay" (IFIBIO), Facultad de Medicina, Buenos Aires, Argentina. [2]Centro Nacional de Control de Calidad de Biológicos (CNCCB), – ANLIS "Dr. Carlos G. Malbrán", Buenos Aires, Argentina.

Shiga toxin from EHEC, causes hemolytic uremic syndrome and acute encephalopathies. The striatum is frequently affected in patients infected with EHEC. In addition to Stx2, LPSis released by the bacteria and may also contribute to this pathology. The aim of this study was to determine: i) whether Stx2 activates the microglia, ii) whether Stx2 induces a demyelination process, iii) whether LPS exacerbates the effect of Stx2, and iv) the participation of TNF-α. NIH male mice were injected intravenously with vehicle (control),Stx2, LPS and Stx2+LPS. The animals were treated either with intraperitoneal(i.p.) injection of Dexamethasone or with saline solution. Mice were intracardially fixed and their brains were subjected to immunofluorescence with an anti-IBA1 antibody to identify the microglia, and an anti-MBP antibody to determine the myelins heath condition. Also SD male rats were injected via intracerebroventricular with Stx2 (20 pg/gr) or Stx2+Etanercept (3.10 ηM, soluble TNF-α receptor) to immunolocalize Stx2 in striatal neurons by immuno-goldelectron microscopy. Stx2+LPS maximally increased microglial activation and decreasedthe expression levels of MBP in comparison to controls ($p<0.05$).Dexamethasone significantly reversed the changes observed following the treatments with the toxins on IBA1 and MBP immunofluorescences. Etanercept reduced the immuno-gold particles that corresponded to Stx2 in striatal neurons($P<0.05$). We concluded that Stx2 induces microglial hyperplasia, hypertrophy and demyelination; LPS enhances the deleterious action of Stx2; Dexamethasone and Etanercept treatments determined a pro-inflammatory compromised generated by these toxins, in particular TFN-α.

203 (974) **DISSECTING THE DIFFERENTIATION PROFILE OF HIPPOCAMPAL NEURONS IN THE VPA RAT MODEL OF AUTISM: AN IN VITRO APPROACH**

Marianela Evelyn Traetta[1, 2], Martin Gabriel Codagnone[1, 2], Nonthue Alejandra Uccelli[1], Sandra Zárate[3], Analía Reinés[1, 2].

[1]Instituto de Biología Celular y Neurociencias Prof. E. De Robertis (IBCN)-UBA-CONICET [2]Cátedra de Farmacología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires [3]Instituto de Investigaciones Biomédicas (INBIOMED)-UBA-CONICET.

Autism spectrum disorders (ASD) are a group of neurodevelopmental disabilities characterized by impairments in social interaction and stereotyped behaviors. Prenatal exposure to valproic acid (VPA), a well validated ASD animal model, mimics the main behavioral and neuroanatomical alterations found in these disorders. Regarding the cellular and molecular changes seen in the hippocampus of VPA rats, we previously reported a decrease in synaptic protein synaptophysin (SYN) and the polysialylated form of the neural cell adhesion molecule (PSA-NCAM). We hypothesized that prenatal VPA exposure may modify neuronal differentiation and/or synaptic formation and maturation either directly by epigenetic changes or indirectly through mechanisms involving other cells. Therefore, we tested whether in vitro postnatal hippocampal neurons from VPA rats show similar synaptic and PSA-NCAM expression patterns as those observed in vivo. An in vitro approach was performed by culturing hippocampal neurons from P1-P2 rat pups after in utero (E 10.5) VPA (500 mg/kg) or saline exposure. In order to study neuronal differentiation and synaptic formation, a time course was performed by fixing neurons at different days in vitro (DIV). During early neuronal differentiation in vitro (DIV 3-5), SYN immunostaining was increased in the VPA group. Notably, neurons from this group showed increased actin immunostaining accompanied with abundant dendritic and axonal filopodia. Not all synaptic proteins were increased since PSD-95 levels were lower than in control neurons. Later in culture (DIV 14), in the VPA group, a reduction was found both in SYN puncta area and number as well as a decrease in PSA-NCAM immunoreactivity. To sum up, we observed transient changes in hippocampal postnatal neurons from VPA animals which correlate with in vivo founding. Our results suggest that the early increase in SYN expression and actin sprouting might well represent a mechanism to promote synapse formation.

204 (887) **ROLE OF ESTRADIOL IN ANIMALS PRENATALLY EXPOSED TO AMPHETAMINE ON STRESS RESPONSE**

AND DOPAMINE RECEPTORS IN THE NUCLEUS ACCUMBENS

Gisela E. Pennacchio[1,2], Flavia Neira[1], Elisa O. Pietrobon[1], Graciela A. Jahn[1], Susana R. Valdez[1,2], Claudia Bregonzio[3], Marta Soaje[1, 4].

[1]Instituto de Medicina y Biología Experimental de Cuyo, (IMBECU) CCT-CONICET, Mendoza- [2]Facultad de Ciencias Exactas y Naturales (FCEN) Universidad Nacional de Cuyo. [3]Instituto de Fisiología, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo. [4]Departamento de Farmacología, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba.

The psychostimulants activate the same neurobiological substrates than stress response. Prenatal exposure to amphetamine (PEA) induces endurable changes that become manifest in the adulthood. These changes can be expressed as an altered central dopaminergic activity. Sex steroids modulate brain dopaminergic systems and may influence the neuroadaptive changes induced by amphetamine. Stress exposure induces dopamine release in the mesolimbic pathway and the activation of hypothalamic-pituitary-adrenal axis. Our aim was to evaluate the effect of estradiol in PEA animals in response to stress and dopaminergic receptors and tyrosine hydroxylase (TH) expression in nucleus accumbens. To this purpose, female rats were treated daily with amphetamine 2,5mg/kg i.p or saline during days 15 to 21 of pregnancy. Their offspring were ovariectomized (OVX) at day 60, treated with estrogen (E_2) (2 x 5ug/rat/24 h) or oil 15 days later, and subjected to immobilization stress during 2 h. Blood and tissue samples were collected to determine corticosterone by RIA and dopamine receptors (D_1R, D_2R, D_3R) and TH expression by real time PCR in extracts of nucleus accumbens. Stress exposure increased corticosterone levels in OVX (basal: 109,19 ±44,57 ng/ml vs stress: 214,41±21,49 ng/ml) and OVX+E_2 (basal: 157,24±33,87 ng/ml vs stress: 364,04±23,75 ng/ml), in both groups PEA blunted the corticosterone response to stress. PEA induced a decrease in basal D_3R expression and an increase in TH expression in response to stress, both responses were E_2 dependent. No change was observed in D_1R and D_2R expression. We conclude that the neuroadaptive response induced by PEA in nucleus accumbens is under estrogen influence. Moreover, an altered stress response should be considered between the neuroadaptive changes induced by PEA.

205 (993) **BEHAVIORAL CHARACTERIZATION OF A SURGICAL MODEL OF MENOPAUSE AND HORMONE REPLACEMENT TREATMENTS IN RATS**

Mercedes Imsen[1], Florencia Merino[1], Martín Codagnone[2], Camila Racana-Narváez[1], Adriana Seilicovich[1], Analía Reinés[2], Sandra Zárate[1].

[1]Instituto de Investigaciones Biomédicas (INBIOMED, UBA-CONICET), Facultad de Medicina, Universidad de Buenos Aires [2]Instituto de Biología Celular y Neurociencia "Prof. E. De Robertis" (IBCN , UBA-CONICET), Facultad de Medicina, Universidad de Buenos Aires.

Ovarian hormone loss during natural or surgical menopause is associated with cognitive impairment, mood alterations and higher risk of age-related disorders. Increasing importance is placed on the translational validity of animal models of human menopause and hormone replacement treatments(HRT) to discern risk *vs.* benefit of such therapeutic cinterventions. Ina previous work, we validated an animal model of long-term ovariectomy(OVX) and early estradiol (E2) andprogesterone (P4) replacement therapy in terms of steady hormone levels within the physiological range and biological effects in peripheral hormone-responsive tissues. In the present work, we aim to characterize our model by analyzing different behavioral parameters modulated by ovarian hormonal status.

To this aim, Wistar adult female rats were ovariectomized (OVX) or sham-operated (SHAM). In parallel, some OVX rats were s.c. implanted with silastic capsules containing 1mg E2and/or 50mg P4. After 12 weeks, the animals were subjected to behavioral tests to evaluate parameters of depression, anxiety and spatial working memory.

Twelve-week ovarian hormone deprivation induced depressive-like behavior and impaired spatial working memory, evaluated in the forced swimming test and the Y maze spontaneous alternation test, respectively. OVX rats also showed enhanced anxiety evaluated by the elevated plus maze test (OVX vs. SHAM, *p<0.05,Student's t test). Long-term HRT differentially impacted on such behaviors, generallyE2 alone having a better outcome than P4 alone or in combination with E2(ANOVA). Remarkably, no alterations in spontaneous locomotion were detected among groups.

Our findings show that depressive and anxiety-related behaviors as well as spatial working memory are affected by hormonal status and validate our model of chronic ovariectomy and HRT to further study the effect of estradiol and progesterone in brain areas highly responsive to ovarian hormones.

206 (952) **EFFECT OF ENDOGENOUS ADENOSINE ON ELECTRICALLY-EVOKED ACH SECRETION AT MAMMALIAN NEUROMUSCULAR JUNCTION (NMJ)**

Javier González Sanabria[1], Maximiliano Hurtado Paso[1], Adriana Losavio[1].

[1]Laboratorio de Neurofisiología, Instituto de Investigaciones Medicas A. Lanari-IDIM, Universidad de Buenos Aires (UBA), Consejo Nacional de Investigaciones Cientificas y Tecnicas (CONICET). Buenos Aires, Argentina.

At mammalian NMJ, ATP is co-released with ACh, and metabolized *via* ecto-nucleotidases to adenosine (AD), which modulates ACh release through presynaptic inhibitory A_1 or facilitatory A_{2A} receptors (R), according to the amounts of extracellular AD. Ouraim was to analyze the effect of endogenous AD on electrically-evoked AChsecretion when the nerve was stimulated at 0.5 Hz (50 pulses), 5 Hz (750pulses) or 50 Hz (750 pulses or 5 bursts of 150 pulses, 20-s inter burst interval). In phrenic-diaphragm preparations (CF1 mice), we studied the action of the specific antagonist or agonist for A_1R (DPCPX 0.1 µM, CCPA 500 nM) and for $A_{2A}R$ (SCH-58261 [SCH] 50nM or PSB-0777 [PSB] 20 nM) upon EPP amplitude. At 0.5 Hz (n=7) and 5 Hz (n=6),DPCPX did not change the amplitude of the 1° EPP, mean EPPs, last 20 EPPs and last20 EPPs/1° EPP ratio, while CCPA (0.5 Hz n=4; 5 Hz n=5) significantly reduced1° EPP amplitude, mean EPP amplitude and last 20 EPPs. At 0.5 Hz, CCPA did not alter last 20 EPPs/1° EPP ratio but at 5 Hz this relation was higher than in control ($p<0.05$). During continuous 50 Hz stimulation, DPCPX (n=6) did not modify 1° EPP amplitude but increased ($p<0.05$) mean EPP amplitude, last 20 EPP amplitude and last 20 EPPs/1° EPP ratio whereas CCPA (n=4) decreased 1° EPP and mean EPP amplitude ($p<0.05$), did not alter last 20 EPPs and increased last 20EPPs/1° EPP ratio ($p<0.05$). During intermittent 50-Hz stimulation the results were similar to those observed at 5 Hz (DPCPX n=8, CCPA n=4). On the other hand, SCH showed no effect on any amplitude parameter and PSB increased($p<0.05$) the amplitude of the 1° EPP, mean EPPs and last 20 EPPs and did not modify last 20 EPPs/1° EPP ratio at all stimulation frequencies (n=4 each). These findings suggest that during continuous high stimulation frequency enough endogenous AD is generated and accumulated in the synaptic cleft able to modulate Ach secretion *via* inhibitory A_1R.However, more AD is needed to activate facilitatory $A_{2A}R$.

207 (1033) **PROGESTERONE AND ESTROGEN PREVENTS THE APPEARANCE OF PREMOTOR AND MOTOR SIGNS IN MALE HEMIPARKINSONIAN RATS.**

Natalia Belén Gonzalez[1], Antonella Rosario Ramona Cáceres Gimenez[1], Maria Belen Mulle Bernedo[1], Sebastian García[1], Riacardo Cabrera[1].

[1]Instituto de Investigaciones Biomédicas - Facultad de Ciencias de la Salud- IMBECU-CONICET

Parkinson's disease is the second most prevalent neurodegenerative disease after Alzheimer. A 6-hydroxydopamine(6-OHDA) lesion simulates the motor deficits that occur due to a depletion of dopamine neurons in the nigrostriatal pathway, and has the advantage of presenting side-biased motor impairment. Some reports concerning motor deficits indicate a favourable response

to steroid administration in hemiparkinsonian animals. The objective was to evaluate the neuroprotection of the neuroactivesteroids, progesterone and estrogen, over motor and pre-motor signs induced bya 6-OHDA lesion. Sprague-Dawley male rats (280 – 320 g) 60 days old were used. Hemiparkinsonism was induced by 6-OHDA stereotaxic lesion in left *corpus striatum*. The experimental groups were: Sham (S), hemiparkinsonian (HP), and hemiparkinsonian treated subcutaneouslywith estrogen and progesterone (HPEP). Pre-motor signs were evaluated by aforce swimming test and motor signs by Raturn. Data were analysed byANOVA-1 and Tukey`s post hoc. A significance decrease ($p<0.001$) was observed in the time spend swimming on the force swimming test between S and HP groups; while there were no significant differences in S vs HPEP. The injection of 6-OHDA induced a significant change in the turning behaviour to the left side in HP ($p<0.001$) and HPEP ($p<0.05$)groups vs the S group. We conclude that the estrogen and progesterone treatment prevents the onset of depression like behaviour in HP rats and reduces the motor signs induced by a 6-OHDAlesion. Estrogen and progesterone exerts a neuroprotective effect against Parkinson disease.

208 (986) **CORPUS CALLOSUM CELLULAR AND STRUCTURAL ALTERATIONS IN THE VPA RAT MODEL OF AUTISM**

Nonthué Alejandra Uccelli[1], Martin Gabriel Codagnone[1, 2], María Victoria Rosato Siri[3], Marianela Evelyn Traetta[1, 2], Juana Pasquini[3], Analía Reinés[1, 2].

[1]Institute of Cell Biology and Neuroscience "Prof. De Robertis" UBA-CONICET, Bs. As., Argentina [2]Department of Pharmacology, FFyB, UBA, Bs. As., Argentina [3]Department of Biological Chemistry, IQUIFIB, FFyB, UBA-CONICET, Bs. As., Argentina.

Autism Spectrum Disorders (ASD) are a group of neurodevelopmental disabilities with unknown biological basis. A long-distance hypoconnectivity and short-distance hyperconnectivity hypothesis has emerged for these disorders. Structural and functional alterations in the corpus callosum (CC),the main structure that contains axonal tracts connecting brain hemispheres, have been reported in ASD patients. Changes in adhesion molecule expression, neuroinflamation and white matter alterations have been described in different brain areas both in ASD patients and animal models.

The aim of this work was to elucidate cellular and molecular alterations in CC sub-regions from rats prenatally exposed to valproic acid (VPA). For this purpose, at postnatal day 35, we studied cellular organization by DAPI staining in CC rostral body and posterior midbody. Also, we evaluated the expression of the neural cell adhesion molecule (NCAM) and its polysialilated form (PSANCAM) and studied glial cells by tomato lectin (microglia), glial fibrillary acidic protein (GFAP, atrocytes), CC1 (oligodendrocytes) and myelin basic protein (MBP) by immunohistochemistry. In VPA rats, we found decreased NCAM expression levels in rostral CC but not in the posterior region, accompanied by a robust diminution in PSA-NCAM levels in both CC sub-regions. We also observed cellular disorganization in the CC from VPA animals but no changes were found in microglia or astroglia. CC1 and MBP immunoreactivity decreased in both CC sub-regions of VPA rats. In summary, our results indicate long-distance connectivity defects as well as a spread reduction in mature oligodendrocytes and myelin content in the absence of microgliosis and astrogliosis in both CC sub-regions studied in VPA rats.

209 (1025) **WHICH COGNITIVE DEFICITS CHARACTERIZE THE ALZHEIMER'S DISEASE RAT MODEL MCGILL-R-THY1-APP?**

Federico Filippin[1], Magali Cecilia Cercato[1], Edgar Kornisiuk[1], Alejandra Aguirre[1], M. Veronica Baez[1,2], Sergio Nicolas Lavaise[1], Tomas gonzalez Garello[1], Nicole Burstein[1], Claudio Cuello[4], Diana Alicia Jerusalinsky[1,3].

[1]Laboratorio de Neuroplasticidad y Neurotoxinas, Instituto de Biología Celular y Neurociencia, CONICET, UBA. Paraguay 2155 3th floor, CABA, Argentina. [2]Universidad de Buenos Aires. Facultad de Medicina. Departamento de Histología, 1UA de Biología Celular, Histología, Embriología y Genética. Buenos Aires, Argentina. [3]Universidad de Buenos Aires. CBC. Buenos Aires, Argentina.

Alzheimer's disease (AD) is the major neurodegenerative disorder affecting aging people. Itis characterized by extracellular amyloid plaques and intracellular neurofibrillary tangles in AD brains. One of the most accepted hypothesis to explain AD is based on Ab peptides overproduction, which aggregates in oligomers (ABOs), then in fibrils and finally in plaques. ABOs bind to or near different postsynaptic receptors causing oxidative stress in neurons by activating several cascades, ending in neuronal death. Circulating ABOs rise inflammation by increasing reactive glia. Altogether, these mechanisms would explain cognitive alterations in AD. We characterized memory deficits in McGill-R-Thy1-APP (TG) rat, which expresses human amyloid precursor protein (hAPP) with two ordinary mutations (Swedish and Indiana) that led to Ab overproduction. About one year old hemizygous TG rats bearing only one copy of the mutated hAPP with increased intracellular levels of AB and their wild type (WT) littermates, were trained in various tasks to discriminate putative aging deficits from those due to transgenicity. TG and WT rats we reevaluated in open field (OF) habituation, step-through inhibitory avoidance(IA), new object location (OL) and recognition (OR). There were no differences between WT and TG rats in OF exploration, both showing long-term memory (LTM) habituation to the environment. TG were unable to remember the IA task in contrast to WT, which performed well 24 h after training. Neither WT nor TG seemed to discriminate OL at variance with younger animals. WT showed short-term memory (STM) and LTM of OR,while TG only showed STM for OR, being unable to form LTM of this task. Hence, this TG rat could not learn/consolidate memories with spatial and/or aversive components like IA and OR. Therefore, it seems to be an appropriate model for investigating early steps of AD and for evaluating potential therapies to repair cognitive dysfunctions in this sort of pathologies.

210 (575) **AGGRESSIVE BEHAVIOUR AND LOCOMOTOR ACTIVITY IN MALE SPRAGUE DAWLEY RATS WITH SEROTONINERGIC DEPLETION**.

Maria Belen Mulle Bernedo, Sebastian García, Ricardo Cabrera.

Instituto de Investigaciones Biomédicas - IMBECU-CONICET- Universidad de Mendoza.

The serotoninergic system modulates appetitive, motivational and aggressive behaviours. The last one is particularly associated with territorial defence and the increase of aggressiveness. The reduction of 5-HT synthesis by drug has proven effective in increasing aggressive behaviour, in strains and species prone toit, and locomotion activity.The aims were: 1- to describe the aggressive behaviour induced by the tryptophanhydroxylase inhibition by parachlorophenylalanine (pCPA) in a strain characterized by low aggressiveness and 2- to examine dose/day response on aggressive behaviour and locomotor activity.Male Sprague-Dawley rats 60 days old were used. The animals were divided into 5groups naïve, saline control and pCPA treated rats (300 mg/kg, i.p) evaluated on the 3rd and 6th day after. We evaluated intermale aggression with smaller males test and 5 minutes before we tested locomotor activity.The aggressive behaviour was measured as the persecution latency time (PL) and presence of tromping, bites, attempted mounts and lateral threats (AB). We also measured non-social interaction (freezing, lying, and sitting), social interaction(sniffing and grooming) and locomotor activity. The test was recorded and the videos analysed with the Kinovea 0.8.15 software. Data were analysed by ANOVA I and II and Tukey's post Hoc test. We observed a significant decrease in PL ($p \leq 0.05$) and a significant increase ($p \leq 0.05$) in non-social interaction between the treated, control and naïve groups. There was a significant increase ($p \leq 0.05$) in AB between the treated group tested on the 3rd and 6th day. There were no significant differences in social interaction and locomotor activity.The depletion of 5-HT affect PL, AB and non-social exploration. AB is related to the day/dose response even in a strain with low aggressiveness. Aggressive behaviour, as we describe, is related both to serotonergic depletion and to dose/day response, while there is no incidence in locomotion activity.

211 (2045) **HIPPOCAMPAL-RELATED BEHAVIORAL ALTERATIONS INDUCED BY PRENATAL STRESS COULD BE PARTIALLY REVERTED BY ENRICHED ENVIRONMENT.**

Mariano Enrique Ramborger[1], Maria Rocio Larreche[1], Ana María Genaro[2], Maria Aurelia Zorrilla-Zubilete[1,2].
[1]Lab. Neuropsicofarmacología, Centro de Estudios Farmacológicos y Botánicos (CEFyBO-CONICET), UBA, Buenos Aires, Argentina. [2]1a. Cátedra de Farmacología, Facultad de Medicina, UBA, Buenos Aires, Argentina.

During neurodevelopment, mice have an increased susceptibility to the deleterious effects of prenatal time insults, which have an impact on memory, learning and anxiety-related behaviors. Some of these changes are associated with an alteration of the HPA axis response. We aim to evaluate the effect of enriched environment (EE) on hippocampal-related behavioral impairment induced by prenatal restraint stress.Pregnant female Balb/c mice were individually restrained 2 hours/day, since the 14th day of gestation until delivery. Offspring were initially divided into different groups: prenatal stress (PS), no prenatal stress (C), in turn each group was treated with EE. The offspring were testing 90 days after birth.Balb/c subjected to PS showed impairment in habituation memory to an Open Field Test, and this was reverted meaningfully by the EE ($p<0.05$). In addition, an increase in anxiety behaviors was observed in PS mice ($p<0.05$), which was reverted by EE in the elevated plus maze test. In an Object in place test, PS performance in the discrimination task was altered when compared to control mice ($p<0.05$), and EE could not revert this disability. Moreover, a significant increase ($p<0.05$)in glucocorticoid receptors (GR) was found in both the hippocampus and lymphoid cells of PS mice, but was reverted by EE, up to control levels. Corticosterone plasma levels were increased in acute stress mice and in PS+acute stress mice, the first showing higher levels. EE has proven successful in reversing this effect in the PS group. In conclusion, these results support the idea that PS induces changes in the HPA axis, with a subsequent altered response to stress exposure in the adult life. The behavioral alteration provoked by PS could be related to higher levels of GR in the hippocampus and lymphatic ganglia. These changes were partially reverted by EE. Finally, it is important to note that lymphocytes could be peripheral markers of HPA alteration induced by PS. Funding PIP 112201301 00409 and UBACyT 20020130100131.

AACYTAL: CUIDADO DE ANIMALES DE LABORATORIO

212 (240) **REFINED METHOD FOR ORAL DRUG ADMINISTRATION WITH SAME EFFICACY AS OROGASTRIC GAVAGE IN A MURINE MODEL OF ACUTE TRYPANOSOMA CRUZI INFECTION**

Julián Ernesto Nicolás Gulin[1], Margarita Bisio[1], Facundo García-Bournissen[1].
[1]Servicio de Parasitología y enfermedad de Chagas, Hospital de Niños "Dr. Ricardo Gutiérrez".

In animal research, refinement refers to modifications of husbandry or experimental procedures to enhance animal well-being and minimize or eliminate pain and distress.

In Chagas disease drug screening, it has been standardize a therapeutic scheme of 20 consecutive days of oral treatment in mice models. The aim of the study was to compare efficacy of orogastric gavage administration versus a refined method consisting in oral administration with disposable plastic tips. The protocol was approved by the Hospital's Teaching and Ethics Committees.

Five weeks-old male BALB/c mice were infected with 1,000 T. cruzi trypomastigotes (VD strain) by intraperitoneal (ip) route. At parasitaemia onset, 12 days post infection, treatment was started with benznidazole (BZ) at 100 mg/kg/day given with disposable tips coupled to an automatic pipette (n = 5) or with a K35 gavage (n = 5) in a final volume of 50 µL. After 20 consecutive days of treatment, an immunosuppression cycle with cyclophosphamide (CYP, 200 mg/ kg; ip) was initiated. At the end of the cycle, animals were euthanized and blood, skeletal muscle and heart were sampled for quantitative PCR (qt-PCR) diagnosis.

No significant differences were found between mean required doses (± SD) to parasitaemia negativization (3.20±1.64 vs. 3.60±1.95, respectively; p= 0.586) or patent parasitaemia mean (expressed in days) (4.80±3.56 vs. 5.20±3.83; p = 0.9131) comparing both administration methods. At the end of the CYP cycle, parasitaemia did not rebound, and all blood samples were negative with qt-PCR and negative or below the quantification limit in skeletal o cardiac muscle, suggesting parasitological cure in both treatment groups.

Oral BZ administration with disposable tips coupled with an automatic pipette did not influence its effectiveness in mice. Applying this refined method could contribute to improve animal welfare, reducing stress and risk of morbidity and mortality associated with traditional orogastric gavage administration methods, without interfering with therapeutical outcome.

213 (356) **THE IACUC OF THE FACULTY OF VETERINARY SCIENCES - UNLP: A CONTRIBUTION TO ANIMAL WELFARE**

Miguel Angel Ayala[1], Fabricio Maschi[1], Silvana Nora Milocco[1], Alicia Antonini[1], Paula Blanco[1], Oscar Robledo[1], Enrique Portiansky[1], Cecilia Carbone[1].
[1]Facultad de Ciencias Veterinarias. Universidad Nacional de La Plata.

The Institutional Animal Care and Use Committee of the Faculty of Veterinary Sciences of the National University of La Plata was created in 2008 (Exp. 600-004104 / 08 Res.129 / 09). This multidisciplinary committee is responsible for evaluating research protocols in which animals are included. It is coordinated by the Secretary of Science and Technology of the institution and conformed by experts in laboratory animals, biostatistics, researchers, a member of the community and an ethicist. In the last three years 72 protocols were evaluated, 18 were research projects, 40 doctoral thesis, 13 projects from other institutions in Argentina and a postdoctoral fellowship. According to the degree of severity of the experimental procedures on animals, protocols are classified into four categories: A, B, C and D. Category A corresponds to experiments which cause little or no discomfort or stress; B: experiments that cause stress or mild pain or pain of short duration; C: those involving pain or significant and unavoidable stress in vertebrate species and D: procedures that cause clinical abnormalities as obvious change of behavioral patterns or attitudes, severe pain near or above the threshold of tolerance pain in non-anaesthetized conscious animals and/or severe or terminal stress. Of the 72 protocols evaluated, 12 corresponded to category A; 46 to B; 13 to C and 1 to category D. IACUC activity has focused not only on the review of protocols but also in the education of their members and scientists using animals. This has impacted on the animal welfare observing a refinement of animal experimental procedures as most protocols were rated as category B.

214 (404) **MORPHOLOGY AND METABOLISM IN FEMALE WISTAR RATS SUBMITTED TO DIFFERENT OVERWEIGHT INDUCTION METHODS**

Carlos Gabriel de Lade[1], Marcella Martins Terra[1], Ana Eliza Andreazzi[1], Vera Maria Peters[1], Vinícius Moreira Gonçalves Costa[1], Mariana Bolotari[1], Martha de Oliveira Guerra[1].
[1]Centro de Biologia da Reprodução. Universidade Federal de Juiz de Fora. Brasil.

Introduction: Overweight/obesity is considered a risk factor for cardiovascular and metabolic diseases and it impacts directly on the population's quality of life and longevity. Researches with animal model have been adopted for a better understanding of the pathophysiology and treatment of this comorbidity. This condition can be induced by litter size reduction or hypercaloric diets and, replicates the nutritional profile of the current population. Objective: To evaluate the effectiveness of litter size reduction and hypercaloric diet on overweight induction and changes on

glucose metabolism in rats obtained from Centro de Biologia da Reprodução/UFJF (CIAEP 0100482013). Methods: Twenty-eight females were divided into 3 groups and followed from birth to 90th-day-old: control litter (CL), 8-12 animals/litter (n=10), reduced litter (RL), 4 animals/litter (n=10) and treated with control diet; litter hypercaloric diet (LHD), 8-12 animals/litter (n=8), treated with hypercaloric diet. All animals were submitted to an oral glucose tolerance (OGTT) and insulin tolerance tests (ITT). At 90 days of age, the animals were weighted, euthanized and the retroperitoneal and perigonadal fats were collected. RESULTS: The CL presented lower average body mass (168±9.5g) thanthe averages RL (191±7.5g) and LHD (197±12.5g) ($p<0.05$). Fat mass was higher in RL (7.6±2g) regarding to CL (5.5±1.4g) and LHD (5.3±1.5g) ($p<0.05$). The LHD showed lower glucose clearance in OGTT, represented by a larger area under the curve (16270±1600) compared to CL (13910±1880) and RL (14150±1340) ($p<0.05$). The plasma glucose disappearance rate constant (K_{ITT}) in ITT did not differ between groups. Conclusion: The models were effective to induce overweight in females at 90 days of age compared to CL. The LHD showed higher change in glucose metabolism during the OGTT compared to other groups.

215 (542) **BEHAVIORAL AND PHYSIOLOGICAL DIFFERENCES BETWEEN TWO METHODS OF SUBCUTANEOUS TUMOR MEASUREMENTS**

Agustina Resasco[1], Ana Cristina Carranza Martin[1,2], Miguel Ayala[1], Cecilia Carbone[1].

[1]Laboratorio de Animales de Experimentación, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata. [2]Instituto de Genética Veterinaria, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata.

The standard method for subcutaneous tumor measurement in immunodeficient mice is through the use of calipers. Nevertheless, this introduces bias and it is stressful for mice. Beside animal welfare considerations, the stress produced during the immobilization may modify both the behavior and physiology of the individuals, and hence experimental results could also be altered. It has been proved that the use of tunnels for the manipulation of this species minimizes anxiety response. We have previously introduced a newmethod for tumor measurement that uses photographs of subcutaneous tumors, which are obtained from videos where mice voluntarily introduce themselves into an acrylic tube containing graph paper that is used as a scale. The aim of this work is to evaluate the physiological and behavioral changes that these two methods induce in order to assess the welfare of mice. We used 20 female and 16 male nude mice with a subcutaneous tumor transplant. Half of them were measured with the caliper and the other half with the non-aversive method. Measurements were made once a week for a period of four weeks. Feces were sampled on a weekly basis for corticosterone determinations and the number of voluntary contacts with the experimenter within a three-minute period was registered.The differences that we obtained in corticosterone concentrations from both methods were not significant ($P>0.05$). Nevertheless, we found that mice were less reluctant to making contact with the experimenter with the non-aversive method and this difference was statistically significant ($P<0.05$). For this reason, we conclude that the non-aversive method would be of preference in experiments where we want to reduce experimental variables to a minimum, especially if we are conducting behavioral assays. It would also be of preference in those cases where the same experimenter cannot take all the measurements or if we want to improve our animals' welfare.

216 (608) **SETTING UP AN INSTITUTIONAL ANIMAL CARE AND USE COMMITEE AT DR. RICARDO GUTIERREZ CHILDREN'S HOSPITAL**

Julián Ernesto Nicolás Gulin[1,4], Mariana Cruz[2,4], Facundo García-Bournissen[1,4], Romina Grinspon[2,4], Susana Nowicki[2,4], Elena De Matteo[3,4].

[1]Servicio de Parasitología y Chagas. [2]Centro de Investigaciones Endocrinológicas "Dr. Cesar Bergadá" (CEDIE). CONICET-FEI-División de Endocrinología. [3]Servicio de Anatomía Patológica. [4]Hospital de Niños "Dr. Ricardo Gutiérrez".

*Buenos Aires, Argentina.*Laboratory animals are an invaluable tool for biomedical research as much of the current scientific knowledge is obtained from preclinical studies. However, animal use entails an ethical and social responsibility, considering alternatives to animal employment, applying strategies to minimize animal use and refining procedures to avoid or reduce pain and stress. Regulations regarding care and use of laboratory animals are increasing, requiring protocol approval to publish and to seek funding from national and international organizations.Currently, there are two Institutes from CONICET at "Dr. Ricardo Gutiérrez" Children's Hospital (HNRG), and several research groups use animals in their projects. Hence, the establishment of an Institutional Animal Care and Use Committee (IACUC) was proposed to evaluate the protocols that include animal use as well as the institutional animal care and use program.The IACUC is formerly composed of professionals from different biomedical areas: physicians, biochemists, veterinarians and laboratory animal technologists. The activities for self and institutional training include: seminars related to the 3Rs principles, bioethics and legislation, experimental design and sample size determination and protocol evaluation provided by IACUC members and special guests. Besides, a form for protocol submission was prepared and made available on-line at the Hospital's Education and Research Committee (CODEI) website. Since its establishment, in March 2016, the IACUC has met 10 times, evaluated four protocols and given one institutional seminar about proper protocol submission. It is expected that the IACUC will support the work of professionals and researchers from HNRG, CODEI and Ethics Committee. One of the priorities of IACUC will be the continuous training for students and researchers from our hospital, thus contributing to obtain reliable and repeatable results within an ethical framework ensuring the welfare of laboratory animals involved.

217 (661) **SETTING UP A MULTIPLEX PCR FOR THE DETECTION OF MYCOPLASMA PULMONIS AND FILOBACTERIUM RODENTIUM (CAR BACILLUS)**

Silvana Nora Milocco(1), Martín Carriquiriborde(1), Florencia Gentil(1), Juan Martín Laborde(1), María del Pilar Cagliada(1), Cecilia Carbone(1).

[1]Laboratorio de Animales de Experimentación (LAE) Facultad de Ciencias Veterinarias UNLP.

The diagnosis of infectious diseases through the technique of polymerase chain reaction (PCR) has revolutionized the field of microbiology. This technique based on the principles of molecular biology offers the possibility of obtaining highly reliable results and shortening delivery times thereof. Particularly, despite these advantages, this method does not replace but complements traditional diagnostic tests. In order to reduce costs and time a variant of PCR technique was developed: the multiplex PCR (mPCR). It consists in the detection of the presence of several microorganisms in a single reaction. This method allows to amplify different target sequences simultaneously in a single tube. Multiplex PCR is a valuable tool in many biological studies but it is a multifaceted procedure that has to be planned and optimised thoroughly to achieve sensible and meaningful results. Especially, primer concentrations have to be adjusted to assure an even amplification of all targeted DNA fragments.

In this paper the development of a mPCR for the detection of *Mycoplasma pulmonis* and *Filobacterium rodentium* in experimental rats and mice is described. These respiratory tract microorganisms are very highly prevalent in animal facilities in Argentina. The presence of these bateria interferes in the tests results when infected animals are used in research. Samples were obtained from macerated lungs by using a DNA extraction kit from tissue. Specific primers were used to identify *M. pulmonis* and *F. rodentium*.

218 (678) **SENTINEL PROGRAM FOR HEALTH MONITORING OF ANIMALS HOUSED IN IVC**

María del Pilar Cagliada[1], Fabricio Alejandro Maschi[1], Juan Martín Laborde[1], Silvana Nora Milocco[1], Martín

Carriquiriborde[1], Flor Gentil[1], Miguel Angel Ayala[1], Cecilia Carbone[1].
[1]Laboratorio de Animales de Experimentación LAE. Facultad de Ciencias Veterinarias. UNLP.

The development of murine models used in biomedical tests, pharmacological studies and quality controls is a fundamental issue in laboratory animals science. It is well known that the use of genetic modified animals (GMA) has contributed with scientific progress, since they are considered a important and indispensable tool. Obtaining transgenic mice involves significant economic efforts compared with the traditional lines and frequently the animals needed to conduct routine health monitoring are not available. Therefore other alternatives have to be considered. One option is the use of sentinel animals which must come from colonies whose microbiological status is completely known. These animals are introduced for three months into the same rack where the GMA are kept, after this period a health control of the sentinels is performed , replacing in this way the transgenic ones. The aim of this study was to evaluate the efficiency of the use of SPF N:NIH(S)-*Foxn1^{nu}* mice *(nude)*, produced at the Laboratory of Experimental Animals LAE - FCV, for the sanitary control of mouse colonies kept in individual ventilated cages (IVC). In order to evaluate the sentinel program efficiency 12 health monitorings were performed in mice from 2 institutions where the animals are housed in IVC; 6 controls were carried out on the sentinel animals and 6 on GMA from the colony. The results showed that the infections found in mice colonies housed in the racks, also were found in sentinels. It was concluded that the use of sentinel program in routine health monitoring is efficient and help to replace the animals under experience, in this case GMA.

219 (836) **BIOINDICATORS OF STRESS IN DIFFERENT WEANING SCHEMES IN CATTLE**

Maria Mercedes Odeon[1, 2], Sebastian Vittone[3], Silvina Soledad Maidana[1, 2, 5], Sonia Alejandra Romera[1, 2, 4, 5].
[1]CONICET [2]CICVyA-INTA Castelar [3]EEA Concepción del Uruguay- INTA [4]Universidad del Salvador [5]Universidad de Morón.

The agricultural sector is changing rapidly due to global trends of globalization, internationalization of markets and multinational trade agreements. One of the emerging issues in this scenario is the animal welfare. The concept of animal welfare is based on the harmonious relationship of the animal to the environment.

A variety of behavioral, physiological, biochemical and immunologic parameters have been proposed to evaluate the responsiveness of the animals to stress. The aim of this study was to evaluate biochemical and physiological parameters as indicators of well-being and stress of cattle under different weaning schemes.The experimental design consists of two groups, one of conventional weaning (6months) and a group of early weaning (1month). Whole blood samples were taken at different times,-7,0 (weaning),1,7,14,21.

Received samples were analyzed in a hematology analyzer, then the plasma was separated and analysis of total plasma protein was performed by the Lowry method, plasma cortisol levels by HPLC.With the results of hematologic counter observed only in the conventional group, a significant decrease in the number of lymphocytes, a significant increase in the number of granulocytes, leading to an increase in the ratio GR L.

We found a significant increase on day 1 post- weaning in both groups equally. Result matching literature refers to increases in concentrations of total proteins in response to acute stress. Levels then drop to day7 and 14 and finally recovers baseline to day 21.

As cortisol levels, we observed a response to stress with large increases on day 1 however, the characteristic peak of the response to acute stress is not seen, but a downward curve most characteristic to chronic stress is observed, being clear response in the conventional weaning group, while in the animals with early weaning seems more difficult to achieve and return to baseline levels.In conclusion we observed that in the parameters analyzed, conventional weanlings act as expected in a stress response, whereas early weaning no such response is observed.

220 (873) **OPTIMIZATION OF PROCESSING FOR BLOOD SAMPLES EVALUATION OF STRESS BIOMARKERS**

Maria Mercedes Odeon[1, 2], Javier Capuccio[3], Sandra Romero[4], Sonia Alejandra Romera[1,2,5,6].
[1]CONICET [2]CICVyA-INTA Castelar [3]EAA Marcos Juarez-INTA, Córdoba [4]INTA IPAF NOA Hornillos, Jujuy [5]Universidad del Salvador [6]Universidad de Morón.

In recent years it is being given to increasing animal welfare standards importance due to the confluence of several factors. The objective measurement of welfare is a complex process, plasma levels of glucocorticoids and behavioral changes have been used as measures of stress, but we need to expand the search for biomarkers in order to control and monitor this state, both production systems and research. In order to evaluate cellular and molecular parameters as indicators of well-being in different species, we set out to develop a efficient method of obtaining sample for such assessment. We worked with samples of lama, swine and chicken. Blood samples were taken with citrate 3.8% (1:10) as an anticoagulant. Then they processed with three different protocols for future evaluation of obtaining white blood cells with each of them in order to maximize the number of white cells obtained, avoiding contamination of red blood cells. It began with 500 ul of blood in each protocol, were performed in duplicate. The difference between protocols mainly consists of the method of red blood cell lysis, as it is the main variable to adjust due to the diversity between species. To analyze the efficiency of each cell counts protocol was performed with trypan blue in Neubauer chamber. To assess purity of the samples obtained an extended cells was performed, they were stained with Giemsa and observed under optical microscope. The general protocol follows this steps:1-Centrifuge 1000g 30min to separate plasma. 2-LYSIS 3-Centrifuge 1200g 5min 4-LYSIS 5-Centrifuge 1200g 5min. 6-Wash with RPMI. 7- Centrifuge 1200g 5min 8- Resuspend cells in RPMI10% FBS. After analyzing the number of cells obtained and the contamination of RBCs is concluded that: in swine the lysis that optimized processing was: NH4Cl lysis Buffer 5 min , 4 ° C. In lama : NH4Cl lysis buffer 15 min , 37 ° C and in the case of chickens no red blood cell lysis was performed but a protocol was followed with ficoll gradient (1.077 g / ml) blood:ficoll 1:2. It was achieved, efficient purification and a good method of cryopreservation of cells at -80 ° C for each species.

221 (2000) **RAT HAIR USE IN DETERMINING PESTICIDES CONSUMED FOR WAY ORAL**

Edwin León Mora[2,4], Ledis Reyes-Moreno[2,4], Freddy Arias-Mora[1], Mario Masís-Mora[3], Maripaz Castro-Murillo[2], Ivannia Ureña-Solera[1,2].
[1]Facultad de Farmacia. Universidad de Costa Rica. [2]Laboratorio de Ensayos Biológicos. Universidad de Costa Rica. [3]Centro de Investigación en Contaminación Ambiental. Universidad de Costa Rica. [4]Departamento de Farmacología, Escuela Medicina. Universidad de Costa Rica.

Introduction: Costa Rica has presented an increment on the pineapple´s monocultives, in 2014 there was 490 809 ha dedicated to crops. This has caused an increment in the use of some pesticides, specially bromacil. The problem with this herbicide is the high mobility in the aquatic ecosystems. The use non-invasive methods, like hair, are alternatives to monitorize the population, and decide if they are in risk.

Objetive:The aim was to develop a methodology for the identification and quantification of bromacil in hair using and animal model. Furthermore, this is to be a pioneer in the analysis of pesticides in hair in Costa Rica as a form of biomonitoring.

Materials and Methods: Twelve Sprague Dawley rats in a block design, was used. An amount of 3,84 mg/kg/day of bromacil in fresh water, were given to the rats for 45 days, while the other rats consumed normal water. Then, at 15 and 45 days into the respective treatment, the hair were cut of the back of each rat, and washed to eliminate external contamination. After that, a bromacil extraction was performed and analyzed with a LC-MS to quantify and identify the presence of bromacil. The results was analyzed

by a Kruskall-Wallis test for analyze all the treatments.Approval by the institutional animal care committee of the Universidad de Costa Rica, number: 2012-2014.

Results: The quantity of the pesticide that could be determined at 15 and 45 days of exposure was (14,32±6,94) µg/kg for females and (17,3 ± 7,31) µg/kg for males, and for 45 days of exposure the quantity of (67,63 ± 30,8) µg/kg for females and (32,985 ±12,76) µg/kg for males and the control doesn´t present an appreciate quantity. The Kruskal-Wallis test reveals that there are significant differences between treatments and the control (p<0,005). Conclusions: We develop a successfully methodology for the identification and quantification of bromacil in hair using an animal model.

222 (2001) **INCIDENCE OF GOOD MANUFACTURING PRACTICES AND GOOD LABORATORY PRACTICE CLINICS NO MACACA MULATTA MANAGEMENT AS BIOMODELS BLOOD DONOR.**

Rebeca Castillo Rodríguez[1], Elizabeth Harris[1], Yosmany Rodriguez[1], Miriam Tamara Garcia Osuna[1], Maikel González[1], Jesús Guerra[1], Axel Mancebo[1], Avelina León[1], Diuris Blanco[1], Amelia Peña [1], Marisel Ronda[1].
[1]National Center for Laboratory Animal Breeding, La Habana Cuba.

Nonhuman Primates (NHP) are considered a kind of inestimable value for their uses in the development of Biotechnology and Pharmaceutical Industry Medical. CENPALAB is the only center in PNH producer for Experimentation Cuba has a colony of Macaca mulatta whose purpose is to use animals in experiments and other activities. This work aims to evaluate the mplementation of the Good Manufacturing Practices (BPP) and Good Clinical Laboratory Practices not in handling this animal model to be used as a blood donor. For the development of the 28 animals of the colony of these animals CENPALAB applying all the techniques described in the documentation work tell yourself, Standard Operating Procedures and Good Manufacturing Practices and the principles of Good Laboratory Practice they were used . As a result it was established this biomodel as a blood donor, the most requested by biotechnology centers in the country.

223 (2033) **CONFORMATION OF AN INSTITUTIONAL COMMITTEE FOR THE CARE AND USE OF EXPERIMENTAL ANIMALS AT THE NATIONAL INSTITUTE OF HUMAN VIRAL DISEASES**

María del Carmen Saavedra[1], Graciela Susana Gamboa[1], Vanesa Karin Mescher[1], Gladys Ethel Calderón[1].
[1]Instituto Nacional de Enfermedades Virales Humanas "Dr. Julio I. Maiztegui" (INEVH).

The INEVH is part of the National Administration of Laboratories and Health Institutes (ANLIS). Its mission is not only to design, coordinate and implement activities of diagnosis, treatment, research, prevention and teaching of human viral diseases but also to achieve the development and production, control and quality assurance of biologicals, vaccines and diagnostic reagents associated with human viral diseases. Some ofits activities involve the use of laboratory animals. Since its beginning, the INEVH has considered aspects related to animal welfare. Given the scientific and ethical progress in appropriate use and care of laboratory animals to minimize or avoid the suffering of them, the INEVH has created an Institutional Committee for the Care and Use of Experimental Animals (CICUAE). In 2011, through an internal arrangement, the director of the INEVH, established the conformation of CICUAE-INEVH which was formed by five of our professionals and three external researchers. The mission of CICUAE-INEVH was defined so as to advise the INEVH's researchers, in compliance with the principle of the 3Rs and animal welfare under current international standards for research, development and teaching. Since 2012 to the present, nine research projects were evaluated belonging to researchers of INEVH and other institutes of ANLIS, to be presented in different areas: grants, magisters and doctoral theses. These projects were evaluated by this Committee in all procedures involving the use of laboratory animals, which were approved incorporating the suggestions of it. We can conclude that the work done by this committee, in addition to supporting human resources training, provides a positive impact on the scientific community.

224 (2037) **IMPROVING TRAINING FOR INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE MEMBERS**

Micaela María Ricca[1], Ma. Emiliana Herrero[2], A. Carolina Mourelle[3].
[1]Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile [2]Instituto Ciencias Básicas y Medicina Experimental Instituto Universitario Hospital Italiano (IUHI) [3]IDEHU-CONICET.

International and national regulations require that Institutional Animal Care and Use Committees (IACUCs) must be established by institutions that use laboratory animals for research or teaching to oversee, evaluate and monitor the institution›s animal care and use program. Composition and functions of Latin American IACUCs are diverse due to the lack of training available. In addition, many Latin American institutions cannot afford to send IACUC members to training courses. Lack of information in Spanish further hampers efforts to promote training. In order to harmonize IACUCs function and training among Latin America IACUC members, the challenge was to develop a Spanish language e-learning training program. The program follows the constructivist model used by Aula Virtual Bioterio for other courses, through debates on animal bioethics, from the macro to the study of specific cases pragmatically. Didactic and interactive educational training were carefully designed for successful completion of each stage for all the attendees. CIOMS/ICLAS International Guiding Principles for Biomedical Research Involving Animals was a key document, and collaboration with AAALAC, SBCAL and ICLAS experts was incorporated into the curriculum. For assessing the results, we asked students to answer an anonymous survey, on a score of excellent, very good, good or regular. Over 61 respondents, 75.4% assessed the proposed program as excellent and 21.3% as very good; about the methodology (totally virtual classes) 67.2% assessed as excellent and 27.9% as very good. Finally, to confirm and check the contribution of this training in order to review and modify previous work experiences. As a result 60.7% scored excellent, 31.1% very good and 6.6% good. In conclusion, an e-learning training program designed and managed properly can be a valuable tool to enable effective approach to training and quality over a large geographic area at low cost for participants individually.

METABOLISMO Y NUTRICIÓN I / METABOLISM AND NUTRITION I

225 (260) **DEVELOPMENT OF A MOUSE MODEL TO STUDY THE ROLE OF INSULIN RECEPTOR (IR) IN PRE-ADIPOCYTE DIFFERENTIATION 3T3-L1 CELL LINE**

María Amparo Lago Huvelle[1], Lucía Soledad Barcos[1,], Michael Schupp[2,], Federico Coluccio Leskow[1],
[1]Universidad de Buenos Aires. Consejo Nacional de Investigaciones Científicas y Técnicas. Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN). Facultad de Ciencias Exactas y Naturales, Buenos Aires, Argentina [2]Institute of Pharmacology, Center for Cardiovascular Research, CCR. Charité University Medicine. Berlin, Germany

The epidemic increase in obesity causing insulin resistance and type 2 diabetes has become a worldwide problem. To better understand the mechanisms that lead to metabolic disorders, it is crucial to develop a better underlying knowledge of the molecular events that regulate adipocyte differentiation. Many positive modulators of this process have been identified, such as the CCAAT/enhancer binding protein (C/EBPβ) and peptide hormones like insulin.The insulin receptor (IR) is transmembrane tyrosine kinase receptor, encoded by a single gene composed of 22 exons. Due to alternative splicing of exon 11, the gene gives rise to two

protein isoforms that differ by a 12-amino-acid insertion: the IR lacking exon 11 (IR-A) and the IR containing exon 11 (IR-B). We hypothesize that IR plays an important role in regulating adipocyte differentiation process.

To address this issue, we have generated two IR KO clones from the 3T3-L1 cell line, using the CRIPSR/Cas9 system, and tested it as an adipocyte differentiation model. We verified accurate genome editing through sequencing and IR protein lacking through WB. These clones were unable to differentiate under standard differentiation protocols.

In order to recover the differentiation capacity in these KO clones, we re-expressed IR-A or IR-B through retroviral infection. Correct isoform expression was determined at mRNA level through RT-PCR. Preliminary results show that they recapitulated some of the molecular events typical of adipocyte differentiation. This was evaluated at the level of early adipogenic markers such as C/EBPβ and in late adipocyte-specific genes, such as the gene encoding aP2, a lipid-binding protein.

Taken together, these results suggest that IR could be modulating important steps of adipocyte differentiation. Further studies are necessary to elucidate the targets of IR action.

226 (809) **DIETARY SUPPLEMENTATION OF OMEGA-3 FATTY ACID PREVENTS THE ALVEOLAR BONE LOSS IN HYPERCHOLESTEROLEMIC RATS WITH EXPERIMENTAL PERIODONTITIS?**

Maria Eugenia Antona[1], Andrea Gloria Ferreira Monteiro[1], Valeria Zago[3], Iana Belén Perez Alcaraz[1], Patricia Mandalunis[2], Cecilia Ramos[1], Silvia María Friedman[1], Elisa Vanesa Macri[1],

[1]Department of Biochemistry, School of Dentistry,University of Buenos Aires. Argentina. [2]Department of Histology and Embryology. School of Dentistry, University of Buenos Aires. Argentina. [3]Lipids Lab. Clinical Biochemistry. School of Pharmacy and Biochemistry INFIBIOC, University of Buenos Aires. Argentina.

Periodontitis (P) is a high prevalent inflammatory infectious disease which may cause alveolar bone resorption and tooth loss. In rats, hypercholesterolemia (HC) causes decreased of bone volume (BV). PUFAn-3 has been recognized for its anti-inflammatory effect but its effectiveness has not been probe under P.

Objective: We evaluated whether the dietary supplementation of fish-oil reduce alveolar bone loss in rats with experimental P.

Adult Wistar rats were fed ad libitum 1 of 3 diets: a commercial (C control,n=24), HC (n=24) or HC plus fish oil(n=24, HCn3). HC was induced by an atherogenic diet for 3 weeks prior to P. At T0, rats were subjected to a unilateral ligature around the right first molar (P, HC+P, HCn3+P).The contralateral first left molars (not ligated) were used as untreated controls (C,HC,HCn3). At the end of each experimental period (T1,T2,T3 & T4), 6 rats per group were euthanized, and blood was drawn for serum lipids profile (mg/dL): triglycerides (TG) and NonHDL-chol. Hemi-mandibles were removed and processed with H&E for histomorphometric evaluation. On digital photomicrographs in interradicular bone, periodontal ligament height (hPL, μm) and BV related to total volume [BV/TV(%)] were evaluated. X-rays were used for periodontal bone support (PBS) measurements.

Results (mean±SD, ANOVA-SNK): HCn3 showed a decreased in NonHDL-chol ($p<0.001$) and the lowest TG ($p<0.001$) from T1 and T2, respectively. At T3 and T4, hPL was found to be significantly higher in HC and HCn3 than C ($p<0.01$). Up to T1, hPL was lower ($p<0.01$) and PBS% higher ($p<0.01$) than HC+P, showing a decreased risk of alveolar bone resorption; but finally, HCn3+P BV/TV(%) level remained between HC+P and P($p<0.05$).Conclusion: HC in adult rats may contribute to the severity of P. Fish oil-enriched diet regulated serum TG and chol levels. Further studies are necessary to demonstrate the effectiveness of PUFAn-3 in the treatment of P. UBACyT 20020150200013BA

227 (819) **KEY ENZYMES AND NUCLEAR RECEPTORS OF HEPATIC LIPID METABOLISM IN DAIRY CATTLE DURING THE TRANSITION PERIOD**

Emmanuel Angeli[1,2], Trionfini Valentina[1], Gareis Natalia Carolina[1], Marelli Belkis[1,3], Hein Gustavo[1,4], Ortega Hugo[1,3],

[1]Laboratorio de Biología Celular y Molecular Aplicada, Instituto de Ciencias Veterinarias del Litoral (ICiVet-Litoral), Universidad Nacional del Litoral (UNL) / Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Esperanza, Santa Fe, Argentina. [2]Cátedra de Practica Hospitalaria de Grandes Animales, Facultad de Ciencias Veterinarias del Litoral, Universidad Nacional del Litoral (UNL), Esperanza, Santa Fe, Argentina. [3]Cátedra de Biología Celular, Facultad de Ciencias Veterinarias del Litoral, Universidad Nacional del Litoral (UNL), Esperanza, Santa Fe, Argentina. [4]Cátedra de Análisis Avanzados de Alimentos, Centro Universitario Gálvez, Universidad Nacional del Litoral (UNL), Gálvez, Santa Fe, Argentina.

The transition period (TP) is the most critical in the lactation of a dairy cow, characterized by a negative energy balance leading to an important lipid mobilization, with increases in the systemic non-esterified fatty acids (NEFA) and beta-hydroxybutyric (BHB) concentrations. While carnitine palmitoyltransferase-1 (CPT1), acyl-Coa oxidase-1 (ACOX1) and the nuclear receptor PPAR-alpha promotes fatty acids oxidation in several tissues, including the liver, diacylglycerol acyltransferase-1 (DGAT1) is involved in triacylglycerol biosynthesis. The aim of this study was to evaluate some pathways of hepatic lipid metabolism during the TP. Blood samples and liver biopsies from dairy cattle (n = 10) were taken at 28 and 14 days prepartum, and also at 4, 14, 28 and 60 days postpartum. Systemic NEFA, BHB, glucose and insulin concentrations were measured. Besides, the liver expressions of fatty acids oxidation (CPT1, ACOX1) and re-esterification (DGAT1) enzymes, and nuclear receptor PPAR-alpha were evaluated by Real Time-PCR and western blot. There were no significant changes ($p > 0.05$) in glycemia and insulinemia. Differences in the BHB and NEFA concentrations were observed at different sampling days, being significant at 4, 14 and 30 days postpartum ($p < 0.05$). The expressions of CPT1 and PPAR-alpha were higher in the postpartum period ($p < 0.05$), with an opposite behavior respect the expression of DGAT1. No differences in the mRNA expression of ACOX1 were observed ($p > 0.05$). These results show significant changes in hepatic lipid metabolism of dairy cows during the TP. These knowledge could represent a useful tool to understand the metabolic behavior in animals with different milk production and to optimize the health of dairy cattle during this period.

228 (849) **THE EFFECT OF INSULIN GROWTH FACTOR 1 ON DELTA-AMINOLEVULINIC SYNTHASE EXPRESSION**

Sandra Milena Mora[1], Leda Maria Oliveri[1], Alcira Maria del Carmen Batlle[1], Victoria Estela Parera[1], Maria Victoria Rossetti[1], Esther Noemi Gerez[1],

[1]Centro de Investigaciones sobre Porfirinas y Porfirias (CIPYP) - CONICET - Hospital de Clìnicas José de San Martín

Acute Intermittent Porphyria (AIP) is an inherited disorder of heme biosynthesis characterized by a reduced activity of Porphobilinogen-deaminase, associated to a marked increase in the expression of the hepatic isoform of delta-aminolevulinic synthase (ALAS1), the first and regulatory enzyme of the pathway, with the accumulation of the neurotoxic precursor ALA. The aim of this study was to investigate if the insulin like growth factor 1 (IGF1) has any role on ALAS1 regulation. IGF1 levels in plasma and ALA levels in urine in 50 AIP patients were determined. In the *in vitro* assays C3A cells were incubated in DMEM medium and treated with IGF1 for 15min at different concentrations (1, 10, 50 nM), in the presence and absence of AG1024/L, a selective inhibitor of the tyrosine fosforilation of the beta subunit of IGF1 receptor. Control cells were treated with the corresponding vehicle.

According to these results patients were divided in three groups: normal IGF1 and ALA; low IGF1 and high ALA; normal IGF1 and high ALA. Reference values: IGF1 values depend on the age (ng/ml); ALAS < 4mg/24h. In the studied population a significant correlation was observed in the group with low IGF1 and high ALA levels ($p < 0.001$). *In vitro* assays, IGF1 treatment

leads to a reduction of 61% in the ALAS1 mRNA basal levels; this effect was reversed by AG1024/L presence.

These results would suggest a significant role of IGF1 on ALAS1 expression levels.

229 (920) **KIDNEY PROTECTION BY (-)-EPICATECHIN SUPPLEMENTATION IN HIGH FAT DIET FED MICE**

Laura Fischerman[1,2], Mónica Galleano[1,2], Patricia I. Oteiza[3], César G. Fraga[1,2],

[1]Instituto de Bioquímica y Medicina Molecular (IBIMOL), UBA-CONICET [2]Cátedra de Fisicoquímica, Facultad de Farmacia y Bioquímica, UBA [3]Department of Nutrition, Department of Environmental Toxicology, University of California, Davis

Obesity is an independent risk factor for the development of cardiovascular disease, type 2 diabetes and other disorders. High amounts of saturated fats appears in part responsible for the increased prevalence and incidence of obesity. Fruit and vegetable consumption has beneficial effects on health, that can be attributed to some particular compounds, based on their chemical structure and population studies. This work aims to elucidate the effects of (–)-epicatechin (EC) on the mechanisms involved in insulin response and oxidative damage in kidney (cortex and medulla) of mice fed a high-fat diet (HFD). For 15 w, two experimental mice (C57BL/*6)* groups received control diet, with (CE) or without EC supplementation (C). Two other groups received HFD (60% of calories from fat), with (EC) and without (EC) supplementation (HFE and HF, respectively). 10 minutes before animal euthanization, insulin was injected to half of the animals of each group. Since the 6th week there was an increased body weight in HF respect to the other groups ($p<0,05$). Serum triglycerides, cholesterol, fasted- and fed- glucose, and fasted- and fed-insulin (measured with commercial kits) were higher in HF group than in C, CE, and HFE ($p<0,05$). Triglycerides in kidney cortex and medulla were similar in the 4 groups. Phosphorylation of Akt was studied by western blot in cortex and medulla homogenates, showing no differences among the four groups with higher levels when stimulated with insulin ($p<0,001$). There was a significant increase in insulin receptor expression in kidney cortex in HF compared to C and HFE groups ($p<0,05$). Protein-4-hydroxynonenal adducts were increased in HF as compared to C and HFE ($p<0,05$) in medulla. These results suggest that EC can modulate insulin response and oxidative stress differentially, as the pathophysiological mechanisms triggered seem to be different in renal cortex and medulla in mice fed HFD.

230 (975) **REMODELING OF VASCULAR PROTEOGLYCANS OF THE EXTRACELULAR MATRIX INDUCED BY HUMAN APOLIPOPROTEIN A-I. PROBABLE ROLE IN THE FUNCTION-CYTOTOXICITY EQUILIBRIUM.**

Aldana R. Bariandarán, [1]Silvana A. Rosu[2], Agustín Blachman[1], Daniela Recchi[3], Andrea Del Carreto [3], M. Alejandra Tricerri[2], Graciela C. Calabrese[1].

[1]Universidad de Buenos Aires- Facultad de Farmacia y Bioquímica- Cátedra de Biología Celular y Molecular. Junín 956 Primer Piso (CABA- C1113AAD, Argentina). [2]Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP), Facultad de Ciencias Médicas, UNLP, Calle 60 y 120, La Plata, CP 1900 [3]Sanatorio Güemes. Servicio de Obstetricia. F. A. de Figueroa 1240, C1180AAX CABA.

Apolipoprotein A-I (apoA-I) is the major constituent of human high density lipoproteins (HDL), which play a key role in reverse cholesterol transport. In addition, apoA-I exhibits antioxidant and anti-inflammatory properties and inhibit the aggregation and neurotoxicity of the amyloid-b peptide. Nevertheless, wild-type apoA-I could also be amyloidogenic, as it was associated to atherosclerosis lesions. Changes in the microenvironment have been suggested to explain protein missfolding and tissue deposition. The aim of this work was to analyze the expression and the chemical structure of proteoglycans (PGs) in human umbilical vein endothelial cells (HUVEC) in the presence of wild type apoA-I. Recombinant apoA-I which behaved as the native protein was employed in the assays. HUVEC were cultured for 24 hs in the presence of increasing doses of apoA-I (1.5–100 µg/mL) to determine the cytotoxicity by MTT assay. After treatment, PGs were characterized through: (1) their core protein mRNA and (2) the levels of glucuronyl C-5 epimerase (DS-epi1/2) mRNA by reverse transcriptase-PCR (RT-PCR). In addition, chicken ovalbumin upstream promoter transcription factor II (COUP-TFII) mRNA was also determined by RT-PCR. No cell cytotoxicity was determined through all the apoA-I doses analyzed. A significant decrease in biglycan and versican CS/DS PGs was detected at 1.5 µg/ml apoA-I ($P<0,05$, $n=3$), whereas a significant increase in decorin CS/DS PG and perlecan heparan sulfate PG was observed compared with the control ($P<0,05$, $n=3$). In addition, a significant decrease in DS-epi1/2 mRNA was detected in HUVEC apoA-I treated cells compared with the control ($P<0,05$ $n=3$). Our results show that in spite of the fact that venous endothelial cell expressed the venous phenotype marker COUP-TFII in the presence of 1.5 µg/ml wild type apoA-I, the apolipoprotein was able to induce a PG remodeling in human endothelial cells with a prevalence in chondroitin sulfate glycosaminoglycan chains.

231 (988) **EFFECT OF ANDROGEN ON THE ANTIOXIDANT DEFENSE SYSTEM IN AORTA OF MALE RAT**

Silvina Alvarez[1], Maria Eugenia Ciminari[2], Valeria Muñoz[2], Silvana Ocaña[2], Eloy Salinas[2], Maria Veronica Perez Chaca[2], Nidia Gomez[1].

[1]Instituto Multidisciplinario de Investigaciones Biológicas San Luis IMIBIO-SL [2]Universidad Nacional de San Luis.

There are many studies of androgens effect on vascular tone and endothelial function, however few of them focuses on reactive oxygen species (ROS) generation and regulation by testosterone. The antioxidant defense system consist of: enzymatic and non-enzymatic compounds. Among the latter we found copper, zinc or selenium. One of the most important functions of zinc is its participation in the antioxidant defense system. The aim of this work was to study the effect of castration on antioxidant parameters in aorta male rats. Moreover, we analyzed the antioxidant defense system when Zn was administered as a non-enzymatic antioxidant. We tested the effect of androgen deprivation on the prooxidant-antioxidant balance 30 days after castration. Wistar male rats (200±20g) were separated in five groups: controls (Co), controls supplemented with zinc (Co+Zn), castrated (Ca), castrated supplemented with testosterone (Ca+T) and castrated supplemented with testosterone and zinc (Ca+T+Zn) for five days. After 30 days rats were killed and aorta was obtained. RNA was extracted by using TRIzol. Nuclear factor-erythroid 2 related factor 2 (nrf-2), Glutathion peroxidase (GPx), and catalase CAT expression were determined by RT-PCR. S28 was the control. Blood pressure was measured before castration and 30 days post castration. Blood cells count was realized. ANOVA was used for statistical analysis. Nrf 2 expression showed a significant increase in Ca ($p<0.05$) but Ca+T group did not reversed the effect. CAT expression did not change in Ca but Ca+T+Zn was significantly higher than Co and Ca rats ($p<0.05$). Gpx expression in Ca, Ca+T and Co+Zn and Ca+T+Zn were higher than Co group ($p<0.05$). Blood pressure did not change after castration and blood cells count didn´t show any difference between treatments. We conclude that androgen deprivation alters the prooxidant-antioxidant balance in aorta. Nrf2 is Zn sensitive, while GPx is influenced by testosterone and Zn.

232 (998) **MCAM KNOCKDOWN INHIBITS 3T3-L1 FIBROBLASTS DIFFERENTIATION TO ADIPOCYTES**

Matías Gabrielli[1,2], María del Carmen Vila[1,2].

[1]Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires (2) IQUIBICEN – CONICET.

Obesity is a chronic disease associated to a dysfunction of the adipose tissue. The study of adipose tissue development and function may help to find new approaches for the treatment of this pathology. 3T3-L1 fibroblasts are one of the most used cell models to

study adipogenesis. In the search for novel genes involved in this process we have previously identified MCAM (melanoma cell adhesion molecule), which mRNA increases during 3T3-L1 fibroblasts differentiation to adipocytes and its levels correlate with the extent of the differentiation achieved. MCAM is a transmembrane protein of the Ig-family which has been involved in different processes such as cell adhesion, signal transduction and cell differentiation. To assess the role of MCAM and the importance of the upregulation of its mRNA during 3T3-L1 cells differentiation, we analyzed the effects of downregulating MCAM mRNA. 3T3-L1 fibroblasts were transduced with lentivirus containing an shRNA sequence directed to MCAM mRNA. We found that MCAM-knockdown cells showed impaired differentiation as evaluated by Oil-Red-O staining of cytosolic lipids. This was associated with lower levels of peroxisome proliferator activated receptor gamma (PPARγ) mRNA, the master gene in adipogenesis, and perilipin mRNA, which is a protein present in the lipid droplet. We have previously observed that the increase in PPARγ mRNA level precedes the increment of MCAM mRNA. Moreover, MCAM mRNA is further increased by treatment with pioglitazone, a PPARγ agonist, suggesting that MCAM may be regulated by PPARγ. Consistently we recently observed that treatment of mature adipocytes with 25 ng/ml TNFα for 48 h, downregulated PPARγ and also decreased MCAM mRNA. In keeping with this, we searched chromatin immunoprecipitation databases and found two putative binding sites for PPARγ near the MCAM gene. Our results indicate that upregulation of MCAM is important in adipogenesis and may be regulated by PPARγ.

233 (1011) **FAT WEIGHT AND LEAN BODY MASS COMPOSITION IN SPRAGUE DAWLEY RATS FED WITH SEMISYNTHETIC AND ISOCALORIC DIETS CONTAINING CHIA SEEDS (SALVIA HISPANICA)**

Gustavo Antonio Martínez[1,2], Joaquín Gabriel Ivona[1,2], Evelyn Maritza Montes Chañi[1,2], Sandaly Oliveira Silva Pacheco[1,2], Fabio Juliano Pacheco[1,2].
[1]Centro de Investigación y Ciencias de la Salud [2]Universidad Adventista del Plata.

Introduction: The intake of chia seeds are thought to provide a variety of nutrients such as polyunsaturated fatty acids, proteins, high soluble fibers and calcium. However, there are few studies conducted in animal models investigating the effect of chia seed consumption on body weight regulation. Objective: This study aims to evaluate the fat and lean body mass of animals fed with a diet containing chia seeds for a large period of time. Materials and methods: Animals: After two weeks of weaning 30 Sprague Dawley rats were distributed into two groups of 15 animals each group, named Control group and Chia group. Diet: Both groups received a diet according to the AIN 93, in the Chia group, however 10% of the diet was replaced by whole chia seeds. Macro- and micronutrients provided by the chia seeds were considered before developing the control diet, ensuring that both groups would receive an isocaloric diet. Data collection: At two different time points of 330 and 405 days, total weights were determined as well as subcutaneous and visceral fat, bone and muscle (fat free weight), and the remaining weight corresponding to solid organs, fluids and viscera. Statistical analysis: To determine the correlation between variables, Pearson´s correlation was used with a 95% confidence interval. Results: It was found that animals in the Chia group presented a significant positive correlation between the variables age-total weight (R = 0.664, p = 0.013), age-fat weight (R = 0.628, p = 0.022) and age-fat free weight (R = 0.695; p = 0.022). However in the control group none of these correlations were identified. Conclusion: Over the course of time, animals in the Chia group simultaneously increased musculoskeletal tissues and adipose tissue in regard to Control group. These results suggest that the intake of chia seeds could be helpful to prevent muscle mass loss during adulthood and elderly age.

234 (1023) **LOW N-6/N-3 RATIO ON A DIET WITH CHIA SEEDS AFFECTS BONE PARAMETERS IN MALE SPRAGUE DAWLEY RATS WHEN COMPARED TO THE AIN-93M DIET**

Joaquín Gabriel Ivona[1, 2], Gustavo Antonio Martínez[1, 2], Evelyn Maritza Montes Chañi[1, 2], Sandaly Oliveira Silva Pacheco[1, 2], Fabio Juliano Pacheco[1, 2].
[1]Centro de Investigación y Ciencias de la Salud [2]Universidad Adventista del Plata.

Introduction: Peak bone mass is a predictive factor in the development of osteoporosis. Nutrition influences bone metabolism. Several studies support a relationship between bone metabolism and the amount and ratio of n-6 and n-3 fatty acids in the diet. Chia seeds (Salvia hispanica L) provide a high proportion of n-3 acids. Objective: Examine and quantify whether whole chia seeds as part of the diet affect bone parameters in rats. Materials and methods: There were used 20 male Sprague Dawley rats that after 2 weeks of weaning were randomly assigned into 2 designs which only varied in their duration. Design 1 lasted 10 months, design 2 lasted 13 months; each had 2 groups of 5 rats each: control group (C) and experimental group (E). Group C received a diet in accordance to the AIN-93M standards, with an n-6/n-3 ratio of 7.46; group E received a diet in which whole chia seeds were included representing 10% of the total mass, and with an n-6/n-3 ratio of 1.07. Both diets were given throughout the project, and they only varied in the quantity and quality of fatty acids and minerals given, due to the insertion of chia seeds as part of diet E. All rats were sacrificed at the end of the project and bone parameters were measured by dual-energy X-ray absorptiometry: bone mineral density (BMD), bone mineral content (BMC), BMD and BMC on total and proximal left tibia. Results: In design 2 (which lasted 405 days) group E obtained significantly higher values ($p<0.02$) for BMC in both total and proximal left tibia. Adding both designs, group E got a positive but not significant difference on BMD values compared to group C ($p=0.052$). No other significant differences were found. Conclusions: Diet E had an n-6/n-3 ratio almost 7 times lower than diet C, but obtained higher BMC values. This supports the idea that diets containing a higher proportion of n-3 fatty acids, as do diets with chia seeds, help achieving a greater peak bone mass and may protect against osteoporosis.

235 (1088) **NUTRITIONAL PATTERN AND PREVALENCE OF METABOLIC DISEASES IN SAN LUIS CITY, ARGENTINA: A FIRST REPORT OF FINDINGS**

Mayra Cortez[1], Ivana Olivero[1], Flavia Luna[1], Florencia Claveles[1], Florencia Barrera[1] , Lucia Gerrero[1], Lucia Lopez[1], Natalia Lozano[1], Ayelen Vallejos-Lucero[1] , Maria Fernanda Bruno[1], Maria Eugenia Dios-Sanz[1], Paula Maggi[1], Silvina Calcagni[1] , Maria Lurdes Pascuali[1] , Laura Aballay[2], Camila Niclis[3], Dario C. Ramirez[4], Sandra Esther Gomez Mejiba[1].
[1]PROICO-100414-School of Health Sciences, National University of San Luis, San Luis, Argentina [2]Statistics and Biostatistics. School of nutrition, National university of Cordoba, Cordoba. Argentina. [3]Institute of Research in Health Sciences. School of Medical Sciences, National University of Córdoba, Córdoba, Argentina. [4]Laboratory of Experimental and Translational Medicine, IMIBIO-SL-CONICET. National University of San Luis [5]Laboratory of Experimental Therapeutics. IMIBIO-SL-CONICET-UNSL, San Luis, Argentina.

The association between nutritional pattern and prevalence of metabolic diseases is of Public Health concern. Herein we performed a cross-sectional study in 103 patients between May 2015-May2016 in men and women between 18-80 years-old in statistically-selected neighborhoods in San Luis City. The nutritional status (Body Mass Index, BMI), anthropometric and sociodemographic data, physical activity (IPAQ short-version forms) and food intake (validated FFQ) were assessed to determine the average high-energy-dense food (HEDF) intake (g /day). Data were shortened by age and gender, and analyzed using the T- and Chi-square tests. The models of multiple-logistic regression were adjusted. This analysis included as responsible variable: presence/absence of overweight (BMI> 25) or obese (BMI> 30); and as co-variables: HEDF intake, gender, age, total-energy value (TEV) and level-of-physical activity. 51% of all patients were either

overweight or obese, of which 22% were obese, and no gender difference was observed. 36% were smokers or ex-smokers. Cardiovascular risk was estimated in 41% with no gender differences. TEV was estimated in 2,768±1,085 Kcal/day and was higher in men than women. HEDF intake was estimated in 385.7±287.2 g/day. This parameter did not show gender differences and highest in patients below 40's. There was a positive association of age with overweight (OR 1.05, IC95% 1.03-1.09) and with obesity (OR 1,77 IC95% 1,03–4,98). However, there was no association between HEDF intake and nutritional status. SFFQ showed that most of half of patients consumed high-fat and -sugar foods. Fry cooking was the preferred cooking method followed by salting and stubbing. Almost 50% patients use artificial sweeteners at least twice-a-day and a low intake of water, fruits and vegetables. In this first stage our data suggest that age, life-style, sweeteners, fries and HEDF intake are associated to increase risk of metabolic diseases. Supported by PROICO 100414/PICT2014-3369.

236 (2016) **DEVELOPMENT OF REFRIGERATION-FREE PROBIOTIC FOODS**

Carlos Bauman[1] , Roberto Grau[1].
[1]Laboratorio de Microbiología [2]Facultad de Ciencias Bioquímicas y Farmacéuticas [3]Universidad Nacional de Rosario [4]FONCyT.

Bacterial spores can tolerate very high and very low temperatures, desiccation and high pressures, changes in pH ant saltiness, solar and UV radiation, enzymatic and detergent attack. And they are in a completely dormant state until they germinate. For these reasons they are potentially suitable for industrial food production and could have an indefinite shelf life. In this study we characterized *Bacillus subtilis* Natto (BSN), which is used to produce the functional probiotic food Natto, a traditional Japanese dish for the last 1,000 years.

We produced Natto in the laboratory. Boiled soy beans were inoculated with BSN spores and incubated at 37 °C for 24 h.

We sprayed BSN spores on yerba mate and assessed whether or not they germinated over time (which would discard this as a commercial product).

We produced granola bars with BSN spores and evaluated their survival to cooking conditions (160 °C, 30 min).

A 2,000-fold increase in viable cells and 50-fold increase in spores relative to the inoculum was obtained in Natto. The vegetative cells reached their peak after 24 h, decreased 10 fold at 48 h and died completely by 120 h. The spores reached their peak at 24 h and remained constant over time.The spore count remained constant after six months of spraying them on the yerba mate. No germination was observed. There was an 80% survival of the BSN spores in the granola bars after cooking. There was no germination after a three-week period and the spore count remained constant.

In both situations, yerba mate and granola bars, the organoleptic properties of these foods were not altered.

Bacterial spores greatly amplify the world of industrial probiotics. And they become a key ingredient to a wide range of potential industrial probiotic food products at low cost and long shelf life.

237 (2021) **SCREENING OF NATURAL EXTRACTS WITH ANTI-OBESITY ACTIVITY**

Maria Rosana Ramirez[1].
[1]El Centro de Investigaciones y Transferencia de Entre Rios – CONICET – Facultad de Ciencias de la Alimentación- UNER.

Obesity is a metabolic disease resulting from the excessive accumulation of body fat, which causes health damage human; with substantial losses in the quality of life. Obesity treatments generally involve a combination of dietary modifications, physical activity, pharmacotherapy, and in some cases, surgery. The pancreatic lipase is one of the most studied targets for chemical compounds acting as anti-obesity agents because it is responsible for the hydrolysis of dietary fats in the intestine. The search for novel glucosidase inhibitors is important owing to their therapeutic potential in the treatment of diabetes, metastatic cancer, lysosomal storage disease, between others disorders. Several glycosidase and lipase inhibitors have been discovered from natural sources showing the potential of plant products as a source of such phytocompounds. Therefore, the objective of this study was to evaluate the inhibitory potential of the various natural extracts on the digestive enzymes β-glucosidase and lipase *in vitro*. The samples were also analyzed for antioxidant capacity, qualitative and quantitative composition of phytocompounds and nutrients. The determination of chemical compounds was performed by spectrophotometric methods. The antioxidant capacity was evaluated by the method of 2,2-diphenyl-1-picrylhydrazyl (DPPH), measuring the absorbance at 517 nm after 30 min. TLC bioautographic methods to detect lipase and β-glucosidase inhibitors in plant extracts were used. Some differences were observed among their chemical compositions and biological activities. Samples were rich in polyphenolics compounds and carotenoids. Additionally, the results suggested that the polyphenolic compounds was a very important factor responsible for the inhibitory activity against β-glucosidase and pancreatic lipase. In conclusion, this work suggests that natural products may be a potential source of dietary fat absorption inhibitors. The broad spectrum of the biological activities from the studied plant extracts can be applied as the guideline for the selection of plant species for additional pharmacological investigations.

238 (2047) **VITAMIN A DEFICIENCY ALTERS HORMONE RECEPTORS AND MORPHOLOGY´S VIRGIN MAMMARY GLAND.**

Miriam Ester Vasquez Gomez[1], Agustina Leonela Orozco Reina[1], Fiorella Campo Verde Arbocco[2], Veronica Fillipa[1], Fabian Mohamed[1], Graciela Jahn[2], Maria Sofia Gimenez[1].
[1]Dpto. Bioqca y Cs Biológicas. UNSL. IMIBIO-SL, San Luis. CONICET. E-mail: eridnere@gmail.com [2]IMBECU, CONICET, Mendoza, Argentina.

Vitamin A (VA) is important to growth of epithelial tissues and promotes the formation of lumen by mammary epithelial cells *in vitro* and suggests that plays a similar role *in vivo*. The effect of vitamin A deficiency (VAD) in the expression of hormone receptor, morphology and proliferation were determined. Wistar female virgin rats were separated into 6 groups: fed with VA diet for 3 months (c3m), VAD diet for 3 months (d3m), VAD diet for 75 days followed by refeeding with VA for 15 days (r3m), VA diet for 6 months (c6m), VAD diet for 6 months (d6m) and VAD diet for 150 days followed by refeeding with VA for 30 days (r6m). The expressions of estrogen receptors (REα and REα), prolactin receptor (RPrl), progesterone receptor B (RPB) were measured by RT-qPCR, fosfocolin citidililtranferase (CTα) by RT-PCR; fatty acids (FA) and unsaturation index of phospholipids (UIP) by FAME; PCNA by immunohistochemistry and morphology by hematoxylin-eosin. The expression of REα increased whereas REα and RPB decreased in d6m respect to c6m and r6m ($p<0.05$). Levels of RPrl, CTα, PCNA and total FA decreased in d3m and d6m respect to controls and refed ($p<0.05$). UIP increase in d3m and d6m with respect to control and refed ($p<0.05$). REα correlate with REα(r:-0.67, $P<0.017$). VAD generate morphological changes in d3m (low alveolar development and fewer alveoli) and d6m (ductal predominance and poorly developed) rats. Our results allow us to infer that a longer VAD, contribute to lower development in the gland. The lower values of RPrl and PCNA, could be the cause of the morphological changes. The variation in FA of phospholipids and decreased CTα, could alter the phospholipid/cholesterol ratio, and together with the increase of UIP, cause changes in membrane and biophysical properties. The 6 months VAD causes changes in REα, REα and RPB levels. The transcriptome alterations of receptors, directly or indirectly, could promotes histoarchitecture changes in the gland.

GENÉTICA / GENETICS

239 (237) **STUDY OF DUPLICATIONS OF THE FUNCTIONAL CYP21A2 GENE CARRYING Q318X IN GENETIC COUNSELLING**

Maria Pia Serra[1], Maria Lorena Viale[1], Andrea Laura Paissan[1], Soledad Lovazzano[1], Analia Valeria Stigliano[1], Rodrigo Garraza[1], Andrea Elina Kozak[1], Patricia Fainstein Day[1].
[1]Hospital Italiano de Buenos Aires.

Congenital Adrenal Hyperplasia (CAH) is an autosomal recessive condition due to a deficiency of one of 5 enzymes involved in the steroidogenesis pathway. The most common form is 21-hydroxylase deficiency.

Different mutations in the *CYP21A2* gene result in variable deficiency of cortisol and aldosterone together with increased synthesis of androgens. The level of residual enzyme activity determines the clinical phenotype that ranges from mild virilization to salt wasting life threatening crisis. Rare haplotypes with Q318X mutations and duplicated *CYP21A2* genes have been reported to occur in different populations to a varying extent. Discrimination between a normal (Q318X mutation on one of the duplicated *CYP21A2* genes) and an affected allele (Q318X mutation without duplicated functional gene) is important for prenatal diagnosis and genetic counselling. Individuals who carried the Q318X mutation on one of the duplicated *CYP21A2* genes are not affected with CAH. Objective To study the genotype of the partner of a woman with non-classic CAH (genotyping:V281L+ Del/Conv) who asked for genetic counselling, according to Q318X mutation and polymorphisms previously found. Methods:The genetic analysis included the amplification by PCR of exons and introns of CYP21A2 gene in four fragments and its sequenciation. The gene copy number was studied by Long-range PCR using primers specific for TNXA and TNXB. Results: We found a duplication of *CYP21A2* gene with heterozygous Q318X mutation on one copy next to TNXB and another non affected copy next to TNXA. Conclusions: Q318X mutation is frequently associated with duplication of the CYP21A2 gene. When this mutation is detected in healthy individuals, the number of copies of CYP21A2 gene must be established The high frequency of gene duplications, as well as novel variations, should be considered since they have an important involvement in carrier testing and genetic counseling.

240 (239) **STUDY OF AIP GENE IN PATIENTS WITH CLINICAL FEATURES OF FAMILIAR ISOLATED PITUITARY ADENOMAS (FIPA): FIRST SERIES IN ARGENTINA**

Maria Lorena Viale[1], Maria Pia Serra[1], Analia Valeria Stigliano[1], Rodrigo Garraza[1], Andrea Elina Kozak[1], Patricia Fainstein Day[1].
[1]Hospital Italiano de Buenos Aires.

Familiar isolated pituitary adenomas (FIPA) encompasses the familiar occurrence of isolated pituitary adenomas outside the setting of syndromic conditions such as MEN1 and Carney's complex, and comprise about 2-3% of pituitary adenomas. About 20% of FIPA have mutations in the aryl hydrocarbon receptor interacting protein gene (*AIP*), usually associated with a worse outcome.It is an autosomal dominantly inherited disease with a penetrance of *AIP* mutation between 15-30%. Objective:To assess the frequency of germinal mutations in *AIP* gene and polymorphisms(SNPs) in a cohort of patients with FIPA or with diagnosis of pituitary macroadenomas under the age of 36. Subjects and Methods:We studied 23 potential carriers and 66 healthy subjects (mean age 34,3 +/- 0,7 years, 50 women) was analyzed as control group.The promoter and exons 1 to 6 and intronic flanking regions were amplified by PCR. The DNA fragments were analyzed by sequencing. Results:We found the following SNPs:c.682C>A (Q228K) in 23 of the 23 patients studied,c.920A>G(Q307R) in 20/23, IVS 3 + 111 C>T in 13/23, c.-810T>G in 1/ 23, c.993+60G>C 3' UTR in 1/ 23 and , c.- 941 A>G in 1/23 this variant has not been previously described in the literature.

We did not find any mutations in the patients. Due to the high frequency of the Q228K and Q307R in the patients group we decided to study them in the control group and we found the Q228K in heterozygous form in 1/66 and 65/66 in homozygous form and the Q307R all in homozygous form. Conclusions:There are limited data on AIP SNPs with potencial functional consequences.In the literature these polymorphisms have been found significantly different between FIPA patients and healthy controls however in our population are equally distributed.Our results suggest that these variants do not have pathological implications.The investigation for mutations in the AIP gene in families with pituitary adenomas is necessary, since it is associated with poor outcome and resistance to treatment.

241 (354) **MOLECULAR CHARACTERIZATION OF GALT GENE IN CHILDREN WITH DECREASED GALACTOSE-1-PHOSPHATE URIDYLTRANSFERASE ACTIVITY**

Carolina Crespo[1], Hernan Eiroa[1], María Inés Otegui[1], Lilien Chertkoff[1], Luis Pablo Gravina[1].
[1]Hospital de Pediatría "Prof. Dr. Juan P. Garrahan".

Introduction: Classical galactosemia is an autosomal recessive inherited metabolic disorder caused by mutations in the galactose-1-phosphate uridyltransferase (*GALT*) gene. GALT enzyme deficiency leads to the accumulation of galactose-1-phosphate in various organs, causing hepatic, renal and cerebral impairment. Over 180 disease-causing mutations have been reported in the *GALT* gene. Diagnosis of galactosemia through analysis of total galactose and/or activity of GALT enzyme is effective, but environmental factors and the high frequency of the Duarte 2 (D2) variant may lead to false positive results, since D2 alleles cause partial deficiency of enzyme activity, meanwhile classical Galactosemia (G) alleles cause total deficiency. Objective: To describe molecular characterization of *GALT* gene in patients with decreased GALT activity, and to correlate molecular results with enzyme activity. Patients and Methods: Twenty four patients with enzyme activity below 9 µmol/h/g Hb (50% of normal value) were included. Q188R mutation was studied by PCR-RFLP. Samples negative or heterozygous for Q188R were sequenced by analysis of the 11 exons and the exon-intron boundaries of the *GALT* gene. Results: Ten different sequence variations were identified, including two novel mutations (p.M1T and p.S222R). The three most common disease-causing mutations were p. Q188R, p.K285N and IVS8-13A>G. They accounted for 16, 9 and 3 of the 48 alleles respectively. N314D Duarte 2 variant appeared in 13 of the 48 alleles. *GALT* genotype correlated with enzyme activity in 90% of patients. Conclusion: This is the first report of mutations in the *GALT* gene in Argentinean patients with decreased GALT activity. Molecular analysis is useful to reduce false positive results, distinguishing D2/G mixed heterozygotes from classical galactosemia G/G homozygotes. This study supports the importance of including the molecular analysis of *GALT* gene in the diagnostic algorithm of galactosemia.

242 (469) **H/ACA RIBONUCLEOPROTEIN COMPLEX EXPRESSION AND THEIR CORRELATION WITH PROGNOSTIC FACTORS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA**

Patricia Carolina Dos Santos[1], Julieta Panero[1], Carmen Stanganelli[2], Flavia Stella[1], Raimundo Bezares[3], Irma Slavutsky[1].
[1]Laboratorio de Genética de Neoplasias Linfoides, Instituto de Medicina Experimental, CONICET-Academia Nacional de Medicina. [2]División Patología Molecular, Instituto de Investigaciones Hematológicas, Academia Nacional de Medicina. [3]Servicio de Hematología, Hospital "Teodoro Álvarez", Buenos Aires, Argentina.

Chronic lymphocytic leukemia (CLL) is a clinically heterogeneous disease. Telomeres are essential structures that protect the ends of linear chromosomes, which functions depend on the telomerase enzyme and telomere associated proteins. Among them, H/ACA ribonucleoprotein (RNP) is composed of four evolutionary conserved proteins: Dyskerin (DKC1), NOP10, NHP2 and GAR1. We have evaluated the expression profile of the H/ACA RNP complex as well as *hTERT* and *hTR* telomerase subunits mRNA levels, in patients with CLL. Results were correlated with telomere length (TL), genetic alterations, *IGHV* mutational status, and clinicopathological characteristics of the disease. The study was performed on mononuclear cells from peripheral blood

samples of 72 CLL patients at diagnosis (31 females; mean age: 67 years) and 14 normal controls. Gene expression and absolute TL measurement was carried out by q-PCR. *IGHV* mutational status were analyzed by RT-PCR and sequencing. Cytogenetic and FISH analysis were also performed. Gene expression analysis showed higher mRNA levels of *hTERT*, *NHP2* and *GAR1*, as well as lower expression of *hTR*, *DKC1* and *NOP10* in patients compared to controls (p=0.043). A significant correlation between *GAR1*, *NOP10* and *NHP2* mRNA levels was detected (p=0.008), supporting a strong interaction among them. Patients with short TL had increased *hTERT* and *DKC1* expression (p=0.03). Higher mRNA levels of *DKC1* and *NHP2* in patients with two or more cytogenetic and FISH anomalies (p=0.04) compared to those with no/one alteration was observed. Increased *hTERT* expression in unmutated *IGHV* cases was also found (p=0.02). No association between gene expression and clinical parameters was found. Our findings show a global modification in the expression of telomere associated genes in CLL being, to our knowledge, the first analysis of *hTR*, *NOP10*, *NHP2* and *GAR1* in this pathology, suggesting a role for these genes in genomic instability and telomere dysfunction in CLL.

243 (519) **NUCLEAR INDUCTION OF TELOMERIC REPEAT-CONTAINING RNA DEPENDS ON MICROTUBULE STABILITY UNDER OXIDATIVE STRESS CONDITIONS**

Natalia Maricel Galigniana[1], Nancy Lorena Charó[1], Ana María Cabanillas[2], Graciela Piwien-Pilipuk[1].

[1]Laboratorio de Arquitectura Nuclear, Instituto de Medicina y Biología Experimental (IByME), CONICET, Buenos Aires, Argentina. [2]Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI)-CONICET, Depto. de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba.

Telomeres are nucleoprotein complexes at the end of linear chromosomes essential for chromosome stability, and they can be transcribed in response to developmental changes and cellular stress conditions. These transcripts are known as telomeric repeat-containing RNA (TERRA). Increasing oxidative stress and inflammation enhance the process of erosion of telomeres with each cycle of replication. In fact, the G-rich telomeric sequence is more susceptible to acute oxidative damage, compared with genomic DNA. However, little is known about how this affects TERRA levels. Since oxidative stress is known to disrupt cytoskeleton integrity and mechanical cues are important regulators of transcriptional programs, our objective was to characterize TERRA levels using different oxidizing agents and to evaluate the possible role of microtubules in this process. We previously found that human embryonic kidney HEK-293T cells undergo induction of TERRAs after 4h treatment with H_2O_2, sodium arsenite or buthionine-sulfoximine, which is prevented by antioxidant treatment with N-acetyl-L-cysteine. In the present study, we show that TERRA induction is mimicked by microtubule disruption using colcemid. Interestingly, microtubule stabilization using taxol also resulted in TERRA induction, suggesting that a delicate mechanotransduction mechanism is involved in TERRA regulation. Cell fractionation experiments showed that TERRAs localize in both nuclear and cytosolic compartments, contrary to classical belief. Nevertheless, TERRA induction was only observed in the nuclear fraction, implying that only the nucleus seems to harbor the newly transcribed TERRAs in response to H_2O_2 treatment. These results indicate that the nuclear functions of TERRAs may be essential in the response of HEK-293T cells to oxidative stress caused by H_2O_2 exposure, possibly in order to contribute to telomere integrity and, hence, genome stability.

244 (560) **UNCOMMON RESULTS OF NF1 MOLECULAR ANALYSIS IN A BIG FAMILY WITH NUMEROUS AFFECTED MEMBERS**

Diana Parma[1,2], Leonela Luce[1,2], Florencia Giliberto[1,2], Liliana Francipane[3], Irene Szijan[1], Marcela Ferrer[4].

[1]Cátedra de Genética, Facultad de Farmacia y Bioquímica, UBA. [2]Instituto de Inmunología, Genética y Metabolismo (INIGEM CONICET-UBA), Facultad de Farmacia y Bioquímica, UBA. [3]División Genética, Hospital de Clínicas "José de San Martín", Facultad de Medicina, UBA. [4]División Neurocirugía, Hospital de Clínicas "José de San Martín", Facultad de Medicina, UBA.

Neurofibromatosis type 1, the most common genetic disorder affecting the human nervous system, is the result of loss-of-function mutations of the tumor suppressor *NF1* gene and inherited in an autosomal dominant fashion. The condition predisposing individuals to the development of neurofibromas, optic nerve gliomas and skeletal abnormalities, is fully penetrant and has a highly variable expression, even within the same family.

The molecular diagnosis of NF1 is still difficult due to the large size of the gene, the existence of pseudogenes, the lack of mutational hotspots and the complex molecular spectrum. We studied familial NF1 in order to identify the family members with the risk of developing the disease. The focus was the molecular testing of the two youngest members (4 years, 2 months) of a family that includes 11 affected individuals out of 19 total relatives.

A simple and highly sensitive methodology was used: segregation analysis of four *NF1* intragenic polymorphic microsatellites (D17S1307, D17S1849, IVS27AC28.4, IVS38GT53.0) and mutational screening using bidirectional DNA sequencing of the *NF1* exons of interest. The analysis of the STRs revealed the at risk haplotype in the 7 affected members studied and a different haplotype in 2 individuals who could be excluded from the risk. The analysis of the two probands (still unaffected children) indicated that one of them carries the at risk haplotype while the other carries the different one.

A recombination event was found between markers D17S1849 and IVS27AC28.4 in two individuals. Interestingly one of them was affected and the other was asymptomatic. These data suggest that the mutation may be located between the markers mentioned above.

The data obtained are important for familial genetic counselling and allow the early diagnosis of predisposition to NF1. The finding of an intragenic recombination is an infrequent event in the NF1 syndrome.

245 (562) **IGHV MUTATIONAL STATUS AND PROGNOSTIC FACTORS IN CHRONIC LYMPHOCYTIC LEUKEMIA**

Carmen Stanganelli[1], Patricia Dos Santos[2], Juana Cabrera[1], Flavia Stella[2], Raimundo Bezares[3], Cecilia Rodriguez[4], Irma Slavutsky[2].

[1]Instituto de Investigaciones Hematológicas, Academia Nacional de Medicina. [2]Instituto de Medicina Experimental. [3]Hospital Álvarez, Buenos Aires. [4]Hospital Nacional de Clínicas, Córdoba.

Chronic lymphocytic leukemia (CLL) is the most common form of adult leukemia in the Western world, associated to a highly variable clinical course. The mutational status of the immunoglobulin variable heavy-chain (*IGHV)* region represents one of the best prognostic markers in this pathology. In this study, we analyzed the association between *IGHV* repertoire and mutational status in a series of 167 Argentinean patients with CLL (103 males; mean age: 65.5 years; 82% at initial stages) and correlate them with cytogenetic and fluorescence in situ hybridization (FISH) alterations as well as clinical parameters. *IGHV* analysis was performed by PCR and sequencing. *IGHV* sequences with less than 98% homology with respect to the germline counterpart were considered as mutated (M), and those with ≥98% were unmutated (UM). Cytogenetic and FISH analysis for13q14, 11q22 and 17p13 deletions and trisomy 12, were performed on peripheral blood lymphocyte samples. Fifty seven percent of patients were M-CLL, and 43% were UM. The *IGHV3-23* gene (10%) was the most commonly used, followed by *IGHV1-69* (9.5%), *IGHV4-34* (9%) and *IGHV3-21*(6.5%). Stereotyped HCDR3 (heavy chain complementary determining region 3) was found in 15% of patients and clusters #2, #4 and #7 were the most frequently found. Normal karyotypes (p=0.047) and deletion 13q14 as a single alteration (p=0.006) were more commonly observed in M-CLL, while trisomy

12 (p=0.01), deletion 17p13 (p=0.001) and two or more alterations by FISH were found associated to UM-CLL (p=0.0001). Forty three percent of patients expressing *IGHV1-69* had deletion 11q22. The analysis of clinical parameters showed significantly higher beta2-microglobulin (p=0.049) and shorter overall survival in UM-CLL patients (50.5 months) compared to M-CLL cases (98.5 months) (p=0.0001). Our results confirm the association of *IGHV* mutational profile and gene rearrangements with specific cytogenetic lesions and different clinical outcome in CLL.

246 (651) **ANALYSIS OF BASAL CHROMOSOMAL ABERRATIONS AND MICRONUCLEUS FREQUENCY IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA.**

Micaela Palmitelli[1, 2], Carmen Stanganelli[3], Patricia Dos Santos[2], Irma Slavutsky [2], Marcela González Cid[1].
[1]Laboratorio de Mutagénesis, Instituto de Medicina Experimental, CONICET-Academia Nacional de Medicina. [2]Laboratorio de Genética de Neoplasias Linfoides, Instituto de Medicina Experimental, CONICET-Academia Nacional de Medicina. [3]División Patología Molecular, Instituto de Investigaciones Hematológicas, Academia Nacional de Medicina, Buenos Aires, Argentina.

Chronic lymphocytic leukemia (CLL) is a B-cell malignancy characterized by a highly heterogeneous clinical evolution. The mutational status of the immunoglobulin heavy-chain variable (IGHV) region permits to divide this pathology in two different subgroups: mutated (M) CLL with less than 98% homology with respect to the germline counterpart, associated to better prognosis, and unmutated (UM) CLL with ≥98% homology, related to a poor outcome. Chromosome aberrations (CA) and micronucleus (MN) techniques represent different forms to evaluate genomic instability; MN provides a measure of both chromosome breakage and chromosome loss. In this study we analyze the basal frequency of CA and MN in 22 CLL patients (13 males; mean age: 64.6 years), and 7 controls. Cytogenetic analysis was performed on stimulated peripheral blood lymphocyte cultures. For each patient, CA was evaluated on 50 metaphases meanwhile 250 interphase nuclei were analyzed for MN frequency. *IGHV* mutational status was determined by PCR and sequencing. Fourteen (63.6%) CLL patients presented metaphases with chromatid breaks, gaps and dicentrics. Analysis of data showed increased MN frequency (2.87±0.38%) as well as the number of metaphases with CA (1.77±0.47) in CLL cases compared to controls (0.4±0.02% and 0.63±0.2, respectively) (p=0.01). When patients were analyzed according to the *IGHV* mutational status, M (10) and UM (12), no significant differences between groups in MN (3.17±2.18% and 2.52±0.98%) and CA (1.10±1.45 and 2.33±2.50, respectively) frequencies were observed. To our knowledge, this is the first study measuring MN and CA in CLL cases. Our results reflect the presence of genomic instability in this pathology and suggest that similar mechanisms may be present in M and UM subgroups of patients.

247 (675) **TEN YEARS OF EXPERIENCE IN MOLECULAR DIAGNOSIS OF FRAGILE X SYNDROME**

Mariana Capelli[1], María Florencia Cantarella[1], Mariana Samara[1], Nazareth Loreti[1], Verónica Ferreiro[1].
[1]Genos S.A.

Fragile X Syndrome (FXS) is a genetic disease mainly caused by an abnormal expansion in the number of the trinucleotide CGG repeats located in the 5' UTR in the fragile X mental retardation 1 gene (FMR1) at Xq27.3. This leads to a hypermethylated region in the gene promoter, therefore silencing it and lowering the expression levels of the fragile X mental retardation 1, a protein involved in synaptic plasticity and maturation.

Normal alleles (N) bare between 6 and 55 repetitions, premutated alleles (PM) may vary between 55 and 200 CGG repeats and generally don't present methylation pattern alterations. The alleles with more than 200 repeats are considered as full-mutated alleles (FM), these last ones are related to the Fragile X Syndrome phenotype.

Our aim was to perform a molecular strategy for the diagnosis of FXS in those patients with intellectual disability and/or developmental delay, submitted to our laboratory.

We analyzed DNA samples from 3538 individuals (2636 males and 902 females) using several molecular tests such as PCR, Fluorescent-PCR assay, TP-PCR, MS-MLPA and Southern Blot. We found 62 males and 20 females carrying a full-mutated allele; and 12 cases of mosaicism: 2 females N/PM/Full and 10 males Pre/Full.

Using the techniques mentioned in whole, we were able to arrive to the right diagnosis of FXS in 92 patients, almost all of them with the characteristic phenotype, and to rule out the diagnosis in several intellectually handicap patients, allowing the correct genetic counseling to the families.

248 (756) **ASSOCIATION OF GENETIC VARIANTS IN FCER1A AND FCER2 GENES AND TOTAL SERUM IGE LEVELS IN CHILDREN WITH ASTHMA**

Gabriel Veneruzzo[1], Verónica Araoz[1], Pablo Gravina[1], Adriana Roy[1], Claudio Castaños[1], Lilien Chertkoff[1], Verónica Giubergia[1].
[1]Hospital de Pediatría SAMIC "Prof. Dr. Juan Pedro Garrahan".

Introduction: Several studies suggest that genetic variants in the genes coding for the high affinity and the low-affinity IgE receptors (*FCER1A* and *FCER2*, respectively) are associated with IgE levels in Caucasian asthmatic and non asthmatic individuals.

Objectives: To determinate the genotype frequency of *FCER1A and FCER2* variants, (rs2427837 and rs28634072 respectively) and to evaluate the association of these variants with IgE levels in children with asthma from Argentina. Methods: Children of both sex, mean age 11.2 years, SD 3.3, with mild, moderate and severe asthma (n: 236) were genotyped for rs2427837 and rs28634072 variants by PCR-RFLP. Total serum IgE level was measured by ELISA and aged-adjusted values were determined for each individual (n: 204). A comparison of the distribution genotype among different populations was performed using X^2 test. Total serum IgE levels were analyzed using Kruskall-Wallis test for each genotype.

Results: The genotype frecuency was: 74.1% GG; 22.4% AG; 3.5% AA for rs2427837 and 68.4% TT; 29.4% CT; 2.2% CC for rs28634072. None of the 2 genotyped variants deviated from Hardy–Weinberg equilibrium. Genotype distribution according to severity group did not show statistical difference. The minoritary allele frequencies for both variants was significantly lower than the observed in other Caucasian populations (A: 0.15 for rs2427837 and C: 0.17 for rs28634072, p<0.0001). A significant difference of IgE serum levels was observed among the genotypes for rs2427837 (p=0.047), with the highest IgE level in the AA group. In contrast, no significant association of rs28634072 with IgE levels was identified.

Conclusion: In this series of patients the frequencies of the minor alleles for both genetic variants were significantly lower than the described in other Caucasian population. The AA genotype for rs2427837 was associated with higher IgE level. This genetic variant appears to contribute to IgE level in asthmatic children from Argentina.

249 (761) **CLINICAL CHARACTERIZATION OF PATIENTS WITH IN-FRAME MUTATIONS IN THE DMD GENE**

María Eugenia Foncuberta[1], Soledad Monges[1], Fabiana Lubieniecki[1], Julieta Mozzoni[1], Angélica Moresco[1], Luis Pablo Gravina[1], Lilien Chertkoff[1].
[1]Hospital de Pediatría "Prof. Dr. Juan P. Garrahan".

The dystrophinopathies include a spectrum of muscle diseases caused by pathogenic variants in *DMD* gene. The severe end of the spectrum is classified as Duchenne muscular dystrophy (DMD) and the mild end include asymptomatic forms with increased CPK. The commonest mutations are intragenic deletions (65%) and duplications (10%); the remaining 25% are small mutations. According to the reading-frame rule, frame shift mutations lead to

the severe DMD phenotype, whereas in-frame mutations lead to less severe phenotypes; nevertheless exceptions to the reading frame rule exist. Objective: to describe the phenotypic features in patients with in-frame mutations in the *DMD* gene. Methods: 335 male patients were studied by MLPA technique. Sequencing of *DMD* gene was performed in 55 patients with negative MLPA, clinical and biopsy compatible with dystrophinopathies. Clinical phenotype, muscle biopsy, age of wheelchair dependence, muscular and cardiac involvement were retrospectively collected. Results: 31 patients presented in-frame mutations (29 deletions, 1 duplication and 1 small deletion). In two cases the mutation encompassed part of the actin-binding domain (ABD), in 15 the ABD and part of the rod domain, 12 revealed deletions affecting the rod domain, 1 case showed a deletion in the cysteine-rich domain and 1 case presented a small deletion in the carboxyl-terminal domain. Regarding the clinical phenotype, the two patients with mutations in the ABD were clinical defined as BMD (Becker muscular dystrophy); 14 patients with ABD and rod domain deletions, and patients with deletions in the cysteine-rich and C-terminal domains were classified as DMD. Patients with deletions involving the rod domain showed a more heterogeneous clinical presentation, from high CPK to BMD. Conclusion: This study provides data about the phenotype characteristics of patients with in-frame mutation in the *DMD* gene that may be important for prognosis and therapeutic aspects of the disease.

250 (777) **MULTIPLE CONGENITAL ANOMALIES (MCA) AND ISOLATED CONGENITAL HEART DISEASE (CHD): PRELIMINARY GENETIC RESULTS IN PATIENTS UNDER A RESEARCH AND DEVELOPMENT CLINICAL PROJECT**

Lucia Soledad Massara[1,2], Marisol Delea[1], Pablo Barbero[1], Maria Paz Bidondo[1], Verónica Cazayous[2], Viviana Cosentino[3], Lilian Furforo[4], Mónica Rittler[4], Liliana Dain[1].
*[1]Centro Nacional de Genética Médica- ANLIS, C.A.B.A. [2]Hospital de Alta Complejidad en Red El Cruce – SAMIC, Pcia. de Buenos Aires. [3]Hospital Interzonal General de Agudos Luisa Cravenna de Gandulfo, Pcia de Buenos Aires. [4]Hospital Materno Infantil Ramón Sardá, C.A.B.A. *The first two authors equally contributed.*

Congenital anomalies (CA) are morphological and/or functional disorders that originate before birth. Affecting 3 to 5% of newborns, they represent the second leading cause of infant mortality in Argentina, after perinatal conditions. The etiology of CA is heterogeneous ranging from genetic to teratogenic factors. However, in approximately 50% of the patients, the underlying causes are unknown. Cases with MCA are those with 2 or more unrelated birth defects. MCA are present in 2,26/1000 births. CHD are the most frequent CA, with a prevalence of 4,06/1000 births.

The goal of this work was to identify chromosomal abnormalities and genomic imbalances as part of an algorithm aiming to study the genetic causes of MCA and isolated CHD cases from our population.

Peripheral blood was obtained from 104 (59 MCA and 45 isolated CHD) patients born between June 2015 and July 2016 in 13 public hospitals participating in the National Network of Congenital Anomalies of Argentina (RENAC). DNA was extracted from all cases and a karyotype was performed in MCA patients. Two MLPA kits (CHD associated regions) were used in patients presented with conotruncal CHD.

A normal karyotype was found in 35/59 MCA patients, while 5/59 presented cytogenetic abnormalities: trisomy 18 (n=2), a 47, XXX, an apparently balanced translocation (t(1;2)(q25;q21)), and a supernumerary marker chromosome. In 19 patients, the karyotype could not be performed.

Conotruncal CHD were present in 18 cases. By MLPA analysis, a typical 22q11 deletion was observed in 1/5 patients with conotruncal CHD as a feature of MCA, and in 2/13 of isolated conotruncal CHD cases. One patient with isolated contruncal CHD presented an imbalance in 15q14.

Our results are in accordance with the expected frequency of chromosomal abnormalities and 22q11 imbalances in this group of patients. An array–CGH and/or NGS will be performed in the near future for selected patients according to the proposed algorithm.

251 (880) **STRUCTURE-BASED PREDICTION OF CYP21A2 NOVEL VARIANTS: A SURVEY OF GENE VARIATIONS**

Carlos David Bruque[1,2], Marisol Delea[1], Leandro Simonetti[2], Juan Orza[1], Noemí Buzzalino[1], Lucía Espeche[1], Belén Benavides[1], Alejandro Nadra[3], Liliana Dain[1,2], Cecilia Fernández[1].
[1]Centro Nacional de Genética Médica, ANLIS. [2]Instituto de Biología y Medicina Experimental, CONICET. [3]Departamento de Química Biológica Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, IQUIBICEN-CONICET.

In a previous work we have developed and validated a procedure with the goal of predicting the effect of newly uncharacterized mutations variants in the *CYP21A2* gene with improved accuracy based on the high identity bovine and human templates. In this work we performed an exhaustive survey of mutations and SNPs (found in general population) in the coding sequence of the *CYP21A2* gene encoding the 21-hydroxylase enzyme. All reported variants were initially classified according to their putative impairment on protein dysfunction and/or location in the structure. We focused our analyses on that affecting protein stability lacking functional assays aiming to predict their residual enzymatic activity (REA). Variants were retrieved from the Human Cytochrome P450 Allele Nomenclature database, dbSNPs, 1000 Genome Database and from the bibliography. The predicted free energy of each of the mutants relative to the wild type counterpart ($\Delta\Delta G$) was calculated by the FoldX algorithm and the REA was estimated as previously described. When available, phenotype of patients and presence of a mutation in the homologous allele were also considered. A total of 343 variants were collected, 148 were presumed to be involved in protein stability and 108 lacked functional assays. For the latest group, in 10/32 mutants the REA was in accordance with the expected one, while in 9 no information was available to perform such correlation. In 52/76 SNPs the predicted REA was above of 75% and these SNPs were considered as non-pathogenic. Our tool allowed us to predict the effect of uncharacterized variants. While most of the SNPs showed no biological implications, some of them may have a deleterious effect.

Since the clinical manifestation of the 21-hydroxylase deficiency depends on the less affected allele, the estimation of the residual activity of a novel variant is important for an accurate genetic counseling.

252 (987) **THE ROLE OF THE GLUCOCORTICOID RECEPTOR IN PANCREATIC PROGENITOR CELL DIFFERENTIATION AND BETA CELL GENESIS**

Silvio Adrián Traba[1,2], Mariya Chhatriwala[3], Ludovic Vallier[3,4], Adali Pecci[1,5], Santiago A. Rodríguez-Seguí[1,2].
[1]Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE-UBA-CONICET), Buenos Aires, Argentina. [2]Departamento de Fisiología, Biología Molecular y Celular (FBMC), Universidad de Buenos Aires, Buenos Aires, Argentina. [3]Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, United Kingdom. [4]Anne McLaren Laboratory for Regenerative Medicine, Department of Surgery and Wellcome Trust - Medical Research Council Cambridge Stem Cell Institute, University of Cambridge, United Kingdom. [5]Departamento de Química Biológica (QB), Universidad de Buenos Aires, Buenos Aires, Argentina.

The in vitro production of functional beta cells for transplantation in type 1 diabetic patients is a long-standing goal to achieve, which would allow the suppression of insulin administration. Current differentiation protocols to derive beta cells from human pluripotent stem cells (hPSCs) artificially mimic the cell signaling events that occur during fetal development, and involve manipulation of signaling pathways that are known to play key and stage-specific roles during pancreas growth and differentiation. Thus, gaining insights into the mechanisms by which novel pathways control this process might significantly impact this field of research. Previous studies have shown that the glucocorticoid receptor (GR) signaling pathway plays an important role in the generation of an appropriate number of beta cells to accomplish an accurate glycemic control in adults, although the underlying

molecular mechanisms still remain largely unexplored. We have recently reported the construction of genomic cis regulatory maps in human pancreatic islets and in the human embryonic pancreas based on ChIP-seq and RNA-seq experiments. In the latter case we used human pancreatic buds that were dissected from 6-7 wpc embryos and cells from a matched differentiation stage that were in vitro derived from hPSCs.

Our analyses led to the discovery that TEAD and YAP are important gene expression regulators that in the embryonic pancreas are in charge of maintaining the MPC phenotype. Further analysis of this resource shows that a subset of the genes that are highly expressed in MPCs could be downregulated upon activation of the GR pathway. Using in vitro mouse and human models of pancreas development, our current efforts are focused in understanding how glucocorticoids affect the MPC gene expression program.

253 (1006) **THE ROLE OF CYTOCHROME P450 ISOENZYMES ON PORPHYRIA CUTANEA TARDA DEVELOPMENT**

Diego Miguel Gordillo[1], Lubna Abou Assali[1], Gabriela Nora Cerbino[1], Laura Sabina Varela[1], Alcira Batlle[1], Victoria Estella Parera[1], María Victoria Rossetti[1].
[1]Centro de Investigaciones sobre Porfirinas y Porfirias (CIPYP) - CONICET - Hospital de Clinicas – UBA.

Porphyria Cutanea Tarda (PCT) is a disorder characterized by a reduction of Uroporphyrinogen decarboxilase (UROD) activity in all tissues or only in liver in hereditary (H-PCT) or acquired (A-PCT) PCT forms, respectively. In both PCT types multiple susceptibility factors, such as iron excess, smoking, estrogens and halogenated polycyclic hydrocarbons, contribute to liver UROD deficiency. It was suggested a role for CYP1A1 and CYP1A2 isoformes in PCT development. We analised three polymorphisims, one in CYP1A2 and two in CYP1A1 in a group of Argentinean PCT patients and in a control group, with the aim of investigating if they could play some role on PCT manifestation. One hundred PCT patients, 37 H-PCT and 63 A-PCT employing PCR-RFLP were analysed and each variant was compared with 73 controls. The Fisher exact test was used to detect differences in alelles and genotype frequencies, odds ratio and 95% confidential interval. For CYP1A2*1F (-734 C>A) the frecuences of A alelle and A/A genotype are higher for both types of PCT vs control group, being the A allele associated to a more transcriptional activity of CYP1A2 gene. There are significant diferences for A/A ($p<0.022$) and A/C ($p<0.022$) vs C/C genotype and also A allele vs C alelle ($p<0.018$) for total PCT vs control group. For CYP1A1*4A (c.4487 C>A), C alelle and C/C genotype are the most frequent for total PCT vs control group. A significant differece for A/A genotype vs C/C genotype ($p<0.028$) and C/A ($p<0.014$) was found. The same was observed in H-PCT vs control group, $p<0.0001$ and $p<0.0005$, respectively. For CYP 1A1 *2C (c.4889 A>G), A/A genotype and A alelle frequencies were higher for all groups. In this case G alelle codified for a more activity enzyme. Significant difference were found only for A-PCT vs control for A/A vs A/G genotypes ($p<0.0016$). So we could concluded that these polymorphisms could be, among others, a risk factor to PCT clinical signs triggering in Argentinean population.

254 (1044) **USE OF DNA OLIGONUCLEOTIDES AND SSDNA APTAMERS AS PROTEIN DETECTION TOOLS COMPLEMENTARY TO WESTERN BLOTS.**

Cristian Jorge A. Asensio[1], Tomás Antonio Santa Coloma[1].
[1]Biomed, CONICET-UCA. Alicia Moreau de Justo 1600. Buenos Aires.

The use of DNA as reagent in the detection of proteins bound to nitrocellulose or other membranes is known as south-western technique and it is equivalent to the much better known western-blot technique that employs antibodies. In here, we proposed to determine if the use of biotinylated DNA oligonucleotides can be a tool complementary (or supplementary) of western blots. We explored the profiles produced by the binding of different oligonucleotides to proteins resolved by SDS-PAGE and present in lysates of different cell lines or organs. We determined the way the oligonucleotides interact with the different proteins including histones. Interestingly, by modulating the composition of the solutions employed for membrane blocking, DNA binding and washing we were able to control which one of the histones is detected preferentially. On the other hand, when the aim was to avoid histone detection, we were also able to prevent it by adding some components to the binding and/or the blocking buffers. Furthermore, we studied the compatibility of the use of oligonucleotides in membranes already employed with antibodies, stripped or not. Besides, we determined the capacity of the oligonucleotides to bind histones and other proteins in the presence of salts and detergents together with the optimal solution for oligonucleotide stripping and membrane re-probing. Finally, to evaluate the usefulness of our technique characterizations in the field of DNA aptamers, we tested a ssDNA aptamer sequence previously published as specific for a human core histone peptide but that had not been selected or validated in the context of a cell lysate or of the other histones. Interestingly, this aptamer was not able to differentiate its intended histone target from the other core histones present in the lysates. We hope that our findings and strategies will improve DNA aptamer selection programs in general as well as to facilitate studies of histones and other proteins in blotting membranes.

255 (1072) **GENOMIC DIAGNOSIS IN PATIENTS WITH INTELLECTUAL DISABILITY BY MLPA AND ARRAY-CGH IN A PUBLIC GENETIC CENTER IN ARGENTINA**

Lucía Daniela Espeche[1], Andrea Solari[1], Marisol Delea[1], Lilian Furforo[1], Carlos David Bruque[1], Belén Benavidez Mori[1], Ma. Ángeles Mori[2], Julián Nevado[2], Sandra Rozental[1].
[1]Centro Nacional de Genética Médica "Dr. Eduardo Castilla", ANLIS, Ministerio de Salud, CABA, Argentina. [2]Instituto de Genética Médica y Molecular, Htal. Univ. La Paz, Madrid, España.

Genomic rearrangements are responsible for a variety of phenotypes. Subtelomeric anomalies (SA) have been reported, using FISH or MLPA, in approximately 5% of patients with unexplained intellectual disability (ID) and dismorphic features. The introduction of arrayCGH has enabled the identification of both subtelomeric and interstitial genomic imbalances, or copy number variants (CNVs), in approximately 10-20% of patients.

The aim of our study was to determine the contribution of SA screening before arrayCGH analysis and the identification of imbalances in patients with ID.

We studied 330 patients with ID for SA with MLPA kits P036 and P070. Of those, 60 were analyzed by arrayCGH (Karyoarray and ISCA 8x60K platforms).

SA were detected in 7% (21/330) of patients with both MLPA kits and the results were inconclusive for 6/330 that showed SA with only one kit. With arrayCGH, interstitial genomic imbalances were detected in 36/60 patients; in 14/36 we identified benign CNVs and in 14/36 variants of uncertain significance. Among the 8/36 patients with CNVs classified as pathogenic (13%), the phenotypes showed common and different features with similar cases reported in the literature. Parental studies facilitated, in some cases, clinical interpretation of the genomic imbalances detected.

Our results demonstrate that the screening for SA by MLPA is not cost effective. The use of arrayCGH allowed us to detect clinically relevant genomic imbalances and to provide new evidence to delineate the phenotype of the imbalances detected.

ONCOLOGÍA II / ONCOLOGY II

256 (236) **LOSS OF TRISTETRAPROLIN (TTP) PROMOTES DEVELOPMENT OF DYSPLASTIC LESIONS IN TONGUE EPITHELIUM**

Micaela N. Stedile[1], Dario M. Ferri[1], Mariela Veggetti[1], Edith Kordon[1], Ana R. Raimondi[1].
[1] Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE) UBA, Conicet.

Oral squamous cell carcinoma (OSCC) constitutes the sixth leading cause of cancer worldwide. The early events in OSCC

carcinogenesis have been less studied and still poorly understood. In this regard, a common feature among relevant RNAs of OSCC and other types of cancer is the presence of regions enriched in adenine and uracil (AREs) situated in 3´untranslated regions (3´UTR). The stability of RNAs with AREs is regulated by a group of proteins called AUBPs (ARE – binding proteins) which bind these regions. Here we focused on a particular AUBP, tristetraprolin (TTP). This protein promotes degradation of mRNAs with AREs in their 3´UTR. Recently, expression of TTP has been reported diminished in cell lines and biopsies of OSCC. Considering this background, we hypothesize that lack of TTP in basal tongue cells produces tissue abnormalities associated to early carcinogenesis events. To test this hypothesis, we generated double transgenic mice in a tissue – specific and conditional manner (K14-CreERtam /TTPloxP+/+ = TTP KO). Tongue tissue from these mice was used to study proliferation and differentiation markers by immunohistochemistry. During the evaluation period (8 months) mice were healthy. However, we found a significant decrease in transgenic mice weight compared with wild type mice (p< 0, 01). Histologic analysis of TTP-KO tongues revealed moderate dysplastic areas which exhibited chronic inflammation with infiltrated macrophages and increased vascularization. Immunohistochemistry of epithelial TTP – KO tongue showed an abnormal expression of PCNA (proliferation marker) and deregulated expression of cytokeratins 1, 6 and 14 (differentiation markers). Particularly, PCNA and cytokeratin 14 were found in suprabasal layers, which are typically expressed in the basal layer of normal tissue. We conclude that lack of TTP is sufficient to deregulate proliferation and differentiation in tongue epithelium and contributes to progressive accumulation of dysplastic changes.

257 (258) **ABSENCE OF TNFR1 ENHANCES INFILTRATION OF CD4 + /CD8 + T-LYMPHOCYTES IN THE TUMOR MICROENVIRONMENT REDUCING SUBCUTANEOUS MELANOMA GROWTH.**

Yamila Isabel Rodriguez[2], Melina Castro[2], Ludmila Campos[2], María Cecilia Della Vedova[2], Dario Ramirez[1,2], Diego Croci Russo[3], Sergio Alvarez[1,2].
[1]Universidad Nacional de San Luis. [2]IMIBIO SL- CONICET. [3]IHEM-CONICET.

A tumor is not simply a mass of genetically modified and identical cells. Neoplastic cells are in close interaction with endothelial, stromal and immune cells by both cell-cell contact and soluble factors in the tumor microenvironment. The participation of natural killer (NK) cells, CD4+ and CD8+ T lymphocytes (TL) and M1 pro-inflammatory macrophages are determinant in cancer regression. However, tumor cells have mechanisms to evade the immune response and modulate the microenvironment allowing the establishment of cell phenotypes that promote tumor growth, invasion and metastasis. Thus, the tumor microenvironment has a crucial role in cancer progression. Indeed, in a model of subcutaneous implantation of B16F1 murine melanoma cells, we have previously shown a diminished tumor growth in tumor necrosis alpha receptor 1 (TNFR1)-KO mice compared to wild type (WT) mice. Here, we aimed to establish the role of immune cells and cytokines in this differential response. The number of M2-like tumor associated macrophages (TAMs; F4/80+ CD206+) and lymphocytic infiltrate (CD4+ CD8+ LT) was evaluated by flow cytometry in tumor samples collected 19 days after inoculation of B16F1 cells. In addition, we estimated CCL-2 and IL-10 levels by QPCR and ELISA respectively. Although expression of CCL-2 (chemokine involved in macrophage recruitment) was significantly higher in tumor tissue from TNFRI-KO mice, no differences in the number or TAMs were observed. Furthermore, reduced levels of IL-10 in TNFRI KO mice were accompanied by a significantly higher lymphocytic infiltrate CD4+ and CD8+. Altogether, these data indicates that absence of TNFRI in C57BL/6 mice enhances antitumor immune responses leading to diminished tumor growth.

258 (269) **MUSCARINIC SIGNALING PROMOTES TUMOR ANGIOGENESIS: ROLE OF MUSCARINIC RECEPTORS EXPRESSION IN NON-TUMORIGENIC BREAST CELLS.**

Paola Martínez Pulido[1], Dileyvic Giambalvo Gomez[1], Manuel Oroño[1], María Gabriela Lombardi[1], María Elena Sales[1].
[1]Centro de Estudios Farmacológicos y Botánicos (CEFYBO).

Angiogenesis is one of the most important steps in tumor progression. The development of new capillaries involves the participation of pro-angiogenic factors like vascular endothelial growth factor-A (VEGF-A) and extracellular matrix remodelation by metalloproteinases (MMP). Our previous works demonstrated that muscarinic acetylcholine receptors (mAChR) are over-expressed in human breast cancer tumors in comparison to normal breast tissue. We reported that non-tumorigenic human breast cells, MCF-10A neither show immunolabelling for mAChR, VEGF-A and MMP-9 nor stimulate angiogenic response in NUDE mice. Here, we developed stable MCF-10A cell lines that express different mAChR subtypes (mAChR-lines) and we investigated the effect of mAChR activation on VEGF-A levels, MMP-9 activity and tumor neovascular response induced in vivo. We demonstrated by Western blot that the transfection of mAChR induced de novo VEGF-A and MMP9 synthesis in comparison to non-transfected MCF-10A cells. Both effects were potentiated by the addition of the cholinergic agonist carbachol (CARB) and reverted by the preincubation with muscarinic antagonists or by silencing mAChRs with specific interference RNA (iRNA). CARB also increased MMP-9 activity in the supernatants of mAChR-lines. We also observed that mAChR-lines inoculated in the skin of NUDE mice induced a strong neovascular response (vessel mean density±SD) (mAChR3:5.0±1.1; mAChR4:6.4±0.7; mAChR3R4:4.8±0.5) and CARB significantly enhanced angiogenic response (mAChR3:6.7±0.8; mAChR4: 8,6±0.9; mAChR3R4: 6.4±0.6) (p<0.05; N=6). The effects on MMP-9 activity and neovascular response were reverted by muscarinic antagonists and iRNA treatment. In conclusion, our results indicate that mAChRs over- expression on non-tumorigenic breast cells induced an angiogenic response mediated by VEGF-A and MMP-9 that may promote tumor progression.

259 (316) **TUMOR AND CIRCULATING CD4+ AND CD8+ LYMPHOCYTES IN CBI- MICE CHALLENGED WITH TRIPLE NEGATIVE MAMMARY ADENOCARCINOMA**

Antonela Del Giúdice[1,], Angela Paula Oviedo[1], Lenadro E Mainetti[1,3], O.Graciela Scharovsky[1,2,3], María J Rico [1,3], Viviana R Rozados[1].
[1]Instituto de Genética Experimental, Facultad de Ciencias Médicas Universidad Nacional de Rosario [2]CIC-UNR [3]CONICET.

Mammary adenocarcinoma M-406 (M-406) is a murine triple negative tumor which, when inoculated s.c. in CBi$^-$ inbred mice is rejected in 100% of the animals, while when inoculated i.v. generates lung metastases in 100% of them. In order to explain this discrepancy and due to the importance of the immune response in tumor and metastases growth, the number of CD4$^+$ and CD8$^+$ lymphocytes in primary tumor, lung metastasis and blood, was determined in tumor bearing mice. CBi$^-$ mice were distributed into two groups that were challenged with M-406: GSC) s.c. inoculum and GIV) i.v. inoculum. Blood samples were taken on day 0 (baseline), from mice of both groups; blood and primary tumor, on day 10, from mice of GSC group, and blood and lung metastases, on day 20, from mice of group GIV. The baseline percentage of circulating CD4$^+$ lymphocytes (assessed by flow cytometry) [median (range), 82.4 (74.5-90.0)] was significantly higher compared to GIV [73.2 (62.3-75.4)] (P<0.05), without differing from GSC [75.8 (52.6-88.0)]. The% of circulating CD8$^+$ lymphocytes showed no differences between groups, baseline [7.7 (4.7- 10.0)], GSC [7.1 (0.8-8.0)] and GIV [5.7 (0.2 -15.7)]. The number of CD4$^+$ [2 (1-2)] and CD8$^+$ lymphocytes [2 (1-2)] in lungs (assessed by immunohistochemistry in 25 fields, 1000X) were significantly lower than those observed in the primary tumor: CD4$^+$ [6.3 (5.5-9.0)], CD8$^+$ [7.5 (2.5-9.5)]. These results allow us to conclude: 1) The lower number of CD4$^+$ and CD8$^+$ lymphocytes in lungs compared to that observed in the primary tumor would explain, at least in part, the presence of lung metastases and the rejection of the

primary tumor observed when the tumor is inoculated s.c. 2) The interaction between the immune system and the tumor cells with metastatic phenotype in CBi^- mice, leads to a decrease of circulating $CD4^+$ lymphocytes which would contribute to the growth of lung metastases.

260 (323) **BENEFITS OF METRONOMIC THERAPY TARGETING MUSCARINIC RECEPTORS IN BREAST CANCER INVOLVES AN INCREMENT IN APOPTOSIS/NECROSIS RATIO AND A DECREMENT IN ABCG2 EXPRESSION**

Alejandro Español[1], Agustina Salem[1], Yamila Sanchez[1], Lucas Laserna[1], María Elena Sales[1].
[1]Centro de Estudios Farmacológicos y Botánicos (CEFYBO-UBA-CONICET).

We demonstrated that muscarinic acetylcholine receptors (mAChR) are expressed in breast tumor cells while they are absent in non-tumorigenic murine and human breast cells. The addition of carbachol (CARB) during 40h stimulates cell death similarly to paclitaxel (PX), a cytostatic drug frequently used in breast cancer treatment. Applying the principles of metronomic therapy, a combination of low concentrations of CARB with PX promoted cell cytotoxicity generating a apoptosis/necrosis ratio 2:1 in murine mammary adenocarcinomas. In the human breast tumor cell line MCF-7, derived from a luminal tumor, the treatment with CARB (10-9M) and PX (10-11M) increased cell death by 47± 6% ($p < 0.001$ vs. control) measured by MTT assay, in a similar manner to 10-6M PX, that also induced death in the non-tumorigenic cell line, normal MCF-10A, an undesirable effect in chemotherapy. By flow cytometry with annexine-V and 7-AAD, we demonstrated that the combination reduced necrosis ($p<0.05$ vs. control) and increased apoptosis ($p<0.05$ vs. control). In addition, we observed that after treatment with CARB plus PX cells were less sensitive to the stimulation with CARB 10-9M during 1h that usually induces proliferation in tumor cells. These effects could be mediated by a reduction of 92 ± 3.05% ($p<0,001$ vs. control) in the expression of ABCG2 protein measured by Western blot. This protein is involved in the resistance to cancer drugs administration because it favors the output of these drugs into the extracellular. We can conclude that the treatment of MCF-7 tumor cells with a metronomic combination of CARB plus PX could be as effective as pharmacological concentrations of PX to produce cell death and to reduce drug resistance in cancer treatment.

261 (329) **UNRAVELING NUCLEAR ERBB-2 ROLE IN RESISTANCE TO ERBB-2-TARGETED THERAPIES IN BREAST CANCER**

Santiago Madera[1], María Florencia Chervo[1], Leandro Venturutti[1], Franco Izzo[1], María Alicia Cortes[2], Violeta Chiauzzi[1], Cecilia Proietti[1], Roxana Schillaci[1], Eduardo Charreau[1], Rosalía Inés Cordo Russo[1], Patricia V. Elizalde[1].
[1]Laboratorio de Mecanismos Moleculares de Carcinogénesis, Instituto de Biología y Medicina Experimental (IBYME - CONICET), Buenos Aires, Argentina [2]Facultad de Medicina, Universidad Nacional del Nordeste (UNNE - CONICET), Corrientes, Argentina.

Membrane overexpression of ErbB-2 (MErbB-2), member of the ErbB family of receptor tyrosine kinases, or its gene amplification occurs in 15-20% of breast cancers (BC) and is associated with poor prognosis. ErbB-2 directed therapies include the monoclonal antibody trastuzumab (Tz) and lapatinib (Lap), a tyrosine kinase inhibitor. Despite their clinical efficiency, many patients do not respond to such therapies and resistance to available drugs is still a major clinical issue. Notably, ErbB-2 migrates to the nucleus (NErbB-2) where it acts as a transcription factor (TF) or as a coactivator of TF. Here we explored the role of NErbB-2 in BC resistant to Tz and Lap. For this purpose, we transfected BC cells with the ErbB-2ΔNLS mutant which is unable to translocate to the nucleus and also acts as a dominant negative inhibitor of endogenous ErbB-2 nuclear translocation, and compare ErbB-2ΔNLS, Tz and Lap effects on MErbB-2-overexpressing human BC cells sensitive (BT-474) or resistant (JIMT-1) to Tz and Lap. Analysis of ErbB-2 subcellular distribution showed that ErbB-2 was mainly located at the plasma membrane in BT-474 cells and that heregulin (HRG), a ligand of ErbBs, induced NErbB-2 localization. In JIMT-1 resistant cells, NErbB-2 was constitutively detected and further enhanced by HRG. Nor Tz neither Lap blocked NErbB-2 presence in BT-474 and JIMT-1. Despite basal proliferation in BT-474 was inhibited by ErbB-2ΔNLS, Tz and Lap, only ErbB-2ΔNLS was able to block HRG-induced proliferation. Moreover, ErbB-2ΔNLS, but no Tz or Lap, inhibited JIMT-1 proliferation. We have previously demonstrated that NErbB-2 modulates BC growth acting as a coactivator of the TF Stat3 and regulating Cyclin D1 (CCND1) expression. We revealed that HRG induces CCND1 expression and that only ErbB-2ΔNLS inhibits its levels in JIMT-1 cells. These findings highlight NErbB-2 as a novel therapeutic strategy in Tz and Lap resistant BC, aiming the ErbB-2 oncogenic pathway unreached by current therapies.

262 (340) **THE HSP90-BINDING COCHAPERONE FK506-BINDING PROTEIN 51 IS A NOVEL MODULATOR OF TELOMERASE ENZYMATIC ACTIVITY**

Nadia Romina Zgajnar[1], Cristina Daneri Becerra[1], Michelle Patiño[1], Mario Daniel Galigniana[1,2].
[1]Instituto de Biología y Medicina Experimental, IBYME-CONICET [2]Departamento de Química Biológica, FCEN-UBA.

Immunophilins (IMMs) are a family of proteins that bind immunosuppressive drugs and show peptidylprolyl isomerase activity (PPIase). FKBP51 is an Hsp90-binding cochaperone able to bind the macrolide FK506, which in turn, abolishes PPIase activity. We have previously demonstrated that FKBP51 is a novel mitochondrial factor with antiapoptotic action that is highly expressed in cancer cell lines and tumour tissues. These systems show high telomerase activity, whose inverse transcriptase subunit, hTERT, is an Hsp90 client protein. Because both proteins, FKBP51 and hTERT, are highly expressed in tumours, and both of them form complexes with Hsp90, we postulated that FKBP51 could form complexes with hTERT regulating its enzymatic activity. Here we show that FKBP51 and its close-related partner FKBP52 (75% similitude) co-immunoprecipitate with hTERT-Hsp90 complexes. Such association is disrupted by the Hsp90-inhibitor radicicol, and due to the overexpression of the TPR-domain of the IMM, which is required to interact with the chaperone. Confocal images show a high colocalization score for hTERT and FKBP51, in particular when various stressing conditions such as oxidative stress, among others, favour the rapid nuclear accumulation of the IMM. When the stimulus persists, hTERT is exported to mitochondria, an event that could be related to the chaperoning action of FKBP51 after releasing hTERT from the telomeric regions where it is anchored. The cytoplasmic hTERT pool not imported to mitochondria (where it shows antiapoptotic actions) is targeted to proteosomal degradation. Importantly, measurements of telomerase activity demonstrated that FKBP51 greatly enhances the enzymatic activity, a key requirement to protect the shortening of chromosome ends and favor the rapid clonal expansion of the cells. In this regard, its close partner FKBP52 is functionally redundant. In short, this study demonstrates for the first time that IMMs are novel regulators of hTERT activity.

263 (357) **RUNX1 PARTICIPATION IN EPITHELIAL MESENCHYMAL TRANSITION IN BREAST CANCER**

Sofia Maria Sosa[1], Maria Sol Recouvreux[2], Luciana Rocha-Viegas[1], Pablo Nicolas Echeverria[3], Natalia Rubinstein[1,4].
[1]Instituto de Fisiologia, Biologia y Neurociencias, UBA-CONICET, Argentina. [2]Instituto Oncologico "Angel H Roffo", Buenos Aires, Argentina. [3]Departamento de Biologia Celular, Universidad de Ciencias III de Ginebra, Suiza. [4]Departamento Fisiologia y Biologia Molecular y Celular-FCEN-UBA, Argentina.

Triple-negative breast cancer (TNBC) is a heterogeneous group of diseases with a well-known association with epithelial-mesenchymal transition (EMT). EMT is a process implicated in the early stages of the metastatic cascade, and involves the cellular conversion to a more invasive (mesenchymal) cell type. Recent

results from our group show that the transcription factor Runx1 is able to up regulate the expression of RSPO3 (oncogene) and down regulate GJA1 (tumor suppressor gene) on breast tumor cell lines in a Foxp3-depending fashion. Others and we showed that Runx1 is necessary for epithelial mammary tumor cell migration and invasion. Furthermore, it was found that RUNX1 protein expression correlates with poor prognosis in TNBC. Runx1 was first described as a hematopoietic TF involved in human leukemia; however, growing research evidences demonstrate that there is an incomplete understanding of its transcriptional activity in breast cancer. The aim of this study was to determine if Runx1 is expressed and active during EMT. Using a gene expression profile database obtained by TGFb-EMT induction model on epithelial cell line, we found that mesenchymal cells express significantly higher levels of Runx1 mRNA, these data was validated by qPCR on NMuMG ($p<0,05$; Control vs TGFb-treated cells). Published ISMARA analysis showed that Runx1 transcriptional activity could be potentially up regulated during EMT. Interestingly, bioinformatics-based data done by our group reveals that SOX4 (master regulator of EMT), FST and GALNT6 (EMT-related genes) are potential targets of Runx1. Finally, by Chip analysis we demonstrated that Runx1 is able to bind to all three-promoter regions on (MDA-231 and LM3) mammary tumor cell lines. Collectively, these evidences prompted us to suggest that Runx1 is induced during TGFb treatment on mammary epithelial cells and its transcriptional activity could be responsible for the epithelial-mesenchymal transition.

264 (373) **THIOSEMICARBAZONES: POTENTIAL ANTITUMOR AGENTS AGAINST BREAST CANCER**

Aldana Solimo[1], Maria Cristina Soraires Santa Cruz[2], Andrea Loaiza Perez[1], Elisa Bal de Kier Joffe[1], Liliana Finkielsztein[2], Mariana Callero[1].
[1] Instituto de Oncología Ángel H. Roffo [2]Química Medicinal, Depto de Farmacología, FFyB, UBA.

Thiosemicarbazones (TSC) are synthetic compounds with antibacterial, anti-viral, anti-fungal and anti-neoplasic activities. Our aim was to investigate the anti-tumor effect of three TSC (T1, T2, T3). Experiments were done using two human breast cancer cell lines MCF-7 and MDA-MB 231. Cytotoxicity was evaluated by MTS assay in order to determine IC50 values. MCF-7 cells (ER+/ PR+/ Her2-) showed the following values T1= 6,47±0,4 mM; T2= 5,7±2,5 mM and T3= 90,97±7,7 mM while in MDA-MB231 cells (ER-/ PR-/ Her2) the IC50 were T1= 3,82±1,3 mM; T2= 4,16±0,9 mM and T3= 16,69±9,3 mM. By etidium bromide and acridine orange stain, we determined that citotoxicity was mediated by apoptosis in both cell lines. Apoptotic cells were quantified by a cytometric TUNEL assay. After a 48 h treatment, we analyzed cell cycle on propidium iodide stained cells by flow cytometry and we detected an increase in Sub G0 cells in both cells lines treated with T1 and T2 (10 mM). MCF-7 cells also showed a diminished colonies-forming capacity under T1 and T2 treatment (10 mM) respect to control cells (90,33±6,1 and 66,6±1,3 vs 270±21.4 colonies, respectively, $p<0,05$). In order to investigate TCS effect on MCF-7 cancer stem cells, we evaluated mamosphere-initiating capacity and we found that treatment with T1 and T2 (10 mM) significantly reduced primary mamosphere number and diameter respect to control cells (60,56±6,4; 151±15,7and 58,9± 5,1 vs 269±13,8 mamospheres/well, $p<0,05$). Respect to MDA-MB 231 cells we evaluated, TCS-modulation on cell migration by a wound healing assay. After a 48 h treatment, T1 and T2 (10 mM) significantly reduced cell migration capacity respect to control cells (13,02 ± 0,2%; 13,35 ± 0,5% vs 30,19 ± 3,7%, respectively, $p<0,05$).

In conclusion, our results show T1 and T2 anti-tumor effects on MCF-7 and MDA-MB 231 cells. Further studies on TCS mechanism of action could place this type of drugs as a possible alternative therapy for breast cancer treatment.

265 (402) **METABOLIC SIGNATURE CHARACTERIZATION IN PROSTATE CANCER MEDIATED BY HEME-OXYGENASE 1**

Nicolás Anselmino[1], Alejandra Páez[1], Javier Cotignola[1], Geraldine Gueron[1], Elba Vazquez[1], Valeria Gabriela Antico Arciuch[1].
[1]Laboratorio de Inflamación y Cáncer, IQUIBICEN/Dpto. Química Biológica, CONICET/FCEN-UBA.

Prostate cancer (PCa) is the second leading cause of cancer-associated death in men, being bone metastases the main cause of mortality. Energetic metabolism alterations have become a new hallmark of cancer, since variations in a single gene can orchestrate changes in metabolic pathways and confer an adaptive advantage. Heme-oxygenase 1 (HO-1) exerts an antitumoral role in PCa inhibiting cell proliferation, migration, tumor growth and angiogenesis. The aim of this work was to assess the role of HO-1 in the metabolic signature of PCa. Through RNA-Seq we found a set of metabolic genes deregulated under pharmacological induction (hemin treatment) or genetic induction of HO-1 in PC3 cells. STAR and ATP5L2 were found upregulated, while HMGCS2, PRODH and ACOT12 were downregulated. These genes encode for steroid hormone metabolism, ATP synthesis, ketogenesis, proline and lipid metabolism. The analysis of the deregulated genes (2-fold) under HO-1 modulation by Gene Ontology revealed alterations in several metabolic pathways such as steroid, tyrosine and lipid metabolism, and ion transport. Bone is the only site of PCa progression, and bone cells are able to produce factors that increase the growth and survival of tumor cells favoring progression. However, the molecular nature of this interaction remains to be elucidated. Our preliminary results performed on co-cultures of PC3 cells (treated or not with hemin) with Raw264.7 (pre-osteoclastic) or MC3T3 (pre-osteoblastic) cells demonstrate that HO-1 is able to direct the metabolic fate of bone precursor cells due to the deregulation of glycolytic genes. HO-1 induction in PC3 cells downregulated the expression of PKM2 and LDHA in co-cultured Raw264.7 and MC3T3 cells ($p< 0.05$). Based on our results, we propose HO-1 as a key regulator of the metabolic status of PCa cells and a powerful mediator capable of redefining the metabolic signature of bone precursor cells, thus, favoring the establishment of a less aggressive phenotype.

266 (413) **TRANSCRIPTOMIC PROFILING OF EPITHELIAL TO MESENCHYMAL TRANSITION BIOMARKERS UNDER HEME-OXYGENASE 1 (HO-1) MODULATION IN PROSTATE CANCER**

Sofía Lage Vickers[1], Federico Schuster[1], Alejandra V. Paez[1], Nicolás Anselmino[1], Emiliano G. Ortiz[1], Javier Cotignola[1], Elba Vazquez[1], Geraldine Gueron[1].
[1]Laboratorio de Inflamación y Cáncer, IQUIBICEN/Dpto. Química Biológica, CONICET/FCEN-UBA.

Epithelial–mesenchymal transition (EMT) is a cellular mechanism long recognized as a central feature of normal development and cancer. During tumorigenesis EMT may increase the motility and invasiveness of cancer cells. This process plays critical roles in the development of the prostate gland. Loss of epithelial markers (e.g. E-cadherin) and gain of mesenchymal markers (e.g N-cadherin) at the leading edge of solid tumors are associated with progression to metastasis. Elevated N-Cadherin has been shown to be a significant predictor of clinical recurrence in prostate cancer patients following radical prostatectomy. We have previously described the anti-tumoral effect of heme oxygenase I (HO-1) in PCa. To assess EMT differential expression in PCa cells overexpressing HO-1 pharmacologically (hemin treatment) or genetically (transfected with pcDNA3HO-1 vector) we performed RNAseq on PC3HO-1 vs. PC3pcDNA3 (empty vector) and PC3 hemin vs. PC3 control. For both comparisons, we obtained putative differential subsets of up-regulated (≥ 2, ≥ 3, ≥ 5, ≥ 8 fold change cut-off) and down-regulated (≤ -2, ≤ -3, ≤ -5, ≤ -8 fold change cut-off) genes. We screened for overlapping up or down regulated genes on same threshold subsets. Results show 92 down (≤ -2, $p<0.05$) and 118 up-regulated (≥ 2, $p<0.05$) overlapping genes. We performed GO analysis identifying pathways in which these differentially expressed genes are involved and enriched in subcategories including cell adhesion, extracellular structure organization, embryonic organ morphogenesis, tissue regeneration and epithelial structure maintenance. We performed a heat map depicting the top key markers related to EMT regulated by HO-1

induction. Of note our results show a significant down regulation for N-cadherin, OB-cadherin, fibronectin, laminin-5, and twist2, markers driving EMT. Our work extends the evidence that HO-1 is implicated in EMT, interrupting the cell plasticity of tumor cells, contributing to an epithelial phenotype in PCa.

267 (415) **TRANSCRIPTION FACTOR MOTIFS IN HO-1 EFFECTOR GENES IN PROSTATE CANCER.**

Federico Schuster[1], Sergio Nemirovsky[1], Jimena Giudice[2], Javier Cotignola[1], Elba Vazquez[1], Geraldine Gueron[1].
[1] Department of Biological Chemistry, University of Buenos Aires / IQUIBICEN CONICET, Buenos Aires. [2] Department of Cell Biology and Physiology, School of Medicine. University of North Carolina at Chapel Hill, Chapel Hill, NC.

Prostate cancer (PCa) is the second leading cause of death by cancer in males worldwide. A chronic inflammatory microenvironment is a decisive factor of PCa progression. The induction of HO-1 represents an essential event in cellular responses to pro-oxidative and pro-inflammatory insults maintaining cellular homeostasis. HO-1 has been proposed to act as a biosensor regulating cell destination. We previously reported by ChIP analysis that HO-1 associates with gene promoters relevant in PCa (PSA, uPA and MMP-9). These results provide a novel function for HO-1 down-modulating AR transcriptional activity. We have also shown that HO-1 physically interacts with transcription factors (TF) such as STAT3 and ZNF589. To screen for potential HO-1 TF partners, we performed RNAseq on PCa cells overexpressing HO-1 pharmacologically (hemin treatment) or genetically (transfected with pcDNA3HO-1 vector). Bioinformatics analyses were performed in order to identify putative TF binding motifs in promoter sequences of HO-1 up- or down-regulated genes. The regulatory regions of up- or down-regulated genes were considered for analysis (1 kb up- and 0.2 kb downstream of TSS). We established a working pipeline where multiple bioinformatics tools were applied to obtain the regulatory regions (MEME suite). A list of TF motifs over-represented in these regulatory region datasets compared to a neutral one (regulatory regions of genes with 1.02 ≥ fold regulation ≥ -1.02) was obtained by Analysis of Motif Enrichment tool (AME). Results rendered lists of TF associated with each data set. The same analysis was done for the overlapping HO-1 modulated genes (pharmacologically and genetically) revealing two key TFs relevant in PCa: JDP2 component of the AP-1 transcription factor and TGFB1I1, co-activator of the androgen receptor. Overall, our results provide further evidence for a novel function for HO-1 as a potential co-regulator of transcription in PCa.

268 (427) **INITIAL CHARACTERIZATION OF MAGEC2 TUMOR PROTEIN**

Franco Pascucci[1], Julieta Laiseca[1], Fernanda Toledo[1], Fatima Ladelfa[1], Martin Monte[1].
[1]Lab. de Oncología Molecular, Dpto Química Biológica; Facultad de Ciencias Exactas y Naturales; IQUIBICEN UBA-CONICET; Universidad de Buenos Aires.

MAGE-I (Melanoma Antigen GEnes) are expressed in tumors, placenta and germ line cells. Since MAGE-I proteins are highly homologous, it has been suggested that could display a redundant function. We and others have presented evidence pointing to specificity rather than redundancy in many cases. In this work we pointed to the initial characterization of MageC2 function by comparing to the activity of already established MAGE-I proteins. We first determined that MageC2 is a nuclear localized protein with a molecular weight of 55KDa approximately. This localization is different to that observed for MageB2 (nucleolar) but similar to MageA2 and MageA11. With the aim to evaluate it function, we compared MageC2 activity to that of MageA2 as inhibitor of p53 and MageA11 as coactivator of the androgen receptor, AR. We performed specific reporter gene assay to analyze the effect of MageC2 on p53 (pG13-LUC reporter) and AR activity (pPSA-LUC reporter). Our results indicated that MageC2 exerts no significant induction on AR (1,3 fold increase) as compared to MageA11 (4,2 fold increase). However, when we tested MageC2 on p53 activity, we observed 3 fold repression, behavior comparable to that of MageA2 (4,1 fold repression). Western blot analysis showed that p53 protein level was not affected in either case. ARF tumor suppressor is a negative regulator of MAGE-I proteins. While MageA11 is degraded by ARF, MageA2 is relocalized to the nucleoli. To understand the effect of ARF on MageC2, we performed immunofluorescence and protein stabilization assays. No changes in cellular localization were detected when MageC2 was co-transfected with GFP-ARF. Interestingly, ARF expression correlated with 50% decrease of MageC2 protein levels. Our preliminary results suggest that the oncogenic potential MageC2 could rely on its ability to inhibit p53. The mechanism of ARF regulation on MageC2 is more likely to involve protein degradation rather that cellular relocalization.

269 (812) **SURVIVIN VARIANTS, EXPRESSION LEVEL AND SUB-CELLULAR LOCALIZATION IN PEDIATRIC HODGKIN LYMPHOMA**

Mario Alejandro Lorenzetti[1], María Jimena Mosna[1], Sandra Colli[2], Paola Andrea Chabay[1], Elena Noemí De Matteo[2], María Victoria Preciado[1].
[1]Laboratorio de Biología Molecular, División Patología, Hospital de Niños Ricardo Gutiérrez. [2]División Patología, Hospital de Niños Ricardo Gutiérrez.

Survivin, a member of the inhibitors of apoptosis family is expressed during fetal development but down regulated in adult tissues. The over expression of survivin has been described in a variety of epithelial tumors, but to a lesser extent in lymphoid malignancies such as pediatric Hodgkin lymphoma (HL). In addition to wild type survivin (svn-wt) 5 splice variants were described, where Svn-wt, Svn-DEx3 and Svn-2B are clinically relevant. Moreover, sub-cellular localization was shown to alter its function as a cell cycle modulator in carcinomas. The aim of the work was to study mRNA expression of survivin major splice variants by RT-qPCR, protein expression pattern within tumor microenvironment as well as its sub cellular localization in a series of 18 pediatric HL by imunofluorescence and correlate it with proliferation and apoptosis markers, IHQ for Ki-67 and TUNEL, respectively. Svn-wt mRNA was detected in 16/18 cases while Svn-2B and Svn-DEx3 mRNA amplified in 12/18 samples; remaining cases were negative. Survivin was expressed within the nucleus in the majority of tumor cells (68-100%, median 88%), identified by double staining with CD30, and to a lesser extent in the surrounding infiltrate (21-80%, median 61%). Moreover, survivin expression, quantified as the integrated optical density of fluorescent signal was significantly stronger in tumor cells vs infiltrating cells (p=0,0003). Ki-67 stained positive in tumor cells (50-100%, median 96%) and no TUNEL+ tumor cells were detected; however, no statistical correlation was found between any survivin variant and Ki-67 or TUNEL. In conclusion, given that survivin is over expressed in the nucleus of tumor cells but that no correlation was observed between the number of survivin+ tumor cells, or the fold change expression in mRNA of any given survivin variant and ki-67+ cells, it is plausible to suggest that survivin could act as one contributing factor to the survival of tumor cells and the pathogenesis of HL.

270 (843) **GLUCOSE 6-PHOSPHATE DEHYDROGENASE INHIBITION AND METFORMIN TREATMENT SINERGIZES TO KILL FIVE HUMAN MELANOMA CELLS**

María Florencia Arbe[1], Gerardo Claudio Glikin[1], Liliana Maria Elena Finocchiaro[1], Marcela Solange Villaverde[1].
[1]Universidad de Buenos Aires, Facultad de Medicina, Instituto de Oncología Ángel H. Roffo, Área de Investigación, Unidad de Transferencia Genética, Buenos Aires, Argentina.

Cancer cells exacerbate not only glycolysis but also TCA cycle and other metabolic pathways including pentose phosphate pathway (PPP) to enhance biosynthetic processes, proliferation and growth. Human malignant melanoma is a highly aggressive and frequently life-threatening skin cancer. In addition, melanoma metabolic rewiring has been previously described. The aim of the present work was to investigate the cytotoxic effects of modulating

different metabolic pathways with metformin (MET, antidiabetic drug), 2-deoxyglucose (2DG, hexokinase inhibitor), dichloroacetic acid (DCA, pyruvate dehydrogenase kinase inhibitor), 6-aminonicotinamide (6-AN, glucose 6-phosphate dehydrogenase inhibitor, G6PD) and methrotrexate (MTX, dihydrofolate reductase inhibitor) on five human melanoma cell lines (hM1, hM2, hM4, hM9 and A375). After 24 h of culture, cells were treated with 2DG (0.5-10 mM), MET (1-15 mM), DCA (0.5-50 mM), 6-AN (1-100 uM) and MTX (1-10000 nM). The antitumor effects of bioenergetic modulation were evaluated by the acidic phosphatase assay (APH), 5 days after treatments. We found diverse responses since each melanoma cell line showed a different profile of sensitivity thus suggesting high metabolic plasticity. Then, we studied what was the effect of combining the metabolic modulators. Interestingly, we found that independently of the individual response, the combination of the G6PD inhibitor (6-AN, 50 uM) and MET (5 mM) extremely decreased the viability ($p<0.0001$) of all evaluated melanoma cell lines. Here we reported for the first time that inhibiting G6PD, the rate limiting enzyme of PPP, drastically increased MET cytotoxic effects. The results reported here support further studies to investigate the mechanism involved in this synergistic effect and its biological relevance.

271 (870) **CELL-MEDIATED IMMUNITY INDUCED BY ACTIVE IMMUNOTHERAPY IN NON SMALL CELL LUNG CANCER PATIENTS VACCINATED WITH RACOTUMOMAB**

Valeria I Segatori[1], Héctor A Cuello[1], Marina Albertó[1], Cynthia A Gulino[1], Daniel F Alonso[1,2], Mariano R Gabri[1,2].
[1]Laboratory of Molecular Oncology, National University of Quilmes, Buenos Aires, Argentina [2] National Council of Scientific and Technical Research (CONICET), Buenos Aires, Argentina.

The strategies based on positive modulation of immune system actually represent therapeutic options with prominent acceptance for cancer patients treatment due to its selectivity and higher tolerance compared to chemotherapy. Racotumomab is an anti-NGc- containing gangliosides anti-idiotype monoclonal antibody that has been aproved in Argentina as an active immunotherapy for the treatment of non small cell lung cancer (NSCLC). The aim of this work is to evaluate if vaccination with racotumomab induces antibody-dependent celular cytotoxicity (ADCC) in NSCLC patients included in a phase III clinical trial. Abs against NGc-gangliosides in patients´ pre and hiperimmune serum samples were first evaluated by FACS using X63 murine myeloma cells (highly positive NGcGM3). The percentage of X63 cells stained with post-vaccination serum samples was significantly higher in comparison with the percentage of cells that were incubated with pre-immune serum ($p<0.05$ ANOVA). We explored ADCC response in serum samples from 21 vaccinated patients. The results indicated that anti-NGc Abs in hiperimmune samples from 18 vaccinated patients elicited a specific cytotoxic activity against X63 cells. We considered a positive serum response when patients developed at least a two-fold increase in tumor cells lysis after vaccination. As expected, when target cells were devoid of NGcGM3 by PDMP treatment, ADCC was significantly decreased ($p<0.05$ two-way ANOVA followed mean CI comparison test), demostrating that vaccination induced an antigen specific celular immune response. For those patients that showed at least a two-fold increase in ADCC activity median survival was 13 months, determined by Kaplan Meier analysis. In contrast, for those that reached a lower cytotoxic response median survival was 9 months. Our data strongly suggest that racotumoab vaccination induces an antigen specific celular immune response in NSCLC patients and it could be associated with longer survival.

272 (891) **RAC3 OVEREXPRESSION INDUCES A TUMOR INICIATING CELL PHENOTYPE**

Laura Panelo[1], Felipe Martín Jaworski[2], María Fernanda Rubio[1], M. Cecilia Lira[1], Francisco Rosa[1], Elba Vazquez [2], Mónica A. Costas[1].
[1]Instituto de Investigaciones Médicas Alfredo Lanari; IDIM-CONICET [2]Facultad de Ciencias Exactas y Naturales; IQUIBICEN-CONICET.

RAC3 is a nuclear receptor coactivator whose expression is almost undetectable in differentiated cells, but it is overexpressed in several tumor types and required to maintain pluripotency in stem cells. We have previously demonstrated that RAC3 overexpression increases proliferation, apoptosis resistance and the invasive capacity of different tumor cell lines. We investigated if RAC3 overexpression in non-tumoral cells could be inducing a CSC-like phenotype. Non-tumoral HEK293 cells were transfected with a RAC3 expression vector (RAC3) or the empty vector (EV) and, then, different CSC parameters were analyzed. We found a significant increase in CD44 protein levels in RAC3 compared with EV ($p<0.05$), in addition to an increased Hoechst efflux compared with EV ($p<0.05$), compatible with an increased chemoresistance. Moreover, RAC3 overexpression enhanced the tumorsphere and clonogenic growth compared with EV ($p<0.01$; $p<0.05$). These characteristics correlated with an increased migratory and invasive behaviour, as well as, an increase in Vimentin expression. Therefore, we investigated if RAC3 cells were capable of generating tumors in Nu/Nu mice. 2x106 RAC3 or EV cells were subcutaneously inoculated and tumor growth subsequently monitored. We found that only RAC3-overexpressing cells were tumorigenic in vivo (9 days; $p<0.01$). However, these tumors disappeared 30 days post-injection. We may conclude that RAC3 overexpression induces CSC-like phenotype but additional signals could be required in vivo to maintain the tumor growth.

273 (894) **CHARACTERIZATION OF THE GLYCOPHENOTYPE AND THE RELATED GLYCOENZYMES IN PEDIATRIC CANCER CELL LINES AND PATIENT´S DERIVED PRIMARY TUMORS**

Héctor Adrián Cuello[1], Marina Alberto[1], Cynthia Gullino[1], Rosario Aschero[2], Laura Galluzzo Mutti[2], Valeria Inés Segatori[1], Fabiana Lubieniecki[2], Mariano Rolando Gabri[1].
[1]Molecular Oncology Laboratory, Quilmes National University, CONICET, Buenos Aires, Argentina. [2]Department of Pathology, Pediatric Hospital "Juan P. Garrahan", Buenos Aires, Argentina.

In cancer, altered expression of glycoenzymes (GE) results in cell surface glycosylation changes, such as falling or rising expression of certain glycans, accumulation of precursors and appearance of incomplete and novel structures. The resulting altered glycophenotype (GP) is a leading factor in the behavior of the malignant cell, having a crucial role in the development of the malignant phenotype. Little is known about the GP in pediatric cancers such as Neuroblastoma (NB), Retinoblastoma (RB), and Medulloblastoma (MB). The aim of this work is to characterize the GP in solid pediatric cancer and its relationship with the GE transcripts levels, in cell lines and in Patient´s Derived Primary Tumor Cell Cultures (PDTCC). GP was evaluated by flow cytometry using monoclonal antibodies against LeX, SLeX, LeA, SLeA, LeY, LeB, Tn, T epitopes, and lectins for glycan branching detection. Transcripts levels of GE were measured by Real-Time PCR with specific primers. Results showed that expression of Lewis glycan family is related to an aggressive tumor GP. We observed high expression of SLeX and Lex in RB Weri-RB1 cell line, which is in accordance with high transcripts levels of ST3Gal3 and C2GnT1. However, Y79 cell line showed a lower or an absent expression of these glycans as well as the GE evaluated. Whereas N-myc amplified NB cell line CHP-212 expressed high levels of SLeX, SleA and LeY, as observed in other N-myc amplified NB cell lines, N-myc non amplified cells did not show these GP. While evaluating metastatic MB PDTCC, we observed that high expression of SLeX occurred in accordance with high levels of ST3Gal3 and C2GNT1 transcripts. We propose that tumor cell lines could be a useful tool to resemble the patient´s tumor GP. Also, regarding the few reports related to the GP and GE expressions in pediatric tumors, our results suggest that there is a substantial modulation of them in relation to the aggressiveness of the cells.

274 (901) **ASSOCIATION BETWEEN OCT-4 EXPRESSION AND CHEMORESISTANCE IN COLORECTAL CSC-LIKE ENRICHED SUBPOPULATIONS**

Rodrigo Lloyd[1,3], Milena Batalla[1], María Belen Cerda[1,2], Florencia Giannoni[1,2], Agustin Giannoni[1], Osvaldo Podhajcer[2,4], Lucía Policastro[1, 2].
[1]Laboratorio de Nanomedicina, Comisión Nacional de Energía Atómica [2]CONICET [3]Instituto Nacional del Cáncer [4] Fundación Instituto Leloir .

Chemoresistance in tumors has been linked to the presence of a small subpopulation of cells named cancer stem cells (CSCs). These cells exhibit several characteristics which allow them to escape and survive from chemotherapeutic drugs, such as overexpression of multidrug resistance transporters, effective antiapoptotic mechanisms and an increase antioxidant capacity to maintain low intracellular levels of reactive oxygen species (ROS). The expression of embryonic genes in this subpopulation is also related to the development of these mechanisms of resistance in some cancers, but this issue is poorly known in colorectal cancer (CRC). The objective of this study was the characterization of an CSC-like enriched cultures derived from tumorspheres generated from the oxaliplatin-resistant T84 CRC cell line developed in our laboratory. We have characterized the expression Oct-4, Sox-2 and Nanog embryonic genes in these cultures, in order to identify potential resistant-associated genes to be silencing by small interference RNA molecules (siRNAs). Our results indicate that cultures derived from tumorspheres exhibit an increased expression of Oct-4 but no differences in the expression of Nanog and Sox-2 genes. Based on these results we choose Oct-4 as a siRNA silencing target by transfection protocols to evaluate it role in chemoresistance. We have observed that cell survival was decreased significantly in Oct-4 silencing CSC-like enriched cultures in presence of oxaliplatin respect non treated cultures. In conclusion Oct-4 might be an effective target to be silenced in the CSCs-like population in CRC in gene therapy protocols to sensitize conventional chemotherapy treatment.

275 (908) **CANCER STEM CELLS IN THE RESISTANCE TO PHOTODYNAMIC THERAPY IN HUMAN GLIOBLASTOMA MULTIFORME AND SQUAMOUS CARCINOMA**

Lucia Belén Rodriguez[1], Maria Laura Vilchez[1], Laura Milla[1], César Prucca[3], Maria Julia Lamberti[1], Rodrigo Palacios[2], Beatriz Caputto[3], Viviana Rivarola[1].
[1]Dpto. de Biología Molecular. Universidad Nacional de Rio Cuarto. [2]Dpto. de Química. Universidad Nacional de Rio Cuarto. [3]Dpto. de Química Biológica. CIQUIBIC. Universidad Nacional de Córdoba.

Photodynamic therapy (PDT) is an oncologic treatment. It is based on the administration of a photosensitizer and its activation through visible light. It is mainly employed to treat skin, bladder, oesophagus and lung cancer. An innovator PDT application is for human glioblastoma multiforme (GBM). The main problem of all anticancer therapies is the recidivism caused by resistant cells. It is proposed that only a small proportion of neoplastic cells, called cancer stem cells (CSC), have the capacity to generate a tumour and resist to oncologic treatments. The objective of the present investigation was determinate if PDT-Me-ALA (pro-drug) resistant cells of T98G and SCC lines have CSC characteristics, as a first step to further search of therapeutic targets. There were determinated in resistant cells, comparing with parental cells (non submitted to PDT): the tumorigenic capacity (immune-depressed mice), the growth ability in 3D cultures (spheroids), the intracellular photosensitizer accumulation after Me-ALA incubation (fluorescence microscopy, flow citometry and spectrofluorometry) and the cell viability after PDT employing ALA and Me-ALA. The resistant population showed CSC characteristics, such as higher tumorigenic capacity, spheroids with higher viability, size and number of cells, lower photosensitizer drug accumulation and higher viability after PDT, respect to parental cells. These results motivated us to continue this investigation studying the CSC molecular ways with the aim to find possible therapeutic targets to enhance the PDT efficacy.

276 (936) **THE ANTI-CK2 PEPTIDE CIGB-300 ELICITS AN ANTITUMOR EFFECT IN VITRO AND INHIBITS METASTATIC DISSEMINATION IN VIVO IN AN AGGRESSIVE MURINE MAMMARY CARCINOMA MODEL**

Carla Sabrina Capobianco[1], Johanna Elena Sidabra[1], Yasser Perera[2], Silvio Perea[2], Daniel Fernando Alonso[1], Hernan Gabriel Farina[1].
[1]Laboratory of Molecular Oncology, Quilmes National University [2]Laboratory of Molecular Oncology, Division of Pharmaceuticals, Center for Genetic Engineering and Biotechnology (CIGB).

CK2 is a holoenzyme overexpressed in several types of cancer. It has more than 300 substrates mainly involved in DNA reparation and replication, chromatin remodeling and cellular growth. In recent years CK2 became an interesting target for anticancer drug development. CIGB-300 is a peptidic inhibitor of CK2, designed to bind to the phospho-acceptor domain of CK2 substrates, impairing the correct phosphorylation by the enzyme. Previously, it was demonstrated that CK2 inhibition was able to significantly reduce primary tumor growth using heterotopic cervix and lung cancer models. Both in vitro and in vivo, CIGB-300 also displayed an important anti-angiogenic and pro-apoptotic effect on tumor cells. Taking into account the fact that breast cancer is one of the main tumor types in which CK2 is overexpressed, and the antitumor effects shown in other tumor types, our focus in this work is to study the effect of CK2 inhibition using CIGB-300 as a modulator of key features of breast cancer cell biology. In this regard, a proliferation and a cell adhesion assay was conducted. In addition, the effect of CIGB-300 treatment in the phosphorylation of the proliferative kinase ERK was measured by Western Blot analysis. In vivo, the effect of intravenous administration in tumor cell dissemination to lung and the growth of local recurrences in an incomplete surgery model was studied. As we expected, CIGB-300 reduced the proliferation (IC50=158 μM) and spreading capability of F3II cancer cells, in addition with a decreased level of ERK phosphorylation. In vivo studies using the syngeneic F3II breast cancer model in Balb/c mice showed a decrease in the number of lung metastases in mice treated systemically with CIGB-300 after primary tumor surgery (Mann Whitney test, $p<0.05$). However, CK2 inhibition did not affect the growth of local recurrences. In summary, in this work we present preliminary evidence that supports the potential of CIGB-300 as an antitumoral agent for breast cancer.

277 (946) **AMINOFLAVONE INDUCES A TH1 PROFILE IN A MICE BREAST CANCER MODEL**

Mariana A Callero[1], Cristina E Rodriguez[1], Aldana M Sólimo[1], ELisa Bal de Kier Joffe[1], Andrea Loaiza Perez[1].
[1]Instituto de Oncología Ángel H. Roffo.

Aminoflavone (AF) is an investigational agent and AhR ligand with potent activity against estrogen receptor positive (ER+) and certain estrogen receptor negative (ER–) breastcancer cells. Considering that AhR has recently been described as a physiologic modulator of immune cells and that we have previously demonstrated that AFP464 exerts immuno-modulating effects that inhibits tumor growth, we decided to further investigate AFP464 action on the immune response in M05 mouse model, a semi-differentiated estrogen-dependent breast adenocarcinoma. Inbred 2–4 month old BALB/c female mice bearing M05 tumor were treated with AFP464 (12 mg/kg, i.p) once daily dosing for a total of 5 days (QD × 5). The first treatment was given when the average size of the tumors was about 1 cm2. After a 21 days treatment, we quantified regulatory T lymphocytes (LTreg) populations from spleen and tumor inflammatory infiltrate by flow cytometry. We found that AFP464 reduced LTreg number respect to control mice both in spleen and tumor infiltrate (1,69 ± 0,2% vs 2,49 ± 0,3% and 0,085 ± 0,05% vs 0,38 ± 0,14%, respectively, $p<0,05$). Besides, we analyzed IFN-g and IL-10 levels in conditioned media from splenocytes co-cultured with senescent LM05-Mix cells (derived from M05 tumor). As a result, we observed a significant increase in IFN-g/ IL-10 relation (2,87 ± 0,6 UA vs 1,28 ± 0,5 UA, $p<0,05$), as a consequence of an increased IFN-g secretion by splenocytes from mice treated with AFP464 respect to control (870,8 ± 101,5 pg vs 533,03 ± 124,9 pg, $p<0,05$). Our results suggest that AFP464 has an antitumor

activity mediated by downregulation of LTreg and a Th1 polarization of splenocytes.

278 (954) **DISTINCTIVE GLYCOSYLATION SIGNATURE AND GALECTIN-1 EXPRESSION IN DIFFERENT HUMAN BREAST CANCER LINES AND TISSUES**

Ramiro M. Perrotta[1], Tomás Dalotto-Moreno[1], Florencia Moses[1], Alejandro J. Cagnoni[1], Karina V. Mariño[1], Diego Croci[2], Gabriel A. Rabinovich[1, 3], Mariana Salatino[1].
[1]Instituto de Biología y Medicina Experimental (IBYME), CONICET, Buenos Aires, Argentina. [2]Instituto de Histología y Embriología (IHEM) "Dr. Mario H. Burgos" CCT CONICET, Mendoza, Argentina. [3]Departamento de Química Biológica, FCEN, UBA, Buenos Aires, Argentina.

Galectins can influence antitumor immune response, angiogenesis and tumor metastasis by interacting with cell surface glycans. Changes in glycosylation observed in malignant cells are specifically sensed by lectins and can be used as disease biomarkers. With the goal of investigating the relevance of galectin-glycan interactions in shaping distinct breast cancer phenotypes, we first selected five human cell lines that model different human breast cancer types. To identify specific glycan structures, we used a panel of biotinylated lectins (Gal1, LEL, SNA, PNA, PHA-L and MAA) and analyzed their binding to the cell surface by flow cytometry. We found that highly aggressive lines, as the triple negative MDA-MB-231 and the HER2-enriched Trastuzumab (TZ)-resistant JIMT-1, showed higher binding of Gal1 ($p<0.001$) and MAA ($p<0.01$) than SKBR3, BT474 (Her2+/TZ-sensitive) and T47D (Luminal A) indicating abundant terminal LacNAc residues and higher α2-3 sialylation. MDA-MB-231 also stained positively for LEL ($p<0.05$) and PNA ($p<0.05$), reflecting abundant poly-LacNAc extensions and asialo-core 1-O-glycans. In contrast, BT474 cell line exhibited higher binding of SNA ($p<0.05$) indicating enrichment of α2-6-linked sialic acid, correlating with a restrictive Gal1 binding phenotype ($p<0.001$). Remarkably, Western blot analysis showed that TZ-resistant JIMT-1 expressed higher levels of Gal1 in comparison to its TZ-sensitive counterparts: SKBR3 and BT474 ($p<0.001$). In addition, T47D and MDA-MB-231 cell lines also showed substantial Gal1 expression. Finally, lectinhistochemistry in paraffin embedded human breast cancer sections revealed a diverse binding of Gal1 and SNA in stroma and epithelial cells, reflecting heterogenous glycan patterns among patients. We conclude that each breast cancer cell line and tissue has a particular "glycosylation signature" that, in association with Gal-1 expression levels, may predict cancer progression or response to targeted therapies.

279 (962) **CHARACTERIZATION OF ACYL-COA SINTHETASE 4 (ACSL4) PROMOTER IN HUMAN BREAST CANCER CELLS**

Melina Andrea Dattilo[1], Yanina Benzo[1], Paula Fernanda Lopez[1], Natalia Morduchowicz[1], Ana Fernanda Castillo[1], Paula Mariana Maloberti[1].
[1]INBIOMED (UBA-CONICET) Departamento de Bioquímica Humana - Facultad de Medicina - Universidad de Buenos Aires.

ACSL4 expression in human breast cancer cells correlates with tumor aggressiveness but its regulation remains unknown. Our objective is to characterize the transcriptional regulation of ACSL4 in breast cancer cells according to their level of aggressiveness (MDA-MB-231 and MCF-7). A 1.8 kb fragment of promoter was cloned upstream Nluc gene, a luminiscent reporter. Deletions were made from both 5' and 3' ends. Results show there is at least one element in 5' region that downregulates the promoter activity in both cell lines. Unidirectional deletion of 3' end shows a positive regulatory 43 bp region only in MDA-MB-231 as responsible of the higher expression of ACSL4. Bioinformatic identification of possible transcription factors involved in this regulation showed consensus sites for RORa in the 5' region and E2FF consensus sites in 3' region of the promoter among others sites like CREB, SP1 and ERSS. Mutations were constructed for consensus sites potentially involved. RORa mutation increased activity in both cell lines suggesting this transcription factor is involved at least in part in the negative regulation of promoter activity in the 5' end in these breast cancer cells. For the 3' region, E2FF mutants sites were obtained within the region related to the differential promoter activity. A transcriptional activity decrease was observed only in MDA-MB-231. Thus E2FF is enhancing at least in part the promoter activity in the 3' end only in MDA-MB-231. Overexpression of ERa in MDA-MB-231 reduced promoter activity, indicating that ERa is a negative regulator of ACSL4 expression. In regard the epigenetic mechanisms, a histone deacetylases inhibitor increases transcription in both cell lines and a cytosine methylation inhibitor increases transcription only in MDA-MB-231. Therefore, with these results we could observe different transcription factors and epigenetic mechanisms that could regulate differentially ACSL4 promoter in both breast cancer cell lines.

280 (966) **ERK5 REGULATED BY ERBB-2 DRIVES PROLIFERATION OF TRIPLE NEGATIVE BREAST CANCER CELLS**

Maria Florencia Chervo[1], Wendy Béguelin[2], Santiago Madera[1], Franco Izzo[1], Mara De Martino[1], Leandro Venturutti[1], Héctor Ramiro Quintá[3], Violeta Alicia Chiauzzi[1], Cecilia Jazmin Proietti[1], Eduardo Charreau[1], Roxana Schillaci[1], Rosalía Inés Cordo Russo[1], Patricia Virginia Elizalde[1].
[1]Laboratorio de Mecanismos Moleculares de Carcinogénesis, Instituto de Biología y Medicina Experimental (IBYME), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Buenos Aires C1428, Argentina. [2]Division of Hematology/Oncology, Department of Medicine, Weill Cornell Medical College, Cornell University. New York, NY 10021, USA. [3]Departamento de Química Biológica, Instituto de Química y Físico Química Biológica, Universidad de Buenos Aires. Buenos Aires C1113AAD, Argentina.

Triple negative breast cancer (TNBC) refers to the group of tumors with poor prognosis without clinically significant levels of estrogen and progesterone receptors, and which lack membrane ErbB-2 (MErbB-2) overexpression or gene amplification. Our hypothesis is that BC defined as TN indeed expresses ErbB-2 which instead of being localized at the membrane is present in the nucleus where it modulates tumor growth. We explored NErbB-2 presence in TNBC using immunofluorescence (IF) and confocal microscopy. We found a strong NErbB-2 expression in a panel of TNBC cell lines (MDA-MB-468, HCC-70, MDA-MB-231 and MDA-MB453). To explore the biological function of NErbB-2, cells were transfected with ErbB-2DNLS mutant which is unable to translocate to the nucleus and acts as dominant negative inhibitor of endogenous NErbB-2 translocation. ErbB-2DNLS abolished NErbB-2 presence and proliferation in TNBC cell lines. Interestingly, we also demonstrated that in vivo blockade of NErbB-2 expression suppresses tumor growth in two pre-clinical models of TNBC. We previously perform a ChIP-Seq study to identify ErbB-2 binding sites in T47D cells treated with HRG, a ligand of ErbBs family. These cells express moderate amounts of MErbB-2 and HRG treatment induces its nuclear migration. In this ChIP-Seq we identified Erk5 as a downstream target of NErbB-2. Here we explored Erk5 expression and function in TNBC. We found that protein and mRNA levels of Erk5 were increased in TNBC cells. Through ChIP assays using primers spanning the ErbB-2 binding site described in our ChIP-Seq, we observed a specific binding of NErbB-2 to the Erk5 promoter in TNBC. Moreover we demonstrated that blockade of Erk5 expression inhibits cell proliferation. This indicates that NErbB-2 may modulate Erk-5 expression, thus leading to proliferation of TNBC. Our results identify NErbB-2 as a key player in TNBC and highlight both NErbB-2 and Erk5 as potential therapeutic targets in these tumors.

INFECTOLOGIA Y PARASITOLOGIA / INFECTOLOGY AND PARASITOLOGY

281 (616) **RELATIONSHIP BETWEEN INFECTIVE DOSE AND RESISTANCE VARIABLES TO TRICHINELLA SPIRALIS**

(TS) IN LINES OF MICE OF THE CBI-IGE STOCK WITH DIFFERENT SUSCEPTIBILITY TO THE PARASITE.

Julieta Belén Friscione[1], Brenda Domenech[1], Ana V. Codina[1,2], Paula Indelman[3], María D. Vasconi[1,3], Lucila I. Hinrichsen[1,2].

[1]instituto De Genética Experimental, Facultad De Ciencias Médicas, Universidad Nacional De Rosario. [2]CIC-UNR, Universidad Nacional De Rosario. [3]Área Parasitología. Facultad De Ciencias Bioquímicas Y Farmacéuticas. Universidad Nacional De Rosario.

In intestinal parasitic infections, the defense mechanisms of the infected host include complex interactions, beginning with the recognition of parasite antigens, and culminating in an inflammatory reaction in the intestinal mucosa, intended to eliminate the worms. Rodents are natural hosts of the nematode *T. spiralis* that causes the food disease trichinellosis. The CBi-IGE mouse lines differ in the response to infection with increasing doses of Ts. To analyze the role of the genotype in the enteral stage of infection, CBi+ (susceptible) and CBi/L (resistant) adult mice were infected with 1 (dose **I**) or 2 (dose **II**) Ts L1 larvae per g BW. They were sacrificed on days 3, 6, and 13 post-infection (n=18 per line) and the small intestine was excised. The intestinal adult parasites recovered were counted and expressed as total number of adults (**nAP**) and Ts female fertility (**Ff**) was calculated, for each mouse, as mean number of newborn larvae released per female after incubating each female in RPMI medium at 37C for 18 h. **nAP** decreased from day 3 to 13 in both genotypes (P<0.05), but only the resistant line could expel the worms completely (median (range), days 3, 6 and 13; CBi+, **I**: 12 (11-14), 6 (6-12), 5(4-14); **II**: 22(12-38), 13(1-48), 1(0-30); CBi/L, **I**: 4(1-7), 5(0-12), 0(0-1); **II**: 15(9-26), 13(3-23), 0(0-4))(P<0.05). **Ff** was affected differently in each line in the period studied (mean±SEM, 6 and 13 days; CBi+, **I**: 37±8,9, 37±3,7); **II**: 42±3,1, 50±4,0; CBi/L, 43±1,6, 24 (one female only); **II**: 41±1,9, 22±3,4) (P<0.05). The susceptible CBi+ line did not modify **Ff** while this variable decreased significantly in the resistant CBi/L (P=0.0010). It is currently accepted that the main determinants of the evolution of intestinal nematode infections are the initial parasite load and genotypes of host and parasite. In this murine model, the results suggest that host genotype plays a key role in that interaction.

282 (636) **STUDY OF TOXOCAROSIS AND ECHINOCOCCOSIS IN ASSOCIATION WITH SOCIO-ENVIRONMENTAL CONDITIONS IN PERIPHERAL AND URBAN COMMUNITIES OF MAR DEL PLATA CITY, GENERAL PUEYRREDON DISTRICT.**

Carla Lavallén[1,2], Mariela Kifer[3], Karina Riesgo[4], Beatriz Brignani[4], Martín Biscaychipi[4], Amalia Rojas[4], Gabriela Colace[4], Estela Chicote[4], Cristian Giuntini[4], Guillermo Denegri[1,2], Marcela Dopchiz[1,2].

[1]Laboratorio de Zoonosis Parasitarias (Facultad de Ciencias Exactas y Naturales - Universidad Nacional de Mar Del Plata). [2]CONICET. [3]Centro de Atención Primaria de la Salud Antártida Argentina (MGP). [4]Centro De Especialidades Médicas y Ambulatorias (MGP).

Parasites zoonoses are a socio-sanitary problem related with high unemployment, poverty and housing issues. Numerous canine endoparasites could infest people developing a disease. Two parasites zoonoses of world and country importance are the toxocarosis produced by *Toxocara* nematodes and the echinococcosis caused by *Echinococcus* larval and adults cestodes. The aim of this study was to evaluate if the differences at socio-environmental conditions were related with the existence of toxocarosis and echinococcosis in peripheral (PC) and urban communities (UC) of Mar de Plata city. Epidemiological surveys were made to 460 persons (PC) and to 172 (UC). The presence of *Toxocara canis* in dogs was evaluated through coproparasitological research in 306 samples (PC) and 46 (UC). Coproantigen tests to the identification of *Echinococcus granulosus* in dogs were done in 109 samples (PC) and 27 (UC). Screening studies including ultrasonography, chest X-ray, ophthalmological examination, blood count and ELISA test were carried on 200 persons (PC) and 114 (UC), for the detection of toxocarosis (T) and cystic echinococcosis (CE). The PC showed poor housing conditions and a the highest frequency of families living in overcrowded conditions (I–F=0,15; p<0,05). The frequencies of dogs infested with *T. canis* were 13,4% in the PC and 8,7% in the UC. The frequencies of coproantigen positive samples were 22,2% in the UC and 17,8% in the PC. The ultrasonography and chest X-ray did not reveal lesions by T or CE. The PC showed the highest frequencies of ELISA reactive children (X^2=29,3; p<0,01) and adults (X^2=24,9; p<0,01). Five of them showed maculopathies. Eosinophilia was observed in reactive people from both communities. Association between reactive ELISA and contact with dogs was observed in the 71,3% of PC and in the 57,1% of UC. Both parasites zoonoses were present in the communities. The PC have more risk factors to the persistence and dissemination of the diseases in the population.

283 (705) **CHARACTERIZATION OF EXTRACELLULAR VESICLES PRODUCED BY ECHINOCOCCUS GRANULOSUS LARVAL STAGE AND THE INTERACTION WITH HOST CELLS**

María Celeste Nicolao[1], Christian Rodrigues Rodriguez[1], Andrea Carina Cumino[1].

[1]CONICET, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar Del Plata.

Human echinococcosis is a zoonotic cestode disease caused by Echinococcus sp. larval stage (protoescoleces- PTS- and cysts or metacestode-MTC-). These helminth parasites lack digestive and excretory system but they have developed active endocytic-exocytic cellular processes to regulate metabolite uptake and excretion. In the present work, we analyzed the cestode extracellular vesicles (EVs) production and their interaction with host cells. Loperamide (Lp) a calcium channel blocker, reduced PTS and MTC viability in a dose-dependent manner showing a significant effect with 10 µM and 25 µM after 24 h of treatment, respectively. Additionaly, using the Fluo3-AM fluorescent probe, a cytosolic calcium level increment (which was considered as an exocytosis stimulus) was determined in Lp-treated parasites in comparison to the controls. Moreover, the acetylcholinesterase activity in culture supernatants was determined as an EVs-release indicator, revealing a higher activity in presence of Lp. In addition, EVs were purified from parasite-culture medium through several centrifugation and ultracentrifugation steps and it was shown that Lp-treatment provoked higher density of vesicles than the control condition. On the other hand, transmission electron microscopy enabled the vesicles morphological characterization and the identification of abundant exosomes. Finally, the EVs-host cell interaction was examined. Purified exosomes were labeled with PKH26 red fluorescent labeling and the macrophages uptake and interaction with hepatic cells were analyzed by confocal microscopy. These results show that Echinococcus granulosus possesses a great vesicular traffic which could be involved in the host immune response.

284 (847) **TLR2 LIGATION IN HUMAN MACROPHAGES TRIGGERS A SPECIFIC, DEFAULT, LATE PHASE AND SUSTAINED CYTOSOLIC RESPONSE.**

Cristian J. A. Asensio[1,2], Rodolfo C. Garcia[1].

[1]ICGEB, Trieste, Italy [2]BIOMED, CONICET- UCA, Buenos Aires, Argentina.

TLR receptors are part of the defence against microorganisms and also endogenous inflammatory insults. It is not completely known how the specific responses of each member of this family are controlled during the concomitant activation of other immune/inflammatory receptors including those of the family. Since different receptors share multiple cytoplasmic signalling proteins along different pathways it remains a main question to know if any specific signalling is possible for each receptor. To study the spatio-temporal regulation of TLR receptors we focussed on the study of the proteome responses of the THP-1 human macrophage cell line. We stimulated these cells with 3 different bacterial species (live and killed) and with pure TLR2/TLR4 ligands. In order to detect

reproducible changes in protein markers we employed novel biochemical techniques to compare the cytoplasmic proteome profiles after different stimuli. We achieved enhanced sensitivity compared with the traditional gel staining techniques. Among many proteome alterations, we detected a specific response to TLR2 ligation in the cytosol showing a very reproducible temporal behaviour. It is a default response which persist for days proportionally to the half life and abundance of TLR2 ligands. Besides, the response is not affected by the concomitant activation of other inflammatory or cell stress pathways. Concomitant bacterial infection or TLR4 activation cannot modify this response to TLR2 ligation. We propose this response as a specific proteomic biomarker of TLR2 ligation. The results suggest that ligation of TLR2, by any ligand regardless of its structure, triggers the same cytosolic response with the participation of microtubules and a chaperone which is post-translationally modified in a reproducible manner and timing. Using novel proteomic strategies we demonstrate that TLR2, with the participation of cytosolic protein complexes, monitors continuously the presence of its ligands in macrophages.

285 (882) **CHITOSAN ENHANCES THE ANTIMICROBIAL EFFECT OF CLOXACILLIN AGAINST STAPHYLOCOCCUS SPP. ISOLATED FROM CHRONIC BOVINE MASTITIS**

Maria Laura Breser[1], Veronica Felipe[1], Maria Soledad Orellano[1], Luciana Bohl, Agustín Conesa[1], Paula Isaac[1], Carina Porporatto[1].

[1]Centro de Investigaciones y Transferencia de Villa María (CIT-UNVM)-Conicet - Universidad Nacional De Villa María.

Staphylococcal mastitis is a major and costly problem of dairy cattle all over the world, and persistent infections are often attributable to biofilm growth of bacteria. The main treatment to attend this infections is the antibiotic therapy, although in many cases has a partial efficacy and cannot completely remove this process. The higher cure rates of antibiotic therapies are during the dry period, in which one of the most commonly used is cloxacillin (Clx). Cationic polymers such chitosan (Ch) have important properties such as antimicrobial agent, so they are a good supplement for use in biomedical applications. In the present work, we analyzed minimum bactericidal concentration (MBC and CFU/mL), metabolic activity (MTT assay) and biofilm eradication (Crystal Violet assay) of chronic bovine isolates, after being cultured in planktonic and biofilm form. Bacteria were cultivated in the presence of different concentrations and combinations of Clx and Ch. Our results indicated that MBC for Clx was higher for biofilm growth than in planktonic cultures (between 20 to 65 times more) (MBC, CFU/mL and MTT assay). The Clx showed no significant inhibition effects over formation and eradication of preformed biofilms (Crystal Violet assay and CFU/mL). The combination of Ch and Clx significantly reduces the antibiotic needed to remove planktonic and biofilm growth (3-5 and 4-10 times, respectively). This combination significantly inhibited biofilm formation and increased eradication of this on dose-dependent manner. In conclusion, our results showed that, bacteria inside biofilms needed significantly higher concentrations of Clx those in planktonic conditions. However, we observed that combination of Clx and Ch could significantly reduce the concentration of antibiotic required to remove bacteria in biofilms, suggesting that the combined administration can be a great strategy for intramammary treatment of chronic bacterial infections.

286 (943) **EFFECTS OF E. COLI O157:H7 ON HUMAN COLONIC EPITHELIAL CELL LINES AND TRANSLOCATION OF SHIGA TOXIN**

Nicolas Ezequiel Garimano[1], Adriana Andrea Albanese[1], Maria Marta Amaral[1], Cristina Adriana Ibarra[1].

[1]Laboratorio de Fisiopatogenia, Departamento de Fisiología, Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO HOUSSAY-CONICET), Facultad De Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina.

Shiga toxin (Stx)-producing Escherichia coli (STEC) strains are responsible of bloody diarrhea (hemorrhagic colitis) and hemolytic uremic syndrome (HUS). STEC O157:H7 is, by far, the most prevalent serotype associated with HUS and Stx2 is the major virulence factor associated for the more severe symptoms of the infection. After passage through the acidic barrier, STEC colonizes the human colon promoting production and absorption of Stx2. However, the mechanisms involved in the pathogenesis of diarrhea mediated by Stx2 are not well known yet. Our aim was to study the cytotoxic effects of STEC O157:H7 on human colonic epithelial cells in order to better understand the means by which Stx2 induces diarrhea and translocate the intestinal barrier. In this study, we examined HCT-8 and Caco-2 viability after incubation with purified Stx2, STEC O157:H7 strain 125/99 (125/99wt), a mutant of 125/99 strain lacking *stx2* gene (125/99Δstx2) and the filtered 125/99wt supernatants. Cells were grown in 96-well culture plate and viability was measured by neutral red uptake after a 24h incubation period under growth arrested condition. We have also evaluated the translocation of purified Stx2 across HCT-8 cultured as monolayers on Millicell cell culture inserts in the presence of 125/99Δstx2. Transepithelial electric resistance was monitored daily during the development of cell culture until confluence was achieved, and after Stx2 treatment to ensure monolayer integrity. Cytotoxicity of collected basal media on Vero cells assays was enhanced by bacterial presence on the luminal side. Furthermore, the cytotoxic effects induced by 125/99wt and 125/99Δstx2 strains on HCT-8 and Caco-2 cell lines were significantly higher than those observed with purified Stx2 and filtered 125/99wt supernatants. These results indicate the importance of bacterial cells in the interaction and translocation of Stx2 through the intestinal barrier to cause HUS.

287 (995) **HBV REPLICATION OF THE MOST PREVALENT SUBGENOTYPES IN ARGENTINA INDUCES APOPTOSIS IN HUMAN HEPATOCYTES**

Mercedes Elizalde[1], Ina Sevic[1], Mora González Lopéz Ledesma[1], Diego Flichman[1], Rodolfo Campos[1], Luciana Barbini[2].

[1]Cátedra de Virología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires. [2]Cátedra de Microbiología Clínica, Departamento de Química, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata.

Introduction. Hepatitis B virus (HBV) causes acute and chronic liver infections. The virus is classified in subgenotypes (sgts), being F1b and F4 the most prevalent in our country. Naturally occurring HBV mutations at the BCP (nts 1762-1764, K130M and V131I at X protein) are associated with mechanisms of pathogenesis. Objectives. Investigate if HBV replication induces different mechanisms of hepatocyte cell death and determine whether this induction is characteristic of the sgts and the X gene variants. Methods. Huh-7 cells were transfected with a mixture of full genome HBV clones, of F1b and F4 sgts with wild type (wt, KV) and mutated (mut, MI) 1762-64 positions, a system previously determined to be replication efficient. Cell mortality was analyzed by trypan blue staining. Cell death by apoptosis was detected by the observation of morphological changes by contrast phase microscopy, acridine orange and ethidium bromide (AO-EB) staining and flow cytometry. Results. Compared to control cells, HBV replication of wt and mut F1b and F4 sgts significantly increased cell mortality, at different post-transfection times. It also induced significant morphological changes (cell shrinkage, chromatin condensation and margination at the nuclear membrane, plasma membrane blebbing and apoptotic bodies) typical of apoptosis and significant increases in the percentage of early and late apoptotic cells (AO-EB staining and flow cytometry). In addition, it was evident that HBV replication of mutant sgts induced higher percentages of apoptotic cells than the wt virus. Conclusions. HBV replication of the most prevalent sgts in Argentina induces human hepatocyte death by apoptosis. These results contribute to describe the molecular mechanisms of HBV pathogenesis in chronic infections.

288 (996) **STUDY OF APOLIPOPROTEIN E GENETIC VARIANTS AND THEIR RELATION WITH DYSLIPEMIAS ASSOCIATED WITH HAART IN HIV-1 INFECTED CHILDREN**

Debora Lujan Alfonso[1], Alicia Vaca[1], Debora Mecikovsky[1], Paula Aulicino[1], Rosa Bologna[1], Andrea Mangano[1].
[1]Hospital de Pediatria Dr. J. P. Garrahan.

Highly active antiretroviral therapy (HAART) has improved the prognosis and quality of life in HIV-1-infected individuals, especially in children. Given the long-term exposure to HAART, a considerable number of patients experience adverse effects as dyslipidemia, associated mainly to Protease Inhibitors (IPs), exhibiting hypertriglyceridemia and hypercholesterolemia, pro-atherogenic profile features. In other hand, the apolipoprotein E (APOE) shows 3 polymorphisms: e2, e3 and e4 alleles, being these genetics variants related to hereditary dyslipemias and could be associated with HAART lipid abnormalities. Taking in mind these data, the aim of this work was to evaluate the association between APOE genotypes and plasmatic cholesterol levels in HIV-infected children under HAART therapy. A retrospective study was performed in a 44 HIV-1 infected children, diagnosed between 2005 and 2015 and selected with 1 plasma total cholesterol (TC) level assessment before and after 1 year of HAART. The APOE genotypes were determined by ARMS-PCR. The T-test was used to compare TC plasma media among groups. The genotype APOE frequencies were E2/E3 6.82%, E2/E4 2.27%, E3/E3 72.73%, E3/E4 18.18% and E2/E2 and E4/E4 did not find. The allelic frequencies were e2, 0.045; e3, 0.852 and e4, 0.102, being similar to the previously reported in the Argentinian population. The pretreatment TC levels between the different genotypes did not show significant differences. While, after the year of HAART a tendency of higher TC levels in the E2/E3 genotype was observed respect to the other genotypes (126±27.8 vs. 214±26.8, p=0.056). This preliminary study suggest that the HIV-infected children who presented the E2/E3 genotype exhibited a predisposition to develop hypercholesterolemia when are treated with IPs.

289 (1009) **THE HIGHLY CONSERVED HA2 PROTEIN OF THE INFLUENZA A VIRUS INDUCES A CROSS PROTECTIVE IMMUNE RESPONSE**

Cecilia Caldevilla[1], Yesica Paredes Rojas[1], Itati Ibañez[1], Nora Mattion[1].
[1]Instituto de Ciencia y Tecnología Dr. César Milstein-CONICET. CABA, Buenos Aires, Argentina.

Influenza virus undergoes continuous evolution by mutation of amino acids present in the exposed head domain of hemagglutinin (HA), which results in poor induction of cross-reactive antibodies against non-matching strains. It has been reported that the highly conserved HA2 stem region of HA is a promising candidate to develop a broad spectrum influenza vaccine. The aim of this study was to evaluate the protection conferred by two immunogens based on the stem region of an HA3 virus. A protein-based and a DNA-based vaccine were generated to investigate their immunogenicity and cross protection potential.Groups of 9 Balb/c mice were immunized intramuscularly with three doses of DNA vaccine (50 ug) or intraperitoneally with purified protein (10 ug). One group received only protein, another only DNA, and two groups received combination of vaccines. A control group received a vaccine containing the complete HA and another only PBS. Challenge experiments were performed with $2LD_{50}$ of mouse-adapted human influenza strains A/Victoria/3/75 (H3N2) and A/Puerto Rico/8/1934 (H1N1) three weeks after the last immunization. Survival, weight loss and viral titer in lungs were determined. High levels of protection (80-100%) were obtained after a lethal challenge with homologous strain H3N2 in groups immunized with protein or with a dose of protein followed by two doses of DNA. Mice vaccinated only with DNA were not protected. As expected, lower levels of protection were observed after challenge with the heterologous strain H1N1. However, mice vaccinated with the HA2 stem region performed better than those immunized with the complete protein. The induced antibodies cross-reacted in vitro with heterosubtypic influenza strains. The statistical analysis confirmed that the use of immunogens based on the conserved regions of the HA protein is of great value for the development of an influenza vaccine capable of broadening the spectrum of protection.

290 (1093) **DEVELOPMENT OF A SIMPLIFIED MOLECULAR TEST OF CONGENITAL CHAGAS DISEASE FOR NEONATAL SCREENING**

Luciana Larocca[1], Fabiana Stolowickz[1], Carolina Carrillo[1], Adrián Vojnov[1].
[1]Instituto de Ciencias y Tecnología Dr. César Milstein - CONICET. División Laboratorio - Hospital General de Agudos Carlos G. Durand.

Introduction. Chagas disease is an American endemic caused by the protozoan parasite *Trypanosoma cruzi*. The estimated number of annual cases of congenital *T. cruzi* infection in Argentina is 1,200, but less than 400 are officially reported due to methodological limitations for early detection. Objectives. To develop a simple test for congenital Chagas detection based on a molecular isothermal amplification (AMI) adapted to simplified neonatal screening practices. Materials & Methods**.** Samples of negative or artificially inoculated blood were dropped onto Guthrie test cards and used as substrate for AMI reaction at predefined conditions (65°C - 40 min). It was determined: a- Sensitivity, compared with a Quantitative Polymerase chain reaction (Q-PCR); and b- Specificity, challenging the reaction with other trypanosomatids, human and yeast DNA.The reaction efficiency pipetting the reagents separately vs a pre-prepared mix, and reading the result by lateral flow dipsticks chromatography (LFD) (field method) vs electrophoresis (analytical method) were also tested. Finally, AMI tests were performed with blood samples of newborn babies delivered from not infected and infected mothers, in order to challenge the simplified AMI test in field conditions. Results. a- Samples artificially inoculated with *T. cruzi*, showed a detection sensitivity by AMI of 1-10 parasites/sample, 1 to 10 times greater than with Q-PCR. The sensitivity was similar in LFD than electrophoresis and in this kind of simplified test, dispensed in mix reaction. b- In all cases, heterologous DNA samples were negative by AMI and Q-PCR. c- 1 of the 20 blood samples of newborn babies delivered from not infected mothers resulted positive by AMI (5%) and 95% resulted negative. Conclusions. Our results indicate that AMI can yield a high specificity and sensitivity using a dried drop of blood in a Guthrie card and pre-prepared mix, with a remarkably simpler trial without expert human resources or complex equipment.

291 (2013) **EVALUATION OF FUNCTIONAL POLYMORPHISMS ASSOCIATED WITH THE EXPRESSION OF IL-10 AS PROGNOSTIC MARKERS OF CLINICAL EVOLUTION IN INDIVIDUALS CHRONICALLY INFECTED WITH TRYPANOSOMA CRUZI.**

Alicia Alejandra Grijalva Hinojos[1], Analia Toledano[2,3], Lucia Gallo Vaulet[3], Roberto Agüero[4], David Di Nardo[1], Elizabeth Bogdanovich[5], Horacio Repetto[4], Daniel Stecher[5], Silvia Repetto[1,5], Catalina Alba Soto[1].
[1]Facultad de Medicina. IMPAM (UBA-Conicet). [2]Departamento de Hemoterapia e Inmunohematología, Hospital de Clínicas José de San Martín. [3]Departamento de Bioquímica Clínica, Hospital de Clínicas José de San Martín. [4]División de Cardiología, Hospital de Clínicas José de San Martín. [5]División de Infectología, Hospital de Clínicas José de San Martín.

Chagas disease is a major health issue as 30% of chronically infected individuals will develop cardiac complications. The anti-inflammatory cytokine IL-10 appears to have a role in delaying the onset of chronic chagasic cardiomyopathy (CCC). Low IL-10 levels are a prominent signature of the switch from an anti-inflammatory profile of asymptomatic patients to a proinflammatory one of patients with CCC. The aim of this study was to determine whether a functional polymorphism of the IL-10 promoter (-1082A<G; rs1800896) could be used as a prognostic marker of evolution to cardiomyopathy in chronically infected patients. A cross-sectional study was conducted and 18 patients at chronic phase of disease with positive serology for *T. cruzi* were enrolled. Positive serology was confirmed by 2 or 3 commercial tests**.** Data were collected from integrated epidemiological, clinical and cardiological history. Cardiological evaluation included EKG,

chest X ray and echocardiography. Patients with CCC from other etiologies were excluded. Genotype and allele frequency of the -1082A<G polymorphism (rs1800896) was evaluated by SSP-PCR. Association between the categorical variables (presence/absence of cardiomyopathy and the 1082A<G polymorphism) was analyzed using Pearson's chi^2 test and relative risk with the SPSS software. P<0.05 was considered significant. Patients' median age was 51.5 years (22-76), being 10 females (55.6%), 14 (82.4%) from Argentina and 3 from Bolivia. CCC was detected in 7 patients (38.9%). We confirmed that patients >50 years had increased relative risk of developing CCC (p<0.025) but found no association between CCC and place of origin or gender. Seven patients displayed the AA and AG genotype, respectively, and 4 of them the GG genotype. No association was found between CCC and the presence any of the 3 genotypes in this small cohort. The study will be extended to a greater number of patients and to other biomarkers related to the regulation of IL-10 expression.

292 (2019) **THE ANAEROBIC PATHOGEN CLOSTRIDIUM PERFRINGENS COLONIZES THE GUT OF THE NEMATODE CAENORHABDITIS ELEGANS.**

Sebastian Claudio Cogliati[1], Cira Crespo[1], Roberto Grau[1].
[1]Laboratorio de Microbiología - Subsuelo Sala 9 - Facultad de Ciencias Bioquímicas y Farmacéuticas - Universidad Nacional de Rosario.

C. perfringens is a pathogenic Gram-positive anaerobic spore forming bacterium. Due to the production of many extracellular toxins, *C. perfringens* is the causative agent of important human diseases such as gas gangrene (GG) and food poisoning (FP). So far, big animals (goats, horses) are used as host models for the study of these diseases. Therefore, due to the involved disadvantages, we analyzed the suitability of the soil translucent nematode *C. elegans* as a model for studying infections caused by *C. perfringens*. This nematode, which feeds on bacteria, has been used previously as a model in the study of host-pathogen interactions. The nematode was co-cultivated with the pathogen in liquid medium. At different times, N2 wild-type worms were taken, mechanically disrupted and clostridial cells were counted. To get the number of spores, the homogenate was heated at 80 °C for 15 min to kill vegetative clostridial cells. A significant increase of colony forming units (CFU) of viable cells and spores of the pathogen inside the worm (3000 +/- 20 CFU per worm) was observed. On the other hand, *C. elegans* fed with FITC-labeled *Clostridium* cells and in vivo fluorescence microscopy confirmed that the pathogen was able to colonize the worm intestine. To determinate the pathogenic effect of *C. perfringens* on *C. elegans*, killing assays were performed. Briefly, 50 worms were seeded on a clostridial lawn developed in BHI medium. Every day, live and dead worms were counted and the percentage of survival determined. It was observed that worms died five times faster in the presence of *Clostridium* than in presence of the control *Escherichia coli* OP50 strain. Furthermore, worms observed by phase contrast microscopy (x40) showed significant morphological differences when compared with worms grown in the presence of OP50 cells (i.e., inflammatory bowel). In conclusion, *C. elegans* could became an enabling model for the study of infections caused by *C. perfringens*.

293 (2020) **CLOSTRIDIUM PERFRINGENS EXPRESSES ITS LETAL TOXINS IN THE GUT OF CAENORHABDITIS ELEGANS.**

Sebastian Claudio Cogliati[1], Cira Crespo[1], Roberto Grau[1].
[1]Laboratorio de Microbiología - Subsuelo Sala 9 - Facultad de Ciencias Bioquímicas y Farmacéuticas - Universidad Nacional de Rosario.

C. perfringens is a bacterial pathogen widely distributed in the environment. It is the main causative agents of diseases in human and animals such as gas gangrene (GG), food poisoning (FP) and antibiotic-associated diarrhea (DA). FP and DA diseases are associated with production of the CPE enterotoxin (enterotoxigenic *C. perfringens* cpe$^+$) during sporulation. For the development of GG, *C. perfringens* produces multiple toxins, including phospholipase C (PLC) and perfringolysin O (PFO). In a previous work, we demonstrated that *C. elegans* is a valid model for the study of infections caused by *C. perfringens*. Therefore, now we analyze the clostridial toxin expression in the nematode during infection. The nematode was co-cultivated with the pathogen in liquid medium. At different times, N2 wild-type worms were taken, mechanically disrupted and clostridial cells were counted. To get the number of spores, the homogenate was heated at 80 °C for 15 min to kill vegetative clostridial cells. A significant increase of colony forming units (CFU) of viable cells and spores of the pathogen inside the worm (3000 +/- 20 CFU per worm) was observed. In addition, human blood (hemolytic activity) and egg yolk (phospholipase activity) were used to measure the activity of PFO and PLC toxins inside the worm, respectively. For both toxins, enzymatic activity was detected. Accordingly, worms fedon clostridial cells harboring reporter fusions (*cpe-gusA*, *pfo-gusA* and *plc-gusA*) to measure toxin gene expression (β-glucuronidase reporter fusion) were used. The β-glucuronidase activity inside the worm was measured and an increase of activity with time was observed. These results correlated well with intestinal colonization by the pathogen. In conclusion, *C. perfringens* not only colonizes and grow in the intestine of the nematode but also produces the necessary toxins for the development of disease.

294 (2040) **IDENTIFICATION OF HAPLOTYPES IN INDIVIDUALS WITH PATHOLOGIES ASSOCIATED TO HUMAN T CELL LYMPHOTROPIC VIRUS TYPE 1 (HTLV-1) INFECTION IN ARGENTINA**

Sindy Anna Fraile Gonzalez[1], Camila Belén Cánepa[1], Mirna Biglione[1], Carolina Andrea Berini[1].
[1]Universidad de Buenos Aires. Consejo Nacional de Investigaciones Científicas y Técnicas. Instituto de Investigaciones Biomédicas en Retrovirus y Sida (INBIRS). Facultad de Medicina. Buenos Aires, Argentina.

Introduction: HTLV-1 is the causative agent of Adult T cell Leukemia/Lymphoma (ATLL) and HTLV-1 associated myelopathy (HAM). We propose to identify the HLA-A alleles related to susceptibility/protection for ATLL and HAM in cases from Argentina.

Materials and methods: A total of 29 cases, 7 with ATLL and 22 with HAM were studied. DNA was extracted, proviral load (PV) determined by qPCR and HLA-A by PCR and sequencing.

Results: The PV was significantly lower in asymptomatics compared to pathologies. Eight polymorphisms of HLA-A were observed: A*02, A*33, A*31, A*29, A*11, A*68, A*23 and A*24. HLA-A*23, A*24 and A*68 were only observed in ATLL, while A*11 and A*29 in HAM. HLA-A*02, A*31 and A*33 were present in both pathologies (p= 0.38; p=0.84 and p=0.36). In HAM, HLA-A*02 was more frequent, followed by A*33, with no significant difference (p=0.09). When comparing polymorphisms with 19 asymptomatics, A*02 was more frequent in the former (p=0.0092) while A*33 in individuals with pathologies (p=0.045). Individuals bearing the A*31 allele had a significantly higher CPV compared to the ones bearing A*02 (p=0.0488).

Discussion: In Argentina, A*02, A*24 and A*26 were observed in seronegative individuals with frequencies of 48.2%, 8.9% and 5.3%, respectively. No significant differences for A*02 and A*24 were observed when compared to our results in individuals with pathologies and asymptomatics; although HLA-A*02 was more frequent in the former, and A*26 was absent. In Jamaica, Japan and Brazil HLA-A*02 was described as protective both for ATLL and HAM, in concordance to our results. HLA-A*33 has been previously described as protector for ATLL in Jamaica, while in our population it was associated to individuals with pathologies, especially with HAM. More studies are necessary to confirm these results and be used as a prognosis guide for the development of the pathologies.

295 (2043) **OPTIMIZATION OF DNA EXTRACTION FROM TRYPANOSOMA CRUZI BY PHENOL-CHLOROFORM-ISOAMYL TECHNIQUE**

Esteban Alberto Actis[1], Fiorella Campo Verde Arboccó[2,3], Mariella Superina[1], Graciela Alma Jahn[2].

[1]Laboratorio de Medicina y Endocrinología de la Fauna Silvestre, IMBECU (CCT Conicet Mendoza, Mendoza, Argentina). [2]Laboratorio de Reproducción y Lactancia, IMBECU (CCT Conicet Mendoza, Mendoza, Argentina). [3]Laboratorio de Hormonas y Biología del Cáncer, IMBECU (CCT Conicet Mendoza, Mendoza, Argentina).

Trypanosoma cruzi is the etiologic agent of Chagas disease, a vector-borne zoonosis widely distributed in the Americas, present in more than 180 species of wild mammals. Eco-epidemiological studies of *T. cruzi* in wildlife require high sensitivity to obtain representative results. A major constraint of these studies is the low amount of blood that can be collected from small mammals, which may also have low parasitemia. Real-time PCR (qPCR) is a useful and sensible diagnostic technique. DNA extraction can be done using commercial kits, which although optimizing and increasing the DNA output, also increase the processing cost. On the other hand, obtainment of detectable quantities of parasitic DNA using conventional extraction techniques such as phenol-chloroform-isoamyl may be inefficient when parasitemia is low, such as in the chronic stage of the disease. We optimized the performance of the phenol-chloroform-isoamyl technique based on the co-precipitating capacity of mammalian euchromatin to increase the extraction output of parasitic DNA. We worked with different dilutions of *T. cruzi* epimastigotes (Dm28c clone), with and without supplemented genomic DNA (obtained from rat liver). We extracted DNA by phenol-chloroform-isoamyl technique and performed qPCR with Taq Brazil polymerase and specific primers (121-122) for the DNA kinetoplast of *T. cruzi*. The correct molecular weight of the amplicon (330 pb) and additional band (≈500pb) was confirmed by 3% agarose gel electrophoresis. In the genomic DNA-supplemented samples, the take-off was 2-3 times lower than without supplemented DNA, indicating that the extraction efficiency and qPCR sensitivity for *T. cruzi* DNA detection were improved. The addition of genomic DNA significantly increased the efficiency of the extraction technique without contributing external components to a conventional extraction. It is therefore a valuable low-cost tool for *T. cruzi* detection in cases with low parasitemia.

296 (2070) **DETERMINATION OF SUSCEPTIBILITY TO PYRAZINAMIDE IN MYCOBACTERIUM TUBERCULOSIS THROUGH MODS CULTURE OF SPUTUM SAMPLES**

Roberto Hugo Alcantara Estela[1], Ricardo Antiparra Villa[1], Robert H Gilman[2], Patricia Fuentes Bonilla[1], Lisette Marin García[1], Janina Campos Tinero[1], Rodolfo Huerta Guillen[1], Mirko Zimic Peralta[1], Patricia Sheen Cortavarría[1].
[1]Universidad Peruana Cayetano Heredia. [2]Johns Hopkins Bloomberg School of Public Health.

Lack of adherence in DOTS strategy is an important cause of tuberculosis (TB) treatment failure. The administration of PZA, the only drug effective against latent TB, without a susceptibility profile can produce serious hepatic side effects. PZA-susceptibility assays are not adequate; lack of reproducibility is the most critical issue. The objective of this study was to design an effective PZA susceptibility test based on MODS culture of sputum sample to evaluate: (i) *Mycobacterium tuberculosis* growth at two concentrations: 400 and 800 µg/mL (MODS-PZA). With this method the susceptibility was determine by the evaluation of the inhibition of growth in cultures with PZA since the first day of incubation; and (ii) the detection of POA produced in three days of incubation: 10th, 13th and 16th (MODS-Wayne). In this method, a PZA-free culture was performed during 7, 10 and 13 days. PZA was added to the culture at day 7th, 10th and 13th, after that an incubation of three additional days was performed to let POA production. Three hundred thirty samples were recollected and processed by bacilocopy and MODS culture. One hundred ninety four samples gave positive MODS culture and valid results for reference tests. A composed standard was used as reference test. Three test were performed to get a composed standard: MGIT, Classic Wayne, and Sequencing of *pnc*A. MODS-PZA reported a sensitivity of more than 75% and specificity more than 80%, with a considerable Kappa index (more than 0.7). Instead, MODS-Wayne had a sensitivity of 90% and a specificity of 95% with considerable Kappa index (0.71). Not significant differences were reported between MODS-PZA and MODS-Wayne regarding sensitivity and specificity. The results showed that both test are promising assay due to its significant values of sensitivity and specificity, low setting costs, and simplicity.

297 (1021) **HBV ANTIVIRAL RESISTANCE MUTATIONS IN HIV-HBV CO-INFECTED PATIENTS.**

Bruno Saldain[1], Mariana Hualde[2], Marina Grand[2], Luciana Barbini[1].
[1]Cátedra de Microbiología Clínica, Departamento de Química, Universidad Nacional de Mar del Plata, Mar del Plata, Argentina. [2] Unidad de Internación de Infectología HIGA "Dr. Oscar E. Alende", Mar del Plata, Argentina.

Antiretroviral treatments for HIV in HIV-HBV co-infected patients can select HBV resistant mutants. The study of HBV resistance mutations in patients with previous treatments help in the selection of drugs for the particular virus infecting and will increase the probability of response. The aim of this work was to identify mutations associated with antiviral HBV resistance in co-infected HBV-HIV patients. The patients included are being treated for their HIV infection (HBVMDQ86 and HBVMDQ87). For sequencing, DNA was extracted from serum samples and a 812 bp segment of the HBV pol gene was amplified by nested PCR, and the product was sequenced. The sequences were phylogeneticlly analysed and genotyped by Neighbor Joining y Maximum Likelihood methods. The translated proteins and the presence of mutations associated with antiviral resistance were analysed. The phylogenetic analysis showed that HBVMDQ86 belongs to F1b and HBVMDQ87 to A genotypes. These results are in agreement with previously reported most prevalent genotypes in Mar del Plata. The analysis of POL proteins showed that HBVMDQ86 presented mutations at the reverse transcriptase) domain: rt202 and rt204, mutations associated with resistance to entecavir and entecavir/lamivudine/telbivudine, respectively. These results can explain that this patient has previously received entecavir for his HBV infection with no success. The sample HBVMDQ87 showed the rt204 and rt181 mutations, associated with lamivudine/aprenavir resistance and a I53T mutation. These results can explain the previous lamivudine unsuccessful treatment of the patient. This work highlights the importance of the information provided by genome sequences before the selection of the most appropriate antiviral drugs and is especially useful in co-infected patients treated for HIV that indirectly selected HBV resistant mutants. In conclusion, both samples showed mutations at POL that can explain the unsuccessful HBV treatments, previously received.

298 (767) **TRYPANOSOMA CRUZI DETECTION IN CORNEA AND OCULAR TISSUE FROM CADAVERIC DONORS**

Marta Inés Starcenbaum Bouchez[1], Elisabeth Cittadino[1], Hector Fontana[1], Juan Miguel Burgos[2].
[1]Banco de Ojos, Hospital Santa Lucía, CABA, Argentina. [2]Instituto de Investigaciones Biotecnológicas IIB-UNSAM, San Martín, Pcia Bs As, Argentina.

During 2016, 576 corneal transplants were performed in Argentina, managed centrally by the INCUCAI, and 3030 patients are waiting for transplant. Since 1999, under Resolution No. 269/99 (INCUCAI) *T. cruzi* seropositive individuals are not accepted as donors who generated, only in 2014, 15 discarded donors because of their serological status. Considering that there are no studies about the presence of parasites in human tissue eyes we analyze the presence of *T. cruzi* in corneas, sclera and eye muscle of two seropositive donors (male, aged 65 and 73, from the provinces of Entre Rios and La Rioja, respectively). The procedure consisted of DNA extractions using commercial kit and subsequent amplification reactions of specific sequences: i) beta actin, to verify the correct DNA extracted quality; ii) hypervariable region of minicircle kinetoplast (kDNA, oligonucleotides 121 and 122) to detect *T. cruzi* DNA; iii) satellite *T. cruzi* region (oligonucleotides TcZ1 and TcZ2) as a confirmatory study. Our observation revealed

the presence of *T. cruzi* DNA in the three tissue types analyzed in both patients (5/8 sclera, 4/4 corneas, and 2/2 samples eye muscles). To analyze parasite populations, and rule out possible contaminations, a comparison of patterns of restriction fragments hypervariable sequence kDNA (PCR-RFLP, double digestion MspI-RsaI) was carried out and analized on polyacrylamide electrophoresis, obtaining specific profiles with high similarities within each patient. These results confirm the presence of *T. cruzi* in the analyzed tissues and, particularly positivity corneas, confirming the risk of being used in transplantation.

Studies have proper authorization of the Ethics Committee INCUCAI, approval by Saint Lucia Eye Hospital, and the Central CIS GCABA registered under No. 156/16.

PRESENTACIÓN DE POSTERS SAI II / SAI POSTER PRESENTATION II

ENFERMEDADES INFECCIOSAS II/ INFECTIOUS DISEASES II

299 (55) **BRUCELLA ABORTUS INFECTION ELICITS HEPATIC STELLATE CELLS (HSC) FIBROSIS THROUGH INFLAMMASOME-DEPENDENT IL-1â PRODUCTION.**

Paula Constanza Arriola Benitez[1], Ayelén Ivana Pesce Viglietti[1], Diego Comerci[2], Guillermo Hernán Giambartolomei[1], Maria Victoria Delpino[1].

[1]Instituto de Inmunología, Genética y Metabolismo (INIGEM-CONICET/UBA). [2]Instituto de Investigaciones Biotecnológicas Dr. Rodolfo A. Ugalde (IIB-INTECH-UNSAM-CONICET).

The liver is affected in human brucellosis. *B. abortus* (*Ba*) triggers on HSC a profibrotic response characterized by inhibition of MMP-9 with concomitant collagen deposition and TGF-β1 secretion in a way that involved a functional T4SS. Taking into account that it has been reported that inflammasome is necessary to induce a fibrotic phenotype in HSC, we hypothesized that *Brucella* infection might create a microenvironment that would promote inflammasome activation and a concomitant profibrogenic phenotype in HSC. Our results indicate that *Ba* infection induces IL-1β secretion (ELISA) by LX-2 cells by a mechanism dependent on a functional T4SS ($p<0.001$). When infection experiments were performed in the presence of glyburide, a compound that inhibits NLRP3 inflammasome, the secretion of IL-1β was significantly inhibited respect to uninfected controls ($p<0.001$). The same effect was observed when infection was performed in the presence of specific caspase-1 inhibitor Ac-YVAD-cmk ($p<0.001$). These results indicate that caspase-1 and NLRP3 are involved in IL-1â secretion by *Ba*-infected LX-2 cells. Then experiments were conducted to determine whether expression of inflammasome components could be upregulated during *Ba* infection. We determine the expression of caspase-1, NLRP3 and ASC by qRT-PCR. Our results indicated that *Ba* infection induces an increase in caspase-1 and NLRP3 mRNA expression ($p<0.01$) but was unable to modified ASC expression. We proposed to determine the role of inflammasome in the induction of a fibrogenic phenotype in LX-2 cells during *Ba* infection. To this end the levels of MMP-9 (zymography), TGF-β (ELISA) and collagen (Sirius red staining) were determined in LX-2 cells that were infected with *Ba* in presence of Ac-YVAD-cmk and glyburide. Both inhibitors were able to reverse the effect of *Ba* infection on LX-2 cells. Taken together this results indicate that *Ba* induce inflammasome activation in HSC with concomitant induction of a fibrotic phenotype.

300 (183) **INTERACTION OF POLYMORPHONUCLEAR AND BACTERIA ISOLATED FROM CHILDREN WITH BRONCHIOLITIS OBLITERANS POST ADENOVIRUS.**

Silvia Orosco[1], Juan Carlos Valdéz[2], Nadia Gobbato[2], Nilda Arias[3], Clara Silva[4], Mirta Rachid[2], Gabriela Castillo[2], María Díaz Zamora[2].

[1]Servicio de Neumología, Hospital del Niño Jesús, Tucumán. [2]Cátedra de Inmunología. Facultad de Bioquímica, Química, Farmacia. Universidad Nacional de Tucumán. [3]Cátedra de Patología Molecular. Facultad de Bioquímica, Química, Farmacia. Universidad Nacional de Tucumán. [4]Cátedra de Bacteriología, Facultad de Bioquímica, Química, Farmacia. Universidad Nacional de Tucumán.

Introduction: Constrictive Bronchiolitis Obliterans (BO) is characterized by inflammation and fibrosis of the bronchioles wall leading to obstruction and sometimes occlusion of the airways. Elevated numbers of neutrophils within the airways are a hallmark of BO. After a viral infection of the lower respiratory tract, polymorphonuclear leukocyte (PMN) is involved in inflammation and tissue damage. This BO form is common in South America. Here, we studied the functionality of PMN isolated from blood of BO patients when are challenged with bacteria frequently isolated from sputa of these patients to determine any alteration related with the pulmonary affectation. Methods: Expectorated sputum and blood were collected from 10 children attending to our hospital diagnosed with medium and severe BO post adenovirus pneumonia. The study protocol was approved by the Ethics Committee of Hospital, and informed consent was obtained from the parents of children. Neutrophils isolated from blood were challenged with *Staphylococcus aureus*, SARM, *Pseudomonas aeruginosa, and Klebsiella pneumoniae* isolated from sputa. Microbicidal activity and spectrofluorometric determination of NETs formation by Sitox Green (DNA extracellular detection) and NO intracellular production by DAF-FM DA were performed. Serum metalloproteinasas were measured by gelatin zymography. Results and Conclusions: In the group of 6 patients with severe BO, 3 patients had stable lung function, neither recurrent infections nor mucus production, and high values of netosis, intracellular production of NO and microbicidal activity. The remaining 3 patients behave like the 4 patients with medium bronchiolitis showing impaired lung function, recurrent infections, low nets formation, NO production and microbicidal activity. The greater reactivity of PMN when challenged with bacteria is related to severe bronchiolitis, without pulmonary infections and more stable lung function than medium bronchiolitis.

301 (224) **B. ABORTUS RNA: A NOVEL VITA-PAMP INVOLVED IN THE DOWN-MODULATION OF MHC-I EXPRESSION ON HUMAN MONOCYTES.**

Maria Ayelen Milillo[1], Lis Noelia Velasquez[1], Aldana Trotta[1], Maria Victoria Delpino[2], Luciana Balboa[1], Guillermo Hernan Giambartolomei[2], Paula Barrionuevo[1].

[1]Instituto de Medicina Experimental (CONICET-Academia Nacional de Medicina). Buenos Aires. Argentina. [2]Instituto de Inmunología, Genética y Metabolismo (CONICET-UBA). Laboratorio de Inmunogenética. Buenos Aires. Argentina.

Brucella abortus elicits a strong Th1 immune response which activates cytotoxic T lymphocytes. However, this pathogen is able to survive inside macrophages and generate a chronic infection. Previously we reported that infection of human monocytes/macrophages with B. abortus inhibits the IFN-□-induced MHC-I cell surface expression. More importantly, we have recently demonstrated that B. abortus RNA, described as a viability-associated (vita)-PAMP, is the bacterial component involved in this phenomenon. Thus, the aim of this study was to further characterize the component, signalling pathways and mechanisms implicated in MHC-I down-modulation. For this, RNases-treated B. abortus RNA was employed to stimulate human monocytic THP-1 cells in the presence of IFN-□ for 48 h. Then, the expression of MHC-I molecules was evaluated by flow cytometry. Surprisingly, completely degraded RNA was still able to inhibit MHC-I expression ($p<0.05$) and it also induced the intracellular retention of these molecules within the Golgi apparatus into the same extent as intact RNA. On the contrary, DNase- and Proteinase K-treated RNA as well as eukaryotic RNA controls elicited no effect. Furthermore, B. abortus RNA also inhibited MCH-I expression on human primary monocytes and murine bone-marrow derived macrophages ($p<0.05$). TLR3 is one of the best known RNA immune receptors, therefore we evaluated whether it could be involved in this phenomenon. Yet, in the presence of a TLR3 inhibitor, B. abortus RNA down-

regulated MHC-I expression. On the other hand, neutralization of the EGFR resulted in partial recovery ($p<0.05$) of RNA-mediated MHC-I inhibition. Overall, these results indicate that the vita-PAMP RNA as well as its degradation products constitute a novel virulence factor whereby B. abortus, by a TLR3 independent mechanism and through the EGFR pathway, inhibit MHC-I expression. Thus, bacteria can hide within infected cells and avoid the immunological surveillance of cytotoxic CD8+ T cells.

302 (225) **CROSSTALK BETWEEN PLATELETS, B. ABORTUS AND IMMUNE CELLS.**

Aldana Trotta[1], María Ayelén Milillo[1], María Victoria Delpino[2], Guillermo Hernán Giambartolomei[2], Roberto Gabriel Pozner[1], Lis Noelia Velásquez[1], Paula Barrionuevo[1].
[1]Instituto de Medicina Experimental (CONICET-Academia Nacional de Medicina). Buenos Aires. Argentina. [2]Instituto de Inmunología, Genética y Metabolismo (CONICET-UBA). Laboratorio de Inmunogenética. Buenos Aires. Argentina.

Brucellosis is an infectious disease elicited by bacteria of the genus *Brucella*. Platelets have been extensively described as mediators of hemostasis and responsible for maintaining vascular integrity. Nevertheless, they have recently got involved in the modulation of innate and adaptive immune responses. We have already demonstrated a crosstalk between *B. abortus* and monocytes. However, the role of platelets during monocyte/macrophage infection by these bacteria remains unknown. The aim of this study was to investigate whether platelets are involved in the development of *Brucella*-mediated infection. To start evaluating this, THP-1 cells (pro-monocytic human cell line) were infected with *B. abortus*-GFP (100:1) in the presence or absence of platelets for 4 h and the effect of platelets on the infectious capacity of *Brucella* was analyzed by confocal microscopy. Our results showed that the presence of platelets stimulated the invasion of monocytes by *B. abortus*. Moreover, we observed that platelets formed complexes solely with infected monocytes. Afterwards, we evaluated the ability of platelets to modulate functional aspects of monocytes during the infection. First, we studied the secretion of immunomodulatory mediators. To address this, THP-1 cells were infected with *B. abortus* in the presence or absence of platelets for 4 or 24 h. The supernatants from infected cells were collected and quantified by ELISA. Next, we studied the expression of adhesion and co-stimulatory molecules on the monocyte surface by flow cytometry. The presence of platelets during monocytes/macrophages infection stimulated IL-1β, IL-8 and MCP-1 secretion ($p<0.01$) while it inhibited the secretion of TNF-α ($p<0.01$). At the same time, platelets stimulated the expression of ICAM-1 (CD54) and CD40 ($p<0.01$). Overall, our results indicate that platelets can modulate the *B. abortus*-mediated infection of monocytes increasing their pro-inflammatory capacity, which could promote the resolution of the infection.

303 (458) **BORDETELLA PERTUSSIS MGTC PLAYS A ROLE IN INTRACELLULAR SURVIVAL BY FACILITATING THE ADAPTATION TO MAGNESIUM LIMITING CONDITIONS AND ACIDIC PH.**

Juan Hilario Cafiero[1], Yanina Lamberti[1], Hugo Valdez[1], María Eugenia Rodriguez[1].
[1]CINDEFI (UNLP - CONICET), Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina.

Bordetella pertussis (*Bp*), the causative agent of whooping cough, survives inside host cells, a process that requires the adaptation of the pathogen to this harsh environment. *Bp* genome contains a homolog of mgtC, a virulence factor of several pathogens that is involved in growth under mildly acidic pH and Mg^{+2} limiting conditions and that is crucial for intracellular survival. The aim of this study was to analyse the role of mgtC in *Bp* intracellular survival. A *Bp*ΔmgtC mutant strain and a complemented strain were constructed and used in parallel with the wild type strain (wt*Bp*) in an infection assay of PMA-differentiated THP-1 cells. Immunofluorescence microscopy showed that there were no differences between the strains in the uptake of bacteria by THP-1 cells. The intracellular survival of these strains inside THP-1 cells was analyzed in a polymyxin B protection assay by CFU counts at 3, 24 and 48 hours post-infection (pi). *Bp*ΔmgtC showed a decrease in intracellular survival at 24 and 48 hours pi ($p<0.05$) as compared with both wt*Bp* and the complemented strain. Accordingly, confocal microscopy studies showed a higher traffic of *Bp*ÄmgtC to lysosomes as showed by a higher colocalization with the dye Lysotracker than wt*Bp* at 24 and 48h pi ($p<0.05$). To characterize the defect in the intracellular survival of *Bp*ÄmgtC, we tested the *in vitro* growth of this strain in different conditions. There were no differences in the growth yield in liquid medium between wt*Bp* and *Bp*ÄmgtC in Mg^{+2} replete conditions. However, *Bp*ÄmgtC showed lower biomass yield under Mg^{+2} limited conditions ($p<0.01$). We further found that mgtC is upregulated in Mg^{+2} starvation ($p<0.001$), as determined by RT-qPCR. *Bp*ÄmgtC showed a lower resistance to mild low pH than wt*Bp* strain ($p<0.01$), suggesting that this gene is also involved in acidic tolerance. Altogether, this data suggest that mgtC is involved in *Bp* adaptation to the endosomal environment and plays a key role in bacterial intracellular survival.

304 (529) **BOVINE – ECHINOCOCCUS GRANULOSUS CELL LINE (EGPE) AS ANTIGENIC SUPPORT FOR HYDATIDOSIS DIAGNOSIS AND FOLLOW UP.**

Andrea Florencia Maglioco[1,2], Melisa S Barbery Venturi[1,2], Jorge Gentile[3], Susana Hernández[3], Oscar Jensen[4], María Laura Gertiser[4], Alicia G Fuchs[1].
[1]Centro de Altos Estudios en Ciencias Humanas y de la Salud, Universidad Abierta Interamericana (UAI) [2]CONICET [3]Hospital Ramón Santamarina, Tandil [4]Centro de Investigación en Zoonosis, Provincia de Chubut.

Introduction: Echinococcus granulosus is the causative agent of hydatid disease (HD), a widely distributed zoonosis in the world. The usual source of antigens used for immunodiagnosis is the hydatid cyst fluid. Differences were observed in the specificity and sensitivity between different studies, perhaps due to the use of a source of non-standardized antigens. In our laboratory, a cell line from bovine protoscolices (EGPE cells) has been established (patent first instance approved INPI P-090102320). In these cells, the antigen B was detected by immunohistochemistry and DCO1 was detected by PCR (Echeverria et al 2010). Aim: to validate the use of antigens from EGPE cells, as a standardized source of antigens, to diagnose HD and monitoring its follow up. Study design: Presence of relevant antigens to HD were evaluated in EGPE, by western blotting. Three different protein mixtures were assayed: proteic extract from short or long culture of EGPE and supernatant. Serum from 13 patients with HD or non HD patients from an endemic zone (Tandil) were used in a case-control study. Six of these HD patients were evaluated 4 years later. Bands detected exclusively by HD patients were taking into account as positive band. Results: The combination of different antigens from the 3 sources allow the diagnosis of 13/13 patients. The three sources have different protein composition. The bands detected by HD patients in short culture antigens were (KDa): 76-78, 70-73, 64-65, 58-60, 52-55, 44-47 and 40-41; in long culture antigens detected were (KDa): 93-94, 78-81, 73-77, 69-72, 63-66, 56-57, 51-55, 46-47 and 40-42; and in supernatant antigens detected were (KDa): 86-90, 79-80, 75-76 and 58-60. These bands were lost in 5/6 HD patients 4 years later ($p<0.05$, Chi-square test). Conclusion: More than one antigen is necessary to HD diagnosis and EGPE proteins would be a very useful source of antigens for HD diagnosis and follow up. This study was approved by UAI ethics committee.

305 (545) **ANTI-PROLIFERATIVE EFFECT OF NON STEROIDAL GLUCOCORTICOID RECEPTOR AGONIST COMPOUND A IN CELLS STIMULATED WITH SEG.**

María Julieta Fernández Lynch[1], Mariángeles Díaz[1], Sofía Noli Truant[1], María Belén Sarratea[1], María Belén Antonoglou[1], Mauricio de Marzi[2], Emilio Malchiodi[1], Marisa Mariel Fernández[1].
[1]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Inmunología-IDEHU (UBA-CONICET),

Buenos Aires, Argentina. [2]Universidad Nacional de Lujan, INEDES, Lujan, Buenos Aires, Argentina.

Bacterial superantigens, such as SEG, are exotoxins that trigger peptide-non-specific T cell proliferation and proinflammatory cytokine release. Immunosuppressants, like glucocorticoids, are used to revert inflammatory actions in superantigen related diseases. However, we have previously reported resistance to dexamethasone by enterotoxin gene cluster and group II toxins in peripheral blood mononuclear cells (PBMCs). Non-steroidal glucocorticoid receptor agonists (SEGRAs), like Compound A, exert their action by transrepressing activation of proinflammatory pathways [Bossher 2005]. Therefore, we studied the pathways involved in glucocorticoid resistance and whether SEGRAs can exert a better immunosuppressive action than dexamethasone. SEG was recombinantly produced in *E. coli* and purified by Ni-NTA. PBMCs were enriched from heparinized blood using Ficoll-Hypaque gradient, cultured for 48 h for proliferation assessment with ^{3}H-thymidine under different stimuli (SEG 10 µg/ml, dexamethasone 10^{-5} M, Compound A 10^{-6} to 10^{-4} M, rapamycin 1-200 nM and BAY 11-7082 1-50 µM.) The effect of SEG on macrophage cell line RAW and T cell line LBR was also assessed by proliferation assays. PBMCs proliferation induced by 10 µg/ml SEG is inhibited by NFκB inhibitor BAY 11-7082 at 10 µM (two way ANOVA $p>0.05$) showing the importance of this inflammatory transcription factor in the superantigen action mechanism. 10 µM compound A also inhibits SEG-induced proliferation showing a better immunosuppressive activity than 10 µM dexamethasone treated cells which show significant proliferation ($p<0.001$ vs $p>0.05$). The study on isolated cell lines shows that SEG is unable to induce proliferation lacking costimulatory signals rendering these pathways an interesting target. Understanding the underlying mechanisms of glucocorticoid resistance in staphylococcal toxin related diseases can help improve treatment outcome in patients, being SEGRAs promising drugs for treating these diseases.

306 (696) **BORDETELLA PERTUSSIS INFECTION AFFECTS IRON HOMEOSTASIS IN MACROPHAGES.**

Jimena Álvarez Hayes[1], Hugo Valdez[1], Bruno Blancá[1], María Eugenia Rodriguez[1].
[1]CINDEFI (UNLP CONICET La Plata), Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina.

We previously showed that Bordetella pertussis (Bp), the etiological agent of whopping cough, is able to survive inside macrophages (M□). It is known that M□ response to intracellular pathogens is associated with an intracellular deprivation of iron levels through different mechanisms that involves iron exportation and scavenging, a phenomenon termed nutritional immunity. Pathogens have developed mechanisms to counteract this effect. In this study we investigated the host cell modulation of iron homeostasis in response to Bp infection and this pathogen influence on such homeostasis. THP-1 M□ were infected with or without (control) live or heat-inactivated Bp (Bp-hi). M□ mRNA was recovered at different times post infection and live intracellular bacteria were determined by FISH staining and confocal microscopy analysis. Expression of *DMT1*, *TFR1*, *FPN1*, *LCN2*, *HAMP*, *HMOX1* and *FTH1* genes, all of them involved in cell iron homeostasis, was evaluated by RT-PCR. We found that the expression of most of them was induced by infection. However, *FPN1* and *LCN2* expression was induced early after infection with Bp-hi but not in cells infected with live bacteria, suggesting that Bp modulates M□ iron homeostasis during infection and that this modulation is dependent of bacterial viability. Since pertussis toxin and adenylate cyclase were found involved in M□ defense response during infection we next analyzed whether any of these toxins are also involved in iron gathering. M□ infections with the Bp (wtBp), BpDCyaA or BpDPTx showed that the expression of genes involved in iron scavenging and exportation was significantly higher in cells infected with BpDCyaA as compare with wtBp infection. Accordingly, we found that wtBp survival 24 h post-infection is higher (35%) than BpDCyaA (17%). Taken together these results suggest that Bp is able to modulate host nutritional immunity response to overcome iron restriction and survival inside M□ and that CyaA is involved in this modulation.

307 (759) **TRICHINELLA SPIRALIS INFECTION AND PROTEIN DEFICIENCY: STUDY OF MAST CELLS, GOBLET CELLS AND SPECIFIC ANTIBODIES IN LUNG AND INTESTINE**.

Cecilia Celeste Vila[1], Maria Priscila Saracino[1], Guido Hernán Falduto[1], Marcela Adriana Calcagno[1], Pablo Cesar Baldi[1], Anabel Pallaro[2], Stella Maris Venturiello[1].
[1]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Inmunología-IDEHU (UBA-CONICET), Buenos Aires, Argentina. [2]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Nutrición, Buenos Aires, Argentina.

We have shown that protein deficient rats infected with *Trichinella spiralis* have a more severe infection, with a delayed expulsion of adult worms (AW). Here we studied the effect of protein deficiency on the appearance of mast and goblet cells (important in anti-*T. spiralis* response), and of antibodies (Abs) against Excretory-Secretory Products from muscle larvae (ML-ESP) and AW (AW-ESP) in lung and intestine during the infection. Weaning Wistar rats received protein-deficient diet (PD) containing 6.5% casein, or a control diet (C, 20% casein). After ten days, both groups were orally infected with ML; non-infected rats served as controls (n=3 per group). At days 3, 6, 9, 13 and 33 post-infection (p.i.) lungs and intestine were obtained. Histological analyses for counting mast cells (Alcian Blue, Safranin) and goblet cells (Haematoxilin-PAS) were performed. Data were analyzed using the two-way ANOVA test. Lung and intestine tissue extracts were obtained at days 6 and 13 p.i. (PERFEXT method) and specific Abs were detected by ELISA. Intestine: Goblet cells increased, compared to non-infected rats, from day 6 p.i. in C group ($p<0.005$) and from day 9 p.i. ($p<0.01$) in PD group. Mast cells increased from day 6 p.i. in C group ($p<0.01$) and from day13 p.i in PD group ($p<0.001$). At day 6 p.i. C and PD had only anti-ML-ESP Abs. At day 13 p.i. C group presented Abs against both stages, but PD only against AW-ESP. Lung: Goblet cells increased from day 3 p.i. in C group ($p<0.01$) and from day 9 p.i. in PD group ($p<0.001$). Mast cells increased from day 3 p.i. in C group ($p<0.01$) and from day 9 p.i. in PD group ($p<0.01$). Both groups had specific Abs against both stages at days 6 and 13 p.i.Protein deficiency seems to affect more the mucosal cellular response than the humoral response to *T. spiralis*. This may explain the delayed expulsion of AW and the severe infection with higher parasite burden in rat receiving the PD diet.

308 (865) **DEVELOPMENT OF A LATERAL FLOW IMMUNOASSAY FOR THE RAPID AND EARLY DIAGNOSIS OF HUMAN LEPTOSPIROSIS.**

Nazarena Pujato[1;2], Paulina Jacob[1;2], Yosena Chiani[1], Fernanda Schmeling[1], Noelia Landolt[1;2], Bibiana Vanasco[1;2].
[1]Instituto Nacional de Enfermedades respiratorias (INER) "Dr. Emilio Coni"- ANLIS "Dr. G. C. Malbran" [2]Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral.

Laboratory diagnosis of human leptospirosis is crucial due to the varied and unspecific symptoms of the disease. However, lack of reliable and simple techniques, produces delay in diagnostic directly affecting patient's health. Because of this, development of a point of care test based in lateral flow immunocromatography (LFI), low cost and easy to perform, is an attractive alternative to improve the diagnosis. In Argentina, scare availability of the inputs required for manufacturing this kind of devices is an important limitation, which gave rise to our purpose: To design a LFI device using national available inputs, for detecting specific IgM antibodies in serum samples from patients with clinically suspected leptospirosis. In the present work, a preliminary strip design matrix was proposed which employs nitrocellulose membrane, filter paper and DAB chromogenic reagent. The antigen selected for specific antibodies detection was the thermorresistant antigen (TR), currently used in routine techniques for leptospirosis diagnosis. Performance of the

designed matrix was evaluated in a double-blind study including 60 serums, previously classified in our National Reference Laboratory as leptospirosis confirmed or discarded cases. The detection assay consists in labeling sample IgM antibodies with an anti-IgM_{humana}/HRP secondary antibody, dispensing them onto the reactive strip and finally developing the result through addition of DAB. Data analysis gave a Younden's index J= 0,83 (IC: 0,71- 0,96), with a sensitivity Se= 83,3% (IC: 69,8- 96,9) and specificity Sp=100% (IC: 97,9- 100). The Kappa coefficient was 0,80 indicating a high concordance between results obtained with the produced LFI tests and the reference test. Results suggest that this preliminary matrix design was very efficient for detecting specific serum antibodies directed to leptospira. Results encourages us to continue working on improving the matrix architecture and achieving a one-step assay with the aim of finally obtained a national test, easily applicable in any health center, which would favorably impact on leptospirosis diagnosis at different levels of the public health system.

309 (893) **INHIBITION OF WNT SIGNALING PATHWAY CONTROLS PARASITE REPLICATION AND INFECTION-INDUCED PATHOLOGY DURING TRYPANOSOMA CRUZI INFECTION.**

Ximena Volpini[1], Laura F. Ambrosio[1], Laura Fozzatti[1], Constanza Insfrán[1], C. Cristina Motrán[1].
[1]Dpto. Bioq. Clínica, Facultad de Ciencias Químicas. UNC. CIBICI-CONICET.

During the very early stages of *T. cruzi* infection, this parasite is found within macrophages (Mo), where its replication can be either inhibited or favored, leading to dissemination to other sites within the body. Experimental evidence indicates that control of *T. cruzi* parasitism during the early phase of infection is critically dependent on effective Mo activation. Wnt signaling, essential for embryonic development, has also recently been involved in the regulation of inflammatory processes. This signaling pathway is induced in Mo by inflammatory stimulus and depending on the composition of Wnt//Frizzled (Fz) complex, non-canonical or Wnt/b-catenin (canonical) pathways are initiated leading to amplify or control the inflammation, respectively. In addition, the role of canonical pathway on effector and regulatory T cells is controversial. We have reported that canonical Wnt signaling is activated in spleen and Mo after *T. cruzi* infection *in vivo* and *in vitro* respectively, with this pathway inhibition in Mo controlling intracellular parasite replication. In this study we tested the hypothesis that the pharmacological modulation of Wnt signaling pathway during *T. cruzi* infection might limit parasite replication and also infection induced-pathology. *In vitro* canonical Wnt pathway inhibition by treatment of infected Mo with iCRT14 or CCT036477 increased the secretion of pro-inflammatory cytokines and was more effective to inhibit parasite replication ($p<0.0001$) tan treatment with IWP-L6, which can also inhibit the non-canonical Wnt pathway. *In vivo* pharmacological inhibition of Wnt signaling by treatment of *T. cruzi*-infected B6 mice with IWP-L6 was able to control the parasitemia ($p<0.01$) and the inflammatory liver pathology ($p<0.05$). These findings suggest that modulation of Wnt pathways may allow the design of new therapeutic strategies to control T. cruzi replication and parasite-induced pathology.

310 (899) **TRYPANOSOMA CRUZI INFECTION: A NOVEL TREATMENT WITH ENDOGENOUS NONTOXIC AHR LIGANDS PROMOTES TREG CELLS DIFFERENTIATION WITHOUT DETRIMENTAL EFFECTS ON PARASITE-SPECIFIC IMMUNITY.**

Laura Fernanda Ambrosio[1], Constanza Insfrán[1], Ximena Volpini[1], Miriam Postan[2], Laura Cervi[1], Claudia Cristina Motrán[1].
[1]Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI)-CONICET-Universidad Nacional de Córdoba. [2]Instituto Nacional de Parasitología Dr. Mario Fatala Chabén/ANLIS/Malbrán, Buenos Aires, Argentina.

During *T. cruzi* infection, both a strong inflammatory and an efficient regulatory responses are essential to restrict parasite replication and prevent immunopathology. Studies in *T. cruzi*-infected B6 mice have shown that despite its ability to control parasite replication they are unable to expand regulatory T cells (Treg), resulting in the premature death of these animals by inflammatory liver failure. The AhR is a ligand-activated transcription factor that plays important roles in several biological processes, including the immune response. In T cells, its function depends on the ligand bound with the xenobiotic TCDD and endogenous agonist ITE and kynurenines promoting Treg and FICZ favoring Th17 differentiation. We have reported that the treatment of B6 mice with TCDD 24hs prior infection was able to control the inflammatory response increasing the% of Treg but also inducing T cell apoptosis, both effects contribute to increase the parasite burden and to diminish the survival. In this study we tested the hypothesis that the activation of AhR signaling using two endogenous nontoxic AhR ligands as ITE and 3-HK (a kynurenine toxic for *T. cruzi*) might control the infection and also the immunopathology. The treatment of *T. cruzi*-infected B6 mice with ITE (days 7, 9 and 11 post infection (pi)) plus 3-HK (from day 5 to 10 pi) induced a significant increase in the% (day 13, $p<0,05$, and day 90 pi, $p<0,01$) and number (day 90 pi, $p<0,01$) of splenic Treg. Also, a significant increase of Treg producing TGF-beta was observed in treated vs control (PBS- and DMSO-treated) mice. Interestingly, at day 17 pi, less% of splenic CD4+ Annexin V+ cells ($p<0,05$) and less inflammatory index in the liver were observed in treated vs control mice, which did not shown significant changes in parasitemia or survival. Our results suggest that 3HK+ITE might be a novel therapeutic treatment able to control the inflammatory response without detrimental effects on parasite-specific immunity.

311 (950) **B CELL RESPONSE IN TRYPANOSOMA CRUZI INFECTION IS IMPAIRED IN THE ABSENCE OF IL-17.**

Facundo Fiocca Vernengo[1], Cristian Gabriel Beccaría[1], Melisa Gorosito Serrán[1], Carolina Lucía Montes[1], Eva Virginia Acosta Rodriguez[1], Adriana Gruppi[1].
[1]CIBICI-CONICET. Departamento de Bioquímica Clínica e Inmunología, FCQ, UNC. Córdoba. Argentina.

B cells and antibodies play a key role in pathogen clearance and host survival during extracellular parasite infections. The Germinal centers (GC) provide a microenvironment that stimulates and regulates the interactions of B cells with follicular Th (Tfh) cells, which provide the cognate help required for the generation of high affinity Ab-producing plasma cells and memory B cells. In the last years, IL-17 has been reported as a key cytokine that promote spontaneous GC formation and autoantibodies production. Our aim was to elucidate the role of IL-17 in the generation of B cell response in T. cruzi infection. For this purpose, IL-17A/F KO and C57BL/6 mice were infected with 15.000 trypomastigotes. We found that IL-17A/F KO were susceptible to infection (p=0,05) as they started dying at day 22-23 post infection (pi). Interestingly, IL-17A/F KO mice showed higher liver and spleen parasitism at day 24pi (p=0,0193 and 0,0006 respectively). By flow cytometry analysis we found that IL-17A/F KO mice had lower frequency (23dpi) and number (14 and 23dpi) of GC B cells than infected control mice (p= 0,0203, 0,0306 and 0,0186 respectively). In addition, B cell activation was affected in IL-17A/F KO mice as they showed higher frequency of naïve B cells. Notably, Bcl-6 expression on B cells was markedly affected on IL-17A/F KO mice. Surprisingly, plasma cell generation was similar in both experimental groups. At the opposite site, T follicular helper (Tfh) and T follicular regulatory (Tfr) cells frequencies were not markedly affected in IL-17A/F KO mice. However, Tfh cells generated in IL-17 deficient mice showed higher levels of the inhibitory molecule 2B4. Finally we found that IL17R expression on B cells was higher than on Tfh in C57BL/6 infected mice. In conclusion, IL-17 deficiency did not affect Tfh but was sufficient to evoke a defective B cell response, particularly impacting on B cells.

312 (1024) **CANDIDA ALBICANS INDUCES IFNB EXPRESSION IN FEMALE GENITAL TRACT EPITHELIAL CELLS.**

Emilse Rodriguez[1], Cecilia Vigezzi[1], María Soledad Miró[1], Gerardo Gatti[1], Paula Alejandra Icely[1], Mariana Maccioni[1] Claudia Elena Sotomayor[1].

[1]CIBICI-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba.

Type I interferons (IFNs-I) constitute a family of pleiotropic cytokines best known for their ability to induce an antiviral state and by coordinating the immune cells involved in antiviral or antibacterial immunity. IFNs-I are produced after virus or bacteria recognition by TLRs and recent studies demonstrate that fungal recognition through CTL receptors also induce activation of the IFNs-I in professional APC. *Candida albicans* (*Ca)* is the most common cause of vulvovaginal candidiasis (VVC) that affects approximately 75% of women worldwide. We aimed to study whether *Ca* recognition induces IFNs-I in epithelial cells of female genital tract in order to establish a possible role during VVC. For this purpose, human cervical epithelial cell line (HeLa) were stimulated with: *Ca* SC5314 strain (infective) (fungus: cell ratio 0.25:1, 0.5:1,1:1, 5:1), *Ca* treated with Amphotericin B (*Ca*-AMB) (non-infective) (5:1), *Ca* DNA complexed with polyethylenimine (*Ca* DNA-PEI), Zymosan and Poly I:C for 24h. IFNb, IRF3, IRF7 and Mx1 mRNA levels were measured by qPCR and cytokine profile (IL1b, IL6, TNFa and TGFb) by ELISA. Poly I:C was able to induce a strong IFNb mRNA expression in HeLa cells ($p<0.01$). Unexpectedly, viable but non-infective *Ca*-AMB was able to induced a high IFNb mRNA expression on epithelial cells ($p<0.05$). Interestingly, *Ca* DNA-PEI delivered into the cytoplasm of HeLa cells also induced higher IFNb mRNA levels compared with untreated cells ($p<0.05$). Poli I:C and *Ca*-AMB induced strong expression of Mx1 mRNA ($p<0,001$). Moreover, infective *Ca* Sc5314 strain, *Ca* DNA-PEI and Poly I:C induced high IL6 levels ($p<0,001$). While TNFa and TGFb amounts were variable among different stimuli, IL1b remained undetectable. These results provide novel and important evidence about the ability of both, *C. albicans* cell wall components and fungal DNA to trigger IFNb production in epithelial cells, and expands the spectrum of immune mediators involved in the local response during VVC.

313 (1043) **INDUCTION OF GALECTIN 1 EXPRESSION IN HIV-INFECTED CD4+ T CELLS MODULATES VIRAL REPLICATION**.

Julia Rubione[1], Gabriel Duette[1], Pehuen Pereyra Gerber[1], Jorge Geffner[1], Karina Mariño[2], Marta Toscano[2], Gabriel Rabinovich[2], Matías Ostrowski[1].
[1]*Instituto de Investigaciones Biomedicas en Retrovirus y Sida* [2]*Instituto de Biología y Medicina Experimental.*

The sequential action of glycosyltransferases and glycosidases are responsible for modifying cell surface´s glycans (cellular glycome). These changes can either expose or mask ligands for endogenous lectins, such as Galectins (Gal). Extracellular Gal-1 has important immunomodulatory functions by selectively deleting Th1 and Th17 cells and enabling the expansion of regulatory T cells. Herein, we show that *in vitro* infection of CD4+ T cells induces the expression and secretion of Gal-1. Moreover, HIV-infected cells exhibit an increased binding of extracellular Gal-1 to their surface suggesting that during HIV infection the cellular glycome is modified. We hypothesize that during HIV replication secreted Gal1 modulates the functionality of CD4+ T cell in an autocrinous manner. We successfully silenced Gal1 expression in primary CD4+ T cells and the model cell line, HeLa. We are currently studying the contribution of this lectin to HIV replication and the immunomodulatory role of Gal1 in the context of HIV infection.

314 (122) **GENERATION OF POLY- AND MONOCLONAL MEMORY T CELL LINES FOR ANTIGEN DISCOVERY IN CHRONIC CHAGAS DISEASE.**

Gonzalo Raúl Acevedo[1], Alcinette Bunying[2], Nazila Sabri[2], Augusto Atienza[3], Valeria Judkowski[2], Clemencia Pinilla[2], Karina Andrea Gómez[1].
[1]*Instituto de Investigaciones en Ingeniería Genética y Biología Molecular "Dr. Héctor N. Torres" (INGEBI-CONICET). Ciudad Autónoma de Buenos Aires, Argentina.* [2]*Torrey Pines Institute for Molecular Studies. San Diego, California, USA.* [3]*Hospital General de Agudos "Dr. Ramos Mejía". Ciudad Autónoma de Buenos Aires, Argentina.*

T cells are a major player in immune response against T. cruzi infection. Still, knowledge on the role of parasite-specific Th cells and their specificities is limited. Patient-derived antigen-specific T cell lines are a key tool for the study of this subject. The aim of this work was the generation of such lines from a chronic Chagas patient's memory CD4 T cells by modulation of in vitro stimulation and culture conditions. CD3+ CD4+ CD45RO+ cells were magnetically sorted from PBMC and submitted to stimulation with T. cruzi lysate (antigen-specific) or PHA (non antigen-specific). At day 27 post-stimulation, cultures showing signs of proliferation were challenged with parasite lysate. Proliferation and Interferon (INF)-gamma secretion were used as a measurement of antigen-specific response. Autologous B-LCL were used as antigen presenting cells. Out of 192 wells initially stimulated with the lysate, 58.3% were steadily expanding at day 27, and most of these (87.5%) showed T. cruzi-specific response. Conversely, 100% of the PHA-stimulated wells reached this checkpoint, but only 37.5% responded to parasitic antigens. Nineteen T. cruzi-specific cultures were selected for further functional characterization by multiplex cytokine secretion assay (INF-gamma, TNF-alpha, GM-CSF, IL-2, -4, -10, -13, -17), and two were selected, on the basis of polyfunctionality, to undergo LDA.Seven hypothetically clonal, antigen-specific CD4 T cell lines were generated, which responded to T. cruzi lysate by INF-gamma and GM-CSF secretion. All of these, as well as the population from which they originated, maintained their specific response capability, even after long-term in vitro culture (225 days). TCR V-beta staining assay demonstrated that at least one of these lines is monoclonal, and expresses a member of the V-beta 5 family, which has a reportedly augmented frequency in chronic Chagas patients' CD4+ T cells.

315 (157) **IN VIVO ROLE OF LANGERHANS CELLS AND IL-17 IMMUNITY IN EXPERIMENTAL M. CANIS SKIN INFECTION.**

Verónica Liliana Burstein[1,2], Lorena Guasconi[1,2], Ignacio Becacecce[1], Martín Theumer[1,2], Mónica Herrero[3], Cristian Mena[1], Diana Masih[1], Laura Chiapello[1,2].
[1]*Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba.* [2]*CIBICI-CONICET.* [3]*Hospital Córdoba.*

Microsporum canis is a dermatophyte fungus highly prevalent in immunocompetent children that causes superficial infections. However, invasion beyond epidermis has also been reported. The *in vivo* role of IL-17 and Langerhans cells (LC) during dermatophytosis is currently unknown. Objective: To determine the impact of IL-17 signaling in experimental dermatophytosis in mice and the role of LC in establishing skin antifungal immunity. Wild type (WT), IL-17RAKO, IL17A/FKO or Lang-EGFPDTR C57BL/6 mice were epicutaneously infected with *M. canis.* For LC depletion, diphtheria toxin was injected 3 days before infection. On 4, 8 and 18 days post-infection (dpi) histopathological analysis, skin fungal burden (HPLC ergosterol quantification) and fungal dissemination were determined. $CD11b^+Ly6G^+$, T cells populations and cytokine production were analyzed (ELISA, FACS) in epidermis or skin-draining lymph node (sdLN) cells. Cytokines were also measured in epidermal sheet explants incubated with *M. canis.* WT mice resolved infection by 20 dpi showing features of human dermatophytosis. The response was mainly characterized by significant neutrophilic skin infiltrate and IL-17-producing $CD4^+$ T cells in sdLNs by 8 dpi. *M. canis* hyphae stimulated IL-17A/F, IL-23, IL-6, IL-12 and IL-10 production by epidermal cells and purified LC promoted only IL-17A/F production by allogeneic lymphocytes. IL-17 deficient mice resolved infection similar to WT, but showed higher skin inflammation and fungal burden and a shift to IFN-ã production in sdLNs, compared to WT mice. *In vivo* IFN-ã blocking partially inhibited the exacerbated cutaneous inflammation and LC depletion in Lang-EGFPDTR mice showed significant decrease in *M. canis* specific IL-17-producing $CD4^+$ T cells. Langerhans cells induce Th17 antifungal immunity. IL-17 signaling protects against *M. canis* infection and the exacerbated Th1 inflammation, but is not involved in PMN recruitment to skin or in control of extracutaneous fungal dissemination.

316 (301) **INTERACTION DECTIN-1/B-GLUCANS IN BRAIN: DELICATE BALANCE BETWEEN CONTROLLING INFLAMMATION AND FUNGAL CONTROL.**

Cecilia Vigezzi[1]; María Soledad Miró[1], María Emilse Rodríguez[1], Paula Icely[1], Claudia Sotomayor[1].
[1]Laboratorio de inmunidad innata a patógenos fúngicos. Facultad de Cs. Químicas. CIBICI-CONICET. Córdoba.

Glucan receptor Dectin-1 is essential for antifungal response to Candida albicans in the periphery, but little is known about its role during Central Nervous System (CNS) infections. b-glucans are widely recognized as immunomodulators. It has been demonstrated in vitro that the activation of microglia through Dectin-1 attenuates the production of proinflammatory cytokines. Our objective was to evaluate the role of this interaction in brain during C. albicans systemic infection. Male C57BL/6 mice were injected with 2,5.106 intra venous C. albicans SC5314 (parental) or SC5314-FKS 645 (glucan synthase mutant strain), and at 4,12,24 and 48h post-infection(pi). Animals were sacrificed and blood, kidney and brain samples were obtained to evaluate fungal burden (CFU), expression of Dectin-1 and IL-1beta (IL-1β) (qPCR), and in situ cytokines secretion (IL-1β, IL6, TNF-alpha, ELISA). Dectin-1 deficient mice (KO) were infected with SC5314 and killed at 4h and 12h. In both strain, fungemia was highest at 4h pi (CFU-p<0.05). Profile of infection of each strain was different. At early time point, the fungal burden was significantly higher in SC5314 strain than in FKS 645 (p4h, 12h <0.05). Interestingly, at 4h pi SC5314 WT infection did not induce proinflammatory cytokines secretion, whereas in infection with FKS-645, local IL-1β and TNFa secretion was significantly increased (p<0.05). TNFα production in Dectin-1KO was significantly higher than in WT animals (p<0.05), while increasing IL-1β was observed as a trend. We also evaluated the ability of the parental and mutant strain to induce cytokine release after incubation with BV2 microglia cell line. IL-1β levels were undetectable at 24h, while higher TNFα release was observed (p<0.05). The absence or decrease of Dectin-1 pathway, promotes a robust proinflammatory reaction in brain that could be relevant to control the fungal growth. This study provides evidences about the immunomodulatory mechanisms of b-glucans in brain.

317 (325) **BRUCELLA ABORTUS-ACTIVATED MICROGLIA INDUCE NEURONAL DEATH THROUGH PRIMARY PHAGOCYTOSIS.**

Ana María Rodriguez[1], María Cruz Miraglia[1], Miriam M. Costa[2], Paula Barrionuevo[3], Vida A Dennis[4], Sergio Costa Oliveira[2], Maria Victoria Delpino[1], Guillermo Giambartolomei[1].
[1]Instituto de Inmunología, Genética y Metabolismo (CONICET/UBA). Hospital de Clínicas "José de San Martín", Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina. [2]Department of Biochemistry and Immunology, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte-Minas Gerais, Brazil. [3]Instituto de Medicina Experimental (CONICET-Academia Nacional de Medicina). Pacheco de Melo 3081. C1425AUM. Buenos Aires. Argentina. [4]Center for NanoBiotechnology Research and Department of Biological Sciences, Alabama State University, USA.

Central nervous system invasion by bacteria of the genus *Brucella* results in an inflammatory disorder called neurobrucellosis. *B. abortus* infects microglia, eliciting their activation and production of pro-inflammatory mediators. Evidence of neurological involvement occurs to varying degrees in nervous systems of patients with neurobrucellosis. The aim of this work was to determine the putative mechanisms involved in this phenomenon. For this, we used murine primary cultures of neurons and microglia to demonstrate that, due to *B. abortus* infection, microglial primary phagocytosis actively induces neuronal death, without inducing neuronal apoptosis. This phenomenon was due to microglia-TLR2 activation by *Brucella* lipoproteins. We demonstrated that *B. abortus*-activated microglia secrete nitric oxide (NO) and increase their phagocytic ability and proliferation (p<0.05). NO induced the exposure of eat-me signal on neurons (phosphatidylserine, PS). Blocking PS-binding protein milk fat globule epidermal growth factor-8 (MFG-E8) interaction, or microglial vitronectin receptor-MFG-E8 interaction was sufficient to prevent neuronal loss without inhibiting microglia activation (p<0.05). Hence, our results demonstrate a novel form of inflammatory neurodegeneration for a bacterial infection, where inflammation cause exposure of eat-me signal on neurons, leading to their death through primary phagocytosis. These results describe part of the mechanisms whereby *B. abortus* could induce neuronal death during neurobrucellosis.

318 (374) **ALTERATIONS IN PULMONARY FUNCTION DURING TRICHINELLA SPIRALIS INFECTION.**

Guido Hernán Falduto[1], Patricio Acosta[2], Cecilia Celeste Vila[1], Fernando Polack[2], Stella Maris Venturiello[1].
[1]Instituto de Estudios de la Inmunidad Humoral (CONICET/UBA), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires. [2]Fundación INFANT.

Experimental parasitic infection caused by *Trichinella spiralis* provokes, in the murine model, an early allergic inflammatory response in the lung, before and during the migratory stage (MS) passage through this organ. The present study aimed to analyze whether this allergic response provokes changes in pulmonary function and related parameters at days 1, 2, 3, 6, 9, 13, 30 post-infection (p.i.). Pulmonary function was assessed by measuring pulmonary resistance (R) at increasing doses of methacholine (cholinergic bronchoconstrictive agent). Lung parenchyma mast cells presence and degranulation were analyzed by: histological stains (toluidine Blue), histamine detection in bronchoalveolar-lavage (BAL; ELISA), presence of CD45+ FcåRI+ lung cells (flow cytometry) and anti-MS IgE in BAL, lung tissue extracts and sera (immunofluorescence assay). Data were analyzed using the one-way ANOVA test. Our results revealed: I- an increased R at days p.i. studied, compared to non-infected animals (p<0.05). Animals infected on days with the highest passage of MS through the lung were the most affected (days 6-9 p.i.). II- A significant increase in mast cells (p<0.05) and CD45+ FcåRI+ lung cells until day 6 p.i. This is in line with the maximum level of histamine detected in BAL at day 9 p.i. (72.20 ± 5.48 ng/ml *vs.* 28.66 ± 4.01 ng/ml in non-infected animals; p=0.0003). III- Anti-MS IgE was detected from day 6 p.i. in the three biological fluids. In summary, inflammatory response triggered by *T. spiralis* alters pulmonary function causing bronchial hyperresponsiveness due to narrowing of airways. Parasite migration, the presence of mast cells and specific IgE cause cell degranulation and increased levels of histamine in BAL. These results contribute to the understanding of lung signs and symptoms observed in human trichinellosis.

319 (378) **LEPTOSPIRA INTERROGANS TRIGGERS MURINE KIDNEY FIBROSIS INDEPENDENTLY OF MACROPHAGES AND GALECTIN 3.**

María Florencia Ferrer[1], Emilia Scharrig Fernández[1], Ricardo Martín Gómez[1].
[1]Laboratory of Animal Viruses, Institute of Biotechnology and Molecular Biology, CCT-La Plata, CONICET-UNLP, 1900 La Plata, Buenos Aires, Argentina.

Leptospirosis is an emergent zoonotic disease worldwide distributed caused by spirochetes of the genus Leptospira. Macrophage influx and galectin 3 production have been suggested as major players driving acute inflammation and chronic fibrosis in many diseases. However, their involvement in the pathogenesis of *Leptospira interrogans* serovoar Copenhageni (LIC)-induced renal nephritis and fibrosis are unknown. Our aim was to characterize the role of macrophages and galectin 3 on survival, clinical course, bacteremia, bacterial burden (by real-time PCR (qPCR) and immunohistochemistry), acute pathology (by routine histologic techniques and qRT-PCR), and chronic fibrosis (by picrosirius red technique and digital quantification) in LIC-induced interstitial nephritis and fibrosis in the kidney by a comparative study of normal and macrophage-depleted mice or in absence of galectin 3. Our results showed that C57/BL6J mice infected with LIC and depleted of macrophages by liposome-encapsulated clodronate treatment

compared with infected untreated mice presented significant higher bacteremia and subacute bacterial burden as well as increased subacute interstitial nephritis and chronic fibrosis, compared with untreated infected mice. In order to study the role of galectin 3 in LIC-induced nephritis and fibrosis, C57BL/6J *Lgals3-/-* and their control littermate mice were used. Disruption of the galectin 3 gene significantly increased acute bacterial burden as well as acute kidney inflammation compared with C57BL/6J wild-type mice. Also, galectin 3 disruption significantly increased chronic bacterial burden and fibrosis with similar chronic inflammation. Taken together, these results suggest that macrophages and galectin 3 have a role in limiting LIC burden although bacterial presence is more important than macrophage presence, galectin 3 levels and/or inflammatory cell exudate in order to trigger chronic kidney fibrosis.

320 (382) **ROLE OF THE SIGNALING OF IL-17A ON THE AUTOPHAGY PROCESS DURING HUMAN TUBERCULOSIS.**

Nancy Liliana Tateosian[1], Joaquín Miguel Pellegrini[1], Nicolás Oscar Amiano[1], Agustán Rolandelli[1], Paula Morelli[1], Florencia Castello[1], Alberto Levi[2], Nicolás Casco[2], Juan Domingo Palmero[2], Maria Isabel Colombo[3], Verónica Edith Garcia[1].

[1]IQUIBICEN- CONICET. Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales. Universidad de Buenos Aires, Argentina. [2]División Tisioneumonología, Hospital FJ Muñiz. Buenos Aires, Argentina. [3]Laboratorio de Biología Celular y Molecular, Instituto de Histología y Embriología (IHEM)-CONICET, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo. Mendoza, Argentina.

An appropriate immune response against *Mycobacterium tuberculosis* (*Mtb*) requires Th1 cytokine responses but IFN-g alone is not enough to the complete bacterial eradication. Actually, Th17 cells have been associated with *Mtb* infection. Autophagy is an immune effector mechanism against intracellular pathogens, positively modulated by Th1 cytokines, and negatively regulated by Th2 cytokines. However, the role of other cytokines on autophagy in tuberculosis patients (TB) remains unknown. Here we study the role of IL-17A on autophagy during active human TB. Monocytes (Mo) from TB patients and healthy donors (HD) were infected with *Mtb* H37Rv pathogenic strain and cultured ± IL-17A (10ng/ml, 24h). Then autophagy levels were analyzed by Flow cytometry and Immunofluorescence Microscopy against LC3-IIB. We observe that IL-17A increased autophagy against *Mtb*-H37Rv in Mo from HD and TB patients that display strong immunity against *Mtb* (HR TB; p<0,01), significantly affecting mycobacterial survival. In contrast, during infection of Mo from patients with severe TB disease and weak immunity to *Mtb* (LR-TB), IL-17A did not modify autophagy levels. To investigate this differential autophagic response, we analyzed IL-17A receptor, with no significant differences in expression. Signaling through IL-17A receptor induces ERK phosphorylation. Actually, stimulation with *Mtb* antigen increased ERK phosphorylation in HR TB (p<0,05), and IL-17A addition further increased this phosphorylation. Furthermore, inhibition of ERK reversed the induced effect of IL-17A as detected by a significant decrease in $CD14^{+}LC3\text{-}II^{+}$cells (p<0,05). In contrast, while stimulation with *Mtb* slightly increased ERK phosphorylation in LR TB, the addition of IL-17A did not modify the phosphorylation of this kinase. Together we demonstrate that IL-17A increases autophagy against *Mtb* in Mo through a mechanism that activates ERK in direct association with disease severity.

321 (441) **HELMINTHOCYTOTOXIC ACTIVITY OF INTESTINAL LAMINA PROPRIA CELL SUSPENSIONS DURING EARLY PHASE OF TRICHINELLOSIS.**

Maria Priscila Saracino[1], Cecilia Celeste Vila[1], Guido Hernán Falduto[1], Marcela Adriana Calcagno[1], Emilio Malchiodi[1], Stella Maris Venturiello[1].

[1]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Inmunología-IDEHU (UBA-CONICET), Buenos Aires, Argentina.

Background. Intestine is the organ where the helminth parasite Trichinella spiralis establishes and reproduces. Here we evaluated the intestinal lamina propria cell suspensions (ILPCS) ability to kill T. spiralis migrant (mL) by an antibody-dependent mechanism (ADCC). Non-infected ILPCS ($ILPCS_{NI}$) or 3, 6 and 13 days p.i. ($ILPCS_{I}$) were compared. Relative percentages of leukocyte in ILPCS were analyzed. Methods. In ADCC assays, mL were incubated with $ILPCS_{NI}$ or $ILPCS_{I}$ and anti-mL cytotoxic serum (CS) or non-infected rat sera as positive and negative controls, respectively. Results are expressed as mL mortality percentages. Leukocyte formula in ILPCS was determined by Giemsa staining. Results. 1. $ILPCS_{NI}$ were able to kill mL only in the presence of specific antibodies (n= 5, one-way ANOVA, p<0.0001); 2. $ILPCS_{I}$ presented a cytotoxic activity per se at all the days p.i. studied; 3. Significant differences in mL mortality percentage were observed between 13 and the other days p.i. ILPCS, being higher on day 13 p.i. (n= 5/day p.i., two-way ANOVA, p<0.03); 4. $ILPCS_{I}$ leukocyte formula showed increased percentages of eosinophils and neutrophils than $ILPCS_{NI}$ (n=5/day p.i., two-way ANOVA, p<0.0001). Conclusions. We report, for the first time, that leukocytes from intestinal lamina propria have heminthocytotoxic activity based in a cellular antibody-independent cytotoxic mechanism against T. spiralis NBL.

322 (471) **MONOCYTES FROM CHAGASIC PATIENTS ARE HYPERINFLAMMATORY: EXPRESSION OF ATP METABOLIC MACHINERY IN DIFFERENT MONOCYTE SUBPOPULATIONS.**

Liliana María Sanmarco[1], Laura Marina Visconti[3], Nicolás Eric Ponce[1,2], Natalia Eberhardt[1], María Cecilia Ramello[1], Diego Fernando Elizondo[1], Natalia Beatriz Spitale[3], María Lola Vozza[3], Germán Andrés Bernardi[3], Angel Ramón Minguez[3], Maria Pilar Aoki[1].

[1]Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI)-CONICET. Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba. [2]Instituto de Investigación Médica Mercedes y Martín Ferreyra. Universidad Nacional de Córdoba. [3]Hospital Nuestra Señora de la Misericordia.

Monocytes represent a heterogeneous population of immune effector cells. In humans, at least three different subsets can be distinguished: classical monocytes (CD14++CD16-), intermediate monocytes (CD14++CD16+), and non-classical monocytes (CD14+CD16++). There is poor agreement on functional properties of the three subsets of monocytes. Taking into account that different human inflammatory diseases evidence monocyte subsets perturbation, the aim of the present work was to characterize the frequency and the properties of different monocyte subpopulations in blood samples from seropositive patients with Chagas disease. Chagasic patients showed increased frequency of IL-1β and nitric oxide (NO)-producing monocytes (p<0.05) concomitant with increased serum levels of IL-1bβ and lower levels of anti-inflammatory IL-10 compared to control donors. However, seropositive and seronegative individuals presented similar frequency of reactive oxygen species (ROS)-producing monocytes. Furthermore, monocytes from seropositive patients seemed to have greater ability to hydrolyze the pro-inflammatory ATP, since they exhibited higher CD39 and CD73 expression than monocytes from seronegative individuals. Similar to bacterial and viral infections, the proportions of classical monocytes decreased (p<0.0001), while the frequency of non-classical subset increased (p<0.01) in chronic chagasic patients. Strikingly, non-classical monocytes presented increased NO and ROS production and less IL-10 production than classical monocytes. The decreased production of IL-10 could be correlated with lower expression of CD39 in non-classical monocytes from control donors. Moreover, CD73 expression were higher in non-classical monocytes. Our data suggest that monocytes from chagasic patients exhibited augmented inflammatory properties. Hence, it is not unusual that they might play important roles in promoting inflammation in human diseases.

323 (980) **ROLE OF KIR GENES IN CHRONIC HCV AND ITS RELEVANCE TO THE CLINIC.**

Ariel Hernán Podhorzer[1], Andrés Machicote[1], Melisa Dirchwolf[2], Santiago Belen[1], Silvina Montal[3], Sebastián Marciano[4], Silvia Paz[2], Graciela Theiler[1], Omar Galdame[4], Gustavo Podesta[3], Hugo Fainboim[2], Leonardo Fainboim[1].
[1]INIGEM-Lab Inmunogenética. Hospital de Clinicas. CONICET-UBA [2]Div. Hepatopatias infecciosas. Hospital. F.J.Muñiz [3]Unidad de Cirugía Hepatobiliar y Trasplante. Hospital Universitario Austral, Pilar [4]Hepatología, Hospital Italiano de Buenos Aires.

Patients carrying KIR2DL3 and his ligand in homozygous have better response to an acute HCV infection. However there are few studies of the role of KIRs in chronic HCV infection. We aim to analyze KIR gen frequency and KIR expression on NK ^{bright}, NK^{dim}, from peripheral blood (PB) and from the liver of HCV patients and healthy donors (HD). Expression of KIR receptors were analyzed by flow cytometry on PB of HD (N=63) HCV (N=33). liver perfusion from HD (N=36) and HCV (N=6). KIR, KIR2DS4 alleles and HLA typing was made by PCR-SSOP technique. In comparison with healthy donors, chronic HCV patients showed an increased frequency of the functional form of the gene KIR2DS4 (KIR2DS4fl; 0.52 vs. 0.42,p<0.05). KIR 2DS1 and 3DL2 expression was increased in PB NK^{dim} cells (15.8% vs. 6.4%, p<0.01; 26.2% vs. 16.7%, p<0.01) and KIR3DL1 was decreased (14.7% vs. 20.9%, p<0.05). Those differences were not detected in the liver. Interestingly, NK cells have a "memory like" phenotype (decrease CD45RA). In PB: 82.1% NK^{brigh} vs. 94.4%, p<0.05; 96.7% NK^{dim} vs. 99.4%, p<0.01. In liver: 65.6% NK^{bright} vs. 94.6%, p<0.001; 81.6% NK^{dim} Vs. 92.0%, p<0.01.The cytotoxic potential of liver NK and T cells was increased in HCV patients (NK: 55.6% vs. 20.2%, p<0.05; T-cells: 53.6% vs. 25.0%, p<0.05. The gene frequency and KIR expression showed significant changes according to clinical condition of HCV individuals: levels of TGP and KIR2DS5(p<0.05), KIR3DL2 and HCV genotype III,(p<0.01),High BMI and KIR3DL2 (p<0.01). Association with progression to cirrhosis in KIR3DL1;(p<0.05) and NKG2A (p<0.05), inflammation (CD94 p<0.05 and NKG2C p<0.05); viral load and KIR2DS3 (p<0.05) and KIR2DS4fl p<0.01), and KIR2DS4fl homozygous and cirrhosis p<0.05). In conclusion, NK cells showed several hallmarks associated with HCV, like the presence of memory like NK cells and the increased frequency of KIR2DS4fl. Of clinical relevance, KIRs alteration was found associated with clinical features of HCV patients.

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324 (298) **INNATE IMMUNITY AND CELL DEATH IN EXPERIMENTAL GLIADIN-INDUCED ENTEROPATHY.**

María Florencia Gómez Castro[1], Paula Carasi[2], Romina Araya[1], Allan Mowat[3], Elena Verdu[4], Fernando Chirdo[1].
[1]Instituto de Estudios Inmunológicos y Fisiopatológicos (IIFP-CONICET/UNLP), Buenos Aires, Argentina [2]Cátedra de Microbiología General, Facultad de Ciencias Exactas (UNLP), Buenos Aires, Argentina [3]Centre for Immunobiology, Institute of Infection, Immunity and Inflammation, University of Glasgow, Scotland, UK. [4]Farncombe Institute, McMaster University, Hamilton, Canada.

Several studies have shown that a set of gluten-derived peptides are immunogenic and induce a potent gluten-specific CD4$^+$ T-cell response in the small intestinal mucosa in celiac disease patients. In previous studies, we showed that gliadin peptide, p31-43, which is not presented in MHC molecules, induces intestinal damage and cell death in mice. The receptor for p31-43 has not been identified yet. Our aim was to assess whether the enteropathy caused by p31-43 is sequence specific and also characterize cell death and signaling pathways involved in vivo, using an experimental murine model. Eight-week-old C57Bl/6 and IFNáR$^{-/-}$ mice received intraluminal injections in proximal small intestine of p31-43 or peptides with the same aminoacidic composition but different sequences at 10µg/ml and 100µg/ml, and PBS as control. We evaluated intestinal damage 16h post treatment analyzing villus height /crypt depth (V/C) ratio and number of intraepithelial lymphocytes (IELs) on H&E stained sections of small intestine. We found that inflammation and epitelial damage were sequence-specific, since decreased V/C ratios and increment in the number of IELs were only observed in p31-43 treated mice at both dosis. Based on the same experimental design we evaluate IFNáR$^{-/-}$ mice and found that indeed, damage was dependent on type I IFNs and observed only in p31-43 treated mice. We also found an increase in the Bax/Bcl2 ratio by PCR and increased number of Anexin V$^+$/PI$^+$ cells by flow cytometry in isolated intestinal epithelial cells of p31-43 treated compared with control mice suggesting pro-apoptotic effects of p31-43 in small intestine. In conclusion, using the experimental model of enteropathy developed in our laboratory we demonstrated that gliadin peptide p31-43 cause enteropathy and cell death by innate mechanisms depending on type I IFN. These sequence specific effects may explain part of the initial phase of celiac disease pathogenesis.

325 (601) **CHARACTERIZATION OF THE TH2-SIGNATURE OF COLORECTAL POLYPS IN ATOPIC CHILDREN.**

Karina Eva Canziani[1], Melisa Pucci Molineris[2], Gabriela Nanfito[3], Luciana Guzmán[3], Norma Balcarce[3], David Díaz Jimenez[4], Marcela García[5], María Eugenia Altamirano[6], Eduardo Cueto Rua[3], Marjorie De la Fuente[4], Glauben Landskron[4], Dominik Meier[2], Marcela Ramello Hermoso[4], Carlos A. Fossati[1], Cecilia Muglia[1], Guillermo H. Docena[1].
[1]Instituto de Estudios Inmunológicos y Fisiopatológicos, Argentina. [2]Instituto de Medicina Traslacional, Trasplante y Bioingeniería-IMeTTyB, Universidad Favaloro, Argentina. [3]Hospital de Niños Sor María Ludovica, Servicio de Gastroenterología, Argentina. [4]Universidad de Chile, Facultad de Medicina, Instituto de Ciencias Biomédicas, Programa de Inmunología, Chile. [5]Hospital de Niños Sor María Ludovica, Servicio de Alergia, Argentina. [6]Hospital de Niños Sor María Ludovica, Servicio de Patología, Argentina.

Although colorectal polyps are mainly found in patients with carcinoma, juvenile polyps are frequently found in pediatric patients with no history of tumor disease. The aim of this work was to characterize the cellular infiltrate of single sessile colorectal polyps in children with rectal bleeding suspected to have a food allergy. Children (44) with rectal bleeding were studied during the 2014-2015 period. Colonoscopy was indicated in all patients, and the following studies were carried out in excised polyps and surrounding tissue (control): H&E staining, immunohistochemistry, confocal microscopy, quantitative RT-PCR, cell proliferation and ELISA. Besides, IgE analysis was carried out by skin prick test (SPT) and serology (ELISA). Polyps were found in 18 out of 44 patients (40.9%), who presented a personal and/or family clinical history of atopy (70.8%), with high level of serum milk-specific IgE. SPT was negative in 94.4% (17/18) patients. Unlike surrounding colonic tissue, the polyp stroma showed a cell infiltrate with eosinophils and mononuclear cells, active germinal centers with proliferating and switching cells, IgE-producing plasma cells, significant high levels of Th2 cytokines (IL-5 and IL-13) and eotaxin-3, and low levels of IFN-γ and IL-17. Polyps had milk-specific T cells, TSLP+ cells, ST2+ cells and IL-33-producing cells. In conclusion, we found an allergic inflammatory infiltrate in colorectal polyps of atopic patients with IgE-producing cells, Th2 cells and active germinal centers. These findings suggest a causal relationship between colorectal polyps and the atopic condition of patients.

326 (105) **CHARACTERIZATION OF IL-33/ST2 AXIS IN DUODENAL MUCOSAE OF CELIAC DISEASE PATIENTS.**

Federico Pérez[1], Carolina N. Ruera[1], David Díaz Jiménez[2], Fernando G. Chrido[1], Marcela Hermoso[2], Luciana Guzmán[2], Laura Garbi[4], Glauben Landskron[2], Marjorie de la Fuente[2].
[1]Instituto de Estudios Inmunológicos y Fisiopatológicos IIFP-CCT La Plata-CONICET. [2]Instituto Milenio en Inmunología e Inmunoterapia. [3]Servicio de Gastroenterología Hospital de Niños "Sor María Ludovica" de La Plata, Argentina. [4]Servicio de Gastroenterología HIGA San Martin, La Plata, Argentina.

Our previous studies showed upregulation of IL-33 alarmin and its receptor ST2 in duodenal mucosa of active Celiac Disease (CD) patients. Since these factors are known to play an important role in many different inflammatory diseases, we went further on to characterize which cells express IL-33 and ST2. Duodenal biopsies and blood samples were collected from paediatric and adult patients during the routine procedure for CD diagnosis. Immunofluorescence microscopy analysis was performed on sections of paraffin-embedded tissues. Serum levels of IL-33 were determined by commercial ELISA. We found significant higher levels of IL-33 (p=0,012) in serum samples of CD patients (n=21) than in healthy population (n=9). In the duodenal *lamina propria* of these patients, we found that IL-33$^+$ cells were mesenchymal cells identified by the expression of: Desmin, CD90, Vimentin, a-Smooth Muscle Actin. Moreover, some of those cells seem to be associated with intestinal vasculature, possibly pericytes or endothelial cells. On the other hand, ST2$^+$ cells were mainly CD138$^+$ plasma cells, and a minor population were CD3$^+$ or CD7$^+$ T lymphocytes. We characterized that IL-33 and ST2 expressing cells in lamina propria of CD patients, were mainly mesenchymal cells and B lymphocytes, respectively. In addition to previous results, we demonstrated that not only ST2 soluble receptor was upregulated in serum of active CD patients, but also the alarmin IL-33. The facts that IL-33 is able to stimulate Th1 lymphocytes, cytotoxic cells as well as to promote actions on wound healing process, and also its function as alarmin allows us to connect the IL-33/ST2 axis and CD pathology.

327 (193) **IMPROVEMENT OF MYELOPOIESIS IN CYCLOPHOSPHAMIDE-IMMUNOSUPPRESSED MICE BY ORAL ADMINISTRATION OF VIABLE OR NON-VIABLE LACTOBACILLUS RHAMNOSUS.**

Susana Salva[1], Andrés Gramajo-López[1], Julio Villena[1], Susana Alvarez[1,2].
[1]Laboratory of Immunobiotechnology, CERELA-CONICET. Tucuman, Argentina. [2]Institute of Applied Biochemistry, Tucuman University. Tucuman, Argentina.

Recent research shows that gut microbiota is able to control immunity in distant tissues through regulation of hematopoiesis at primary immune sites as bone marrow (BM). We demonstrated that the dietary supplementation with probiotic lactic acid bacteria (LAB) improves steady-state and emergency granulopoiesis, the respiratory innate immune response and the resistance against respiratory pathogens in immunosupressed hosts. While the viability of the LAB is an important factor to achieve optimal protective effects, it is possible to stimulate immunity using non-viable LAB. The aim of this work was to evaluate whether viable or non-viable immunobiotic *Lactobacillus rhamnosus* CRL1505 (LrV or LrNV) was able to reduce alterations induced by cyclophosphamide (Cy) on BM hematopoietic stem cells (HSCs), myeloid and erythroid multipotent precursors (MMP and EMP), through the balance between apoptosis and cellular roliferation. Adult Swiss-mice were orally treated with LrV or LrNV (10^8 cells/d/mouse) during 5 consecutive days. On day 6, lactobacilli-treated and untreated control mice received one intraperitoneally dose of Cy (150 mg/kg). Cy decreased HSCs (Lin$^-$Sca$^-$1$^+$c-Kit$^+$), MMP (Gr-1$^+$Ly6G$^+$Ly6C$^-$) and EMP (Terr117$^+$), triggered cell apoptosis at 12h and decreased cell proliferation in BM. Lactobacilli treatments were able to significantly promote early recovery of HSCs and MMP. Both LrV and LrNV treatments did not prevent damage by apoptosis of these populations. However, the preventive oral administration of *L. rhamnosus* was able to increase the proliferative capacity of BM cells measured by BrdU incorporation. These findings reveal that *L. rhamnosus* CRL1505 is able to accelerate the recovery of Cy-induced immunosuppression. For the first time we demonstrate that a LAB directs innate immune cell development via promoting myelopoiesis. Non-viable *L. rhamnosus* could be a good and safe resource for reducing chemotherapy-induced immunosuppression in cancer patients.

328 **(676) ISOLATION AND MODULATION OF PATHOGEN-SPECIFIC T CELLS FROM PATIENTS WITH INFLAMMATORY BOWEL DISEASES.**

Renata Curciarello[1], Canziani Karina[1], María de los Ángeles Serradell[2], Martín Rumbo[1], Ayelén Hugo[3], Andrés Rocca[4], Santiago Brayer[4], Alicia Sambuelli[4], Martin Yantorno[5], Gustavo Correa[5], Néstor Chopita[5], Guillermo H. Docena[1], Cecilia I. Muglia[1].
[1]Instituto de Estudios Inmunológicos y Fisiopatológicos (IIFP), CONICET y Universidad Nacional de La Plata, La Plata [2]Cátedra de Microbiología, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina [3]Centro de Investigación y Desarrollo en Tecnología de Alimentos (CIDCA), CONICET y Universidad Nacional de La Plata, La Plata [4]Hospital de Gastroenterolgía Dr Carlos Bonorino Udaondo, Ciudad Autónoma de Buenos Aires [5]Hospital Interzonal General de Agudos General José de San Martín, La Plata.

Inflammatory bowel diseases (IBD) are a complex group of pathologies amongst which ulcerative colitis (UC) and Crohn´s disease (CD) are the most conspicuous. Lamina propria T cells (LPTC) are key cells in IBD pathogenesis, contributing to mucosal inflammation by secreting pro-inflammatory cytokines. It has been shown that LPTC in IBD are resistant to apoptosis. Kefir is a fermented milk that has been used in Eastern Europe with health-promoting properties. The aim of this work was to isolate and establish primary cultures of LPTC from IBD patients and modulate their pro-inflammatory response using microorganisms from kefir.Colonic biopsies from IBD patients (N=8, 5 CD and 3 UC) were washed in HBSS medium with EDTA and DTT, and digested by collagenase and DNAse treatment. In order to select pathogen-specific T lymphocytes, cells were cultured with extracts of enteroadhesive (EA) *Escherichia coli* and IL-2 for 10 days. Microorganisms from kefir (*Lactobacillus kefiri* and *Enterococcus durans*) were evaluated for their modulation capacity on LPTC by proliferation assays (flow cytometry with CFSE) and cytokine secretion (TNF and IL-10 by ELISA) of T cell lines stimulated with anti-CD3 and anti-CD28 followed by culture with probiotic bacteria. LPTC lines specific for EA *E. coli* were developed for all patients. Cell proliferation of activated lymphocytes decreased with the presence of *L. kefiri* and *E. durans* (proliferation index: 3.0±0.54 vs 1.3±0.33 and 0.56±0.28 respectively; unstimulated control: 1.02±0.03). Besides, TNF secretion was dampened in activated LPTC incubated with *L. kefiri* compared to medium (P□0.05). No significant differences were observed for IL-10 secretion. Our results show that probiotics strains from kefir modulate pathogen specific activated T cells form IBD patients. These promising results could provide the basis for future therapies in IBD patients.

329 (693) **REGULATORY IMMUNE MECHANISM MEDIATED BY PROBIOTICS TO CONTROL ALLERGY DEVELOPMENT**.

Eva María del Mar Velez[1], Carolina Maldonado Galdeano[1,2], Ivanna Novotny Nuñez[3], Silvia Correa[3], Gabriela Perdigon[1,2].
[1]CERELA-CONICET, Tucumán [2]Cat. De Inmunología, Fac. De Bqca, Qca. Y Fcia, UNT [3]CIBICI-CONICET, Córdoba.

In previous work we found in a mouse respiratory allergy model to ovoalbumin (OVA), that the intake of a probiotic fermented milk (PFM) lowers specific IgE and increases IgG (mainly IgG2a); also influences IL-10 and IFN-γ production in lungs. Our aim was to establish if the origin of the regulation observed was due to regulatory Tcells (Treg) or induction of Th1 balance. Adult BALB/c mice were split into 5 groups: Normal-Control(NC), Basal(B=5days-PFM), OVA-Sensitization-Control(SC), Previous (PT=5d-PFM+OVA+H_2O) and Continuous feeding(CT=5d-PFM+OVA+PFM); SC, PT and CT were sensitized with 3 OVA injections and 7 days with OVA aerosols exposure. At 7 and 15 days-post-sensitization (dPS) and 2 days post-re-stimulus (dPR) mice were sacrificed; lymphocytes were purified from lungs to analyze CD4/CD25/Foxp3 markers for Treg cells by flow cytometry. Also, lungs tissue was reserved for confocal microscopy to study co-expression of CD4 Tcells with IL-10 and IFN-γ and for IL-12 production in lungs supernatant by ELISA. Treg cells were significantly reduced in the 3 sensitized groups in comparison with NC and B ($p \leq 0.05$) in all the studied

times. Confocal microscopy studies showed that $CD4^+$/IL-10^+ cells in lungs were increased in SC and CT groups compared to NC; $CD4^+$/IFN-γ^+ cells were increased in those animals with continuous intake of PFM at 7dPS compared to NC and SC. IL-12 was increased in CT in all the studied times. We confirm that the regulatory mechanism to control allergy development was due to the induction of a Th1 balance instead of Treg cells but without exacerbating Th1 response. IL-12 levels activate Th1 cells to produce IFN-γ to inhibit IL-4 (Th2). These findings are relevant and they show how a PFM can help treating or preventing allergies and contributing to the nutritional state.

330 (709) **BIOLOGICAL RHYTHMS IN THE REGULATORY/ TOLEROGENIC FUNCTIONS OF THE IMMUNE ACTIVITY IN THE GUT**.

Bibiana E. Barrios[1], Ivanna Novotny Nuñez[1], Lisa Maccio Maretto[1], Silvia G. Correa[1].
[1]CIBICI-CONICET-Clinical Biochemistry Department. Faculty of Chemical Sciences. National University of Córdoba. Córdoba- Argentina.

In the gut, the circadian clock orchestrates temporal organization of many aspects of physiology including immunity. As intestinal environment modulates the phenotype and function of immune cells, we wonder if over a 24h period there are variations in the activation or function of cells that could affect tolerance. We evaluated activation status of CD4+ and CD8+ lymphocytes and numbers of Foxp3+ regulatory T cells (Foxp3+ Treg) in mesenteric lymph nodes (MLN) from undisturbed C57BL/6 mice at 6, 12, 18 and 24h CT. We found that both CD4+ and CD8+ T cells bearing the markers CD62L+CD69+ oscillated in 24h period time with maximum at 6/18h and minimum at 12h CT ($p<0.05$); similar observations were found with CD4+CD62L+CD69- cells. We also found a significant increment in the absolute number of Foxp3+ Treg cells at 18h with minimum at 6h ($p<0.05$) and an overall increase in the frequency of this subpopulation in the light phase of the cycle (12 and 18 h). Since intestinal environment also modulates the activity of dendritic cells (DC), we examined the ability of DC isolated from afferent lymphatics at 6, 12, 18 and 24h CT to induce Foxp3+ Tregs and the expression of gut homing molecules, $\alpha4\beta7$ integrin and CCL25 receptor chemokine, CCR9. When co-cultured with MLN CD4+Foxp3- cells sorted from GFP-Foxp3 mice, DC isolated at 18h showed higher ability to induce in CD4+ cells the expression of Foxp3 as well as $\alpha4\beta7$ and CCR9 homing receptors. These results show that the activation of T cells fluctuate along the dark and ligh cycle phases. These variations can be explained by changes in both the potential of tolerogenic DC and the frequency of Foxp3+ Treg cells. Together these circadian oscillations could contribute to greater plasticity in the balance immunity / tolerance in intestinal mucosa.

331 (795) **LTC4 MODULATES AN INFLAMMATORY RESPONSE AT THE GUT LEVEL VIA THE ACTIVATION OF CYSTEINYL LEUKOTRIENE RECEPTOR 1**.

Julieta Alcain[1], Soledad Gori[1], Gabriela Salamone[1], Silvia Vanzulli[2], Gabriela Mora[3], Mónica Vermeulen[1].
[1]Laboratorio de Células Presentadoras de Antígeno y Respuesta Inflamatoria, IMEX-CONICET, Academia Nacional de Medicina. [2]Centro de Estudios Oncológicos (CEO), Academia Nacional de Medicina. [3]Hospital Dr. Cosme Argerich, Laboratorio de Alergia e Inmunología.

Leukotriene C4 (LTC4) is an important lipid mediator involved in the genesis of chronic inflammatory responses. Its effects are mediated through the cysteinyl receptor 1 and 2 (CysLTR1/2). In previous works, it has been demonstrated that LTC4 is able to modulate chemotaxis of dendritic cells (DC) and their activation state, with the ability to interfere with their maturation. Here, we studied the capacity of LTC4 to break the gut tolerance. For this, BALB/c mice were orally inoculated with a single dose of PBS (Ct) or LTC4 (0.01 µM in 100µl of PBS) at the beginning of Ovalbumin tolerance (100 mg/day in drinking water for 5 days). After 12 days, histological examination of small intestine showed that the intestine was extensively modified by the exposure to LTC4, mainly evidenced through the shortening of villi, increased protrusion of goblet cells and edema in the lamina propria, together with a marked recruitment of leukocytes, mainly eosinophilic-granulocytes. Then, we decided to evaluate the populations recruited in mesenteric lymph node after treatment. In the first place, LTC4 inhibited the $CD11c^+$ $CD103^+$ dendritic cells (DC), the main population associated to mucosal tolerance (mean% $CD11c^+CD103^+$ DC ± SEM: PBS 2.1 ± 0.25, LTC4 0.92 ± 0.1, $p \leq 0.05$, n = 4). Also, we observed a greater expression of CysLTR1compared to CysLTR2 (mean band ratio/b-actin; CysLTR1 vs CysLTR2: 1.2 ± 0.08; 0.4 ± 0.03; * $p<0.05$, n=4) in the small intestine of mice which was not affected by the induction of tolerance. Finally, LTC4 was able to induce the production of $CD4^+$IL-17^+ lymphocytes in the lymph nodes of tolerated mice. Interestingly, the use of montelukast, an antagonist of CysLTR1, was able to reverse the induction of Th17 profile (mean% $CD4^+$IL-17^+ cells ± SEM: PBS, 0.5 ± 0.04; LTC4, 13 ± 1.34; MK 0.6 ± 0.05) along with the recruitment of neutrophils, however it had no significant effect on gut morphology. In conclusion, LTC4 modulates gut homeostasis partially via CysLTR1.

332 (64) **CHARACTERIZATION OF IL10 AND IL17 SECRETING B CELLS IN HUMAN TONSILS.**

Lindybeth Sarmiento[1], Ariel Billordo[1], Plácida Baz[1], Andrés Blanco[1], Pablo Fernández[1], Eloísa Arana[1].
[1]INIGEM (UBA-CONICET) Instituto Otorrinolaringológico Arauz (IORL).

Tonsils are secondary lymphoid organs which are mostly B-cell (Bc) maturation and differentiation sites. They show similarities with lymph nodes and could participate as effector organs of local systemic type as well as inductive sites of mucosal immunity. Their immune actions must be tightly regulated to balance the protection against virulent germs and the tolerance to harmless flora and innocuous Ags in food and air. Despite the logic of such presumption, and the relevance of the issue in light of the importance gained by the mucosal route of vaccine administration, the potential role of tonsils in oral tolerance induction has not been completely elucidated so far. As it is conceivable that alteration of the immune equilibrium activation/regulation may trigger recurrent tonsillitis and hypertrophy leading in turn to tonsillectomy, the aim of our work was to test the ability of tonsillar Bc to secrete IL10 (regulatory cytokine) and IL17 (pro-inflammatory cytokine). The frequency of tonsillar IL10 secreting Bc (8,9%±3% of total Bc, B10) and IL17 secreting Bc (11,9%±5% of total Bc, B17) was determined by FACS upon 72 hs stimulation *ex vivo,* via TLR9 and CD40L. Also, we demonstrated that IL10 secreting B cells were enriched within both the IgM memory ($CD19^+CD24^{high}CD27^+CD38^{+low}$) and the transitional T3 ($CD19^+CD24^{int}CD27^-CD38^+$) B-cell subsets, in agreement with previous reports on Bc isolated from PBMC. Interestingly, we found co-expression of IL10 and IL17 following the indicated stimulation and alternative ones (*i.e* IL2+IL4, different time points). On the other hand, we did not detect any co-expression pattern with other pro-inflammatory cytokines (IL6). Finally, we established a negative correlation between the percentage of B10 and B17 populations and the fraction of proliferating $CD3^+$ (autologous) in co-culture. Importantly, we postulate that these findings might apply to other mucosal human Bc, as in gut.

333 (610) **SYSTEMIC IMMUNE RESPONSE AFTER ORAL PROBIOTICS ADMINISTRATION AGAINST SALMONELLA TYPHIMURIUM**

José María Lemme Dumit[1,2], Silvia Inés Cazorla[1], Gabriela Perdigón[1,2], Carolina Maldonado Galdeano[1,2].
[1]Laboratorio de Inmunología, Centro de Referencia para Lactobacilos (CERELA-CONICET). [2]Cátedra de Inmunología, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán.

Lactobacillus casei CRL431, *Lactobacillus paracasei* CNCM I-1518, and their cell walls (CW431 and CW1518, respectively) improves innate immune response and exert an immunomodulatory effect in healthy mice. The aim of the current study was to

determine whether CRL431, CW431, CNCMI-1518, and CW1518 act as a potential mucosal adjuvant of *Salmonella* Typhimurium attenuated by heat-shock (HSST), and would be able to protect healthy mice against a lethal oral challenge with *Salmonella*.

BALB/c mice were supplemented 7d with probiotics, and intragastric sensitization with HSST was performed during 3 spaced days. After 3 weeks one group was challenged with 100 µL of *Salmonella* (10^9 CFU/mL) (*S*G), another group received probiotics supplementation again during 7d, after that, mice were sensitized with once dose of HSST, and 7d later werechallenged with *Salmonella* (rs*S*G). Control groups (CG and rsCG, respectively) received the same treatment without the pathogen challenged. Translocation of microorganisms was evaluated. The immune response was determined by *in vivo* and *in vitro* assays.Probiotic supplementation, did not show increase of the CFU/g of organs compared with infection groups ($p<0.05$). Delayed-type hypersensitivity test did not show differences in *S*G and rs*S*G compared to control groups. Increased levels of IFN-γ ($p<0.001$), and decreased levels of TNF-α ($p<0.001$), IL-10 ($p<0.001$), and IL-6 ($p<0.05$) were found in *S*G compared to CG. Serum anti-*Salmonella* IgG titers did not show increase compared to infection group ($p<0.05$). Total and specific SIgA did not increase in *S*G compared to CG ($p<0.05$). All test groups showed an increase in the opsonophagocytic uptake of macrophages compared to non-immune sera ($p<0.05$).

Our results showed that oral probiotics administration was not able to induce a systemic immune response to *Salmonella.* However, these findings reinforce the important effect that probiotics have over the innate immune response and their immunoregulatory roles.

334 (623) **NASAL PRIMING WITH PEPTYDOGLICAN OF PROBIOTIC STRAINS CAN IMPROVE THE ADAPTIVE IMMUNE RESPONSE IN MALNOURISHED MICE**

Yanina Noralí Kolling[1], Susana Salva[1], Julio Villena[1], Susana Alvarez[1,2].
[1]*Laboratory of Immunobiotechnology, Reference Centre for Lactobacilli (CERELA-CONICET). Tucuman, Argentina.* [2]*Institute of Applied Biochemistry, Tucuman University. Tucuman, Argentina.*

Lactobacillus rhamnosus CRL1505 peptidoglycan (Pg05) increases the infection resistance against *Streptococcus pneumoniae* (Sp) and improves innate immune response in immunocompromised-malnourished mice. The aim of this work was the comparative study of the effect of three peptidoglycans on the behavior of cellular and humoral components of adaptive immune response against Sp. In addition to Pg05, peptidoglycans from probiotic *L. plantarum* CRL1506 (Pg06) and the non-immunomodulatory control *L. rhamnosus* CRL534 (Pg534) were evaluated.Weaned mice were malnourished with a protein-free diet for 21d and then received a balanced diet during 7d (BD group) or BD with nasal supplementation of Pg05, Pg06 or Pg534 during the last 2d. On d8, all groups were infected with Sp. The T and B cell populations and the production of Th1/Th2 cytokines and specific antibodies were studied. After infection, the number of thymus T cells ($CD4^-CD8^-$, $CD4^-CD25^+$, $CD4^+CD8^+$) increased in the BD group, but the values of the Pg05 group were higher than those of BD group. In the spleens and the lungs, $CD3^+CD4^+$ and $CD3^+CD8^+$ cells augmented, but only Pg05 induced a bigger increase than the BD groups. The Pg05 showed a rise in the values of Th1/Th2 cytokines in broncho-alveolar lavages (BAL) and serum at 10dpi compared with the BD group. The Pg05 group showed an increase of pro-pre B and immature B cells in bone marrow as well as mature B cells in spleen and lung. Moreover, the Pg05 mice had higher levels of BAL and serum anti-pneumococcal antibodies and improved opsonophagocytotic activity than the others groups. These particular findings demonstrate that immunomodulatory effect of the Pg05 is a strain specific property and opens interesting questions regarding the molecular differences between peptidoglycans. Cellular fractions could be an interesting alternative as mucosal adjuvants, especially in immunocompromised hosts in which the use of live bacteria might be unsafe.

335 (707) **EFFECT OF YOGURT WITH OR WITHOUT PROBIOTIC ADDITION ON BODY COMPOSITION CHANGES AND IMMUNE SYSTEM IN AN OBESE MODEL.**

María Florencia Balcells[1], Gabriela Perdigón[1,2], Carolina Maldonado Galdeano[1,2].
[1]*Centro de Referencia para Lactobacilos, CERELA-CONICET, Tucumán, Argentina* [2]*Cátedra De Inmunología, Instituto De Microbiología, Facultad de Bioquíca, Química Y Farmacia, Universidad Nacional De Tucumán.*

Aim: to study the effect of feeding with conventional yogurt or yogurt probiotic added on body weight, biochemical parameters, improvement on the intestinal immune system in an obese model. Males adults BALB/c mice were divided into 2 groups: Normal Control (NC) that received a conventional balanced diet and water ad libitum, Obese Control (OC) fed with a high-fat diet. The OC group was divided in sub-groups according to the dietary supplement administered: low-fat milk (OM), conventional yogurt (OY) and probiotic yogurt (OP) for 8 weeks. Body weight was determined 3 times per week. Animals were sacrificed at 30 and 60 days (30d and 60d) and samples were (serum, small intestinal fluid, large intestine(I), liver and spleen), It was determined glucose(GLU), total cholesterol(TC), HDL, triglycerides(TG), IgA+ cells, IL10, INFγ, IL6, IgA, intestinal microbiota and translocation. Results showed that OC and OM reached peak body weight at 4 weeks, while OY and OP after 6 week. Difference in GLU levels were obtained at 30d between NC and OC. TC and HDL were lowering in OY and OP compared to OC and OM at 30d. TG was increased at 30d in NC, OC and OY decreasing at 60d. Enterobacteria population showed significantly increases in OM at 30 and 60d. No change was observed in the translocation of enterobacteria and lactobacilli in spleen. In liver lactobacilli traslocation increased significantly in OM and OY at 30d. Enterobacteria traslocation increase in OM in time assayed. IgA + cells, total IgA and INFγ increased in OY and OP at 30 and 60d being more significant at 60d. IL6 significantly decreased in OC compared with NC at 30 and 60d and the yogurt supplementation normalized IL6 levels. IL10 was significantly decreased in OC and increase with yogurt supplementation. These results show the beneficial effect of yogurt and probiotic yogurt on the intestinal immune response and improvement of the intestinal barrier in obesity, one of the most important chronic diseases.

336 (728) **CELLULAR AND MOLECULAR EVENTOS INVOLVED IN THE ANTI-TUMORAL EFFECT OF LIPOTEICHOIC ACID FROM LACTOBACILLUS RHAMNOSUS GG.**

Adrián Friedrich[1], Valeria Campo[1], Paula Manuelli[1], Juliana Leoni[1], Mariela Laura Paz[1], Daniel González Maglio[1].
[1]*Instituto de Estudios de la Inmunidad Humoral (IDEHU-CONICET-UBA).*

In previous reports our lab has proven the anti-tumoral effect of orally administered lipoteichoic acid (LTA) from *Lactobacillus rhamnosus* GG (LGG) on an Ultraviolet B (UVB)-induced non-melanoma skin cancer mouse model. The aim of the present work was to evaluate underlying mechanisms involved in this anti-tumoral effect. We tested the modulatory capacity of orally administered LTA regarded to gene transcription in Peyer's patches (PP), small intestine free from PP (SI) and mesenteric lymph nodes (MLN) by PCR. After a single dose of 100 µg LTA the transcription of TNF-α, IL-10 and IL-1β in SI and IFN-γ in PP was increased at 16hs. At 24 hs we observed a significant increase in TNF-α and IL-12p35 transcription in PP. Finally, at 48 hs a significant increase in IFN-γ transcription was observed in MLN. In order to evaluate the capacity of orally administered LTA to revert UVB-induced immunosuppression, we performed a hypersensitivity assay to oxazolone after a 12 dose treatment in irradiated and control mice. We observed that UVB-irradiated and LTA-treated mice showed a partial reversion of immunosuppression after oxazolone challenge compared with irradiated and PBS-treated ones. At last, in order to evaluate the anti-tumoral capacity of LTA in an UV-independent model, we tested a multiple doses schedule treatment in animals that already had developed skin tumors but were no longer exposed to UVB irradiation. Animals with visible and multiple skin tumors were administered

with LTA by gastric gavage every other day. We demonstrated that LTA is capable to significantly reduce the tumor number and total tumor area after 4 weeks of treatment and that this reduction was abolished by the suspension of the treatment. In conclusion, LTA from LGG has proven to produce several *in vivo* effects after one or multiple oral administrations. This could be useful to a better understanding of the interactions between molecules isolated from probiotic bacteria and host immune response.

337 (768) **ORAL ADMINISTRATION OF PROBIOTICS INCREASE ANTIMICROBIAL PEPTIDES PRODUCTION BY PANETH CELLS.**

Silvia Inés Cazorla[1], José María Lemme Dumit[1], Gabriela Perdigon[1], Carolina Maldonado Galdeano[1].
[1]Laboratorio de Inmunología, Centro de Referencia para Lactobacilos (CERELA-CONICET) [2]Cátedra de Inmunología, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán.

The huge amount of intestinal bacteria represents a continuing threat to the intestinal barrier. In order to meet this challenge, gut epithelial produce a lot of antimicrobial peptides (AMP) that act at the forefront of innate immunity. The aim of this work was to explore whether AMP production by Paneth cells is influenced by probiotics administration, in order to avoid the imbalance of intestinal microbiota and preserve intestinal barrier. Groups of 45 days old BALB/c mice received *Lactobacillus casei* CRL 431 (LC 431), *Lactobacillus casei* CNCM I-1518 (LC 1518) or water upon 7 and 5 days, respectively. At the end of this time mice were sacrificed and samples of intestinal fluids, small and large intestine sections were taken. In mice fed with the probiotics bacteria, an increase in the number of Paneth cells were observed after hematoxylin and eosin stain of small intestine. The increase in the microbicidal activity against the pathogens *S. aureus* and *S. thyphimurium* by these intestinal fluids was observed respect to the group that received conventional diet ($p<0.05$), after plating dilutions of the incubation mixtures and determinate the number of CFU the next day. Interestingly, a slightly increase in the microbicidal activity was observed against the probiotics bacteria. These results were in parallel with those observed by transmission electron microscopy. Strong damage of the bacterial cell with leakage of cytoplasmic content, and cellular fragmentation were observed in *Salmonella* and *S. aureus* after exposing to intestinal fluids of animals fed with probiotics. Finally, we detected a slightly higher amount of anaerobes and lactobacillus bacteria, as part of the colon microbiota in LC 431 and LC 1518 animals compared to mice with a conventional diet. These results support that probiotcs modulate antimicrobial peptides production by Paneth cells to preserved epithelial barrier. This will be other mechanism by which probiotic protects against infectious diseases.

338 (2068) **ASSESSMENT OF THE MECHANISMS INVOLVED IN HOST ADAPTATION OF ENTEROHEMORRAGIC ESCHERICHIA COLI (EHEC).**

Romina Fernandez-Brando[1], Martín Gómez[1], Andrea Brubalia[1], Gonzalo Pineda[1], María Victoria Ramos[1], Cristina Ibarra[3], David Gally[2], Marina Palermo[1].
[1]Laboratorio de Patogénesis e Inmunología de Procesos Infecciosos, Instituto de Medicina Experimental (IMEX)-CONICET, Academia Nacional de Medicina [2]Division of Infection and Immunity, The Roslin Institute, University of Edinburgh, [3]Laboratorio de Fisiopatogenia, Facultad de Medicina, UBA, Buenos Aires, Argentina.

Although the production of Shiga toxin by enterohemorragic *Escherichia coli* (EHEC) determines Hemolytic Uremic Syndrome (HUS) onset, factors that modulate intestinal colonization are key components in pathogenesis and host mucosal immune response. We showed previously that the passage of a clinically isolated EHEC strain (125/99) through the gastrointestinal tract of mice increases its pathogenicity in mice, and that stool-recovered strains (125R and 125RR) induce a more generalized and persistent colonization than the parent strain (Fernandez-Brando et al, 2012). We aimed at elucidating the underlying mechanism involved in the pathogenesis and bacterial adaptation to the intestinal environment of mice. We assessed the global transcription profile by microarray and found more than 100 differentially expressed genes in 125RR strain: small RNAs (sRNA), proteins from the type three secretion system, several enzymes, membrane transporters and receptors and several putative transcripts. We confirmed the augmented expression of EspB and fliC ($p<0.05$) and the diminished expression of ECs1537/1561 ($p<0.05$) by qPCR. We also demonstrated the augmented expression of EspD by western blot, which could explain the greater colonization of stool-recovered strains. In an attempt to elucidate targets for sRNA regulation we studied acid resistance mechanisms, since arcZ, rprA, and ryhB are involved in that mechanism. The 125RR strain showed an increased survival at pH 2.5 for 1 h ($p<0.05$), which could determine a lower infectious dose. Given the importance of motility in surpassing the mucus barrier in the mucosal environment and the finding of the augmented expression of flic, we tested the motility phenotype in semisolid agar. The 125RR strain showed an increased motility compared to 125/99 and 125R ($p<0.01$). These results suggest that the stool-recovered strain is more proficient to deal with the murine mucosal barrier thus leading to the onset of HUS characteristic symptoms in mice.

339 (396) **ASSESSMENT OF THE IGA AND IGG RESPONSE AGAINST COMMENSAL MICROBIOTA DURING THE DEVELOPMENT OF COLITIS INDUCED BY DEXTRAN SULFATE SODIUM IN MICE.**

Lisa Maccio Maretto[1], Ivanna Novotny Nuñez[1], Bibiana E Barrios[1], Silvia G. Correa[1].
[1]CIBICI-CONICET. Departamento de Bioquímica Clínica. Facultad de Ciencias Químicas. Universidad Nacional de Córdoba. Córdoba. Argentina.

Intestinal macrophages (MΦs) represent the most abundant population of resident phagocytic cells in the gut. They can be identified by the expression of either CD11b, F4/80 or CD64 markers and play an important role in the intestinal immunity. Recently, it has been shown that IgG against commensal microbiota may contribute to the intestinal homeostasis. Herein, we evaluated the canonical IgA response as well as the production of IgG to luminal microorganisms in untreated WT mice (control group) or after 5 days of administrating dextran sulfate sodium (colitis group). During the 7 day-period of colitis development we collected feces from both groups and we analyzed IgG+ and IgA+ bacteria by flow cytometry. On day 7 of the colitis we isolated mononuclear cells from lamina propria by mild collagenase digestion and we evaluated a) frequency of CD138+IgG+ plasmatic cells and b) uptake of immune complexes (IgG-Ag) in CD11b+ F4/80+ permeabilized MΦs. Compared with controls the percentage of IgA-coated bacteria did not vary in mice with colitis while the percentage of IgG-coated microorganisms was significantly lower with the ongoing inflammation ($p<0.01$). Interestingly, the frequency of bacteria coated with both antibodies increased markedly already on day 2 and this increment persisted on days 4 and 5 ($p<0.05$), when the barrier permeability is critically affected. The frequency of CD138+IgG+ plasmatic cells in lamina propria of WT and colitis groups was similar while a reduction in the frequency of CD11b+ MΦs with intracellular IgG was observed ($p=0.027$); the intracellular IgG in CD11b+ MΦs evaluated as MFI was also reduced by the intestinal inflammation ($p=0.012$). These results show that CD138+ IgG+ are constitutively located in lamina propia and that their frequency is not modified in colitis. Fast changes in the proportion of IgG in the lumen and immune complexes in phagocytic CD11b+ cells may become sensitive markers of the ongoing acute intestinal inflammation.

PRESENTACION DE POSTERS SAIC III / SAIC POSTER PRESENTATION III
NEUROCIENCIAS II/ NEUROSCIENCES II

340 (84) INVOLVEMENT OF AUTOPHAGIC AND LYSOSOMAL PATHWAYS UNDER MANGANESE-INDUCED OXIDATIVE STRESS IN MICROGLIAL CELLS

Soledad Porte Alcon[1], Roxana Mayra Gorojod[1], Agustina Alaimo[1], Monica Lidia Kotler[1].
[1]Lab. Disfunción en Enfermedades Neurodegenerativas y Nano-Medicina, Dpto de Qca. Biológica-FCEyN-UBA, IQUIBICEN-CONICET.

Manganese(Mn) overexposure causes a neurodegenerative disease known as Manganism. It has been reported that Mn activates the microglia triggering the production ofinflammatory mediators (ROS/RNS and TNF-α)which could promote neuronal death. In this context, the proper elimination of dead cells by microglia is crucial. This phagocytic activity depends on the lysosomal compartment where internalized material is digested. However, under oxidative stress conditions lysosomes are severely affected. As a consequence, the hydrolytic enzymes are released into the cytosol triggering several celldeath pathways. We aim to study the role of lysosomes in Mn-induced microglialcell death. Also, the possible activation of the autophagic pathway wasassessed. We previously demonstrated that Mn (250 and 750μM; 24h) generates oxidative stress in murine BV2 microglial cells. In the present work lysosomes amount and size were analyzed by Lysotracker Red DND-99 staining (fluorescence microscopy and Flow Cytometry). Results indicated that Mn increases the number of these acidic vesicles (250μM: 147%, $p<0.001$; 750μM: 139%, $p<0.001$) as well as the cellular complexity (250μM: 26%, $p<0.05$; 750μM: 45%, $p<0.05$) without affecting their size. Next, we studied the involvement of lysosomal cathepsins in Mn-induced cell death. Both cathepsin B (Ca-074 Me) and D (Pepstatin A)inhibitors prevented the cytotoxic effect of 250μM Mn (8.7% and 8.8%,respectively, $p<0.001$). Moreover, we analyzed the autophagy-related proteins expression by immunoblotting. Mn induced an increment in LC3-II and p62 expression levels (250μM: 1.25 and 3 fold; 750μM Mn: 2 and 7 fold, respectively). Our findings suggest that Mn causes lysosomal alterations and autophagic pathway activation in BV2 microglial cells.

341 (**160) HISTONE DEACETYLASE SIRT6 PARTICIPATES IN EARLY NEURODEGENERATIVE EVENTS IN DIABETIC RETINOPATHY**

Emilia Soledad Bogni[1], Ada Yeste[2], Francisco J Quintana[2], Raul Mostoslavsky[3], Dafne Magali Silberman[1].
[1]Centro de Estudios Farmacológicos y Botánicos (CEFYBO-CONICET), 1ª Cátedra de Farmacología, Facultad de Medicina, UBA, Buenos Aires, Argentina. [2]Brigham and Women's Hospital, Harvard Medical School, Boston, USA. [3]The Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, USA

Diabetic retinopathy(DR) is the leading cause of blindness worldwide. Recent research has demonstrated that DR is not only a microvascular disease but may be a result of neurodegenerative processes. Current investigation points to the role of histone acetyl transferases and deacetylasesas regulators of key genes linked to diabetes. SIRT6, a NAD-dependentdeacylase, modulates aging, cancer, energy metabolism, neurodegeneration and DNA repair. In previous studies we showed that SIRT6 deficiency causes major retinal transmission defects related to changes in expression of glycolytic genes and glutamate receptors, as well as elevated levels of apoptosis in inner retina cells. Given the importance of glucose availability for retina function and the critical role for SIRT6 in modulating glycolysis, the main goal of this work was to analyze SIRT6 involvement in the molecular machinery that regulates the development of experimental DR. Using the non-obese diabetic (NOD) mouse strain that spontaneously develops type 1 Diabetes, we determined by Western blot and RT-PCR that high glucose concentrations (>200mg/dl) induced increased VEGF ($p<0,01$) and decreased BDNF retinal levels ($p<0,01$).Moreover, FOXO1 levels, known for modulating gluconeogenesis, were increased in retinas from NOD diabetic vs. control mice. Additionally, significantly lower SIRT6 levels ($p<0,05$) associated with increased H3K56acetylation levels ($p<0,01$) were shown in retinas from NOD diabetic mice compared to controls. Interestingly, retinas from SIRT6-KO mice showed are semblance to diabetic retinas exhibiting lower levels of BDNF and increased levels of VEGF and FOXO1. Noteworthy, immunofluorescence studies showed that GFAP levels were increased in NOD diabetic but not in SIRT6-KOretinas. Our results suggest that neurodegenerative events regulated epigenetically may occur early during diabetes development preceding proliferative, vascular and histological changes observed later in a diabetic retina.

342 (211) **PRE-EXPOSURE TO DIAZEPAM ADMINISTRATION CONTEX DURING CHRONIC TREATMENT PREVENTS WITHDRAWAL EXPRESSION: CORRELATION WITH LOW NNOS LEVELS IN HIPPOCAMPUS.**

Emilce Artur de la Villarmois[1], Mariela Fernanda Perez[1].
[1]IFEC-CONICET; Departamento de Farmacología, Facultad de Ciencias Químicas, UNC.

Benzodiazepines are commonly prescribed for anxiety and sleep disorders. However, prolonged administration may lead to dependence with evident withdrawal syndrome. We previously demonstrated that a hippocampal(HP)-dependent associative learning process underlie diazepam (DZ) dependence. Also, nitric oxide (NO) participates in DZ dependence; and it is synthesized by neuronal NO synthase (nNOS) upon activation of glutamate NMDA receptors, which are crucial players in HP synaptic plasticity. The aim of the present work is to develop a therapeutic strategy to prevent withdrawal expression by interfering with the learning process underlying DZ dependence, and to reveal possible HP NO-dependent mechanisms underlying DZ dependence. For this purpose we administered DZ (5mg/kg/day i.p) to male wistar rats along 18 days, the last 5days they were pre-exposed to DZ administration context and 48 hs after the last DZ administration animals were evaluated in the plus-maze test to evidence an "anxiety-like behavior" as a sign of the withdrawal syndrome. Animals where then sacrificed for assessment of HP synaptic plasticity and nNOS expression by extracellular multi-unitary recordings and western blot respectively. Our results show that animals without pre-exposure expressed anxiety, evidenced an increased HP plasticity (measured as a lower threshold to generate LTP) and HP NOS-1 expression comparable to control group. Contrary, animals pre-exposed to the DZ administration context did not show anxiety or enhanced HP synaptic transmission, but a significant reduction in nNOS expression was observed when compared to controls. In conclusion, we can hypothesize that manipulation of the contextual cues presented during DZ administration may be considered an effective non-pharmacological tool to prevent the withdrawal syndrome. The mechanisms underlying this effect could be related to reduction in HP glutamate transmission, principally associated to reduced NO levels.

343 (318) **EFFECTS OF SELECTIVE ESTROGEN RECEPTOR AGONISTS ON ALTERED HYPOTHALAMIC PARAMETERS OF SPONTANEOUSLY HYPERTENSIVE RATS.**

Julieta Correa[1], Maria Elvira Brocca[1], Florencia Labombarda[1,2], Paulina Roig[1], Alejandro F. De Nicola[1,2], Luciana Pietranera[1,2].
[1]Instituto de Biología y Medicina Experimental. CONICET. [2]Depto. de Bioquímica Humana. Fac. de Medicina. UBA

Hippocampal neuropathology is a recognized feature of the spontaneously hypertensive rat(SHR). Previous studies have found that SHR present abnormalities in the hippocampus consisting of decreased cell proliferation in the dentate gyrus(DG), astroglial reactivity and decreased neuronal density in the hilus of theDG. Hypertensive rats also present a hyperfunction of hypothalamic neuropeptides, as shown by increased expression of the vasoactive neuropeptide arginine vasopressin (AVP) in the paraventricular nucleus (PVN) and hyperresponse of AVP following mineralocorticoid treatment. Additionally, the SHR shows increased expression of the mineralocorticoid receptor (MR) both in the hippocampus and the PVN. These abnormalities are reversed by exogenous administration of estradiol, anactive neuroprotective and hypotensive factor. Two types of estradiol receptors(ER) are expressed in the hypothalamus. To elucidate which ER subtype is involved in the

hypothalamic effects, we used agonists of the ER (propylpyrazole triol, PPT) and the ER (diarylpropionitrite, DPN) given s.c. during 2 weeks at 2.5 mg/kg every other day to 4 month old male SHR. We measured the expression of AVP mRNA in the hypothalamus using real time PCR. We found that hypertensive animals have increased expression of AVP mRNA in the hypothalamus ($p<0.05$). Treatment with both specific agonists PPT and DPN significantly reversed this increment($p<0.05$). Pro-inflammatory cytokines, such as TNFα can also stimulate AVP in the hypothalamus. Therefore, we measured TNFα mRNA in SHR and found in this strain a slight, although not significant increase of TNF expression. However, treatment with both PPT and DPN reduced TNFα mRNA expression in the hypothalamus of SHR($p<0.05$). These data indicate that both estradiol classical receptors are involved in the reduction of AVP overexpression. These effects may be due to direct effects upon the AVP gene or by down-regulation of TNFα expression.

344 (353) **ENHANCEMENT OF GLUCOCORTICOID RECEPTOR TRANSCRIPTIONAL ACTIVITY BY CB1 CANNABINOID RECEPTOR SIGNALING. ITS ROLE ON MEMORY CONSOLIDATION OF AVERSIVE EXPERIENCES**

Gina Granja-Galeano[1], Ana Paula Dominguez-Rubio[2], Carlos Daniel Zappia[1], María Zorrilla-Zubilete[3], Carlos Fitzsimons[4], Ana Franchi[2], Federico Monczor[1].
[1]Instituto de Investigaciones Farmacológicas (ININFA), FFyB, UBA, CONICET. Argentina. [2]Centro de Estudios Farmacológicos y Botánicos (CEFyBo), FMED, UBA, CONICET. Argentina. [3]Cátedra de Farmacología, FMED, UBA. Argentina. [4]Swammerdam Institute for Life Sciences, University of Amsterdam. The Netherlands

The cannabinoid receptor type 1 (CB_1)and the glucocorticoid receptor (GR) are coexpressed in several brain areas involved in learning and memory, including the hippocampus, a brain area involved in spatial and episodic memory. Glucocorticoids mediate the behavioral consequences of stress, such as memory consolidation associated with emotionally arousing events. The downstream immediate-early gene Egr1 is a GR-responsive gene involved in memory consolidation. It has been suggested that CB_1signaling modulates GR-mediated transcriptional activity. Here, we studied the effect of CB_1signaling on GR-mediated transcriptional activity related to memory consolidation of aversive events. For in-vitro studies, we used the HT22 neuronal cell line, derived from murine hippocampus. In these cells, induction of Egr1 by the GR agonist Dexamethasone (Dex) was potentiated by the selectiveCB_1agonist meta-anandamide (300%, $p<0.05$). To assess whether this potentiation was present in-vivo, we studied the expression of Egr1in the hippocampus of male CD1 mice carrying a knock out mutation for the CB_1receptor gene (CB_1KO)and their wild type littermates (WT). Dex (1,5 mg/kg; i.p.) produced a two-fold increase in Egr1 6h after injection, in WT mice. Co-treatment with the CB_1agonist HU210 (0,1 mg/kg,i.p.) induced a threefold increment, whereas the CB_1antagonist AM251 (1 mg/kg)completely blocked Dex-induced Egr1 expression. Consistently, in KOmice Dex did not induced Egr1 expression, and cotreatment with HU210or AM251 had no effect either. When associative memory was evaluated with a passive-avoidance task, Dex treatment immediately after a 0,2mA for 2 s of shock, facilitated memory consolidation in WT mice. On the other hand, KO mice were unable to consolidate associative memory neither under basal conditions nor after Dex treatment. Our results suggest that CB_1signaling modulates GR transcriptional activity and is needed in memory consolidation of aversive experiences.

345 (496) **ROLE OF ALPHA-SYNUCLEIN IN THE MITOCHONDRIAL DYNAMICS AND AUTOPHAGY. IMPLICATIONS FOR PARKINSON'S DISEASE**

Jimena Hebe Martínez[1], Agustina Alaimo[1], Roxana Mayra Gorojod, Mónica Lidia Kotler[1].
[1]Laboratorio de Disfunción Celular en Enfermedades Neurodegenerativas y Nanomedicina Departamento de Química Biológica. Facultad de Ciencias Exactas y Naturales. Universidad de Buenos Aires IQUIBICEN-CONICET

Parkinson disease (PD) is a neurodegenerative pathology of the central nervous system affecting 1% of the population over 60 years old. This disease is characterized by motor deficiency, rest tremor, and the selective loss of dopaminergic neurons of the *Substantia Nigra Pars Compacta.* One of the key molecules involved in PD development is a small protein named -synuclein. In pathological conditions, α-synuclein is overexpressed and associated to mitochondria. This interaction could trigger the mitochondrial impairment and reactive oxygen species generation both events involved in PD. This study hypothesized that -synuclein impairs the mitochondrial function involving processes such as metabolic imbalance, mitochondrial dynamics and autophagy. The present work studied the effect of this protein inSH-SY5Ycells overexpressing α-synuclein. We proved that this protein diminished cell viability (34±5%and 31±7% vs control, $p<0,05$ and $p<0,01$by ATP and LDH assays respectively) and reduced ATP production (49±5%vs control, $p<0,01$, ATP production assay)with a concomitant increase in the reactive oxygen species generation (270±39%, $p<0,001$ and 17±9%, $p<0,05$ for reactive peroxides and mitochondrial superoxide evaluated with DCFH-DA and MitoSOX Red assays respectively). In addition, α-synuclein was able to trigger mitochondrial membrane dissipation, imbalance in fission-fusion equilibrium, autophagy and mitophagy. In these cases the analysis were made by fluorescence microscopy withMitoTracker Red CMXRos, western blot for LC3-II and immunofluorescence againstLC3 and TOM20 followed by a Manders colocalization analysis. Both autophagy and mitophagy could act as a rescue cellular mechanisms by decreasing cell death and damaged mitochondria. These results add new evidence to the knowledge of the effects of α-synuclein on the mitochondrial functionality and represent a contribution to the understanding of the processes leading to development of PD.

346 (606) **TOLL-LIKE 4 RECEPTOR CONTROLS ASTROGLIAL CONVERSION TO THE PROINFLAMMATORY PHENOTYPE.**

Gerardo Rosciszewski[1], Vanesa Cadena[1], Alejandro Villarreal[2], Thierry Roger[3], Javier Ramos[4]
[1]Instituto de Biologia Celular y Neurociencias Prof. E. De Robertis UBA-CONICET, [2]Institute for Anatomy and Cell Biology, University of Freiburg, Freiburg, [3]Centre Hospitalier Universitaire Vaudois, Lausanne

Astrocytes are starting to be recognized as a facultative immunocompetent cell type able to participate in innate immunity response in the Central Nervous System (CNS). Astrocytes react to brain injury with a generic response known as reactive gliosis, which involves activation of multiple intracellular pathways including several that may be beneficial for neuronal survival. However, by unknown mechanisms reactive astrocytes can polarize into a proinflammatory phenotype that induces neurodegeneration. We hypothesized that astroglial polarization to the proinflammatory phenotype involves excessive activation of TLR4 and downstream NFκB. Using in vitro primary astroglial cell cultures exposed to oxygen-glucose deprivation (OGD) and rat brains exposed to ischemia by cortical devascularization, we observed that both paradigms induce TLR4 expression in astrocytes. In vivo, confocal images showed an early increase in TLR4 expression in the ischemic core and penumbra. Then, we exposed enriched astrocytic culture to OGD for 6 h, and 16 h later we treated them with lipopolysaccharide (LPS). OGD-exposed astrocytes responded to LPS with a significantly increased response of stellation compared with astrocytes exposed to control conditions. Conversely, primary astrocytes obtained from TLR4 KO, but not those obtained from TLR2 KO animals, showed reduced NFκB activation when exposed to LPS. Gain of function studies using plasmidmediated TLR4 overexpression exacerbated astroglial response to LPS by producing a sustained NFkB activation and increased levels of proinflammatory IL1B and TNF-α; while mock-transfected astrocytes showed a peak of NFκB activation and lesser proinflammatory cytokines level by qPCR. We conclude that reactive astrocytes exposed to ischemia in vitro and in vivo express higher TLR4 levels and its activation by TLR4 ligands such as LPS induces astroglial polarization to

the proinflammatory-neurodegenerative profile probably facilitating neuronal death.

347 (958) **ROLE OF GALECTIN 1 ON EXPERIMENTAL ALZHEIMER´S DISEASE. EFFECT OF TREATMENT ON AMYLOID DEPOSITION, NEUROVASCULAR UNIT AND COGNITIVE PERFORMANCE**

Carlos Javier Pomilio[1;2], Juan Beauquis[1;2], Ángeles Vinuesa[1;2], Melisa Bentivegna[1;2], David Clauser[1;2], Rosa Morales[3], Gabriel Rabinovich[2;3], Flavia Eugenia Saravia[1;2].
[1]Laboratorio de Neurobiología del Envejecimiento IBYME CONICET, [2]Departamento de Química Biológica FCEN UBA, [3]Laboratorio de Inmunopatología IBYME CONICET

Alzheimer´s disease (AD) is the leading cause of dementia in the elderly. Amyloid deposits and neurofibrillary tangles are among the principal hallmarks in the cortex and the hippocampus, brain target areas of this devastating disease that lacks effective treatment. Neuroinflammation early compromise the components of the neurovascular unit in AD. Galectin-1 (Gal 1) is a glycan binding protein which is proposed to have several properties on immune and endothelial cells in the periphery and also in the central nervous system. Firstly, we found that astrocytes surrounding amyloid plaques were Gal1+ in the hippocampus of PDAPPJ20 mouse, transgenic (Tg) model of AD. Then, we administered Gal1 (i.p.9 injections of 100ug/dose) or vehicle during 3 weeks to 12 month old Tg mice, when they exhibited evident plaque deposition. Age-matched non transgenic mice were used as controls. Gal1 treated group improved the performance in the Novel Object Location Recognition cognitive test compared with the vehicle group. The plaque load, assessed as Congo Red + area on hippocampal slices, diminished in a significant manner in Gal1 treated mice, reaching almost 70% decrease ($p<0.05$). Iba1+ microglial cells in the hilus of the dentate gyrus exhibited less reactivity-measured as soma size- after Gal 1 treatment ($p<0.05$) suggestive of a Gal 1 modulatory effect on this cell population involved in amyloid clearance through phagocytosis. Moreover, the perivascular amyloid deposition decreased in Tg mice receiving Gal 1, visualized by tomato lectin labeling for microvasculature combined with A immunofluorescence. Our results showed a possible relevant role for Gal 1 in this neurodegenerative disease at multiple levels, including cellular and cognitive aspects. Additional in vitro experiments are in progress to identify the associated mechanism of action of Gal 1 modulating the neuroinflammatory status in the hippocampus of AD mice.

348 (978) **RESTORATION OF INJURIED NERVOUS SYSTEM BY IMMUNOPHILIN LIGANDS**

Cristina del Rosario Daneri Becerra[1], Nadia Romina Zgajnar[1], Michelle Patiño[1], Mario Daniel Galigniana[1,2].
[1]Instituto de Biología y Medicina Experimental, IBYME-CONICET, [2]Departamento de Química Biológica, FCEN-UBA

Immunophilins (IMMs) are proteins classified as cyclophilins (CyP) when they bind cyclosporine A (CsA),or FK506-Binding Proteins (FKBPs) when they bind the macrolide FK506. Thenervous system expresses high levels of IMMs, but their biological roles are poorly understood. Previously, we reported that FK506 favours neurodifferentiation in an FKBP52-dependent fashion, whereas FKBP51 is an antagonistic factor. Organotypic prefrontal brain slices and spinal cord slices treated with FK506 showed high induction of FKBP52 expression, whereas treatments with CsA induced the expression of CyP17 in brain, but not in spinal cord. To test the neuroregenerative properties of both IMM ligands, in this study we subjected mice to surgical transection of the mid thoracic spinal cord. Locomotion recovery was evaluated according to the BMS (Basso Mouse Scale) test for three weeks of treatment with daily (s.c.) doses of 0.1 mg/kg CsA or FK506. In both cases, locomotor function was significantly recovered. No differences were observed inKO mice for FKBP51 or FKBP52 versus wild type controls. However FKBP51 KO mice showed stronger recovery, whereas FKBP52 KO mice recovered at lower rate, confirming in vivo that both IMMs are antagonistically related to neuroregeneration. Grip strength, balance and motor coordination were evaluate din drug-treated mice by Rotarod performance test, and locomotor behaviour was analyzed using ANY-Maze Video Tracking System byStoelting Co. Results led to equal conclusions as the BMS test. To test the putative neuroprotective action of these drugs against neurocortical brain hypoxia, mice were injecte dwith 2 µl 50 mM $CoCl_2$ in the frontoparietal cortex using a stereotaxic apparatus. Rotarod tests showed recovery of motor coordination in both CsA- and FK506-treated mice, although the ANY-Maze analysis demonstrated that FK506-treated mice have a better performance. We conclude that IMMs activated by their specific ligands play key neurotrophic roles.

349 (1003) **FEMALE DISTINCTIVE BEHAVIOURAL PROFILE IN THE VPA RAT MODEL: DIVING IN THE GENDER DIFFERENCES OF AUTISM SPECTRUM DISORDERS**

Martin Codagnone[1, 2], Manuel Molina[1], María José Malleville Corpa[1], Marianela Evelyn Traetta[1, 2], Nonthué Alejandra Uccelli[1], Analía Reinés[1, 2].
[1]Instituto de Biología Celular y Neurociencias "Prof. de Robertis" IBCN, Universidad de Buenos Aires-CONICET, [2]Cátedra de Farmacología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires

Autism spectrum disorders (ASD) are a group of severe neurodevelopmental disabilities with an early onset and no clear neurobiological basis. ASD are characterized by pervasive impairments in social interactions, deficits in verbal and non-verbal communication, and stereotyped behaviours. Although incidence of ASD is four times higher in boys than in girls, gender differences have been out of scope. The rat model of autism induced by prenatal exposure to Valproic Acid (VPA) presents in males, behavioural and neuroanatomical alterations similar to those seen inautistic patients. Since gender differences in the VPA rat model have not been addressed, the aim of this work was to characterize the behavioural profile off emale VPA rats (FVPA). At early postnatal days (PND) 7-15, FVPA showed growth and maturational deficits similar to the ones exhibited by male VPA rats (MVPA).FVPA not only displayed delayed eye opening but a lower body weight. They alsoshowed altered negative geotaxis, higher latencies to nest seeking response and a deficit in swimming performance. Similar to MVPA, FVPA matched control performance by the end of the early postnatal period. However, at PND 30-35, FVPA behaviour differed from the one of MVPA. Although FVPA showed reduced interactions in the social play behaviour test, they exhibited distinctive pinning features. Contrary to MVPAthat showed an exploratory deficit and increased stereotypical activities, FVPAmatched control female behaviour. Notably, at this stage (PND 35), FVPA hippocampal cytoarchitecture alterations resemble those seen in MVPA. To sum up, FVPA exhibit a distinctive behavioural profile characterized by a maturational delay and strong social impairment but normal exploratory and stereotypical activity. Understanding gender differences in ASD can lead the way to unravel the basis of social impairment and to come up with novel therapeutic targets for these disorders.

350 (190) **AMPHETAMINE-INDUCED SENSITIZATION AS AN ANIMAL MODEL OF SCHIZOPHRENIA: BEHAVIORAL CHARACTERIZATION AND ROLE OF ANGIOTENSIN II AT1 RECEPTORS**

Osvaldo Martin Basmadjian[1], Victoria Belen Occhieppo[1], Natalia Andrea Marchese[1], Gustavo Baiardi[2], Claudia Bregonzio[1].
[1]Instituto de Farmacología Experimental Córdoba (IFEC-CONICET) Departamento de Farmacología. Facultad de Ciencias Químicas Universidad Nacional de Córdoba, Córdoba, Argentina. [2]Laboratorio de Neurofarmacología, (IIBYT-CONICET) Universidad Nacional de Córdoba Facultad de Ciencias Químicas, Universidad Católica de Córdoba, Córdoba, Argentina

Amphetamine (amph) exposure induces adaptive responses observed as increased dopaminergic reactivity in mesolimbic

pathway concomitant with decreased dopaminergic reactivity in the mesocortical one. Similar alterations have been described in the pathology of schizophrenia. Forthis reason, amph-induced sensitization is a validated animal model of schizophrenia;resembling described positive signs and cognitive deficits. Brain AngiotensinII, through AT_1 receptors (AT_1-R), modulates dopaminergicneurotransmission in limbic areas. Previously, we found that behavioral neuroadaptativeresponses to amph involved AT_1-R activation. The aim for the presentwork was to study AT_1-R involvement in behavioral responses in an animal model of schizophrenia using amph-induced sensitization. Male Wistar rats (250-320g), at standard laboratory conditions, were administered with AT_1-Rantagonist Candesartan/vehicle (3 mg/kg p.o., day 1-10) and amph/saline (2.5mg/kg i.p., day 6-10). On day 31 we evaluated the locomotor sensitized response to saline/amph challenge (0.5 mg/kg i.p.) and the working memory (Y-maze test).Data were analyzed with two-way ANOVA followed by Bonferroni test and t-test. We corroborated that the amph protocol used induces behavioral sensitization to amphchallenge and cognitive deficit, observed as a decreased spontaneous alternations in the Y-maze. Moreover, it was found an increased susceptibility to stress, expressed as augmented locomotor activity, 30 minutes after saline injection. So, we conclude that this protocol was effective to elicit some of the described signs of schizophrenia. Interestingly, the AT_1-Rantagonist blunted the neuroadaptative responses to the psychostimulant. Since the available therapeutic treatments have low efficacy and high incidence of side-effects, new pharmacological approaches become necessary. However, further studies are needed to postulate the AT_1-R antagonists as an alternative pharmacological tool.

351 (200) **ALTERATIONS IN MITOCHONDRIAL BIOENERGETICS AND NITRIC OXIDE METABOLISM DUE TO ACUTE ALCOHOL EXPOSURE IN MOUSE BRAIN CORTEX SYNAPTOSOMES AND NON-SYNAPTIC MITOCHONDRIA**

Analia Karadayian[1], Analia Czerniczyniec[1], Paulina Lombardi[1], Juanita Bustamante[2], Silvia Lores Arnaiz[1].
[1]Instituto de Bioquímica y Medicina Molecular (IBIMOL)-Universidad de Buenos Aires (UBA), CONICET, Fisicoquímica, Facultad de Farmacia y Bioquímica, Buenos Aires, Argentina. [2]Centro de Altos Estudios en Ciencias Humanas y de la Salud (CAECIHS), Universidad Abierta Interamericana, Buenos Aires, Argentina

The abuse of alcohol consumption is a type of alcohol-related disorder causing anumber of serious consequences to health. Recently, we demonstrated that alcohol hangover (AH) induced a strong oxidative stress in brain cortex. Specifically, synaptosomes (S) resulted to be more affected due to AH than non-synaptic mitochondria (NSm). The aim of the present work was to study mitochondrial bioenergetics and NO metabolism at the beginning of AH in brain cortex NSm andS. Swiss male mice were treated with a single i.p. injection of saline (controlgroup) or ethanol (3.8 g/kg; AH group). Biochemical assays were performed 6 h post-treatment when blood alcohol concentration was close to zero (AH start). Mitochondrial respiration, respiratory complexes activity, mitochondrial transmembrane potential, UCP-2 expression were determined. Also, nNOS expression, NOS activity and NO levels were assayed. Results showed a significant decrease in respiratory function in both subcellular fractions ($p<0.05$). The enzymaticactivity of complex I-III and II-III was 21% and 33% decreased respectively inS while no changes were observed in NSm. Complex IV activity was 48% and 42% decreased in S and NSm respectively. Mitochondrial membrane potential was 20% and 15%decreased in S and NSm respectively. UCP-2 expression was 49% increased in Sand 31% decreased in NSm. On the other hand, NOS expression was unaltered in Sand 37% decreased in NSm. Contrary to this result, NOS activity and NO levels were decreased in S ($p<0.05$) while both parameters remained unchanged in NSm. It could be concluded that both synaptosomes and non-synaptic mitochondria exhibited alterations in mitochondrial function being more pronounced in synaptosomes than in non-synaptic mitochondria. The oxidative stress previously found in synaptosomes could be associated with the induction of UCP-2 as a mechanism to decrease oxygen free radicals generation by modulating mitochondrial membrane potential.

352 (244) **ACUTE ACETAMINOPHEN (APAP) INTOXICATION INDUCED SELECTIVE NEUROCHEMICAL IMPAIRMENT OF THE FUNCTION OF DOPAMINE SYSTEM IN RAT BRAIN STRUCTURES**

María Belén Vigo
[1]ININFA, Instituto de Investigaciones Farmacológicas, UBA-CONICET, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires- Ciudad Autónoma de Buenos Aires, Argentina [2]INQUIFIB, Instituto de Química y Fisicoquímica Biológica. UBA-CONICET. Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires- Ciudad Autónoma de Buenos Aires, Argentina. [3]Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT, United States. [4]Cátedra de Fisiopatología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires. Ciudad Autónoma de Buenos Aires, Argentina

APAP overdoses, produce hepatotoxicity and acute liver failure (ALF). Brain damage produced after APAP intoxication is usually the outcome of hepatic encephalopaty, secondary to ALF. We previously reported decreases in locomotor activity (~50%) during APAP intoxication resulting in liver damage that does not progress to ALF. Therefore, the aim of the present work was to evaluate the effect of acute APAP intoxication on dopamine (DA) system in pre-frontal cortex (Pfc), cortex (Cx), hippocampus (Hp) and striatum (St) in rats. For this purpose, male Wistar rats were dosed with APAP (1g/kg; i.p., n=5) or vehicle (n=5). 24h later, brain areas were processed to measure DA levels by HPLC. Another batch of animals were processed to evaluated the nuclear translocation of Nrf2 (oxidative stress sensor), astrocyte marker (Glial fibrilar protein; Gfap), and neuronal marker (Neurofilament heavy; NF200) expression in Pfc, Cx, Hp and St by Western blotting. Results: APAP significantly decreased DA levels in Pfc by 80% $p<0.01$, in Hp by 91.82% $p<0.05$ and St by 50.32% $p<0.01$, while no changes in Cx were observed. Nrf2 translocation was higher by 150% in Pfc $p<0.01$, 42% in Cx $p<0.05$ and 138% $p<0.05$ in Hp with APAP treatment with no changes in St. Nf200 significantly decreased by 66% in Pfc $p<0.05$, 39% in St $p<0.05$ and with decreasing trend that not reach significance in Hp and Cx. By contrast GFAP was significantly increased by 199% $p<0.05$, 70% $p<0.05$ and 9% $p<0.05$ in Hp, St and Pfc respectively, with a tendency toward increase in Cx. Conclusions: these data indicate that the the APAP-induced hypolocomotion we previously reported may be associated with dowregulation in DA levels in Pfc, Hp and St; probably due to endocannabinoid system (CB1 receptors) activation by AM404, a brain APAP metabolite. Additionally we demonstrate that APAP intoxication produces astrogliosis and a decreased in neurons processes, in the absence of ALF, most likely due to direct toxic effects of APAP in brain.

353 (509) **ESTROGEN CHRONIC ADMINISTRATION EFFECTS ON MOTOR BEHAVIORAL ACTIVITY ASSESSED BY BIOINFORMATICS TOOLS ON DIFFERENT STAGES OF A NEURODEGERATIVE PROCESS IN ADULT MALE RATS.**

María Paula Bonaccorso Marinelli[1], Franco Emiliano Nieto Grimalt[2], Mauricio José Ledesma[2], Ricardo Jorge Cabrera[1].
[1]INBIOMED- IMBECU-CONICET, [2]Universidad de Mendoza

We analyze estrogen actions *in vivo* in adult hemi parkinsonian male rats lesioned with 6-hydroxydopamine (6-OHDA) by bioinformatic assessing motor behavior on different stages of the neurodegenerative process. On postnatal day 60 (PND), 20 rats were injected with 6-OHDA in left corpus striatum (HP). From PND67 to 77, 10 HP rats received chronic treatment with 17b-Estradiol (E= 0.1 µg/kg/day s.c) and 10 got oil as vehicle. Other group (C) n=9 had the same surgery without 6-OHDA and oil treatment. Motor behavioral activity of all groups was video recorded

and evaluated with automated softwares. Open Field Test (OFT) was conducted on PND74 without drug induction (NDI) and, on PND88 after amphetamine injection (AMPH). After 48h Forced Swimming Test (FST) NDI was evaluated.OFT total time moving after AMPH significantly increased ($p<0.001$) in all groups. Time spent in corners NDI was less ($p<0.05$) in HP+E in comparison with HP and C. After AMPH this parameter decreased ($p<0.01$) but the difference between all groups remained. Border entries after AMPH were significantly high ($p<0.05$) in all groups, and even higher ($p<0.001$) for HP+E.Time spent in the centre was high in HP+E NDI and after AMPH in comparison with HP and C, but not statistically significant. Walking track plots were significantly different ($p<0.05$). HP walk near to the borders, while HP+E and C have random walking. HP and HP+E increased($p<0.05$) average speed and total distance travelled in comparison to C in FST. Swimming track plots were significantly different ($p<0.05$). HP+E and C have random swimming mode, while HP's is concentric. We conclude that E treatment has beneficial effects on motor behavior as it improves distance, velocity and motility. Also there is a big difference when using bioinformatics to assess ethological behavior in animal models. These techniques decrease operator errors due to direct observation and handling, early and acutely detect parameters and increase data amount speed and quality.

354 (694) **ABSENCE OF ENDOGENOUS GALECTIN-1 DOES NOT AFFECT NORMAL AXONAL DEVELOPMENT**

Hector Ramiro Quinta[1], Francisco Barrantes[2], Juana Maria Pasquini[1].

[1]Departamento de Química Biológica Patológica-IQUIFIB-UBA-CONICET. [2]Laboratory of Molecular Neurobiology, BIOMED UCA-CONICET, Buenos Aires

During embryonic stages, axonal development is crucial to establish neuronalconnections, which among other things will allow normal locomotor activities.We have demonstrated that Galectin-1 (Gal-1) in its dimeric form promotes reactivation of actin cytoskeleton dynamics, leading to axonal re-growth aftera spinal cord injury (Quinta et al.,2016). Incontrast, monomeric Gal-1, corresponds to endogenous lectin concentrations,<7mM; does not promote the repair phenomenon (Quinta et al.,2014). Taking into account that endogenous Gal-1 (e-Gal-1) does not modify actin cytoskeleton dynamics, we set to determine whether the complete absence of e-Gal-1 affects axonal development. Using embryonic *lgals-1*$^{-/-}$mice we determined the 3D axonal development, axons number and their length relative to w*ild type* (*WT*) and *lgals-3*$^{-/-}$ embryos. Towards this purpose we applied acombination of novel techniques which allowed us to image in 3D the axonal morphology in whole embryos rendered transparent with the *CLEARING* method in combination with 1-photon microscopy (Quinta et al., 2015).Moreover, we identified single axons and its sprouting deep in the tissue at high magnification without tissue sectioning by combining epifluorescence microscopy under high power LED illumination followed by serial image section deconvolution and 3D reconstruction. Our results show that the absence of e-Gal-1did not affect the normal axonal development; axonal length did not differ from that observed in *WT* and *lgals-3*$^{-/-}$ embryos. Moreover, no significant differences were observed in the number of axons from dorsal root ganglia and in the shape of growth cones. Finally, the presence ofPlexinA4, the main axonal guidance protein, did not change in the absence of e-Gal-1. The conclusion from this series of studies is that the absence of e-Gal-1 does not modify normal axonal development whose effect could be due to e-Gal-1 cannot affect the axonal cytoskeleton dynamics, in full agreement with our previous results.

355 (749) **CHARACTERIZATION (TREM -2) EXPRESSION PROFILE AND ROLE IN EXPERIMENTAL BRAIN ISCHEMIA**

Vanesa Cadena[1], Gerardo Rosciszewski[1], Javier Ramos[1].

[1]Instituto de Biologia Celular y Neurociencias Prof. E. De Robertis UBA-CONICET

After brain injury, DAMP are released from necrotic cells and activate toll-like receptors (TLR) in professional innate immunity cells and astrocytes. Astrocytes respond to the injury with a typical phenomenon known as reactive gliosis, and thereafter they can polarize towards a proinflammatory phenotype that induces neurodegeneration. The mechanisms leading to the proinflammatory-neurodegenerative glial profile are unknown but evidences are indicating that overactivation of TLR and downstream NFkB signaling can be involved. TREM-2 and its adaptor DAP12 down-regulate TLR signaling, and thus it is proposed to participate in the fine-tuning of the inflammatory response in professional immune cells. Using an experimental model of focal brain ischemia by unilateral cortical devascularization and astroglial-enriched cell culture, we studied here the expression of TREM-2 and the cellular consequences of its activation. We observed that TREM-2 is expressed primarily in microglia, but also in penumbral astrocytes after brain ischemia. In vitro, TREM-2 is expressed in rat astrocytes-enriched cell cultures exposed to hypoxia or oxygen-glucose deprivation (OGD, in vitro model of ischemia) but also facilitated by PAMP (LPS) or DAMP (HMGB-1) treatment. Further, we transfected astrocytes and overexpressed TREM-2 and DAP12 and observed that TREM-2 overexpressing astrocytes show reduced NFkB activation after LPS exposure. We conclude that TREM-2 represents an interesting target to limit the astroglial response to inflammatory clues and to prevent the conversion to the proinflammatory neurodegenerative phenotype.

METABOLISMO Y NUTRICIÓN II/ METABOLISM AND NUTRITION II

356 (393) **ENDOTHELIAL LIPASE AS A SUPPORTING ACTOR OF LIPOPROTEIN LIPASE IN INSULIN RESISTANCE**

Magalí Barchuk[1,2], Verónica Miksztowicz[1,2,3], Valeria Zago[1,2,3], Carolina Olano[1,2], Silvia Friedman[4], Laura Schreier[1,2], Gabriela Berg[1,2,3].

[1]Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Departamento de Bioquímica Clínica. Cátedra de Bioquímica Clínica I. Buenos Aires, Argentina. [2]Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Instituto de Fisiopatología y Bioquímica Clínica (INFIBIOC). Buenos Aires, Argentina. [3]Universidad de Buenos Aires. CONICET. Facultad de Farmacia y Bioquímica. Buenos Aires, Argentina. [4]Universidad de Buenos Aires. Facultad de Odontología. Cátedra de Bioquímica General y Bucal. Buenos Aires, Argentina

Lipoprotein lipase (LPL) and endothelial lipase (EL) are enzymes involved in lipoproteins metabolism. Insulin-resistant states (IR) may alter the expression of these enzymes in an opposite manner, decreasing LPL and increasing EL, modifying lipoprotein profile. Until now, no data has been reported about EL activity in IR affected tissues. Our aim was to evaluate LPL and EL activity in adipose tissue and heart, and its association with lipoprotein profile in a diet induced obesity model. Methods and results: Male Wistar rats (180-200 g) were fed with standard diet (Control, n=8) or standard diet plus 40% fat (HFD, n=8) during 14 weeks. The study was approved by the Ethic Committee-FFYB (UBA). Glucose, lipoprotein profile and IR markers were measured in serum. Epididymal adipose tissue (AT) and heart LPL (as TG-hidrolase) and EL (as phospholipase) activity were assessed by radiometric assays. In addition AT PPARg expression was measured. HFD presented higher TG/HDL-chol ($p=0.025$), glucose ($p<0.001$) and free fatty acids (FFA) levels ($p=0.017$). EL activity was increased in heart ($p=0.044$) and AT ($p=0.039$) from HFD, meanwhile LPL activity was decreased in both tissues ($p<0.001$ and $p=0.047$, respectively). In AT both enzymes activities were inversely correlated ($p=0.05$). EL activity from AT was directly associated with FFA ($p=0.03$) and with atherogenic lipoproteins ($p=0.008$); meanwhile LPL inversely correlated with body weight ($p=0.015$). PPARg expression in AT tended to be decreased in HFD ($p=0.06$) without association with lipases activities. Conclusions: This is the first time that EL activity is reported in heart and AT in an obese model. Given the inverse association between both enzymes activities it could be suggested

that the increase in EL activity could be an alternative pathway for free fatty acids uptake in tissues with decreased LPL activity. Associations of EL with atherogenic lipoproteins and FFA supports the negative role of this enzyme in IR states.

357 (394) **ANALYSIS OF FKBP51, FKBP52 AND HSP70 IN BROWN ADIPOSE TISSUE IN RESPONSE TO EXPOSURE OF MICE TO COLD**

Maria Itati Rodriguez Ceschan[1], Graciela Piwien Pilipuk[1].
[1]Instituto de Biología y Medicina Experimental (IBYME)

Glucocorticoids play an important role in adipose tissue *via* the glucocorticoid receptor that forms a heterocomplex with Hsp90-Hsp70 and a high molecular weight immunophilin (INM) FKBP51 or FKBP52. We have shown that FKBP51 is highly expressed in white (WAT) and brown (BAT) adipose tissue in mice, localizing mainly in mitochondria. In contrast, FKBP52 is only expressed in BAT. Thus, we focused our study of the INMs in BAT. Mice were exposed 1 or 4 h at 4°C or kept at 25 C, and the expression of the INMs was analyzed by WB. FKBP51 increases its expression in BAT while decreases in WAT of mice exposed to cold. FKBP51 is resolved at least in three bands on WB corresponding to forms with different degrees of phosphorylation. Exposure of mice to cold increases the bands with faster electrophoretic mobility, suggesting that in BAT FKBP51 may be dephosphorylated. In addition, FKBP52 also increases its level of expression in BAT upon exposure of mice to cold. Since FKBP51 is present in mitochondria, we investigated whether the INM interacts with uncoupling protein (UCP)1, a BAT-specific protein that catalyzes a proton leak across the inner mitochondrial membrane. When FKBP51 was immunoprecipitated from mitochondria isolated from BAT, UCP1 co-immunoprecipitated indicating that they interact. The stress-response chaperone Hsp70 is present in the intra- and extracellular compartments, the former associated to anti-inflammatory actions while the latter to pro-inflammatory pathways. We find a rapid increase of Hsp70 in BAT, as reported; however an increase in Hsp70 expression is also found in WAT after 1h of exposure to cold. Interestingly, Hsp70 is almost undetectable in BAT and WAT after 4 h of cold exposure raising the possibility Hsp70 may be secreted to exert its action in the extracellular compartment in response to cold. Taken together these results, Hsp70 and the INMs show rapid changes upon exposure to cold, and FKBP51 may possibly participate in the control of UCP1.

358 (397) **GELATINASES BEHAVIOR IN ADIPOSE TISSUE, HEART AND LIVER IN A DIET INDUCED OBESITY MODEL**

Magalí Barchuk[1,2], Celina Morales[4], Valeria Zago[1,2,3], Silvia Friedman[5], Laura Schreier[1,2], Verónica Miksztowicz[1,2,3], Gabriela Berg[1,2,3].
[1]Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Departamento de Bioquímica Clínica. Cátedra de Bioquímica Clínica I. Buenos Aires, Argentina. [2]Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Instituto de Fisiopatología y Bioquímica Clínica (INFIBIOC). Buenos Aires, Argentina. [3]Universidad de Buenos Aires. CONICET. Facultad de Farmacia y Bioquímica. Buenos Aires, Argentina. [4]Universidad de Buenos Aires. Facultad de Medicina. Instituto de Fisiopatología Cardiovascular, Departamento de Patología. Buenos Aires, Argentina. [5]Universidad de Buenos Aires. Facultad de Odontología. Cátedra de Bioquímica General y Bucal. Buenos Aires, Argentina

Matrix metalloproteinases (MMPs) constitute a family of endopeptidases synthesized by multiple cell types. MMPs may remodel the tissue parenchyma during the process of adipose tissue (AT) expansion, as well as liver and heart fibrosis. Insulin-resistance (IR) may alter MMPs behavior, however there are scarce data about the effect of obesity on MMP-2 and -9 activity in these tissues. Our aim was to determine whether expanded visceral adipose tissue, liver and myocardium steatosis modify MMP-2 and -9 behavior. Methods and results: Male Wistar rats (180-200 g) were fed with standard diet (Control, n=8) or standard diet plus 40% fat (HFD, n=8) throughout 14 weeks. The study was approved by the Ethic Committee-FFYB (UBA). Glucose, lipoprotein profile and IR markers were measured in serum. In epididymal AT vascular density, size and adipocyte density and PPARg expression were determined. Fat depots in liver and heart were evaluated through histologic analysis. MMP-2 and -9 activity was measured in the three tissues and plasma by zymography. HFD presented higher TG/HDL-chol ($p=0.025$), glucose ($p<0.001$) and free fatty acid levels ($p=0.017$). Compared to Control, AT from HFD presented higher adipocyte size ($p<0.001$), lower adipocyte density ($p=0.042$) and vascular density ($p=0.05$), this one not significant after normalizing by adipocyte number. Fat depots were observed in liver and heart from HFD. HFD AT presented increased MMP-9 activity ($p=0.018$) and a tendency to decrease PPARg expression ($p=0.06$), inversely correlated ($p=0.06$). MMP-9 activity showed a tendency to increase in heart from HFD, no activity was detected in plasma and liver. No differences in MMP-2 activity were observed in the studied tissues. Conclusion: obesity induced by HFD promotes an increase in MMP-9 activity mainly in AT, unreflected in plasma. This behavior is not observed in MMP-2 activity. In obesity, PPARg could be involved in the negative regulation of MMP-9.

359 (405) **LIPOPROTEIN LIPASE ACTIVITY IS ENHANCED IN HUMAN EPICARDIAL ADIPOSE TISSUE**

Magalí Barchuk[1,2], Graciela López[1,2], Miguel Rubio[4], Daniel Yñon[4], Julio Baldi[4], Laura Schreier[1,2], Verónica Miksztowicz[1,2,3], Gabriela Berg[1,2,3].
[1]Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Departamento de Bioquímica Clínica. Cátedra de Bioquímica Clínica I. Buenos Aires, Argentina. [2]Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Instituto de Fisiopatología y Bioquímica Clínica (INFIBIOC). Buenos Aires, Argentina. [3]Universidad de Buenos Aires. CONICET. Facultad de Farmacia y Bioquímica. Buenos Aires, Argentina. [4]Universidad de Buenos Aires. Hospital de Clínicas "José de San Martín". División de Cirugía Cardiovascular. Buenos Aires, Argentina

Epicardial adipose tissue (EAT) is a visceral AT surrounding and infiltrating myocardium and coronary arteries. An increase of EAT volume is a predictor of coronary artery disease (CAD) and represents a chronic inflammatory injury for the myocardium. In EAT little is known about lipoprotein lipase (LPL) behavior, enzyme involved in lipolysis of circulating lipoproteins and responsible for fatty acid deposition in AT. Our aim was to evaluate LPL activity and its inhibitor of activity, angiopoietin like- 4 (ANGPTL4), and activator of expression PPARg, in EAT from CAD patients. Methods and results: in EAT and subcutaneous adipose tissue (SAT) from patients undergoing coronary artery bypass graft (CAD, n=18) or valve replacement (No CAD, n=13) LPL activity (by radiometric assay) was evaluated. In a subgroup of patients ANGPTL4 and PPARg expression were evaluated. The study was approved by the Ethic Committee of the Hospital de Clínicas, UBA. In both groups there were no differences in age, body mass index (BMI) or lipoprotein profile. LPL activity (uU/mg) was higher in EAT than SAT in both groups ($p<0.001$); meanwhile ANGPTL4 expression was lower ($p=0.018$) without difference in PPARg levels. No differences were observed in any of these studied parameters between groups. LPL activity negatively correlated with ANGPTL4 expression in SAT ($p=0.039$). In CAD, EAT LPL negatively correlated with TG/HDL-chol ($p=0.03$). When patients were divided according to BMI, surprisingly LPL activity was higher in over than normal weight patients ($p=0.045$). Conclusions: EAT LPL activity would be complex regulated and had not been reported until now. Our results indicate that the regulation of LPL would be through its activity and not its expression. The increase in LPL activity in overweight patients although the inverse association with TG/HDL-chol index (surrogate marker of insulin-resistance) indicates that further studies are necessary to understand LPL behavior in EAT.

360 (421) **LIVER X RECEPTOR MAY REGULATE FATTY ACID SYNTHASE IN MAMMARY EPITHELIAL CELLS DURING LACTATION**

Diego Yair Grinman[1], Valeria Pilar Careaga[2,3], Michael Rudolph[5], Paul S. MacLean[5], Marta Silvia Maier[2,3], Adali Pecci[1,4]
[1]Instituto de Fisiología, Biología Molecular y Neurociencias IFIByNE-UBA-CONICET. [2]Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, UBA. [3]Unidad de Microanálisis y Métodos Físicos Aplicados a la Química Orgánica UMYMFOR-UBA-CONICET. [4]Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, UBA. [5]Division of Endocrinology, Metabolism and Diabetes, University of Colorado School of Medicine

Liver x receptors (LXRs) a/b are transcription factors that act as cholesterol sensors. When upregulated, intracellular cholesterol is metabolized to oxysterols, the natural ligands of the LXRs. Activated LXRs enhance the expression of enzymes that control lipid synthesis and transport and even modulate the inflammatory response. Gene expression studies revealed that activation of fatty acid synthesis in the mammary epithelium occurs in response to parturition and the onset of lactation. This increased expression of lipogenic genes is significantly larger than their relative expression in the liver, supporting the hypothesis that the mammary gland is undergoing changes to fulfill milk lipid levels. Our aim was to identify whether the LXRs regulate the expression of lipogenic genes in epithelial mammary cells and thus to study its participation in milk lipid production and transport during lactation. We´ve found that in the normal mouse mammary epithelial cell line (HC11) LXRs activation induces Fatty Acid Synthase (FAS) and ABCA1, an ATP-dependent cholesterol transporter expression. We also identified that, in purified mouse mammary gland epithelial cells (MECs), LXRa's protein levels increase along with lactation progress. Moreover, incubation of MECs from lactating day 5 mammary glands with the LXR synthetic agonist GW3965 (GW) induced FAS expression 2.96±0.21 fold vs. non treated cells. Finally, we performed FAME analysis on milk collected from lactating mothers treated for 24h with GW or vehicle to quantify the milk lipid profile. In concordance with FAS induction, we found that the amount of C16:0 (palmitic acid), the main product of FAS activity, increased in GW treated mice suggesting that LXRa may regulate the *de novo* lipogenesis in the mammary epithelium. The better characterization of LXR's effects will provide relevant data on milk fat production mechanism that would gain interest in dairy industry due to their putative capacity to modify lipid composition.

361 (521) **BEYOND HDL LEVELS, ALTERATIONS IN HDL SUB-FRACTIONS IN METABOLIC SYNDROME ARE LINKED TO THE INFLAMMATORY STATE**

Diego Lucero[1, 2, 3], Lita Freeman[4], Maureen Sampson[4], Gisela Gualano[5], Graciela I López[1, 2], Eduardo Fassio[5], Alan T Remaley[4], Laura Schreier[1, 2].
[1]Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Dpto de Bioquímica Clínica. Laboratorio de Lípidos y Aterosclerosis. [2]Universidad de Buenos Aires. Instituto de Fisiopatología y Bioquímica Clínica (INFIBIOC). [3]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) [4]Lipoprotein Metabolism Section. National Heart, Lung and Blood Institute. National Institutes of Health. Bethesda. USA. [5]Departamento de Gastroenterología. Hospital Nacional "Prof. A. Posadas", Buenos Aires, Argentina

In a previous study we have demonstrated that insulin-resistance (IR) is related to low HDL cholesterol (HDL-c) and qualitative alterations in HDL sub-fractions, linked to apparent higher HDL functionality. Also, IR is characterized by a chronic and low grade inflammatory state, contributing to a greater atherogenic risk. Aim: to study the association between the inflammatory state in IR and the quanti-qualitative alterations of HDL occurring in IR. Methods: We studied 30 metabolic syndrome (MetS) patients (ATPIII) and 15 controls. In fasting serum we measured lipid profile, high sensitivity-"C" reactive protein (hs-CRP) -as inflammatory marker-, glucose and insulin for calculation of HOMA-IR. HDL size, HDL particle number (HDLp) and sub-fraction profile were determined by nuclear magnetic resonance. Nascent pre-β1-HDL was assessed by 2D-electrophoresis. Results: MetS showed the characteristics of IR, with increased triglyceride levels and HOMA-IR and lower HDL-c levels ($p<0.01$). Moreover, the inflammatory status in MetS was evidenced by higher hs-CRP (median (range), 3.56(0.88-10.00) vs. 1.04(0.36-2.60) mg/L; $p=0.01$). The HDL sub-fraction analysis showed that MetS presented a low average HDL size, reduced number of HDLp ($p=0.01$) and, of note, increased pre-β1-HDL ($p=0.04$), suggesting a less efficient HDL maturation process in IR. Interestingly, hs-CRP negatively correlated with HDLp ($r=-0.41$; $p=0.01$), being at the expense of small HDL ($r= -0.42$; $p=0.01$). Hs-CRP positively correlated with pre-β1-HDL ($r=0.78$, $p=0.001$). All remained significant after adjusting by HOMA-IR and HDL-c ($p<0.045$). Conclusion: Results suggest that in MetS quanti-qualitative alterations in HDL, that might be consequence of a slower HDL maturation process with accumulation of pre-β1-HDL, are associated to the inflammatory state of IR, independently of HDL-c. The inflammatory state in IR could be explained in part by the decrease in small HDL particles of known anti-inflammatory actions.

362 (596) **CATALASE AND SUPEROXIDE DISMUTASE BEHAVIOR IN EPICARDIAL ADIPOSE TISSUE FROM PATIENTS WITH CORONARY ARTERY DISEASE**

Veronica Miksztowicz[1,2,3], Esteban M Repetto[3,4], Magalí Barchuk[1,2], Graciela López[1,2], J Baldi[5], Cora Cymeryng[3,4], Laura Schreier[1,2], Miguel Rubio[5], Gabriela Berg[1,2,3].
[1]Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Departamento de Bioquímica Clínica. Cátedra de Bioquímica Clínica I. Buenos Aires, Argentina. [2]Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Instituto de Fisiopatología y Bioquímica Clínica (INFIBIOC). Buenos Aires, Argentina. [3]Universidad de Buenos Aires. CONICET. Facultad de Farmacia y Bioquímica. Buenos Aires, Argentina. [4]Universidad de Buenos Aires. Facultad de Medicina. Departamento de Bioquímica Humana. Buenos Aires, Argentina. [5]Universidad de Buenos Aires. Hospital de Clínicas "José de San Martín". División de Cirugía Cardiovascular. Buenos Aires, Argentina

Epicardial adipose tissue (EAT) is a visceral fat adjacent to coronary arteries and myocardium. EAT is considered an endocrine organ, and its volume an independent risk factor for coronary artery disease (CAD). Catalase (CAT) and Superoxide Dismutase (SOD) are antioxidant enzymes that protect cells against potentially harmful effects of reactive oxygen species. Until now, there is no evidence about the behavior of these enzymes in EAT from patients with CAD. Aim: to evaluate CAT and SOD activity in EAT from patients with CAD and their relationship with insulin-resistance (IR). Subjects and Methods: Peripheral venous blood, EAT and subcutaneous adipose tissue (SAT) were obtained from patients undergoing heart surgery for coronary artery bypass graft (CAD, N=10) or valve replacement (No CAD, N=10). In serum glucose, lipids and lipoproteins profile was assessed. In EAT and SAT, CAT activity was evaluated by decomposition of H_2O_2 at 240 nm, and SOD activity according to pyrogallol autoxidation method at 420 nm. The study was approved by the Ethic Committee of the Hospital de Clínicas, UBA. Results: In both groups there were no differences in age, body mass index, glucose or lipoprotein profile. In EAT from CAD patients, SOD activity was decreased compared to No CAD ($p=0.024$) without differences in CAT activity. SOD and CAT activities were not associated with lipoprotein profile, however both enzymes showed a negative correlation with TG/HDL-chol as IR marker ($p=0.01$). Moreover, in EAT, CAT activity was decreased in patients with TG/HDL-chol ≥ 3 ($p=0.038$). In both groups, no differences in SOD and CAT activity was found between EAT and SAT. Conclusions: this is the first time that CAT and SOD activities are reported in EAT. Our findings propose a possible regulation of these enzymes by IR in EAT, suggesting an increase in oxidative stress in this tissue; future studies are necessary to explain the regulation of both enzymes.

363 (604) **CONJUGATED LINOLEIC ACID (CLA) ISOMERS DIFFERENTIALLY AFFECT TRIACYLGLYCERIDE METABOLISM IN RATS FED AT RECOMMENDED AND HIGH LEVELS OF DIETARY LIPIDS**

Paola Guadalupe Illesca[1], Jimena Verónica Lavandera[1,2], Carolina Daniela Gerstner[1,2], Marcela Aida Gonzalez[1], Claudio Adrián Bernal[1,2].

[1]Departamento de Ciencias Biológicas. Facultad de Bioquímica y Ciencias Biológicas. Universidad Nacional del Litoral. Ciudad Universitaria. Paraje El Pozo s/n. Santa Fe. Argentina. [2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Santa Fe. Argentina

Different nutritional studies in humans as well as in animal experimental models have demonstrated health benefits of CLA consumption. Nevertheless, some detrimental effects of CLA have been reported. The aim of this study was to evaluate the retention of CLA in different tissues and the potential effects on parameters related to the triacylglycerol (TAG) regulation at recommended and high levels of dietary lipids. Male Wistar rats were fed for 30 days with diets containing 7% or 20% lipid, supplemented or not with CLA (groups:C7-C20-CLA7-CLA20). We analyzed: serum and tissues CLA retention, serum and liver TAG levels, hepatic TAG secretion rate (TAG-SR) *in vivo*, enzymatic activities of: epididymal adipose tissue (EAT) and muscle lipoprotein lipase (LPL) activities and liver acetyl-CoA carboxylase (ACC), and the liver gene expression of: sterol regulatory element-binding protein-1c (SREBP-1c), fatty acid synthase (FAS), stearoyl-CoA desaturase-1 (SCD1), peroxisome proliferator-activated receptor-alpha (PPAR-α) and carnitine palmitoil transferase-Ia (CPT-Ia). CLA isomers were differentially retained in the samples analyzed (adipose tissue> liver> serum). CLA7 presented hypertriglyceridemia related to an enhanced TAG-SR. The increase of liver TAG in CLA7 group was associated with augmented ACC activity and reduced gene expression of PPAR-α and CPT-Ia. CLA did not potentiate the liver TAG accumulation observed in C20 related to an increased TAG-SR and lowered SCD1 and SREBP-1c expression. Supplementation with CLA showed different responses on lipid metabolism in rats depending on the dietary lipids levels. These results provide new knowledge about the mechanisms involved in TAG regulation by dietary fatty acid isomers.

364 **(617) EFFECTS OF THERMALLY OXIDIZED SUNFLOWER OIL AND CHOLESTEROL ON BONE MASS AND BIOMECHANICAL COMPETENCE, IN GROWING RATS**

Estefania Alsina[1], Andrea Ferreira Montero[1], Bozzini Clarisa[2], Margarita Olivera Carrion[3], Andrea Stranges[1], Patricia Boyer[2], Elisa Vanesa Macri[1], Silvia Friedman[1].

[1]Department of Biochemistry. Faculty of Dentistry.University of Buenos Aires. [2]Department of Physiology.Faculty of Dentistry.University of Buenos Aires. [3]Department of Bromatology. Faculty of Biochemistry. University of Buenos Aires

Previously we demonstrated the negative effect of consuming cholesterol-rich diets on serum lipids and bone quality. The present study investigated the effect of consuming thermally oxidized vegetable oil and cholesterol (chol) on bone mass and biomechanical competence, in rats. Male weaning Wistar rats (n=18;21±1d) were randomly assigned to 1 of 3 groups according to the lipid source supplemented a commercial diet (13% w/w). Rats were fed ad libitum for 8 wk. SFO received sunflower oil; SFOx, SFO oxidized frying oil at 180 °C for 40 hours and SFOx-chol (SFOx plus chol). Body weights and lengths (Wt and L) were recorded. At T=8wk, rats were euthanized. Total skeleton bone mineral content/Wt (BMC;mg/g), density (BMD;mg/cm2) and subareas: lumbar spine, tibiae and femur by DXA were determined. Serum total chol (mg%) and bone biomechanical competence (structural and geometrical properties and elasticity) were assessed. Results (mean ±SD.Anova-SNK). Hypercholesterolemia was found in SFO-chol, p<0.001. SFOx and SFOx-chol showed decreased final Wt (p=0.001), L (p=0.002) and BMD (p=0.001). BMC/Wt was low in SFOx-chol (p=0.038). Lumbar spine BMD was increased in SFO (p=0.013) but no differences were found in BMC/Wt (p=0.187). Femur BMD was higher in SFO (p=0.011) and BMC/Wt vs. SFOx-chol (p=0.025). No differences were found in BMD tibiae (p=0.139) but BMC/Wt was high in SFO (p=0.038). Structural (load-bearing capacity (Wf;p=0.001), yielding load (Wy;p=0.008) and diaphyseal stiffness (Wy/dy;p=0.000); geometric (cross sectional bone area; p=0.009, moment of inertia of the fracture section; p=0.001) and material properties (Young's modulus of elasticity; p=0.001) were negatively affected in SFOx groups. Conclusion: During growth, consuming thermally oxidized vegetable oil negative induce significant changes in bone mass and spacial distribution affecting the intrinsic bone quality, despite the presence of hypercholesterolemia. UBACyT: 20020130100506BA

365 (639) **INFLUENCE OF DIETS WITH DIFFERENT PROFILE OF UNSATURATED FATTY ACIDS ON DHA CONTENT AND CICLOOXIGENASES EXPRESSION IN BRAIN. EFFECTS OF TRANS FATTY ACID**

Marcela Aida González[1], Jimena Verónica Lavandera[1], Juliana Sain[1], Verónica Reus[3], Ana Clara Fariña[1], Larisa Carrera[2], Claudio Adrián Bernal[1].

[1]Bromatología y Nutrición – FBCB - Universidad Nacional del Litoral - Santa Fe – Argentina. Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) [2]Facultad de Ciencias Médicas. UNL. Santa Fe - Argentina

The levels of docosahexaenoic acid (DHA ,22:6n-3), araquidonic acid (AA, 20:4n-6) and anti and pro inflammatory lipids mediators derivated from these polyunsaturated fatty acid (PUFA) for the action of enzymes, like cicloxygenases are critical to the structure and normal brain function. Trans fatty acid (TFA) presents in the diet could be incorporated into neuronal membranes affecting metabolic process. The objective was evaluate in mice brain, the effect of diets with different composition of unsaturated FA with or without TFA, on the DHA content, TFA retention, cicloxygenases (COX_1, COX_2, and transcriptional factor PPARα mRNA levels, and also histological brain changes. Male CF1 mice (22g) were fed (120 days) with diets based on the AIN-93G (American Institute of Nutrition) with different MUFA relation n-3/n-6/n-9: Rappessed (R) (10.9/19.0/63.2), Corn (C) (0.9/53.3/31.3) and Olive oil (O); with or without 0,75% TFA: Rt, Ct and Ot, respectively. AG composition was determinated by gas chromatography and COX_1, COX_2 and PPARα mRNA levels, by real time PCR. Histological serial sections were stained with hematoxylin-eosine. Result were analyzed by One-Way ANOVA, followed by Scheffé (p<0.05). TFA retention was 0.96^a(Ot), 0.81^b(Ct) y 0.56^c(Rt). DHA levels (%), was higher in animals fed with R vs C and O (20% y 16%, p<0.05), and was lower the PUFA n-6/n-3 ratio (32% y 29%, p<0.05), respectively. COX2 mRNA levels diminished in groups fed O (55%) and R (46%) vs C. PPARα was increased in R (34%, p<0,05) vs O and C. The addition of TFA not modified the mRNA levels of these parameters. Histological sections showed lymphocytes presence in relation with the ependymal lining cells of the brain surface in animals fed O and C. The results obtained showed that the type of FA in the diet rather than TFA supplementation could modulate inflammatory response in brain.

366 (857) **PARACRINE CROSSTALK BETWEEN PERIVASCULAR ADIPOSE TISSUE AND SMOOTH MUSCLE CELLS IN FRUCTOSE FED- APOLIPOPROTEIN-DEFICIENT MICE**

Jimena Beatriz Cejas[1], Isabel María Quesada[1,2], Analía Redondo[1,2], Rodrigo Serrano[1], Claudia Magdalena Castro[1,2].

[1]Área Química Biológica, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo. [2]Laboratorio de Biología Vascular, IMBECU, CONICET,CCT Mendoza

Perivascular adipose tissue (PVAT) release growth factors and adipokines that could have direct paracrine effects on the underlying vessel. Type 2 diabetes can affect PVAT causing abnormal fat cells and infiltration of inflammatory cells, that produce an imbalance between PVAT derived factors. We set out to study the effect of PVAT secretomes obtained from C57BL/6 mice (WT), Apolipoprotein-E deficient-mice (ApoE-KO) and fructose- fed ApoE-KO mice on cultured vascular smooth muscle cells (VSMC). Secretomes were obtained from 400 mg of aortas adipose tissue in

1 ml of DMEM/F-12 at 37° C for 4 hours. Treatment with ApoEKO-PVAT secretomes showed a significant increase in VSMC proliferation compared to WT-PVAT secretomes (P <0.01). Oxidative stress was determined by a fluorometric probe. It was noted that either PVAT-secretome from WT or ApoE-KO mice produced a slight increase in reactive oxygen species (ROS) generation compared with the control group, while the secretome from fructose-fed ApoE-KO mice significantly increased oxidative stress compared to other groups (P<0.0001). These observed effects were greatly diminished when secretomes were incubated with SOD or mixture SOD + catalase, suggesting that secretomes already contain ROS that directly impact on VSMC. Our findings could indicate an active role of PVAT in the pathogenesis of cardiovascular diseases associated with insulin resistance and diabetes.

GENÉTICA / GENETICS

367 (354) **MOLECULAR CHARACTERIZATION OF GALT GENE IN CHILDREN WITH DECREASED GALACTOSE-1-PHOSPHATE URIDYLTRANSFERASE ACTIVITY**

Carolina Crespo[1], Hernan Eiroa[1], María Inés Otegui[1], Lilien Chertkoff[1], Luis Pablo Gravina[1].
[1]Hospital de Pediatría "Prof. Dr. Juan P. Garrahan"

Introduction: Classical galactosemia is an autosomal recessive inherited metabolic disorder caused by mutations in the galactose-1-phosphate uridyltransferase (*GALT*) gene. GALT enzyme deficiency leads to the accumulation of galactose-1-phosphate in various organs, causing hepatic, renal and cerebral impairment. Over 180 disease-causing mutations have been reported in the *GALT* gene. Diagnosis of galactosemia through analysis of total galactose and/or activity of GALT enzyme is effective, but environmental factors and the high frequency of the Duarte 2 (D2) variant may lead to false positive results, since D2 alleles cause partial deficiency of enzyme activity, meanwhile classical Galactosemia (G) alleles cause total deficiency. Objective: To describe molecular characterization of *GALT* gene in patients with decreased GALT activity, and to correlate molecular results with enzyme activity. Patients and Methods: Twenty four patients with enzyme activity below 9 µmol/h/g Hb (50% of normal value) were included. Q188R mutation was studied by PCR-RFLP. Samples negative or heterozygous for Q188R were sequenced by analysis of the 11 exons and the exon-intron boundaries of the *GALT* gene. Results: Ten different sequence variations were identified, including two novel mutations (p.M1T and p.S222R). The three most common disease-causing mutations were p. Q188R, p.K285N and IVS8-13A>G. They accounted for 16, 9 and 3 of the 48 alleles respectively. N314D Duarte 2 variant appeared in 13 of the 48 alleles. *GALT* genotype correlated with enzyme activity in 90% of patients. Conclusion: This is the first report of mutations in the *GALT* gene in Argentinean patients with decreased GALT activity. Molecular analysis is useful to reduce false positive results, distinguishing D2/G mixed heterozygotes from classical galactosemia G/G homozygotes. This study supports the importance of including the molecular analysis of *GALT* gene in the diagnostic algorithm of galactosemia.

368 (324) **GENOMIC STUDY OF MITOCHONDRIAL DNA AND NUCLEAR GENES CODING FOR MITOCHONDRIAL PROTEINS IN PROSTATE CANCER**

Federico Pablo Cantor[1], Conrado Marco Olivieri[1], Geraldine Gueron[1], Elba Vazquez[1], Javier Cotignola[1].
[1]Laboratorio de Inflamación y Cáncer, IQUIBICEN/Dpto. de Química Biológica, CONICET/FCEN-UBA

Prostate cancer (PCa) is the second most frequently type of cancer and the sixth cause of cancer-related deaths in men. Prognostic markers for disease outcome include the Gleason score, TNM stage, pre-surgical PSA and prostatectomy margin involvement. However, these parameters fail to identify some aggressive tumors and patients with good clinical prognosis still recur and develop metastasis. Several studies show that tumors display mitochondrial dysfunction and that the mitochondrial DNA (mtDNA) is frequently mutated. We aim to study mitochondrial alterations at genomic level using public data from RNAseq and expression microarrays in order to identify molecular markers that improve PCa prognosis. We observed significant differential expression between Gleason scores 3+4 and 4+3 (FECH, ACACA, LDHD). We also found 268/1158 nuclear genes coding mitochondrial proteins differentially expressed between tumors from patients with and without biochemical relapse (e.g. SLC25A1, FIS1). In order to determine whether the analysis of gene expression improves PCa relapse prediction, we performed ROC curves using current clinico-pathological prognostic features and included the 5 most significant differentially expressed genes. The Area Under Curve (AUC) for the clinico-pathological markers was 0.81. The inclusion of gene expression increased the AUC to 0.98. The analysis of mtDNA mutations showed that 55% of tumors with Gleason $\geq$7 had mutations in COX2, while none of the tumors with Gleason<7 had COX2 mutated (p=0.02). Data from gene expression microarrays showed significant differential expression between adjacent non-tumoral and healthy normal tissues (RDH11, AGR2), primary tumor and healthy normal tissue (PINK1, SLC25A4), and metastasis and primary tumor (ATP5A1, AUH). These results demonstrate that mtDNA and nuclear genes coding for mitochondrial proteins are altered during PCa development and progression, and that the analysis of gene expression might improve PCa prognosis.

369 (332) **BAALC EXPRESSION AS A PREDICTOR OF PRIMARY RESPONSE TO TREATMENT IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA**

Mercedes Abbate[1], Daiana Leonardi[1], Javier Nahuel Brandani[1], Geraldine Gueron[1], Elba Vazquez[1], Javier Cotignola[1].
[1]IQUIBICEN, CONICET / DPTO. QUÍMICA BIOLÓGICA, FCEN, UBA

Current treatment protocols for Acute Lymphoblastic Leukemia (ALL) include an initial stratification of patients into groups of risk for disease relapse in order to receive the most appropriate treatment protocol and to reduce the number of relapses and therapy failure. This stratification is based on biochemical and cytogenetic parameters at diagnosis, and the early response to treatment determined by the minimal residual disease. However, there are still some patients that recur in all risk groups. Many studies suggest that the inclusion of gene variants and expression profiling during initial stratification is a promising field. It has been reported an association between BAALC (brain and acute leukemia, cytoplasmatic) over-expression and poor prognosis in Acute Myeloid Leukemia. Thus, we aimed to evaluate the prognostic value of BAALC in childhood ALL. We studied the expression levels of AALC using public repositories of expression microarrays and a Bioconductor-based analysis. All studies included pediatric ALL samples. In two studies (GSE39339, GSE141618) we found that poor/non-responders (>25% of blast in bone marrow (M3) after the first cycle of chemotherapy) expressed higher BAALC levels at diagnosis (fold-change (FC)=5.3; p=0.002; and FC=3.1; p=0.01; respectively) compared with good responders (bone marrow M1/M2). In addition, patients who failed to achieve complete remission during induction showed a 4-fold increase in BAALC expression compared to patients who reached complete remission and relapsed within 4 years (p=0.01). In other studies (GSE19143, GSE5820, GSE646_647) that analyzed if primary ALL samples were sensitive or resistant to ex vivo treatment with glucocorticoids we observed a trend for higher BAALC expression in resistant samples (FC=1.6; p=0.05). These results suggest that the inclusion of BAALC expression profile at diagnosis of childhood ALL could improve the initial stratification and, in consequence, improve therapy response and survival.

370 (349) **DMD GENE SMALL MUTATION SCREENING BY WHOLE EXOME SEQUENCING**

Leonela Natalia Luce[1,2], Diana Parma[2], Alberto Penas Steinhardt[3,4], Irene Szijan[2], Florencia Giliberto[1,2].
[1]Universidad de Buenos Aires. CONICET. Instituto de Inmunología, Genética y Metabolismo (INIGEM). Buenos

Aires, Argentina. [2]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica. Departamento de Microbiología, Inmunología y Biotecnología. Cátedra de Genética. Buenos Aires, Argentina. [3]Universidad de Buenos Aires. CONICET. Instituto de Estudios de la Inmunidad Humoral (IDEHU). Buenos Aires, Argentina. [4]Universidad Nacional de Lujan. Departamento de Ciencias Básicas. Luján, Buenos Aires, Argentina

Duchenne Muscular Dystrophy (DMD) and Becker Muscular Dystrophy (BMD) are X-linked genetic diseases caused by mutations in the *DMD* gene. DMD is a severe dystrophy that affects 1:3500 born males, whereas BMD is less severe and affects 1:18000. Molecular alterations responsible for DMD/DMB are gross deletions and duplications in 70% of cases and small mutations in the remaining 30%. While large rearrangements are identified by MLPA, point mutations are detected by gene sequencing. This study aimed to detect small mutations in the *DMD* gene by Whole Exome Sequencing (WES) in 9 boys with presumptive clinical diagnosis of DMD/DMB and a woman obligate carrier. WES was performed by Macrogen service and the pathogenicity of the identified variants was determined according to: its presence in a DMD mutation database; its absence in results of sequence consortiums such as 1000genomes and Hapmap; and online predictive softwares (polyphen, SIFT and Mutationtaster). We have found in the affected boys a range between 7-18 sequence variants in the *DMD* gene, when in the only woman studied we detected 33. We were able to identify the disease causing mutations with 100% certainty in 9 cases which consisted in 5 nonsense mutations, 1 frameshift deletion, 2 frameshift duplications and 1 altering a donor consensus splicing site. In 2 of these cases, we also found a missense mutation predicted to have a negative impact on the dystrophin protein. In the remaining case, we have identified an in-frame deletion determined as a Variant of Uncertain Significance (VUS). Finally, we can conclude that this screening methodology was able to detect small mutations in the *DMD* gene in 9/10 individuals, allowing confirmation of the diagnosis in the boys. Further studies should be performed in order to establish the pathogenicity of the VUS. The importance of this work relies on the fact that is the first one applying WES for DMD/DMB molecular diagnosis in Argentina.

371 (483) **THE ASSOCIATION BETWEEN THE KLF14 RS4731702 SNP AND THE LIPID PROFILE IN TYPE 2 DIABETES MELLITUS PATIENTS: A STUDY IN SAN LUIS CITY, SAN LUIS, ARGENTINA**

Micaela Fernanda Alvarez[1], Miriam Vasquez Gomez[1], Irma Inés Gonzalez[1], Gustavo Fernández[1], Susana Siewert[1].
[1]Universidad Nacional de San Luis

Type 2 diabetes mellitus (T2DM) is a chronic disease also known as non-insulin-dependent diabetes. It accounts for more than 90% of all diabetes cases in the global population. A cluster of metabolic disturbances that include insulin resistance, abdominal obesity, hyperglycemia, hypertension, and dyslipidemia are the hallmark of T2DM. The single nucleotide polymorphism (SNP) rs4731702 in the KLF14 transcription factor gene has been associated with T2DM and HDL-c concentrations. The present study was aimed at determining the distribution of rs4731702 SNP genotypes and evaluate its association with serum lipid profile in T2DM patients in a population at San Luis province, Argentina. A total of 73 volunteers (26 T2DM patients and 47 healthy controls) participated in this study. Biochemical measurements were done with commercial enzymatic kits Based on the GenBank sequence of human *KLF14*, accession number NT_007933.15, we designed a pair of outer primers and two allele-specific inner primers using the Tetra Primer ARMS-PCR, in a free access web (http://cedar.genetics.soton.ac.uk). The KLF14 genotypes distribution among study groups was not significantly different. In T2DM patients, there was a difference in HDL-c values and TC/HDL-c ratio, with T carriers of the rs4731702 SNP (non C/C) having higher HLD-c and lower TC/HDL-c ratio values than C/C homozygotes (p=0.0047 and 0.0041, respectively). HDL-c levels were higher in diabetic patients, without dyslipidemia, carring the genotype non-C/C (p=0.023). Conversely, TC and LDL-c levels were lower in diabetic patients, without dyslipidemia, in non-C/C carriers (p<0.001). The results of the current study showed that T2DM patients having the C/C genotype of KLF14 (rs4731702) may have a worsen insulin resistance and be susceptible to more serious pathophysiological consequences regarding lipid metabolism. The genotyping of the rs4731702 can be of high predictive and interventional value of cardiovascular complications in T2DM.

372 (568) **A MULTISTEP APPROACH FOR THE ANALYSIS OF HEREDITARY HEARING LOSS GENES IN DEAF PATIENTS: LOOKING FOR A NEEDLE IN A HAYSTACK**

Viviana Karina Dalamón[1], Paula Ines Buonfiglio[1], Vanesa Lotersztein[2], Ernesto Goldschmidt[3], Ana Belen Elgoyhen[1].
[1]Instituto de Investigaciones en Ingeniería Genética y Biología Molecular, INGEBI / CONICET. [2]Servicio de Genética, Hospital de Clinicas "José de San Martín". [3]Servicio de Genética, Hospital General de Agudos "Dr. Juan A Fernandez"

Hereditary Hearing Loss (HHL) is a common trait affecting 1 in 2000 children. It is characterized by the presence of a large genetic heterogeneity, and to date, over 100 different genes have been identified worldwide. In order to overcome this problem, we designed a multistep strategy: Step 1) screening of frequent mutations in 12 HHL genes by direct sequencing; Step 2) screening of 120 HHL genes by targeted -sequencing (TS); Step 3) validation of identified mutations by *in-silico* studies and design of functional *in-vivo* analysis. A total of 1150 samples were analyzed; 600 from non-syndromic unrelated Argentinean deaf patients and 550 from relatives and siblings. The first step of the study consisted in investigating and reporting the spectrum and frequency of reported mutations in *GJB2, GJB6, OTOF, MT-RNR1, PJVK, TECTA, EYA4, EYA1, SIX1, TMC1, COL4A5* and *COCH* genes in deaf patients from Argentina. In step 2, 7 samples were candidates for following analysis through a commercial optimized platform of massive sequencing for exons of 120 genes known to cause HHL. After genome variants annotation, data were filtered according to quality value, allele frequency, pathogenicity prediction and conservation score. Direct sequencing allowed us to detect 44 different sequence variations in 252 of the 600 patients (42%) located in genes *GJB2, GJB6, OTOF and EYA1.* TS and massive sequencing of 120 HHL genes in 7 samples showed 37 variations in 22 different genes. All of the patients were positively characterized leading to the identification of pathogenic variants. Nevertheless, as many genes were analyzed, in some patients we detected 2, 4 or 8 mutated genes, making genotype/phenotype relationship difficult to assess. We show in the present study some clearcut results, others that are uncertain, and also a third set requiring further analysis or subsequent functional studies to establish which mutations underlay the pathology. Functional studies of some of the identified mutations using Zebra fish models followed by an accurate phenotypic characterization are under way. These findings clearly highlight the importance of genetic studies followed by in silico and in-vivo validation to better understand the genetic basis of HHL.

373 (587) **TOWARDS THE GENERATION OF A DATABASE DESIGNED FOR HEALTH SPECIALISTS: THE 21-HYDOXYLASE DEFICIENCY AS A CASE STUDY**

Leandro Simonetti[1], Carlos D. Bruque[1, 2], Francisco Pisciottano[2], Cecilia S. Fernández[1], Alejandro Nadra[3], Liliana Dain[1, 2].
[1]Centro Nacional de Genética Médica, ANLIS, Buenos Aires, Argentina. [2]Instituto de Biología y Medicina Experimental, CONICET, Buenos Aires, Argentina. [3]Departamento de Química Biológica Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, IQUIBICEN-CONICET, Buenos Aires, Argentina

Linking the effects of genetic variations (GVs) to their functional outcomes is a major issue in bioinformatics, especially now that human next-generation sequencing projects generate millions of previously unknown single nucleotide variants. These GVs can

be related to many pathological conditions and may influence susceptibility to disease and drug treatment. GVs are compiled in databases (DBs) that offer useful information, but are mostly aimed at investigators, lacking data regarding the effects of GVs in human health. Also, many times these effects are unknown because they get masked by heterozygosity. That's why over the years, many tools have been developed to predict the effect of variations. Towards the generation of a DB designed for health specialists in the human genetics field, we decided to start compiling all the sequence, experimental and predictive information regarding the GVs of the *CYP21A2* gene, which encodes the enzyme 21-hydroxylase. The deficiency in this enzyme accounts for 90-95% of the congenital adrenal hyperplasia cases, and has been throughly studied in our laboratory. The most comprehensive DB regarding the clinical effects of variations in the *CYP21A2* gene is CYPallels, which contains 202 GVs, but hasn't been updated since 2010 and mostly accounts for GVs that come from clinical cases. To update and expand this data, we explored different on-line DBs as well as our own data, and found ~750 GVs, with 532 reported after 2010. From the total of GVs, ~350 affects the coding region and ~400 are found in introns and untranslated regions. An effect on human health is reported for only ~240 DVs, classified as benign or as related to a form of adrenal hyperplasia. The tools developed to acquire and clean-up the data for *CYP21A2*, will be used to build a DB which, in conjunction with state of the art prediction tools, will offer health professionals useful information regarding genetic disorders.

374 (702) **CGH ARRAY: EXPERIENCE IN THE DIAGNOSIS OF PATIENTS WITH CONGENITAL ANOMALIES WITH AND WITHOUT INTELLECTUAL DISABILITIES**

María Florencia Cantarella[1], Mariana Capelli[1], Mariana Samara[1], Nazareth Loreti[1], Moya Graciela[1], Verónica Ferreiro[1].
[1]Genos S.A.

Congenital anomalies represent 1 to 3% of newborn, and in Argentina are de first and second leading cause of infant mortality according to region. They also contribute to a significant percentage (4-35%) of birth defects and intellectual disabilities. CGH Array is a molecular test which detects clinically significant imbalances of the human genome, by comparative genomic hybridization (CGH). This study allows to describe changes in the number of copies of sequences larger than 100Kb and exonic changes in selected genes of the nuclear genome, detect deletions in the mitochondrial genome and analyze up to 120K SNPs. In order to establish the diagnosis of patients with congenital anomalies with previous inconclusive studies, 300 DNA samples were analyzed. For the analysis it was used the test developed by Kleberg Cytogenetics Laboratory del Baylor College of Medicine, using v8.1.1.4x180K and v8.3.2x400K+SNPs slides manufactured by Agilent Technologies. The analysis of results was adviced by the authorities of Baylor Miraca Genetics Laboratory. We found genetic alterations in a total of 118 patients (39.3%). In 55 patients the alteration were pathogenic, in 55 were variants of uncertain significance and in 10 patients the alterations were benign. CMA technique allowed detection and characterization of genetic alterations in a large number of cases with congenital anomalies, therefore enabling the correct diagnosis and genetic counseling of the families involved.

ONCOLOGÍA II/ ONCOLOGY II

375 (770) **HUMANIN INCREASES THE RESISTANCE OF BREAST CANCER CELLS TO CHEMOTHERAPY**

María Florencia Gottardo[1], Mariela Alejandra Moreno Ayala[1], Mercedes Imsen[1], Antonela Asad[1], Matias Luis Pidre[2], Victor Romanowski[2], Marianela Candolfi[1], Adriana Seilicovich[1].
[1]Instituto de Investigaciones Biomédicas (INBIOMED, UBA-CONICET), Facultad de Medicina, Universidad de Buenos Aires [2]Instituto de Biotecnología y Biología Molecular (IBBM, CCT-CONICET - LA PLATA) Centro científico tecnológico, La Plata

Humanin (HN) is a mitochondrial derived peptide with potent cytoprotective action in many cell types. It has been described that endogenous and exogenous HN can protect normal tissues against toxic effects of chemotherapy. However, the role of this peptide in tumor development is not well known. We have previously shown that HN is expressed in murine mammary carcinoma cells (4T1) and exerts antiapoptotic and mitogenic actions against various insults such as serum deprivation or TNF-α. The aim of this study was to investigate whether HN affects the sensitivity of tumor cells to chemotherapeutic agents. HN (10 μM) hampered the antiproliferative effect of doxorubicin (DOXO, 500 nM) on 4T1 cells in vitro (by ELISA BrdU; C=0.55±0.04; DOXO=0.34±0.02*, HN=0.52±0.02, DOXO+HN=0.53±0.01, *p<0.05, ANOVA). Also, we evaluated the in vivo effect of HN on tumor progression in BALB/c mice bearing established 4T1 sc tumors. Mice received 1 injection/w. of DOXO (100 μg/mouse) and 3 injections/w. of HN (10 μg/mouse) for 2 w. HN per se increased tumor growth and inhibited the antiproliferative effect of DOXO treatment (p<0.05). Also, HN administration increased the number of lung metastasis (p<0.05, ANOVA follow by Tukey). In addition, we detected HN expression in human breast carcinoma cells (MDA-MB-231), where HN inhibited the antiproliferative effect of DOXO in vitro (C=0.46±0.02; DOXO=0.20±0.01*, HN=0.51±0.02, DOXO/HN=0.30±0.01, *p<0.05, ANOVA). We also evaluated the action of endogenous HN using a specific HN shRNA in a clonogenic assay. Inhibition of endogenous HN decreased the clonogenic capacity of MDA-MB-231 cells incubated in presence of DOXO. Our results show that HN increases the resistance of both human and murine mammary tumor cells to a chemotherapeutic drug, suggesting that HN could be a therapeutic target in the cytotoxic treatment for breast cancer.

376 (724) **THERAPEUTIC BLOCKADE OF FOXP3 IN A MURINE BREAST CANCER MODEL**

Mariela Alejandra Moreno Ayala[1], Maria Florencia Gottardo[1], Mercedes Imsen[1], Antonela Sofia Asad[1], Flavia Zanetti[2], Elisa Bal de Kier Joffé[3], Noelia Casares[4], Juan Jose Lasarte[4], Adriana Seilicovich[1], Marianela Candolfi[1].
[1]Instituto de Investigaciones Biomédicas (INBIOMED, UBA-CONICET), Facultad de Medicina, Universidad de Buenos Aires [2]Instituto Cesar Milstein (CONICET) [3]Instituto de Oncología Angel H. Roffo [4] Universidad de Pamplona, Navarra, España

Regulatory T cells (Tregs) have been involved in the relatively low efficacy of antitumor vaccines in cancer patients. Our previous results indicate that prophylactic antitumor dendritic cell (DC) vaccines improve the survival of murine models of breast cancer. However, DC vaccines administered to tumor-bearing mice induce the expansion of Tregs and lack efficacy in this therapeutic setting. We aimed to improve the antitumor effect of DC vaccines using a cell penetrating peptide (P60) that inhibits Foxp3, a transcription factor required for Treg function. Mice bearing established LM3 or 4T1 breast tumors were treated sc with a DC vaccine loaded with tumor cell lysate and stimulated with CpG. Mice were daily treated with ip injections of P60 or a control peptide. We found that although therapeutic DC vaccines alone did not affect tumor growth, P60 alone or in combination with DC vaccines significantly reduced tumor growth rate (p<0.05). While mono-therapy led to long-term survival in less than 30% of the animals, over 70% of mice treated with vaccine+P60 survived 100 d after tumor inoculation (p<0.05). As we already described expression of Foxp3 in breast tumor cells, we evaluated whether the antitumor effect of P60 was an immune-mediated or a direct antitumor effect. In immuno-compromised animals bearing established tumors, we found that daily treatment with P60 for 7 d significantly delayed tumor growth (doubling time: C: 5 d, P60: 6 d, p<0.05), suggesting that Foxp3 plays a trophic role that facilitates tumor progression. However, P60 did not elicit long-term survival in these mice, suggesting that the immune-mediated effects of the treatment are required for therapeutic efficacy. Our results suggest that Foxp3 blockade could have a dual therapeutic

benefit on tumor regression when used in combination with antitumor vaccines, both by inhibition of Treg function as well as through a direct antitumor effect in Foxp3-expressing tumor cells.

377 (196) **FGFR2 MODULATES THE METASTATIC POTENTIAL OF BREAST CANCER CELLS**

María Sol Rodriguez[1], Isabel Lüthy[1], Claudia Lanari[1], Cecilia Pérez Piñero[1].
[1]Instituto de Biología y Medicina Experimental

We have previously shown that FGF2 activates FGFR2 and progesterone receptors (PR) increasing the proliferation of breast cancer cells suggesting that the activation of the FGF-FGFR axis might be important in the acquisition of hormone independence. It has been reported that FGF2 induces RUNX2 expression and high levels of RUNX2 were detected in mammary carcinomas which acquired a hormone independent phenotype. The aim of this study was to evaluate the effect of constitutively active FGFR2 on a human breast cancer cell line. IBH-6 cells express estrogen receptor (ER), PR and FGFR 1-4, RUNX2 and they grow in vivo in NSG mice without hormone supply giving rise to invasive carcinomas. We have already shown that IBH-6 cells with silenced FGFR2 give rise to smaller tumors, showed a less aggressive phenotype, a lower proliferation index and a decrease in CCND1 and RUNX2 expression compared with control tumors. In this study we transfected IBH-6 cells with a constitutively active FGFR2 (R2CA) or with a control plasmid. Control cells showed a higher proliferation index in vitro when treated with FGF2 ($p<0.05$), and a diminished proliferation index when treated with an FGFR inhibitor ($p<0.001$). R2CA cells did not respond to either treatment. However, an increase in RUNX2 expression and in the pERK/ERK ratio was observed in these cells as compared to control cells. In addition, 3.10^6 experimental or control cells were s.c. inoculated into the flank of NSG mice. The growth rate was similar for both xenografts, but lung metastases were only detected in animals bearing R2CA tumors. We propose that FGFR2 activating the RUNX2 pathway increases tumor progression. The results reported here emphasize the use of FGFR2 inhibitors in combination with standard therapy in breast cancer.

378 (215) **CYCLIN A EXPRESSION IN EXPERIMENTAL MODELS OF BREAST CANCER WITH DIFFERENT LEVELS OF PROGESTERONE RECEPTOR ISOFORMS**

Marina Ayre[1], Melina E. Bilinski[1], Britta M. Jacobsen[2], Claudia Lanari[1], Victoria T. Fabris[1].
[1]Instituto de Biología y Medicina Experimental, Buenos Aires [2]University of Colorado Anschutz Medical Campus, Colorado, USA

Deregulation of cyclins expression is frequently found in breast cancer and has been associated with aneuploidy. We have previously reported an overexpression of cyclin A in two aneuploid antiprogestin resistant tumors derived from C4-HD, a mammary carcinoma induced by medroxyprogesterone acetate (MPA) that shows a progestin dependent pattern of tumor growth and high levels of progesterone receptors (PR). Taking into account that antiprogestin sensitive tumors show higher levels of isoform A of progesterone receptor (PRA) than isoform B (PRB) and that antiprogestin resistant variants show the opposite ratio, we decided to study the expression of cyclin A in other experimental models of breast cancer bearing different ploidy levels and different PRA/PRB ratios. An increase in cyclin A expression was observed by Western blots in the acquired resistant variant C7-2-HIR and in the constitutive resistant C7-HI tumor (1.7 fold and 1.5 fold, respectively) compared to the responsive tumor C7-2-HI ($p<0.05$). The immunohistochemical (IHC) analysis of paraffin sections of the tumors confirmed these results, showing a higher percentage of cyclin A positive nuclei in both resistant tumors, suggesting an association of cyclin A expression with endocrine resistance and with the PRA/PRB ratio. Next we evaluated the expression of cyclin A in T47D human breast cancer cells that express both PR isoforms and in T47D-YA or T47D-YB cells that express either PRA or PRB respectively. Immunofluorescence studies show an increase in cyclin A nuclear expression in T47D-YB cells as compared to T47D cells ($p<0.001$) or T47D-YA ($p<0.01$) respectively. Similar results were observed by IHC in paraffin sections of xenografts derived from these cells inoculated into NSG mice. These results suggest an association between cyclin A expression and PR isoform ratio. Studies are in progress to determine the interplay between both signaling pathways.

379 (39) **MECHANISMS UNDERLYING THE ANTITUMOR EFFECTS OF CHEMO GENE THERAPY IN HUMAN MELANOMA**

Chiara Fondello[1], Lucrecia Agnetti[1], Gerardo Glikin[1], Liliana M. E. Finocchiaro[1].
[1]Instituto de Oncología Ángel H. Roffo

Malignant melanoma is an extremely aggressive form of skin cancer whose incidence continues to increase worldwide. Here, we established and characterized three human melanoma cell lines derived from surgically excised melanoma tumors, to evaluate the therapeutic potential of the combination of bleomycin (BLM) with human interferon-β (hIFNβ) gene or herpes simplex virus thymidine kinase/ganciclovir suicide gene (SG) lipofection. After acridine orange/ethidium bromide (AO/EB) staining, double stained cells (yellow) were found after hIFNβ or SG lipofection. Control lipofection (βgal) of BLM produced late apoptotic/necrotic events, which enhanced with GS/BLM and hIFNB/BLM treatments. The loss of cell membrane integrity produced by our treatments was confirmed by flow cytometry. Through DNA damage determination, we found that our treatments incremented the subG_0 fraction confirming apoptosis/necrosis. This genotoxicity exhibited a direct correlation with the significant rise of the intracellular reactive oxygen species (ROS) levels. In addition, ROS generation correlated ($p < 0.01$) with the percentage of cells with low mitochondrial membrane potential ($\Delta\psi$m). A significant correlation was found between the cytotoxic effect of chemo – gene therapy and the fraction of cells exhibiting: i) cell membrane compromise ($p < 0.01$), ii) DNA damage ($p < 0.005$), iii) high ROS levels ($p < 0.05$) and iv) low $\Delta\psi$m ($p < 0.01$). These results, in addition to the fact that the cytotoxic effects could not be reverted with antioxidants (L-NAC), suggest that BLM and genetic treatments induced a mitochondria-dependent apoptosis/necrosis, by both, depolarizing mitochondria and activating a sustained increase in ROS generation. The three human melanoma cell lines presented response diversity to our chemo-gene treatments. However, a pattern emerging from our findings strongly suggeststhat, compared to a single treatment, the combination of gene transfer and BLM may have greater antitumor efficacy.

380 (199) **DECREASED EXPRESSION LEVELS OF RAC3 COACTIVATOR SENSITIZES COLORECTAL CANCER CELLS TO THE EFFECT OF CHEMOTHERAPEUTIC DRUGS**

María Cecilia Lira[1], Francisco Damián Rosa[1], Laura Carolina Panelo[1], Pablo Javier Azurmendi[2], Alejandro Fabián Celía[2], Leonardo Paz[2], Adrián Sambresqui[2], M Virginillo[2], Juan B Palmitano[2], Cecilia Salazar[2], Mónica Alejandra Costas[1], María Fernanda Rubio[1].
[1]IDIM CONICET-UBA [2]Instituto de Investigaciones Médicas Dr. Alfredo Lanari UBA

Colorectal cancer (CRC) is one of the most commonly diagnosed cancers with high prevalence. Our group studies the role of RAC3 coactivator in tumor development and previously demonstrated that RAC3 overexpression contributes to cell proliferation, inhibition of apoptosis and autophagy. The aim of this study was to evaluate the RAC3 expression levels in different CRC cell lines and determine their sensitivity to chemotherapeutic drugs. The expression levels of RAC3 were determined by qPCR and Western blot in three human CRC cell lines (HT29, HCT116 and LoVo). RAC3 expression was higher in HT29> HCT116> LoVo (44> 9.6> 1-fold respect LoVo by qPCR). Once characterized the RAC3 expression levels, we studied sensitivity to drugs used to treat CRC such as 5-fluorouracil (5FU 0-200 mM) and oxaliplatin (Oxa 0-100 mM). Cell viability was determined by crystal violet staining and the EC50 was calculated for each cell type

(Oxa: EC50 HT29 0.85±0.2 mM, HCT116 0.6±0.3 mM and LoVo 0.05±0.02 mM; 5FU: EC50 HCT116 4.5±0.6 mM, LoVo 0.62±0.2 mM, HT29 did not respond to treatment with 5FU in the doses used). We observed that Lovo were more sensitive to treatment with these drugs. To study whether sensitivity observed in the different CRC lines was due to the expression levels of RAC3, the HCT116 cell line was transfected with an shRNA for RAC3 (shRAC3) and performed qPCR to validate the knockdown efficiency (shRAC3 0.08-fold respect HCT116 control). Compared to the control, the shRAC3-transfected group displayed significantly decreased viability (5FU: control 4.5±0.6 mM vs shRAC3 2.4±0.2 mM and Oxa: control 0.6±0.3 mM vs shRAC3 0.18±0.1 mM). In conclusion, our results show that the expression levels of RAC3 influence sensitivity to chemotherapeutic drugs. Therefore, the knowing of the RAC3 expression levels in tumoral samples could be probably important in order to design new improved therapeutic strategies.

381 (287) **SPHINGOLIPID CERAMIDE-1-PHOSPHATE (C1P) PROTECTS AGAINST CYCLOPHOSPHAMIDE-INDUCED TOXICITY AND DEPLETION OF MACROPHAGES**

Natalia Pascuali[1], Leopoldina Scotti[1], Mariana Di Pietro[1], Marta Tesone[1,2], Griselda Irusta[1], Dalhia Abramovich[1], Fernanda Parborell[1], Antonio Gomez Muñoz[3].
[1]Instituto de Biología y Medicina Experimental (IByME-CONICET) [2]Departamento de Química Biológica, Fac. Ciencias Exactas, Universidad de Buenos Aires. [3]Departamento de Bioquímica y Biología Molecular, Fac. Cs. y Tecnología, Universidad del País Vasco (UPV/EHU), Bilbao, España

Gonadotoxicity is a well-known side effect of alkylating agents such as cyclophosphamide, which cause oocyte and follicular destruction. They are also immunosuppresors, leading to depletion of immune cells like macrophages, which are the most frequent immune cells in the ovary and modulate follicle development, ovulation, and luteinization. We have previously shown that the bioactive sphingolipid C1P, which has a proangiogenic, anti-apoptotic role, can protect ovarian cells from cyclophosphamide-induced toxicity in an in vivo model. Hence, we propose it as a potential protective molecule for macrophages as well. We studied whether ceramide-1-phosphate can promote macrophage protection against damage due to cyclophosphamide. We used an in vitro approach, employing J774A.1 macrophages and the activated metabolite of cyclophosphamide, perfosfamide. Cells were treated with perfosfamide (2–20 μM) and/or C1P (2-8 μM) for different time points. Cell viability was estimated via the MTS colorimetric assay and the Trypan Blue exclusion method. Cell death was evaluated by FACS analysis of Annexin V/PI staining and IL-6 production was assessed by ELISA. Cytotoxicity by MTS test was found to be dose-dependent. Marked cytotoxic effects were observed at perfosfamide concentrations from 5 μM to 20 μM, while C1P (4-8 μM) increased cell viability in perfosfamide-treated macrophages ($p<0.05$). The IC50 of perfosfamide was calculated (7.5 μM). The Trypan Blue assay confirmed that C1P partially blocks the perfosfamide-induced decrease in viability ($p<0.05$). We observed by FACS analysis that macrophages undergo apoptosis when incubated with perfosfamide, while C1P partially protects them from this effect ($p<0.05$). IL-6 production was not detected in supernatants of perfosfamide-treated macrophages nor in untreated cells at 6 hs. This is the first study that shows that C1P is involved in the protection of macrophages under chemotherapy. Since their role is essential for ovarian function, preserving them could provide a novel strategy against gonadotoxicity.

382 (315) **TRANSLATIONAL MEDICINE: PRECLINICAL DEVELOPMENT AND OPTIMIZATION OF NEW ANTITUMOR AGENTSCA**

María Virginia Giolito[1], Patricia Cornier[4], Carina Delpiccolo[4], Dora Boggián[4], Mauricio Menacho-Márquez[1,2], Leandro Ernesto Mainetti[1,2], O.Graciela Scharovsky[1,2,3], Ernesto Mata[4], Viviana Rosa Rozados[1,2], María José Rico[1,2].
[1]Instituto de Genética Experimental/IGE, Facultad de Ciencias Médicas, UNR [2]CONICET [3]CIC-UNR [4]Facultad de Química, Bioquímica y Farmacia. UNR. IQUIR-CONICET. Rosario, Argentina

The development of new antitumor agents is, presently, a demand for the public health due to the demonstrated side effects of the known antineoplastic drugs, to the development of drug resistance and, also, to the lack of effective therapies for some types of cancer and for the treatment of metastasis. The purpose of this study was to evaluate the antitumor activity of the synthesised compounds (aminoacyl/peptidyl penicilines, dihidroisocumarines, propagilamines, stilbenes, -lactamase and oxadiazoles) and their mechanisms of action. The inhibition of the proliferation, measured with the tetrazolium salt WST-1 reagent, of the murine 4T1 mammary carcinoma cell line was evaluated by incubating them for 48 h with RPMI medium+10%FBS in the presence of decreasing concentrations (100 to 10 μM) of the compounds. According to the obtained results, there were chosen 6/44 compounds: 22i, 266, 171, 4210, 3151 and 6, which have shown antiproliferative effect, of, at least, 50% with respect to the control, at the lowest doses (25 and 10 μM). It was evaluated the migratory capacity by scratch-wound assays of 4T1 cells in the presence of the chosen compounds. Compared to the control group without treatment, 4T1 cells showed a lower migratory capacity when cultured during 4 h with 22i compound ($P<0.05$), and during 7 h with 22i, 4210, 171 ($P<0.001$) and 266 ($P<0.01$). In preliminary assays, the apoptosis/necrosis of the treated cells was determined by flow cytometry. Low doses of the compounds 22i, 171, 4210 and 6 showed higher% of apoptotic cells with respect to the control cells. These encouraging results prompted us to study the effect of the compounds on cell lines of different tumor types and/or different origin (human). Also, we are designing in vivo studies in order to test both, the therapeutic effect and the toxicity of the treatment. The chosen compounds would be promising agents for continuing the preclinic studies in the way to a putative translation to the clinic.

383 (425) **SOLUBLE GUANYLYL CYCLASE ALPHA1 SUBUNIT IS A NOVEL KEY MEDIATOR OF PROLIFERATION IN SEVERAL ESTROGEN-DEPENDENT HUMAN TUMOR CELL LINES**

Agustina Gurruchaga[1], Sonia Alejandra Ronchetti[1], Georgina Cordeiro[1], Analía Gabriela Ricci[2], Beatriz Haydée Duvilanski[1], Jimena Paula Cabilla[1].
[1]INBIOMED (UBA-CONICET), Facultad de Medicina, Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina. [2] IByME-CONICET, Vuelta de Obligado 2490, Ciudad Autónoma de Buenos Aires, Argentina

The nitric oxide receptor soluble guanylyl cyclase (sGC) is a heterodimer composed by two subunits, alpha and beta, and catalyzes cGMP formation. We have shown that E2 exerts opposite effects on these sGC subunits, increasing both alpha1 (a1) levels and decreasing beta1 (b1) expression in vitro and in vivo. Besides, a1 increase has been strongly associated with cell proliferation and tumor progression in androgen-dependent tumor cell line LNCaP. On the other hand, b1 would be involved in cell cycle arrest and high levels of b1 are associated with better prognosis in breast cancer. The aim of the present work was to investigate the role of sGC a1 subunit in cell proliferation. Estrogen-responsive breast cancer MCF-7 and endometrial tumor cell line ECC-1 were used. a1 expression was silenced through siRNA specific sequences using scramble sequences as control. Cells were incubated with or without 1 nM E2 for 48 h. a1, b1 and PCNA protein levels were measured by western blot. Cell proliferation was assessed by BrdU incorporation. a1 knock-down reduced PCNA levels in ECC-1 and MCF-7 (50% and 70% of decrease vs. respective control). Surprisingly, a1 siRNA-transfected cells showed a significantly augment in b1 protein levels (ECC-1: 2.5, MCF-7: 3.6 fold-increase). Moreover, a1 silencing reduced E2-stimulated cell proliferation in ECC-1 cells evidenced by a decrease in PCNA protein levels (% of C, E2: 275.9±2**, a1 siRNA: 60±5*, a1 siRNA: 69.2±6*, *p<0.05, **p<0.01 vs. respective C) and BrdU incorporation (BrdU labelling index,% of C; E2: 159.6*; a1 siRNA: 27.2**, a1 siRNA+E2: 35.6##, *p<0.05,**p<0.01 vs. C, ##p<0.01 vs. E2). Our results show for the

first time that sGC a1 subunit participates in cell proliferation of hormone-dependent tumors in basal and E2-stimulated conditions. In this way, sGC a1 might be proposed as a novel, key mediator of cell proliferation, which underscores its potential as therapeutic target and/or prognosis marker in hormone-dependent tumors.

384 (505) **META-TYROSINE AND ORTHO-TYROSINE: PARALLEL STUDY OF THEIR ANTI-METASTATIC EFFECTS AND THEIR EFFECTS ON NORMAL TISSUES**

Ariel Ramiro Strazza [1], Daniela Romina Montagna[1], Paula Chiarella[1], Roberto Meiss[1], Graciela Dran[1], Raúl Ruggiero[1].
[1]IMEX-CONICET ANM

In a former paper, we demonstrated that serum meta-tyrosine (m-tyr) and ortho-tyrosine (o-tyr), two isomers of tyrosine absent in normal proteins, were responsible for the phenomenon of concomitant tumor resistance (CR) in which a tumor-bearing host inhibits the growth of secondary tumor implants. Herein we have compared the effects of both isomers on the growth of metastases produced by the highly metastatic LMM3 tumor in parallel with their effects on normal organs and functions in the body. BALB/c mice bearing a s.c. LMM3 tumor for 25 days were divided in three groups that received, between 25 and 45 days, a daily i.v. injection of 33 mg/kg of m-tyr (n=7), o-tyr (n=7) or saline (n=15). A fourth group (n=10) was sacrificed at day 25 to evaluate the number of metastases at the onset of treatment. At day 45, all treated and control mice were sacrificed and metastases counted. Number of metastases (Median [range]) at day 25 was 25.0 [22-28]. At day 45 the number of metastases was: Control Group = 60.0 [44-100]; O-tyr Group = 29.0 [15-63] ($p < 0.05$); M-tyr Group = 17.3 [12-33] ($p < 0.001$). In parallel, three groups of normal mice received a similar schedule of m-tyr (n=6), o-tyr (n=6) or saline (n=6) and after 21 days a sample of mice was sacrificed and different organs were investigated. Neither histologic nor cytologic alterations were detected even when some organs with high rate of renewal such as skin, bone marrow and small intestine were studied. Hematologic cell populations in blood and lymphoid populations in lymph nodes and spleen were not altered either, as evaluated by microscopic observation and flow cytometry. Similarly, m-tyr-treated and o-tyr-treated mice did not display neither lower humoral and cellular immune responses nor alterations in the profile of serum glucose, transaminases, creatinine, uric acid and urea meaning that both tyrosine isomers exhibit strong anti-metastatic effects without any apparent toxic-side effects on the organism.

385 (546) **P-GLYCOPROTEIN 1 MEDIATES GALECTIN-1-INDUCED RESISTANCE TO DOXORUBICIN IN HEPATOCELLULAR CARCINOMA (HCC) CELLS**

Pablo Carabias[1], María Lorena Bacigalupo[1], Silvina Otero[1], Nicolas Saffioti[1], María Teresa Elola[1], Carlota Wolfenstein[1], Juan Pablo Rossi[1], Gabriel Adrián Rabinovich[2], María Victoria Espelt[1], María Fernanda Troncoso[1].
[1]IQUIFIB, Facultad de Farmacia y Bioquímica, UBA [2]IByME-CONICET

Galectin-1 (Gal1), a β-galactoside-binding protein, is overexpressed in HCC and it is related to tumor aggressiveness. P-glycoprotein 1 (Pgp), also known as multidrug resistance protein 1 (MDR1), is an ATP-dependent drug efflux pump. Its overexpression in tumor cells decreases intracellular chemotherapeutic drug concentration, showing a multidrug resistant phenotype. Previously, we reported that Gal1 overexpression in human HCC HepG2 cells (HepG2Gal1) reduces apoptosis induced by the chemotherapeutic drugs camptothecin and doxorubicin (DOX). Also, we described that HepG2Gal1 cells show increased levels of Pgp protein expression. Our aim was to evaluate if Gal1 overexpression reduces intracellular DOX levels in HepG2 cells, and to confirm Pgp involvement on Gal1-mediated resistance to DOX-induced cell death. By fluorescence techniques we found a significant decrease in intracellular DOX concentration (pmol/μM total protein, min after treatment) in HepG2Gal1 cells compared with HepG2 cells (1.2±0.1 vs 1.8±0.1, 30; 1.7±0.4 vs 2.9±0.5, 60; 2.3±0.5 vs 4.5±1, 90; 3.1±0.7 vs 5.6±1.2, 120). Co-incubation of HepG2 or HepG2Gal1 cells for 24h with DOX (2μM) and verapamil (20μM), a Pgp inhibitor, diminished cell viability (MTT) compared with cells incubated only with DOX, respectively (HepG2, 37.4±5.1% vs 54±5.3%; HepG2Gal1, 60.1±3.4% vs 77.8±2.5%). Similar results were obtained silencing Pgp expression in HepG2Gal1 cells with siRNA (Scr+DOX 61.1±2.7% vs siRNA+DOX 40.8±7.2%). However, probenecid treatment (250μM), a multidrug resistance-associated protein 2 (MRP2) inhibitor, did not change DOX-treated cell viability (HepG2, 51.5±9.3% vs 53.1±6.7%; HepG2Gal1, 69.0±9.8% vs 78.1±12.3%). Thus, Gal1-overexpressing HepG2 cells accumulate less intracellular DOX, showing a resistant phenotype. Moreover, Pgp inhibition, but not MRP2, or Pgp specific expression silencing sensitizes HepG2 cells to DOX, suggesting the involvement of Pgp in Gal1-mediated resistance to DOX-induced cell death.

386 (754) **METFORMIN AND PROPRANOLOL COMBINATION USED FOR TREATMENT OF M-406 MAMMARY TUMOR**

María Virginia Baglioni[1,2], Nahuel Cesatti Laluce[1], Angela Paula Oviedo[1], Viviana R Rozados[1], O. Graciela Scharovsky[1,2,3], María José Rico[1,2], Mauricio Ariel Menacho Márquez[1,2].
[1]Instituto de Genética Experimental, Facultad de Ciencias Médicas, UNR [2]CONICET [3]CIC-UNR

Drug repositioning refers to the use of drugs designed for other uses that showed an antitumor effect. Metformin (M) is an antidiabetic drug, and propranolol (P) is a b-blocker. We recently found that M+P combination could be effective for triple negative breast cancer (TNBC) treatment. In this work we evaluated the effect of M and P on proliferation, apoptosis, vascularization, and modulation of the immune system (IS). Also, we evaluated the use of M+P combination as adjuvant therapy. To this aim, mice were challenged s.c. with M-406 tumor; 3 days later they were divided in 4 groups and treated in the drinking water as follows: C) no treatment; M) 2g/l M; P) 25 mg/l P; M+P) combined treatment. When tumors were exponentially growing, they were excised and used for immunohistochemistry (IHC) or disrupted and used for flow cytometry. IHC analysis indicated that there was a significant decrease in proliferation for all treated groups (Ki67 IHC; $P<0.001$), a significant increase in apoptosis in tumors from M+P group (TUNEL staining; $P<0.05$), and no differences in vascularization (CD31 IHC). Evaluation of the effect of M+P on IS revealed an increase on circulating Treg cells (CD4+,Foxp3+; $P<0.05$) and intratumoral CD4+, Treg and Th17 cells ($P<0.05$). In order to analyze the possible use of M+P as adjuvant therapy, tumors were surgically removed when they reached 100 mm3 and mice were randomly divided in two groups (C and M+P). When the first mouse showed signs of metastatic disease, mice were euthanized, lungs were stained to highlight metastasis. We found that 50% of control animals developed metastasis while no metastatic node was evident in M+P group. Our results suggest that M+P reduced proliferation and induced apoptosis of tumor cells, promoting an increase of intratumoral immune cells populations; and this combination could be of interest as adjuvant therapy for TNBC, although further studies should be done for a putative translation of these results to the clinic.

387 (760) **DRUG REPOSITIONING FOR CANCER TREATMENT: METRONOMIC THERAPY WITH CHLOROQUINE AND PROPRANOLOL**

Georgina Soledad Reynoso[1], María Virginia Baglioni[1,2], María José Rico[1,2], Viviana R Rozados[1], O. Graciela Scharovsky[1,2,3], Mauricio Ariel Menacho Márquez[1,2].
[1]Instituto de Genética Experimental, Facultad de Ciencias Médicas, UNR[2] CONICET [3]CIC-UNR

Drug repositioning or repurposing in oncology refers to the use of drugs originally formulated to other indications that showed antitumor potential. In this work we made a screening of repurposing drugs that included metformin (diabetes treatment), propranolol (P; indicated to treat hypertension), doxycycline (antibiotic), chloroquine (C; malaria treatment or prevention), DHEA (sexual hormones precursor) and orlistat (obesity treatment). The antitumor potential of these drugs was evaluated in vitro in a murine triple negative breast cancer (TNBC) model (4T1 cells) through standard viability assays, either individually or in a combined manner. From the combinations tested, C+P resulted attractive as it showed a strong growth inhibition even combining low doses of both drugs

(P<0.01). Also, the combination of C+P resulted effective in decreasing proliferation of other two TNBC cells: M-406 (derived from mice tumors) and MDA-MB-231 (human cells). To further explore the potential antitumor effect of this combination on 4T1 cells, we evaluated the effect of C+P on the ability of cells in colonies formation, migration and apoptosis. We observed that both drugs were able to inhibit growth on a dose dependent manner (C: P<0.001; P: P<0.05 for all the doses tested); decreased clonogenic capability (C+P: P<0,001); and increased apoptosis (C+P: P<0.001). Combination of C+P showed a slight, but not significant, effect on normal cell migration. C+P combination resulted more effective than any individual treatment for all the studied processes. Altogether, our results suggest that: the therapy with repositioned drugs might be of interest for TNBC treatment; C and P would have antitumor effect through, at least, viability decrease and apoptosis increase; the combined treatment might have an additive effect.

388 (643) **PHARMACOLOGICAL INHIBITION OF RAC1-PAK1 AXIS RESTORES TAMOXIFEN SENSITIVITY IN HUMAN RESISTANT BREAST CANCER CELLS**

Nazareno Gonzalez[1], Georgina A. Cardama[1], María J. Comin[2, 3], Valeria I. Segatori[1], Marina Pifano[1], Daniel F. Alonso[1, 3], Daniel E. Gomez[1, 3], Pablo Lorenzano Menna[1, 3].
[1]Laboratory of Molecular Oncology, National University of Quilmes, Buenos Aires, Argentina [2]Laboratory of Organic Synthesis, Center of Research and Development in Chemistry, National Institute of Industrial Technology (INTI), San Martín, Argentina. [3]National Council of Scientific and Technical Research (CONICET), Buenos Aires, Argentina

Tamoxifen is a standard endocrine therapy for estrogen receptor positive breast cancer patients. Despite its success, development of resistance mechanisms is still a serious clinical problem. Deregulation of survival signaling pathways play a key role in tamoxifen resistance, being upregulation of Rac1-PAK1 signaling pathway one of the most important. With the aim of evaluating the role of Rac1 in acquired endocrine resistance, we developed the breast cancer cell model MCF7::C1199 by transfecting the hormone-dependent cell line MCF7 with a constitutively active version of the Rac1 GEF Tiam1. Overexpression of this construct enhanced Rac1 activity. MCF7::C1199 cells not only showed distinctive features of Rac1-regulated process as increased migration (p<0.05) and proliferation rates (17.8 hours versus 23.7 hours doubling time, in comparison with control cells), but also showed that upregulation of Rac1 activity triggered a hormone-independent growth, since MCF7::C1199 cells were not able to respond to estrogen stimulus. Moreover, MCF7::C1199 cells displayed a tamoxifen resistant phenotype, showing that modifications in breast cancer cells that promote progression from hormone-dependent to hormone-independent growth alter their response to endocrine therapy. PAK1 is the main downstream effector of Rac1 and has been involved in acquired Tam resistance. We showed that PAK1 activity increases in response to tamoxifen in MCF7::C1199 cells, increasing phosphorylation levels of estrogen receptor at Ser305, a key phosphorylation site involved in tamoxifen resistance. Finally, we evaluated the effect of 1A-116, a specific Rac1 inhibitor developed by our group, in MCF7::C1199 cells. 1A-116 restored tamoxifen anti-proliferative effects, switched off PAK1 activity and decreased estrogen receptor phospho-Ser305 levels. In conclusion, these results show that inhibition of Rac1-PAK1 signaling pathway may provide benefits to revert resistance mechanisms in endocrine therapies

INFECTOLOGÍA Y PARASITOLOGÍA / INFECTOLOGY AND PARASITOLOGY

389 (291) **NEW POTENTIAL DRUG TARGETS OF NEGLECTED DISEASES PRODUCED BY CESTODE PARASITES: HISTONE DEACETYLASES ENZYMES**

Hugo Rolando Vaca[1], Federico Camicia[1], Lucas Maldonado[1], Natalia Macchiarolli[1], Laura Kamenetzky[1], Marcela Cucher[1], Mara Rosenzvit[1].
[1]Laboratorio de Biología Molecular de Hidatidosis IMPAM-UBA-CONICET, Buenos Aires, Argentina

Cystic hydatidosis and cysticercosis, caused by the cestode parasites *Echinococcus granulosus* and *Taenia solium* respectively, are considered neglected diseases and a priority for de WHO. These zoonoses represent significant problems in human and animal health in South America. Because the only drugs available for the treatment are albenzadole and praziquantel and the possible emergence of resistant parasites, it is very important to identify new drugs against these parasites. The cestode parasites have complex life cycles and show a remarkable phenotypic plasticity that involves a complex system of control of gene expression that is associated in trematodes which changes in chromatin structure. In this work we propose to study epigenetic mechanism in cestodes parasites, principally the histone deacetylase enzyme (HDAC) as a target of drugs for parasitic diseases. Bioinformatic analyses performed in recently available cestode genomes showed that HDACs are present in *Echinococcus granulosus sensu stricto G1, Echinococcus canadensis G7, Echinococcus multilocularis, T. solium and Mesocestoides corti*. In addition, growth of *M. Corti* (model of cestodes parasites) in the presence of generic HDAC inhibitor showed a decrease in viability, measured by alamar blue reduction, and phenotypic alterations compared with untreated parasites. DNA amplifications performed by RT-PCR resulted in PCR products that were cloned and characterized by sequence analyses as HDAC8s in *E. Canadensis* and *M. corti*. The sequence conservation of these enzymes with the homologs in the trematode *Schistosoma mansoni* suggests that the drugs designed against *S. Mansoni* HDACs could be effective against cestodes. This is currently being tested in our laboratory. The characterization of HDACs in cestodes will help to understand the particular development features of parasite cestodes and provide new candidate therapeutic targets against neglected parasitic diseases.

390 (350) **THERAPEUTIC EFFICACY OF ALBENDAZOLE (ABZ) MICROCRYSTAL FORMULATIONS ADMINISTERED DURING THE PARENTERAL STAGE OF TRICHINELLA SPIRALIS INFECTION TO CBI-IGE MICE RESISTANT TO THE PARASITE**

Ana V. Codina[1,2,*], Josefina Priotti[3,4,*], Mercedes Locret[1], Darío Leonardi[3,4], María C. Lamas[3,4], María D. Vasconi[1,5], Lucila I. Hinrichsen[1,2].
*[1]Instituto de Genética Experimental, Facultad de Ciencias Médicas, UNR. [2]CIC-UNR. [3]Departamento de Farmacia, Facultad de Ciencias Bioquímicas y Farmacéuticas, UNR. [4]IQUIR-CONICET. [5]Área Parasitología, Facultad de Ciencias Bioquímicas y Farmacéuticas, UNR. **(*) Contributed Equally To This Work***

ABZ is the most extensively selected drug for oral treatment of trichinellosis, a parasitic disease caused by the helminth *Trichinella spp*. It is classified as Class II by the Biopharmaceutical Classification System because of its extremely poor aqueous solubility which limits oral absorption. Several strategies have been used to improve the solubility and dissolution rate of ABZ, as the bottom-up technology using different polymers to obtain ABZ microcrystal formulations. The analysis of the *in vitro* antiparasitic activity of these formulations in the adult form of *Trichinella spiralis* (Ts) allowed selecting hydroxyethylcellulose (S4A) and chitosan (S10A) based microcrystals as the most active. The aim of this research was to compare the *in vivo* efficacy of S4A and S10A in the parenteral stage of Ts infection, on the muscular encysted parasite stage. Adult mice of the resistant line CBi/L (CBi-IGE stock) orally infected with 2 L1 larvae/g BW were divided into four groups (n=8 per group): control (*C*), treated with ABZ (*ABZ*), with S4A (*S4*) or S10A (*S10*). Treated mice were given a daily oral dose (30 mg ABZ/kg BW) on days 27, 28 and 29 post-infection, and were euthanized seven days after the last dose. Muscle worm burden (number of L1 larvae/g muscle weight) and number of dead larvae, identified with the methylene blue supravital staining technique, were studied. The control group showed a lower percentage of dead larvae than the treated groups (median, range: 17%, 0-29; *ABZ* 32%, 0-37; *S4* 31%, 14-33; *S10* 50%, 14-88) though only *S10* was significantly different (P<0.05). *S10* also increased larval mortality compared to *ABZ* (P=0.0279). Chitosan-based microcrys-

tals might be an effective way of improving oral bioavailability and therapeutic activity using low doses of ABZ, which implies a lower degree of toxicity for the host.

391 (695) **NVOLVEMENT OF AMPK IN THE PHARMACOLOGICAL STIMULATION OF THE AUTOPHAGIC PATHWAY IN ECHINOCOCCUS MULTILOCULARIS**

Julia Alexandra Loos[1], Raphaël Duvoisin[2], Klaus Brehm[2], Andrea Carina Cumino[1].
[1]National University Of Mar del Plata, Argentine. [2]University Of Würzburg, Germany

Echinococcosis is a neglected zoonotic disease caused by infection with the larval stage of tapeworms within the genus *Echinococcus* spp. In order to develop strategies for echinococcosis treatment and control, it is necessary to highlight basic studies on the parasite larval stage and to identify possible new molecular targets, particularly in those signal transduction pathways on which parasite cells depend for their survival. The AMP-activated protein kinase (AMPK) is one of the central regulators of metabolism in eukaryotes and plays a critical role in the cell growth and autophagy regulation. The most thoroughly described mechanism by which AMPK regulates these cellular processes is through suppression of the TOR pathway. Recently, it was demonstrated that, Ander *in vitro* conditions, *E. granulosus* larval stage is susceptible to metformin (Met) and that this drug indirectly activates Eg-AMPK, as a consequence of cellular energy charge depletion. Therefore this kinase could be highly relevant to *Echinococcus* spp. Here, we initiated a functional analysis of AMPK in the *E. multilocularis* experimental model. By *in toto* immunolocalization assays, we detected the expression and subcellular localization of Em-AMPK and Em-LC3 (autophagy marker) in the germinal layer of *in vitro* obtained metacestodes vesicles. Interestingly, a different expression pattern of Eg-LC3 was observed between control and Met-treated samples. In addition, by qPCR analysis, we found that Met produced a dose-dependent increase in Em-*lc*3 transcript levels in parasite primary cells. Subsequently, using RNA interference, we achieved a significant reduction in transcript abundance and protein expression levels for Em-*ampk*1. **The Em-AMPK knock**down partially prevented the inductor effect of the drug on the Em-*lc3*expression. Therefore, AMPK might be to some extent necessary for the regulation of Met-stimulated autopahgic process in *Echinococcus* spp. However, further studies are needed to clarify this issue.

392 (737) **LIPID DROPLETS DYNAMICS AND THE RESPONSE TO PHARMACOLOGICAL TREATMENT IN ECHINOCOCCUS GRANULOSUS**

Valeria Dávila[1], Andrea Cumino[2].
[1]National University Of Mar Del Plata, Argentine. [2]CONICET

Uptake of fatty acids seems to be crucial for Echinococcus spp. since cestode lack the ability to synthesize fatty acids and cholesterol de novo, and, as flukes, have lost many genes associated with the peroxisome, essential in the occurrence of fatty acid oxidation. Lipid droplets (LDs) are conserved organelles for intracellular neutral lipid storage with high metabolic activity, whose functions are not limited to passive stock of lipids, being the maintenance of endoplasmic reticulum (ER) homeostasis a critical one. Mobilization of lipids in LDs occurs through lipolysis and autophagy. Here, we attempt to characterize these organelles and their dynamic in response to different drugs by confocal and electron transmission microscopy. We identified a high basal level of LDs in Echinococcus larval stage, possible due to its low UPR capacity and high sensibility ER stress. In close connection with ER, we identified smaller LD associated with nuclei compared with those in the cytoplasm. Moreover, changes on size, number and cell distribution of LDs during metformin, rapamycin and indomethacin treatments were detected. We also verified the power of oleic acid (18:9 n-1, monosaturated fatty acid), the most abundant fatty acid in the diet and serum of human host, as a potential autophagic inductor and promoter of LDs formation. On the other hand, expression of hormone sensible and lisosomal acid lipases (Eg-lal1, Eg-lal2 and Eg-hsl), different autophagy-related genes (atg1 to atg18), FoxO and TFBE were analyzed by RT-PCR and qPCR from drug-treated protoscoleces and metacestodes. Finally the colocalization of Eg-Atg8-Alexa-Fluor 488 and bodipy 493/503 by in toto immunofluorescence was performed in protoscoleces, suggesting the importance of autophagy in the regulation of LDs. Our results allowed us to conclude that Echinococcus lipid store can be modulated by different metabolic and pharmacological conditions.

393 (1051) **NANOANTIBODIES AS A SENSITIVE AND COST-EFFICIENT TOOL FOR DENGUE DIAGNOSTIC**

Yesica Luciana Paredes Rojas[1], Cristian Malnero[1], Cecilia Caldevilla[1], Cybele Garcia[2], Nora Mattion[1], Lorena Itati Ibañez[1].
[1]Instituto de Ciencia y Tecnología Dr. César Milstein (CEVAN)-CONICET. Caba, Buenos Aires, Argentina. [2]Laboratorio de Estrategias Antivirales, Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. IQUIBICEN-CONICET. Ciudad Universitaria. Ciudad Autónoma de Buenos Aires

Dengue is an important viral disease transmitted by mosquitoes, and in recent years it has become endemic in Argentina. High levels of the Dengue virus protein NS1 has been detected in the sera of infected patients, and for this reason this protein has been used for the development of diagnostic kits. Nanoantibodies (NAbs), which are fragments of heavy chain antibodies obtained from camelids, are a potentially useful tool for Dengue detection. These small molecules typically exhibit strong specificity and affinity for different antigens, are thermostable and can be easily produced in microorganisms at a low cost. Most importantly they can be straightforwardly modified by standard molecular biology techniques. Given the need for innovative and affordable dengue detection systems, here we report the production of NAbs with ability to recognize the NS1 protein of Dengue virus. For this purpose, two llamas were immunized with purified and inactivated supernatant of Dengue infected cells containing high levels of NS1 protein. Total RNA was isolated from peripheral blood. After cDNA synthesis, part of the genes encoding antibody heavy chains were amplified by PCR and cloned into the pHEN4 phage display vector. This plasmid was transformed into TG1 E. coli cells, which were later infected with the M13K07 helper phage. Selection of NS1 specific binders was carried out by phage display. NAbs were separated from TG1 periplasm by osmotic shock, and specific binding to purified NS1 was tested by ELISA. Two libraries of about 2x108 transformants with more than 88% of inserts of the right size were obtained. After 3 rounds of panning, 192 candidate clones were isolated and amplified in TG1 cells. Periplasmic extraction of NAbs allowed us to determine that isolated candidates specifically recognize NS1 protein of Dengue virus. Selected clones will be modified and produced at a large scale to generate a Dengue NS1 detection kit.

394 (128) **ROLE OF A PPAR GAMMA AGONIST AND MACROPHAGES IN CARDIAC NEOVASCULARIZATION IN AN EXPERIMENTAL MODEL OF CHAGAS' DISEASE**

Federico Nicolás Penas[1] , Ana Dmytrenko[2], Gerardo Adrían Mirkin[1] , Ágata Carolina Cevey[1], María Jimena Rada[1] , María Elena Sales[2], Nora Beatriz Goren[1].
[1]Instituto de Investigaciones en Microbiología y Parasitología Médica (IMPAM-UBA, CONICET). [2]Centro de Estudios Farmacológicos y Botánicos (CEFYBO-UBA, CONICET)

Chagas disease, caused by *Trypanosoma cruzi* (*Tc*), involves a persistent inflammatory response affecting the heart as a main target, where it causes serious alterations. Macrophages (Mp) are involved in the clearance of infection. Upon parasite uptake, Mp increase inflammatory mediators, leading to parasite killing. Yet, inflammatory response exacerbation may lead to tissue damage. PPARg are ligand-dependent nuclear transcription factors that, besides regulating lipid and carbohydrate metabolism, have important anti-inflammatory effects. PPARg has been involved in

Mp polarization from M1 to M2 phenotype, yet its role in *Tc* infection has not been fully elucidated. We evaluated the role of HP24, a synthetic PPARg ligand, in cardiac angiogenesis in an experimental model of Chagas' disease. HP24 treatment neither changes parasitemia nor survival (P>0.05). Also, body weight remains similar to that of untreated mice. Then, we studied the effect of HP24 in Mp. Treatment increased the expression of VEGFA and Arg I, and decreased the expression of NOS2 as assesed by Western blot (Wb, P<0.05). *Ex vivo* co-cultures of Mp from *Tc*-infected and treated mice and heart explants from infected mice showed increased VEGFA, CD31 and Arg I expression in the latter (P<0.05). HP24 decreases collagen deposits in heart (Picrosirius red staining, P<0.05). Next, we analyzed the expression of CD31, VEGFA and eNOS in hearts by immunostaining and Wb. We found that HP24 increases expression of these markers (P<0.05). Angiogenesis assays in mouse skin showed that Mp from infected and treated mice, improved angiogenesis (P<0.05). Moreover, the skin of normal mice transferred with Mp from *Tc*-infected and treated mice showed increased expression of CD31 and VEGFA (Wb, P<0.05). Altogether, our results demonstrate that HP24 induces the expression of proangiogenic markers in Mp and heart tissue and suggest a potential use of PPARg agonists to control noxious cardiac remodelling in infected mice.

395 (818) **INVASIVE ABILITY OF DIFFERENT STAPHYLOCOCCUS AUREUS STRAINS ISOLATED FROM BOVINE INTRAMAMMARY INFECTIONS SELECTED ACCORDING GENOTYPIC PROFILES AND CLINICAL BEHAVIOR**

Sofia Clara Sacco[1,2], María Sol Renna[1], Melisa Belén Lovato[1], Celina Baravalle[1], Pereyra Elizabet Amanda Lorena[1], Luis Fernando Calvinho[3], Bibiana Elisabet Dallard[1].
[1]Laboratorio de Biología Celular y Molecular Aplicada, Instituto de Ciencias Veterinarias del Litoral (ICIVET-Litoral), Universidad Nacional del Litoral (UNL) / Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Esperanza, Santa Fe, Argentina. [2]Patología Veterinaria, Departamento de Preclínicas, Facultad de Ciencias Veterinarias del Litoral, Universidad Nacional del Litoral (Unl), Esperanza, Santa Fe, Argentina. [3]Estación Experimental Agropecuaria, Inta Rafaela

The aim of the study was to evaluate the invasion ability in a bovine mammary epithelial cell line (MAC-T) of different *Staphylococcus aureus* strains isolated from bovine intramammary infections (IMI), selected according genotypic and clinical outcome. Seven *S. aureus* strains with different genetic virulence profile (*ica, bap, agr, blaZ, cap*), biofilm production capacity and clinical behavior were used. Three strains (806, 3, 179) were isolated only once from a mammary quarter and not re-isolated in all lactation period (nonpersistent-NP strains). Three strains (5011, 5128, 037) were obtained from the same mammary quarter in three or more consecutive monthly milk samplings over a period of six months (persistent-P strains). All isolates were tested by pulsed field gel electrophoresis showing different pulsotypes. Strain V329 was used as *bap*-positive and strong biofilm producer. Firstly, MAC-T cells were incubated with two strains subjected to two different growth times (2 h, exponential phase) and (16 h, plateau phase). No differences were observed in the number of intracellular CFU/ml recovered between strains obtained from 2 or 16 h of bacterial growth. The number of intracellular UFC/ml recovered from co-culture with *S. aureus* strain obtained after 2 h of bacterial growth showed lower standard deviation than those recovered from co-culture obtained after 16 h. Hence, this condition was selected to compared the invasive ability between 7 selected strains. We observed significant differences (p<0,05) in the intracelular CFU/ml recovered among *S. aureus* evaluated strains. Strain 806 showed a lower invasion ability compared with other strains (p<0,05). This ability could be associated with the presence of *cap8* gene and *agr*-type (*agrII*). Strain 3 showed the highest invasion ability compared with other *S. aureus* strains (p<0,05). No associations were observed between invasion ability and strain clinical behavior (NP or P).

396 (997) **LOCATION STUDY OF ERMTR GENE IN STREPTOCOCCUS AGALACTIAE**

Marina Gisel Novosak[1], Agustin Moya Alvarez[2], Fernando Javier Bobadilla[1], Iliana Julieta Cortese[1], Domingo Javier Liotta[2], Margarita Ester Lazceski[1].
[1]Instituto de Biotecnología Misiones "Dra. María Ebe Reca" (Inbiomis). Facultad de Ciencias Exactas, Químicas y Naturales. Universidad Nacional de Misiones. Posadas, Misiones. [2]Laboratorio de Biología Molecular Aplicada (Labimap). Facultad de Ciencias Exactas, Químicas y Naturales. Universidad Nacional de Misiones. Posadas, Misiones

Streptococcus agalactiae (GBS) is the leading cause of severe invasive infections in infants less than three months. Meningitis, pneumonia and sepsis are the leading cadres in these children. These infections are described as the most serious that an individual may suffer in their first hours of life, the maternal transmission is the main route of infection (95%). Although intrapartum antibiotic prophylaxis (IAP) with penicillin can significantly decrease neonatal GBS diseases, rates of resistance to antibiotics recommended for pregnant women allergic to penicillin, such as clindamycin and erythromycin, have increased. Among the mechanisms of resistance are changes in the target site mediated by *erm* genes frequently associated with transposons (conjugative and non-conjugative) on chromosome or plasmid. The genomic location of these genes is relevant for its potential interspecific horizontal transmission. The aim of this study was to determine the location of *ermTR* gene, one of the most reported in GBS. Three strains of GBS were selected for their history of phenotypic resistance and the presence of *ermTR* gene. The identification of GBS strains was confirmed by conventional biochemical tests. The extraction and purification of plasmid, cromosomal and total DNA were performed with commercial kits. The extraction control and/or chromosomal plasmids contamination was monitored by PCR with target in the *16S* gene. Assays location of *ermTR* gene was performed using specific PCR. In the development of PCR for *ermTR* gene amplification a single sharp band of the expected size was obtained. Location assays show the presence of this *ermTR* gene into the chromosomal DNA of all the strains. In conclusion, the chromosomal location of the *ermTR* gene was determined and is necessary to continue with further studies of genomic context to contribute to the knowledge of resistance to macrolides and lincosamides.

PRESENTACION DE POSTERS SAFE II / SAFE POSTER PRESENTATION II

FARMACOCINÉTICA / PHARMACOKINETICS

397 (94) **ESTABLISHMENT OF CELL LINES DERIVED FROM PATIENTS WITH METASTATIC RETINOBLASTOMA AND THEIR IN VITRO SENSITIVITY TO MAINSTAY CHEMOTHERAPY**

Santiago Zugbi[1], María del Rosario Aschero[2], Ursula Winter[1,5], Ana Torbidoni[1,5], Eduardo Cafferata[3,5], Osvaldo Podhajcer[3,5], Guillermo Chantada[4], Paula Schaiquevich[1,5].
[1]Unidad de Farmacocinética Clínica, Hospital de Pediatría JP Garrahan. [2]Servicio de Patología, Hospital de Pediatría JP Garran. [3]Laboratorio de Terapia Molecular y Celular, Fundación Instituto Leloir. [4]Servicio de Hemato-Oncología, Hospital de Pediatría JP Garrahan. [5]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

Retinoblastoma is a solid tumor that arises during the development of the retina. Despite that intraocular retinoblastoma is a highly curable, patients with metastatic disease need aggressive treatments and if the tumor spreads into the CNS the disease is usually fatal. We aimed to establish in vitro models of metastatic retinoblastoma and evaluate the sensitivity of the tumor cells.

Lymph node tumor samples were obtained from a metastatic patient heavily pretreated at the time of tumor progression (LNP) and during the autopsy (LNA) at Hospital Garrahan under an

approved protocol. Patient samples were injected s.c. in athymic nude mice and after the tumors grew, were removed and processed to obtain a cell suspension. Once established in neural stem cell medium, 20.000cells/well were cultured and exposed to increasing concentrations of melphalan (0.01 to 327.5µM). Cell viability was assessed using MTT. The concentration of melphalan that caused a 50% cell decrease (IC50%) was calculated and compared to those previously obtained from commercial cells (WERI-Rb1) and from cells derived from non-metastatic patients, naive of treatment (007) or multi-treated (008).

Primary retinoblastoma cell lines were established both from LNP and LNA; both grew as typical neurospheres and marked positive to cone-rod homeobox (CRX) transcription factor. The median (range) IC50% of melphalan for LNP and LNA was 78.7µM (105.7-53.8) and 89.6µM (95.7-69.6), respectively ($p>0.05$). These IC50 are clearly high compared to values (median) previously observed in commercial cells (WERI-RB1: 11.9µM) and a cell line derived from a non-metastatic patient (007: 0.3µM; 008: 4.9µM) cell lines ($p<0.05$). We were able to obtain primary cell lines from metastatic tumors that were characterized for their pharmacological sensitivity as evidenced by their higher IC50 compared to commercial and non-metastatic derived cell lines. These models have a direct impact in translational research of retinoblastoma.

398 (736) **DISPOSAL AND DEPLETION MARBOFLOXACIN TISSUE RESIDUES IN RAINBOW TROUT IN SUMMER CONDITIONS**

Guillermo Prieto[1] , María Paula Tonini[1], Carlos Errecalde[1], Miguel Mancini[2], Natalia Urzúa Pizarro[1], Carlos Luders[3], Sergio Salas[4].

[1]Farmacología FAV, UNRC. [2]Acuicultura, FAV, UNRC. [3]Farmacología, Universidad Católica de Temuco, Chile. [4]Criadero Boca de Río, Villa Dolores.

Marbofloxacin, a second generation fluoroquinolone is active against Gram-negative, some Gram-positive and mycoplasma bacteria, it constitutes an alternative in aquaculture, as background in mammals indicates good oral absorption and broad tissue distribution. Because there are no studies in trout, this study aims to characterize its disposition in skin and muscle by oral application and withdrawal period in summer conditions. Rainbow trout (*Oncorhynchus mykiss*), randomly selected, weighty 326.3 ± 44.1 are given marbofloxacin 2 mg/kg orally. Serie 1 (n=42) with food and Serie 2 (n=42) fasting for 12 hours prior-administration and 3 hours post-administration. The animals placed in batches (n=3) in 500 liters of water at 19.3 ± 0.5 °C whit renewal rate of 2.2. At different times up to 120 hours post application each batch is euthanized by exsanguination extracting skin and muscle sample, that are then stored at -20 °C. Preparative assay consisted of liquid-liquid extraction of the analyte whit mcg of sample, 200 microliters water, 800 microliters solution of water: methanol: perchloric 50 :50 : 2 v/v/v and enrofloxacin as internal standard. Then it was centrifuged at 13.500 rpm 25´at 4 °C. Separation and quantification were done by HPLC by isocratic elution on reverse phase using C-18 column and a fluorescence detector at 295 nm excitation and 490 nm emission, mobile phase composed of water, acetronitrile and triethylamine (79: 19: 1 v/v/v) at pH 3. Average temporal concentrations are analyzed PK solution 2.0 software. Withdrawal period are established using residual values with WT 1.4 program with 95% confidence. The results indicate that the presence of food delays absorption and reduces tissue disposition and Cmáx, but provides greater permanence, reflected by the withdrawal period expressed in Acumulated Thermal Units (ATU) in muscle: 65.8 (fasting) and 95.9 (food) and skin: 149.04 (fasting) and 230.1 (food).

399 (742) **EFFECT OF FLUNIXIN ON MARBOFLOXACIN PHARMACOKINETICS IN CALVES**

Carlos Errecalde[1], Guillermo Prieto[1], Natalia Urzúa Pizarro[1], María Paula Tonini[1], Carlos Luders[2] 1. Farmacología, FAV, UNRC. 2. Farmacología, Universidad Católica de Temuco, Chile.

Marbofloxacin is a second generation fluoroquinolone with concentration dependent bactericidal activity against Gram-negative, some Gram-positive and mycoplasma organisms due to theinteraction with DNA gyrase enzyme. The study was conducted to determine the plasma pharmacokinetics of marbofloxacin alone and associated to flunixin to assess whether the pharmacokinetics of marbofloxacin is modified by flunixin in weaning calves. In a crossover design, each animal group A (n=3) received 6 mg/kg intramuscular marbofloxacin. Group B (n=3) received marbofloxacin 6 mg/kg plus 2.2 mg/kg intramuscular flunixin meglumine. Treatments were exchanged after 2 weeks. Blood samples from each animal were collected in heparinized tubes at different times up to 24 hours post application, they were immediately centrifuged and stored at -20 °C until HPLC analysis. Preparative analyte assay consisted of liquid-liquid extraction using 200 microliters of plasma, 200 microliters of water, 800 microliters of methanol and enrofloxacin as internal standard, stirred 30" in vortex then centrifuged 30´ at 13,500 rpm at 4 °C. Separation and quantification were performed by isocratic elution on reverse phase, at 0.8 ml per minute flow rate, 50 microliter injection volume, pre- column, C-18 octadecylsilane column, fluorescence detector at 295 nm excitation and 490 nm emission and mobile phase composed of water, acetonitrile and triethylamine (79: 19: 1 v/v/v), pH 3. Plasma concentration data were analyzed by PK Solution 2.0 software. The plasma concentration data were analyzed by PK Solution 2.0 software. The results indicate that intramuscular marbofloxacin has rapid absorption and distribution from the central compartment with moderate retention in body tissues. Applying Mann-Whitney test, it verified that the co-administration of flunixin not significantly changed ($P> 0.05$) the kinetic parameters of marbofloxacin.

400 (765) **QUINIDINE IN EPILEPSY: INDIVIDUAL THERAPEUTIC MONITORING**

M. Cecilia Kravetz[1], Guillermo F. Bramuglia[1,2], Damian A. Cuadrado[1], Esteban E. Otamendi[1], Silvia Tenembaum[3], M. Sylvia Viola[1].

[1]Cátedra de Farmacología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires. [2]Fundación Investigar, Buenos Aires, Argentina. [3]Servicio de Neurología, Hospital Garrahan.

Migrating Partial Seizures of Infancy (MPSI) is an epileptic encephalopathy characterized by an early onset and focal seizures, associated to alterations in genes codifying ion channels such as KCNT1, and antiepileptic drug resistance. Quinidine (QND) is an antiarrhythmic that modifies the potassium channel (KCN) conductance. Since KCNT1 is expressed in cardiomyocytes and neurones, QND was proposed as a new alternative treatment for patients with MPSI. QND adverse events, mainly prolonged QT segment, justify follow up with QND serum monitoring and ECG. The objective of this work was to study QND plasma levels in one KCNT1 mutated patient, with the aim to propose a strategy for QND therapeutic monitoring in MPSI patients. Our laboratory developed a HPLC-UV detection method to measure QND in serum samples (linearity 0.5-10 mcg/mL, LQ 0.5mcg/mL). Samples were drawn before next dose, after achieving steady-state. ECG was performed at least one week later after QND increase.

A 4 years old girl with a KCNT1 mutation characterized using next generation sequencing with 25-30 seizures/day, prescribed with QND and topiramate (TPM) was studied.

During 2 months QND serum levels remained below 0.5 mcg/mL while QND increased (400 to 640 mg/day) and TPM was reduced (200 to 100 mg/day). QND doses were further increased (560-720 mg/day) and its levels augmented to 1.0 mcg/mL when TPM dose was decreased from 100 to 20 mg/day. At QND 1000 mg/day serum level increased 60% when TPM was stopped. QND doses do not correlate with serum levels while TPM doses were above 100 mg/day.

Prolonged QT (0.48 and 0.51 s) was observed with 640 and 1000 mg/day of QND and control of seizures was achieved at 1 mcg/mL (reference range:2-5 ug/ml). The results showed QND/TPM pharmacokinetic interaction and suggest that individual therapeutic concentrations could be necessary to achieve in order to improve QND therapy in pediatric patients with KCNT1 mutation.

401 (874) **CEFUROXIME PLASMA PHARMACOKINETICS AND URINE ELIMINATION AFTER INTRAVENOUS ADMINISTRATION TO CATS**.

Paula Mercedes Lorenzini[1], Sabrina Mariela Passini[1], Laura Montoya[1], Martín Pablo Lupi[1], María Fabiana Landoni[2], Gabriela Alejandra Albarellos[1].

1. Universidad de Buenos Aires, Facultad de Ciencias Veterinarias, Cátedra de Farmacología. 2. Universidad Nacional de La Plata, Facultad de Ciencias Veterinarias, Cátedra de Farmacología.

Cefuroxime is a second generation cephalosporin active against gram-negative (Enterobacteriaceae), gram-positive (streptococci, staphylococci) and anaerobic pathogens. MIC breakpoint for susceptible enterobacteria is ≤8 mcg/mL. In human beings and in domestic animals it is used for the treatment of many soft tissue infections and, also, for antibiotic prophylaxis in abdominal surgical procedures. Cefuroxime is widely distributed and is eliminated mainly through renal mechanisms (glomerular filtration and active tubular secretion) as the active drug. Cefuroxime pharmacokinetics was described in some domestic animals (dogs) and in human beings, but, there is not published information about its pharmacokinetic profile in cats. The aim of the present study was to characterize cefuroxime plasma pharmacokinetic and cefuroxime urine elimination after a single intravenous administration to domestic cats.

Five healthy adult cats (3.44±0.65 kg b.w.) received 20 mg/kg of cefuroxime by IV route. Blood samples were withdrawn at pre-established times and total urine production was collected at 1 hour intervals. Blood and urine sampling lasted for 8 hours after antibiotic administration. Cefuroxime concentrations in plasma and urine were determined by microbiological assay (Kokuria rhizophila ATCC 9341, LOQ: 0.78 mcg/mL). Main pharmacokinetic parameters were estimated by conventional methods by using a PC software (Phoenix® WinNonlin® 6.3, 2005-2012, Certara, L.P.).

Estimated pharmacokinetic parameters for IV cefuroxime (20 mg/kg) in cats were: Maximum plasma concentration, Cp(0): 135,46±81,42 mcg/ml. Distribution volume at steady state, Vd(ss): 0.31±0.08 L/kg. Total body clearance, ClB: 0.20±0.06 L*kg*h, elimination half-life, t½: 1.25±0.15 h andmean residence time, MRT: 1,59±0.19 h. After 8 h, 78.09±24.59% of cefuroxime was recovered from urine. Cefuroxime urine concentrations were in the range of 2277.57±1436.48 mcg/mL (0-1 h) and 93.71±70.73 mcg/mL (7-8 h). For a T>MIC=60% (MIC?4 mcg/mL), cefuroxime should be administered every 12 h to optimise antibacterial efficacy in cats. This dosage interval, also is appropriate for urinary infections due to susceptible microorganisms.

402 (889) **HPLC/UV ANALYTICAL METHOD VALIDATION FOR THE DETERMINATION OF CLINDAMYCIN IN CAT PLASMA.**

Sabrina Mariela Passini[1], Laura Montoya[1], Martín Pablo Lupi[1], Paula Mercedes Lorenzini[1], Gabriela Alejandra Albarellos[1].

[1]Universidad de Buenos Aires, Facultad de Ciencias Veterinarias, Cátedra de Farmacología

Clindamycin is a lincosamide antibiotic commonly used in veterinary medicine. The purpose of this work was to develope and validate an HPLC/UV method for the determination of clindamycin in cat plasma, to be applied in future pharmacokinetics assays. Two techniques were combined to develop this method, the sample preparation was carried out according Batzias (Batzias 2004) and, chromatographic conditions were adapted from Mifsud´s work (Mifsud 2014). Analysis was carried out using an HPLC/UV detector seted a t 195 nm wavelenght. The antibiotic was separated on a C_{18} reversed-phase column (25cm*4.5mm*5mc) with ward column. Clindamycin phosphate standard curves were prepared in blank plasma from cats (10-0.63mcg/ml) and in mobile phase (20-0.10 mcg/ml). Standard curves were prepared each day to obtain the validation parameters: selectivity,linearity, precision, accuracy, LOQ (VC<20%) and recovery, according to ICH guidelines. Cat spiked plasma was desproteinized with acetonitrile (1ml) followed by vortex-mixing and centrifugation. The supernatant was separated and extracted with the addition of 6 mL of dichloromethane, vorted-mixing again and centrifuged. The lower layer was transfered to a new glass tube and evaporated with N_2 at 45°C. It was later reconstituted in 0.5 ml of mobile phase. The chromatographic conditions were: mobile phase: phosphate buffer(pH=2.9)/acetonitrile (72:28), the system was operated isocratically at a flow rate of 1ml/min. Blank cat plasma was running to test selectivity, no peaks were detected at clindamycin retention time. The method was linear (R^2=0.99) between 20–0.10mcg/ml. The LOQ was set at 0.1mcg/ml (VC12.78%). The intraday and interday precision was VC<5% and VC<10% respectively. The accuracy of the method was in between 80-120%, the recovery 74.11±3.9%. This method meets all validation requireements for the aforementioned parameters and can be used to determine clindamycin concentrations in cat plasma for pharmacokinetic studies.

403 (1061) **MODULATION OF ABC EFFLUX TRANSPORTERS ALONG INTESTINAL MUCOSA AFTER CHRONIC INTAKE OF ERUCA VESICARIA.**

Martín Ignacio Roma[1], Victoria Schiariti Lampropulos[2], Marcela López Nigro[2], Carballo Marta[2], Peroni Roxana Noemí[1].

[1]Instituto de Investigaciones Farmacológicas (ININFA). Facultad de Farmacia y Bioquímica. Universidad de Buenos Aires. CONICET. E-mail: rperoni@ffyb.uba.ar. [2]Universidad de Buenos Aires.Facultad de Farmacia y Bioquímica. INFIBIOC - Dpto. Bioquímica Clínica - CIGETOX-Citogenética Humana y Genética Toxicológica. Junín 956 (1113), Ciudad Autónoma de Buenos Aires. Argentina.

Due to public interest in cancer prevention and alternative therapies, the consumption of nutraceuticals and herbal supplements containing biocompounds is widespread. Cruciferous species, like Eruca vesicaria (arugula commercial variety), contain phytochemicals that could interact with intestinal ATP-binding cassette(ABC) transporters, which expel xenobiotics back to the intestinal lumen, affecting pharmacokinetics, substances bioavailability and resulting possibly in diet-drug interactions. Therefore, the aim of this study is to analyze the expression of ABCB1, ABCG2 and ABCC2 along the intestinal tract in a murine model of chronic intake of E.vesicaria leaves juice.

Adult male and female Swiss mice (n=16) were randomly separated for administration of rocket leaves juice at a dose representing the human average intake (1.40 g/kg b.w.; daily by gavage during 14 days). The ABC transporters expression levels in the intestinal mucosa were assessed by Western-blotting. In the small intestine proximal section, namely proximal and distal jejunum, there were no expression profiles modifications of ABC transporters neither in male nor in female mice. An exception was the ABCC2 expression significant increase(p<0,005) in proximal jejunum in males. In the small intestine last portion(ileum) and colon, a significant increase of ABCB1(p<0,05) and ABCG2(p<0,05) was observed for males and females. On the contrary, ABCC2 levels markedly decreased(p<0,05 and p<0,001 for males and females, respectively).

Present results suggest that repeated intake of rocket leaves alters the ABC transporters expression in intestine distal regions and may modify the bioavailability of substrates mainly absorbed at this point as well as the penetration of carcinogens in the ileum-colonic mucosa.

Our ongoing trials are focused in the isolation and characterization of those modulatory compounds present in rocket leaves that may be prototypes of potential chemopreventive drugs. Support: PIP00499(13-15).

FARMACODINÁMICA / PHARMACODYNAMICS

404 (669) **TARGETING THE TARGET: ELUCIDATING T44BF MECHANISM OF ACTION, A LEAD COMPOUND IDENTIFIED FROM A PHENOTYPIC SCREENING.**

Maia Cabrera[1], Natalia Gómez[1], Federico Remes Lenicov[2], Emiliana Echeverria[1], Ángela Rodriguez[3], Carina Shayo[3], Albertina Moglioni[4], Natalia Fernández[1,4], Carlos Davio[1].

[1]Instituto de Investigaciones Farmacológicas, Facultad de Farmacia y Bioquímica, UBA, ININFA-CONICET-UBA. [2]Instituto de Investigaciones Biomédicas en Retrovirus y SIDA, Facultad de Medicina, INBIRS-UBA-CONICET. [3]Instituto de Biología y Medicina Experimental-CONICET. [4]Cátedra de Química Medicinal, FFyB, UBA.

Focusing on the extensively studied family of thiosemicarbazone (TSC) compounds, we have previously identified by phenotypic screening 4,4'-dimethoxybenzophenone thiosemicarbazone (T44Bf) as a promising pharmacological compound in a panel of human leukemia cell lines. Previous results indicate that T44Bf-mediated antiproliferative effects are associated with mitotic arrest, followed by programmed cell death. Since target deconvolution broadly improves drug discovery programs seeking to optimize and progress lead compounds obtained from phenotypic screening, the aim of this work was to delve into the mechanism of action of T44Bf that will allow its further rational optimization. Present findings indicate that T44Bf mitotic arrest is a reversible event as was evidenced by G0/G1 subpopulation levels reestablishment after two hours of T44Bf removal ($p<0.01$). Furthermore, this arrest is consistent with sustained activation of spindle assembly checkpoint (SAC) as was determined by increase of cyclin B1 and downregulation of cyclin A ($p<0.01$) suggesting a potential interference on microtubule dynamincs. To monitor T44Bf potential effects on microtubule dynamics, we performed a biochemical assay employing purified soluble polymerized tubulin and proteins associated to microtubules obtained from U937 cell line. The results showed that treatment with T44Bf 20μM significantly inhibits microtubule polymerization ($p<0.001$). Our results demonstrate that T44Bf mechanism of action involves microtubule polymerization. These results correlate with data generated using both biochemical and cell cycle assays, and further validate the microtubule polymerization assay as a specific and reproducible assay for its further rational optimization.

FARMACOEPIDEMIOLOGIA / /PHARMACOEPIDEMIOLOGY

405 (1032) **USE OF ANALGESIC AND ANTIBIOTIC MEDICATION IN CHILDREN UNDERGOING SURGICAL PROCEDURES**

Maria Teresa Rocha, Ana Lisa Carauni, Sergio Daniel Morales, Isabel Hartman, Maria Eugenia Horna, Lorena Dos Santos.

Facultad de Medicina Universidad Nacional del Nordeste.

The administration of analgesics and antibiotics is an essential component in the practice of pediatric surgery. These drugs should be selected with rational criteria in order to provide safe and effective care. The aim of this study was to evaluate the rationality of analgesics and antibiotics used in children undergoing surgeries of the gastrointestinal tract. An observational, descriptive cross-sectional study of analgesics and antibiotics used in children undergoing surgery in a referral hospital of the Corrientes city was performed. The variables were: demographic variables (age: expressedcategories, sex), presumptive diagnosis (according to the IC10 classification), antibiotic prophylaxis used before surgery, analgesic medication before and after surgery (drugs doses, routes of administration, pharmaceutical presentation, duration of the treatment). Data were collected twice a week during seven months. One hundred and six patients undergoing surgery were recorded, 39 of them corresponding to surgeries of the gastrointestinal tract. Most of the patients were children of school (n = 11) and preschool (n = 11) ages, followed by infants (n = 10), adolescents (n = 6) and neonates (n = 1); mostly male (60%). The most frequent diagnoses were: hernias (n = 22), generalized peritonitis (n = 4) and cholecystitis (n = 4). The analgesic used in both, pre-surgical and after surgery events, was dipyrone, 20mg / kg / day. Antibiotics were: cephalexin (50-100 mg / kg / day), ampicillin (100 mg / kg / day) + gentamicin (5 mg / kg / day); both in neonates and infants. In preschool children gentamicin + metronidazole (30mg / kg / day), albendazole (30 mg / kg / day) were used. Special attention to the use of dipyrone as an analgesic and ampicillin + gentamicin as antibiotic prophylaxis in neonates and infants, should be taken because there is no evidence worldwide about the use of this drugs as first-line in pediatric patients.

FARMACOGENÓMICA / PHARMACOGENOMICS

406 (1048) **EVALUATION OF P53 EXPRESSION IN ANIMAL MODEL OF GASTRIC INJURY SUBJECTED TO A COWPEA PROTEIN DIET**

Julieta Basterra[1], María Belén Quijano[1], Gerardo Marcelo Andino[1], María Victoria Avanza[3], Maria Carla Zimmermann[1].

1. Laboratorio de Medicina Genómica. Facultad de Medicina-UNNE. CONICET. 2. Cátedra de Farmacología. Facultad de Medicina-UNNE. 3. Facultad de Ciencias Exactas y Agrimensura. UNNE-CONICET.

In the last 20 years, the use of protein concentrates from beans has increased due to their high nutritive value. Cowpea (Vigna unguiculata), is a leguminous cultivated in the NEA region of Argentina. Cowpea presents 17-28% of proteins, and is believed to be involved in the regenerative process of tissues. The use of cowpea to repair the gastric epithelium has being previously tested in our lab in experimental animals with gastric injuries. In the present work we have studied the expression of p53 protein. p53 oncogene is involved in the vast majority of cancers, such as Lynch syndrome and others of gastrointestinal origin. The main aim of this work was to analyze the expression of p53 in experimental animals subjected to gastric injury with ethanol and food supplementation with cowpea seeds. 7 weeks old Balb-c male mice divided in five experimental groups were analyzed. Dietary supplements were elaborated in the School of Pharmacy and Biochemistry, UBA, containing proteins from cowpea. A single dose of ethanol 96° (10ml/kg) was administrated intragastrically. Animals from each experimental group were monitored throughout the experience. They were anesthetized and sacrificed and the stomach and intestine were extracted for histological and immunohistochemical analysis. In all cases, national and international bioethical standards (ANMAT N° 5330/97) were followed. Experimental group from dietary supplement with animal protein and dietary supplement cowpea, showed histologically normal development of the studied organs. Groups that were subjected to induction of gastric injury by ethanol, presented according to the case, histological variations, such as alteration of mucous cell membranes, dehydration and cytotoxic effects. Interestingly, ethylic alcohol + dietary supplement cowpea group showed a significant decline in the damage induced by ethylic alcohol. Minimal changes were seen in p53 expression by immunohistochemically technique. These results lead us to conclude that, although the dietary supplement cowpea might have an important role in the protection of gastric tissue, intervening positively in this process, it is necessary to deepen the study of p53 by alternative methodologies. This is why we propose as the next stage of our work to study the mRNA expression of p53 in the same experimental model

407 (1091) **ERYTHROPOYETIN RECEPTOR EXPRESION IN BREAST TUMOR ANIMAL MODEL**

Maria Jose Benitez Peressi[1], Mariela Inés Profeta[1], Ángel Alsina[1], María Lorena Dos Santos[1], María Carla Zimmermann[1].

[1]Laboratorio De Medicina Genomica Facultad De Medicina UNNE CONICET. [2]Cátedra De Farmacología Facultad De Medicina UNNE.

Erythropoietin (EPO) and its receptor (EPOR) were established to be essential for definitive erythropoiesis. Recently, new impetus, tools, and model systems have emerged to re-examine EPO/EPOR actions. This includes indications that EPO affects significantly more than standard erythroblast survival pathways; increasing evidence that EPO/EPOR signaling pathway can mediated the worsening of tumorigenesis. The main aim of this work was to analyze the expression of EPOR in an animal model of breast cancer. We have previously established in our laboratory

an experimental model of mammary tumors. Female Balb-c mice, 8 weeks of age, were subjected to tumor induction. Briefly, five 1mg/kg consecutive doses of 7,12-Dimethyl Benzanthracene (DMBA) was administered intragastrically (i.g). Subsequently, a single dose of 10 mg/kg of medroxyprogesterone acetate (MPA) was given. Additionally, weekly doses of 2000 IU of rhEPO were subcutaneously administered. Animals from each experimental group were monitored throughout the experience and hematological parameters were determined at each time of the experimental protocol. Animals were sacrificed and mammary fat pads were removed for histological and immunohistochemical analysis. The control group presented normal hematologic and histological values. Interestingly, the erythropoietin treated groups showed and increased EPOR immunohistochemical staining in most mammary tumors related to low or none EPOR expression of the normal control group. The results lead us to conclude that there are controversies regarding the effects that rhEPO can produce in a tumor environment, providing an impetus to the review of the EPO-EPOR biology.

FARMACOLOGÍA / PHARMACOLOGY

408 (1068) **COMBINED THERAPY WITH BENZNIDAZOLE AND MILTEFOSINE IN AN ACUTE MURINE MODEL OF TRYPANOSOMA CRUZI INFECTION**

Julian Ernesto Gulin[1], Margarita Bisio[1], Jaime Altcheh[1], Facundo Garcia Bournissen[1].
[1]Servicio de Parasitología y enfermedad de Chagas, Hospital de Niños "Dr. Ricardo Gutiérrez", Buenos Aires, Argentina.

Treatment options for Chagas disease are limited to benznidazole (BZ) and nifurtimox (NFX). New treatments are not available due to high cost of developing active molecules. Drug repurposing is a cost-effective solution to fulfill current needs of better and safer therapy and drug combination is astrategy to improve efficacy reducing treatment time and doses, with lower side effects rates.

We have previously assessed anti-*T. cruzi* activity of miltefosine (MLT) both *in vitro* and *in vivo* withpromissory results. The aim of this work was to determine the *in vivo* efficacy of MLT and the synergism between MLT and BZ.

Six weeks old BALB/c female mice were infected with 500 *T. cruzi* trypomastigotes (VD strain; DTUTcVI) by intraperitoneal (ip) route. At parasitemia onset, 5 mice/group were randomly assigned to the following treatments: Non-treated (NT); BZ 5 mg/kg; MLT 25 mg/kg; MLT 25 mg/kg+BZ 5 mg/kg; MLT 50 mg/kg+BZ 50 mg/kg; BZ 100 mg/kg. Treatment was administered orally for 20 consecutive days. Effects on parasitemia, mortality and parasitic load in blood were recorded.

Parasitemia reached $3.8x10^5$ trypomastigotes/mL in NT group, but decreased significantly (p=0.002)in all treatment groups, with 76-94% parasite reduction. The effect on parasitemia was similar to anycombined therapy and any treatment prevented mortality. At the end of therapy, animals treated with BZ 5 mg/kg or MLT 25 mg/kg alone or in combination still exhibited parasitemia. However, animals treated with BZ 100 mg/kg or BZ 50 mg/kg+MLT 50 mg/kg remained negative. These groups were immunosuppressed with cyclophosphamide (CYP). At the end of the CYP cycle, 40% of BZ 100 mg/kg group had parasite reactivation but none from BZ 50 mg/kg+MLT 50 mg/kg. qPCR revealedthat 60% of animals treated with BZ alone and 20% of mice treated with BZ and MLT had parasite in circulation.

These results suggest an additive effect of BZ 50 mg/kg combined with MLT 50 mg/kg in an acutemurine model of *T. cruzi* infection.

409 (1074) **EVALUATION OF SDF-1/CXCR4 EXPRESSION IN HUMAN HER2/NEU POSITIVE BREAST CANCER SAMPLES**

María de los Ángeles Martínez[1], Silvana Beatriz Larroza[1], María del Rosario Mariana Gómez-Pescié[1], Lorena Dos Santos Antola[2], María Carla Zimmermann[1,2].
[1]Laboratorio de Medicina Genómica, Facultad de Medicina, Universidad Nacional del Nordeste. [2]Cátedra de Farmacología, Facultad de Medicina, Universidad Nacional del Nordeste.

Stromal Derived Factor-1 (SDF-1) is a chemokine whose membrane receptor CXCR4 can be found in breast tumor and the metastatic sites of the primary tumor. The signaling pathway of this chemokine and its receptor is involved and is capable to induce directional migration of cells due to a chemokine gradient. Recently, it has been observed that CXCR4 works as a mediator in the migration of tumor cells to specific organs and t it could be correlated with expression of the Human Epidermal Growth Factor Receptor-2 (HER2/neu) in breast tumors. HER2/neu is a transmembrane protein, also known as ErbB-2, which has been implicated in many types of human cancers, specifically breast cancer, in which it is used as a prognostic factor. We hypothesized that the overexpression of HER2/neu activates the SDF-1/CXCR4 axis in HER2/neu positive breast tumors, and that transactivation enhances tumor progression, malignancy and metastasis capacity. The aim of this work was to investigate the association between SDF-1/CXCR4 and HER2/neu expression in breast cancer paraffin sections. We carried out an RT-qPCR of human breast tumor paraffin samples to determine the SDF-1 and CXCR4 expression. We, additionally, performed the same SDF-1/CXCR4 RT-qPCR in some of the metastatic sites of the primary tumor. Also, analysis of HER2/neu expression in these samples were determined by immunohistochemistry. Most HER2/neu positive breast tumor paraffin samples were found to express high levels of either SDF-1 of CXCR4. Some of the metastatic sites presented higher levels of this chemokine and its receptor expression, while others presented no expression at all. Further investigation is needed, particularly in HER2/neu negative samples, in order to achieve acceptable information to validate our hypothesis.

410 (1082) **STEVENS JOHNSON SYNDROME SECONDARY TO ADMINISTRATION OF ANTIEPILEPTICS DRUGS**

Lorena Dos Santos[1], Maria Eugenia Horna[1], Isabel Hartman[1] Pablo Spada[1], Maria Teresa Rocha[1], Sergio Daniel Morales[1]
[1]Facultad de Medicina Universidad Nacional del Nordeste.

Stevens Johnson Syndrome (SJS) is idiopathic in 25.5% of the patients but can also becaused by certain drugs (phenobarbital, carbamazepine, valproic acid). The Antiepileptic drugs hypersensitivity syndrome is a serious adverse drug reaction (ADR), initially described with aromatic antiepileptics such as phenytoin, carbamazepine, phenobarbital and primidone. Objective: To identifySJS as an adverse reaction of antiepileptic drugs notified to the Regional Pharmacovigilance Centre (CRF-UNNE). We performed an observational descriptive, cross-sectional study. All notifications of the CRF-UNNE database were included. The ADRs were classified considering: severity (mild, moderate, severe) and the mechanism of appearance (type A and B Rawlins and Thompson classification). From the 200 notifications of ADR by antiepileptic drugs, 2% (4) were SJS. Average age: 35 years old, female / male ratio: 1/1. Antiepileptic drugs were involved in SSJ according to the Anatomical-Therapeutical-Chemical classification (ATC-2010) N03 code: N03AA barbiturates and derivatives (phenobarbital N03AA02), N03AB hydantoin derivatives (N03AB02 phenytoin), N03AX other antiepileptics (lamotrigine N03AX09). According to severity: 10% were severe (1 fatal case of SJS). According to Rawlins and Thompson classification all cases of Stevens Johnson were type B. They were other SJS cases not related to antiepileptic drugs. They may occur with drugs other than antiepileptics that also act at the Central Nervous System.

411 (418) **IN VIVO EFFECT OF 5-FLUOROURACIL ALONE OR IN COMBINATION WITH ALBENDAZOLE AGAINST ECHINOCOCCUS GRANULOSUS**

Patricia Eugenia Pensel[1,3], Gabriela Ullio Gamboa[2,3], Jean Pierre Benoit[4], María Celina Elissondo[1,3].
[1]Laboratorio de Zoonosis Parasitarias, Fac. Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata,

Argentina. [2]Laboratorio de Farmacotecnia, Fac. Ciencias Químicas, Universidad Nacional de Córdoba, Argentina. UNITEFA. [3]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina. [4]INSERM U1066, MINT-Micro et Nanomédecines biomimétiques, IBS-CHU Angers, 49933 Angers cedex 9. France.

Cystic echinococcosis (CE) is a zoonotic disease caused by the larval stage of *Echinococcusgranulosus*. The drugs commonly used against CE are benzimidazoles. Unfortunately, 20%-40% of cases do not respond favourably to such chemotherapy. Consequently, the search of new therapeutic alternatives such as the use of anticancer drugs has increased. In previous works, we reported the *in vitro* effect of 5-fluorouracil (5-FU) combined with albendazole (ABZ) on the larval stage of *E. granulosus*. The combination of 5-FU + ABZ had a stronger effect than that did both drugs alone. The goal of this study was to compare the clinical efficacy of 5-FU alone or combinated with ABZ in mice infected with *E. granulosus*.

The procedures and the protocols involving experimental animals were approved by the Institutional Animal Care and Use Committee (RD 148/15) of the FEyN UNMdP. Female CF-1 mice (n=50) infected with *E. granulosus* were allocated into the following groups: 1) Unmedicated control group; 2) Saline control group, 3) ABZ group; 4) 5-FU group; 5) ABZ + 5-FU group. ABZ suspensión (5 mg/kg) was administered daily during 30 days by oral route. 5-FU (10 mg/kg) was injected in the tail vein weekly for 5 consecutive weeks. All treatments resulted in a statistically significant reduction on the cysts weight compared to those obtained for unmedicated mice (*P<0,01*). Although the parasite weights recovered from 5-FU group was lower related to ABZ group, the results closely missed statistical significance (*P>0,05*). Co-administration of 5-FU with ABZ did not enhance the *in vivo* efficacy of drugs alone related to the cyst weight (*P>0,05*). However, SEM studies revealed greater damage extensión after treatment with 5-FU + ABZ compared to the monotherapy. In conclusión, co-treatment of 5-FU with ABZ improved the *in vivo* effect of monotherapy only at ultrastructural level. Since 5-FU did not cause toxic effect, further *in vivo* studies will be performed by adjusting the dosage and frequency of treatment.

412 (453) **MRP4 ASSOCIATION WITH 5FU AND GEMCITABINE RESISTANCE IN PANCREATIC CANCER**.

Nicolás Di Siervi[1], Agustín Yaneff[1], Ana Sahores[1], Maria May[2], Alejandro Carozzo[1], Carina Shayo[2], Natalia Gomez[1], Carlos Davio[1].

[1]Instituto de Investigaciones Farmacológicas, Facultad de Farmacia y Bioquímica, UBA, ININFA-CONICET-UBA. [2]Instituto de Biología y Medicina Experimental-CONICET.

Pancreatic ductal adenocarcinoma (PDAC) ranks among the most lethal of human malignancies due to several factors: there are no early detection markers, has extensive local tumor invasion, early systemic dissemination, and extremely poor response to chemotherapy and radiation treatment. Gemcitabine (GEM) became the reference regimen for advanced pancreatic cancer after a randomized trial showed significant improvement in the median overall survival as compared withfluorouracil (5FU) administered as an intravenous bolus. This chemotherapy treatment reduces primary tumor burden, but patients have an overall survival not longer than six months. Our laboratory has validated a membrane transporter (MRP4) as a new therapeutic target in pancreatic cancer and its association with cAMP efflux. Both gene silencing (shRNA) and pharmacological inhibition (MK571) leads to lower tumorigenicity, malignancy and proliferation in PDAC models both on in vivo and in vitro assays. The hypothesis of our work is that 5FU and GEM cause an adaptation of the tumor, overexpressing MRP4, increasing their level of invasion and malignancy. We performed a kinetic cAMP transport assay to assess MRP4 activity in the presence of these two chemotherapeutic agents in PANC-1 cell line, concluding that they do not significantly affect the flow of cAMP. In proliferation concentration-response curves, neither MK571 nor MRP4 gene specific silencing modified 5FU or GEM sensitivity after 72 hours treatment. Interestingly, we observed an increase in MRP4 mRNA levels of up to six times (p<0,01) in cells treated 96 hours with IC50 concentrations of these chemotherapeutic agents. Therefore, these results may explain one of the causes of chemotherapy failure, despite of decreasing the size of the primary tumor. The adaptation of neoplastic cells by treatment with 5FU or GEM involved an increase of MRP4 conferring them greater invasiveness and aggressive behavior.

FARMACOLOGÍA CLINICA / /CLINICAL PHARMACOLOGY

413 (261) **PROGNOSTIC VALUE OF SPECKLE TRACKING IN MYOCARDIAL ASSESSMENT IN SEPSIS**

Juan Pablo Ricarte Bratti[1,2], Nilda Yolanda Brizuela[1], Marcelo Urinovsky[2], Miguel Angel Tibaldi[2], Eduardo Moreyra (h)[2], Andres Menara[1].

[1]Cátedra Farmacología General . Medicina. Facultad De Ciencias Médicas. Universidad Nacional De Córdoba. [2]Sanatorio Allende – Córdoba.

Introduction and objectives: In sepsis the impact of left ventricular dysfunction on prognosis is uncertain. Speckle tracking echocardiography (STE) is a novel and sensitive method forassessing ventricular function, capable of unmasking myocardial dysfunction not appreciated with conventional echocardiography. The objective of this study was to assess STE in septic patients and determine if it is associated with mortality.

Methods: Between April 2015 and March 2016, patients ≥ 18 years of age admitted to the intensive care unit with the diagnosis of sepsis and without previous cardiomyopathy were prospectively imaged by transthoracic echocardiography. Myocardial function was assessed by conventional measures and STE. Mortality was assessed over 30 days.

Results: A total of 14 patients were included and the 30-day mortality rate was 25.9%.The ejection fraction (EF) estimated by conventional methods as M-Mode and Simpson method was similar between the group of patients that died and those who survived. The ventricular function determined by Global STE was similar between patients who died and those who survived (– 14% ± 4 vs – 17% ± 2, p = 0.118). No significant differences were observed in others echocardiographic variables.

Conclusion: In patients with sepsis the evaluation of ventricular function with Global STE wasn´t more sensitive than EF by conventional methods to detect dysfunction and wasn´t associated with higher 30-day mortality. Larger trials will be necessary to corroborate these findings.

414 (322) **USE OF DISCRETE EVENT SIMULATION (DEM) TO EVALUATE THE CLINICAL PRACTICES IN NEOVASCULAR AGE-RELATED MACULAR DEGENERATION**

Juan Pablo Real[1], Jose D. Luna[2], Santiago D Palma[1],

[1]Dpto. Farmacia, Facultad de Ciencias Quimicas-UNC. UNITEFA (CONICET). Córdoba, Argentina [2]Centro Privado de Ojos Romagosa SA - Fundación VER, Córdoba, Argentina

Neovascualar Age-related Macular Degeneration (nAMD) is the leading cause of severe visual loss in the developed world. Intravitreal anti vascular endothelial growth factors (anti-VEGFs) are effective but involve substantial resource burden.Since the initiation of anti-VEGF therapy, different treatment regimens have been developed attempting to provide comparable visual result,but with a fewer number of injections or visits.

Purpose: To evaluate the outcomes and direct medical cost of different regimen treatments in managing nAMD.

Methods: We developed a discrete event simulation model reproducing the long-term evolution of 5000 patients with nAMD. The model allows the calculation of the cost-effectiveness of treatment of AMD with two different therapeutic regimens: pro re nata (PRN) and Treat and Extend (TAE). The simulation was performed using 3 available drugs: Ranibizumab (RNB),Bevacizumab (BVZ) and Aflibercept (AFL). Data on effectiveness, rate of visual loss without treatment, the vision-related quality of life, time with VEGF

suppression and time to functional recurrence were identified through systematic literature searches. The rate of adherence of patients to the controls and the time delay in access to drugs were obtained from the database of local patient studies.

Result: On ten years of simulated treatment, costs associated with the BVZ regimens, TAE and PRN, were U$26236 and U$20636, with 4,30 and 3,91QALYs,respectively.Cost associated with RNB TAE and RNB PRN,were U$102750 and U$ 71139, with 4.36 and 3.95 QALY, respectively.Finaly, the costs associated with the AFL regimens TAE and PRN,were U$79283 and U$76658, with 4.49 and 4.06 QALY respectively.TAE obtain the better therapeutic results regardless of the drug used. TAE can be considered cost-effective compared to PRN when the drug used is BVZ and AFB.

Conclusion: DEM is a useful tool in the analysis of clinical practices For this case, TAE is the most cost effective regimen except when RNZ is used

415 (783) **ASSESSMENT OF THE EFFICACY AND MODULATION OF THE IMMUNE RESPONSE OF THE INACTIVATED STRAIN ENTEROCOCCUS FAECALIS CECT7121 IN DIARRHEA FOALS**

Virginia Margarita Rivulgo[1], Monica Sparo[2], Guadalupe de Yaniz[3], Paula Dominguez[1], Gaston Delpech[1], Sergio Sanchez Bruni.

1. Departamento de Fisiopatología , UE CIVETAN -CONICET, FCV, UNCPBA, Tandil, Argentina, Campus Universitario. 2. Microbiología Clínica, ESCS-UNCPBA, Olavarria, Argentina. 3. Área de Patología FCV, UNCPBA, Tandil, Argentina, Campus Universitario.

The immune system of foals is a continuous challenge from birth. Up to 60% of foals develope diarrhea in the first months of life. The secretory immunoglobulin A (IgA) plays the role of antibody in mucosal adaptive immunity. Some benefic live bacteria exert a general immune stimulation making control against pathogens. The main goal of this study was to assess the efficacy of inactivated benefic strain *Enterococcus faecalis* CECT7121 on the immune response in the gastrointestinal tract of newborn foals. This study was designed in an observational, prospective, crossover design, taken the concentrations of Ig A obtained from the saliva, as surrogate marker of the intestinal IgAconcentrations.

Thirty seven foals were divided in two groups. Animals of the control group I (n=22) received theplacebo formulation (distilled water) by oral route. The experimental Group II (n=15), were orally treated with a formulation containing the inactivated *E. faecalis* CECT7121 in a concentrationof 1 x10^9 UFC. Both treatments were carried out throughout 6 days.

The presence and duration of diarrhea was recording for animals of each group. Representativesaliva samples were taken in whole foals by swabbing. The technique used for determining the IgA concentrations was the Quantitative Radial Immunodiffusion.

The Ig A concentrations was assessed as Areas Under the concentration vs. time Curve (AUC). The AUC values obtained for the control group (46.34 ± 2.8 mg/dL) was significantly (*p value <0.0001*)lower than that obtained for the experimental group (76.34 ± 4.9 mg/dL). Those values of Ig A obtained for the experimental group correlated with decrease of diarrhea in 35% . In conclusion, thehigher IgA values found in saliva after given the formulation with inactivated bacteria in animals of theexperimental group, correlated with a relevant immunomodulatory activity with diarrhea decrease.

416 (1084) **KNOWLEDGE ABOUT MEDICINAL PLANTS IN DOCTORS OF CORDOBA, ARGENTINA**

Melina Garcia Gerbaudo[1,2,3], Maria Susana Hernandez Caffot[1,2,3], Nuri Ponce[1,2,3], Carlota Grigorjev[1,2,3], Nilda Brizuela[1,2,3].

1. Universidad Nacional de Córdoba. 2. Facultad de Ciencias Médicas. 3. Cátedra de Farmacología General - Escuela práctica.

Introduction. An increase in consumption of medicinal plants in recent years, either prescribed by physicians or self-prescribed by patients, has been observed, . This practice may arise adverse reactions, intoxication, overdose, or interactions with other drugs since they are often used in combination. Objective. To assess the knowledge about effectiveness, risks and frequency of prescription, as well as the acceptance of medicinal plants frequently used in our city, among doctors of Cordoba. Methods. A prospective, descriptive, cross-sectional study was carried out during June and July of 2016 to resident physicians, specialists or family doctors working in public or private Institutions in Cordoba. A random, anonymous, voluntary and semistructured survey was used. The sample consisted of 165 respondents, 52,3% women and 47,7% men. The ages were between 25 and 69 years.

Results. Among all respondents 74% does not prescribe medicinal plants in their daily practice but 51%consider them effective. The 61% responded that the p hytotherapy is not safer than therapy with conventional drugs. Regarding knowledge on the correctproposed indication coincidences were as follow: Manzanilla as antihaemorrhoidal 44%, Ambay as antitussive 30%, Poleo as antispasmodic 20%, Valeriana as hypnotic 82% and Cola Horse as diuretic 31,2%. 53% of the respondents failed to know the adverse reactions that can produce medicinal plants and the 57% didn´t know contraindications of medicinal herbs during pregnancy. The majority ignored whether ANMAT authorizes phytotherapic products and laws regulating their use.

Conclusions. According to the results, most doctors didn´t know either the appropriate use neither the adverse events that may produce the phytotherapy, which can be a determinant in not considering them as potential therapeutic resources in their daily practice. We believe that an adequate training by our Department of Pharmacology both to physicians and to the general community can be key strategy that may contribute to proper and rational use of phytotherapics.

417 (2059) **EFFECT OF NSAID ANALGESICS ADMINISTERED ALONE OR IN COMBINATION WITH LOCAL ANAESTHETICS ON THE PAIN ASSOCIATED WITH CASTRATION IN PIGLETS**

Ignacio Otero, Sabrina Passini, Gabriela Albarellos, Agustina Monfrinottti, Martin Lupi, Marcelo Acerbo, Laura Montoya.

Facultad de Ciencias Veterinarias, Universidad de Buenos Aires.

In intensive pig production, surgical castration without anesthesia is performed at approximately the first week of life. However, in extensive production this surgical practice is usually performed with animals of a higher age range (post-weaning). The aim of this study is to include the use of NSAIDs (non steroidal anti-inflammatory drugs) such as meloxicam or flunixin and compare them with the use of local anesthetics, in piglets castration of 60 days. 37 male piglets from 2 months of life were used (23,5±4.07kg). The animals were castrated and previously separated into five groups randomly. B: Lidocaine HCl (Lidocaine2%, Richmond) Intratesticular, 3ml. C: Meloxicam 0.4 mg/kg (Meloxivet John Martin) IM, D: Flunixin (Flumeg, Over) 2,2 mg/kg IM, E: Meloxicam + Lidocaine, F: Flunixin +Lidocaine. All protocols were administered 10-15 minutes prior to surgery. It was measured before, during and after castration, the presence of vocalization, fore and hind legs moves, tremors, urination or defecation, rectal temperatura and glycemia. Behavioral variables were assigned by a score (presence: 1, Absence: 0) and were added so as to calculate an overall score (TGS). The results obtained were compared using ANOVA and Tukey post test to determine differences between treatments ($P \leq 0.05$).The animals showed no adverse effects after using different protocols. The blood glucose showed no significant differences. Similar results were obtained with the temperatura and TSG intrasurgical. The parameters observed post castration (TSG 30 and 60 minutes) showed no significant differences between treatments for any of the monitored times.

According to the results, the inclusion of the NSAIDs to the protocol showed no advantages over the use of local anaesthetic alone. Instead of our results, we should continue with these studies to try to reduce the doses of depressant drugs in this algid surgical intervention. In the other hand, obtain less stressed animals with better weight gain.

418 (445) **PHARMACOEPIDEMIOLOGY OF TACROLIMUS IN PEDIATRIC LIVER TRANSPLANTATION**

Natalia Riva[1], Paulo Cáceres-Guido [1], Marcela Rousseau [2], Marcelo Dip [3], Esteban Halac[3], Oscar Imventarza [3], Paula Schaiquevich[4].

[1]Unidad de Farmacocinética Clínica, Hospital de Pediatría Prof. Dr. JP Garrahan. [2]Farmacia, Hospital de Pediatría Prof. Dr. JP Garrahan. [3]Trasplante Hepático, Hospital de Pediatría Prof. Dr. JP Garrahan. [4]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)

In pediatric transplantation, it is challenging to find the balance between efficacy and toxicity, since serious consequences exist if the FK target range is missed. Low FK blood concentrations may result in graft function impairment or acute rejection, while over-immunosuppression may increase the risk of adverse events (AE). We retrospectively evaluated safety and efficacy of tacrolimus (FK) in a large pediatric liver transplant cohort in Latin-America.

During 2-year follow-up, we analyzed data from patients who underwent liver transplantation in the period 2010-2012 and recorded FK exposure, AE and acute rejection episodes. AE were classified according causality and severity. Exposure to FK before and during AE was compared using Wilcoxon matched pairs test. Kaplan-Meier curves were used for survival analysis.

In total 46 out of 72 patients experienced 69 AE, standing (incidence) hypomagnesemia (49%), nephrotoxicity (22%), post-transplant lymphoproliferative disease (6%) and hypertension (6%). Furthermore, 43% and 89% of AE were classified as moderate or severe, and probable or definitive, respectively. In addition, 65% of patients presented one or more acute rejection episodes. Besides, the 12-month acute rejection-free survival was 41%(CI, 30.1%-53.1%). Finally, a significant difference was observed in FK-trough concentrations before and during hypomagnesemia($p<0.05$) and nephrotoxicity($p<0.05$).

For the next decade, FK will remain the first choice immunosuppressive agent, and therefore optimization of FK-based immunosuppressive therapy is of high importance. Data suggest that children experience AE, even with low-FK doses and supports therapeutic monitoring to reduce the risk of AE in this vulnerable population.

SISTEMAS CARDIOVASCULAR Y RESPIRATORIO / CARDIOVASCULAR AND RESPIRATORY SYSTEMS

419 (149) **INVOLVEMENT OF SPINALLY RELEASED GABA IN THE REGULATION OF BLOOD PRESSURE**

Stella Maris Celuch.

Instituto de Investigaciones Farmacológicas (ININFA) (UBA-CONICET)

Electrophysiological studies showed that the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) decreases the excitability of sympathetic preganglionic neurons (SPN). The inhibitory action of GABA at SPN was also examined by the observation of the cardiovascular effects of intrathecally (i.t.) administered agonists and antagonists for GABA receptors. Since there is little information about the role of GABA in the effects of other neurotransmitters that regulate the blood pressure at the level of the spinal cord, the aim of this study was to analyze the participation of GABA in the hypotensive effects of i.t. administered adrenaline (ADRE, 30 nmol) and apomorphine (APO, 50 nmol) in pentobarbital-anesthetized male Sprague-Dowley rats.The hypotensive response to ADRE (-18.2±3.1 mmHg, n=8) was prevented by both the GABAA-receptor antagonist bicuculline (BIC 5 nmol, it; n=4) and the GABA synthesis inhibitor 3-mercaptopropionic acid (MPA, 60 mg/kg, i.p.; n=6). On the other hand, the GABA uptake inhibitor nipecotic acid (NIP, 2.3 µmol, i.t.; n=5) enhanced the effect of ADRE. Similarly to ADRE, the hypotensive response to APO (-16.0 ± 2.8 mmHg; n=7) was abolished by MPA and BIC (n=5), although it was unmodified by NIP (n=6). The response to APO was instead increased by diazepam (105 nmol, it; n=4), a drug that potentiate the effects of GABA by binding to the allosteric benzodiazepine site at GABAA-receptor. Diazepam did not modify the hypotensive effect ofADRE. It is suggested that the hypotensive effects caused by neurotransmitters/neuromodulators such as catecholamines and dopamine at the spinal cord involve the release of GABA at the surroundings of SPN. Supported by CONICET (PIP 201201-00425).

420 (745) **EFFECTS OF CAPTOPRIL DURING LAST WEEK OF GESTATION IN PUPS LUNG DEVELOPMENT.**

Rocio Ayelem Conforti[1], Florencia Martina Soler Garcia[1, 2], Susana Ines Sanchez[1], Gladys Maria Ciuffo[1, 2], Lucia Beatriz Fuentes[1, 2].

[1]Universidad Nacional De San Luis. [2]Imibio-Sl-Conicet.

Lung development is a complex process that involves coordinated action of multiple genes and theparticipation of numerous transcription and growth factors. The rat lung organogenesis begins in the embryonic stages and ending two weeks postnatal. Angiotensin II is a multifunctional hormone that manifests its properties by interacting with two major subtypes of cell surface receptors $AT_1$and AT_2. Different components of renin angiotensin system (RAS) are expressed in development lung suggesting a possible involvement during lung development. Angiotensin converting enzyme (ACE) inhibitors are able to modify the actions of the RAS and are indicated for the treatment of hypertension. When ACE inhibitors were used in the second half of pregnancy, they can cause several abnormalities: fetal growth retardation, pulmonary hypoplasia and others. The aim of this study was to test the effects of Captopril during last week of gestation in rat's lung development. Pregnant rats at 13 day of pregnancy were treated with captopril (2,85 mg/kg/day) and isotonic vehicle solution as control and pup`s lung were examined at day 0, 8 and 15(P0, P8, P15). Lung development was evaluated by histological analysis. Histopathology of the pup's lungs shows altered development of the lung parenchyma, conducting airways and pulmonary vasculature. The evaluation in distal parenchyma from captopril treated group showed impaired alveolar formation, enlargement of distal airway space at P8 y P15 ($p<0.001$). In addition, we observed hyperplasia of airway smooth muscle, an increase in vessel walls. The accumulation of fluid within alveoli and bronchioles is in agreement with increase goblet cells in bronchioles ($p<0.01$). The major finding of our study was a clear adverse effect of prenatal treatment with captopril on the postnatal lung histomorphology that closely resemble new bronchopulmonary dysplasia in premature newborns. The effects produced by ACE inhibition during late pregnancy suggest participation of the RAS in the development lung process.

421 (757) **PRENATAL ACE INHIBITORS MODIFY THE IMMUNOFLUORESCENCE OF ANGIOTENSIN II RECEPTORS DURING POSTNATAL LUNG DEVELOPMENT.**

Florencia Martina Soler Garcia[1, 2], Rocio Ayelem Conforti[1], Susana Ines Sanchez[1], Gladys Maria Ciuffo[1, 2], Lucia Beatriz Fuentes[1, 2].

[1]Universidad Nacional De San Luis. [2]Imibio-Sl-Conicet.

The renin–angiotensin system (RAS) is a classical endocrine system regulating blood pressure, electrolyte and fluid homeostasis. The activity and distribution of RAS are developmentally regulated in a tissue-specific manner, and angiotensin II (Ang II) can act as a modulator of growth in a variety of cells and tissues. Different components of the RAS, including angiotensin-converting enzyme (ACE) and both angiotensin type 1 (AT_1) and type 2 (AT_2) receptors, are expressed in lung and several evidences from the literature demonstrated a possible involvement during lung development. However, RAS components expression pattern, during postnatal lung morphogenesis, is largely unknown. ACE inhibitors are among the most widely prescribed antihypertensive agents, but when used in the second half of pregnancy, they can cause several abnormalities.The aim of this study was to test the effects of prenatal ACE inhibition on the presence and subtype distribution of angiotensin II receptors postnatal lung development.

Pregnant Wistar rats (230-250g) on the 13th day of pregnancy were randomly assigned to three experimental groups:

control (n=6), Captopril (n=6) and Enalapril treated (n=6) rats. Mini-osmotic pumps were implanted subcutaneously, containing sterile isotonic saline solution (control), Enalapril or Captopril in a dose 2,85 mg/kg/day. The in vivo effects of prenatal exposure ACE inhibitors were evaluated at different postnatal ages: 0 (P0), 8 (P8) and 15 (P15) on the presence and localization of Ang II receptors by immunofluorescence.

AT_1 receptors were localized in the stroma underlying the epithelium of bronchioles and vascular smooth muscle cells in control y treated animals at the different ages studied. The AT_1 levels were modify in bronchioles, Enalapril treatment produced a significant decrease at P0 ($p<0.001$) and increase significantly at P8 ($p<0.05$), Captopril decrease significantly at P8 and P15 ($p<0.001$). In vessels, AT_1 receptors decrease significantly at P0 in animals treatment ($p<0.001$) and increase at P15 ($p< 0.001$). In contrast, the AT_2 receptor was observed on the bronchiolesepithelial cell brush border and vessels walls at different stages. AT_2 subtype levels were increased in Enalapril treatment and decrease significantly with captopril at P0 ($p<0.001$). In the vessels both treatment decrease significantly this receptor at P0 and increase at P8 and P15 ($p<0.001$).

These findings demonstrated that Ang II receptors were present in postnatal lung development. The ACE inhibitors no modify the localization of Ang II receptors in lung tissues but causes changes in levels of both subtype receptors suggesting essential participation of Ang II in the expression of its receptors during lung maturation process.

422 (810) **CARDIOPROTECTION OF HYPOTHYROIDISM ON TWO DEGREES OF STUNNING BY ISCHEMIA/REPERFUSION AND MITOCHONDRIAL ROLE IN RAT HEARTS: ENERGETICAL STUD**

María Inés Ragone[1], María Lara Lazarte[1], Lucía Clavellino[1] Matias Bayley[1] Alicia E Consolini[1]
Farmacología-GFEYEC, Depto de Cs. Biológicas. Facultad de Ciencias Exactas, UNLP. 47 y 115, La Plata

Hypothyroidism (HypoT) could worse angor, since thyroid hormones affect cardiac metabolism. In this work we studied the influence of HypoT on the energetics of rat hearts exposed to two degrees of stunning by ischemia-reperfusion (I/R): moderate and severe, without infarct. HypoT was induced by drinking methimazole 0.02% for 15 days. Isolated hearts were perfused inside a calorimeter at 37 °C to measure left ventricular pressure (LVP, in mmHg) and total heat flow (Ht, in mW) during exposition to moderate I/R (I/Rm, 20 min I-45 min R) or severe (I/Rs, 30 minI/45min R). Protocols were done in HypoT and euthyroid (EuT) rats. In I/Rm, HypoT improved the postischemic contractile recovery (PICR) to 92±5% (vs 69±6% EuT-m, $p<0.05$) and reduced diastolic contracture (+ΔLVEDP), without changing the muscle economy (P/Ht). But in I/Rs, HypoT improved still more the PICR to 54.5±6.0% (vs 11.6±4.7% EuT-s) and the P/Ht to 2.9±0.4 (vs 1.0±0.4) and reduced the +ΔLVEDP. Clonazepam (mNCX inhibitor) reduced PICR and P/Ht and increased ΔLVEDP in both HypoT models, I/Rm and I/Rs. When ischemic hearts were reperfused with Krebs-36 mM Na^+-caffeine 10 mM (K-caff-low-Na, to release SR Ca^{2+} minimizing the NCX efflux) the initial rise of contracture (ΔLVP) was decreased in HypoT vs EuT with I/Rm, without changing the area under curves (AUC-ΔLVP, AUC-Ht). But when non-ischemic HypoT hearts were perfused with K-caff-low-Na the AUC-ΔLVP was higher than in I/Rm, because of a slow relaxation, and AUC-Ht was lower. In isolated non-ischemic cardiomyocytes loaded with Fluo-4 ($[Ca^{2+}]c$) or with Rhod-2 ($[Ca^{2+}]m$) the exposition to K-caff-low-Na transiently increased F/Fo-Fluo-4 more quickly in HypoT than EuT cells, with similar pattern of F/Fo-Rhod-2. Results suggest that: a) HypoT reduced the stunning more in severe than moderate I/R, b) Hypo-I/R reduced SR release and stimulated mitochondrial Ca^{2+} uptake; c) mNCX is important for cardioprotection in HypoT hearts. *UNLP-X-642.*

423 (831) **INFLUENCE OF AGE ON THE STUNNING BY ISCHEMIA/REPERFUSION AND EFFECTS OF GENISTEIN ON RAT HEARTS: MECHANO-ENERGETICAL STUDY**

Germán Andrés Colareda[1], Patricia Bonazzola[1, 2], María Inés Ragone[1] , Alicia E. Consolini[1]
[1]Farmacología-GFEYEC, Depto de Cs. Biológicas. Facultad de Ciencias Exactas, UNLP. 47 y 115 (1900) La Plata. [2]ININCA. Facultad de Medicina, UBA-CONICET.

Age may be a cardiac risk for angor. We studied the influence of age on the energetic of rat hearts exposed to two degrees of stunning by ischemia-reperfusion (I/R): moderate and severe, without infarct. Isolated hearts from >20 months aged female rats (AgF) were perfused inside a calorimeter at 37°C to measure left ventricular pressure (LVP, in mmHg) and total heat flow (Ht, in mW) during exposition to moderate I/R (I/Rm, 20 min I-45 min R) or severe (I/Rs, 30 minI/45min R). Effects were compared with those of hearts from young female (YF) and male (YM) rats. In I/Rm, the postischaemic contractile recovery (PICR) was lower in AgF than in Y especially at the start of R (24.8±4.8% vs 77.7±4.9% $p<0.05$), as well as muscle economy (P/Ht of 1.1±0.2 vs 4.1±0.8 mmHg/(mW/g)). Diastolic contracture (+ΔLVEDP) was higher in AgF than Y (64.7±6.6 vs 7.7±1.9 mmHg). In I/Rs, the PICR was similar in AgF than Y (13.3±3.0% vs 14.5±2.4% respectively) as well as P/Ht. In vivo administration of genistein (Gst, 3 mg/kg via SC 24 hours before I/R) improved PICR in I/Rs to 63.9±6.2% and 34.1±5.1% for AgF and YF respectively, and P/Ht (3.2±0.4 and 1.0±0.2 mmHg/(mW/g)) as well. But Gst improved PICR of YM (to 50.8±4.9%) and P/Ht (to 3.0±0.7) more than those of YF. The cardioprotection of Gst on YM was not due to a higher SR Ca store, since it did not change either ΔLVP or ΔHt induced by reperfusion with 10mM caffeine-low Na^+ (36mM). Cardioprotection neither was due to activation of PI3K-Akt since the inhibitor wortmannin did not reduce PICR in YM hearts treated with Gst. Results suggest that: a) Age reduced cardioprotection more in severe than in moderate I/R, b) Gst was more cardioprotective in estrogenic deficiency (YM and AgF) than in YF. *UNLP-X-642.*

PRESENTACION DE POSTERS SAIC IV / SAIC POSTER PRESENTATION IV

REPRODUCCIÓN I / REPRODUCTION I

424 (89) **ELLAGIC ACID AND RETINOIC ACID: TWO NATURAL COMPOUNDS THAT AFFECT ENDOMETRIAL CELL GROWTH**

Bárbara Andrea Mc Cormack[1,2], Daniela Madanes[1,2], Luciana Ferella[1,2], Mariela Bilotas[1,2], Javier Singla[4], Analía Ricci[1,2], Rosa Inés Barañao[1,2].
[1]Instituto de Biología y Medicina Experimental (IBYME). [2]Fundación Instituto de Biología y Medicina Experimental (FIBYME). [3]CONICET. [4]Hospital de Clínicas "José de San Martín".

Introduction: Current treatments for endometriosis (EDT) focus on analgesic-driven pain relief, removal of implants through laparoscopic surgery and prevention of recurrence through continuous hormonal treatments. As these strategies are not fully efficient, new long-term therapies with no or minimal adverse effects are continuously under research.

Objective: Our work provides a novel approach in the treatment of this disease, taking advantage of natural products which have already shown satisfactory results in cancer research: ellagic acid (EA) and retinoic acid (RA). In vitro, we propose to assess their action on the high rate of ectopic endometrial tissue proliferation which is one of the most relevant aspects in EDT development.

Materials and Methods: We conducted a series of in vitro tests. We worked with epithelial and stromal cultures and two cell lines: human endometrial stromal (T-HESCs) and endometrial adenocarcinoma epithelial (ECC-1) cells. After treatment, cell proliferation was evaluated by the WST-1 assay or ethanol-fixed and stained with Propidium Iodide for evaluating the cell cycle by flow cytometry.

Results: We observed that treatment with 10uM RA for 24 and 48 hours significantly inhibited T-HESC cell proliferation

(p<0.01, p<0.05, respectively) T-HESC. In the same way, 24 and 48 hours of 100uM EA treatment (p<0.01) and 24 hours 10uM RA treatment (p<0.001), inhibited ECC-1 cell proliferation. We obtained similar results with stromal and epithelial primary cell cultures derived from endometrial biopsies of healthy and EDT patients (p<0.01). Moreover, flow cytometry revealed a tendency towards cell cycle arrest when treating ECC-1 cells for 24 and 48 hours with RA or EA, showed, as well as T-HESC cells for 24 hours with RA.Conclusions: Based on these results, we highlight the importance of further research on the mechanism of action of these natural compounds, as they may represent a novel and promising therapeutic treatment for EDT.

425 (88) **LEPTIN ACTION ON TROPHOBLASTIC CELLS SURVIVAL UNDER HYPOXIA CONDITION**

Paula Balestrini[1], Ayelen Toro[1], Mariana Jaime[2], Bernardo Maskin[2], Cecilia Varone[1].
[1]Departamento de Química Biológica, UBA-FCEN, IQUIBICEN-CONICET. [2]Hospital "Profesor A. Posadas".

Leptin is produced by placenta and has many roles as an autocrine hormone. We have previously demonstrated that leptin promotes proliferation and survival of trophoblastic cells. In the present work we aimed to study the mechanisms that mediate the effect of leptin in placental apoptosis induced by cobalt chloride (CoCl2), a hypoxia mimicking agent. We determined whether leptin modulates p53 expression and activity of placental cells, and also studied the regulation of HIF-1α. Swan-71 cells, cultured under standard conditions, as well as, human placenta explants were used. Placental cells and explants were treated with $CoCl_2$ (50, 100 and 250 µM) to induce apoptosis and with or without 100 ng/ml of recombinant leptin. Western blot and transfection assays with a p53 reporter constructs vectors were carried out. All procedures were approved by ethical review committee at the A Posadas National Hospital. In order to prove CoCl2 effect, we studied HIF-1α expression and we confirmed that the treatment with 100 µM of CoCl2 induced its expression (p<0,05) and leptin seems to reduced HIF-1α levels. Next we evaluated p53 expression on placental cells treated with CoCl2 and found that p53 expression is altered by $CoCl_2$ treatment in a dose and time dependent manner. In these conditions leptin reduced the expression of p53 (p<0,05). These findings suggest that leptin is capable to protect placental cells in hypoxia conditions.

426 (101) **LEPTIN PROTECTS TROPHOBLASTIC CELLS FROM UV RADIATION-INDUCED APOPTOSIS**

Ayelen Toro[1], Antonio Pérez-Pérez[2], Bernardo Maskin[3], Víctor Sánchez-Margalet[2], Cecilia Varone[1].
[1]Departamento de Química Biológica, FCEN-UBA, IQUIBICEN-CONICET. [2]Departamento de Bioquímica Médica y Biología Molecular, Facultad de Medicina, Universidad de Sevilla. [3]Hospital Nacional "Profesor A. Posadas".

It is well established that leptin modulates angiogenesis, growth, immunomodulation as well as anti-apoptotic effects in the placenta. In recent studies we demonstrated that leptin promotes survival of placental cells involving the regulation of different intrinsic pathway factors under stress conditions such as serum deprivation, hyperthermia and acidosis. Moreover we also proved that leptin exerts a negative regulatory effect on p53 levels. In this context we aimed to study leptin effect on trophoblastic cells exposed to UV radiation since p53 plays a pivotal role in UV damage response. Swan-71 cells were exposed to UV radiation (1, 2 and 4 mJ/cm2) and treated with or without recombinant leptin (50, 100 and 250 ng/ml) during 5 h. Western blot were carried out to evaluate leptin action on anti- and pro-apoptotic intermediaries. We demonstrated that p53 expression was increased by UV exposure (p<0,05). We also proved that UV exposure generated an increment of JNK phosphorylation (pJNK), a typical UV damage marker. Leptin treatment reduced p53 expression and pJNK phosphorylation in cells exposed to UV radiation. In order to evidence leptin anti-apoptotic effect, we studied the expression of several proteins involved in the apoptotic pathways. We found that leptin decreased Caspase-3 activation, cleaved PARP-1 (cPARP-1), Bax and Bid levels (p<0,001). Mdm-2 and p53 phosphorylation (pS46p53) levels were also modulated by leptin, confirming that leptin participates on p53 axis regulation. All these findings suggest that leptin is able to protect placental cells against UV damage, reinforcing a leptin anti-apoptotic effect.

427 (100) **ASSESSMENT OF THE ROLE OF HYPOXIA INDUCIBLE FACTORS (HIFS) IN FSH REGULATION OF SERTOLI CELL PROLIFERATION.**

María Noel Galardo[1], Agostina Gorga[1], Mariana Regueira[1], Gustavo Marcelo Rindone[1], Eliana Herminia Pellizzari[1], María del Carmen Camberos[1], Selva Beatriz Cigorraga[1], María Fernanda Riera[1], Silvina Beatriz Meroni[1].
1Centro de Investigaciones Endocrinológicas "Dr. César Bergadá" (CEDIE) CONICET - FEI - División de Endocrinología, Hospital de Niños Ricardo Gutiérrez

The final number of Sertoli cells (SC) reached during the proliferative periods determines sperm production capacity in adulthood. It is well known that FSH is the major SC mitogen; however, little is known about the transcription factors involved in the regulation of SC proliferation. On the other hand, Hypoxia Inducible Factors (HIFs) are master regulators of cell growth. HIFs consist of a constitutive HIFbeta (HIFb) subunit and a HIFalpha (HIFa) subunit. HIF transcriptional activity is regulated through the abundance of HIFa subunits. To date, three HIFa isoforms have been described. The association of each HIFa subunit with HIFb subunit constitutes three active transcription factors: HIF1, HIF2 and HIF3, which interact with consensus hypoxia response element in the promoter region of target genes. Besides, regulation by hormones of HIF transcriptional activity under normoxic conditions was demonstrated. The aim of this work was to investigate whether HIFs participate in the regulation of rat SC proliferation by FSH. SC obtained from 8-day old rats were maintained under basal (B) conditions or stimulated with FSH 100 ng/ml in the absence or presence of a pharmacological agent that promotes HIFa subunit degradation -LW6. BrdU incorporation, HIF transcriptional activity by gene reporter assay and HIF1a, HIF2a, HIF3a and cyclin D1 (CCND1) expression by RT-qPCR were evaluated. Results are expressed as mean±SD of three independent experiments (*P<0.05 vs. B). It was observed that FSH increased HIF transcriptional activity (B:15653±445, FSH:27438±1979*RLU) and HIF2a mRNA levels (31.0±2.5*fold variation vs.B) without modifying either HIF1a or HIF3a expression. In addition LW6 inhibited the FSH effect on CCND1 expression (FSH:1.7±0.2*, FSH+LW6:1.2±0.1 fold variation vs.B) and BrdU incorporation (B:13.9±1.9, FSH:26.9±9.7*, FSH+LW6:12.4±3.2%BrdU-positive cells). These results suggest that HIFs might be involved in the regulation of SC proliferation by FSH. PICT2014-0945.

428 (111) **ACTIVATION OF PPARGAMMA (PPARG) REGULATES SERTOLI CELL NUTRITIONAL FUNCTION**

Agostina Gorga[1], Gustavo Marcelo Rindone[1], Mariana Regueira[1], Eliana Herminia Pellizzari[1], Maria del Carmen Camberos[1], Selva Beatriz Cigorraga[1], María Fernanda Riera[1], María Noel Galardo[1], Silvina Beatriz Meroni[1].
[1]Centro de Investigaciones Endocrinológicas "Dr. César Bergadá" (CEDIE) - CONICET - FEI - División de Endocrinología, Hospital de Niños Ricardo Gutiérrez.

Sertoli cells (SC) provide the structural and nutritional support for germ cell development. Studies on SC glucose metabolism have shown that this cell type actively metabolizes glucose and converts it to lactate, which is an important source of energy for germ cells. Furthermore, it has been demonstrated that SC can oxidize FA and it is assumed that this metabolic process fulfills the energy requirements of SC. FA are stored as triacylglycerides (TAG) within lipid droplets (LD). Recently, we have demonstrated that the transcription factor PPARg participates in the expression of genes involved in the regulation of lipid storage as TAG in LD. In this metabolic context, the simultaneous regulation of FA storage and lactate production may be relevant to seminiferous

tubule physiology. The aim of this work was to study the participation of PPARg activation in the regulation of lactate production in SC. Cultures of SC obtained from 20-day-old rats were incubated for different periods of time without (basal, B) or with Rosiglitazone -RSG, pharmacological activator of PPARg. Results are expressed as mean±SD of three independent experiments obtained in 48h incubations with 10µM RSG (*p<0.05). We observed that RSG increased lactate production (B: 2.5±0.3; RSG: 7.9±1.3*µg/µgDNA) and glucose consumption (B: 35±3; RSG: 81±5*µg/µgDNA). Next, we wondered which mechanisms might be contributing to the increase in lactate production observed after PPARg activation. To this respect, we observed that RSG increased glucose uptake (B: 1055±49; RSG: 1340±57*dpm/µgDNA) accompanied by an increment in Glucose Transporter 2 expression. Nevertheless, lactate dehydrogenase (LDH) activity and LDH A subunit expression were not modified by RSG. Altogether, these results suggest that PPARg, in addition of regulating lipid storage, participates in the regulation of lactate production in order to ensure the simultaneous provision of energy to Sertoli cells and germ cells.

429 (103) **STUDY OF CHANGES IN SPERMIOGENESIS OF RABBITS CAUSED BY HYPERCHOLESTEROLEMIA ACQUIRED BY HIGH FAT DIETS**

Layla Yamila Simón[1,2,3], Abi Karenina Funes[1,2], Regina Lucia Colombo[1,2], Estefanía Saez Lancellotti[1,2,3], Miguel Fornés[1,2,3].

[1]IHEM - CONICET – Mendoza. [2]Facultad de Ciencias Médicas - UNCuyo – Mendoza. [3]Facultad de Ciencias Médicas - Universidad del Aconcagua – Mendoza.

High fat diets generate acquired hypercholesterolemia (HC) in New Zealand rabbits. These HC bugs have seminal and sperm alterations, specifically morphological abnormalities increase. During spermiogenesis, the Golgi/acroplaxome/manchette maturation is associated with the final shape of spermatozoa. The aim of this work was to describe the mechanism related with sperm morphological changes described in HC rabbits. In order to do this work, adult rabbits were fed with balanced meal (NormoCholesterolemic Rabbits, NCR); or with fat-enriched balanced meal, suplemented with 14% (w/w) of grease (HyperCholesterolemic Rabbits, HCR). Twice a month, semen samples were obtained and a complete sperm analisis was made. Spermatozoa were fixed, stained and analyzed under light microscopy to corroborate morphological alterations already described. Then, testis were obtained and analyzed under light and electron microscopy. In order to study Golgi/acroplaxome/ manchette development in specific seminipherous epithelial stadiums of the spermatogenesis (VI to VIII), these stadiums were isolated and observed under fluorescence microcopy. At sperm level, the morphological abnormalities on head and insertion point of tail increased on HCR group after 6 months of high fat diets, comparing with NCR group. At testicular level, histological changes were observed as small light areas (lipid droplets) between spermatogenic cells, seminipherous epithelial detachment, and lax and asymmetric acrosomes, on HCR condition. At seminipherous epithelial cells level, spermiogenesis alterations were shown: nucleus condensation abnormalities, misplaced tails presence, acrosomic vesicles abnormalities, cytoplasmic whirls, manchette alterations with depolarized microtubules and oblique traction. These results suggest that hypercholesterolemia has a negative effect that finally affects spermiogenesis, expressed as teratozoospermia.

430 (145) **APOPTOTIC GERM CELLS (AGC) AND FSH REGULATE PERILIPINS (PLINS) EXPRESSION AND LIPID DROPLETS (LD) FORMATION IN SERTOLI CELLS (SC)**

Mariana Regueira[1], Agostina Gorga[1], Gustavo Marcelo Rindone[1], María Noel Galardo[1], Eliana Herminia Pellizzari[1], María del Carmen Camberos[1], Selva Beatriz Cigorraga[1], María Fernanda Riera[1], Silvina Beatriz Meroni[1].

[1]Centro de Investigaciones Endocrinológicas "Dr. César Bergadá" (CEDIE) División de Endocrinología, Hospital de Niños Ricardo Gutiérrez – FEI – CONICET.

SC actively metabolize glucose into lactate, which is the major source of energy for germ cells (GC), and use fatty acids (FA) -stored as triacyglycerides (TAG) in LD- as its own energy source. It is known that LD levels increase when SC engulf and phagocyte AGC. Also, it has been shown that FSH increases the incorporation of acetate into TAG stored within LD, however, it is unknown whether FSH increases LD levels. PLINs are proteins that participate in the formation and maintenance of LD. The regulation of mechanisms involved in PLIN expression and LD formation may be relevant to SC physiology. The general objective of this work was to analyze possible effects of AGC and FSH in LD formation. Specifically, determine the effect of FSH on LD levels and of FSH and AGC on PLIN expression. For this purpose, CS were maintained under Basal (B) conditions or stimulated with FSH for 48h. FSH promoted an increase in LD content (1.5±0.2* number of LD/SC) and an increase in PLIN1 mRNA levels (3.6±0.3*). Additionally, SC cultures obtained from 20-day-old rats were maintained under B conditions or co-cultured with AGC for 48h. AGC were generated by incubating GC obtained from 31-day-old rats in the absence of SC for 48h, a condition known to initiate apoptosis. SC co-cultured with AGC showed an increase in the mRNA levels of PLIN 1, 2 and 3 (2.2±0.2*, 1.7±0.3* and 1.9±0.4*, respectively). All results are expressed as fold-increase, mean±SD, n=3; *p<0.05 vs. B. Altogether, these results demonstrate that FSH and AGC can regulate the expression of genes involved in LD formation in SC. These observations reveal different physiological mechanisms involved in the regulation of LD formation, which are essential to sustain SC energetic metabolism.

431 (144) **EXPLORING THE ROLES OF EPINEPHRINE IN HUMAN TESTICULAR PERITUBULAR CELLS**

Soledad Paola Rossi[1,2,3], Verónica Rey-Ares[3], Mónica Beatriz Frungieri[1], Artur Mayerhofer[3].

[1]Instituto de Biología y Medicina Experimental, Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina. [2]Departamento de Bioquímica Humana, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina. [3]BioMedizinisches Centrum (BMC), Ludwig-Maximilians-University (LMU), Cell Biology, Anatomy III, Planegg, Germany.

Catecholamines reach the testes via its sympathetic innervation and the blood stream. They mediate stress-responses via receptors and may be are involved in the regulation of testicular function. We determined by RT-PCR that Human Testicular Peritubular Cells (HTPCs) isolated from infertile patients express β1-, β1- and β2- adrenergic receptors. The aim of this study was to investigate if HTPCs are possible targets of catecholamines and to test their influence on cell viability, smooth muscle markers, pro-inflammatory factors and CXCL12 expression. Epinephrine (Epi; 10nM, 100nM, 1µM and 10 µM) has a trophic effect and significantly increases metabolic activity (determined by ATP assay) on HTPCs, after 24 h of incubation. Epi and 10µM salbutamol (S; agonist β2) and phenylephrine (P; agonist β1) decreased calponin and smooth muscle actin (SMA) mRNA expression after 24 h incubation (qPCR: calponin/GAPDH: B:1.00±0.09; Epi:0.63±0.08*; S:0.68±0.12*; P:0.63±0.06*; SMA/GAPDH: B:1.00±0.08; Epi:0.39±0.10*; S:0.51±0.02*; P:0.52±0.02*; X±SEM; * P<0.05). Moreover, Epi and P increased the mRNA expression of pro-inflammatory factors, IL-6 and COX2, after 3 h incubation (qPCR: IL6/GAPDH: B:1.00±0.10; Epi:2.11±0.35*; P:1.79±0.09*; COX2/GAPDH: B:1.00±0.09; Epi:3.42±0.07*; P: 2.94±1.35*; X±SEM; * P<0.05). Epi also decreased CXCL12 mRNA expression (qPCR: CXCL12/GAPDH 24 h, B:1.00±0.09; Epi:0.67±0.05*; 3 h, B:1.00±0.08; Epi:0.70±0.05*; X±SEM; * P<0.05). Our data, although preliminary, reveal that Epi targets HTPCs, has growth promoting activities, exerts inflammatory actions (IL6 and COX2) and may influence contractility markers. Given that Sertoli and spermatogonial stem cells express CXCL12 receptors, the action of Epi on CXCL12 expression may have consequences for the microenvironment and the spermatogonial stem cells niche and, subsequently, for spermatogenesis. Hence, Epi and stress may be involved in the development of human male sub- or infertility.

432 (206) **DIRECT EFFECT OF METFORMIN ON GRANULOSA CELLS: WIDENING THE USES OF THIS DRUG IN POLYCYSTIC OVARY SYNDROME.**

Mariana Di Pietro[1], Leopoldina Scottil[1], Griselda Irusta[1], Natalia Pascuali[1], Camila Pazos[1], Marta Tesone[1], Fernanda Parborell[1], Dalhia Abramovich[1].
[1]Instituto de Biología y Medicina Experimental (IByME-CONICET)

Polycystic Ovary Syndrome (PCOS) is a frequent pathology that affects more than 5% of women of reproductive age. Its symptoms are heterogeneous and range from chronic anovulation, oligo- or amenorrhea and hyperandrogenism to obesity and insulin resistance. In addition, PCOS is characterized by abnormalities in angiogenesis and alteration in the levels of angiogenic factors.

Metformin has been introduced in the treatment of PCOS to manage insulin resistance and hyperglycemia. Besides its metabolic effects, metformin has been shown to improve ovulation, pregnancy and live birth rates in PCOS patients. Metformin mechanism of action on the ovary is not known and a direct effect of this drug on follicular cells cannot be discarded. Metformin can get into the cell by passive diffusion or through the Organic Cation Transporters (OCTs).

Hypothesis: Metformin can act directly on granulosa cells modulating angiogenic factor synthesis.

Methods: Granulosa cells were isolated from DES-treated prepubertal Sprague Dawley rat ovaries. Cells were seeded onto plastic plates precoated with rat tail collagen with 10% bovine fetal serum. After 24, medium was replaced by serum-free medium and the cells were stimulated with different concentrations of metformin (0; 0.01 and 0.1 mM).

Results: Metformin increased the levels of phospho-AMPK and decreased the levels of VEGF in granulosa cells. Furthermore, we demonstrated for the first time the presence of OCT2 and OCT3 in granulosa cells.

Conclusion: Our results suggest that metformin acts directly on granulosa cells modulating their function and angiogenic factor systhesis. This drug could be getting into the cell through its transporters OCT2 and OCT3. The study of metformin mechanism of action will allow the revision of its indication and uses, leading to a direct benefit to patients trying to conceive.

433 (164) **APOPTOSIS IN LONG TERM DIABETIC MOUSE UTERUS: AN ANALYSIS OF INTRINSIC AND EXTRINSIC PATHWAY OF APOPTOSIS**

Analia Meilerman Abuelafia[1,2], Nicolas Fraunhoffer[1, 2], Karen Roman[1], Paula D´amico[1], Lila Blanco[1], Cecilia Sturla[1], Guido De Gregorio[1], Alfredo Vitullo[2], Marcela Barrios[1].
[1]Carrera de Medicina, Universidad Maimónides. [2]CEBBAD, Universidad Maimónides.

Type I diabetes mellitus (T1D) accounts for about 10% of all cases of diabetes. It is a multifactorial autoinmune disease for which susceptibility is determined by genetic, environmental and immunological factors. Emerging evidence has shown that diabetic mice uterus presented severe atrophy, glands reduction, fibrosis and destruction of smooth muscle cells. These changes would be related with an increase of apoptosis. The objective of this study was to analyze the balance between the apoptosis pathways in diabetic mice uterus. 25 BALB/c female mice (age 30 days; 15 diabetic and 10 controls) were used in this assay. To generate a T1D model, female mice received five injections of streptozotocin at a dose of 60 mg/kg, during 5 consecutive days. Animals were selected as diabetic when glucose level was >200 mg/dl. Weight and food intake was recorded weekly both in diabetic and control animals. Three diabetic and 2 control animals were sacrificed at day 15, 20, 40, 70 and 80 post-treatment. The uteri were removed and weighed; one part was fixed in 4% PFA for immunohistochemistry and immunofluorescence and another one was frozen at -80 °C for Western Blot analysis. The proteins studied were: Bax, Bcl-2, cleaved Bid, Fas/Fas-L system, cleaved caspase 8 and active caspase 3. Bcl-2 expression was constant at all time-points, with no differences between control and diabetic mice. Bax and extrinsic apoptosis pathway markers (Fas, Fas-L, cleaved Bid and cleaved caspase 8) showed strong cytoplasmatic immunostaining in endometrial glands and smooth muscle cell of diabetic mice uterus at all time-points analyzed. Active caspase 3 was positive in glands and smooth muscle cells of diabetic mice uterus with constant levels of expression in all time-points. In conclusion our results show that during the streptozotocin - induced T1D, both intrinsic and extrinsic apoptosis pathway are involved in the endometrial degeneration by promoting glandular and smooth muscle cell death.

434 (253) **CHANGES IN SERUM URIC ACID LEVELS DURING NORMAL AND PATHOLOGICAL PREGNANCIES**

Ana Irene Corominas[1,2,3].
[1]Hospital Nacional Prof. A Posadas. [2]Laboratorio de Biología de la Reproducción, IFIBIO-CONICET. [3]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Ciencias Biológicas.

Determination of serum uric acid (SUA) is used to monitor maternal renal function during pregnancy. Despite its great biological variability in non pregnant and in pregnant healthy women, most laboratories reported as reference range, those of non pregnant women, a fact that promotes misinterpretation of the results. Our aim was to describe the range of SUA in pregnant women throughout pregnancy in normal and pathological conditions. We conducted a prospective approach in which we studied all patients who attended her pregnancy in the Hospital Posadas during 2014. SUA (mg/dl), urea and creatinine were measured along gestation and were evaluated in the context of normal or pathological pregnancy. In diabetic pregnant women (n=164) SUA increased statistically after the 30th week compared to normal pregnancy (3.92±0.85 vs 3.56±0.77, p=0.014); but at the end of pregnancy, SUA were similar to normal ones.

In women with gestational hypertension without preeclampsia (PE) (n=32) we observed a continuous increase of SUA levels but the difference between the end and the beginning of pregnancy was lower than 1.5. In women with PE (n=40), SUA increased progressively with a difference between the 30th week and the beginning of gestation higher than 1.5 (p=0.0001) and the highest increased at the end of the pregnancy (3.92±0.95 vs 6.04±1.40, p<0.0001). In women with intrauterine growth retardation (IUGR) without PE (n=22) SUA were similar to those of normal pregnancy, however in PE with IUGR (n=6), SUA increased significantly after the 35th week (3.93±0.47 vs 7.10±0.56, p=0.0019), more than any other obstetric situation. In all cases, urea and creatinine showed normal values, confirming that patients had no renal compromise. In conclusion, we suggest that the range of SUA in normal pregnant women is narrow throughout gestation. Thus, an increase in the levels of SUA may be useful to define risk groups to detect clinical changes involved in the manifestation of pregnancy disorders.

435 (276) **GABAERGIC SYSTEM IS INVOLVED IN GNRH MODULATION DURING GESTATION IN THE SOUTH AMERICAN PLAINS VIZCACHA (LAGOSTOMUS MAXIMUS).**

Pablo Ignacio Felipe Inserra[1,2], Santiago Elias Charif[1,2], Mariela Giacchino[1,2], Sofía Proietto[1], Alejandro Raúl Schmidt[1,2], Noelia Di Giorgio[2,3], María Clara Corso[1], Julia Halperin[1,2], Victoria Lux-Lantos[2,3], Alfredo Daniel Vitullo[1,2], Verónica Berta Dorfman[1,2].
[1]Centro de Estudios Biomédicos, Biotecnológicos, Ambientales y Diagnóstico (CEBBAD). Universidad Maimónides, Buenos Aires, Argentina. [2]CONICET, Argentina. [3]Instituto de Biología y Medicina Experimental (IByME), Buenos Aires, Argentina.

GABA is the major inhibitory neurotransmitter. GABAergic system is involved in the modulation of expression and delivery of gonadotropin-releasing hormone (GnRH), the central regulator of fertility in mammals. The South American plains vizcacha, Lagostomus maximus, shows peculiar reproductive features like

massive poliovulation and ovulation at mid-gestation. The aim of this work was to study the involvement of GABAergic and glutamatergic systems on GnRH regulation related to the reproductive stage. Hypothalami of non-pregnant non-ovulating (NPNO), non-pregnant ovulating (NPO), early-pregnant (EP) and mid-pregnant (MP) female vizcachas (n=6 per group) were used to study GABAA, NMDA and GLUR5 expression by Western-blot, GnRH content by RIA, GnRH pulsatility under GABA or glutamate (GLU) modulation by RIA, and serum estradiol (E2) and progesterone (P4) by ELISA. A significant decrease ($p<0.05$) in GABAA expression was observed in NPO and MP vs NPNO and EP females whereas variations among groups were not detected in NMDA or GLUR5 expression. GABAA displayed an opposite pattern of expression than the observed for E2, P4 and GnRH levels, with significant variations ($p<0.05$) throughout the reproductive cycle. To investigate GABA action on GnRH, hypothalamic explants were incubated with GABA, GABA+GABAA antagonist or GABA+GABAB antagonist, and GnRH secretion was determined. GnRH pulsatility frequency was significantly decreased by GABA related to control (CTL), whereas both GABAA and GABAB antagonists reverted GABA effects ($p<0.05$). However, GnRH total mass delivered was significantly increased by GABA vs CTL. No differences on GnRH pulsatility were detected when hypothalami were incubated with GLU or GLU combined with no-NMDA antagonist. These results suggest that the increment in GnRH expression at mid pregnancy is enabled by a decrease in GABAergic system that would elicit the activation of the hypothalamic-hypophyseal-ovary axis and the ovulatory process of mid-gestation.

436 (285) **MTOR/ AKT/ FOXO1 PATHWAY IS ALTERED IN THE HEART OF THE OFFSPRING FROM DIABETIC RATS**

Daiana Fornes[1], Sabrina Roberti[1], Hugo Sato[1], Alicia Jawerbaum[1], Romina Higa[1].

1. Centro de Estudios Farmacológicos y Botánicos (CEFYBO-CONICET-UBA).

Maternal diabetes programs cardiovascular alterations in the adult offspring, however very little is known about the mechanisms involved. Mammalian target of rapamycin (mTOR) is a key kinase involved in cellular growth, metabolism and survival, being part of complex 1 and 2 (mTORC1 and 2). Alterations in mTORC2 through Akt signaling pathways are involved in cardiac failure mechanisms. Besides, FoxO1, a transcription factor whose activation is modulated by the mTORC2 pathway, participates in cellular oxidative homeostasis, metabolism and survival of cardiomyocites, and its overactivation is related with cardiac dysfunction in humans and experimental models of diabetes. FoxO1 activation can be inhibited by Akt phosphorylation that induces FoxO1 inactivation by nuclear exportation. Our objective was to evaluate mTOR levels and Akt and FoxO1 phosphorylation status in the cardiac ventricle of male offspring from control and diabetic rats. Methodology: Pregestational mild diabetic rats were obtained by neonatal streptozotocin administration and were mated with healthy males. In the heart of adult offspring mTOR, Akt and FoxO1 levels and phosphorylation were evaluated by Western blot. Results: mTOR levels were found decreased in the heart of the offspring from diabetic rats ($p<0.05$). The ratio between phospho-Akt and total Akt levels was decreased ($p<0.05$). Moreover, while total FoxO1 levels were increased ($p<0.05$), the ratio between phospho-FoxO1 and total FoxO1 was decreased ($p<0.05$) in the heart of the offspring from diabetic rats when compared to controls. Conclusion: The reduction of the mTOR/ Akt pathway activation related to the increased availability of non-phosphorylated (active) FoxO1 observed, is probably involved in the alterations present in the heart of the adult offspring from diabetic rats.

437. (290) IGF SYSTEM: INVOLVEMENT IN THE OVARIAN FOLLICULAR PERSISTENCE IN COW.

Fernanda M Rodríguez[1], Emilia Huber[1], Natalia C Gareis[1], Antonella Stassi[1], Natalia R Salvetti[1], Hugo H Ortega[1], Florencia Rey[1].

1. Laboratorio de Biología Celular y Molecular, Instituto de Ciencias Veterinarias del Litoral (ICiVet-Litoral), Universidad Nacional del Litoral (UNL) / Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Esperanza, Santa Fe, Argentina.

Ovarian functions are controlled by several hormones as insulin-like growth factor (IGF) system. It has been postulated that alterations in IGF system would be involved in cystic ovarian disease (COD) development. The aim of this study was to evaluate the role of members of IGF system that were altered in COD, in the establishment of bovine persistent ovarian follicles. Thus, IGF1, its receptor IGFR1, the binding protein IGFBP4 and the hydrolytic enzyme PAPP-A were evaluated in ovarian samples. Estral cycles of non-lactating Holstein cows (n=25) were synchronized and then, animals were divided in five groups: control (without hormonal treatment) and 0 (day of ovulation), 5, 10 and 15 days of follicular persistence. Persistent follicles were obtained from cows treated with long-term subluteal progesterone administration using an intravaginal progesterone-releasing device. Concentrations of IGF1 determined by radioimmunoassay and levels IGFBP4 evaluated by western blot (WB) in follicular fluid (FF), were similar in all the stages of persistence and preovulatory follicles. IGFR1 expression analyzed by immunohistochemistry (IHC) was higher in the granulosa of persistent follicles of P0, P10 and P15 ($p<0.05$) than in preovulatory follicles. IGFBP4 protein expression analyzed by IHC was greater in all the stages of persistence than in preovulatory follicles ($p<0.05$). No differences were detected by WB in PAPP-A secreted to FF in all persistent follicles compared with preovulatory follicles. Results of this study showed that several changes previously determined in animals with COD are presented at initial stages of follicular persistence. These alterations in IGF system affect the normal ovarian follicular development and could lead to the development of the COD. These data confirm an essential role of the IGF system in the pathogenesis of COD in cattle.

438 (821) **ALTERED EXPRESSION OF STEROID HORMONES RECEPTORS COREGULATORS DURING BOVINE CYSTIC OVARIAN DISEASE DEVELOPMENT**

Ulises Sebastián Notaro[1], Emilia Huber[1], Natalia Salvetti[1], Hugo Ortega[1], Florencia Rey[1], Fernanda Rodriguez[1], Sebastián Recce[2], Fabián Barberis[2].

1. Laboratorio de Biología Celular y Molecular Aplicada, Instituto de Ciencias Veterinarias del Litoral (ICiVet-Litoral), Universidad Nacional del Litoral (UNL) / Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Esperanza, Santa Fe, Argentina. 2. Cátedra de Genética Veterinaria, Facultad de Ciencias Veterinarias del Litoral, Universidad Nacional del Litoral (UNL), Esperanza, Santa Fe, Argentina.

Steroid hormones regulate important reproduction events through its nuclear receptors. Steroid hormones receptors are ligand regulated transcription factors which modulate genic transcription through molecular mechanisms associated with coregulatory proteins (coactivators and corepresors). The endocrine profile, growth dynamics, and histologic characteristics of persistent ovarian follicles are analogous to those of spontaneous cysts. Cystic Ovarian Disease (COD) is a major factor contributing to poor reproductive efficiency of lactating dairy cow. Our purpose was to study the protein expression of Steroid Receptor Coactivator-1 (SRC-1) and corepressors Receptor-interacting Protein 140 (RIP140) and Ligand dependent nuclear receptor Corepressor (LCOR) in the ovaries of healthy cows (Control group) and in an experimental model of follicular persistence induced by low levels of progesterone. This was achieved by indirect immunohistochemistry and digital image analysis of granulosa cells (GC) and theca cells (TC) in different follicular categories: antral follicles of the control group as reference structure (AC), persistent follicles of groups with 0 (P0, expected day of ovulation), 5 (P5), 10 (P10) and 15 (P15) days of persistence. Expression of SRC-1 was lower in AC than in P0 and P15, in both GC and TC ($p<0.05$). Expression of LCOR was higher in GC of AC and P0 than P10 ($p<0.05$), with no differences in TC ($p>0.05$). Expression of RIP140 was similar in all analyzed follicular categories ($p>0.05$), in both GC and TC.

These results suggest that changes in the expression of coregulatory proteins can lead to altered response to steroid hormones, and thus contribute to the pathogenesis of ovarian alterations such as follicular persistence and COD.

439 (844) **MATERNAL ADMINISTRATION OF RESVERATROL PROTECTS FROM LIPOPOLYSACCHARIDE-INDUCED PRETERM LABOR**

Fernando Correa[1], María Victoria Bariani[1], Ana Paula Domínguez Rubio[1], Manuel Luis Wolfson[1], Julieta Aisemberg[1], Ana María Franchi[1].
1. Centro de Estudios Farmacológicos y Botánicos (CEFyBO - CONICET/UBA).

Preterm birth, defined as infants born alive before 37 weeks of pregnancy are completed, is one of the main causes of perinatal morbidity and mortality worldwide. According to the World Health Organization, an estimated 15 million babies are born prematurely every year and this number is rising. This represents 1 in 10 infants globally. Maternal inflammation/infections are among of the most common causes of preterm onset of labor. We have developed in our lab a murine model of lipopolysaccharide (LPS)-induced preterm labor. Resveratrol, a naturally occurring polyphenol, has been shown to exert anti-inflammatory effects. In the present study, we sought to investigate whether resveratrol was able to prevent the initiation of the preterm labor induced by LPS, as well as the molecular mechanisms involved. We found that, indeed, resveratrol prevented LPS-induced preterm labor in 64% of the cases. Moreover, maternal administration of resveratrol resulted in a downregulation of LPS-induced proinflammatory mediators such as COX-2 and iNOS ($P<0.05$ in each case). Interestingly, resveratrol also modulated the altered prostaglandin and endocannabinoid profile in the uterus from LPS-treated mice, suggesting that resveratrol exerts its tocolytic effects at multiple levels.

440 (917) **INVOLVEMENT OF NITRIC OXIDE AND TNF-α IN CELL DEATH AND PROLIFERATION OF GC-1 SPERMATOGONIA CELL LINE**

María Eugenia Ferreiro[1], Cinthia Soledad Mendez[1], Jimena Ferraris[1], Candela Gonzalez[2], Cristian Marcelo Sobarzo[1], Patricia Verónica Jacobo[1], Livia Lustig[1], María Susana Theas[1].
[1]Instituto de Investigaciones Biomédicas (UBA-CONICET), [2]Centro de Estudios Biomédicos, Biotecnológicos, Ambientales y Diagnóstico. Universidad Maimónides.

Nitric oxide (NO) and Tumor Necrosis Factor alpha (TNF-α) are pleiotropic factors that display multiple biological functions. In experimental autoimmune orchitis (EAO), a rat model of chronic inflammation associated to infertility, spermatocytes die by apoptosis induced by high levels of NO and TNF-α while spermatogonia that persist in the basal compartment of the seminiferous tubules are arrested. We propose that these factors are also involved in spermatogonia cell cycle control in EAO. To evaluate this hypothesis we explored the effect of NO and TNF-α/TNFR system on proliferation and death of the spermatogonia GC-1 cell line. DETA-Nonoate (DETA, a NO donor) reduced GC-1 viability evaluated by the colorimetric assay of MTS (mean±SEM, media: 0.499±0.013; DETA 2mM:0.344±0.024**; DETA 0.5mM:0.522±0.019; DETA 0.05mM:0.580±0.033 **p<0.01vs media) and by Trypan blue dye exclusion assay (1.7 fold vs media). We demonstrated by immunofluorescence that GC-1 expressed TNFR1. TNF-α reduced GC-1 viability (MTS, mean±SEM, media:0.468±0.059; TNF-α 50ng/ml:0.330±0.024*; 20ng/ml:0.282±0.006*; 10ng/ml:0.273±0.006*; *p<0.05 vs media) and reduced the% of GC-1 cells in S phase determined by flow cytometric analysis of cell cycle (mean±SEM, media: G1:27.55±0.050, S:58.10±,1.300 G2:14.350±1.250; TNF-α 50ng/ml: G1:37.230±1.200, S:40.470±6.100, G2:22.37±4.490). Our results showed that NO and TNF-α are involved in the balance of proliferation and death of GC-1 cells; suggesting that these proinflammatory factors might control spermatogonia cycle progression in the testis under chronic inflammation.

441 (909) **CYP17 HUMAN TESTICULAR EXPRESSION INCREASES INDEPENDENTLY TO GONADOTROPIN STIMULATION AND IN ABSENCE OF LOCAL CHANGES IN GROWTH HORMONE (GH)-IGF1-INSULIN AXIS AT LATE PREPUBERTAL PERIOD**

Paula Aliberti[1,2,3], Nora Isabel Saraco[1,2,3], Maria Sonia Baquedano[1,2], Marco Aurelio Rivarola[1,2], Roxana Marino[1], Alicia Belgorosky[1,2], Esperanza Berensztein[1].
1. Servicio de Endocrinología, Hospital de Pediatría Garrahan, Buenos Aires. 2. CONICET. 3. Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires.

In normal boys, insulin resistance, serum IGF1 and bio-available serum testosterone levels increase in late prepuberty and puberty, while this physiological changes and puberty onset are delayed in GH deficiency or resistance. Stimulation of primary human immature testicular cell cultures with IGF1 increases the expression of steroidogenic enzymes (Guercio G 2002, Belgorosky A 1987, Laron Z 1999, Berensztein EB 2008). Hypothesis: local changes of the GH-IGF-insulin axis might have a role in the testicular interstitium maturation previous to pubertal LH stimulation.

Objective: to evaluate the ontogenesis of GH-IGF-insulin axis and steroidogenic enzymes expression in human prepubertal testes. Human prepubertal testes, confirmed by histology analysis, were collected at time of necropsy from 18 subjects without endocrine or metabolic diseases. The expression of GHR, IGF1, IGF1R, INSR,

CYP17 and 3bHSD were assessed by real time PCR. Samples were divided in two groups: 1) Infancy, which includes the postnatal testicular activation stage, n=9 (Age: median 2 months, range 2 days - 12 months), and 2) Childhood, n=9 (Age: median 6 years, range 2 – 9 years). Mean expression of each gene was compared between groups. Even though all genes were expressed in all tissues, only IGF1R expression was significantly higher in Infancy than in Childhood ($p=0.01$); a similar tendency was observed for CYP17. In Childhood, a significant positive correlation between CYP17 expression with age was found ($p=0.03$, $r=0.7$). Although IGF1R and CYP17 are highly express in the infant testis, which might suggest a role in steroidogenesis during the postnatal testicular activation stage, local expression of the GH-IGF-Insulin axis components do not seem to be involved in the gonadotropin independent interstitium maturation detected in late prepuberty. It can be propose that the increment of CYP17 expression could be related to the physiological peripheral changes of the GH-IGF-insulin axis components.

FARMACOLOGÍA / FARMACOLOGY

442 (1082) **STEVENS JOHNSON SYNDROME SECONDARY TO ADMINISTRATION OF ANTIEPILEPTICS DRUGS**

Lorena Dos Santos[1], María Eugenia Horna[1], Isabel Hartman[1], Pablo Spada[1], María Teresa Rocha[1]
1. Facultad De Medicina Universidad Nacional Del Nordeste

Stevens Johnson Syndrome (SJS) is idiopathic in 25.5% of the patients but can also be caused by certain drugs (phenobarbital, carbamazepine, valproic acid). The Antiepileptic drugs hypersensitivity syndrome is a serious adverse drug reaction (ADR), initially described with aromatic antiepileptics such as phenytoin, carbamazepine, phenobarbital and primidone. Objective: To identify SJS as an adverse reaction of antiepileptic drugs notified to the Regional Pharmacovigilance Centre (CRF-UNNE). Observational descriptive, cross-sectional Study. All notifications of the CRF-UNNE database were included. The ADRs were classified considering: severity (mild, moderate, severe) and the mechanism of appearance (type A and B Rawlins and Thompson classification). From the 200 notifications of ADR by antiepileptic drugs, 2% (4) were SJS. Average age: 35 years old, female / male ratio: 1/1. Antiepileptic drugs were involved in SSJ according to the Anatomical Therapeutical-Chemical classification (ATC-2010) N03 code: N03AA barbiturates and derivatives (phenobarbital N03AA02), N03AB hydantoin derivatives (N03AB02 phenytoin),

N03AX other antiepileptics (lamotrigine N03AX09). According to severity: 10% were severe (1 fatal case of SJS). According to Rawlins and Thompson classification all cases of Stevens Johnson were type B. They were other SJS cases not related to antiepileptic drugs. They may occur with drugs other than antiepileptics that also act at the Central Nervous System.

443 (77) **DRUG REPOSITIONING IN CANCER THERAPY: THE ANTIVIRAL RIBAVIRIN MODEL AND EIF4E INHIBITION**

Luciano Anselmino[1,4], Silvina Richard[2,3,4], Verónica Martínez Marignac[1,4.]

1. Laboratorio Interdisciplinario De Biología Y Genética Molecular (IBIOGEM). Centro De Investigaciones Científicas Y De Transferencia Tecnológica A La Producción (Cicyttp-Conicet). Diamante, Entre Ríos, Argentina. 2. Laboratory Of Cytogenetic And Mutagenesis, Multidisciplinary Institute Of Cell Biology. 3. Department Of Science And Technology, National University Of Quilmes, Buenos Aires. 4. CONICET, Scientific Research Council, Province Of Buenos Aires And National Council Of Science And Technology, Argentina

The search of targeted therapy among drugs and compounds in clinical use for diseases others than cancer is an strategy of low cost and probable of fast track to phase I and II clinical studies. In this regards, the repurposing of Ribavirin, an antiviral with more than 40 years of use in the clinic against several DNA and RNA viruses, in cancer has been proposed since it is a specific inhibitor of inositide5'monophosphate dehydrogenase (IMPDH) and an inhibitor of the eukaryotic translation initiation factor 4E (eIF4E) activity. IMPDH expression and activity as well as eIF4E are deregulated in many cancers. Both metabolic and signalling pathways have been reported to have important roles in the development and progression of hematological malignancies and to be overexpressed in colorectal cancer (CRC). Objective: to study the effect of Ribavirin on Oxaliplatin, a commonly used drug in CRC therapy, in two commercially available cell lines (HT29 nd HCT116) by Sulforhodamine B (SRB) cytotoxicity assays. Results: drug combination showed that a clinically achievable concentration of Ribavirin (10uM) resulted in a significantly synergistic effect on Oxaliplatin in both cell lines tested with the sensitization index (R) of 2.93 ± 0.3 for HT29 and 1.71 ± 0.2 for HCT116, respectively. Conclusions: we showed sensitization to Oxaliplatin by targeting the capdependent translation pathway using Ribavirin. Our findings suggested that the metabolic changes induced by Oxaliplatin were dependent on downstream of PI3K/AKT pathway in particular mTOR/eIF4E pathway in HT29 and HCT116 cell lines making eIF4E dependent translation a targetable pathway in CRC under this treatment. More studies are needed in order to pursuit a phase I clinical trial as it is the case in CML and CLL leukemia where Ribavirin has been reported as a reliable approach in therapy.

444 (80) **ACUTE EFFECT OF GRANULOCYTE COLONY STIMULATING FACTOR (G-CSF) ON RAT VENTRICULAR FUNCTION AND ELECTROCARDIOGRAM**

María Cecilia Villa Etchegoyen[1], Patricia Bonazzola[2], Guillermo Alberto Keller[1], Roberto Alejandro Diez[1], Guillermo Di Girolamo[1]

1. Universidad De Buenos Aires - Facultad De Medicina - Segunda Cátedra De Farmacología Universidad De Buenos Aires. 2. Facultad De Medicina - Instituto De Investigaciones Cardiológicas " Prof Dr. Alberto C. Taquini"

Introduction: Among requirements for marketing authorization of drugs, guideline ICH S7B asks for *in vivo and in vitro* non-clinical evaluation to determine their arrhythmogenic potential through prolongation of ventricular repolarization assessed by QT interval in the ECG. For old products, approved decades ago, such evaluation is usually incomplete. This is the case for at least some recombinant G-CSF products. Objective: To evaluate the effect of recombinant G-CSF (glycosylated and non-glycosylated forms) on ventricular function and ECG of rats, and its relationship with concentration. Material and methods: isolated heart of female Wistar–Kyoto rats (n=25) perfused with Krebs solution were studied with Langendorff technique. Drugs were recombinant G-CSF (filgrastim and lenograstim, between 10 and 800 ng/ml; 10 to 20 ng is the expected peak after SC administration) and moxifloxacin (1 to 5 µg/ml, as positive control. QTc was calculated with Bazzet formula and expressed as absolute value, or ΔQTc and%ΔQTc. Variables calculated were left ventricular pressure (PVI, presented as absolute or percentual value, respect to perfusion without drug), maximum rate of contraction and relaxation (+dV/dT and -dV/dT, respectively) and basal pressure (PR). Results: only filgrastim increased significantly absolute QTc at 400 and 800 ng/ml. The 3 drugs reached ΔQTc over 20 ms but only filgrastrim increased significantly this variable between 100 and 800 ng/ml. All drugs decreased PVI, reaching 28.1% respect to control with filgrastim. Krebs and lenograstim did not change +dV/dT or -dV/dT whereas moxifloxacin and filgrastim reduced both variables; they also increased PR in a dose-dependent manner. Conclusion: In spite of both being agonists of G-CSF receptor, filgrastim showed higher effect on ventricular repolarization than lenograstim.

445 (162) **EFFECT OF GLUCOCORTICOIDS ON THE STRUCTURE AND ACTIVITY OF AN EXOGENOUS PULMONARY SURFACTANT IN A NEW PHARMACEUTICAL FORMULATION**

Alejandra Noemi Cimato[1], Andres Hoyos Obando[1], Graciela Facorro[1], Margarita Martinez Sarrasague[1]

1. Cátedra De Física, Facultad De Farmacia y Bioquímica, UBA.

Introduction: Glucocorticoids (GCs) are commonly used to treat chronic lung disease. The incorporation of GCs in the membranes of an exogenous pulmonary surfactant (EPS) could be a novel alternative management to avoid serious side effects of systemic administration and improve drug delivery. The EPS would act as a carrier with therapeutic effect, so it is essential that GCsincorporated into the EPS not affect its biophysical properties. It is known that cholesterol (Cho) generates loss of surfactant capacity, and since the GCs are Cho biochemically derived, they may behave similarly. Objective: To develop a pharmaceutical product combining an exogenous pulmonarysurfactant (PROSURF) and a GC: beclomethasone (Be), budesonide (Bu) or fluticasone (Flu) and to determine how much GC is incorporated into the membranes of the surfactant, and if their presence in the bilayers alter the structure and/or the activity of the EPS. Methods: Samples: EPS-GC (PL = 10 mg / ml; GC 1-3 mg/ml). Controls: EPS and EPS-Cho. The conformational changes and rigidity were evaluated by electron paramagnetic resonance spectroscopy. GCs incorporated into EPS were determined by UV absorption and by polarized light microscopy. Surfactant activity was measured with a pulsating bubble surfactometer. Results and Discussion: Be and Bu were incorporated into EPS up to 1 mg/ml. Crystals of these two GCs were observed at higher concentrations. Be and Bu caused minimal changes in the rigidity of the polar region and increased surface tension, but these changes were not enough to inactivate the surfactant. Flu was incorporated up to 2 mg/ml, and did not significantly alter the biophysical properties at any concentration. Conclusions: All GCs tested could be incorporated into EPS up to 10% causing no detrimental effects on EPS functionality, but EPS-Flu seems to be the most favorable association. These novel combination EPS-GCs might be a promising strategy in the therapy of pulmonary diseases.

446 (235) **DISTRIBUTION AND CONCORDANCE OF N-ACETYLTRANSFERASE GENOTYPE AND PHENOTYPE IN ARGENTINE ADULT INDIVIDUALS UNDER ISONIAZID TREATMENT**

Guillermo Alberto Keller[1], Lucas Fabian[1], Matías Gomez[1], Roberto Alejandro Diez[1], Guillermo Di Girolamo[1]

1. Centro de Vigilancia y Seguridad de Medicamentos, Departamento de Farmacología y Toxicología, Facultad de Medicina, Universidad de Buenos Aires.

Introduction: NAT2 is a polymorphic enzyme involved in the metabolism of several drug. Its activity varies widely between

individuals and ethnic groups and has been associated with susceptibility to adverse reactions and lack of efficacy in relation to the individual phenotype. Hypothesis: Changes in NAT2 enzyme activity are correlated to drug concentrations that can cause potentially toxic effects and / or lack of efficacy. Methods: Adult patients under isoniazid treatment for tuberculosis were included in a epidemiological cross sectional study. Distribution of NAT2 status was classified by genotype (Blum Technique) in rapid (two wild-type alleles, wt), intermediate (only one wt allele) and slow acetylators (both mutated alleles). Metabolic ratio (MR) was determines using acetyl-isoniazid (AcINH) / isoniazid (INH) concentrations two hours after INH intake. Phenotype was classified with reported limits (0.48 and 0.78) and through antimodes determination. Genotype/Phenotype correlation was evaluated with Pearson analysis. Results: 100 patients were included for analysis (53 males). The mean ± SD (min-max) age was 34±9 (25-44) years, weight 68±14 (42-98) Kg and body mass index of 25±3 (18,6-39,1) Kg/m^2. 15 subjects were homozygous for wt allele (rapid acetylators), 46 heterozygous (intermediate acetylators) and 39 slow acetylators. The isoniazid received dose was 4,59±0,88 (3,05-5,99) mg/Kg/day. Pharmacokinetic parameters at 2 hours were: INH 0,29±0,25 (0,02-1,16) µg/mL, AcINH 0,40±0,21 (0,02-0,90) µg/mL and MR 2,82±2,55 (0,02-9,71). Evaluation with classic MR parameters were inconsistent with obtained genotype, meanwhile reinterpretation using antimodes of the trimodal distribution (1.2 and 5.0) led to high genotype/phenotype correlation (>98%, P<0.05). Discussion: Genotyping alone provided an efficient, accurate method of analysis for acetylator status. Meanwhile phenotype seems to be more useful for predicting the risk of toxic or ineffective concentrations.

447 (250) **EVALUATION OF T(P-E) INTERVAL CHANGES INDUCED BY MEPERIDINE IN STANDARD CLINICAL SETTINGS**

Guillermo Alberto Keller[1], María Cecilia Villa Etchegoyen[1], Nicolás Fernandez[2], Nancy Mónica Olivera[2], Patricia Noemí Quiroga[2], Roberto Alejandro Diez[1], Guillermo Di Girolamo[1]
1. Centro De Vigilancia Y Seguridad De Medicamentos, Departamento De Farmacología Y Toxicología, Facultad De Medicina, Universidad De Buenos Aires. 2. Centro De Asesoramiento Toxicológico Analítico (Cenatoxa). Cátedra De Toxicología Y Química Legal, Facultad De Farmacia Y Bioquímica, Universidad De Buenos Aires.

Introduction: The Tp-e interval has been reported as a better parameter for ventricular arrhythmia and sudden death prediction in clinical trials. However, no data about Tp-e changes in standard clinical settings had been reported. Objective: To evaluate Tp-e interval changes in a previously studied sample of patients under standard clinical treatment with a known QT-prolonging drug (meperidine). Methods: Patients with meperidine analgesic indication were included. A basal (previous to treatment) and intratreatment (at steady state) ECG were done for each patient. Basal and intratreatment QT,QTc, dQTc (difference between intratreatment and basal QTc), Tp-e interval and Tp-e/QT coefficient were measured. Correlation analysis was done between ecg variables and plasma drug concentrations. Results: 58 patients were studied (43.1% males). All patients received a (mean ± SD [range]) meperidine dose of 304 ± 133[120-480] mg/day. Meperidine and normeperidine concentrations were 369 ± 60[265-519] and 49 ± 17[15-78] ng/mL respectively. Intra-treatment control results were QTcB 370 ± 30 [305-433], ΔQTcB +9 ± 42 [-90 to +136], Tp-e 63± 14 (40-110), Tp-e/QT 0,19 ± 0,03 (0,15-0,29) ms. Meperidine concentration correlated with QTc (R>0.36) interval, ΔQTc (R>0.69), Tp-e (0,51) and Tp-e/QT (0,47), but the correlation was even better for normeperidine concentration, QTc (R>0.52), ΔQTc (R>0.81), Tp-e (0,58) and Tp-e/QT (0,50). 15 patients (25.86%) presented ΔQTc values > 30, and 8 patients (13.79%) showed ΔQTc > 60. Only 6 patients presented Tp-e/QT>0,23 (10.34). Renal failure was associated ΔQTc > 30 ms (RR 3.74, IC95% 1.73-8.10) and ΔQTc > 60 ms, (RR 4.27, IC95% 1.26-14.48). No patient developed arrhythmias during the study. Conclusions: Tp-e presents a good correlations with meperidine concentrations and its lesser prolongation pattern can explicit the lack of frequent arrhythmogenic effects associated with meperidine treatment in clinical practice.

448 (364) **ANTINOCICEPTIVE EFFECTS OF AMITRIPTYLINE ARE POTENTIATED BY OMEGA-3 FATTY ACIDS COMBINED TREATMENT IN RATS**

Maria Eugenia Toledo[1], Valentina Barral[1], Carlos Horacio Laino[1]
1. Instituto De Biotecnología. Centro De Investigación E Innovación Tecnológica (CENIIT). Universidad Nacional De La Rioja

From the 1960s up to now, tricyclic antidepressants have been prescribed for chronic pain syndromes with successful results. Amitriptyline (AMI) should be considered an analgesic drug of first choice in the treatment of various painful conditions, but in many clinical situations their use is limited by its adverse effects. Research shows that omega-3 fatty acids (O-3 FA) reduce inflammation pain associated with a variety of conditions. Recently, studies in our laboratory have demonstrated that chronic co-treatment of morphine and O-3 FA (DHA and EPA) had a greater antinociceptive effect than morphine alone at the same dose, rendering an additive effect. The aims of the current study were to examine the effects of combinations of amitriptyline with O-3 FA to determine whether these two drugs could produce additive or synergistic effects after the chronic treatment in the hot plate test (HPT) in Wistar rats. Also, we evaluated the locomotor activity of the experimental groups, using the Open Field Test as an assessment of the sedative effect of AMI. We found that compared to control, O-3 FA increased the response latencies following acute and chronic administration, indicating that O-3 FA has antinociceptive effects in rats. AMI (20 mg/kg/day) increased response latency and co-administration of chronic O-3 FA and AMI revealed a higher antinociceptive efficacy than the individual treatments in HPT. Additionally, O-3 FA decreased the sedative effects of AMI. It leads to the conclusion that O-3 FA have antinociceptive effects per se, and the combination of AMI and O-3 FA has more analgesic efficacy than AMI alone in HPT. These data might contribute to new therapeutic approaches and may mean higher response rates and lower side-effects associated with AMI treatment. More studies are required to understand the action mechanism underlying the use of the combination of AMI and O-3 FA.

449 (535) **COMPARISON STUDY OF PLASMA COENZYME Q10 AND OTHER ANTIOXIDANTS LEVELS IN HEALTHY SUBJECTS SUPPLEMENTED WITH DIFFERENT COENZYME Q10 FORMULATIONS**

Paula Samassa[1], Manuela Martinefski[1], Fabian Buontempo[1], Christian Höcht[2], Silvia Lucangioli[1,3], Valeria Tripodi[1,3]
1. Departamento De Tecnología Farmacéutica, Facultad De Farmacia Y Bioquímica, Universidad De Buenos Aires, Buenos Aires, Argentina. 2. Departamento De Farmacología, Facultad De Farmacia Y Bioquímica, Universidad De Buenos Aires, Buenos Aires, Argentina. [3]consejo Nacional De Investigaciones Científicas Y Tecnológicas, CONICET, Buenos Aires, Argentina

Coenzyme Q10 (CoQ10) is a lipophilic molecule considered an antioxidantagent. CoQ10 deficiency is involved in mitochondrial, cardiological anddegenerative muscle and neuronal disease. Most patients with these deficiencies have shown clinical improvement with oral CoQ10 supplementation, especially in infants. The aim of this work was to conduct a preliminary comparison of the plasma CoQ10 levels after a single dose and one week supplementation with two different CoQ10 formulation: commercial grade CoQ10 powder (solid formulation) and a new oil-in-water liquid emulsion. An additional objective was to evaluate the effect of 1-week CoQ10 supplementation at the levels of other antioxidants such as vitamins A, E and C. Six healthy individuals participated in a randomized, crossover, open,consecutive design, with a 2-week washout period. Pharmacokinetic parameters have been assessed after single and multiple intakes of 250 mg CoQ10 given daily

for 1-week. The differences in the pharmacokinetic parameters of maximum plasma CoQ10 concentration, area under the curve between 0-360 and 0-4 h and elimination half-life were statistically significant ($p<0.01$) between formulations, with a relative bioavailability of 489% increase of liquid emulsion over solid CoQ10 formulation. A multiple dose supplementation increased plasma CoQ10 levels in both formulations, liquid emulsion performing better (2.4- vs 3.9-fold for solid and liquid formulation, respectively) without modifications on other antioxidants. Furthermore, the plasma CoQ10 at 7th day was statistically different between formulations ($p < 0.05$). The results obtained have shown that liquid emulsion improves thebioavailability of CoQ10 respect to solid form which not only facilitates theindividualized administration for the infant but in turn could increase the therapeutic efficacy, which should be confirmed by further studies.

450 (595) **GLYCEROL (GLY) INHIBITS HEPATOCELLULAR CARCINOMA CELL PROLIFERATION BY REACTIVE OXYGEN SPECIES (ROS) GENERATION.**

Alejo Matías Capiglioni[1], Florencia Lorenzatti[1], Quiroga Ariel Darío[1], Parody Juan Pablo[1], María Cristina Carrillo[1], Raúl Alberto Marinelli[1], María Paula Ceballos[1], María de Lujan Alvarez[1]

1.Instituto De Fisiología Experimental (IFISE)

In previous studies our group reported antiproliferative and pro-apoptotic effects of GLY administration in an in vivo preneoplasic model. We postulate GLY as a possible ROS inductor that works as a signaling molecule after its metabolization to GLY phosphate (GlyP). Aim: To begin to study the antiproliferative mechanisms of GLY using hepatocarcinoma HepG2 cells. Methods and Results: MTT assay was used to determine the effects of GLY on HepG2 cell line proliferation. The dose-response curve revealed that cell proliferation was diminished in a dose dependent manner (IC50= 0.5% v/v). The primary cellular metabolite of GLY is GlyP. To analyze this metabolic fate of GLY, HepG2 cells were incubated at 37 ^{0}C for 72 h with 0.5% and 1% GLY and GlyP generation was measured by a colorimetric reaction. GlyP levels showed a 47.5%* and 21.0%* increase in cells treated with GLY 0.5% and 1%, respectively, compared to control cells. It has been reported that subsequent GlyP metabolism induces ROS generation. To determine the possible occurrence of this phenomenon, after 72 h of treatment, cells were incubated 30 min with 2',7'-Dichlorofluorescein diacetate (DCF-DA). Inside the cells, DCF-DA molecules were hydrolyzed by esterases, and reacted with intracellular ROS, producing a fluorescent product that can be measured fluorometrically. GLY caused a raise of 20%* in ROS production at both concentrations tested (*$p<0.05$). Conclusion: Cell proliferation was significantly decreased after 72 h of GLY treatment in a dose-dependent manner. GlyP levels were higher in those cells exposed to the treatment, indicating that GLY is metabolized into GlyP, which could be further involved in mitochondrial ROS production. It is well-known that ROS generation can induce antiproliferative and cell death pathways that may explain this phenomenon. Additional studies are needed to elucidate the precise mechanisms involved in the antiproliferative action of GLY in HepG2 cells.

451 (609) **EFFECT OF SIRTUIN 1 (SIRT1) INHIBITORS ON CELLULAR VIABILITY AND APOPTOSIS IN HEPATOCELLULAR CARCINOMA (HCC) CELL LINES. POSSIBLE ASSOCIATION WITH THE EXPRESSION OF ABC TRANSPORTERS.**

Giulia Decándido[1], Ariel D. Quiroga[1], María de Luján Alvarez[1], Juan Pablo Parody[1], Flavia Lambertucci[1], Aldo D. Mottino[1], María Cristina Carrillo[1], María Paula Ceballos[1]

1. Instituto De Fisiología Experimental (IFISE) – CONICET

Multidrug resistance (MDR) in patients with HCC frequently results from upregulation of ABC transporters. SIRT1 deacetylase is overexpressed in this pathology and is associated with tumoral progression and MDR by inducing the expression of the ABC transporter p-glycoprotein (p-gp). Inprevious studies we found a dose dependent cytotoxic effect of the SIRT1 inhibitors Cambinol (Camb) and Ex527 (Ex) in HepG2 and Huh7 cell lines, with Huh7 showing the highest response. Aim: to analyze if SIRT1 activity blockage could be beneficial during HCC therapy. Methods: HepG2 and Huh7 cells were treated for 72 h with 50 μM Camb (Camb50) or 40 μM Ex (Ex40) and viability (MTT), apoptosis (FITC Annexin V) and protein levels of p-gp and MRP3 (western blot) were assayed. Results: SIRT1 inhibitors significantly reduced viability only in Huh7 cells (Camb50: -35%*, Ex40: - 7%*). Camb50 did not modify apoptosis in HepG2 cells but significantly increased early (+65%*) and late (+45%*) apoptosis in Huh7 cells. Ex40 induced early apoptosis in both cell lines (HepG2: +168%*, Huh7: +118%*). Only Camb50 generated a significant fall in p-gp levels (-66%*) in HepG2 cells, whereas both drugs downregulated MRP3 (Camb50: -40%*, Ex40: -37%*) in this cell line. In Huh7 cells, Ex40 significantly reduced MRP3 levels (-30%*) while Camb50 significantly increased its expression (+135%*). Lastly, a significant raise was observed in p-gp levels with Camb50 (+711%*) and Ex40 (+277%*) in Huh7. *$p<0.05$ vs. control cells. Conclusions: Both inhibitors diminished viabilityand increased apoptosis in Huh7, whereas in HepG2 only Ex40 was capable of increasing early apoptosis without changing cellular viability. The modulation of ABC transporters expression was different in both HCC cell lines. Additional studies are necessary to understand this discrepancy and to support the potential application of SIRT1 inhibition during HCC therapy. Finally, this isthe first study that analyzes MRP3 regulation by SIRT1.

452 (918) **PHARMACOKINETIC AND PHARMACODYNAMIC PROPERTIES OF CARVEDILOL IN COARCTED HYPERTENSIVE RATS**

María Corina Litterio[1], Susana Beatriz Gorzalczany[2], Mónica Galleano[1,3], Christian Höcht[2,4], César Guillermo Fraga[1,3], Carlos Alberto Taira[2,4]

1.Cátedra de Fisicoquímica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires. 2.Cátedra de Farmacología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires. 3.Instituto de Bioquímica y Biología Molecular, Universidad de Buenos Aires, CONICET. 4.Instituto de Fisiopatología y Bioquímica Clínica, Universidad de Buenos Aires, CONICET.

Beta blockers have been traditionally considered as the first line therapy for hypertension. The aim of this study was to evaluate pharmacokinetics and cardiovascular effects of carvedilol (a beta blocker of third generation) in a hypertension model of aortic coarctation. Wistar male rats (380-420 g body weight) were subjected to coarctation of abdominal aorta (CoA) or a sham operation (SHAM). 7 days after the surgery, rats were injected i.v. with vehicle or carvedilol 5 mg kg^{-1} and blood pressure (BP) and heart rate were recorded immediately. At that time, CoA rats showed a significant loss of body weight and a lower left kidney weight/body weight ratio respect to SHAM rats. Basal mean arterial pressure of hypertensive rats (166±8 mmHg) was significantly higher than that of the sham operated rats (116±4 mmHg). After intravenous administration of carvedilol a biexponential decay of plasma carvedilol levels was found in both experimental groups compatible with a pharmacokinetic two-compartment model. The chronotropic response as well as the hypotensive response to carvedilol were not significantly different comparing CoA and SHAM rats. When studied the frequency components of blood pressure variability (BPV) (very low frequency-VLF, low frequency-LF and high frequency-HF), CoA rats showed greater BPV in the VLF range compared with SHAM animals, suggesting a compromise of renin-angiotensin system and myogenic vascular function in the regulation of BP. However, no difference was found in LF and HF variability between groups. On the other hand, carvedilol administration significantly reduced BPV in the three studied domains in SHAM and CoA rats. In conclusion, carvedilol exhibited increased hypotensive response in both experimental groups as a consequence of a greater inhibition of vascular sympathetic activity, although we did not find an overactivity of this system in this model of hypertension.

453 (934) **VALIDATION OF THE PHARMACOGENETIC DOSE ADJUSTMENT ALGORITHM FOR THE INITIAL DOSE OF ACENOCOUMAROL**

Esteban Jauregui[1], Paula Scibona[1], Carolina Vazquez[1], María Orlova[1], Jorge Arbelbide[1], Ventura Simonovich[1], Waldo Belloso[1]
1. Hospital Italiano De Buenos Aires

Introduction: Coumarin anticoagulants require a strict dosing control through INR (International Normalized Ratio) in order to prevent lack or excess of pharmacological effects. Usually fixed initial dose is prescribed to adults. Previous reports have shown pharmacogenetic approaches towards an initial adjustment of warfarin dose. We have previously developed an algorithm including clinical and pharmacogenetics data to individualize initial dosing of acenocoumarol, the most extensively used oral anticoagulant in our country. Objectives: To validate the acenocoumarol initial dosing adjustment algorithm previously derived in a different cohort of patients requiring chronic oral anticoagulation. In addition we analyzed the behavior of the algorithm when a new polymorphism (CYP4F2 alleles) was added. Methods: Nested cross-sectional study that included patients under anticoagulation with stable doses of acenocoumarol (3 consecutives INR values between 2-3 at time of enrollment). Genotyping of CYP2C9 (1/1 normal, 1/2 and 1/3 intermediate, 2/3, 2/2 and 3/3 low expression alleles), CYP4F2 (rs2108622 vs any other genotype) and VKORC-1 (A haplotype > susceptible to OAC, no A haplotypes considered normal) were conducted through PCR/RFLP techniques. Algorithm (with and without CYP4F2) was analyzed in its ability to predict the actual dose required for anticoagulation. Results: 40 patients were included in the validation cohort. Median age, weight and amiodarone use were similar to the observed in the derivation cohort. The algorithm was applied to each patient and compared with the actual acenocoumarol dose required. All patients had a stabilized dose of oral anticoagulant. Conclusions: Initial dose of acenocoumarol can be individualized through an algorithm including both clinical and pharmacogenetic data. This “a priori” adjustment may prevent the risks of adverse effects of acenocoumarol during the initial weeks of oral anticoagulation, being subsequently complemented by INR.

NANOMEDR POSTER PRESENTATION / NANOMEDICINE SESSION VI POSTERS: PRIZE TO THE BEST POSTER IN NANOMEDICINE
I. Drug Delivery & Targeting

454 (262) **BIOLOGICAL AND TOXIC EFFECTS OF BILIARY SALTS USED IN THE DEVELOPMENT OF NANOPARTICULATE SYSTEMS FOR DRUG DELIVERY.**

Gándola, Yamila B.[1,2], Luschnat, Tania T.[1]; Fontana, C.[1]; Gonzalez, L.[1, 2].
1. Departamento de Química Biológica Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956 C1113AAD, Ciudad Autónoma de Buenos Aires, Argentina.[2]Instituto de Química y Fisicoquímica Biológica (IQUIFIB)-CONICET.

Biliary acids (BA) are bioactive molecules, mainly implicated in fat diet absorption and digestion, which have multiple metabolic actions. Salts derived from BA are used for the design of severalpharmaceutical nanoparticulated systems. However, high levels of BA have been associated with tumorogenesis of the colon, oesophagus, stomach, pancreas and breast tissue. Considering the multiple biological actions of BA, their association with cancer pathology and their use in pharmaceutical technology, it is highly relevant to study their biological and toxic effects over cancer cells. The objective of this work was to analyze the molecular and cellular effects ofsodium cholate and deoxycholate salts (SC and SDC) over the epithelial breast cancer cells MCF-7. For this purpose, the effects of bile salts over cell proliferation and apoptosis, and the activation of signaling molecules involved in cell cycle and survival promotion were studied. MCF-7 cells were incubated with different concentrations of each bile salt during 24 and 48 hours and cell viability wasassed by a colorimetric assay. Results showed that both biliary salts induced an increase in cell viability at low doses, but citotoxic effects were evidenced when cells were incubated with high SC and SDC concentrations. Cleavage of PARP, which is a hallmark of apoptosis, was found to increase 24hours after incubation with the highest SC concentration tested. Kinetic assays of Akt and Erk1/2 phosphorylation evidenced that citotoxic concentrations of SC and SDC induced a strong and sustained activation of both signalling mediators, while low SC and SDC concentrations only promoted a slight activation of Akt. In conclusion, biliary salts have a dosage-dependent effect over the MCF-7 cells viability. Citotoxicity induced by high bile salts concentrations could be associated to the marked and sustained activation of signalling mediators involved in cell proliferation and survival.

455 (381) **SOLID SILICA-FUNCTIONALIZED MAGNETIC NANOPARTICLES AS DRUG TARGETING DEVICES: DRUG INCORPORATION AND ENDOTHELIAL CYTOTOXICITY.**

Agotegaray, Mariela[1]; Campelo, Adrián[2]; Massheimer, Virginia[2]; Lassalle, Verónica[1]1INQUISUR - CONICET. **De**partamento de Química. Universidad Nacional del Sur.
[2]INBIOSUR - CONICET. Deparrtamento de Química. Universidad Nacional del Sur.

Introduction: Solid silica (Si) coated magnetic nanoparticles (MNPs) are potential devices for drug targeting. Stability and biocompatibility of silica turn these systems feasible forbiomedical applications althoughdrug loading is a challenge due to silica inertia. Objective: Synthesis, characterization and Diclofenac (Dic) incorporation onto citric acid(CA)-functionalized magnetite(MG) coated with Siand 3-aminopropyltriethoxysilane(APTES). Evaluation of cytotoxicity on rat aortic endothelial cells (EC). Design: MG/CA NPs were obtained by co-precipitation. Si and APTES functionalization was performed bya modified Stöber process, prolonged for 12h at room temperature, using ratios MG/CA:TEOS:APTES=(1:0.5:2; 1:1:2; 1:2:2), named 1, 2, and 3. They were characterized by FTIR, DLS, z potential and HR-TEM microscopy. Dic was bonded by N,N´-dicyclohexylcarbodiimideand studied by UV-Vis spectroscopy. Primary cultures of EC were exposed for 48h to final concentrations of 1, 10 and 100µg/mL of MNPs and Dic-loaded MNPs. Cell viability was studied by MTT assay and by the capacity to produce NO (DAN assay). Results: 1 and 2 presented Dh of 400.0±10.0nm and 520±10.0nm; z of 27.9±5.78mV and21.9±6.02mV in aqueous dispersions. 3 was polydisperse with z 32.6±5.81mV. HR-TEM micrographs showed matrix dispersed structures for the MNPs. Formulation 2 incorporated approx. 40% of Dic. Cell viability was not affected at 10µg/mL or below for all the samples ($p<0.001$). Basal NO production was not altered at any of the assayed concentrations for allMNPs except for Dic loaded nanocarrier at 100µg/mL ($p<0.001$). Conclusion: Synthesis of Si/APTES functionalized MNPs rendered aqueous dispersible formulations dependant on precursors´ ratio and concentrations. Diclofenac was covalently incorporated. EC cytotoxicity was dose and drug dependent. No adverse effects for unloaded MNPs were observed. These novel nanocarriers may be suitable for drug targeting.

456 (383) **BETA-CYCLODENTRIN COATED MAGNETIC NANOPARTICLES: NEW INSIGHTS FOR THE LOCALIZED TREATMENT OF DIVERSE PATHOLOGIES.**

Agotegaray, Mariela[1]; García, Elba [1]; Campelo, Adrián[2]; Massheimer, Virginia [2]; Lassalle Verónica[1]
[1]INQUISUR - CONICET. Departamento de Química. Universidad Nacional del Sur. [2]INBIOSUR - CONICET. Departamento de Biología, Bioquímica y Farmacia. Universidad Nacional del Sur.

Introduction Magnetic nanoparticles (MNPs) provide new insights for targeted drug delivery to aspecific site in the organism by an external magnetic field. Coating of MNPs improvesbiocompatibility and provides a platform to attach drugs for diverse purposes.

Objective Synthesis, characterization and evaluation of endothelial cytotoxicity of nano-systemscomposed of magnetite (MG),

oleic acid(OA) and betacyclodextrin (BCD) as nano-devices for drug targeting.

Design OA stabilized MG MNPs were synthesized by co-precipitation. A dispersion of MAG-OA (0.5 mg/mL) in hexane was mixed with aqueous solution of BCD in different MNPs:BCD ratios (1:1; 1:2; 1:3, named MG-OA-BCD1, MG-OA-BCD2, MG-OA-BCD3) for 24h at room temperature. The organic phase was separated; the content of the aqueous phase was extracted with Nd magnet, washed and dried. Samples were studied by FTIR, DLS to determine hydrodynamic diameter (Dh) and surface charge(z); TEM and HR-TEM. Primary cultures of Rat aortic endothelial cells (EC)were exposed 48h to final concentrations of 1, 10 and 100 µg/mL of MG-OA-BCD1 and MG-OA-BCD2. Cell viability was evaluated by MTT assay and by the capacity to produce NO (DAN assay).

Results FTIR demonstrated the incorporation of BCD on MG-OA. Aqueous monodisperse MG-OA-BCD1, MG-OA-BCD2, MG-OA-BCD3 presented Dh of 589.3±42.71nm, 370.3±6.00nm, 555.4±8.50nm and z of 2.27±6.14mV, 10.3±4.85mV, 14.4±5.12mV respectively. HR-TEM micrographs showed almost-spherical shaped MNPs. Synthesis of MG-OA-BCD3 was not reproducible. Cell viability was not affected at doses of 10µg/mL for MG-OA-BCD1 and 1 µg/mL for MG-OA-BCD2($p<0.001$). Basal NO production was not altered at any of the concentrations for both MNPs.

Conclusion The synthesis of nano MAG-OA-BCD was successful. Physicochemical properties depend on BCD concentration. EC cytotoxicity of MNPs was dose dependent with no adverse effects at concentrations below 10µg/ml. The novel nanosystems may possibly be suitable for biomedical applications.

457 (420) **A NEW ALTERNATIVE FOR VITAMIN D CONTROLLED RELEASE BASED IN POLYMERSOMES**

Besada, Lucas N.[1,2]; Cortizo, Ana M.[1]; Cortizo María S.[2.]
1Laboratorio de Investigaciones en Osteopatías y Metabolismo Mineral (LIOMM), Departamento de Cs. Biológicas, Facultad de Cs. Exactas, UNLP, La Plata, Argentina 2Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA), Dpto. de Química, Fac. Cs. Exactas, UNLP, CCT- La Plata, CONICET CC 16, Suc. 4. Argentina.

Vitamin D plays a key role in mineral homeostasis, exerts potent effects on cell growth and differentiation, and modulates the immune response. Oral bioavailability of vitamian D is affected when there are alterations in intestinal absorption caused by pathophysiologic conditions or by dietary or pharmacological interactions. Controlled release systems of drugs are innovative methods in the pharmaceutical area, to improve bioavailability, efficacy and safety of transported drugs. Polymer vesicles prepared by self-assembly techniques have attracted increasing interest in recent years as result of their application such as delivery drugs vehicles.

In the present study we synthesize amphiphilic tri-block copolymer (PBzV-b-PEG-b-PBzV) through controlled radical polymerization (RAFT). Then polymersomes, vesicles composed of polymer bilayer, were formed using solvent injection method with ultrasonication. Simultaneous encapsulation of hydrophilic (propidium iodide) and hydrophobic (rodamin 123) fluorescent dyes was performed. In order to characterize the synthetized copolymer, size exclusion chromatography (SEC) and Infrared Spectroscopy were used. The membrane morphology of the polymersomes was investigated using fluorescent microscopy and inverted confocal laser scanning microscopy. The results showed that the synthesized copolymer has a suitable size and expected functional groups. The microscopic analysis of the polymerosomes shows the presence of two distinct regions in the vesicular structure. The hydrofobic dye was confined to the wall of polymersomes, which consist of hybrophobic PBzVchains, whereas hydrophilic dye was localized in the aqueous core of them. In conclusion, the synthesized copolymer is suitable for the formation of polymeric vesicles and the system could be used in the future to encapsulate vitamin D in the wall of polymersomes to build a controlled release system of this metabolite in order to improve the efficiency of current treatments.

458 (447) **EFFECT OF DIFFERENT ANTI-LEISHMANIAL DRUGS ON THE BEHAVIOR OF LIPOSOMES: ELASTICITY AND PENETRATION THROUGH HUMAN SKIN.**

Peralta, María F.[1]; Guzmán Maria L.[2]; Pérez, Ana P.[3]; Apezteguia, G.[3]; Olivero, María E.[2]; Romero E.[3], Carrer, Dolores C.[1]
1. Instituto de Investigación Médica Mercedes y Martín Ferreyra - INIMEC, CONICET, Universidad Nacional de Córdoba. 2. Departamento de Farmacia. Facultad de Ciencias Químicas - UNITEFA, CONICET, Universidad Nacional de Córdoba. 3. Programa de Nanomedicinas, Universidad Nacional de Quilmes.

Cutaneous Leishmaniasis is a parasitic orphan disease which causes ulcerative injuries and may induce serious complications. Topical treatments would minimize drugs side effects and enhance patient compliance. We propose the use of AmphotericinB (AmB), Imiquimod (Im) or Indole (Ind) carried on Soy phosphatidylcholine: sodium cholate liposomes for topical administration. The literature shows that liposomes penetration in the skin is related to their flexibility. We hypothesize a correlation between the drugs penetration and the liposomes flexibility. Hence, we measured the effect of the candidate drugs on the liposomes flexibility and their skin permeation capabilities. Liposomes flexibility was studied by extrusion. Skin penetration assays were performed in Franz Cells with abdominal human skin. The drugs permeability coefficient (Kp) was calculated. The quantity of drug retained in epidermis and dermis and the penetration depth were determined by validated methods. Drug suspensions at equivalent concentrations of the liposomes were used as references. D penetration ratio (DR) (liposome D µg/ reference D µg) was calculated for comparison purposes. The incorporation of Im did not produce significant changes in the flexibility of liposomes (ANOVA, $p>0.05$), however an increase in flexibility was determined from Ind-liposomes. Formulations containing AmB could not be extruded at the working pressure, possibly due to a decrease in liposomes elasticity. All of the drugs assayed reached the dermis. Kp could only be determined for Ind systems. Ind from liposomes penetrates deeply into the skin in comparison with its reference (DR=1.7), with most of the drug being found in dermis. Im penetrated moderately into the skin, with most of the drug being found in epidermis and a significant amount found in dermis (DR=0.8). AmB was mostly retained in epidermis, with very little amount of drug found in dermis (DR=0.1). Liposome-containing drugs showed a flexibility with the order Ind>Im>AmB. This order correlates with drugs penetration into the skin.

459 (618) **UTILIZATION OF NANOPARTICLES FOR SPECIFIC TARGETING OF ACTIVATED GLIAL CELLS.**

Murta, Veronica[1]; Schilrreff, Priscila[2]; Seib, Mariana[2], María José Morrilla[(2)], Alberto Javier Ramos[(1)]
1. Instituto de Biología Celular y Neurociencias "Profesor E. De Robertis", Facultad de Medicina (UBA), CONICET, Buenos Aires, Argentina. 2. Programa de Nanomedicinas, Universidad Nacional de Quilmes-CONICET, Buenos Aires, Argentina.

An unequivocal association between neurodegeneration and exacerbated immune activation has arisen for most central nervous system (CNS) disorders, and the conversion of glial cells into the proinflammatory phenotype is associated with increased neuronal death. Therefore, regulation of glial activation seems a suitable strategy to reduce CNS damage in different scenarios, including brain ischemia.Unfortunately, this therapeutic strategy is challenging due to the difficulties for some drugs to access the CNS, and possible neurotoxic effect as a result of undesired impact on neurons and other cell types. Given that some polyamidoamine (PAMAM) dendrimers were shown to be incorporated specifically by glial cells, our objective was to design nanoparticles suitable for targeted drug delivery to reactive glial cell.

Considering that the activation of NFkB has been associated with glial proinflammatoryphenotype, *we hypothesize that targeted inhibition of the NFkB pathway in activated glial cells would diminish this phenotype*. In the present work we used

rat primary cell cultures, a reporter (FITC) nanoparticle, and sulfasalazine (SFZ) loaded nanoparticles. We found that a new type of core-shell tectodendrimer (G5G2.5 PAMAM) is time- and dose-dependently incorporated by astrocytes and microglia. Interestingly, the exposure to oxygen and glucose deprivation (OGD), an *in vitro* model of ischemia, specifically increased astroglial uptake of the G5G2.5-FITC dendrimer. Moreover, hippocampal neurons co-cultured with glia did not incorporate it. However, when analyzing the effect of the SFZ-loaded tectodendrimer, we encountered an up-regulation of the NFkB pathway, which was not compensated by the drug. In conclusion, even though the G5G2.5 showed a marked preference for activated astroglia and microglia, making it suitable as a specific drug carrier for reactive glial cells, its action on the NFkB pathway would make it unsuitable for preventing proinflammatory glial phenotype.

460 (720) **INTERACTION OF NANOPARTICLES-TUMOR SPECIFIC MONOCLONAL ANTIBODIES COMPLEX WITH POLYMORPHONUCLEAR CELLS.**

Mitarotonda, Romina[1]; Giorgi, Exequiel[1]; Cerny, Natacha[1]; Fernandez, Marisa[3]; Desimone, Martín[2]; De Marzi, Mauricio[1].

1. Laboratorio de Inmunología, Instituto de Ecología y Desarrollo Sustentable (INEDES) UNLu-CONICET y Departamento de Ciencias Básicas, Universidad Nacional de Luján, Luján, Buenos Aires, Argentina. 2. Universidad de Buenos Aires. IQUIMEFA-CONICET. Facultad de Farmacia y Bioquímica. Cátedra de Química Analítica Instrumental. Buenos Aires, Argentina. 3. Universidad de Buenos Aires. IDEHU-CONICET. Facultad de Farmacia y Bioquímica. Buenos Aires, Argentina

Cancer can be treated by surgery, chemotherapy, radiation therapy, hormonal therapy, and target therapy. However, these treatments often have severe side effects that limit their efficacy. To improve therapy efficiency, tumor specific monoclonal antibodies (mAb) are currently used. For instance, anti-HER-2 mAb Trastuzumab (TZ) used to treat breast cancer has demonstrated remarkable benefits to cancer patients. Nanoparticle-based treatments for cancer therapy represent a promising strategyto enhance therapeutic outcomes by reducing off-target side effects. We developedseveral techniques to prepare silica nanoparticles hollow (SiO_2NPs) that carrying on the surface TZ (TZNPs). This is a nanoparticle able to carry drugs or immunomodulatory molecules in the hollow and it would be directed to the tumor microenvironment by the presence of the antibody. As it has been shown that neutrophils may play a crucial role in the tumor microenvironment including both promoting or inhibiting tumor growth, we evaluated the effect of this new tool on thesecells. We evaluated the production of TGFβ as protumor N2 phenotype and theproduction of IL-1β and nitric oxide (NO) as phenotype N1 antitumor to inducingimmunogenic cell death. In order to study the effect of NP on neutrophils, thecells were incubated for 4 h and the supernatants were obtained. Neutrophils in presence of SiO_2NPs produced high concentrations of NO and IL-1β compared to control. Furthermore, concentrations of TGFβ were not significant. Moreover, the mAb TZ produced a significant increase of IL-1β, a slight increase of TGF and similar values of NO with respect to control cells. Finally, we evaluated TZNPs observing similar levels of TGFβ, IL-1β and NO in comparison to control cells.These results demonstrate that TZNPS does not activate the protumor N2 phenotype. Therefore, TZNPs could be a safe tool for the treatment of illness by recognizing specific tumor cells and transporting effectors molecules.

461 (885) **STABILITY AND COMPATIBILITY EVALUATION OF A NOVEL CATANIONIC UNILAMELLAR VESICLES IN BIOLOGICAL SYSTEMS AS POTENTIAL DRUG NANOCARRIER.**

Stagnoli, Antonela S.[1,2]; Correa, Nestor M.[2]; Luna, Maria A.[2]; Farias, Ezequiel Ma.[1,2]; Villa, Cristian C.[2]; Niebylski, Ana M.[1]

1Departamento de Biología Molecular, Facultad de Ciencias Exactas, Físico-químicas y Naturales, UNRC. 2 Departamento de Química, Facultad de Ciencias Exactas, Físico-químicas y Naturales, UNRC

Catanionic surfactants arise from combining certain anionic and cationic surfactants.They have unique characteristics because their ability to form unilamellarvesicles spontaneously, which is advantageous in drug vehiculization. Despite theirpotential benefits, little is known about their effects on human health. The goal of this work was to develop and characterize a novel catanionic vesicles and evaluate its stability and compatibility in biological systems. The AOT-BHD vesicles were obtained by the combination of the anionic surfactant: sodium bis (2-ethylhexyl) sulfosuccinate (AOT) and the cationic: benzyl dimethyl hexadecylammonio chloride (BHDC). Vesicles stability was evaluated in culture medium (DMEM), saline solution (S), phosphate buffer (PBS), albumin (7% W/V) and at acid medium (pH=2.0) by dynamic light scattering. Cell-vesicles interaction was evaluated with confocal microscopy. Toxicity was evaluated by reduction of tetrazolium salts (MTT), erythrocyte resistance (RG), Trypan blue (TB) and Lethal Dose 50 (LD_{50}) in mice. The vesicles maintained their size and polydispersity at pH=2.0 for only 90 min, in albumin and DMEM solutions. In PBS and S an increase of the vesicle sizes were obtained, probably because of electrostatic interaction with the salts. Polydispersity was maintained which denotes that the vesicles are bigger but stables. TB and the MTT assays showed citotoxicity at concentrations higher than 0.05 mg/ml, but only 2 mg/ml concentration decreased the RG. Confocal microscopy showed vesicles incorporation inside the cell by endocytosis. LD_{50} value obtained was 114.9 mg/kg. These results indicate that vesicles are stable in different solution although in saline media there is an electrostatic interaction with the salts. The resistance at acidic medium, the vesicle capacity of penetrate into the cell and their harmlessness at concentration lower than 0.05 mg/ml, makes the AOT-BHD vesicles a very promising candidate for oral drug delivery.

462 (905) **CHARACTERIZATION AND INTERACTION WITH CELLS OF NANOHYBRIDS: ZnO NANORODS - ULTRADEFORMABLE NANOVESICLES.**

Perez, Ana P.[1]; Apezteguia, Gustavo[1]; Romero, Eder[1].

1. Nanomedicine Research Program-2 (NRP-2) /3R-Center for Anti-inflammatory Nanomedicines (3R-CAN)- National University of Quilmes (UNQ)-CONICET. Argentina.

The efficient penetration of adjuvant and antigen across the skin can generate antigen-specific protective immune reactions required for development of a topical needle free vaccine. The objective of this work was to design an adjuvant system combining the ability of ZnO nanorods (NRZnO) to generate reactive oxygen species (ROS), and the ability of ultradeformable nanovesicles (ultradeformable liposomes and archaeosomes- UDL and UDA, respectively) to penetrate skin. This combination would facilitate the access of NRZnO to the viable epidermis triggering an immune response against an antigen associated. In this work, NRZnO suspensions and nanohybrids (NRZnO-UDL and NRZnO-UDA) were prepared and characterized regarding colloidal stability- and interaction with cells *in vitro*. The aqueous suspension of NRZnO presented reproducible structural characteristics in terms of zeta potential, absorption of UV light, hydrodynamic size and polydispersity, remained stable against changes in ionic strength and pH neutral to basic and generated ROS. However, large aggregates were formed after storage at 4°C for 7 to 28 days. In contrast, not formation of aggregates was found after storage of nanohybrids, indicating that the combination with nanovesicles prevents aggregation of NRZnO. On the other hand, cytotoxicity, uptake and proinflammatory cytokine production were performed in keratinocytes HaCaT and macrophages J774A.1 and THP-1. Only macrophages were able to uptake NRZnO. Finally, incubation of macrophages with NRZnO induced the production of proinflammatory cytokines IL-1β in THP-1 and TNF-α in THP-1 and J774. However, cytokines were not released or potentiated after incubation with nanohybrids, probably due to a high quantity of nanovesicles compared to the quantity of NRZnO used, which could decrease uptake of NRZnO. In conclusion, nanohybrids

were effective to achieve colloidal stability but cytokines release was not potentiated compared to NRZnO.

463 (907) **NANOSTRUCTURED LIPID CARRIERS DECORATED WITH ARCHAEAL LIPIDS FOR TARGETING TO MACROPHAGES OF THE INFLAMED MUCOSA.**

Higa, Leticia[1]; Jerez, Horacio[1]; Briski, Andres[1]; Romero, Eder L.[1]; Morilla, Maria J.[1]
1. Nanomedicine Research Program-2 (NRP-2) /3R-Center for Anti-inflammatory Nanomedicines (3R-CAN)- National University of Quilmes (UNQ)-CONICET. Argentina

Inflammatory bowel diseases (IBD) such as Crohn's disease and ulcerative colitis are disorders of the gastrointestinal tract, characterized by chronic inflammation and epithelial injury induced by the uncontrolled activation of the mucosal immune system. We hypothesize safer therapies could rely on developing macrophages-targeted drug delivery systems capable of specifically delivering high doses of anti-inflammatory and anti-oxidant drugs with minimal exposure of healthy or distant tissues via oral administration.In the present work, we developed nanostructured lipid carriers (NLC) made of a core composed of a mixture of solid (compritol) and liquid (neutral archaeal) lipids stabilized by a shell of polar archaeal lipids and Tween 80, containing dexamethasone. Neutral and polar archaeal lipids of the hyperhalophile archaea *Halorubrum tebenquichense* provided the nanoparticles with antioxidant activity, stability under gastrointestinal conditions and the advantage to be uptaken by macrophages via scavenger receptor class A, respectively. NLC of 100 -300 nm, Z-potential from -38 to -50 mV, entrapment efficiency of 37%, were stable over 31 days of storage at 4°C. DSC showed a decrease of melting point of compritol and low recrystallinity index, indicating the liquid lipid was dispersed within the compritol matrix.TEM micrographs showed spherical shape nanoparticles. No cytotoxicity of NLC (20- 200 µg/ml compritol) on macrophages and epithelial cells were revealed after 24h of incubation. NLC were highly uptaken by macrophages and epithelial cells *in vitro*. Besides, NLC showed higher antioxidant potential than β-caroten and showed high anti-inflammatory activity as measured by reduced production of TNF-α in *lipopolysaccharide* stimulated macrophages. These results suggest that NLC prepared with neutral and polar archaeal lipid are candidates as macrophages-targeted drug delivery systems that combined anti-inflammatory and anti-oxidant activities.

464 (922) **MUNA: MULTIEFFECTOR NANOVESICLES AS POTENT READY TO USE VACCINE ADJUVANTS AGAINST NEGLECTED DISEASES.**

Parra, Federico L.[1]; Caimi, Ayelén T.[1]; Cargnelutti, Diego E.[2]; Vermeulen, Mónica[3]; Petray, Patricia B.[4]; Romero, Eder L.[1]
1. Nanomedicine Research Program-2 (NRP-2)/3R-Center for Anti-inflammatory Nanomedicines (3R-CAN), National University of Quilmes(UNQ)-CONICET, Argentina. 2. Institute of Medicine and Experimental Biology of Cuyo (IMBECU)-CONICET, Mendoza, Argentina. 3. Institute of Experimental Medicine (IMEX)-CONICET, National Academy of Science, Buenos Aires, Argentina. 4. Institute of Medical Microbiology and Parasitology (IMPaM-CONICET), Faculty of Medicine, University of Buenos Aires, Argentina

Chagas and leishmaniasis, two neglected diseases caused by the protozoans *Trypanosoma cruzi* and *Leishmania sp.* respectively, affect more than 8 million people of populations living in poverty and without adequate sanitation. Currently, the development of effective vaccines is still an outstanding bill and adjuvants are the key elements to stimulate and potentiate the immune response.

In this work, imiquimod (IMQ), a Toll Like Receptor 7 (TLR7) ligand, was incorporated into archaeosomes (ARC), nanovesicles made of archaeolipids that contain ligands of scavenger receptors. The fundamentals underlying this design are that ARC will be greedily recognised and endocyted by antigen presenting cells in comparison with conventional liposomes and the IMQ will access in a greater quantity to the endosomal TLR7 causing a stronger immune response. Furthermore, the capacity of ARC with IMQ (ARC-IMQ) to act as vaccine adjuvants against *L. amazonensis* and *T. cruzi* was tested *in vivo* in mice models. In one experiment, formulations of ARC-IMQ, ARC and IMQ alone and Montanide ISA 763 (a commercial adjuvant approved for animal's vaccines but not humans) were mixed with total leishmania antigen and administrated subcutaneously in Balb/c mice three times, one every 15 days. In other experiment, the same immunization protocol was tested in C3H mice but the formulations administrated were ARC-IMQ and IMQ alone mixed with total homogenate of *T. cruzi* (TH), ARC-IMQ and TH free. On day 35, the humoral immune response induced by each formulation was evaluated. Finally, four weeks after the last administration the immune cell populations were analysed by flow cytometry.

The results demonstrate that ARC-IMQ can elicit better antigen specific IgG antibody response in comparison with ARC or IMQ alone and with Montanide and more activation of CD4, CD8 and B lymphocytes than ARC.

In conclusion, despite further preclinic investigation is needed to understand the mechanism of action, ARC-IMQ could be used as vaccine adjuvants against neglected diseases.

465 (937) **STRUCTURALLY MODIFIED (SM) ARCHEOSOMES FOR "STEALTH" MACROPHAGE TARGETING: FIRST STEPS TOWARDS THE EFFICIENT DESIGN OF CARDIOVASCULAR NANOMEDICINES**

Jerez, Horacio E.[1]; Bakas, Laura S.[2]; Romero, Eder L.[1]
1. Nanomedicine Research Program-2 (NRP-2) /3R-Center for Anti-inflammatory Nanomedicines (3R-CAN)- National University of Quilmes (UNQ)-CONICET. Argentina. 2. Biochemical Research Institute of La Plata (INIBIOLP), CCT-La Plata, CONICET. Faculty of Medical Sciences. National University of La Plata. Argentina.

Developing an anti-atheromatous therapeutic strategy mediated by a nano-drug delivery system capable of recognize and eliminate the foam cells in the atheromatous plaque, constitute a formidable technological challenge. To that aim, a special "non biological complex drug" (nbcd) has to be designed, capable of circulating for a prolonged time period, minimizing the plasma protein corona -in order to avoid its recognition and elimination by Kupffer cells in the liver-, lacking of any immune reactogenicity, maintaining its colloidal stability during circulation and retaining its active pharmaceutical principle (api) after dilution. Finally, it has to be actively recognised only by its target cells. The importance of this sequence of pre-clinical steps lies in its use as a predictive tool of suitability: failing in accomplishing one single of these requirements means the whole strategy has to be re-designed. In this work, we present for the first time the concept of structural modification of nanovesicles prepared from archaeolipids extracted from the archaea Halorubrum tebenquichense, by inclusion of cholesterol and cholesteryl hemisuccinate in the archaeolipid matrix. The resultant sm-archaeosomes (determined by fluorescence anisotropy of laurdan) were loaded with the bisphosphonate alendronate and tested for their aggregation in blood and plasma of BALB/c mice, retention of alendronate after 1/500 and 1/1000 dilution in blood, and J774A.1/HaCat cells uptake in the absence or presence of blood/plasma. Our results suggest that sm-archaeosomes and conventional archaeosomes or pegylated nanoliposomes, retained drug and colloidal stability in front of a strong dilution, and were actively taken up only by macrophages, the only cell type that received a massive delivery of carried apoptotic drug. Sm-archaeosomes thus, can be presented as the first step towards a macrophage-targeted nano-drug delivery system compatible with industrial scaling up and GMP methods.

466 (965) **DERMATAN SULFATE/CHITOSAN POLYELECTROLYTE COMPLEX FOR TARGETING AGENTS TO THE VESSEL WALL.**

Vasta, Mercedes[1,3]; Funez, Florencia[1,3]; Glisoni, Romina[1]; Sosnik, Alejandro[2]; Calabrese, Graciela C.[1,3]
1Universidad de Buenos Aires. CONICET. Instituto de Nanobiotecnología (NANOBIOTEC). Buenos Aires, Argentina,

2. Laboratory of PharmaceuticalNanomaterialsScience, Department of MaterialsScience and Engineering, Technion-Israel Institute of TechnologyTechnion City, Haifa, Israel, 3. Universidad de Buenos Aires- Facultad de Farmacia y Bioquímica- Cátedra de Biología Celular y Molecular. Junín 956 Primer Piso (CABA- C1113AAD, Argentina).

There is still no pharmaceutical treatment that directly targets the blood vessel wallinstead of just controlling the risk factors in cardiovascular disease. We have produced polyelectrolyte complexes (PECs) by a simple and reproducible polyelectrolyte complexation method between low molecular mass dermatan sulfate (LMMDS) (polyanionic polysaccharide) and chitosan (CS) (polycationic polysaccharide). We have reported that the uptake of PECs by microvascular endothelial cells was specific. The aim of the present work was to analyze the binding of PECs to normal and injured endothelial cells. PECs were prepared employing CS labeled with fluorescein isothiocyanate (FITC-PECs). The size (D_h), size distribution (PDI) and zeta potential (Z-potential) of PECs were determined by dynamic light scattering (DLS). Murine heart microvascular cells (H5V) were incubated with FITC-PECs (10 µg/mL, according to their LMMDS concentration) for 30, 60, 120 and 180 min in the absence or in the presence of 37.5 µg/mL LMMDS. Lipopolysaccharide treatment (1.5 µg/ml) was performed for 3 hs to induce cell injury. Fluorescence images from a minimum of 10 fields were collected from each sample. FITC-PECs, produced by the ionotropic gelification method, exhibited a main size distribution of 644 ± 81 nm (98%) and an average PDI value of 0.398 ± 0.043 (n= 3). After lipopolysaccharide treatment PECs uptake increased around three times compared with control cells (n=3). H5V cells presented a homogeneous green dotted signal after incubating with PECs for 30-60 min; restricted to certain areas after 120-180 min. The presence of a high but nontoxic dose of LMMDS blocked the uptake of FITC-PECs at all incubation time. Our *in vitro* results show that (1) the uptake of CS-LMMDS PECs was increased in injured endothelium and (2) LMMDS of the PECs could be associated to the specific binding to endothelial cell, what also suggests that CD44 receptor could be responsible for the specific interaction.

467 (968) **ANTIMICROBIAL AND CELL VIABILITY MEASUREMENT OF CHITOSAN NANOPARTICLES TO BE APPLIED AS THERAPY IN BOVINE MASTITIS.**

Orellano, María S.[1,2]; Bohl , Luciana P.[1]; Isaac, Paula[1]; Breser, María L.[1]; Falcone, Ruben D. [2], Porporatto, Carina[1].
[1]Centro de Investigaciones y Transferencia (Conicet), Universidad Nacional de Villa María, Villa María, Córdoba, Argentina. [2]Departamento de Química, Facultad de Cs. Exactas Fisicoquímicas y Naturales, Universidad Nacional de Río Cuarto, Rio Cuarto, Córdoba, Argentina.

Staphylococcus is the genus commonly isolated from bovine mastitis in many countries, which can express several virulence factors, including biofilm formation associated with persistent infections. The difficulty in eradication and the increasing concerns on antibiotics usages underscore the interest in developing new tools to control staphylococcal mastitis. The use of nanomaterials in medicine has enabled the development of more effective therapies. The purpose of this study was to evaluate the antimicrobial activity and anti-biofilm capacity of chitosan nanoparticles (ChNP) against bovine mastitis isolates, as well as their cytotoxic effect on bovine mammary epithelial cells (MAC-T), macrophages (BoMAC) and kidney cells (MDBK). ChNP were obtained using reverse micelles (RM) as nanoreactors and prepared by cross-linking reaction of glutaraldehyde and native Ch inside of n-heptane/sodium 1,4-bis-2 ethylhexylsulfosuccinate (AOT)/water. ChNP were characterized by dynamic light scattering showed a size of 134nm. Minimum inhibitory (MIC) and bactericidal (MBC) concentrations of ChNP were determined against *S. aureus* (29313 and V329) and *S. xylosus* (1007) strains, and the biofilm biomass and cell viability were quantified spectrophotometrically after staining with crystal violet and MTT. The results show that ChNP has lower MIC and MBC values and stronger ability to inhibit biofilm formation of the bacteria tested than Ch (200 vs 1600 µg/ml; p <0.5). The treatment of biofilm with ChNP for 24 h resulted in a significant decrease in the metabolic activity at 12,5 µg/ml relative to that of the untreated control; while a residual metabolic activity of less than 20% at 200 µg/ml (V329) and 800 µg/ml (1007). ChNPs were not cytotoxic to MAC-T, BoMAC and MDBK cells at concentrations below to 200 µg/ml after 24h of treatment. ChNP have antimicrobial properties and low cytotoxicity for bovine cells, resulting in promising candidates for applications in the control of bovine mastitis.

468 (976) **COMPLEXATION OF AMIODARONE AND NIMODIPINE WITH HYDROXYPROPYL-BETA CYCLODEXTRIN AS POTENTIAL ANTI-LEISHMANIA AGENTS IN A COMBINATION FORMULA WITH HEXADECYLPHOSPHOCHOLINE.**

Glisoni, Romina[1]; Delgado Murcia, Lucy G.[2]; Sosnik, Alejandro[3].
1. Universidad de Buenos Aires. CONICET. Instituto de Nanobiotecnología (NANOBIOTEC). Buenos Aires, Argentina. 2. Research Group in Immunotoxicology, Department of Pharmacy, National University of Colombia, Bogotá, Colombia. 3. Department of Materials Science and Engineering, Technion-Israel Institute of Technology, Haifa, Israel.

Introduction/Goal. Leishmaniasis is a zoonotic disease caused by the parasites infection of the genus Leishmania which induces cutaneous, mucocutaneous or visceral involvements. In the world, there are more than 350 million people at risk of disease and each year about 2 million new cases are reported. The treatment can be performed with pentavalent antimonial, amphotericin B, pentamidine or hexadecylphosphocholine (HPC), which can throw serious adverse events and increased resistance, why it is urgent the research for new therapeutic tools or improving the existing ones. The amiodarone (AMI) and nimodipine (NMP) drugs have shown *in vitro* activity against trypanosomatides but *in vivo* activity against L. panamensis, the main causative agent of cutaneous leishmaniasis in Colombia,has not been yet evaluated. Because both drugs have a very poor solubility inaqueous media (260 and 0.5µg/mL, respectively) and before the evaluation of its effectiveness in an *in vi*vo model of infection, it is that it was investigated the complexation ability of HPβCD to improve the aqueous solubility of AMI and NMP as an approach to develop a pre-nanoformulation for topical treatment of cutaneous leishmaniasis in combination with HPC, which it is well known to be irritant topically.

Outstanding Results. Increments up to 37 and 271 times for AMI and NMP were achieved in the presence of HPβCD (10% w/v). On the other hand, the complexes obtained were fully characterized by different techniques (size and Z-pot by DLS, ATR/FTIR, XRD and SEM). The average size ranged from 145-175 nm (PDI=0.09-0.62). The physical stability of the complexes in solution (4°C) was preserved up to 1 month.

Conclusions. The solubility of AMI and NMP in aqueous medium has been successfully increased by complexation with HPβCD. HPC meanwhile could also be complexed. Overall results support the next stage of evaluation in an *in vivo* model of infection of Leishmania with potential additional pharmacokinetic advantages.

469 (1046) **THE INTERACTION OF PH SENSITIVE LIPOSOMES WITH LUNG SURFACTANT IN MONOLAYER BIOMIMETIC SYSTEMS.**

Altube, Maria J[1]; Cutro Andrea[2]; Disalvo Anibal[2]; Romero Eder L[1].
1. Programa de Nanomedicinas, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes. 2. Laboratorio de Biointerfases y Sistemas Biomimeticos, Centro de Investigacion y Transferencia de Santiago del Estero (CITSE/UNSE/CONICET)

The pH sensitive archaeosomes (ApH) are nanovesicles made of polar archaeolipids extracted from hyperhalophile archaebacteria, dioleylphosphatidylethanolamine and cholesterylhemisuccinate. In a previous work, we have shown that ApH efficiently deliver its aqueous content to the cytoplasm of alveolar macrophages and lung epithelial cells in vitro. Moreover, ApH were structurally more resistant to nebulization than conventional pH sensitive liposomes[1]. Those features make ApH excellent candidates for delivering drugs

to the lungs. However, in an in vivo context, inhaled ApH must first interact with the pulmonary surfactant (PS) lining layer that covers the internal surface of the alveolus and provides the low surface tension at the air-liquid interface. Interactions with the PS film determine the subsequent retention and translocation of the inhaled ApH and hence their potential activity on target cells. The interaction of pH sensitive liposomes with Prosurf, a natural PS, was studiedby measuring changes of pressure and compressibility at different initial surface pressures after addition of nanoliposomes by injection, in the aqueous subphase, or nebulization onto the PS monolayer. The results obtained show that ApH produce the highest increase of surface pressure in PS monolayers. This behavior is dependent of the membrane packaging and nanoliposome concentration, the higher interaction was determined with a loosy PS monolayer and with the highest ApH concentration. Also, the ApH incorporation rate into PS monolayer has been affected by the way of its addition, when it was nebulized onto the monolayer the pressure increase rate was higher than when it was injected in the monolayer subphase. Since inhaled ApH would interact with the monolayer from the air phase, this second model would be more suitable. 1-Altube, Maria Julia, et al. “Surviving nebulization-induced stress: dexamethasone in pH-sensitive archaeosomes.” Nanomedicine 11 (2016): 2103-2117.

470 (1049) **DEVELOPMENT OF A DRUG DELIVERY SYSTEM (DDS) BASED ON POLYMERIC NANOPARTICLES: THE POSSIBILITY OF AN ORAL ADMINISTRATION ROUTE FOR INTERFERON ALPHA.**

Imperiale, Julieta[3]; Cánepa C; Berini C[1]; Gherardi Magdalena[1]; Lewicki, Marianela[2]; Acosta, Gabriela B[3].; Sosnik, A[4].; Biglione, Mirna[1]

1. Institute of Biomedical Research on Retroviruses and AIDS (INBIRS), UBA-CONICET, Buenos Aires, Argentina. 2. Instituto de Investigaciones en Microbiología y Parasitología Médica, CONICET, Buenos Aires, Argentina. 3. Institute of Pharmacological Research (ININFA), National Scientific and Technical Research Council, School of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina. 4. Laboratory of Pharmaceutical Nanomaterials Science, Technion, Haifa, Israel.

Interferon alpha (IFNa) is a protein drug used to treat oncological diseases and viralinfections. Owing to its sensitivity to enzymatic degradation and limited absorption in the gastrointestinal tract, pegilated IFNa is administered via parenteral route once weekly which is associated with pain, allergic reactions and poor patient compliance. To overcome these problems, the design of a suitable drug delivery system (DDS) able to protect the drug in order to administer it orally would lead not only to greater acceptance and adherence to the treatment but also to a better quality of life for patients. In this context, we prepared IFNa2b loaded chitosan nanoparticles (IFN CS NPs) by ionotropic gelation method. Infrared spectra supported the formation of CS NPs. The amount of CS that formed NPs, colorimetrically determined, was 95.5%. Size, determined by dynamic light scattering (DLS), showed a bimodal distribution; the mean sizes were 381.7 ± 35.2 nm and 50.17± 6.96 for blank CS NPs, 353.0 ± 31.2 nm and 42.49 ± 23.75 for IFNa-loaded ones. The polidispersity index was 0.472 ± 0.030 and 0.407 ± 0.010, while the zeta potential (Z-Pot) 31.4 ± 4.6 mV and 31.8 ± 1.7 mV, respectively. The Z-Pot value suggests not only a net positive surface charge but also physical stability of the DDS as was confirmed at 4 and 25°C for 30 days by DLS results. The encapsulation efficiency was 99.5%. Transmission electron microscopy confirmed the size obtained by DLS results. The antiviral activity of encapsulated IFN determined in Vesicular Stomatitis Virus (VSV) infected MDBK cells, was comparable to commercial IFN. Preliminary pharmacokinetic studies in Balb/C mice showed absorption of IFNa2b after oral administration of IFN loaded CS NPs in opposition to different studies in which the drug was not detected in plasma following administration of free drug. These promising CS NPs show great potential for application in oral delivery of IFNa2b allowing an enhancement of patient compliance.

471 (1053) **TOPICAL VACCINATION WITH SUPER-STABLE READY TO USE NANOVESICLES**

Caimi, Ayelen T[1]; Parra, Federico[1]; De Farias, Marcelo A.[2]; Portugal, Rodrigo[2]; Perez, Ana P.[1]; Romero, Eder L.[1]; Morilla, Maria J.[1]

1. Nanomedicine Research Program-2 (NRP-2) /3R-Center for Anti-inflammatory Nanomedicines (3R-CAN)- National University of Quilmes (UNQ)-CONICET. Argentina. 2. Cryo-Electron Microscopy Group (CME), Brazilian Nanotechnology National Laboratory (LNNano)

Topical vaccination has the potential to make vaccine delivery more equitable, safer and as efficient as parenteral vaccination. Topical vaccination however, is challenged by the presence of the stratum corneum interposed between antigens/adjuvants and the skin-associated lymphoid tissue. Ultradeformable archaeosomes (UDA), vesicles made of polar archaeolipids (TPA) from the hyperhalophile archaea *Halorubrum tebenquichense* (mixture of sn 2,3 ether linked saturated archaeolipids) plus sodium cholate, that can penetrate the intact stratum corneum, could pave the way for an efficient topical vaccination. In this work we submitted UDA to heat stress of sterilization and to lyophilisation. Then we screened the immunogenicity of the model antigen ovalbumin (OVA) associated to UDA in different ways (suspended in the aqueous space, adsorbed to the surface and administered separated) upon topically applied to Balb-c mice; and the adjuvant properties of sterilized and lyophilized UDA was determined. Results showed that after autoclaving the population size and polydispersity index (PDI) of UDA were conserved; in contrast, UDL increased its size by 5 folds. Lyophilized UDA with optimum lyoprotectant combination (0.068% w/v glucose -2.5% v/v glycerol), conserved the size and PDI after 4 months at 40°C; in contrast, UDL increased by 8 folds the size. Surprisingly we found that topical administration of UDA with antigen encapsulated in the aqueous space or adsorbed to the surface elicited the same immune response. Finally, we showed that sterilized and lyophilized UDA retain adjuvant activity upon mixing with antigen. In conclusion, the presence of TPA in UDA provides the colloidal stability needed to be autoclaved, lyophilized and stored under cold-free conditions (super-stable). Furthermore, UDA have the potential to function as an adjuvant when mixed with antigen solutions that does not require the complex steps of antigen loading (ready to use).

472 (1075) **ARCHAEOSOMES IMPROVE REDOX AND INFLAMMATORY STATE IN AORTA SMOOTH MUSCLE CELLS CULTIVATED IN VITRO**

Quesada, Isabel[1,2]; Cejas, Jimena[2]; Arlandi, Marcos[2]; Castro, Claudia[1,2]; Romero, Eder L.[3],

1. Instituto de Medicina y Biología Experimental de Cuyo (IMBECU-CONICET). 2. Facultad de Ciencias Médicas - Universidad Nacional de Cuyo (3) Nanomedicine Research Program, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes.

Oxidative stress is an imbalance between reactive oxygen species (ROS) production and the antioxidant defense system. NADPH-oxidase, a potent source of superoxide anions (O2·-) in the vascular wall, is directly implicated in atherogenesis. Angiotensin II stimulates ROS production through NADPH oxidase activation. ROS regulate vascular function, modulating cell growth, apoptosis, migration, inflammation, secretion, and production of extracelular matrix. Antioxidants and agents that disrupt ROS production derived from NADPH oxidase reverse vascular remodeling, improve endothelial function and reduce inflammation. However, clinical studies on antioxidant therapies have been disappointingly negative. Nanomedicine could help solve this problem. Archaeosome vesicles (ARQ) are nanoparticles composed of polar lipids unique to the Domain Archaea. We hypothesize that ARQ possibly have antiinflammatory and antioxidant properties without the need to be decorated with specific ligands to be captured by vascular smooth muscle cells (VSMC) from the aorta artery wall. All ARQ concentrations tested were non-toxic in in vitro cultivated VSMC. In basal cultured condition, we found no effect in ROS produc-

tion. However, when VSMC are forced to produce ROS adding angiotensin II, ARQ prevented its increased. In addition, ARQ were able to significantly downregulate Nox4 and $p47^{phox}$ genes in VSMC. ARQ also tended to upregulate the anti-inflammatory adipokine Adiponectin and downregulate the pro-inflammatory adipokine Resistin. These results suggest that ARQ decrease ROS production probably by downregulating Nox4 and $p47^{phox}$ genes and could have an anti-inflammatory action by increasing Adiponectin mRNA levels and downregulating Resistin.

473 (2028) **NOVEL ANTICANCER FLUOROPHENAZINE WITHIN POLYMERIC NANOMICELLES: SYNTHESIS, PHYSICOCHEMICAL CHARACTERIZATION AND IN VITRO EVALUATION OF THE ANTITUMOR EFFECTIVENESS**

Lecot, Nicole[1]; Oddone, Natalia[2]; Cabral, Pablo[1]; Glisoni, Romina[3]; Cerecetto, Hugo[1,4]; González, Mercedes[4].
1. Laboratorio de Radiofarmacia, Centro de Investigaciones Nucleares. Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay. 2. Laboratory of Cell Signalling and Nanobiology. Institut of Biological Research Clemente Estable, MEC, Uruguay. 3. NANOBIOTEC UBA-CONICET. Department of Pharmaceutical Technology, Faculty of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina. 4. Grupo de Química Medicinal, Laboratorio de Química Orgánica, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay.

The breast cancer is the most frequent in women, with an increase in developing countries mainly because the diagnosis is made at late stages. Breast cancer is a solid tumor that is characterized by a high degree of hypoxic zones. We recently discovered a new derivative of phenazine (FNZ; N^5,N^{10}-dioxide of 2-amino-7(8)-fluorophenazine), that acts as a selective agent in hypoxic conditions, regarded as a lead in treating solid tumors. To enhance FNZ-tumor disposition and penetration into inner tumor regions and concurrently prolong overall circulation time to favor the accumulation in tumor tissue via enhanced permeation and retencion effect (EPR effect), we proposed its vehiculization into polymeric nanomicelles (PMs), based of PEO-PPO copolymers. The encapsulation efficiency (EE) and the physicochemical characterization of FNZ-loadednanomicelles (T1307-FNZ, F127-FNZ and T1307:F127-FNZ) were evaluated in orderto define reproducibility in terms of in vitro parameters. PMs (10% w/v) increased the solubility of FNZ in 3-4 times (EE=52-78%) with an improved stability for the mixed ones (T1307:F127). The nanosystems were characterized for the particle size (two marked populations with 7-8 and 40-45 nm with narrow size distribution) and Zeta potential (-4 mV) determined by dynamic light scattering (DLS). On the other hand, free-FNZ and FNZ-loaded PMs were evaluated in their capability to inhibit in vitro 4T1 murine mammary tumor cells. The cell viability assay was performed usingthe MTT methodology (48h). FNZ showed antitumor efficacy at concentrationhigher than 20μM. The new systems, i.e. T1307-FNZ, F127-FNZ and T1307:F127-FNZ, displayed better in vitro behavior. Finally, more extensive studies on the antitumor effectiveness of FNZ-loaded polymeric nanocarriers are required, these preliminary results indicate that these PEO-PPO nanomicelles and loaded with FNZ are potential candidates as therapeutic agents in breast cancer treatment.

II. DIAGNOSIS & IMAGING

474 (411) **MAGNETIC NANOPARTICLES STABILIZED WITH ORGANIC ACIDS AS SPECIFIC CONTRAST AGENTS FOR ATHEROSCLEROSIS DIAGNOSIS**

Montiel Schneider, Maria G.[1]; Agotegaray, Mariela[1]; Lassalle, Verónica[1]
1. INQUISUR - CONICET. Departamento de Química. Universidad Nacional del Sur.

Introduction: Atherosclerosis and its vascular complications, are the main cause of death in the western world. Identification of vulnerable plaques which are prone to rupture, releasing thrombus into circulation is essential for a better care of patients presenting this pathology. Magnetic nanoparticlesare being employed as efficient tools to the earlier and selective detection of unstable plaques by Magnetic Resonance Imaging along the last years. Objetive: Design and characterize water dispersible iron oxide magnetic nanoparticles with suitable coatings in order to be efficiently employed in the detection of atherosclerotic plaques by MRI. The goal is to induce the accumulation of nanoparticles in macrophages, calcifying microvesicles presents in atherosclerotic plaques, according to previous reports. Design of experiment: Iron oxide magnetic nanoparticlescoated with tartaric, malic and ascorbic acid have been synthesized by the co-precipitation method with some modifications.Results: Acid coated nanoparticles were obtained with hydrodynamic diameters ranged between 95-278 nm, depending on the kind of acid and synthesis conditions. All formulations showed good water stability for several days. TEM images revealed that magnetic cores were about 6-10 nm. FTIR spectra of already all NPs prepared, displayed the characteristic Fe-O bands associated to magnetic iron oxides. Despite all formulations are interesting for the proposal of the work, the use of ascorbic acid as coated is, perhaps, the most attractive because of its antioxidant properties which could be useful to prevent LDL oxidation. Besides, by changing the reaction conditions, dehydroascorbic acid was obtained as coating. This compound is very important in terms of its possibilities to penetrate the blood brain barrier. Conclusions: Highly hydrophilic magnetic nanoparticles coated with specific organic acids were obtained. They remain stable for several weeks and exhibit appropriate size for biological applications. In vitro biological assays are currently in development.

475 (763) **EVALUATION OF RADIOLABELED TPGS-BASED NANOMICELLES AS IMAGING PROBES. IN VIVO CHARACTERIZATION IN HEALTHY AND TUMOR BEARING ANIMALS.**

Tesan, Fiorella C.; Giaquinta, Diego; Nicoud, Melisa; Moretton, Marcela; Medina, Vanina; Chiapetta, Diego; Zubillaga, Marcela B.; Salgueiro, María J.
1. Consejo Nacional de Investigaciones Científicas y Técnicas. Buenos Aires, Argentina. 2. Consejo Nacional de Investigaciones Científicas y Técnicas. Instituto de Investigaciones Biomédicas. Pontificia Universidad Católica Argentina. Buenos Aires, Argentina. 3. Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Fisicomatemática, Cátedra de Física. Buenos Aires, Argentina. 4. Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Tecnología Farmacéutica, Cátedra de Tecnología Farmacéutica I. Buenos Aires, Argentina.

TPGS-based nanomicelles (TBN) is a system that has been used for delivery of therapeutic drugs. The aim of this work is to label TBN with a gamma emitting nuclide as ^{99m}Tc and evaluate its potential use as an imaging probe for cancer diagnosis. Radiolabelling of TBN with ^{99m}Tc was performed by the direct method. Radiochemical impurities (free ^{99m}Tc and radiocolloids) were assessed by thin layer chromatography and filtration. Size and morphology of radiolabeled nanomicelles were characterized by dynamic light scattering and by transmission electronic microscopy respectively. Pharmacokinetic studies were performed in healthy animals by two different assays: a 24h plasmatic concentration curve and biodistribution (BD) by means of small animal imaging (static and dynamic studies) after intravenous administration (1mCi/animal) of the radiolabeled probe. Regions of interest were drawn over organs of interest in the static images acquired in order to get semi quantitative results of the radioactive micelles BD. To evaluate the tumor uptake, two animal models of breast cáncer were used: N-nitroso-N-methyl-urea induced mammary adenocarcinoma in Sprague Dawley rats and a syngeneic subcutaneous tumor (4T1 breast cell line) in balb-c mice. The uptake ratio (Tumor/Background) was calculated. Plasmatic concentration over time in healthy animals resulted in a two phase decay curve (R^2=0.85;$\%ID_0$=41.46; PI=1.984; K_{fast}=2.052h^{-1}; K_{slow}=0.3379h^{-}

[1]).%Total activity in BD studies for 1 and 12 hs post injection were: 6.1±0.5; 3.9±0.1 soft tissue, 1.2±0.2; 1±0.1 bone, 1.5±0.6; 0.7±0.3 heart, 16.6±1.3; 26.5±1.7 kidneys, 8.6±1.1; 11.1±0.1 liver. T/B was between 30 and 80 in the images performed in the chemically induced tumors. However no uptake was appreciated in images of the 4T1 tumors in mice. Further studies must be conducted to establish the quantity of TBN to be administered for in vivo protocols. Animal procedures were approved by the CICUAL of the FFYB, UBA (Res CD 3761/13).

476 (856) **OBTENTION OF SPECIFIC SINGLE DOMAIN ANTIBODIES DERIVED FROM CAMELIDS (VHH OR NANOBODIES) AGAINST PLASMA BIOMARKERS OF ALZHEIMER'S DISEASE FOR DIAGNOSTIC APPLICATION.**

Joaquin Ignacio Bozzo[1]; Marina Gallo Calderon[1]; Nora Marta Mattion[1]; Vanina Grippo[1].
1. Instituto de Ciencia y Tecnología Dr. César Milstein, CONICET.

Alzheimer's disease (AD) affects Nervous Central System before clinical symptoms are fully expressed. Currently AD diagnosis depends on clinical evaluations, and there is no reliable early diagnostic test. Direct measure of plasma biomarkers for AD, such as alpha-1-antitrypsin (A1AT) and alpha-2-macroglobulin (A2M), could lead to an earlier and more sensitive diagnosis. Nowadays, single domain antibodies derived from camelids (VHH or nanobodies) represent a very useful tool with unique characteristics, making VHH more suitable for sensitive and specific diagnosis. For this reason, we aimed to obtain and produce specific VHH against blood-based protein biomarkers for AD, A1AT and A2M, with potential application in the development of a simple and early diagnostic test.In this work, two llamas were immunized with A2M and A1AT proteins obtaining a high humoral response in both animals. Two VHH libraries were constructed starting from peripheral blood lymphocytes from both llamas. Library quality controls were performed to corroborate full-length VHH insert and repertoire variability. A high percentage of full-length clones (97.6% and 100%) and at least 35 different fingerprinting profiles were determined for each library. Then, phages that expressed in its surface specific VHH against both biomarkers were selected by Phage Display methodology. After two-round selection, specific reactivity of 45 and 10 selected clones for A2M and A1AT respectively, was confirmed by ELISA. Four most reactive nanobodieswere subcloned into pHEN6 vector and expressed as soluble protein in E. coli. VHH expression was confirmed by Western Blot detecting a unique band of approximately 15 kD as expected. Finally, specific biomarker recognition by expressed VHH was confirmed by ELISA and Western Blot. In conclusion, specific Nanobodiesagainst blood-based protein biomarkers of AD, A2M and A1AT, were obtained, expressed and preliminarily characterized for potential application in AD diagnosis.

III. Tissue Engineering

477 (79) **NANOSTRUCTURED FUMARATE COPOLYMER/ CHITOSAN BASED SCAFFOLD DESIGNED FOR BONE TISSUE ENGINEERING.**

Lastra, Maria L.[1,2,3]; Blaszczyk-Lezak, Iwona[3];Molinuelo, Maria S.[1]; Cortizo, Maria S.[2]; Mijangos, Carmen[3].
1. Laboratorio de Investigaciones en Osteopatías y Metabolismo Mineral (LIOMM), Departamento de Cs. Biológicas, Facultad de Cs. Exactas, UNLP, La Plata, Argentina. 2. Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA), Dpto. de Química, Fac. Cs. Exactas, UNLP, CCT- La Plata, CONICET CC 16, Suc. 4. Argentina. [3]Instituto de Ciencia y Tecnología de Polímeros, CSIC, Juan de la Cierva 3, 28006 Madrid, España.

Nanostructured polymers (NP) have recently emerged as promising candidates to efficiently achieve tissue reparation. NP has been proposed to efficiently mimic the natural extracellular matrix (ECM) and thus promote tissue healing. On the other hand,successful tissue regeneration requires scaffolds made of materials with good biocompatibility and low cytotoxicity. It is well accepted that natural polymers or its blends with synthetic polymers present better biocompatibility properties than synthetic polymers alone. Thus we hypothesizes that nanostructured matrices based on natural polymers will improve stem cells (BMPC) development and osteogenicmaturation. To prove our hypothesis we designed nanostructured matrices based on fumaric copolymer and chitosan (diisopropylfumarate-vinyl acetate copolymer-chitosan crosslinked with borax, PFVH-Ch) using an anodized aluminum oxide template of two different pore diameter (170 µm (SN170) and 300 µm (SN300)). Characterization of the polymer was performed by ATR-FTIR, DSC and TGA. The polymer alone or crosslinked were stable at the infiltration conditions (melting the precursor film by wetting method). The nanostructured PFVH-Chwas studied by confocal Raman spectroscopy and SEM. BMPC were used for biocompatibility study (MTT assay) and osteogenic studies (Collagen (Col1) and Mineral production). Our studies show that BMPC adhere better on SN170 than on SN300. However, after 24h BMPC proliferate best on SN300. Osteogenic development showed that BMPC produced low levels of Col1, but it was able to mineralize the ECM. But there were no differences between SN170 and SN300. Additionally the toxicity of the biomaterial was evaluated using a model of macrophages in culture. We found that the nanostructuration of the matrix caused low cytotoxicity as it was measured by IL1□, TNF□ and NO production. Altogether our results suggest that nanostructuration of the polymers would improve osteogenic BMPC development and maturation.

478 (283) **CYTOTOXICITY ASSESSMENT OF HYDROXYAPATITE NANOPARTICLES IN NORMAL AND TUMOR CELL LINES.**

Gorojod, Roxana M.[1]; Alaimo Agustina[1]; Porte Alcon, Soledad[1]; Dittler, Maria L.[2]; Gonzalez, Mónica C.[2]; Kotler, Mónica L.[1].
1. Universidad de Buenos Aires. Consejo Nacional de Investigaciones Científicas y Técnicas. Instituto de Química Biológica, Ciencias Exactas y Naturales (IQUIBICEN). Facultad de Ciencias Exactas y Naturales, Departamento de Química Biológica, Laboratorio de Disfunción Celular en Enfermedades Neurodegenerativas y Nanomedicina. 2. Universidad Nacional de La Plata. Consejo Nacional de Investigaciones Científicas y Técnicas. Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA). Facultad de Ciencias Exactas, Departamento de Química, Laboratorio de Química y Fotoquímica de Nanobiomateriales.

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$; HAP) is an essential component of the human bone inorganic phase. NanoscaledHAP (nHAP) presents emergent properties in comparison to its bulk counterpart, including an inhibitory effect on the proliferation of some types of tumor cells. However, little is known regarding to the potential adverse effects on human health. In this study, we aimed to determine the cytotoxicity of nHAP in both normal and tumor cell lines. For this purpose, we evaluated cell viability (MTT assay) after exposure to nHAP (50-200µg/ml, 24-72h) in both normal (fibroblasts NIH/3T3, microglia BV2 and retinal pigment epithelial cells ARPE-19) and tumor cell lines (astroglia C6, liver HepG2, lung A549 and epithelial HEp-2). Some cellular lines were selected for additional evaluation: lysosomal and nuclear integrity (LysotrackerRed DND-99 and Hoechst 33258 staining, respectively) and cell proliferation by clonogenicassay and Ki67 immunolabeling. Data obtained demonstrated a cytotoxic effect in C6 and HEp-2 cells after 24h exposure (C6: 100µg/ml: 14±5% $p<0.05$, 200µg/ml: 22±4% $p<0.01$; HEp-2: 100µg/ml: 17±2% $p<0.001$, 200µg/ml: 13±22 $p<0.01$). Cell death in A549 cells was detected after 72h exposure (50µg/ml: 15±3%$p<0.01$, 100µg/ml: 13±1% $p<0.01$, 200µg/ml: 12±2% $p<0.01$). Therefore, nuclear and lysosomal integrity were determined exposing C6, HEp-2 and A549 cells and the non-sensitive ARPE-19 to nHAP for 48h. We detected an increase in lysosomal number for all cell types

($p<0.05$) without alterations in nuclear morphology. Moreover, we also observed a decrease in cell proliferation markers for C6 cells. Evidence obtained suggested that nHAP would be more toxic for tumor cells highlighting the importance of deeper investigation.

479 (969) **EVALUATION OF ANTIBIOTIC LOADED SILICA CORE-SHELL NANOPARTICLES AND COLLAGEN TYPE I NANOCOMPOSITE AS DRESSINGS TO PREVENT INFECTION IN CHRONIC WOUNDS.**

Mebert, Andrea M. [1, 2]; Alvaréz, Gisela S.[2]; Aime, Carole[3]; Coradin, Thibaud[3]; Desimone, Martín F.[1, 2]
1. Instituto de Metabolismo y Química del Fármaco (IQUIMEFA - CONICET). 2. Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires (FFyB – UBA. 3. Sorbonne Universités, UPMC Univ Paris 06, CNRS, College de France, UMR 7574, Laboratoire de Chimie de la Matiere Condensee de Paris, Paris, France.

Silica core-shell nanoparticles and collagen type I nanocomposite hydrogels are evaluated as medicated dressings. It is known that collagen is a key component ofa healing wound, however due to the large porosity of the scaffold compared to drug dimensions, the kinetics of release is usually too fast. In this sense, the use of nanocomposite materials where the drugs are associated with nanoparticles embedded in the scaffold has attracted increasing attention. In the present work, 300 nm silica core–shell particles (0.5 M SiO_2), having the capability to simultaneously deliver two topical antibiotics, gentamicin sulphate and sodium rifamycin, are combined with concentrated collagen type I (5 mg mL^{-1}) and evaluated as dressings to prevent infection in chronic wounds. By scanning electron microscopy (SEM) imaging, a dense fibrillar collagen network with its typical periodic banding pattern and an homogenous particle distribution were observed. A sustained antibacterial effect was observed for over 10 days when the nanocomposites were evaluated in vitro assays using Staphylococcus aureus for the antibiograms. In all cases, double loaded nanocomposite (gentamicin and rifamycin) showed greater activity than those only loaded with gentamicin, being indicative that both drugs are released during the experiment term. An unloaded composite showed no antibacterial activity. In vivo studies biocompatibility in subdermal implants and antibacterial efficiency were evaluated in male Wistar rats. A significant difference was found between the double loaded and unloaded (control) composite effect in the treatment on staphylococcal infection, the loaded composite lowered the number of bacteria in around two and a half colony forming unit (CFU) per cm^2 rat skin.

480 (1019) **SILICIFIED COLLAGEN MATERIALS: MODULATION OF THE IN VITRO AND IN VIVO RESPONSE THROUGH SURFACE MODIFICATION TECHNIQUES.**

Foglia, Maria L.[1]; Mitarotonda, Romina[2]; De Marzi, Mauricio[2]; Desimone, Martin F.[1]
1. Universidad de Buenos Aires. IQUIMEFA-CONICET. Facultad de Farmacia y Bioquímica. Cátedra de Química Analítica Instrumental. Buenos Aires, Argentina. 2. Laboratorio de Inmunología, Instituto de Ecología y Desarrollo Sustentable (INEDES) UNLu-CONICET y Departamento de Ciencias Básicas, Universidad Nacional de Luján, Luján, Buenos Aires, Argentina.

Collagen derived materials offer distinct advantages over synthetic polymers, taking into account their natural inherited biocompatibility and potentially interesting mechanical properties. However, given the extraction procedure, the mechanical properties frequently need to be enhanced through the use of different crosslinking methods. Aldehydes are often used for the stabilization of biomaterials, though the crosslinking slightly alters the protein´s surface reactivity hence calling for new biocompatibility studies. At the same time, silicate modification of natural polymers has gained interest within the biomaterials field for their strengthening potential and ease of manipulation, giving rise to different surfaces and bulk materials. Within the present work, diluted collagen gels modified with glutaraldehyde (ColGA) and glutaraldehyde and an aminosilane (ColGASi) were evaluated in vitro and in vivo and the results obtained were compared to those derived from unmodified collagen matrices (Col). In vitro assays focused on the interaction of the materials with elements present in human blood whereas in vivo assays evaluated their ability to support cell proliferation and angiogenesis for a period of 30 days in a rodent model. The exposure of platelet-rich plasma (PRP) of the above mentioned materials led to differential platelet aggregation in the presence of epinephrine. On the other hand, in vivo an increased cellular infiltration was observed along with new blood vessel formation in those matrices containing silicified collagen, while glutaraldehyde fixed collagen induced a foreign body reaction and appeared surrounded by a fibrous capsule after 30 days of subcutaneous implantation. Overall, the results obtained show that the silicification of collagen has advantages not only through the enhancement of its mechanical properties but also through the stimulation of the integration of the material with the surrounding tissue.

ENDOCRINOLOGÍA I/ ENDOCRINOLOGY I

481 (71) **ANDROGEN RECEPTOR GENE: MOLECULAR ANALYSIS IN A SERIES OF 35 INDEX PATIENTS WITH 46, XY DISORDER OF SEX DEVELOPMENT**

Maria Sol Touzon[1], Natalia Pérez Garrido[1], Roxana Marino[1], Pablo Ramírez[1], Mariana Costanzo[1], Gabriela Guercio[1], Marco A Rivarola[1], Alicia Belgorosky[1].
1. Endocrine Service, Hospital de Pediatría Garrahan, Buenos Aires, Argentina.

Background: Androgen insensitivity syndrome (AIS) is the most frequent monogenic cause of 46,XY disorders of sex development (DSD), an X-linked recessive condition. Mutations in androgen receptor (AR) gene are associated with a wide phenotypic spectrum, ranging from complete androgen insensitivity syndrome (CAIS) to a partial form (PAIS). Objetive: To characterize the contribution of the AR gene to the molecular cause of 46,XY DSD in our population. Clinical cases: We studied 35 non related 46,XY DSD patients, with different clinical and hormonal characteristics, adecquate testosterone production and no evidence of gonadal disgenesis in whom the AR gene was the first candidate to molecular analysis. Methods: AR exons (1 to 8) and intron boundaries were direct sequenced in all patients and in 13 family members (65% of affected patients). Results: AR gene mutations were found in 20 individuals (57% of index patients), of whom 11 (55%) were CAIS and 9 (45%) PAIS. Eighteen different mutations were found: 11,1% located in N-terminal transactivational domain; 11,1% in the DNA-binding domain (DBD); 72,2% in the C-terminal ligand binding domain (LBD) and 5,6% were gross deletions . Eleven mutations (61%) had been previously described and 7 (39%) were novel. Somatic mosaicism was present in four individuals (20% of AR-mutated gene patients). Ten percent (10%) of androgen insensitivity syndrome patients had other affected relatives. Conclusions: Androgen receptor gene mutations are the main cause of 46,XY DSD. Mutations in the AR gene are distributed throughout the gene with a preponderance located in the ligand binding domain. The most severe mutations are generally associated with a CAIS phenotype, but the correlation is less defined in PAIS. Some patients with severe mutation display a partial androgen insensitivity syndrome phenotype, which is explained by somatic mosaicism.

482 (129) **OXIDATIVE STRESS IS INVOLVED IN THE INCREASE IN POMC EXPRESSION BY SUPRAPHYSIOLOGICAL GLUCOSE LEVELS IN A CORTICOTROPH CELL LINE**

Juan Salvador Calanni[1], María Mercau Esteban Martin Repetto[1], Carolina Veronica Vecino, Morena Wiszniewski[1], Silvia Sanchez Puch[1], Cora Beatriz Cymeryng[1].
1. Departamento de Bioquímica Humana, Facultad de Cs. Médicas, UBA, CEFYBO-CONICET.

Glucocorticoids (GCs) are the ultimate effectors of the hypothalamus pituitary adrenal axis (HPA) exerting regulatory effects on

carbohydrate and lipid metabolism on multiple target tissues. An increasing number of studies suggest that hyperactivation of the HPA could be involved in the etiology of the metabolic syndrome. In order to examine this issue, we previously studied the effects of a hypercaloric diet (30% sucrose in the drinking water, SRD) on the HPA function in rats, in early stages of the development of IR, when no significant decrease in insulin sensitivity was detected by an insulin tolerance test. Our results showed increased circulating levels of ACTH and corticosterone in these animals. Analysis of pituitary tissues indicated higher levels of POMC and oxidative stress (OxS) parameters in rats of the SRD group. The aim of the following studies was to analyze the mechanisms involved in the regulation of POMC expression in corticotroph cells. Luciferase activity was determined in a corticotroph cell line (AtT20) transfected with the reporter plasmids POMC-LUC or HO1-LUC and incubated with 10 mM glucose. Generation of reactive oxygen species was also measured in these cells. Our results indicated that luciferase activity, determined in cells transfected with POMC-LUC, was significantly increased in the presence of 10mM glucose ($p<0.001$). Under the same conditions we also observed an increased production of ROS, measured with 2□,7□¬dichlorofluorescein diacetate ($p<0.001$) and induction of HO-1 (HO1-LUC), a cytoprotective enzyme ($p<0.001$). Finally, the stimulation of POMC-LUC by 10mM glucose was prevented by the addition of the antioxidant N-¬acetylcysteine ($p<0.05$). Our results led us to conclude that increased expression of POMC observed in corticotroph cells could be a consequence of the generation of oxidative stress triggered within the cells by their exposure to supraphysiological levels of glucose.

483 (167) **CHANGES IN BLOOD PRESSURE IN MALE RATS IN CONDITIONS OF HYPERURICAEMIA AND HIGH-FRUCTOSE DIET**

María Jimena Soutelo[1], Yanina Alejandra Samaniego[1], Carlos Reyes Toso[2], Javier Torres Batan[2], Osvaldo Juan Ponzo[1].

1. Laboratorio de Endocrinología. Dpto. de Fisiología. Facultad de Medicina. UBA. 2. Laboratorio de Reactividad Vascular. Dpto. de Fisiología. Facultad de Medicina. UBA.

High-fructose diet intake and hyperuricemia may increase the onset of cardiovascular disease. OBJECTIVE: to evaluate the effect of these metabolic conditions on blood pressure and vascular morphology. MATERIAL AND METHODS: Adult male Wistar rats (n=7/group), were divided into four groups receiving during 5 weeks: a) Control (C): standard commercial diet and water, b) Fructose (F): control diet plus 10% fructose in the drinking water, c) Oxonic acid (OA): control diet and water plus the uricase inhibitor OA (750 mg/kg/d), d) Fructose and Oxonic Acid (FOA): control diet with 10% (w/v) fructose in the drinking water plus oxonic acid. An average value from three readings systolic blood pressure (SBP) was measured at basal, 2nd week and 4th week treatment, in conscious rats by a validated volume-based tail-cuff method, after they had become acclimatized. At 5-week plasmatic uric acid and renal arteriole media/lumen (M/L) ratio were measured, using for each arteriole, the outline of the vessel and its internal lumen, by computer analysis to calculate the total arteriolar medial area. Statistical analysis by two-way ANOVA and Bonferroni multiple-comparison post test was performed, considering significant $p<0.05$. RESULTS: All animals had similar SBP at the beginning of the experiment. At 2nd week a significant ($p<0.001$) SBP increase was observed in F, OA and FOA vs C. The increment in OA and FOA was significantly higher ($p<0.01$) vs (F) at 2nd and 4th weeks. And at 4th weeks the SBP increment was higher ($p<0.01$) in FOA vs OA groups. Plasmatic uric acid were significantly higher in OA ($p<0.05$) and FOA ($p<0.01$) (C: 0.97±0.04, OA: 1.27±0.14, FOA: 1.49±0.1 mg/dl). The M/L ratio was greater in OA ($p<0.05$) and FOA ($p<0.001$) when compared to C group. CONCLUSION: Hyperuricemia and high-fructose diet conditions, increase SBP together with vascular changes shown by the arteriolar M/L ratio, being these effects more relevant in animals with both conditions simultaneously (FOA).

484 (213) **HYPOTHALAMIC CB1 RECEPTOR ACTIVATION INHIBITS THE INFLAMMATORY RESPONSE INDUCED BY ENDOTOXIC STRESS IN DETRIMENTAL OF THE REPRODUCTIVE FUNCTION.**

Pablo Nicolás Surkin[1,2,3], Sofia Ludmila Gallino[3], Maria Emilia Di Rosso[4], Julia Irene Astrauskas[2], Ana Maria Genaro[4], Andrea De Laurentiis[1], Javier Fernández-Solari[1,2].

1.CONICET. 2.Facultad de Odontología, UBA. 3.CEFyBO (UBA/CONICET). 4.BIOMED-UCA-CONICET.

It is known that endotoxic stress (ES) inhibits reproductive function, and that endocannabinoids (EC) modulate sexual hormones. We hypothesize that ES inhibits reproductive axis via EC system. Thus, the objective of this study was to evaluate the participation of the hypothamic cannabinoid receptor CB1 (CB1R) on hypothalamic-pituitary-gonadal axis in rats submitted to ES. Sprague Dawley rats (n=6/group) were implanted with intracerebroventricular (icv) cannulas at the cerebral lateral ventricle by stereotaxis. 6 days after surgery a silastic catheter was introduced in the right jugular vein. The next day, rats were treated via icv with CB1R antagonist, (AM251, 500ng/5ul) or saline, followed by an intraperitoneal (ip) injection of LPS (5mg/kg) or saline 15 min later. Blood samples were obtained at -30, 30, 60, 90 and 180min from LPS injection. Plasma TNFα by ELISA and LH by RIA were determined. In other experiment, rats equally treated were euthanized 90 or 180min post LPS injection and medial basal hypothalamus (MBH) and adenohypophysis (AH) removed. The tissues from 90 min treated rats were used for qPCR of IL1β, TNFα, TRPV, CB1R, and GnIH (the last only in MBH). Ex vivo experiments were performed with 180min explants to measure GnRH (in MBH) by RIA and TNFα (in MBH and AH) by ELISA in media. Results: Plasma TNF□ increased and LH decreased in LPS treated rats ($p<0.05$). LPS increased IL1β and TNFα mRNA in AH ($p<0.05$). Higher levels of GnIH mRNA and decreased GnRH release were observed in MBH ($p<0.05$). Hypothalamic CB1R blockade partially prevented the inhibitory effect of LPS on plasma LH and GnRH ($p<0.05$), but enhanced plasma TNFα rise and increased IL1β and TNFα mRNA in MBH ($p<0.05$). The blockade also augmented TRPV mRNA in AH ($p<0.05$). Our results suggest that ES inhibits reproductive axis at least in part via CB1R that also participates in homeostasis by modulating inflammation. Thus EC system participates in neuroimmunoendocrine mechanisms underlying fertility.

485 (251) **EVALUATION OF STAGE 58 OF THE METAMORPHOSIS OF XENOPUS LAEVIS BY EXPOSURE TO UNDERGROUND WATER IN A MODEL OF CHRONIC TOXICITY**

Maria Fernanda Modarelli[1], Osvaldo Juan Ponzo[1].

1. Laboratorio de Endocrinología. Dpto. de Fisiología. Facultad de Medicina. UBA.

Contaminated underground water can transporter substances that have proven thyroid disrupter action. Amphibians, to their aquatic larval development, are mainly used as bioassays, observing changes during different stages of metamorphosis. Objectives: Assessed by chronic toxicity bioassay with larvae of one of Xenopus Laevis nida, underground water as a vehicle of thyroid disrupting substances affecting one of the critical stages in the metamorphosis of amphibians. Xenopus Laevis larvae were used for the experiment. They were divided in three groups: a) Control (C): immersed in filtered tap water (n=13), b) Treated (T): immersed in underground water of 30 meters depth of the city of Glew (n=18), c) Positive control (CP): with water containing 0.007mg/l potassium perchlorate (n=18). After 15 days of exposure step 58 of the metamorphosis was analyzed morphological and histological according to Nieuwkoop and Faber criteria. Morphological changes evaluated were: total body length and length of hindlimbs. Histological changes evaluated according to EPA guidelines were: colloid volume, degrees of hyperplasia, follicular epithelium height.

There were no significant differences in total body length and length of hindlimbs between the three groups. The mortality group recorded was 10% in the (T) group exclusively ($p<0.0001$).

It has been observed changes in thyroid gland histoarchitecture degrees hyperplasia that was I in (C), II in (T) and II in (CP). This changes was very significant (p<0.0001) between (C) and the other two groups (T y CP). With respect to colloid volume in step 58, there was increase in (CP) and more over in (T) groups vs (C) (p<0.0001). With respect to epithelial height there was seen similar results (p<0.0001).

In conclusion, in stage 58 of the larval development of Xenopus laevis, the disruptive action of underground water in the study area was manifested by alteration of thyroid histoarchitecture.

486 (232) **PHARMACOLOGICAL AUGMENTATION OF ENDOCANNABINOID SIGNALING REDUCES THE STRESS RESPONSE TO IMMOBILIZATION**

Sofía Ludmila Gallino[1], Pablo Nicolás Surkin[2], Carlos Ezequiel Ríos[2], Fernando Correa[1], Javier Fernandez-Solari[2], Andrea De Laurentiis[1].

1. Centro de Estudios Farmacológicos y Botánicos, CEFYBO-CONICET-UBA. Facultad de Medicina, UBA. 2. Cátedra de Fisiología, Facultad de Odontología, UBA.

Stress activates the hypothalamic-pituitary-adrenal (HPA) axis increasing glucocorticoids and several neurotransmitters. Components of the endocannabinoid (eCB) system (ECS) are present throughout the HPA and both systems interact extensively in the regulation of stress response. The study focuses on ECS regulation of acute neurogenic stress response. Sprague-Dawley male rats were immobilized for 30 min. Each group received intracerebroventricular (icv): anandamide (AEA, 50ng/5ul), or the inhibitor of FAAH (URB597, 50ugr/5ul), or antagonists for CB1 (AM251, 500ng/5ul) or CB2 (AM630, 500ng/5ul) or vehicle 15min before stress. Others received intraperitoneal (ip) Methanandamide (2.5mg/kg), AM251 (2mg/kg), URB597 (0.3mg/kg) or vehicle. Corticosterone (CORT) by RIA and the hypothalamic nitric oxide synthase (NOS) activity were determined. Control and stressed rats were sacrificed 4hs after stress, CORT and hypothalamic and adrenal CB1, CB2 and TRPV1 mRNAs determined by PCR and qPCR. Adrenals from control rats were incubated in buffer Krebs (30 min) with ACTH (10-9M) alone or with AEA (10-9M) or URB597 (3uM) or AM251 (10-5M) or vehicle. Media CORT and tissue NOS activity were determined. Our results show that AEA blocks the increase of CORT at central and peripheral levels (p<0.001). AEA inhibits CORT release through hypothalamic CB1 and CB2 receptors (p<0.05) and CB1 and TRPV1 in adrenal (p<0.001), being necessary the action of both. Nitric oxide participates as a mediator of AEA action at hypothalamic and adrenal levels. These data indicate that an eCB tone provides a steady-state inhibition of HPA axis activity, which, when disrupted, activates the HPA axis and then eCB act to end the stress response allowing homeostasis. Therefore we conclude that the ECS is activated by noxious stimuli and functions to buffer or dampen the endocrine effects of stress suggesting that pharmacological augmentation of eCB signaling could serve to the treatment of anxiety-related disorders.

487 (272) **EFFECT OF A MONOCLONAL ANTIBODY AGAINST HUMAN CHORIONIC GONADOTROPIN (HCG) ON THE PHENOTYPE OF MALE AND FEMALE OFFSPRING OF TRANSGENIC MICE**

Agustina Marcial Lopez[1], Laura D Ratner[1], María Andrea Camilletti[1], Graciela Díaz[1], Ricardo Calandra[1], Susana Rulli[1].

1. Instituto de Biología y Medicina Experimental (IByME - CONICET)

Human chorionic gonadotropin (hCG) is normally secreted by the placenta of humans and primates, but is absent in mice. It stimulates the production of progesterone by the corpus luteum, and is involved in many other functions during placentation and fetal development. Transgenic female mice secreting hCG (hCGβ+) are infertile, obese, produce high levels of progesterone, testosterone and prolactin, and develop pituitary and mammary tumors. Males are fertile, but show increased body weight and reduced testis size. We have previously recovered fertility from hCGβ+ females by a treatment with a monoclonal antibody anti-hCG with neutralizing capacity (mAB-hCG; SAIC 2015). The objective of this study was to analyze the influence of the mAB-hCG treatment, which spans the gestational state of hCGβ+ mothers, on the reproductive phenotype of the offspring. hCGβ+ females treated with mAB-hCG (300 µg/mouse/dose; once a week from 5 weeks until two months of age), were mated with wild-type (WT) males two days after the last injection, and male and female offspring were studied at adulthood. Body, gonadal and pituitary weight, prolactin levels, fertility studies, and the presence of tumors were analyzed. hCGβ+ mice were used as controls. Female hCGβ+ offspring showed no differences in the body weight. Ovarian and pituitary weight, as well as prolactin levels were reduced, as compared with control hCGβ+ (p<0.05). Fertility was recovered in 50% of hCGβ+ females. Male hCGβ+ offspring showed a reduced body weight and increased testis size (p<0.05). These results showed that the neutralization of hCG by mAB-hCG from early stages of gestation had an impact on the offspring's transgenic phenotype. Male offspring showed a normalization of the parameters studied, whereas in females, the phenotype was partially affected, thus suggesting differences between sexes in the way of response to the treatment. Further studies are necessary to understand the mechanism involved in this phenomenon.

488 (275) **PROLACTIN STIMULATES STEROIDOGENESIS BY POTENTIATING CYP19 AND 3ßHSD EXPRESSIONS IN OVARIES OF LAGOSTOMUS MAXIMUS (RODENTIA: CHINCHILLIDAE)**

Santiago Andrés Cortasa[1,2], Alejandro Raúl Schmidt, Santiago Elías Charif, Sofía Proietto, María Clara Corso, Pablo Ignacio Felipe Inserra, Alfredo Daniel Vitullo, Verónica Berta Dorfman, Julia Halperin

1Centro de Estudios Biomédicos, Biotecnológicos, Ambientales y Diagnóstico (CEBBAD). Universidad Maimónides. 2. CONICET.

Ovarian steroid production is critical for the normal development and function of several distant tissues including uterus, breast, skeleton and brain.Particularly for murines, luteal progesterone (Pg) roduction is essential to sustain pregnancy and this process is mainly modulated by prolactin (PRL). Our model species exhibit an ovulatory event at mid-gestation that dramatically raises the circulating Pg. In order to investigate the regulation of ovarian steroidogenesis in L. maximus we set up an ex-vivo method for culturing ovarian explants. After surgery, whole ovaries were cut in explants of 2mm side and cultured with treatment media (TM) which was collected after 24, 48, 72 and 96h to determine Pg and estradiol (E2) content. Explants were either fixed in PFA4% for immunohistochemical analysis or stored at - 80°C for PCR. ELISA results showed that both Pg and E2 release are sustained along the whole experiment. Tissue integrity was confirmed by histological examination with hematoxylin-eosin. To evaluate the effect of PRL over steroidogenesis, ovarian explants were cultured for 24h with TM and increasing doses of lactating mothers`serum (LS). Both Pg and E2 content increased significantly (p<0.05) with the treatment. To confirm that such stimulation was due mainly to PRL, we repeated the experiment in the presence of AG490 (Jak-STAT inhibitor) or estrogen receptors antagonists (ERant). Pg and E2 release were notably inhibited by AG490 whereas ERant did not induce changes (p<0.05). PCR for PRL-receptor (PRLR) and for aromatase (CYP19) and 3ßHSD, two key enzymes for ovarian steroidogenesis, showed increased levels for the three genes in explants cultured with LS and those values notably diminished in the presence of AG490. CYP19 and PRLR protein expression showed the same pattern than their transcripts. Our data strongly indicate that PRL stimulates CYP19 and 3ßHSD in L. maximus, and suggest a positive loop by increasing PRLR expression after stimulation with PRL.

489 (278) **ROLE OF PI3K/AKT AND P38 MAPK IN THE APOPTOTIC AND ANTIPROLIFERATIVE ACTIONS OF PROLACTIN ON LACTOTROPES**

Nataly de Dios[1], Santiago Orrillo[1], Martin Irizarri[1], Florencia Gottardo[1], Florence Boutillo[2], Adriana Seilicovich[1], Vicent Goffin[2], Daniel Pisera[1], Jimena Ferraris[1].

1. Institute of Biomedical Research, University of Buenos Aires-CONICET, Buenos Aires, Argentina. 2. Inserm U845 - Institut Necker Enfants Malades (INEM), Faculté de Médecine, Université Paris Descartes, Paris, France.

Prolactin (PRL) induces apoptosis and inhibits proliferation of lactotropes, regulating anterior pituitary homeostasis. Our results suggest that those effects are mediated by JAK2/STAT5 and ERK1/2 pathways. As the PRL receptor (PRLR) can activate other cascades, we now evaluated whether the actions of PRL on lactotropes depends on the modulation of PI3K/AKT and P38 MAPK, two pathways known to regulate lactotropes homeostasis. To that aim we used the somatolactotrope cell line GH3. Since these cells secrete PRL constitutively, the paracrine/autocrine effect of PRL was studied using a PRLR antagonist (Δ1–9-G129R-hPRL, APRLR, 5µg/ml, 6h). We determined P38 and Akt phosphorylation in the presence or absence of the APRLR (0-240´) by western blot. APRLR did not change significantly Akt phosphorylation but it decreased the p-P38 MAPK. In other experiments, cells were incubated with APRLR in the presence or absence of Akt or P38 inhibitors. Inhibition of PI3K/Akt (LY94007, 10 µM, 6h) increased GH3 cell apoptosis (TUNEL-positive), and inhibited the antiapoptotic effect exerted by the APRLR (C:1.2%, APRLR 0.7% LY: 2.6%, APRLR+LY: 1.7% $p<0.01$ χ^2). PI3K inhibitor did not affect per se the proliferation of GH3 cells but impaired the APRLR effect (BrdU+ cells) (C:43%, APRLR 52.7% LY: 43.2%,APRLR+LY: 37.7% $p<0.01$ χ^2). These results suggest that PI3K/Akt is involved in the apoptotic and antiproliferative effects of PRL. P38 inhibitor (SB 203580, 5µM, 6h) impaired the antiapoptotic effect induced by the APRLR (C: 1.8%, APRLR 0.6% SB: 2.2%, APRLR+SB: 2.2% $p<0.01$ χ^2). Also, it increased GH3 cells proliferation (BrdU + cells) per se and reduced the proliferative effect of APRLR (C: 39.8%, APRLR: 43.3% SB: 52.2%, APRLR+SB: 41.3% $p<0.01$), suggesting that this kinase is involved in the effects exerted by PRL. These results suggest that PRLR activation in lactotropes could modulate PI3K/AKT and P38 MAPK pathways inducing apoptosis and decreasing proliferation of lactotropes.

490 (293) **EFFECTS OF THYROGLOBULIN SPONTANEOUS MUTATIONS LOCALIZED WITHIN THE REGION I ON ITS INTRACELLULAR DISTRIBUTION AND SECRETION**

Cintia E. Citterio[1,2,3], Sofia Siffo[1,2], Christian M. Moya[1,2], Carina M. Rivolta[1,2], Osvaldo Rey[2], Peter Arvan[3], Héctor M. Targovnik[1,2].

1. Instituto de Inmunología, Genética y Metabolismo (INIGEM), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) y Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina. 2. Cátedra de Genética, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina. 3. Division of Metabolism, Endocrinology & Diabetes, Department of Internal Medicine, University of Michigan, Ann Arbor, U.S.A.

Thyroglobulin (TG), the matrix for thyroid hormone synthesis, is a large glycoprotein (330 kDa monomer) containing 4 structural and functional regions. TG region I is composed of multiple type-1 Cys reach repeats plus linker and hinge segment and its folding is the rate-limiting step of TG maturation. Defective TG synthesis cause congenital hypothyroidism; the incidence is 1:100,000 births.

The aim was to characterize the effects of rat TG spontaneous mutations in the region I and their sequential reversion to the wild type sequence, on TG intracellular distribution and secretion.

The analysis of TG triple mutant p.L571P, p.Q676R and p.Q907R (mutTG) in a previously validated expression system of eukaryotic cell monolayers followed by Western Blot with anti-TG antibody, showed that mutTG was expressed but not secreted to the culture media. Moreover, immunofluorescence images showed massive intracellular accumulation of mutTG. The double mutant p.L571P/p.Q907R or p.L571P/p.Q676R, obtained by reversion of a single mutation p.Q676R or p.Q907R in mutTG, respectively, still did not allow the secretion of TG. This could indicate that the aforementioned TG mutants do not complete their post-translational modifications, therefore are not secreted and thyroid hormonogenesis would be impaired by the lack of TG arrival to the follicular lumen. However, the reversion of the single mutation p.L571P in the double mutant p.Q676R/p.Q907R allowed the secretion of TG. This result was confirmed in the TG triple reverted clone (obtained by sequential reversion of single mutations). Interestingly, p.L517P falls in the linker of TG, which has been previously reported to belong to the critical segment of TG (interval between linker and hinge segments of region I) involved in the final acquisition of secretory competence of TG.

Overall, this work is a contribution to the understanding of the implications of multiple mutations on the TG intracellular transport and secretion.

491 (296) **OVARIAN, HYPOPHYSEAL AND HYPOTHALAMIC HORMONES COORDINATE THEIR ACTIONS FOR MODULATION OF MAMMARY GLAND GROWTH AND REMODELING ALONG THE REPRODUCTIVE CYCLE OF LAGOSTOMUS MAXIMUS**

María Clara Corso[1], S. Proietto[1], S. Cortasa[1,2], P.I.F. Inserra[1,2], S.E. Charif[1,2], AR Schmidt[1,2], N Di Giorgio[2,3], V Lux-Lantos[2,3], AD Vitullo[1,2], VB Dorfman[1,2], J Halperin[1,2].

1. Centro de Estudios Biomédicos, Biotecnológicos, Ambientales y Diagnóstico (CEBBAD). Universidad Maimónides, Buenos Aires, Argentina. 2. CONICET, Argentina. 3. Instituto de Biología y Medicina Experimental (IByME), Buenos Aires, Argentina.

Progesterone (P4) and prolactin (PRL) are master regulators of mammary gland (MG) post-pubertal development. Even though MG morphology of females' vizcachas is similar to that described in other rodents, our model species exhibit a unique reproductive feature: a dramatic rise in circulating P4 at mid-gestation due to an ovulatory event which distinguish L. maximus from most mammals. In order to investigate PRL and P4 modulation of MG tissue remodeling in L.maximus, we studied by immunohistochemistry and by PCR the expression of PRL receptor (PRLR), P4 receptor (PR), VEGF, PCNA, vimentin, Bax and FasL in MG at different stages of the reproductive cycle: resting (rt), pregnancy (pg), lactating (lc), regressing (rg). We measured apoptosis levels by TUNEL. Circulating P4 (ELISA) and hypothalamic GnRH (RIA) were determined as well. PRLR increased its epithelial alveolar expression along pg and lc and markedly dropped in rg ($p<0.05$). High PRLR levels at pg and lc were accompanied by increased expression of both VEGF and PCNA which is expected in a growing tissue. VEGF decreased at rg coinciding with PR levels and with the highest levels of apoptosis measured among groups ($p<0.05$). Curiously, PCNA still exhibited an important expression in rg where there is a notable decrease of alveolar epithelial cells due to apoptosis. However, we also found high levels of vimentin that could be attributed to a high proliferation of fibroblast typical for this tissue remodeling stage and that would be in agreement with the high PCNA data. Although rg exhibited the highest apoptosis, some pro-apoptotic markers as FasL and Bax did not show differences among stages ($p>0.05$) and this could be attributed to the tight sequential expression that this factors exhibit along the rg stage. Finally, hypothalamic GnRH was slightly higher in rg compared to lc which coincides with a recent report showing that GnRH is necessary for MG regression and its expression is suppressed by PRL during lactation.

492 (299) **ANALYSIS OF THREE NOVEL MUTATIONS INTO THYROGLOBULIN GENE ASSOCIATED WITH GOITER AND HYPOTHYROIDISM**

Sofia Siffo[1,2], Cintia E. Citterio[1,2], Exequiela Adrover[1,2], Ana Chiesa[3], Mirta B. Miras[4], Verónica Gonzalez[5], Jacques Weill[6], Rogelio Gonzalez-Sarmiento[7], Carina M. Rivolta[1,2,7], Héctor M. Targovnik[1,2,7].

1. Instituto de Inmunología, Genética y Metabolismo (INIGEM), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) y Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina. 2. Cátedra de Genética, Facultad de Farmacia y Bioquímica,

Universidad de Buenos Aires, Buenos Aires, Argentina. 3. Centro de Investigaciones Endocrinológicas, CEDIE-CONICET, División Endocrinología, Hospital de Niños "Ricardo Gutiérrez", Buenos Aires, Argentina. 4. Servicio de Endocrinología, Hospital de Niños "Santísima Trinidad", Córdoba, Argentina. 5. Servicio de Endocrinología, Hospital de Niños "Sor María Ludovica", La Plata, Argentina 6. Clinique de Pédiatrie, Hôpital Jeanne de Flandre, Centre Hospitalier Regional Universitaire de Lille, Lille, France. 7. Unidad de Medicina Molecular-Departamento de Medicina, IBMCC and IBSAL. Universidad de Salamanca-CSIC, Salamanca, España.

Thyroid dyshormonogenesis due to thyroglobulin (TG) gene mutations have an estimated incidence of approximately 1 in 100,000 newborns. The clinical spectrum ranges from euthyroid to mild or severe hypothyroidism. The majority of patients have congenital goiter or goiter appearing shortly after birth. Human TG gene is a single copy gene, 270 kb long which maps on chromosome 8q24 and contains an 8.5-kb coding sequence divided into 48 exons. Up to now, 62 inactivating mutations in the TG gene have been identified in patients with congenital goiter.

The 180 bp of the promoter region and all 48 exons of the TG gene, including splicing signals and the flanking intronic regions were amplified using the primers and PCR conditions reported elsewhere. TG PCR fragments were sequenced using sense and antisense specific primers or M13 universal primers.

The purpose of the present study was to identify and characterize new mutations in the TG gene. We report 7 patients from 6 unrelated families with goiter, hypothyroidism and low levels of serum TG. All patients underwent clinical, biochemical and imaging evaluation. Molecular analyses revealed three novel inactivating TG mutations: c.5560G>T [p.E1835X, exon 30], c.7084G>C [p.A2343P,exon 41] and c. 7093T>C [p.W2346R, exon 41], and four previously reported mutations: c.378C>A [p.Y107X] c.886C>T [p.R277X], c.1351C>T [p.R432X] and c.7006C>T [p.R2317X]. One patient was homozygous for p.W2346R mutation, four were compound heterozygous mutations and the remaining two siblings from another family with typical phenotype had a single p.E1835X mutated allele. p.E1835X includes regions I, II and only a part of region III of TG. Lacks all the carboxyl-terminal hormonogenic sites.

In conclusion, our results confirm the genetic heterogeneity of TG defects and the pathophysiological importance of altered TG folding as a consequency of truncated TG proteins and missense mutations located in ACHE-like domain.

493 (305) **OPPOSITE ACTIONS OF MEDROXYPROGESTERONE ACETATE (MPA) ON BONE AND VASCULAR CELLS**

Pablo Hernan Cutini[1], Virginia Laura Massheimer[1].

1. Instituto de Ciencias Biológicas y Biomédicas del Sur (INBIOSUR), Universidad Nacional del Sur (UNS), CONICET, Departamento de Biología, Bioquímica y Farmacia, Cátedra de Bioquímica Clínica II, Bahía Blanca, Argentina.

A high bone turnover isassociated with increased cardiovascular mortality in aging. Hormone replacement therapy combined with progestins is proposed as an alternative choice in prevention of cardiovascular diseases. Vascular calcification (VCa) mainly involves vascular smooth muscle cells (VSMC) transdifferentiation to bone lineage. The aim of this work was to investigate the role of MPA on osteoblasts and osteoblasts derived from VSMC, as well as its impact on vascular calcification. The following murine cell cultures were used: a) VSMC; b) VSMC induced to osteoblastic transdifferentiation (VSMC-OB) in osteogenic medium (β-glycerophosphate 5 mM; CaCl2 and 4 mM); c) calvarial osteoblasts (OB). VSMC proliferation and migration, and inducible muscle nitric oxide (NO) synthesis are early events that conduct to VCa. We found that 96 h treatment with the progestin (10 nM) inhibited the VSMC growth (17.6% vs control, p<0.05, MTT assay) and does not alter VSMC migration (wound healing assays). On bone cells, treatment of OB with MPA (10 nM) induced a significant increase in both calcium levels (HCl leaching of calcium) and alkaline phosphatase (ALP) activity (426±61 vs 659±14 mg calcium/g protein, control vs MPA, p<0.02; ALP: 41±4 vs 158±26 IU/g protein, control vs MPA, p<0.02). VSMC and VSMC-OB were characterized by measurement specific bone markers (RT-PCR). RUNX2 and TNAP (tissue non-specific alkaline phosphatase) were detected only in VSMC-OB but not in native VSMC. Long treatment of VSMC-OB with MPA showed a significant reduction in ALP activity with respect to control (6.50±0.19 vs 14.05±0.15 x10-3 IU, control vs MPA 10nM, p<0.001) as well as in extracellular calcium deposition (alizarin staining). Indeed 24 h treatment with MPA (10 nM) markedly inhibited NO production (37% vs control, p<0.01). In conclusion, although MPA exerts opposite effects on OB and VSMC-OB, their impact could represent favorable actions in order to promote bone growth and vessel remodeling.

494 (313) **NEUROESTRADIOL IS INVOLVED IN GNRH MODULATION DURING PREGNANCY IN THE SOUTH AMERICAN PLAINS VIZCACHA, LAGOSTOMUS MAXIMUS**

Santiago Elías Charif[1,3], Pablo Ignacio Felipe Inserra[1,3], Noelia Paula Di Giorgio[2,3], Alejandro Raúl Schmidt[1,3], Julia Halperin[1,3], Victoria Lux-Lantos[2,3], Candela Rocío González[1, 3], Alfredo Daniel Vitullo[1, 3], Verónica Berta Dorfman[1, 3].

1. Centro de Estudios Biomédicos, Biotecnológicos, Ambientales y Diagnóstico (CEBBAD), Universidad Maimónides, Ciudad Autónoma de Buenos Aires, Argentina. 2. Laboratorio de Neuroendocrinología, Instituto de Biología y Medicina Experimental (IByME)-CONICET, Ciudad Autónoma de Buenos Aires, Argentina. [3]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

Neuroendocrine areas involved in the reproductive control have shown aromatase (ARO) expression and activity suggesting local synthesis of estradiol (E2). Gonadotropin-releasing hormone (GnRH) surge is affected by E2 availability. The South American plains vizcacha, Lagostomus maximus, shows ovulation up to 800 oocytes per reproductive cycle and ovulation at mid-gestation. The aim of this work was to analyze hypothalamic expression of ARO in the vizcacha at different gestational time-points, and its relationship with GnRH expression and delivery. Hypothalami of non-pregnant non-ovulating (NPNO), early-pregnant (EP), mid-pregnant (MP), and term-pregnant (TP) female vizcachas (n=6 per group) were used to study ARO and GnRH mRNA by qPCR and protein expression by Western-blot or RIA respectively; hypothalamic colocalization of ARO and GnRH was evaluated by immunofluorescence and confocal microscopy, and GnRH pulsatility under ARO action using letrozole (ARO specific inhibitor) by RIA. A significant increment in ARO expression (p<0.05) was detected in MP vs other groups, and these results were correlated with GnRH expression. Cytoplasmic localization of ARO and GnRH was observed in neurons of preoptic area (POA) and supraoptic nucleus (SON). All GnRH neurons showed ARO expression and the quantification of neurons co-expressing both proteins did not show statistical differences among groups. To investigate neuroE2 action on GnRH pulsatility, hypothalamic explants were treated with letrozole and GnRH secretion was evaluated. Total GnRH secretion was significantly decreased by letrozole vs. control; however, pulsatile frequency did not change. The correlation between GnRH and aromatase expression at mid-gestation, and their co-localization in the hypothalamic neurons suggests the modulation GnRH expression by neuroE2 as part of a reproductive strategy of the vizcacha to assure GnRH synthesis during pregnancy.

495 (338) **RELATIOSHIP BETWEEN UNDERCARBOXYLATED OSTEOCALCIN AND MENOPAUSAL STATUS AND/OR BODY MASS INDEX IN NORMOGLUCEMIC ADULT WOMEN**

Francisco Duran[1], Graciela Brito[2], Dana Watson[2], Beatriz Oliveri[1], Carlos Gonzalez Infantino[4], Liliana Zago[3], Susana Zeni[1].

1. Bone metabolic Disease Lab INIGEM CONICET/UBA. 2. Nutrition Dep. La Matanza Univ. 3. Nutrition Dep. Bioch and Pharmacol School UBA. 4. Nutrition Dep. Clinical Hospital. Medicine School, UBA.

Bone is an endocrine organ because osteoblasts secrete osteocalcin (OC) in a fully carboxylated form, specific protein that has a great affinity for hydroxyapatite. Resorption of the bone matrix by osteoclasts results in the release and decarboxylation of bound carboxylated osteocalcin (ucOC). This is the active form that mediates the glucose homeostasis increasing the realease of Insulin. Fat tissue controls the process through the secretion of leptin. The present study evaluated ucOC, OC, leptin and insulin levels in 226 overweight and obese normoglucemic women (20-86 años) (glycaemia: 80 a 110 mg/dl; glycosylate hemoglobin A1c < 5,7%). Women were divided according to the menopausal status in: premenopausal (PM<45 years); peri menopausal (PeM45 to 50 years); early menopausal (EM50 to 65 years) and postmenopausal (M >65 years). Overweight and/or degree of obesity was established according to body mass index (BMI); the insulin resistance according to HOMA-IR. Glucose and HbA1c were determined by automatic methods and ucOC, OC total y leptin and insulin by ELISA commercial kits. Results (mean±SD and *: p<0.05 vs. overweight; (**) p<0.05 vs obesity I degree) in the next order: ucOC; OC; insulin; leptin.

Overweight PM:

1.96±1.08; 9.2±3.9; 12.6±3.3; 2.19±0.50 PeM: 2.75±1.22; 5.8±1.1; 9.7±1.7; 1.44±0.70; EM: 2.21±1.34; 7.3±1.7; 10.0±1.1; 1.74±0.34; M: 3.71±1.89; 5.4±1.4; 10.1±2.5;1.28±0.16

Obesity degree I PM:

2.41±0.98*; 11.5±1.9*; 17.8±4.3*; 2.59±0.41; PeM 2.93±0.78*; 7.8±2.4*; 18.2±2.4*; 2.13±0.93*; EM 3.25±0.96*; 10.4±1.5*; 23.2±5.2*; 2.56±0.48*; M3.81±0.96; 11.4±3.2*; 17.5±6.3*; 2.47±0.81*

Obesity degree II PM:3.51±0.41*,**; 17.5±6.4*,**; 45.8±8.8*,**; 4.85±1.61*,**; PeM:4.31±0.22*,**; 13.1±2.5*,**; 23.1±6.14*,**; 3.32±0.81*,**; EM4.11±0.6*,**; 10.3±1.7*;17.1±4.4*,**;2.41±0.44*; M:5.52±1.7*,**; 12.8±1.8*,**; 36.0±8.2*,*; 2.93±0.47*

(*): p<0.05 vs. overweight; (**) p<0.05 vs. obesity degree I.

For a same menopausal status, ucOC, insulin, leptin and-HOMA-IR increased with the increase in BMI. As the OC did not change, the ucOC/OC percentage tended to increase (data no shown). For a same BMI, the lowest ucOC levels and highest Insulin and HOMA-IR were observed in premenopausal women. Conclusions: the present results in normoglycemic women suggest that the insulin resistance increases as ucOC increases, as a result of the increase in BMI and consequently the greater circulating levels of leptin.

496 (911) **ROLE OF NADPH OXIDASE NOX4 ON THYROID FUNCTION.**

Romina Oglio[1], Leonardo Salvarredi[1], Luciano Rossich[1], Mario Pisarev[1,2], Guillermo Juvenal[1, 2], Lisa Thomasz[1, 2].
1. Comisión Nacional de Energía Atómica, 2. CONICET.

It has been described that TSH or excess iodine increase reactive oxygen species (ROS) production in thyroid cell cultures. Recent observations suggest that the NADPH oxidase NOX4 play an important role in redox cell signaling in thyroid gland. Moreover, NOX4 is prominently expressed in a number of tumors such as papillary thyroid cancers and recent studies underscored NOX4 as a new key effector of TGF-B1 in cancers. Furthermore, NOX4 gene expression has been demonstrated in thyroid of diabetic mellitus rats, which is a consequence of low-serum TSH and Insulin. This is interesting, since insulin is critical for thyroid growth. OBJETIVE: To study the contribution of NOX4 on thyroid growth and function. RESULTS: To determine the cellular source of ROS generated by excess of iodine (KI 100uM) in proliferating FRTL-5 cell line, we used diphenyleneiodonium (DPI), which inhibits NADPH oxidase. DPI completely blocked the increase of ROS induced by excess of iodine (p<0.01). Significantly, KI (100 µM) treatment increased NOX4 protein expression in FRTL-5 cells (p<0.05). Using NOX4-targeted siRNA, we observed a decrease on ROS production induced by KI (p<0.01). Since iodine inhibits some thyroid specific genes expression, siRNA targeted knock-down of NOX4 return to normal mRNA values of NIS, PAX8 and TPO in KI (10 and 100 µM) treated FRTL-5 cells. We analyzed the insulin and TSH effect on NOX4, TGF-B1 and NIS gene expression by real-time PCR in FRTL-5: 0.01 NIS(P<0.01); 9,1 NOX4(P<0.05); 1,9 TGF-B1(P<0.05) fold change in medium without TSH and insulin versus normal condition (+TSH+INS). CONCLUSION: Our results suggest that NOX4 could play a role on thyroid autoregulation. We demonstrated that EC treatment with 20nM DHEA produces an inhibition on platelet adhesion to endothelium (24h - 25%below Cont p<0.05), and decreases endothelium dependent platelet aggregation (60min - 15%below cont p<0.05) in a nitric oxide dependent manner, since preincubation with NAME annulated this effect (p<0.01). EC proliferation studies (MTT assay) showed that 24h treatment with DHEA stimulates cell growth (32, 22 and 12% above Cont 2, 20 and 200nM DHEA p<0.05). Indeed, using wound healing assays, we found that the steroid also promotes cell motility (9±2, 25±8 Cont, 20nM DHEA migrating cells/field p<0.01). The expression of uPA, tPA and androgen receptor (AR) was measured by inmunoblot. To that end, EC were treated for 12 to 48h with 20 or 200nM DHEA. The steroid enhances the expression of both factors (30-80% above control p<0.05). The androgen receptor expression was also increased, suggesting that DHEA mechanism of action could involve AR. Finally, in rat aortic ring angiogenesis assays, we observed that DHEA treatment promotes EC sprouting and capillary like tube formation (30% above control, p<0.05). The presented results show that DHEA exerts a direct action on EC, contributing to the prevention of vascular injury and promoting angiogenesis.

ONCOLOGIA III/ ONCOLOGY III

497 (871) **YERBA MATE (ILEX PARAGUARIENSIS): EVALUATION OF ANTI-TUMORAL EFFECT IN DIFFERENTS EXPERIMENTAL MODELS**

Rocío Soledad García Lázaro [1], Humberto Lamdan[1], Lorena Caligiuri[1], Norailys Lorenzo[1], Daniel Alonso[1], Hernán Gabriel Farina[1].
1. Laboratorio de Oncología Molecular, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes

Yerba Mate has many active phytochemicals with anti-tumor potential. Previously we reported that a yerba mate enriched polyphenols extract was able to inhibit the proliferation of colon (CT26) and breast (MDA-MB 231) tumor cells in a dose-dependent manner, with IC50 values of 0.7 and 2 mM, respectively. Also we reported that this extract showed negative modulatory effect on cell adhesion and migration of tumor cells. The aim of this study was to evaluate the effects of yerba mate extract on specific steps of tumor progression using in vitro and in vivo models. The invasive capacity of CT26 tumor cells was reduced by 30% using a treatment for 18 h of yerba mate extract. We tested the ability of yerba mate extract to interfere in processes such as angiogenesis, tumor growth and latency, using differents in vivo assays. In all in vivo protocols the extract was administrated to Balb/C via the drinking water before and after the subcutaneous inoculation of CT26 tumor cells. The results showed that yerba mate extract reduced tumor vascularization, increased tumor latency and decreased tumor growth. Finally, the tumor cells panel evaluated with yerba mate extract was extending to human colon (COLO205) and lung (H125) cells. Yerba mate extract inhibited the proliferation of these cell lines showing IC50 values of 1.3 and 0.56 mM, respectively. These results highlight the anti-tumoral properties of yerba mate and they would be the starting point to identify fractions and/or molecules responsible for these effects in order to design new anti-cancer drugs.

498 (431) **EPIGENETIC REGULATION OF CLCA2 BY CTBP1, HDACS, ZEB1 AND MIRNAS IMPACTS ON PROSTATE CANCER CELL ADHESION**

Juliana Porretti[1], Cintia Massillo[1], Guillermo Nicolás Dalton[1], Miranda Raquel Victoria Lantelme[2], Lucía Abril Segura[2], Ana María Cabanillas[3], Paola De Luca[1], Adriana De Siervi[1].
1. Laboratorio de Oncología Molecular y Nuevos Blancos Terapéuticos - IByME - CONICET 2. Escuela ORT 3. Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional Córdoba. CIBICI-CONICET

Epidemiologic studies demonstrated that metabolic syndrome (MeS) increased prostate cancer (PCa) risk and aggressiveness. Our previous studies showed that MeS and the C-terminal binding protein 1 (CtBP1) cooperate to induce PCa growth in mice. Genome-wide expression profiles and Gene Set Enrichment Analysis (GSEA) from these tumors, identified to chloride channel acccessory 2 (CLCA2) as a key CtBP1-repressed gene. CLCA2 was described as a tumor suppressor gene that links cell adhesion to cytosolic signaling proteins implicated in breast cancer EMT. We also found that CtBP1 associates to CLCA2 promoter and represses its expression in vivo and in vitro. The goal of this work was to understand the biological relevance and the molecular mechanisms whereby the transcriptional co-regulator CtBP1 represses CLCA2. PC3 cells were co- transfected with CtBP1, the promoter CLCA2 reporter plasmid and the Zn Finger E-box binding homeobox 1 (ZEB1), p65 or different histone deacetylases (HDACs) expression plasmids. We found that CLCA2 promoter activity was not modulated by p65, whereas ZEB1, HDAC2 or HDAC3 together with CtBP1 synergistically repressed CLCA2 promoter activity. Moreover, the HDAC inhibitor (TSA) significantly increased CLCA2 promoter activity and expression. We hybridized a miRNA expression microarray from PCa xenografts grown in MeS mice. After normalization and bioinformatic data analysis, we found several miRNAs that target CLCA2. Among them, the CtBP1-induced hsa-miR-196b, stands out for its role in oral and gastric cancer cell invasion. Thus, we assessed miR196b/CLCA2 role in cell adhesion. miR-196b overexpression decreased PC3 cell adhesion, while CLCA2 promoted cell adhesion in these cells. In turn, CLCA2 depletion dramatically decreased cell adhesion. In summary, this study describes for the first time that CLCA2 is epigenetically regulated by HDACs, ZEB1, miRNAs and CtBP1 in PCa cells modulating PCa cell adhesion.

499 (2012) **HISTONE DEMETHYLASE LSD1 IS FOUND TO BE DOWNREGULATED IN ACUTE LYMPHOBLASTIC LEUKEMIA CELLS**

Dario Ruiz Ciancio[1], Mauricio Vargas[1], Juan Pablo Marquez[1], Martín Alejandro Bruno[1], Maria Belen Mestre[1],
1. Laboratorio de Epigenética. Facultad de Ciencias Médicas, Universidad Católica de Cuyo.

Acute lymphoblastic leukemia (ALL) is a malignant disorder characterized by clonal proliferation of early B- and T- lymphocyte progenitors. ALL is the most common hematologic malignancy in children. Despite having a lower incidence in adult patients, it is one of the leading causes of mortality between hematologic malignancies. Up to 25% of children and more than 50% of adults suffer a relapse of the disease and its survival prognosis worsens significantly. Epigenetics is defined as the study of heritable changes in gene expression that are not due to changes in the primary DNA sequence. DNA methylation, histone modifications and noncoding RNA are all examples of epigenetic factors, and also they may be implicated in the pathogenesis of ALL. Lysine specific demethylase 1 (LSD1, also named KDM1A) is a histone demethylase that can regulates gene expression by removing the monomethyl and dimethyl groups from H3K4 and H3K9. Recent genetic approaches revealed that the expression levels and function of LSD1 are tightly regulated in undifferentiated hematopoietic stem/progenitor cells (HSPCs) and their deregulation underlies the development of hematologic malignancies. In this way, the primary objective of this study is to investigate the expression of LSD1 in ALL cells. Although LSD1 expression is elevated in most type of cancers, such us breast cancers or even acute myeloid leukemia; in this study, our results demonstrate that LSD1 is found to be downregulated in B-cell acute lymphoblastic leukemia. These results were confirmed by Western blot analysis and Real Time PCR. Thus, these novel results could provide an insight into new strategies for early diagnosis and effective treatment of the disease.

500 (433) **MECHANISMS TRIGGERED BY CALCITRIOL AND MENADIONE ON BREAST CANCER CELLS**

Solange Natali Guizzardi[1], Gabriela Picotto[1], Valeria Rodriguez[1], Luciana Bohl[2], Nori Tolosa de Talamoni[1].

1. Cátedra de Bioquímica y Biología Molecular, Facultad de Ciencias Médicas; Universidad Nacional de Córdoba. INICSA (CONICET-UNC), Córdoba. 2. Universidad Nacional de Villa María; CIT (VILLA MARIA CONICET)

Calcitriol regulates proliferation, differentiation, angiogenesis and cell death of breast cancer cells. Since calcitriol produces hypercalcemic effects in vivo as a side effect, our working hypothesis was that the combination of calcitriol with menadione (MEN), a glutathione (GSH) depleting drug, would enhance 1,25 (OH)2D3 (D) antiproliferative effects without undesirable side elevations of serum calcium. The aim of the present study was to investigate about the mechanisms involved in the antiproliferative action of the combined treatment. MCF-7 cells were treated with 100 nM D, 10 µM MEN, both drugs or vehicle (ethanol) for 96 hours. Intracellular calcium concentration and mitochondrial membrane potential were evaluated by flow cytometry. Superoxide anion and nitric oxide (NO) levels were measured by spectrophotometry. The expression of TRPV6 and PMCA1b mRNA levels were determined by quantitative real-time PCR and acidic vesicular organelles (AVOs) formation, by fluorescence microscopy. Statistical analyses were performed by ANOVA / Bonferroni. Both D and the combined treatment increased NO production. Superoxide anion levels, mitochondrial membrane permeability, intracellular calcium concentration and TRPV6 and PMCA1b mRNA levels were enhanced only by the combined treatment, as compared to controls. The increment of AVOs formation suggests activation of a cell death process. In conclusion, MEN increases the effect of calcitriol on MCF-7 cells through oxidative and nitrosative stress, alteration in intracellular Ca2+concentration and changes of the expression of molecules closely related to calcium regulation, thus inducing cell death pathways.

501 (463) **CTBP1 FUNCTIONS AS A SWITCH TO CONTROL AROMATASE TRANSCRIPTION IN RESPONSE TO THE METABOLIC STATUS OF THE PROSTATE CANCER CELLS**

Cintia Massillo[1], Guillermo Nicolás Dalton[1], Juliana Porretti[1], Paola De Luca[1], Adriana De Siervi[1].
1.Laboratorio de Oncología Molecular y Nuevos Blancos Terapéuticos – IByME- CONICET

The normal growth and development of the prostate requires the action of estrogens and estrogens receptors (ER) α and β. Estrogen-related pathways are clearly important in the development and progression of hormone-dependent cancers such as prostate cancer (PCa), but the role of ERβ remains controversial. The production of estrogens from androgens is mediated by the aromatase enzyme. Aberrant expression of aromatase plays a critical role in PCa development and progression. Metabolic syndrome (MeS) causes sex hormone imbalance and has been identified as a risk factor for PCa. Recently, we found that C-terminal binding protein (CtBP1), a transcriptional co-repressor of tumor suppressor genes, is a novel molecular link between MeS and PCa. We developed a MeS mice model that were inoculated with PC3 stable CtBP1 depleted or control cells. MeS mice showed hormone imbalance and high levels of intratumor estradiol. Interestingly, CtBP1 strongly repressed aromatase expression in these xenografts. The aim of this study was to understand the transcriptional regulation mechanism of aromatase mediated by CtBP1 in a MeS/PCa model. By chromatin immunoprecipitation (ChIP), we determined that CtBP1, p300 and ER associate to aromatase promoter in PC3 cells. Using gene reporter assays, we found that CtBP1 and p300 synergistically repress, while ER activates, aromatase promoter activity. Interestingly, estradiol exposure of PC3 cells, released CtBP1 from the aromatase promoter triggering its expression. Furthermore, we found that estradiol dramatically increased the viability of the LNCaP cells and its derivative C4-2, both sensitive to androgens. However, estradiol induced G1 phase arrest in androgen insensitive PC3 cells. In summary, CtBP1 represses aromatase expression en PCa. Nevertheless MeS induces estradiol, releases CtBP1 and activates aromatase expression, which in turn, increases prostate tumor cell proliferation in androgen sensitive cells.

502 (470) **ZEB1 IS MODIFIED POST-TRANSLATIONALLY BY SUMOYLATION**

Maria Victoria Vaglienti[1, 2], Maria Candelaria Llorens[1, 2], Ana Maria Cabanillas[1, 2].
1 ·Dpto Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional Córdoba. 2. CIBICI-CONICET

ZEB1 (Zn Finger E-box binding Homeobox) is a key transcription factor for Epithelial Mesenchymal Transition which not only induces an aberrant motility triggering dissemination and metastasis in cancer cells, but also confers stemness properties. ZEB1 is associated to metastasis initiation, aggressive behavior, treatment resistance and poor prognosis in lung, pancreas and breast cancers. ZEB1 is target of post translational modifications and the presence of many consensus sites for SUMOylation on its sequence suggests a possible role of this addition in the regulation of ZEB1. The SUMOylation is a covalent modification that adds the SUMO protein (Small Ubiquitin-like Modifier) to a Lysin residue (K) in a consensus motif ΨKX(D/E), where Ψ is an hydrophobic aminoacid. SUMOylation regulates a variety of activities such as protein subcellular localization and stability, transcriptional regulation and others. Our goal was to determine whether ZEB1 was SUMOylated (by in vivo assays) and what K residues were modified. An in silico comparison using SUMOplot identified 7 K residues with high score on ZEB1. The experimental approach chosen was to use small fragments of ZEB1 fused to GFP with all the potential SUMO sites: 1(K88, K175), 2 (K327, K473), 3 (K175), 4 (K473, K495, K635), 5 (K752) and 6 (K473,K495,K635,K752). HEK293T cells were cotransfected by lipofection with expression vectors (EV) of His6x-SUMO1 or His6x-$SUMO_2$, Ubc-9 and the EV of full length ZEB1 or the GFP clones. His6x tagged proteins were purified from cell lysates in Ni2+-NTA-agarose affinity columns, immunoblotted and developed with anti ZEB1 and anti GFP antibodies. The results showed that ZEB1 can be SUMOylated in different K sites. We also verified the colocalization of ZEB1 and SUMO-1 by immunofluorescence of HEK293T cells transfected with EV of SUMO-HA, Ubc9, ZEB1 and mutant deletion clones. The results suggest that ZEB1 could be modified by SUMOylation which could affect its oncogenic role.

503 (520) **EFFECT OF BLOCKING OF CTLA-4 IMMUNE CHECKPOINT ON THE GROWTH OF TWO MURINE TUMORS DISPLAYING DIFFERENT IMMUNOGENICITY.**

Daniela Romina Montagna, Ariel Ramiro Strazza, Paula Chiarella, Graciela I. Dran, Raúl Alejandro Ruggiero.

504 (524) **THE TRIAZOLIL AMINOACYL (PEPTIDYL) PENICILLIN TAP6 INHIBITS CELLULAR ADHESION AND MIGRATION IN A METASTATIC MELANOMA CELL LINE**

Elizabeth Barrionuevo[1], Patricia G. Cornier[2], Carina M. L. Delpiccolo[2], Dora B. Boggián[2], Ernesto G. Mata[2], Leonor P. Roguin[1], Viviana Blank[1].
1. Instituto de Química y Fisicoquímica Biológicas, Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires. 2. Instituto de Química Rosario, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario

Melanoma is a very aggressive form of skin cancer, with increased metastatic potential and high resistance to cytotoxic agents. In a previous work, we found that a penicillin derivative named TAP6, formed by a penicillin linked to the dipeptide Phe-Leu through a triazole group, was a potent antitumor agent against B16F0 murine melanoma cells. In order to study if this agent exhibits antimetastatic properties, we initially investigated the effect of TAP6 on the proliferation of different metastatic cell lines and we found that murine melanoma B16F10 cells were the most sensitive to TAP6 (IC50 7.5±2 μM). When B16F10 cells were subjected to the wound healing assay, results showed that a 5 μM concentration of TAP6 decreased the percentage of wound closure (58±6%, 54±10% and 42±5%, $p<0.005$) after 18h, 24h and 48h, respectively. It must be noticed that melanoma migration assays were performed with non-cytotoxic concentrations of TAP6. When we studied TAP6 effect on cell adhesion, we found a concentration-dependent reduction in the number of cells bound to a plastic surface after 1 h of incubation, being the percentage of adherent cells 62±6% and 38±8%, at 20 μM and 40 μM of TAP6, respectively ($p<0.001$). In addition, after incubating B16F10 cells for 18 h in the presence of 10 μM TAP6, a significant decrease of the expression levels of metalloproteinases 2 and 9 was observed ($p<0.01$). We further started to investigate the involvement of Wnt/β-catenin pathway on TAP6-induced biological effects. Results obtained by Western blot assays showed that 18 h treatment caused a 90% reduction in the total content of β-catenin ($p<0.005$). In conclusion, we demonstrated that TAP6 exhibits antimetastatic activity through the inhibition of cell adhesion and migration, and the reduction of the expression of metalloproteinases and β-catenin. Based on these findings, we will continue studying this penicillin derivative as a potential candidate for the treatment of metastatic melanoma.

505 (541) **ON THE TRAIT "UNDETECTABLE IMMUNOGENICITY" OF MURINE SPONTANEOUS TUMORS**

Daniela Romina Montagna[1], Ariel Ramiro Strazza[1], Paula Chiarella[1], Graciela I. Dran[1], Raúl Alejandro Ruggiero[1].
1. IMEX-CONICET-Academia Nacional de Medicina

Most murine tumors of spontaneous origin display no evidence of immunogenicity, a feature putatively shared with many human tumors. Herein, using three murine tumors exhibiting undetectable immunogenicity called CEI, C7HI and LMM3, we have investigated whether that feature can be attributed to absence of tumor antigens or to the existence of tolerogenic mechanisms that prevent such antigens from initiating an antitumor immune response (IR). To discriminate between both alternatives and extending preliminary observations, we used the strategy to mix in vitro lethally irradiated (LI) tumor cells from the non-immunogenic tumors with LI cells from a strongly immunogenic chemically-induced tumor (called MC-C). The mixture was then inoculated s.c. in mice 14 and 7 days before the challenge with different doses of live cells from the non-immunogenic tumors. DT50 (mean ± SE) of CEI tumor: Control Group: 13,000 ± 2,000; Group pretreated with LI CEI cells: 8,200 ± 2,000; Group pretreated with LI MC-C cells: 9,800 ± 1,500; Group pretreated with the mixture of LI CEI cells + LI MC-C cells: 52,000 ± 5,000 ($p < 0.02$ vs. Control, mean of two experiments). Similar results were obtained with C7HI and LMM3 tumors, that is, the pretreatment with the mixture produced a slight but consistent immunizing effect against the growth of the three non-immunogenic tumors and this effect was not associated with cross antigenicity between MC-C and the other tumors. Additional data revealed that the non-immunogenic tumors produced low levels of HGMB and Hsp60 danger signals for dendritic cell (DC) maturation while, in contrast, MC-C tumor produced high levels of those danger signals. Taking into account that anti-tumor IRs are depending on DC maturation, these results suggested that our non-immunogenic murine tumors bear specific tumor antigens which on their own cannot initiate an anti-tumor immune response but in the context of maturational signals of DC provided by LI MC-C tumor cells can do.

506 (543) **ABERRANT RET RECEPTOR EXPRESSION IN THE MAMMARY GLAND CAUSES TUMORS AND DEVELOPMENTAL DEFECTS**

Albana Gattelli[1,2], Tim Roloff[2], Robert Cardiff[3], Charles Perou[4], Edith Kordon[1], Lewis Chodosh[5], Nancy Hynes[2].
1. IFIBYNE-CONICET, University of Buenos Aires (UBA), Buenos Aires, Argentina. 2. Friedrich Miescher Institute for Biomedical Research (FMI), Basel, Switzerland. 3. Center for Genomic Pathology, School of Medicine, University of California Davis (UCD), Davis, USA. 4. Department of Genetics, University of North Carolina (UNC), Chapel Hill, USA. 5. Parelman School of Medicine, University of Pennsylvania (UPENN), Philadelphia, USA

The receptor tyrosine kinase (RTK) Ret, a key oncoprotein in thyroid carcinomas due to gain-of-function mutations, has also

been implicated in other types of cancers. Recently, Ret copy number gains and mutations have been reported at low frequencies in breast tumors. Furthermore, we and others have reported that Ret is overexpressed in about 40% of human tumors and this correlates with poor patient prognosis. Using a transgenic mouse model with the MMTV promoter controlling Ret expression in the doxycycline-inducible system, we show that overexpression of wild type (WT) Ret in the mammary epithelium produces hyperplasias and mammary tumors displaying a solid morphology that recapitulates features of human solid ductal carcinoma in situ. Moreover, Ret-induced tumors express ErbB2 and are estrogen receptor positive. Importantly Ret-induced tumors rapidly regress after doxycycline withdrawal indicating that Ret is the driving oncoprotein. Using next generation sequencing (NGS) we examined levels of transcripts in these tumors. We found that Stat signaling pathways could contribute to Ret-driven tumorigenesis. It is well known that RTKs, which are implicated in breast cancer, e.g. the ErbB receptors, also have roles in normal development. We found that Ret is highly expressed in mid-lactation. Indeed, Ret appears to have a role in the post-lactation transition to involution, where Stat pathways are crucial. Interestingly, when Ret is induced early in lactation we observe enhanced kinetics of involution. The involution period is well known to drive cancer progression. Thus, our results suggest that if Ret expression is deregulated during the lactation-involution transition this might contribute to breast cancer development.

507 (544) **CRX MRNA AS A BIOMARKER FOR RETINOBLASTOMA CELL IN METASTATIC SITES AND IN PATIENT-DERIVED XENOGRAFT**

María del Rosario Aschero[1], Santiago Zugbi[2], Úrsula Winter[2], Paula Schaiquevich[2], Fabiana Lubieniecki[1], Ana Torbidoni[2], Guillermo Chantada[3].
1. Servicio de Anatomía Patología, Hospital de Pediatría JP Garrahan. 2. Unidad de Farmacocinética Clínica, Hospital de Pediatría JP Garrahan. 3. Servicio de Hemato-Oncología, Hospital de Pediatría JP Garrahan

Introduction: The cone-rod homeobox (CRX) transcription factor is expressed in retinoblastoma tumors and in normal retina, but not in other tissues. We used it as specific biomarker for detection of retinoblastoma cells in samples from patients with metastatic retinoblastoma. Objectives: To confirm the presence of retinoblastoma cells in samples from metastatic patients and in patient-derived xenografts (PDXs) using CRX detection. Material and Methods: Extraocular samples were taken from metastatic retinoblastoma patients (n=3) and those with tumor cells invasion were inoculated in the flank of nude mice (patient-derived xenograft: PDX). After tumors grew, they were removed and processed. RNA from the samples wasextracted and mRNA of CRX was detected by retrotranscription follow by Real Time PCR. Results: Patient1: Samples from orbital tumor (OT) and cervical lymph node (CLN) were both positives for CRX, confirming the presence of retinoblastoma cells. CRX expression in peripheral blood (PB), bone marrow (BM) and cerebrospinal fluid (CSF) was negative, dismissing the presence of retinoblastoma cells. PDXs were obtained from OT and CLN and after growing, we confirmed its retinoblastoma nature through CRX detection that was positive for both PDXs. Patient2: Samples from PB, BM and CSF were evaluated for presence of retinoblastoma cell. CSF was positive for CRX, confirming the presence of retinoblastoma cells. This sample was inoculated in a nude mouse, and after growing its retinoblastoma nature was confirmed through CRX presence. Patient 3: Retinoblastoma cells were detected in OT and in BM through CRX positivity. Both tissues were inoculated in nude mice, but only OT grew. The retinoblastoma nature of the PDX was confirmed through CRX presence. Conclusions: We successfully obtain PDXs from extraocular retinoblastoma patients. CRX is expressed in retinoblastoma cells also in PDXs, being a useful tool for confirming the retinoblastoma cells in PDXs and metastatic sites.

508 (249) **ASSOCIATION OF EXPRESSION TO ERBB-2 NUCLEAR WITH KIDNEY CANCER ADVANCED**

Maria Alicia Cortés[1], Rosalia Cordo Russo[2], Nadia Carla Mabel Barbás[3], Mariano Alberto Quenardelle[4], Santiago Madera[2], Florencia Chervo[2], Violeta Chiauzzi[2], Eduardo Hernan Charreau[2], Rosana Gerometta[1], Patricia V. Elizalde[2].
1. CONICET- Facultad de Medicina, Universidad Nacional del Nordeste. 2. Laboratorio de mecanismos moleculares de carcinogénesis, Instituto de Biología y Medicina Experimental (IBYME- CONICET) 3. Servicio de Anatomía Patológica, Hospital Dr. José R. Vidal, Corrientes, 4.Facultad de Medicina, Universidad Nacional del Nordeste

The renal cell carcinoma is refractory to chemotherapy and radiotherapy. The major subtype of RCC that metastasize are clear cell and papillary RCC. When it is in advanced stages, decreased survival, and not always respond to kinase inhibitor therapy. In this context, we explored the expression and localization of ErbB-2 and its relationship with the advance RCC. The techniques used were immunohistochemistry, Western Blot and Inmunocytofluorescence confocal microscopy. We selected 50 paraffin- embedded tissue samples from a cohort of archived CCR from the files of the Histolopathology of Vidal Hospital, Corrientes from 2005 to 2015. The experiment in vitro basal and HRG stimulation they were performed with cell lines 786-O (RCC) and HEK-293 (Embryonic Kidney). Our results show that, the expression to membrane ErbB-2 (MErbB-2) is of 40% (20/50) only Clear cell RCC, are not expression MErbB-2 in Oncocytoma and Papilar RCC. However, it is not related to the stage and histological grade (Chi-square test P=0.8, Stage I vs III y IV). We show that nuclear ErbB2 (NErbB-2) is correlated with histologic grade (Fuhrman Nuclear Grade, FNG) (Chi-square test **P=0.007 FNG 1 vs 2 y 3). Here, we explored ErbB3 expression because we found in the correlation analysis ErbB3 and ErbB2 mRNA expression (TCGA Renal datasets in Oncomine Research Edition). Our results show relationship between NErbB-3 and NErbB-2, in tissue samples and in vitro in 786-O cells with HRG stimulation. Furthermore, In vitro experiments confocal show NErbB2 and NErbB3 with HRG in 786-O (t-test **P=0.01). Our findings showed ErbB-2 is implicated in the carcinogenesis renal. Is very important to localization ErbB-2, because in the clear cell renal cell carcinoma, ErbB-2 nuclear It is associated with poor prognosis. Our challenge is elucidate the molecular mechanisms of partner ErbB-2/ErbB-3 in renal cell carcinoma.

509 (300) **FORKHEAD BOX FACTORS (FOX) MODULATE GLUCOCORTICOID AND ANDROGEN RECEPTORS ACTIVITY IN PROSTATE CANCER**

Javier Nahuel Brandani[1], Daiana Leonardi[1], Mercedes Abbate[1], Elba Vazquez[1], Javier Cotignola[1].
1. Laboratorio de Inflamación y Cáncer, IQUIBICEN/Dpto. Química Biológica, CONICET/FCEN-UBA

Deregulation of hormonal receptors expression is responsible for the development and progression of prostate cancer (PCa). The Androgen receptor (AR) is involved in the PCa progression. The glucocorticoid receptor (GR) might also have oncogenic and/or tumor suppressor activities depending on the presence/absence of AR and other cofactors such as the forkhead box (FOX) family. We previously showed that the expression of FOXA1, FOXM1, FOXO1 and FOXO3 could be modulated by testosterone and dexamethasone (dex); conditions that simulate different stages of progression and treatment of PCa. We aim to deepen in the knowledge of these mechanisms using PC3 (AR-, GR+) and C4-2B (AR+,GR+) PCa cell lines as in vitro models. Cells were transfected with FOXA1 or FOXM1 shRNAs to knockdown gene expression. Diminished cell proliferation and altered cellular morphology were observed in FOX-knockdown cells. PC3 cells were also transfected with a constitutive active AR which was found to modulate FOXs expression. To study the transcriptional activity of GR, cells were transfected with a reporter plasmid containing 3 glucocorticoid response elements. Dex treatment resulted in an increment in luciferase levels ($p<0.001$) in both cell lines. Interestingly, testosterone also induced luciferase expression in the PC3 ($p<0.05$). FOXA1 down-regulation resulted in a lack of Dex or testosterone effect, showing the involvement of FOXA1 in AR and

GR activities. Finally, the expression of the FOXs was analyzed using gene expression microarray data from public repositories. We found that FOXA1 was over-expressed (>2-fold) in PCa-adjacent normal tissue compared to normal prostate, suggesting that FOXA1 altered expression could be an early event in PCa development. The expression of other FOXs was also altered during PCa progression. These results demonstrate that AR modulates FOXs expression, that FOXA1 is needed for GR-induced transcription and their involvement during PCa development and progression.

510 (842) **EVALUATION OF ANTITUMORAL EFFECT OF HRYDROALCOHOLIC BLUEBERRY EXTRACT AGAINST BREAST, COLON AND LUNG CANCER CELLS**

Rocío Soledad García Lázaro[1], Humberto Lamdan[1], Norailys Lorenzo[1], Lorena Caligiuri[1], Daniel Alonso[1], Hernán Gabriel Farina[1]

1. Laboratorio de Oncología Molecular, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes

Blueberries belong to the Vaccinium genus. This fruit is characterized by a high amount of phytochemicals and antioxidants mainly represented by phenolic compounds. It was reported that these polyphenols can inhibit the growth and proliferation of tumor cells. In this work we evaluated the effect of a hydro-alcoholic blueberry- enriched polyphenols extract on the tumor progression steps in experimental models of breast (MDA-MB 231), colon (CT26) and lung (H125) cancer. The extract was able to inhibit the proliferation of CT26 and H125 cells with IC50 concentrations of 0.1 mg/L and 0.2 mg/L, respectively. Both, adhesion and migration of CT26 and MDA-MB 231 tumor cells were also modulated by the blueberry extract. The extract reduced by 60% the invasive ability of CT26 colon cancer cells. Latency, tumor growth and anti-angiogenic capacity of the extract were studied using an in vivo model of Balb/C mice. Althought the results were not statistically significant, we observed a delay in latency and tumor growth and also a trend to reduced blood vessels, in blueberry extract-treated group. The results show that blueberry extract can modulate in vitro growth, adhesion and migration of tumor cells. The in vivo results encourage the search for management protocols that can maximize the anti-tumor potential of blueberries.

511 (981) **CHARACTERIZATION OF ACYL-COA SINTHETASE 4 (ACSL4) PROMOTER IN HUMAN BREAST CANCER CELLS**

Melina Andrea Dattilo, Yanina Benzo, Paula Fernanda Lopez, Natalia Noemi Morduchowicz, Ana Fernanda Castillo, Paula Mariana Maloberti.

512 (1005) **CHEMO-GENE TREATMENTS IN CANINE AND FELINE MELANOMA CELLS**

Lucrecia Agnetti[1], Chiara Fondello[1], Marcela S. Villaverde[1], Gerardo C. Glikin[1], Liliana M.E. Finocchiaro[1].

1. Unidad de Transferencia Genética, Área Investigación, Instituto de Oncología "Ángel H. Roffo", Facultad de Medicina, Universidad de Buenos Aires, Argentina

Gene therapy with interferon-β (IFN-β) gene or herpes simplex virus thymidine kinase/ganciclovir - Suicide Gene (SG) - showed variable effects on different melanoma cell lines generated in our laboratory. Then, we sought to enhance its effect by combining those genes with bleomycin (BLM), a glycopeptide antibiotic with antineoplastic effects due to its endonuclease activity. It was previously demonstrated the high cytotoxic effect generated by such treatments on canine and feline melanoma cell lines, encouraging the further study of this therapy. We found a significant correlation between the extent of the cytotoxic response of the combination gene treatments/BLM with the fraction of cells that showed: i) DNA damage (SubG0/G1, $p<0,05$), and ii) increased intracellular levels of reactive oxygen species (ROS, $p<0,05$), both determined by flow cytometry (IP and DCF). Some morphological changes were observed with dual treatments, as an increase in cell size, cell flattening, and a marked increment in the cell internal granularity, indicating a probable senescent phenotype of those cells that apparently survived the treatments. This finding was consistent with the high fraction of cells that showed an increase in senescence-associated β-galactosidase (SA β-Gal) activity, a known marker of this condition ($p<0,05$ with respect to unlipofected control cells). This would indicate that at least 50% of the remaining cells would be in an irreversible state in which they can no longer divide. Those treatments that significantly increased SA β-Gal activity, also decreased the clonogenic capacity of the surviving cells ($p<0,05$). It is worth to note that the colony-forming ability almost disappeared after treatment with BLM alone or combined with genes. Altogether, the results presented here encourage further studies to evaluate the combined chemo-gene treatments clinical potential.

513 (1008) **SURVIVAL ANALYSIS OF HEAT SHOCK PROTEINS IN BREAST CANCER WITH FOCUS ON MOLECULAR SUBTYPES**

Martín Eduardo Guerrero Gimenez[1], Felipe Carlos Martin Zoppino[1], Gisela Natalia Castro[1], Daniel Ramón Ciocca[1].

1. Instituto de Medicina y Biología experimental de Cuyo (IMBECU)

Aim: To analyze the clinical correlations of mRNA levels of the 95 Heat Shock Proteins (HSP) family members in breast cancer subtypes (Luminal A, Luminal B, HER2-enriched, Basal-like and Normal-like). Methods: The data used in this study was programmatically extracted from the publicly available data set of mammary adenocarcinoma from The Cancer Genome Atlas Project (TCGA). Standardized and non-standardized gene expression levels from 1097 tumor samples and 114 normal breast tissue data available in the RNASeqV2 platform were obtained. Given the expression levels of 50 different specific genes PAM50 classification was performed to classify the samples in five intrinsic molecular subtypes. To assess differential gene expression (DGE) between normal tissue and tumor samples we implemented DESeq2 analysis were log2 Fold change values were obtained associated with exact p-values and False Discovery Rate values (FDR). SAMseq was used as a screening method for determining whether changes in gene expression are significantly associated with survival. Kaplan Meier curves for each clinically significant HSP were generated to analyze overall survival. To assess the effect of several known prognostic predictors (ER, lymph node status, age, etc.) Cox proportional hazard ratio multivariate analysis was performed. Results: We have found HSP clusters that are specifically down-regulated while others appeared specifically up-regulated in breast cancer subtypes. After analyzing survival depend on HSP levels, we identified novel HSPs that show correlations with the clinical outcome of the cancer patients. Conclusions: We report the complexity of the expression of the HSPs in breast cancer, reporting in a large dataset their expression levels according to the genetic subtypes. Novel HSPs not previously related with breast cancer have been associated to this disease. The expression level of certain HSPs are in relation with the survival of patients.

514 (1063) **NEW THERAPEUTIC STRATEGIES FOR HEPATOCELLULAR CARCINOMA BASED ON EPIGENETIC TARGETING**

Juan Bayo[1], Esteban Fiore[1], Alejandrina Real[1], Mariana Malvicini[1], Estanislao Peixoto[1], Marcelo Rodriguez[1], Sofia Gomez-Bustillo[1], Mariana Garcia[1], Guillermo Mazzolini[1]

1. Gene Therapy Laboratory, Instituto en investigaciones en Medicina Translacional-CONICET. Facultad de Ciencias Biomédicas, Universidad Austral, Av. Pte. Perón 1500 (B1629AHJ) Derqui-Pilar, Buenos Aires, Argentina

Hepatocellular carcinoma (HCC) is a health problem worldwide and new therapeutic strategies are urgently needed. The hepatocarcinogenesis process involves several genetic and epigenetic alterations. In particular, deregulation of epigenetic enzymes which catalyze post-translational modification of histones could affect DNA accessibility resulting in changes on gene transcription, DNA repair and cell replication. The aim of this work was to identify epigenetic enzymes related with poor HCC patients prognosis and target them with specific inhibitors. Public clinical and

RNA-Seq data of tumors patients with HCC and adjacent tissue from The Cancer Genome Atlas (TCGA) were analysed using the cBioPortal and the FireBrowser portals. Analysis of 97 epigenetic enzymes using the TCGA data showed a significantly poorer survival for patients bearing HCC that expressed high levels of KDM5B (Jumonji demethylase), LSD-1 (histone demethylase) and EZH2 (histone methyltransferase). Interestingly, KDM5B and EZH2 are overexpressed in HCC in comparison with non-tumor tissues. Then, we evaluated 3 specific inhibitors (JIB-04 for KDM5B, GSK-LSD1 for LSD-1 and DZNEP for EZH2) as therapeutic strategy for HCC in vitro. We performed MTT cell viability assay in a panel of 7 HCC cell lines. The range of concentrations that reduce to the 50 percent the viability of cells (IC50) after 4 days of drug exposure showed a stronger antitumoral effect for JIB-04 (20-250 nM) than for GSK-LSD1 (120-650 nM) or DZNEP (140nM-878μM). Moreover, apoptosis and cell cycle analysis showed that JIB-04 induced a strong cell cycle arrest and an increase in the percentage of apoptotic cells in HCC cell lines. Finally, qPCR of HCC cells treated with JIB-04 showed the downregulation of proliferative (CCNB1, PCNA, SKP2) and the upregulation of antiproliferative/proapoptotic genes (DDIT4 and CCNG2). Our results indicate that epigenetic enzymes are interesting targets for therapeutic strategies against HCC. Moreover, specific inhibitors are promising tools to develop new HCC therapies.

515 (1069) **INVOLVEMENT OF HISTAMINE H4 RECEPTOR IN THE HISTAMINE-INDUCED MODULATION OF TUMOR GROWTH AND ANTITUMOR IMMUNITY IN 4T1 TRIPLE NEGATIVE BREAST CANCER**

Helena Andrea Sterle[1], Melisa Nicoud[1,2], Diego Martinel Lamas[1,2], Juan Carlos Perazzo[3], Graciela Cremaschi[1,2], Vanina Medina[1,2]

1. Institute for Biomedical Research (BIOMED), School of Medical Sciences, Pontifical Catholic University of Argentina (UCA), and the National Scientific and Technical Research Council (CONICET), Buenos Aires, Argentina; 2. Laboratory of Radioisotopes, School of Pharmacy and Biochemistry, University of Buenos Aires, Junín 956 PB, Buenos Aires, Argentina; 3. Department of Pathophysiology, School of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina

Histamine is known to regulate cellular immune responses, affecting maturation and activity of lymphocytes and myeloid-derived suppressor cells (MDSC). However, its role in the regulation of antitumor immunity has not been well established. The aim of the present work was to investigate the influence of the lack of histamine H4 receptor (H4R) on the tumorigenic capacity, tumor cell dissemination and the antitumor immunity in response to histamine in a triple negative breast cancer (TNBC) model. For that purpose, we evaluated tumor growth parameters and the composition of splenic and lymph node immune subsets in syngeneic H4R knockout (H4R KO) and wild-type (WT) mice, inoculated orthotopically with 4T1 murine TNBC cells. Percentages of CD4+, CD8+, CD19+, CD3+, CD11b+Gr1+ and NK1.1+ cells were quantified in spleens and lymph nodes by flow cytometry. In vitro results indicate that histamine (0.1-10 μM) reduced the cell proliferation levels of the 4T1 TNBC, evaluated by the clonogenic assay ($p<0.01$) and BrdU-incorporation ($p<0.05$), but non-significant apoptosis was detected with this treatment. The latency period showed no significant differences between WT and KO mice, while tumor growth was reduced in KO mice. Subcutaneous administration of histamine (1 mg/kg.day) decreased the tumor volume ($p<0.05$) and weight ($p<0.05$) only in KO mice. Most of the animals showed lung metastases and non-significant differences were observed upon histamine treatment. Interestingly, histamine reduced the percentage of CD4+ cells in spleens ($p<0.05$) and lymph nodes ($p<0.05$) of WT but not of KO mice. Histamine further induced a reduction of the percentage of myeloid-derived suppressor cells (CD11b+Gr1+) in spleens ($p<0.05$) from KO but not from WT mice. In conclusion, the present study demonstrates that histamine regulates the growth of TNBC. The H4R and the modulation of tumor immunity seem to be involved in histamine effects.

516 (1078) **CLOZAPINE, A NEW THERAPEUTIC APPROACH FOR BREAST CANCER?**

Melisa Beatriz Nicoud[1,2], Diego Martinel Lamas[1,2], Helena Sterle[1], Juan Carlos Perazzo[3], Graciela Cremaschi[1,2], Vanina Medina[1,2].

1. Institute for Biomedical Research (BIOMED), School of Medical Sciences, Pontifical Catholic University of Argentina (UCA), and the National Scientific and Technical Research Council (CONICET), Buenos Aires, Argentina. 2. Laboratory of Radioisotopes, School of Pharmacy and Biochemistry, University of Buenos Aires, Junín 956 PB, Buenos Aires, Argentina. 3. Department of Pathophysiology, School of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina

Previous data provide evidence supporting the anticancer effects of antipsychotics that could partially explain the lower incidence of cancer in patients with schizophrenia compared with the general population. We have previously demonstrated that clozapine inhibited proliferation of human breast cancer and melanoma cells. The aim of this work was to investigate the in vitro and in vivo antitumor activity of clozapine in 4T1 triple negative breast cancer (TNBC) model. For that purpose, the effect of clozapine on processes associated with cell death and survival, metabolism of ROS and DNA damage was studied in murine 4T1 breast cancer cells. Clozapine effects on tumor progression was further investigated in syngeneic mice, inoculated orthotopically with 4T1 murine TNBC cells. Results indicate that clozapine inhibited clonogenic proliferation and produced a 2-fold decrease in BrdU incorporation of 4T1 cells ($P<0.01$). This effect was associated with an increase in the differentiation marker Nile red evaluated by flow cytometry. Accordingly, in vivo treatment of 4T1 tumors with clozapine (1 mg/kg.day) reduced tumor weight (1.3±0.1 vs. 2.1±0.3 g, $P<0.05$) and volume (1.3±0.1 g vs. 1.9±0.3 cm3, $P<0.05$). Histopathological studies demonstrate that tumors of the clozapine-treated group were microscopically homogeneous with extended areas of differentiation (e.g. glands). We conclude that clozapine produces antitumoral effects in vitro and in mouse syngeneic model of TNBC, suggesting that it could be a potential therapeutic agent in the treatment of breast cancer.

517 (2005) **FIRST REPORT OF ABSCOPAL EFFECT OF BORON NEUTRON CAPTURE THERAPY (BNCT)**

Verónica A. Trivillin[1,2], Emilian Cesar Cayetano Pozzi[1], Lucas Colombo[2,3], Silvia Inés Thorp[1], Marcela Alejandra Garabalino[1], Andrea Monti Hughes[1], Sara Josefina González[1,2], Rubén Oscar Farías[1], Paula Curotto[1], Gustavo Alberto Santa Cruz[1], Daniel Germán Carando[2,4], Amanda Elena Schwint[1,2]

1. Comisión Nacional de Energía Atómica (CNEA). 2. Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). 3. Instituto de Oncología Angel H. Roffo. 4. Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires

The Abscopal effect would inhibit tumor growth at a site distant from the primary site of standard radiotherapy via immunologic mechanisms. BNCT combines selective tumor uptake of 10B compounds and neutron irradiation. The aim of the present study was to evaluate, for the first time, the potential Abscopal effect of BNCT. Twenty-six BDIX rats were inoculated sc with 1x106 DHD/K12/TRb syngeneic colon cancer cells in the right hind flank. Three weeks post-inoculation, twelve tumor-bearing rats were injected with borono-phenyl-alanine (BPA) iv. The right leg bearing the tumor nodule was locally irradiated 3 h post-administration of BPA at RA-3 at an absorbed dose of 7.8 Gy to skin. An additional group of 14 tumor bearing rats were left untreated and used as control. Two weeks post-BNCT, 1x106 DHD/K12/TRb cells were injected sc in the contralateral left hind flank of each of the 26 BDIX rats. Tumor volume in the right leg was determined pre-BNCT and once a week post-BNCT for 7 weeks in BNCT treated and untreated animals. Likewise, tumor volume was measured weekly in the contralateral left flank. The potential inhibitory effect on tumor

development in the left leg, induced by a positive response to BNCT of the tumor in the right leg, was used as an indicator of Abscopal effect of BNCT. Animal experiments were approved by IACUC. At 7 weeks post-BNCT, tumor volume in the left leg was smaller (albeit not significantly) in animals treated with BNCT in the right leg, than in untreated animals (164 ± 163 mm3, n=12 vs 254 ± 251 mm3, n=14 respectively). Within the BNCT group, a statistically significant reduction was observed in left tumor volume in animals whose right leg tumor responded to BNCT vs non-responding animals, i.e. 13 ± 15 mm3, n=5 vs 271 ± 251 mm3, n=7 (Student´s t test, p=0.0013). The present study, albeit preliminary and performed in a simple animal model, provides proof of principle that the response of a tumor to BNCT is capable of inducing an Abscopal effect.

518 (2006) **COFILIN-1 AND ASSOCIATED GENES RELATIONSHIP WITH MELANOMA READIORESISTANCE**

Francisco Martin Garcia[1,2], Candelaria Bracalente[1,3], Camila Zuccato[1], Tatiana Miller[1,2], Vanesa Biolatti[1], Beatriz Molinari[1,3], Fabio Klamt[5], Mauro Castro[4], Irene Ibañez[1,3], Hebe Duran[1,2,3]

1. Comisión Nacional de Energía Atómica. 2. Universidad Nacional de San Martín. 3. CONICET. 4. Universidade Federal do Parana. 5. Universidade Federal do Rio Grande do Sul

Melanoma arises from the malignant transformation of melanocytes and its incidence has increased in recent decades. Is highly metastatic and in advanced stages have a poor prognosis due to the lack of response to chemo- and radiotherapies. The processes of invasion and metastasis, as well as resistance to therapies, are decisive on the clinical outcome of patients. While these processes have been studied as independent, in recent years it has been described a superposition of such mechanisms. In this context, detection of groups of proteins involved in both processes could define predictive and prognostic molecular markers that could be valuable to establish the appropriate therapeutic strategy and develop new therapies aimed at molecular targets. One of most relevant protein in cytoskeletal regulation is cofilin-1 (CFL1), which plays a central role in the migration process. CFL1 increased levels have been associated with the degree of aggressiveness in various cancers. Furthermore, it has been suggested as a marker of invasion, and also to predict resistance to chemotherapy. We have studied in a melanoma model the relationship between expression levels of proteins associated with regulation of the actin cytoskeleton and migration and metastatic phenotype. Preliminary experiments suggest involvement of these proteins in the modulation of radiorresistance. In the current work CFL1 was valued as a predictive marker in melanoma radiotherapy, by analyzing correlation of CFL1 expression and cells intrinsic radiorresistance. Moreover, the network of functionally associated genes to CFL1 was studied, to detect differentially expressed genes in association with cells radioresistance. Results lead us to propose CFL1 as a possible predictive marker, although we need further studies. In other hand, several genes were identified as potential modulators of CFL1 activity. Thereby, we aim to elucidate their relationship with CFL1, as well as the mechanisms.

519 (977) **EFFECTS OF HIGH FAT DIET ON MAMMARY GLAND DUCT EPITHELIUM AND NEOPLASTIC DISEASE PROGRESSION IN A METABOLIC SYNDROME MICE MODEL**

Georgina Daniela Scalise[1], Paula Farré[1], Guillermo Nicolás Dalton[1], Juliana Porretti[1], Cintia Masillo[1], Roberto Pablo Meiss[2,], Paola De Luca[1], Adriana De Siervi[1].

1. Laboratorio de Oncología Molecular y Nuevos Blancos Terapéuticos, IBYME -CONICET. 2. Departamento de Patología, Instituto de Estudios Oncológicos, Academia Nacional de Medicina

Metabolic syndrome (MeS) is a cluster of pathophysiological disorders that comprises at least three of the following factors: abdominal obesity, elevated triglycerides, dyslipidemia, high blood pressure and elevated serum glucose levels. Several studies associated MeS with increased risk for several cancer types, including breast cancer. The aim of this work was to assess the effect of high fat diet (HFD) on mammary gland epithelium development and lung and liver metastasis in mice. We generated a MeS model feeding mice with high fat diet (HFD) for 10 weeks. Control diet (CD)-fed animals were maintained at the same conditions. MDA-MB-231 breast tumor cells were implanted on their mammary fat pad. After four weeks, tumors were surgically removed. Two weeks after surgery mice were sacrificed, and breast, liver and lung samples were collected for histopathological analysis. In addition, in the autopsy, representative samples of other unexpected findings were harvested. We found that 44% of mammary ducts from HFD mice were covered with prominent epithelial cells with nuclear pseudostratification and columnar changes. None of the animals in the control diet (CD) group developed these changes. We found lung metastasis in 20% of the HFD fed mice, while CD group showed no metastasis. We also found liver metastasis in 20% of the HFD fed mice, and only 10% on the CD group. In conclusion, HFD induced early proliferative changes in the mammary duct epithelium, which can be a sign of a preneoplastic condition. The same group of mice had increased number of metastasis and more extended neoplastic disease than mice fed with CD. Altogether, our findings reveal that HFD induces proliferative changes in breast ducts, as well as progression of neoplastic disease.

520 (589) **RHBDD2 AND WWOX PROTEIN INTERACTION AS MODULATOR MECHANISM OF PROLIFERATION AND DIFFERENTIATION OF NORMAL AND NEOPLASTIC BREAST CELLS**

Valeria Alejandra Ferretti[1], Romina Canzoneri[1], Ezequiel Lacunza[1], Edith Kordon[2], Claudio Marcelo Aldaz[3], Martín Abba[1].

1. CINIBA, Facultad de Ciencias Médicas, Universidad Nacional de La Plata, Argentina. 2. LEGMA, IFIBYNE-CONICET, Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina. 3. University of Texas – MD Anderson Cancer Center, Science Park – Research Division, Smithville, TX – USA.

The Rhomboid gene family constitutes a heterogeneous group of proteases / pseudoproteases that are conserved throughout evolution, involved in different biological processes such as EGFR, TNF, ERAD and UPR signaling pathways among others. We previously demonstrated that RHBDD2 is a cancer related gene amplified and overexpressed in advanced breast cancer. On the other and, The WW domain containing protein WWOX has been postulated as a tumor suppressor in breast and other cancers. WWOX modulates the TGFβ signaling pathway via direct WW-domain mediated binding and potential cytoplasmic sequestration of SMAD3 protein. The aim of this study was to characterize the RHBDD2-WWOX protein interaction and their biological relevance over the TGFβ signaling pathway in normal and breast cancer cells. Functional proteomics analysis using TAP/MS allowed us to identify a RHBDD2 – WWOX protein-protein interaction in HEK293 cancer cells. This result was validated in normal (HC11) and breast cancer cell lines (MCF7, T47D, MDAMB231) by Co-immunoprecipitation (Co-IP) and western-blot. In addition, co-localization analysis corroborate the previous finding, demonstrating that such protein-protein interaction occurs at the Golgi complex. HC11 mouse mammary cell line was employed to evaluate Rhbdd2 and Wwox expression at mRNA and protein levels by RT-PCR and Co-IP/WB methods respectively. We detected a high Rhbdd2 expression at the proliferative stage of the HC11 cells with a dramatic decrease at the competent and differentiated stages, while Wwox expression was expressed at the differentiated stage. Oligo-microarray based analysis of MDMBA231 RHBDD2 silenced cells (siRNA) suggest that RHBDD2 could be involved in the modulation of TGFβ signalling pathway among other bioprocess via sequestration of WWOX protein at the Golgi complex. This study show that the RHBDD2-WWOX protein interaction is associated with the modulation of the mam-

mary proliferation and differentiation process, suggesting that this interaction might play a relevant role in the normal development and neoplastic progression.

TOXICOLOGÍA / TOXICOLOGY

521 (247) **COPPER AND IRON TOXICITY IN RAT LIVER ISOLATED MITOCHONDRIA: OXIDATIVE STRESS, ANTIOXIDANT PROTECTION AND REDOX HOMEOSTASIS.**

Rosario Natalia Musacco Sebio[1], Christian Martín Saporito Magriñá[1], Juan Manuel Acosta[1], Sofía Bajicoff[1], Paola Paredes Fleitas[1], Sofía Reynoso[1], Alberto Boveris[1,2], Marisa Gabriela Repetto[1,2]

1. Universidad De Buenos Aires, Facultad De Farmacia Y Bioquímica, Cátedra De Química General E Inorgánica. Junín 956 (C1113aad), Buenos Aires, Argentina. 2. Consejo Nacional De Investigaciones Científicas Y Técnicas, Instituto De Bioquímica Y Medicina Molecular (IBIMOL-UBA-CONICET).

Introduction: The transition metals copper (Cu) and iron (Cu) are necessary at low concentration for different vital functions whereas at higher concentrations they become toxic. Wilson's disease and Haemochromatosis are examples of Cu and Fe toxicity. Here, the pathophysiology proposed in both cases involves the ability of the metals to produce hydroxyl radical (OH•) through the Fenton/Haber-Weiss reaction. Hypothesis: Cu and Fe are able to produce oxidative damage by OH• formation as well as direct action of the metals on biomolecules. Methodology: Rat liver isolated mitochondria were exposed to Cu and Fe overloads. Mitochondrial function (oxygen consumption assessed in aClark-type electrode), phospholipid peroxidation (TBARS) and total thiol content (reaction with DTNB) were determined. Results: Both metals are able to decrease the mitochondrial function, much lower concentrations were needed for Cu to become toxic. The decrease of the oxygen consumption and respiratory control was considerably more pronounced when mitochondria were exposed toconditions of high $H_2O_2/O_2^{\cdot}$ production, showing the participation of these species in metal toxicity. Both metals induced phospholipid peroxidation in a concentration-dependent manner but, interestingly, only when substrates for oxygen consumption were available. Regarding Cu, the antioxidant glutathione (GSH) was the most efficient in preventing mitochondrial dysfunction and phospholipid oxidation. Regarding Fe, these protective effects were only observed upon butylated hydroxytoluene (BHT) supplementation. Cu, but not Fe, is able to react in a direct and stoichiometric way with the mitochondrial thiol groups. Conclusion: Cu and Fe toxicity present at some point a common toxic mechanism of OH• generation through Fenton reaction. However, Cu may also participate in intracellular oxidative mechanism by direct reaction with protein thiol groups and, specially, GSH.

522 (280) **ANGIOGENIC POTENTIAL OF AN ORGANOCHLORINE PESTICIDE IN BREAST CANCER. IN VITRO AND IN VIVO STUDIES.**

Carolina Pontillo[1], Lorena Zárate[1], Alejandro Español[2], Noelia Miret[1], Florencia Chiappini[1], Claudia Cocca[3], Laura Álvarez[1], Diana Kleiman de Pisarev[1], María Elena Sales[2], Andrea Randi[1]

1. Universidad De Buenos Aires, Facultad De Medicina, Departamento De Bioquímica Humana, Laboratorio De Efectos Biológicos De Contaminantes Ambientales, Buenos Aires, Argentina. 2. Laboratorio De Inmunofarmacología Tumoral, Centro De Estudios Farmacológicos Y Botánicos (CEFYBO), Facultad De Medicina, Universidad De Buenos Aires, Buenos Aires, Argentina. 3. Laboratorio De Radioisótopos, Facultad De Farmacia Y Bioquímica, Universidad De Buenos Aires, Buenos Aires, Argentina.

Epidemiological studies report that exposure to organochlorine pesticides like hexachlorobenzene (HCB) increases risk of cancer. We demonstrated that HCB induces proliferation, migration, and invasion in human breast cancer cells as well as enhances neovasculogenesis in mammary endothelial cells. Vascular endothelial growth factor (VEGF) and cyclooxygenase-2 (COX-2) promote carcinogenesis, tumor proliferation and angiogenesis. Nitric oxide (NO) is implicated in cancer biology and tumor progression. Inducible nitric oxide synthase (NOS2) is an important mediator of tumor aggressiveness, however, it should be noted that other isoforms, NOS1 and NOS3, are also implicated. This study examined the HCB action on breast cancer angiogenesis using a xenograft model in mice exposed to HCB (0.3, 3 and 30 mg/kg body weight, b.w.), with the human breast cancer cell line MCF-7 (+ERα), and, MCF-7 cells treated with HCB (0.005, 0.05, 0.5 and 5 µM). Results: HCB (3 mg/kg b.w.) stimulated the angiogenic switch evaluated as the number of vessels/mm^2 (71% $p<0.001$) and increased VEGF expression (80% $p<0.01$) in mice skin. In MCF-7 cells, the pesticide increased COX-2 protein levels at all assayed doses (120% and 130% $p<0.01$; 110% $p<0.05$ and 190% $p<0.001$). Furthermore, 0.005 and 0.05 µM HCB stimulated VEGF secretion (100% $p<0.05$ and 180% $p<0.001$), however 0.5 and 5 µM HCB increased VEGF expression (60% $p<0.05$ and 70% $p<0.01$) in MCF-7 lysates. Besides, 0.005 and 0.05 µM HCB decreased NO production (44% $p<0.05$; 39% $p<0.05$) and NOS3 protein levels (34% $p<0.001$; 54% $p<0.001$). NOS1 levels were reduced at 5 µM HCB (61% $p<0.01$), while NOS2 expression was not affected. In conclusion, our results demonstrate that HCB stimulates VEGF secretion in vitro and in vivo, promoting angiogenesis. Altogether, these data highlight that HCB stimulates cellular angiogenic processes promoting mammary carcinogenesis.

523 (277) **THE ROLE OF GLUTATHIONE AS A PROTECTIVE MOLECULE IN COPPER OVERLOAD**

Christian Saporito Magriñá[1], Christoph Borner[2], Marisa Gabriela Repetto[1,3].

1. Universidad De Buenos Aires, Facultad De Farmacia Y Bioquimica, Catedra De Quimica General E Inorganica. 2. Albert-Ludwigs-Universität Freiburg, Zentrum Für Biochemie Und Molekulare Zellforschung (Zbmz), Institut Für Molekulare Medizin Und Zellforschung. 3. Consejo Nacional De Investigaciones Científicas Y Tecnicas, Instituto De Bioquímica Y Medicina Molecular (IBIMOL) (UBA-CONICET)

Wilson Disease is characterized by high copper (Cu) content in the liver and different organs due to a defective extrusion of the metal into the bile. The enhanced intracellular concentration of Cu ends up in the demise of the cell. However, the molecular events responsible for Cu toxicity are largely unknown. Objective: To outline the mechanism of toxicity of Cu and identify endogenous protective molecules in Cu overload. Methodology: Cell viability (FACS) and –SH content (reaction with DTNB) were assessed after Cu exposure in HCT116, BEAS-2B, Hela, MEFs and Hepa1-6 cells. Results: Cells show large differences in LD50. In cells exposed to LD50, the onset of cell death takes place after 10 hours. Cu oxidized –SH groups of reduced glutathione (GSH), N-acetylcysteine (NAC) and cysteine in vitro. However, during the first 7 hours of Cu exposure, intracellular GSH is not significantly oxidized in any cell line. In accordance, the addition of NAC (membrane permeable –SH) has no effect on the survival of the cell. The lack of effect of NAC on cell viability implies that the oxidation of cell surface –SH groups by Cu has no direct impact on the survival of the cell. However, the addition of GSH or GSSG to the medium largely improves cell survival upon Cu overload. This protection is likely due to the formation of complexes between Cu and GSH or GSSG, thus preventing the metal entrance into the cytosol. However, given the high GSH content, this reaction likely takes place also intracellularly. Likewise, GSH depletion by BSO drastically potentiates cell death induced by Cu while –SH replenishment by NAC in GSH depleted cells brings no improvement to the survival. Conclusion: Cu oxidation of cell surface –SH groups has no direct impact on cell survival. Even when Cu is present at toxic concentrations, cells are in control the intracellular –SH state. Nevertheless, GSH performs as a major protective molecule in Cu overload although, independently of the reduced –SH group.

524 (284) **CHANGES IN CELL MIGRATION AND AROMATASE EXPRESSION IN HUMAN ENDOMETRIAL STROMAL CELLS INDUCED BY ORGANOCHLORINE PESTICIDE HEXACHLOROBENZENE**

Florencia Chiappini[1], Leandro Ceballos[1], Carolina Pontillo[1], Noelia Miret[1], Mariana Farina[2], Andrea Randi[1]
1. Universidad De Buenos Aires, Facultad De Medicina, Departamento De Bioquímica Humana, Laboratorio De Efectos Biológicos De Contaminantes Ambientales, Buenos Aires, Argentina. 2. Conicet-Universidad De Buenos Aires, Centro De Estudios Farmacológicos Y Botánicos (CEFYBO), Laboratorio De Fisiopatología Placentaria, Buenos Aires, Argentina.

Endometriosis is an estrogen dependent gynecologic disease with lasting implications for many women's fertility, and overall quality of life. Exposure to organochlorines can interfere with both hormonal regulation and immune function to promote endometriosis. Hexachlorobenzene (HCB) is a pesticide that induces toxic reproductive effects in laboratory animals. Aromatase activity is increased in eutopic and ectopic endometrial of women with endometriosis. Aromatase expression in endometriosis cells is induced via cyclooxygenase-2 (COX-2)-prostaglandin E_2 (PGE_2) pathway. Estrogen receptor α ($ER\alpha$) is the major mediator of estrogenic effects on stimulation of proliferation and induction of progesterone receptor (PR) expression. PRA and PRB serve an anti-inflammatory role in the uterus by antagonizing COX-2 expression. Degradation of extracellular matrix by metalloproteinases (MMPs) is a basic step in the migration and invasiveness in endometriosis. Recently, we demonstrated that HCB enhances COX-2 expression, MMP2 and 9 activation and PGE_2 secretion in human endometrial stromal cells. The present study examined the HCB effect in human endometrial stromal cell line T-HESC on cell migration (scratch motility assay), aromatase expression (WB), and $ER\alpha$ and PRA protein levels (WB). T-HESC cells were exposed to HCB (0.005, 0.05, 0.5 and 5 µM) or vehicle for 24 h. Results showed that the pesticide increased the cell migration ratio (47%, $p<0.05$; 81%, 77% and 96%, $p<0.001$) in a dose response manner. HCB (0.005,0.05 and 0.5 µM) enhanced aromatase expression (82%, $p<0.01$; 56%, $p<0.05$ and 67%, $p<0.01$). Besides, we found that only 0.5 µM HCB increased $ER\alpha$ levels (89%, $p<0.05$), while PRA protein expression was decreased at 5 µM HCB (55%, $p<0.05$). Our results indicate that HCB exposure could contribute to endometriosis development by modifying cell migration, aromatase expression,and alterations in $Er\alpha$ and PRA protein levels in human endometrial cells T-HESC.

525 (327) **LONG TERM ADVERSE EFFECTS ON FEMALE FERTILITY BY NEONATAL EXPOSURE TO ENDOSULFAN AND GLYPHOSATE IN RATS.**

Marlise Luciana Guerrero Schimpf[1], Paola Inés Ingaramo[1], María Mercedes Milesi[1], Enrique Hugo Luque[1], Jorgelina Varayoud[1]
1. Instituto De Salud Y Ambiente Del Litoral, Facultad De Bioquímica Y Ciencias Biológicas, Universidad Nacional Del Litoral - Consejo Nacional De Investigaciones Científicas Y Técnicas (Conicet), Santa Fe, Argentina.

Pesticide exposure has been associated with increased risk of long-lasting female reproductive disorders such as altered cyclicity, decreased conception rates and increased pregnancy loss. However, the impact of the exposure to pesticide mixtures has received little attention. The aim ofthe present study was to evaluate the reproductive effects of neonatal exposureto a mixture of low doses of endosulfan and glyphosate commercial formulations. Newborn female rats received on postnatal days (PND) 1, 3, 5, and 7, by s.c injection, a commercial formulation of endosulfan at 600 µg/kg/day (END, n=30), a glyphosate based herbicide at 2 mg/kg/day (GBH, n=30) or a mixture of both pesticides at the before mentioned doses (END+GBH, n=27). The control animals were injected with the vehicle (C=22). On PND90, rats were mated with males of proven fertility and the pregnancy rate was evaluated. Sperm-positive females were sacrificed on gestational day 19 to assess the following reproductive parameters: number of corpora lutea (nCL), implantation sites (nIS) and resorption sites (nRS). The END-group showed a decrease of the nIS while the GBH exposed animals evidenced a higher nRS when compared with the control group. Finally, the binary mixture END+GBH revealed an increase in the nRS, as observed in GBH-group. Developmental exposure to END and GBH commercial formulations induced subfertility as a consequence of pre-implantation and post-implantation loss, respectively. Co-exposure to both formulations led to post-implantation loss indicating a predominant effect of GBH formulation. These results draw attention to the need of more studies on the potential reproductive impact of pesticide mixtures.

526 (361) **EFFECTS OF PERINATAL EXPOSURE TO A GLYPHOSATE BASED HERBICIDE ON THE MAMMARY GLAND OF PRE AND POSTPUBERTAL MALE RATS.**

Ayelen L. Gomez[1], Gabriela A. Altamirano[1,2], Melisa B. Delconte[1], Eduardo Masat[2], Mario R. Osti[2], Enrique H. Luque[1], Mónica Muñoz-de-Toro[1,2], Laura Kass[1,2].
1. Instituto De Salud Y Ambiente Del Litoral (ISAL, UNL-CONICET), Facultad De Bioquímica Y Ciencias Biológicas, Universidad Nacional Del Litoral. 2. Cátedra De Patología Humana, Facultad De Bioquímica Y Ciencias Biológicas, Universidad Nacional Del Litoral.

Glyphosate exposure during critical periods of development induced adverse effects on the reproductive system of male rats, suggesting an endocrine disruption action. In addition, it has been reported that the male rat mammary gland (MG) is affected by the action of endocrine disruptors. Here, we evaluated whether perinatal exposure to a glyphosate based herbicide (GBH) modifies the MG development of pre and postpubertal male animals. Pregnant rats (F0) were exposed orally through the diet to vehicle (C, saline solution) or 350 mg/kg/day of GBH from gestational day 9 until weaning. On postnatal day 21 (PND21) and PND60 F1 males were sacrificed and MG and blood samples were collected. The MG samples were processed for whole mount (WM) or kept at -80°C for RT-PCR analysis. On PND21 and PND60 serum levels of testosterone (T) were measured and WMs were analyzed. On PND60, the mRNA expression of estrogen receptor alpha (ESR1) and androgen receptor (AR) was also evaluated. The treatment with GBH produced no signs of abnormalmaternal or nursing behaviors, or changes in the body weight gain or pellet consumption of the F0 dams. The length of gestation was unaltered, and no gross malformations were observed in the F1 pups at delivery or weaning. No differences were observed in T serum levels between experimental groups and T concentration was higher on PND60 (C: 1.93±0.43 ng/ml; GBH: 1.83±0.60 ng/ml) compared to PND21 (C: 0.27±0.06; GBH: 0.27±0.12 ng/ml). On PND21, the treatment with GBH reduced MG total area. In contrast, on PND60, total area, longitudinal growth and perimeter were increased in GBH-exposed group; no differences were observed in the epithelial area. In addition, GBH treatment decreased the mRNA expression of ESR1 on PND60 animals, whereas AR mRNA levels were increased. Our results demonstrate that perinatal exposure to GBH alters MG morphology and gene expression long after exposure ended and suggest that the effects may be due to endocrine disruption.

527 (359) **OXIDATIVE STRESS IMBALANCE AFTER PARTICULATE AMBIENT AIR POLLUTION EXPOSURE IN A CHRONIC NUTRITIONAL STRESS MODEL**

Melisa Lidia Amelia Kurtz[1], Francisco Astort[1], Christian Esteban Lezón[2], Graciela Champin[2], Sebastian Ariel Ferraro[1], Silvia María Friedman[3], Deborah Ruth Tasat[1,4], Patricia Mónica Boyer[4]
1. Centro De Estudios En Salud Y Medio Ambiente, Escuela De Ciencia Y Tecnología, Universidad Nacional De San Martin. 2. Catedra De Fisiología, Facultad De Odontología, Universidad De Buenos Aires, Buenos Aires, Argentina. 3. Catedra De Bioquímica, Facultad De Odontología, Universidad De Buenos Aires, Buenos Aires, Argentina. 4. Catedra De Histología, Facultad De Odontología, Universidad De Buenos Aires, Buenos Aires, Argentina.

Air pollution does not affect everyone equally. Therefore, we sought to study in growing animals under chronic nutritional stress (CNS), the oxidative metabolism after acute exposure to Residual Oil Fly Ash-ROFA, a known surrogate ambient air particle pollution. Twenty-eight weanling male Wistar rats were randomized in two groups regarding food intake: 1) ad libitum food intake (Control-C), or 2) 80% of the amount of food consumed by control (CNS). After 4 weeks animals were intranasally instilled either with ROFA (1mg/kg BW) or saline solution defining 4 groups: C, CNS, C+ROFA, CNS+ROFA. After 24h, total cell number (TCN), differential cell count (DCC) and superoxide anion (O2.-) generation were examined in broncheoalveolar lavage; superoxide dismutase (SOD) and catalase (CAT) antioxidant enzymes activity, in lung homogenates; and number of white cells (BWC)/mm^3 was determined in blood. TCN augmented after ROFA exposure being only significant for C+ROFA group (C:6.9±1.5 vs C+ROFA:17.0±3.1. p<0.05; CNS:5.3±0.6 vs. CNS+ROFA: 9.7±1.6, ns). DCC analysis showed no differences in cell distribution between C and CNS groups. However, ROFA induced both in C and CNS an increase in the polymorphonuclear cells percentage (%PMN). C+ROFA animals depicted a higher% PMN (50%) when compared to the CNS+ROFA (40%) group. O_2.- generation significantly increased in all groups when compared to C. Again, ROFA exposure increases O_2.- generation being higher in C+ROFA than in CNS+ROFA (C+ROFA:68.5±12.6, CNS+ROFA:47.17±12.5, p<0.001). Accordingly, SOD activity diminishes both in C+ROFA and CNS+ROFA (p<0.001) with respect to controls. Irrespective of the treatment, the number BWC was markedly reduced in the CNS group (p<0.001). Our results show that ROFA provokes an oxidative imbalance regardless the nutritional state of the animal. Nevertheless, CNS animals respond less to particulate ambient air pollution what may be consequence of an immunosuppression state.

528 (467) **HEPATOTOXICITY INDUCED BY ACUTE EXPOSURE TO BUENOS AIRES URBAN AIR PARTICLES AND RESIDUAL OIL FLY ASH IN YOUNG AND MIDDLE-AGED MICE**

Nadia Soledad Orona[1], Francisco Astort[1], Sebastian Ariel Ferraro[1], Guillermo Alberto Maglione[1,2], M Martin[3], Fernando Brites[3], Patricia Mandalunis[2], Celina Morales[4], Deborah Tasat[1,2]

1. Universidad Nacional de San Martín, Escuela de Ciencia y Tecnología. Centro de Estudios en Salud y Medio Ambiente. 2. Universidad de Buenos Aires, Facultad de Odontología, Departamento de Histología y Embriología. 3. Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Laboratorio de Lípidos y Lipoproteínas. 4. Universidad de Buenos Aires, Facultad de Medicina, Departamento de Patología, Instituto de Fisiopatología Cardiovascular.

Adverse health effects of air particulate matter (PM) are associated to respiratory and cardiovascular diseases. PM doesn't affect equally to all people, being life stage a putative parameter that can influence PM-related health effects. Regarding particle size, ultrafine particles can be translocated from the lungs into the circulation and extrapulmonary organs among which the liver isincluded. Liver plays a central role in the metabolism and excretion of xenobiotics which makes it highly susceptible to their adverse and toxic effects. The aim of this study was to analyze in the liver of young and middle-aged mice the acute biological impact of Urban Air Particles from Buenos Aires (UAP-BA) in comparison with Residual Oil Fly Ash (ROFA), an ambient particle surrogate. Young (3 month old) and middle-aged (10 months old) BALB/c mice were exposed by intranasal instillation with UAP-BA or ROFA (1mg/kg BW). We evaluated at 3, 24 or 48h post-instillation liver morphology, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), two serum enzymatic biomarkers indicative of hepatocellular toxicity. In young mice, UAP-BA and ROFA induced serum AST activity augmentation only at 3h post-instillation when compared to controls (C:149.4±44.3 vs. UAP-BA$_3$:700.4±452.6, ROFA$_3$:365.2±112.8 U/L, p<0.05). ALT activity increased over time after UAP-BA exposure, while ROFA caused an increase only at the last time point evaluated (p<0.001). In middle-aged mice a significant (p<0.05) increase in serum activities for both enzymes was observed in UAP-BA and ROFA exposed mice only at 3h in comparison to controls. Accordingly, regardless the PM employed, liver histology showed morphological alterations like necrosis foci, moderate inflammatory infiltration and mixed steatohepatitis. In conclusion, we suggest that liver physiology in young and middle-aged inhabitants living in Latin American megacities, like Buenos Aires, may be modulated by urban air particle pollution.

529 (683) **LONG-TERM EFFECTS OF NEONATAL EXPOSURE TO ENVIRONMENTAL RELEVANT DOSE OF GLYPHOSATE-BASED HERBICIDE ON UTERINE DECIDUALIZATION**

Paola Inés Ingaramo[1], Jorgelina Varayoud[1], Marlise Guerrero Schimpf[1], María Mercedes Milesi[1], Mónica Muñoz de Toro[1], Enrique Hugo Luque[1]

1. Instituto De Salud Y Ambiente Del Litoral (ISAL), Fac. De Bioquímica Y Cs. Biológicas, UNL-CONICET .

In Argentina, glyphosate-based herbicides (GBH) are commonly used to control weeds. Although regulatory agencies have asserted that are relatively safe, reports from our laboratory suggest that GBH may have endocrine disrupting effects. The aim of present work was to evaluate the long-term effects of neonatal exposure to a GBH assessing parameters related to uterine decidualization in rats. Female Wistar pups received saline solution (control, C) or an environmental relevant dose of GBH (2 mg/kg) by sc injection on postnatal day (PND) 1, 3, 5 and 7. On PND90 female rats were mated with fertile non-exposed male. Pregnant rats were sacrificed on gestation day (GD) 19 and GD9 to evaluate molecular markers in the implantation sites (IS). Desmin immunostaining was used as a marker of decidualization. The newly formed blood vessels were detected by nestin immunostaining. The Wnt5a, Wn7a and β-catenin expressions were evaluated in IS for their key roles on decidualization. On GD19, GBH group showed a high incidence of fetal resorptions. On GD9, a significant decrease in the decidualized area (DA) (C: 1.55±0.32 mm2 vs GBH: 0.71±0.18 mm2, p<0.05) linked to a decreased caliber of newly formed vessels was found at the IS of the GBH group (C: 0.06±0.01 µm vs GBH: 0.04±0.01 µm, p<0.05). Moreover, Wnt5a expression decreased on antimesometrial zone of IS of GBH group (C: 7.12±0.66 vs GBH: 4.88±0.43, p<0.05) and Wnt7a expression was downregulated in luminal epithelium of mesometrial zone (C: 10.32±1.86 vs GBH: 3.46±1.57, p<0.05). No changes were observed in β-catenin expression between the groups. Long-term effect of neonatal exposure to GBH was evidenced by an increased incidence of abortion associated with altered decidualization process. A decreased DA associated with impaired blood supply in GBH exposed rats may be due to an altered Wnts activation pathway. These mechanisms may be responsible for the high incidence of abortion in GBH treated rats.

530 (473) **EXPOSURE TO LOW LEVELS OF FLUORIDE DURING PREGNANCY AND LACTATION IMPAIRS MEMORY AND AFFECTS CHOLINERGIC NEUROTRANSMISSION IN ADULT RATS**

Bartos Mariana[1], Gumilar Fernanda[1], Gallegos Cristina[1], Bras Cristina[1], Dominguez Sergio[1], Mónaco Nina[1], Bouzat Cecilia[2], Esandi María del Carmen[2], Cancela Liliana[3] and Minetti Alejandra[1]

1. Toxicology Laboratory. INBIOSUR-CONICET, UNS, Bahía Blanca, Buenos Aires. 2. Laboratory of Electrophysiology. INIBIBB-CONICET, Bahía Blanca, Buenos Aires. 3. Departament of Pharmacology. IFEC-CONICET, UNC, Córdoba.

It is known that exposure to high concentrations of Fluoride (F) produces deleterious health effects in human population. In the last years it has been concluded that low concentrations of F may have adverse health effects as well. Transplacental passage of F and its incorporation into fetal tissues has been demonstrated. Literature is poor on the effects of the exposure to low F doses

during pregnancy and lactation on the central nervous system. The purpose of the present study was to evaluate in adult female offspring rats exposed to low F concentrations during pregnancy and lactation, short-term memory (STM), long-term memory (LTM) and expression levels of neuronal a7 nicotinic receptors (nAChR) in hippocampus. Pregnant female rats were exposed to 10 mg/L F in drinking water during pregnancy and lactation. In the 90-day-old offspring, the short-term memory (STM) and long-term memory (LTM) were determined using step-down inhibitory avoidance task. Rats were trained in a step-down inhibitory avoidance paradigm during which stepping-down from a platform presented in a given context was associated with a footshock. Test sessions were carried out 1.5 h (STM) and 24 h (LTM) after training. In the test sessions, the increase in the step-down latency was used as measure of retention. Results show that the exposition to 10 mg/L F during pregnancy and lactation produced in the female offspring a significant impairment in the retention of STM and LTM compared to the control group. mRNA level of a7 nAChR in hippocampus, area involved in memory formation, was determinate by RT-qPCR. We found a significant decrease of a7 nAChR expression in the Fluoride exposed group. In conclusion, the early exposure to low levels of F impairs the retention ofmemory. Our results suggest that the decrease in the expression of a7 nAChR inhippocampus could be responsible of memory impairment.

531 (1042) **DIOXIN-LIKE ENVIRONMENTAL TOXIC ALTERS VASCULAR FUNCTION AND BLOOD PRESSURE IN RATS**

Susana Gorzalczany[1], Maria Ines Roson[2], Giselle Romero Caimi[3], Patricia Bonazola[2], Diana Kleiman[3], Rocio Castilla[2], Laura Alvarez[3]

1. Catedra De Farmacología, Facultad De Farmacia Y Bioquímica, UBA. 2. Instituto De Investigaciones Cardiológicas Prof. Dr. Alberto Taquíni (ININCA) UBA-CONICET. 3. Laboratorio De Efectos Biológicos De Contaminantes Ambientales, Dpto De Bioquímica Humana, Facultad De Medicina, UBA.

Hexachlorobenzene (HCB) is an organochlorine pesticide that induces endocrine dysfunction and alters blood pressure (BP) in female Wistar rats, as well as thyroid hormones (TH), deiodinase II (DII) and estrogen receptor alpha (ERα), factors involved in vascular tone and BP regulation. This study evaluated the effect of HCB on vascular hemodynamics in male Wistar rats to elucidate its mechanism of action in in vivo and ex vivo models. In vivo: rats were gavage-administered HCB (5 to 500 mg/kg bw) every 3 days for 45 days. Ex vivo: aorta rings of control rats were treated with HCB (0.05 and 5μM) for 30 min. The in vivo protocol assessed BP and biochemical markers. Both protocols evaluated histological, biochemical and physiological parameters. In vivo: HCB (500 mg/kg) increased BP (25%, $p<0.05$) and aortic wall thickness (27%, $p<0.05$), and decreased cell number per area (18%, $p<0.05$). The toxic also decreased serum T_4 (28%, $p<0.05$), aortic DII mRNA (41%, $p<0.001$), ERα and eNOS expression (29 and 26%, respectively, $p<0.05$), and increased aorta TGF-b1 mRNA and ATI receptor levels (39 and 31%, respectively, $p<0.01$). HCB-treated groups showed decreased acetylcholine (Ach) aortic relaxation (91.2±3.9, 71.5±3.9, 73.9±3.6, for control, HCB 5mg/kg, HCB 500mg/kg respectively, $p<0.01$), without changes in phenylephrine (F) contraction or nitroprusside (N) relaxation. In vitro: Aortic rings treated with HCB showed a decrease in the maximum contraction to F (in mg 3705±167, 2367±149, 2650±155 for control, HCB 0.05μM, HCB 5μM, respectively, $p<0.01$), without alterations in relaxation to N. Relaxation to Ach tended to decrease with both HCB doses. HCB generates hypertension and alters endothelium-dependent relaxation in male rats, without changes in arterial contraction. DII, ERα and eNOS could be in part involved in the decrease in relaxation, while TGF-b1 and AT1 receptor may be involved in vascular hypertrophy. These findings may explain the increase in BP in HCB-treated rats.

532 (495) **NEONATAL EXPOSURE OF EWE LAMBS TO A GLYPHOSATE-BASED HERBICIDE ALTERS THE EXPRESSION OF PROTEINS INVOLVED IN UTERINE DEVELOPMENT AND DIFFERENTIATION**

Ramiro Alarcón[1], María Mercedes Milesi[1], Oscar Rivera[2], Gisela Dioguardi[2], Norberto Belmonte[2], Mónica Muñoz-de-Toro[1], Enrique Hugo Luque[1]

1. Instituto De Salud Y Ambiente Del Litoral (ISAL), Facultad De Bioquímica Y Ciencias Biológicas, CONICET-Universidad Nacional Del Litoral. 2. Instituto De Investigación Agropecuaria, Ambiente Y Salud (IIPAAS), Facultad De Ciencias Agrarias, Universidad Nacional De Lomas De Zamora.

Glyphosate-based herbicides (GBH) are one of the most employed agrochemicals around the world. Recently, we showed that, in the rat, developmental exposure to a GBH alters uterine organogenetic differentiation showing hyperplasia in prepubertal samples and inducing post-implantation embryo loss. No reports are available regarding GBH effects on the reproductive system of the ewe lamb. This study investigates the effects of a brief postnatal exposure to a GBH on the morphology and differentiation of the prepubertal sheep uterus. Ewe lambs (Frizone breed) were sc injected from postnatal day 1 (PND1) to PND14 with saline solution (vehicle) or 2 mg/Kg/day of a GBH. On PND45 ewe lambs were hysterectomized and uterine samples were paraffin-embedded. In trichrome stained sections morphological parameters were evaluated: luminal epithelial height, glandular density, thickness of the subepithelial stroma and myometrium. Immunohistochemistry was performed to quantify the uterine expression of Ki-67 (cellular proliferation marker) and proteins involved in uterine development and differentiation: estrogen receptor α (ERα), progesterone receptor (PR), Wnt7a and β-catenin. GBH treatment did not alter uterine morphology, but reduced the proliferation rate in the subepithelial stroma, and in the luminal and glandular epithelium. The decrease in cell proliferation was associated with a downregulation of ERα expression in the same compartments. PR expression was increased in the glandular epithelium and in the subepithelial stroma, and downregulated in the luminal epithelium. GBH group also showed a decrease in Wnt7a expression in the subepithelial stroma and a downregulation in β-catenin expression in the luminal and glandular epithelium. Exposure to a low dose of a GBH during early postnatal development decreased uterine proliferation and disrupted the expression of morphoregulatory proteins in prepubertal sheep. All these alterations might impair female fertility at adulthood.

533 (734) **MORPHOLOGICAL AND MOLECULAR ANALYSIS OF MYOMETRIAL DIFFERENTIATION DURING EARLY PREGNANCY IN RATS NEONATALLY EXPOSED TO ENDOSULFAN**

Ramiro Alarcón[1], Jorgelina Varayoud[1], Mónica Muñoz-de-Toro[1], Enrique Hugo Luque[1], María Mercedes Milesi[1]

1. Instituto De Salud Y Ambiente Del Litoral (ISAL), Facultad De Bioquímica Y Ciencias Biológicas, CONICET-Universidad Nacional Del Litoral.

During early pregnancy, the myometrium undergoes a notable increase in proliferation, which is critical for implantation. Alterations of myometrial morphogenesis during development may lead to reproductive anomalies at adulthood. In previous studies we found that neonatal exposure to the pesticide endosulfan alters the expression of the morphoregulatory genes Hoxa10 and Wnt7a in the myometrium of prepubertal rats, and induces implantation failures at adulthood. This study investigates the long-term effects of neonatal exposure to endosulfan on myometrium differentiation during the peri-implantation period (gestational day 5, GD5). Newborn female rats were treated by s.c. injections every 48 h from postnatal day 1 (PND1) to PND7 with corn oil (vehicle), diethylstilbestrol 0.2 μg/Kg/day (DES, endocrine disruptor control) and endosulfan 600 μg/Kg/day (Endo600). On PND90, females were mated and on GD5 the uteri were obtained, fixed and paraffin-embedded. The thickness of circular (cM) and longitudinal (lM) myometrium, as well as, the relative area occupied by blood vessels were evaluated in hematoxylin-eosin stained sections. Protein expression of Ki-67

(as a cell proliferation marker), Wnt7a, and Hoxa10 were evaluated in the myometrium by immunohistochemistry. DES treatment increased the thickness of the cM and the relative area occupied by blood vessels, and enlarged intercellular spaces, suggesting the presence of edema. Contrarily, Endo600 group showed a decrease in the thickness of cM and IM, in association with lower cell proliferation. The treatment with endosulfan decreased the expression of Hoxa10 in the IM, and downregulated Wnt7a in both myometrial layers. Endosulfan-induced Hoxa10 and Wnt7a deregulation in the myometrium might be responsible for the lower proliferation rate, which might be in turn the cause of lower myometrial thickness. These morphological and molecular changes could promote the endosulfan-induced implantation failures.

534 (526) **POSTNATAL EXPOSURE TO ENDOSULFAN INTERFERES WITH THE NORMAL DEVELOPMENT OF THE MALE RAT MAMMARY GLAND.**

Gabriela A Altamirano[1, 2], Melisa Delconte[1], Ayelén L Gomez[1], Eduardo Masat[2], Ramiro Alarcón[1], Verónica L Bosquiazzo[1], Enrique H Luque[1], Mónica Muñoz-de-Toro[1, 2], Laura Kass[1, 2]

1. Instituto De Salud Y Ambiente Del Litoral, (ISAL, UNL-CONICET), Facultad De Bioquímica Y Cs. Biológicas, Universidad Nacional Del Litoral. 2. Cátedra De Patología Humana, Facultad De Bioquímica Y Cs. Biológicas, Universidad Nacional Del Litoral

Endosulfan is one of the organochlorine pesticides that can act as an estrogen agonist and, it has been shown that stimulates the proliferation of the human mammary tumor cell line MCF-7 in vitro. The male rat mammary gland (MG) is sensitive to endocrine disruptors and, in the present study, our aim was to evaluate whether postnatal exposure to endosulfan modifies MG development in pre and postpubertal male rats. From postnatal day 1 (PND1) toPND7, male animals were injected subcutaneously every 48 h with: a) corn oil vehicle, control) or b) 600 µg/kg body weight of endosulfan. On PND21 and PND60, blood and MG samples were collected. MG were processed for whole mount (WM) or paraffin embedded. Testosterone (T) serum levels, MG histo-morphology, collagen fibers organization, proliferation index, and the expression of estrogen (ESR1) and androgen receptor (AR) were evaluated. On PND21, MG area, perimeter, longitudinal growth and number of terminal end buds (TEBs) were higher in endosulfan-exposed than in control rats. Changes in histological sections such as alveolar development and increased presence of organized collagen in the stroma parallel those observed in the WM. Also, epithelial ESR1 expression was higher in endosulfan-exposed (42.7±1.7%) than in control rats (33.2±1.7%, $p<0.05$). On PND60, an increased longitudinal growth and number of terminal structures were correlated with a higher proliferation index in endosulfan-exposed (6.8±0.7%) than in control animals (4.9±0.3%; $p<0.05$). More developed lobuloalveolar structures in addition to hyperplastic ducts surrounded by dense stroma, rich in collagen fibers, were observed in endosulfan-treated rats. On the other hand, T levels and AR expression were similar between groups. In conclusion, our results show that the exposure to endosulfan in the first week of life interferes with the normal development of the MG and induces pre-malignant lesions in postpubertal male rats.

535 (735) **THE PESTICIDE CHLORPYRIFOS INDUCES ESTROGEN RECEPTOR ALPHA-DEPENDENT AND -INDEPENDENT CELL SIGNALING PATHWAYS IN THE BREAST CANCER CELL LINE MCF-7.**

Clara Ventura[1,3], María Rosa Ramos Nieto[1], Noelia Miret[2], Andrea Randi[2], Mariel Núñez[1], Claudia Cocca[1,4]

1. Laboratorio De Radioisótopos. Facultad De Farmacia Y Bioquímica. UBA. 2. Laboratorio De Efectos Biológicos De Contaminantes Ambientales. FMED. UBA. 3. IMBICE. UNLP-CIC-CONICET [4] IQUIFIB. UBA-CONICET

Chlorpyrifos (CPF) is one of the most widely used pesticides worldwide. We demonstrated that relevant environmental concentrations of CPF induce MCF-7 breast cancer cell proliferation via estrogen receptor alpha (ERα) phosphorylation. However, there are not evidences indicating if CPF activates another cell signaling pathways that lead to ERα-phosphorylation. Objective: Our aim was to study the effect of CPF on cell signaling related to ERα and the role on CPF-induced cell proliferation in MCF-7 cells. Methods: Cells were exposed to CPF (0.05 or 50 µM) for different periods. Protein phosphorylation was assayed by Western Blot using specific antibodies or by immunoprecipitation and blotting using anti-phosphotyrosine antibody. Cell proliferation was measured using mitotic index after 10 h of exposure. c-SRC and ERα participation was evaluated using specific inhibitors (1µM PP2 or 1 nM ICI 182,780, respectively). Results: We observed a slight increment of 418-tyrosine phosphorylation of c-SRC induced by 0.05 µM CPF after 1 h of exposure, however, cell proliferation induced by this concentration of the pesticide could not be reverted by PP2. We have also detected an increment of β-subunit Insulin-like growth factor 1-receptor (IGF-1Rβ) phosphorylation promoted by 0.05 µM CPF until 30 minutes of exposure ($p<0.05$). An increment of the IGF-1R-substrate IRS-1 expression was also detected after 24 h of exposure to 0.05 µM CPF ($p<0.05$). All these actions were abolished by ICI 182,780. Furthermore, we found an increment of 9-serine phosphorylation of GSK-3β induced by 0.05 µM CPF after 5 ($p<0,01$), 30 ($p<0,05$) and 60 ($p<0,05$) minutes of exposure. Interestingly, this action was not impeded by ICI 182,780. Conclusions: Our results indicate that 0.05 µM CPF is able to activate different cell pathways as IGF-IR signaling, which could be associated to the estrogenic action of the pesticide, and the GSK-3β signaling which would not be ERα-related.

536 (789) **CADMIUM EXPOSITION DURING GESTATION (20G) AND LACTANCY (PND15) ALTERS ANTIOXIDANT DEFENSE. STUDY OF EFFECT OF SOY PROTEIN AS A PROTEIN SOURCE.**

Veronica Biaggio [1], Karina Altamirano [2], Silvana Piguillem [2], Maria Veronica Perez Chaca [2], Maria Cecilia Della Vedova[1], Maria Sofia Gimenez [1]

1. Instituto multidisciplinario de investigaciones biológicas (IMIBIO)-CONICET-San Luis, 2. Universidad Nacional de San Luis - Área de Morfología.

Introduction: Exposure to toxic metals during pregnancy is one factor that alters the fetal environment and consequently increases the risk of metabolic disorders in adulthood. Cadmium (Cd) accumulates in the placenta inducing low birth weight and oxidative stress. However, little is known aboutthe effect of soy protein during pregnancy and lactation. Our objective was to evaluate the possible protective role of soy protein consumption versus Cd mechanisms by which exerts its toxicity. Methods: we worked with 4 lots of female Wistar rats; 2 lots received casein and two lots soy, as a protein source. 1 lot in each group received tap water and the other 15ppm of Cd indrinking water during pregnancy (20G) and 15 days after birth (PND15). We determined Cd concentration and TBARS, nitrites, carbonyls, proteins and total antioxidant capacity (TAC) levels. Brain tissue total RNA was extracted and RT-PCR was performed using the following primers: MT I; MT II; MT III; Nrf-2; NOX-2, SOD and GPx-1. Sections of brain tissue were performed to histology study. Results: at 20G and PND15 Cd concentration increased in both intoxicated groups ($p<0.001$). Carbonyls, proteins and TAC values, did not show significant difference in all groups studied. In Soy-Cd group MDA and nitrites levels and the expression of Nrf-2; SOD, MT I and MTIII increased ($p <0.05$, $p <0.001$, $p <0.05$, $p <0.05$, $p <0.01$ and $p <0.001$). While expression of mRNA NOX-2 and MT II decreased ($p <0.01$ and $p <0.001$). Morphological studies reveal the brain cortex area with little differentiation. Conclusion: it is known, that the presence of cadmium in the tissues induces stress oxidativo. Maternal exposure to Cd in the drinking water during gestation and 15 days after birth with soy as protein source in the diet, results into significant changes in the activities of antioxidant enzymes in brain.

537 (921) **BUENOS AIRES PARTICULATE MATTER ACTIVATES MAPK AND INCREASES MUC1 ON HUMAN CONJUNCTIVA**

Julia Tau[1], Agustina Tesone[1], Romina Lasagni Vitar[2], Alejandro Berra[1].
1. Laboratorio De Investigaciones Oculares, Departamento De Patología, Facultad De Medicina, UBA, Buenos Aires, Argentina. 2. Instituto De Bioquímica Y Medicina Molecular (IBIMOL), Universidad De Buenos Aires (UBA)- CONICET. Química General E Inorgánica, Facultad De Farmacia Y Bioquímica, Buenos Aires, Argentina.

Our group has previously demonstratedthat subjects from São Paulo, Brasil, exposed to high levels of urbanair pollution reported more ocular discomfort symptoms and presented greatertear film instability compeared to subjects exposed to lower levels. Usually,ophthalmologists do not regard air pollution as a possible cause of the aforementioned alterations. Also, we have demonstrated that Buenos AiresParticulate Matter (PM-BA), at 100 µg/mL, significantly decreased the proliferationand secretion of IL-8, but increased the release of IL-6 on human conjunctival epithelial cells (IOBA-NHC). Mitogen Activated Protein Kinases (MAPK) is a pathway that can trigger the secretion of the pro-inflammatory cytokine IL-6. Besides the immune response, mucins contribute to the protection of the ocular surface against allergens, pathogens, extracellular molecules, abrasive stress and drying. The aim of thepresent work was to evaluate the activation of the Extracellular activated kinase (Erk) and the c-Jun N-terminal Kinase (JNK); and the expression of the cell surface-associated mucins 1 (MUC1) on IOBA-NHC incubated with PM-BA. The PM-BA was collected on PTFE filters by a Gent Stacked Filter Unit Sampler. IOBA-NHC cells were incubated with 100µg/mL of PM-BA in growth media for24Hs. Erk, JNK and MUC1 expression was evaluated by western blot. PM-BA significantly induced activation of Erk and JNK pathways and increased the expression of MUC1 on IOBA-NHC cells ($p<0.05$). These findings suggest that human conjunctival epithelial cells incubated with PM-BA for 24Hs activate Erk and JNK pathways, which may trigger an immune response mediated by IL-6. In addition, the over expression of MUC1 may be an adaptive response to enhance the clearance of the particles in order to protect the ocular surface against the abrasive stress.

803 (1013) **CHRONIC INTOXICATION WITH CADMIUM INDUCES HYPERTENSION TOGETHER WITH OXIDATIVE STRESS AND APOPTOSIS IN RAT AORTA**

Silvina Monica Alvarez[1], Gabriel Giezi Boldrini[1], Glenda Daniela Martin Molinero[1], Silvana Piguillem[2], Nidia Noemi Gomez[1,2], Maria Sofia Gimenez[1],
[1]Laboratorio de Nutricion y Medio Ambiente, IMIBIO-CONICET, UNSL. San Luis. [2]Laboratorio de Morfologia, UNSL. San Luis

Cadmium (Cd) is a toxic metal and an important environmentalcontaminant. We studied its effects on blood pressure, histoarchitecture,oxidative stress and apoptosis markers of rat aorta. Male Wistar rats wereused: 1 group received regular water (control-Co) and the other, 15 ppm of Cdin the drinking water for 60 days (Cd). During the treatment, blood pressurewas measured with a CODA system. Total RNA was isolated with Trizol and cDNAwas obtained. Nrf2 factor, NOX2, p47, GPx, SOD, NFKB, p53, Transforming growth factor beta (TGF-β), VCAM, BAX, Bcl -2 and FAS ligand were determined by PCR. BAX/Bcl-2 ratio was calculated. S28 was used as control. Aortas were fixed, sectioned, stained, and examined for evidence of injury. Cd induced significant increase in systolic and diastolic blood pressure ($p<0.05$). NOX2 showed a significant increase ($p<0.001$) in the Cd group even though p47 did not show differences. Nrf2 showed a significant decrease in Cd ($p<0.05$) while GPx did not show differences and SOD significantly increased in the Cd group ($p<0.05$). NFKB and TGF-β expression did not change while VCAM, p53, FAS ligand and BAX/Bcl-2 ratio showed a significant increase in Cd group ($p<0.05$ in both). Regarding the morphology, irregular luminal layers of endothelial cell linings were observed in aortas of Cd-treated animal. In this group, light microscopy revealed structural changes in tunica intima cells, exhibiting clearer and bigger cytoplasms than cells from Co aortas. Cells of the tunica media in close contact with the intima also showed these alterations. This shows that Cd induces hypertension, oxidative stress, changes in the expression of apoptosis markers, together with architectural changes in the aorta.

538 (1045) **THE ROLE OF STATINS AS POTENTIAL TOOLS FOR HEPATOCARCINOGENESIS THERAPY**

Ezequiel Ridruejo[1], Giselle Romero Caimi[2], Maria Jesus Obregon[3], Noelia Miret[2], Diana Kleiman[2], Laura Alvarez[2]
1. Hepatology Section, Department Of Medicine. Centro De Educación Médica E Investigaciones Clínicas "Norberto Quirno" (CEMIC). Ciudad Autónoma De Buenos Aires. Argentina. 2. Laboratory Of Biological Effects Of Environmental Pollutants, Department Of Human Biochemistry, Faculty Of Medicine, University Of Buenos Aires. Ciudad Autónoma De Buenos Aires. Argentina. 3. Department Of Molecular Physiopathology, Instituto De Investigaciones Biomedicas (Centro Mixto CSIC-UAM). Madrid, Spain.

Hepatocellular carcinoma (HCC) represents 90% of liver tumors. Statins, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (HMG-CoAR), have been used in the treatment of different tumors. Anti-tumoral activity may be mediated by transforming growth factor-β1 (TGF-β1), epidermalgrowth factor (EGF) and thyroid hormones (TH) regulation. The aim of our study is to determine the molecular mechanism of action of statins, involved in the prevention of HCC. We used Hep-G2 cells and an initiation-promotion model in rats, diethylnitrosamine (DEN), 100 mg/kg bw, and hexachlorobenzene (HCB, 100 mg/kg bw) to develop preneoplasic liver foci. In vivo we evaluated: 1- proliferating cell nuclear antigen (PCNA) levels in liver focal and non focal areas, (IH); 2- HMG-CoAR and TGF-β1 mRNA content (RT-PCR) and cholesterol serum levels; 3- total and phosphorylated Src levels, (WB); 4- TH and deiodinase I (DI) and III mRNA levels. In vitro we evaluated the dose dependent effects of atorvastatin (AT) and simvastatin (SM) on: 1- PCNA and total and phosphorylated Src (WB); 2-TGF-β1 and HMG-CoA levels (RT-PCR). Results: in focal areas we have shown an increase in PCNA (60%, $p \leq 0{,}001$), HMG-CoAR (31%, $p \leq 0{,}01$), cholesterol (28%, $p \leq 0{,}05$), TGF-β1 (35%, $p \leq 0{,}01$), and phosphorylated Src (39%, $p \leq 0{,}01$) levels. DI levels were reduced 41% ($p \leq 0{,}01$) and DIII increased 30% ($p \leq 0{,}01$). Tisular T4 increased 38% ($p \leq 0{,}01$) and tisular T3 was reduced 37% ($p \leq 0{,}01$). In vitro, the inducing effect of HCB (5uM) on:HMG-CoAR mRNA levels was reduced 29% and 38% with AT (20 y 30 mM), and 20% and 31% with SM (10 y 20 µM). HCB effect on increased PCNA, TGF-β1, and phosphorylated Src levels, and decreased DI were antagonized by maximun doses of AT (52, 32, 41, 42%) and SM (58, 36, 40,39%), respectively of its maximum effect. Pre-treatment with TGF- β1 inhibitor (SB431542, 10 uM) prevented HCB effect on above mentioned parameters. Conclusion: statins (AT and SM) revert HCB induced proliferative effects on Hep-G2 cells. TGF-β1, c-Src and TH may be the statins molecular targets in HCC treatment.

539 (2002) **TOXICOLOGICAL SAFETY EVALUATION OF ACM T1H BY INTRAVENOUSLY ROUTE IN CENP: BEAG DOGS.**

Yana Gonzalez Torres[1], Axel Mancebo Rodríguez[1], Eric Acosta Lago[1], Iliana Sosa Testé[1], Avelina León Goñi[1], Diuris Blanco Gámez[1], Consuelo Gonzalez Triana[1], Ailemys Curbelo Valiente[1], Dasha Fuentes Morales[1], Angel Raymundo Casacó Parada[6].
1. CENPALAB. Centro Nacional Para La Producción De Animales De Laboratorio 2. Laboratorio De Electrofisiología. Centro Nacional De Investigaciones Científicas. 3. Centro De Investigaciones Clínicas. Centro Nacional De Investigaciones Científicas. 4. Centro De Investigaciones Médico Quirúrgicas. CIMEQ 5. Hospital Manuel Fajardo 6. Centro De Inmunología Molecular. CIM

The humanized AcM T1h is an antibody produced by the Center of Molecular Immunology. This product is proposed for the treatment of Rheumatoid Arthritis. The objective of this study was

to evaluate the toxicological safety of the repeated endovenous administration (12 weeks) of AcM T1h to Cenp:BEAG dogs. We establish two experimental groups Control and Treated (4X), of six animalseach, 3 per sex. Measurements included clinical observations, body weight, rectal temperature, clinical signs (cardiac and respiratory frequencies, arterial pressure and pulse), electrocardiogram, ophthalmological evaluations, electrophysiological evaluations, neurological exams, and hematological and clinical chemistry. The study ended with 100% survival. The results showed thatbody weight, body weight gains and rectal temperature were not affected by the AcM T1h. There were found significant differences between groups in cardiac and respiratory frequencies, but apparently not related with the test substance administration, since all values were found to be within those reported as normal for dogs. Hematological and serum chemistry was unaffected by the testsubstance. The results obtained showed that the AcM T1h is safe in the used biomodel.

PRESENTACION DE POSTERS SAI III / SAI POSTERS PRESENTATION III
Regulación de la respuesta immune / Regulation of the immune response

540 (744) **AMNIOTIC FLUID CONTAINS FETAL IMMATURE B-LYMPHOCYTES CAPABLE TO GIVE RISE TO FULLY COMPETENT B CELLS.**

Natalin Valeff[2], Imke Bommer[1], Lorena Juriol[2], Damián Muzzio[1], Marek Zygmunt[1], Federico Jensen[1,2,3].
1. Research Laboratory, Department of Obstetrics and Gynecology, University of Greifswald, Greifswald, Germany. 2. Laboratory for Immunology of Pregnancy, Center for Pharmacological and Botanical Studies (CEFYBO-CONICET-UBA), Buenos Aires, Argentina. 3. Institute of Health Sciences, National University Arturo Jauretche, Buenos Aires, Argentina.

Albeit diverse components of the innate immunity have been found in amniotic fluid (AF), the presence of B-cells was not investigated so far. B-lymphocytes are classified in B1 and B2 B cells. While B2 B cells are continuously generated through the post-natal life, B1 B cells are mainly originated during embryonic life from precursors ($CD19^+B220^-$) present in the yolk sac and fetal liver. The aim of this work was to make a full characterization of the B-cells found in the AF.C57BL/6 ($H2^b$) females were mated with BALB/c ($H2^d$) males and sacrificed at day 14 of pregnancy. B cells were magnetically isolated from AF. $CD19^+$ isolated B cells were further stained with specific antibodies against B220, CD93, IgM and $H2^d$ for phenotypic characterization using flow cytometry. To analyze the maturation capacity, activation and cytokines/antibodies production, AF isolated $CD19^+$ B cells were co-cultured with a bone marrow stromal cell line in the presence of IL-7 and Flt-3 for 10 days and the phenotype was analyzed again. Additionally, some cells were further stimulated for an extra 24 h with LPS. PMA/ionomycin were added during the last 5 h of culture. Levels of cytokines and immunoglobulin were measured in the supernatants. Phenotypic analysis of AF isolated $CD19^+$ cells permitted the identification of two populations of fetal B cells: an immature population ($CD19^+B220^-IgM^-$, ≈35%) and a more mature population ($CD19^+B220^+IgM^+$, ≈45%). Importantly, all $CD19^+$ B cells expressed $H2^d$ denoting fetal origin. After 10 days in culture the majority of $CD19^+$ B cells co-expressed B220 and IgM while down-regulated the immature marker CD93. Besides, stimulated AF B cells produced TNFα and IL-10. Interestingly, both, LPS stimulated and non-stimulated B cells, spontaneously produced IgM. We demonstrated here the existence of fetal immature B cells in the AF that are fully competent to continue their development and produce antibodies spontaneously as well as cytokines upon activation.

541 (851) **EXTRACTS OF AN ARGENTINIAN PLANT USED IN ANTIRHEUMATIC FOLK MEDICINE INHIBITED NFAT NUCLEAR TRANSLOCATION IN MURINE MACROPHAGES, ACTING AT OESTROGEN RECEPTORS ALPHA.**

Franco Mangone[1], Luciana Salaverry[1,2], Agustina Sotelo[1,2], Ailén Díaz[1,2], Ana Rugna[2], Teresa Gentile[1,2], Estela Rey-Roldán[1,2], Marisa Castro[1,2], Andrea Canellada[1,2].
1. CONICET- UBA, Instituto de Estudios de la Inmunidad Humoral Prof. Ricardo A Margni 2. Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Inmunología.

Smilax campestris Griseb is widely distributed in the north of Argentine and it is used as antirheumatic folk medicine. We previously investigated the effect of aqueous extracts of smilax (SM) on the osteoclast differentiation of murine macrophages. We found that SM diminished RANKL (RL)-induced osteoclast-like cells, cathepsin K mRNA expression and MMP activity, in a dose dependent manner, without affecting RAW 264.7 cell proliferation. RL-induced osteoclastogenesis involves the activation of NFAT signaling in macrophages. It has been described that plant extracts inhibit osteoclastogenesis acting at oestrogen receptors (ER). The aim of the current study was: 1) To determine whether SM inhibit the activation of NFAT in RAW cells; 2) To investigate whether the SM inhibition involved ER. We also analyzed the effect of SM on murine bone marrow derived osteoclast precursors (BMD OC). RAW cells were cultured 16 h with SM (10-100-1000 ng/ml) or estradiol (E2, 10-7; 10-8 M) and then cultured 1 h with phorbol myristate acetate plus calcium ionophore (PIo) to induce the nuclear traslocation of NFAT. To analyze ER involvement, cells were incubated 1 h with ERa or ERb antagonist (1 and 5 uM) prior to SM treatment. We measured NFAT translocation by western blot. BMD OC were obtained by incubating BMD mononuclear cells during 7 days with RL plus MCSF in the presence or not of SM. MMPs secretion to culture supernatants was assessed by zymography. Treatment of RAW cells with SM or E2 diminished the NFAT translocation in a dose dependent manner. Inhibition of 90.1±4.5% ($p<0.001$) was found with SM 100 ng/ml. E2 (10-7 M)% inhibition was 55.0±15.9 ($p<0.05$). The inhibition by SM of NFAT translocation was diminished with 5 uM of Era antagonist ($p<0.05$), while antagonist of ERb had no effect. We found that SM inhibited the secretion of MMPs ($p<0.05$) by BMD OC. We conclude that inhibition of NFAT traslocation by SM acting at ERa could be involved in their effect on osteoclastogenesis.

542 (859) **HIGH FREQUENCY AND PHENOTYPE CHARACTERISTICS OF LIVER CD8+HLA-DR+ TREG CELLS: POTENTIAL ROLE OF IFN-G.**

Andres P Machicote[1], Santiago Belen[1], Ariel Billordo[1], Plácida Baz[1], Lourdes Arruvito[1], Ariel Podhorzer[1], Leonardo Fainboim[1].
1. Instituto de Inmunología, Genética y Metabolismo (INIGEM), Hospital de Clínicas "José de San Martín", Buenos Aires, Argentina.

We have shown that CD8+ T cells from both adult peripheral blood and umbilical cord blood mononuclear cells constitutively expressing HLA-DR, represent a natural human CD8+ regulatory T cell subset (CD8+DR+Tregs), acting through cell-cell contact and the participation of CTLA-4. The liver shows a high frequency of CD8+ cells, including those expressing HLA-DR. The aim was to investigate the difference in phenotypes of CD8+ cells from peripheral blood and liver, focusing on their role in the modulation of the hepatic immune response. The differential phenotype of CD8+HLA-DR+ and CD8+HLA-DR- in blood (n=16) and liver perfusion (n=13) was analyzed by flow cytometry with a wide panel of cell membrane markers and receptors for quemokines. IFN-ã secretion was measured in vitro after PMA/Ionomycin stimulation. In comparison with CD8+HLA-DR- cells, an increased phenotype of central memory (CD45RO+CD27+CD28+, $p<0.001$) and effector memory (CD45R0+CD27-CD28-,$p<0.01$) was detected in blood CD8+HLA-DR+. Conversely, hepatic CD8+HLA-DR+ and CD8+HLA-DR- showed similar memory phenotype patterns. Within both blood and liver ($p<0.001$ and $p<0.05$, respectively), CD8+HLA-DR+ cells contain lower number of naïve cells (CD45RA+CD27+CD28+) in comparison with their HLA-DR- counterpart. However, liver naïve CD8+HLA-DR+ cells showed a significant decrease in the

expression of the lymph node homing receptor CCR7 (p<0.05). In addition, CD8+HLA-DR+ cells showed a higher secretion of IFN-ã (p<0.01). Interestingly, the expression of CCR5 -a known receptor for IFN-ã inducible ligands- was increased in hepatic CD8+HLA-DR+ cells in comparison with CD8+HLA-DR- and CD4+ cells (p<0.05). The high frequency of CD8+HLA-DR+ cells in the liver appears to be associated with a selective recruitment to the liver, possibly across CCR5 and the loss of CCR7 by naïve CD8+HLA-DR+ cells. It remains to be elucidated how the high increase of IFN-ã secretion is associated with their modulatory capacity of the immune response.

543 (154) **IL-10 IS REQUIRED FOR CD8+ T CELL EXPANSION AND INDUCTION OF EFFECTOR FUNCTIONS DURING ACUTE TRYPANOSOMA CRUZI INFECTION.**

Cristian G. Miranda[1,2], Agustina M. Pino Martínez[1], Estela I. Batalla[1,2], Alicia Grijalva[1], Stella M. González Cappa[1,2], Catalina D. Alba Soto[1,2].

1. IMPAM (UBA-CONICET) 2. Departamento de Microbiología, Parasitología e Inmunología. Facultad de Medicina. Universidad de Buenos Aires.

CD8+ cytotoxic T cells have a central role against intracellular stages of the protozoan Trypanosoma cruzi. While studying the impact of IL-10 on the induction of effector mechanisms, we found that IL-10 deficient (IL-10KO) mice fail to increase the pool of splenic CD8+ T cells (p<0.05), a feature of acute T. cruzi infection. Here, we analyzed the participation of IL-10 on the expansion and functional activation of CD8+ T cells in response to acute infection. IL-10KO and wild type (WT) mice were infected with K98, a myotropic T. cruzi strain which produce chronic infection. At 21 days post-infection, splenic CD8+ T cells from IL-10KO mice exhibited reduced proliferative capacity than their uninfected counterparts (p<0.05) and increased apoptosis compared to infected WT mice (p<0.05). Infection also raised the percentage of CD8+ T cells expressing IL-2R á chain (CD25) and Fas (CD95) without differences between WT and IL-10KO mice. Regarding their functional phenotype, CD8+ T cells from infected IL-10KO mice displayed lower IFNã production and CD107a transient expression than their WT counterparts (p<0.05). Likewise, they drastically reduced IL-2 production compared to uninfected mice (p<0.01) as well as their T. cruzi-specific in vivo cytotoxicity (p<0.05). In addition, CD8+ T cells from IL-10KO infected mice expressed significantly higher levels of the inhibitory receptor PD-1 compared to WT infected mice (p<0.05). Taken these results together, the presence of IL-10 appears to be required for the expansion of effector CD8+ T cells committed to control T. cruzi infection. In absence of IL-10, CD8+ T cells display a phenotype suggestive of premature exhaustion according to their low proliferation, cytokine production, enhanced apoptosis and PD-1 expression. Further experiments will be conducted to confirm these findings that attribute a stimulatory function to a cytokine long-recognized for its regulatory properties.

544 (222) **DIFFERENTIAL EXPRESSION OF INHIBITORY RECEPTORS BY REGULATORY T CELLS FROM MICE WITH DIFFERENT SUSCEPTIBILITY TO AUTOIMMUNITY.**

Gloria Janet Godoy[1], Florencia Salazar[1], Rubén Dario Motrich[1], Virginia Elena Rivero[1].

1. CIBICI-CONICET. Facultad de Ciencias Químicas. Universidad Nacional de Córdoba. Argentina.

NOD mice are characterized for their high susceptibility to develop spontaneous and induced autoimmune diseases such as diabetes, thyroiditis, sialilitis and prostatitis. This increased susceptibility has been suggested to be related to defects in the number and/or the functionality of regulatory T cells (Tregs). Herein, we performed a comparative phenotypic analysis of Tregs from NOD, C57BL/6 and BALB /c mice. For that, using FACS, we purified spleen $CD4^+CD25^{hi}$ (Tregs) and $CD4^+CD25^-CD62L^{hi}$ (naïve) cells from the different mice strains under study and performed Tregs induction and activation assays following standard protocols. After performing Tregs activation assays, which consisted in the incubation of $CD4^+CD25^{hi}$cells with a-CD3 Abs and rIL-2 for 72 h, the expression of inhibitory receptors was analyzed. Lower cell frequencies expressing LAG-3, PDL-1 and PD-1, with also decreased mean fluorescence intensities (MFI) values, were found in Tregs from NOD mice when compared with C57BL/6 and BALB /c mice. Moreover, Tregs from C57BL/6, NOD and BALB/c mice showed high, médium and almost null TIM-3 expression, respectively. On the other hand, results from Tregs induction assays, which consisted in the incubation of $CD4^+CD25^-CD62L^{hi}$ cells with rTGFb and rIL-2 for 5 days, revealed lower cell frequencies expressing Foxp3, CD25, CTLA-4, LAG-3, LAP-1, CD39, PDL-1 and TIM-3, again with decreased MIF values, in Tregs from NOD mice when compared with C57BL/6 and BALB /c mice. These results indicate that Tregs from NOD mice have defects in both, their induction and activation abilities. These impairments to express inhibitory receptors could be related to defective functionality and, consequently, to the high susceptibility to develop autoimmune diseases observed in this strain.

545 (282) **MYELOID-DERIVED SUPPRESSOR CELL GENERATION IS REDUCED BY ALL-TRANS-RETINOIC ACID IN LIPOPOLYSACCHARIDE-IMMUNOSUPPRESSED MICE BY DECREASING PROLIFERATION OF HEMATOPOIETIC PRECURSOR CELLS.**

Daiana Martire-Greco[1], Nahuel Rodriguez-Rodrigues[1], Luis Castillo[1], María Belén Vecchione[2], Marcelo de Campos Nebel[1], Marlina Córdoba Moreno[1], Roberto Meiss[1], Martin Amadeo Isturiz[1], Verónica Inés Landoni[1], Gabriela Cristina Fernández[1].

1. Instituto de Medicina Experimental (IMEX-CONICET). Academia Nacional de Medicina. [2]Instituto de Investigaciones Biomédicas en Retrovirus y SIDA (U.B.A.-CONICET).

All-trans-retinoic acid (ATRA) is a derivative of vitamin A with anti-proliferative properties. Immunosuppression by lipopolysaccharide (LPS) inoculation stimulates myelopoiesis with an expansion of myeloid-derived suppressor cells (MDSC). Our aim was to investigate if ATRA was able to modulate MDSC generation by regulating myelopoiesis in murine hematopoietic organs: spleen (spl) and bone marrow (BM). LPS-induced immunosuppression was developed by subcutaneous inoculations of increasing doses of LPS (IS) and ATRA intraperitoneal (IS+A). We found that ATRA decreased the number of CD34+ precursor cells, increased in IS mice (number of CD34+cells ($x10^5$) in spl, Control:7,1±1,5; IS:20,1±6,2*; IS+A:8,1±1,2# and in BM Control:5,3±0,1; IS:14,3±2,7*; IS+A:6,1±0,5#,*vs. Control and #vs. IS p<0,05). We did not find any differences in apoptosis of cells precursor or in the differentiation to their mature counterparts. However, ATRA was able to reduce precursor proliferation, as assessed by a reduction in the area of colony forming units (CFU) generated from CD34+ cells (CFU area ($x10^2$) in spl Control:5,3±0,1; IS:25±3,1*; IS+A:3,7±0,2# and in BM Control:6,5±0,1; IS:15±0,5*; IS+A:3,1±0,2#,*vs. Control and #vs. IS p<0,05). Also, ATRA decreased incorporation of ^{3}H-thymidine (CPM ($x10^3$) in spl Control:2,8±0,1; IS:4,1±0,2*; IS+A:1,5±0,1# and BM Control:1,2±0,2; IS:2,2±0,1*; IS+A:1,4±0,2#,*vs. Control and #vs. IS p<0,05). Along with these findings, ATRA administration to IS mice decreased the number of immature MDSC in spl (number of MDSC ($x10^5$), Control:0,8±0,1; IS:4,2±0,5*; IS+A:1,6±0,3#,*vs. Control and #vs. IS p<0,05), with a restoration of T lymphocyte proliferation (CPM ($x10^3$),Control:2,5±0,2; IS:0,5±0,03*; IS+A:2,3±0,4#, *vs. Control and #vs. IS p<0,05). Our results indicate that ATRA is able to abolish LPS-induced myelopoiesis, affecting the proliferation of precursor cells and decreasing MDSC generation, having a direct impact on the improvement of immune competence.

546 (590) **ABNORMAL/DYSFUNCTIONAL DENDRITIC CELL GENERATION FROM BONE MARROW OF LIPOPOLYSACCHARIDE-IMMUNOSUPPRESSED MICE.**

Veronica Ines Landoni[1], Nahuel Rodriguez-Rodrigues[1], Paula Chiarella[1], Daiana Martire-Greco[1], Luis Castillo[1], Pablo Schierloh[1], Martin Amadeo Isturiz[1], Gabriela Cristina Fernandez[1].

1. Instituto de Medicina Experimental (IMEX)-CONICET-Academia Nacional de Medicina de Buenos Aires, Argentina.

An immunosuppressed state is elicited in some patient at late phases of sepsis, evidenced by a reduced T cell response and antibody production. A loss of dendritic cells (DC) in blood and lymph nodes (LN) has been also described in septic patients. Considering this, we hypothesized that alterations in the number/function of DC may contribute to the immunosuppression (IS) associated to sepsis. Repetitive, increasing doses of LPS in mice mimics this state of IS. The aim of this work was to evaluate the phenotype and functional state of DC from IS mice. We found that the number of DC (CD11c+) in LN of IS mice was lower compare to Control (Ctrol). Then we traced DC migration to LN by the FITC skin painting method. We found a reduced number of DC (CD11c+FITC+) mobilized to LN in IS mice ($p<0.005$). In order to exclude the influence of the IS context on the LN homing of DC, we performed migratory assays injecting Ctrol mice (s.c.) with DC differentiated in culture with GM-CSF from Ctrol and IS bone marrow (BM) progenitors, and loaded with OVA-FITC. The number of FITC+DC found in the draining LN was lower when IS DC were used for injection ($p<0.005$). To investigate the basis of the decreased number of DC mobilized to LN in IS, we evaluated the phenotype of DC generated after in vitro differentiation from Ctrol or IS BM precursors. We found a reduced number of DC originated from IS BM precursors ($p<0.05$). Moreover, an increased number of MHCII high DC was obtained from IS BM cells ($p<0.01$). Consistently with this more mature phenotype, DC from IS BM showed a reduced ability to increase MHCII expression when stimulated with LPS as a maturation stimulus ($p<0.005$). These results indicate an abnormal generation of DC from BM precursor from LPS-IS mice. The precocious generated mature phenotype of DC in IS may affect the ability to up-take antigens and, in consequence, to migrate to LN. This may contribute to the immune derangements observed in post-sepsis immunosuppression.

547 (1090) **EFFECT OF DIFFERENT T. CRUZI STAGES IN ANTIGEN PRESENTING CELLS IN VITRO AND IN VIVO.**

Brenda Celeste Gutiérrez[1], Estela Lamel[1], Marcel Ramirez[2], Stella Maris González Cappa[1], Carolina Poncini[1].
1. IMPaM, UBA-CONICET. [2]FioCruz, Rio de Janeiro, Brasil.

Previous studies demonstrated that trypomastigotes (Tp) purified from blood regulate the activation and functionality of bone-marrow derived (BM) dendritic cells (DCs). In addition, in vivo we described the tolerogenic properties of myeloid DCs in the experimental model of infection with a highly virulent strain inoculated intradermally (Poncini 2008 and 2015). Naturally-occurring, the infection occurs when metacyclic (m)Tp reach mucosa or the feeding wound. Here, we analysed the effect of blood (b)Tp or mTp in BMDCs or in DC-cell line XS106 (Mohan 2005) in vitro and the effect of both parasite stages in vivo. By flow cytometry, we observed that XS106 cell-line presented basal activation with intermediate to high expression of CD86, CD40 and MHCII markers that are not affected by Tp ($p<0,05$). In addition, basal production of IL-10 was detected in the presence or absence of Tp that is downregulated by LPS ($p<0,05$). Regarding cell infection, bTp were able to multiply inside both BMDCs and XS106. However, parasitecell circle was aberrant in XS106 DCs. On the contrary, mTp differentiated in vitro displayed low capacity to infect both BM and XS106 DCs. The intradermal infection with bTp and mTp showed slight differences in relation to cell recruitment into skin at 3 and 7 days post-injection. However, when mice were infected with mTp, no circulating parasites were detected during 50 days of screening. When animals inoculated or not (controls) with mTp were challenged with bTp, only mTp-injected showed low parasitemia and 100% of survival versus 100% of mortality in the other group. Analysis of splenic cell populationsdemonstrated enhanced activation in antigen presenting cells in mTp injected animals versus controls. These results suggest that bTp and mTp triggers different immune responses at the very beginning of the infection.

548 (151) **RECRUITMENT OF IMMUNOCOMPLEX-ACTIVATED NEUTROPHILS MODULATE T CELL RESPONSE IN LYMPH NODES.**

Sofía Daiana Castell[1], María Florencia Harman[1], Victor Gabriel Morón[1], Belkys Angélica Maletto[1], María Cristina Pistoresi[1].
1. CIBICI-CONICET- Facultad de Ciencias Químicas- UNC-Córdoba- Argentina.

Previously we demonstrated that immunocomplexes (IC) generated by injecting OVA in the footpad of immunized mice that have anti-OVA antibodies, induce neutrophils-OVA$^+$ migration to draining popliteal lymph nodes (D-poLNs). In the present study we evaluate the influence of IC-activated neutrophils on T-cell response in lymph nodes. C57BL/6 mice were immunized with OVA emulsified in Freund's complete adjuvant and 15 days later boosted with OVA emulsified in Freund's incomplete adjuvant. Ten days after last immunization they were injected with OVA-FITC in the footpad and D-poLNs were obtained at different times (between 3 and 48 h); saline solution was injected on footpad corresponding to control non-draining popliteal lymph nodes (ND-poLNs). The different cell populations in the lymph nodes were characterized by flow cytometry. Three hours after footpad injection, an increased number of OVA-FITC$^+$ Ly6G^{hi} neutrophils was observed in D-poLNs compared to ND-poLNs ($p<0.05$). The highest levels of OVA-FITC$^+$ neutrophils were detected 6 h after OVA injection ($p<0.001$), at 12 h the number of neutrophils decreased to basal levels. However, total number of D-poLN cells increase after 24 h of OVA injection ($p<0.01$). At24 h we observed an increase of dendritc cells (CD11c$^+$) in D-poLNs compared to ND-poLNs ($p<0.01$). At 48 h we observed an increase of CD4 T cells ($p<0.01$), particularly effector CD4 T-cells (CD44$^+$ CD62L$^-$) ($p<0.01$) with respect to ND-poLNs. Besides, we observed higher percentage of Ki67$^+$ CD4 T-cells in immunized mice 24 h after OVA injection ($p<0.001$), indicating that CD4 T-cells have higher levels of proliferation after recruitment of IC-activated neutrophils. These results suggest that neutrophils recruitment in draining popliteal lymph nodes could be modulating the T-cell response.

549 (337) **IMPACT OF THE RETICULAR STRESS AND UNFOLDED PROTEIN RESPONSE ON THE INFLAMMATORY RESPONSE DURING THE IMPLANTATION PERIOD.**

Elizabeth Soczewski[1], Esteban Grasso[1], Laura Fernández[1], Lucila Gallino[1], Claudia Pérez Leirós[1], Rosanna Ramhorst[1].
1. Immunopharmacology Laboratory, IQUIBICEN, University of Buenos Aires, CONICET, Argentina.

Embryo implantation in humans involves the generation of an inflammatory response associated with invasion of the blastocyst in the decidua. This inflammatory response is sterile and could be induced by endogenous ligands released during tissue remodeling; however, it is still unclear whether other processes are involved. At decidualization, cells undergo reticular stress (RS) and unfolded protein response (UPR), which will allow them to expand their endoplasmic reticulum with the corresponding machinery for protein folding. Here, we focus on the impact of RS and UPR on endometrial and decidual cells and whether they induce a physiological sterile inflammatory response through IL-1beta. We used an in vitro model of human decidualization represented by Human endometrial stromal cell line (HESC) treated or not with VIP 10-7M (vasoactive intestinal peptide known to induce decidualization) or with medroxiprogesterone (MPA 10-6M) and dbcAMP (2,5 10-3M) during 8 days. First, we evaluated the RS by the expression of the 3 sensors, ATF6, PERK and IRE1. Decidualized cells in the presence of VIP or MPA increased the expression of ATF6 and PERK while did not modulate IRE1. This effect was also observed in the presence of Thapsigargin (Tg 2ug/ml) for both sensors ($p<0.05$ Student t-test). We evaluated the modulation of TXNIP expression, a kinase/RNase downstream RS able to activate the inflammasome. TXNIP expression significantly increased in VIP and MPA-decidualized cells as in the presence of Tg, evaluated by qRT-PCR ($p<0.05$ Student t-test). Finally, this increase was asociated with IL-1beta increased intracellular production in cells

decidualized by VIP and MPA in comparison with non-decidualized cells. The present results suggest that human decidualization process is accompained by a physiological RS that might be associated with an increase of IL-1beta production generating a sterile inflammatory response.

550 (607) **MODULATOR EFFECTS OF THE ESSENTIAL OIL OF MINTHOSTACHYS VERTICILLATA ON MACROPHAGE FUNCTIONS.**

Ivana Dalila Montironi[1], Debora Decote-Ricardo[2], Isabel Ferreira La Roque de Freitas[2], Célio Geraldo Freire-de-Lima[3], Elina Beatriz Reinoso[1], Laura Noelia Cariddi[1].

1. Departamento de Microbiología e Inmunología, Universidad Nacional de Río Cuarto-Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Argentina. 2. Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, Rio de Janeiro, Brazil. 3. Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

Minthostachys verticillata is an Argentinean medicinal plant and its essential oil showed immunostimulatory activity on human T and B cells and anti-inflammatory properties. The aim of this study was to characterize the effect of essential oil on macrophage phagocytosis. RAW 264.7 line was used. Cells were exposed to different concentrations of essential oil (EO) (0, 0.5, 5, 50, 60 and 75 µg/ml) at different times in order to evaluate the effect on cell proliferation, adherence and phagocytosis. For assess adhesion and phagocytosis, a suspension of yeast Saccharomyces cerevisiae was added to each well in a 10:1 relation. Cells with only D-MEM and DMSO 0.1% in D-MEM complete were used as control. The results showed that EO treatment did not induce cell proliferation. A significant dose-dependent increase was observed in the number of macrophages that adhered yeast ($p < 0.001$) in the number of yeast adhering macrophages ($p < 0.001$) and in the number of yeast phagocyted ($p<0.05$) demonstrating a modulatory effect of EO on the mechanisms of adhesion and phagocytosis of these cells. Dosage of nitric oxide (NO) and reactive oxygen species (ROS) was also performed after cell treatment with EO, LPS and IFN-ã. No increase was observed in NO production compared to control cells treated 24 h with EO and then stimulated with LPS and IFN-ã or those treated with all three compounds simultaneously. However, it was lower than observed in treatments with LPS or IFN-ã alone ($p<0.001$) suggesting a possible anti-inflammatory effect of EO. In the meantime, ROS production was increased by the presence of EO in all treatments ($p<0.001$) showing that this oil activated microbicide mechanism in macrophages. These results demonstrate the immunomodulatory potential of M. verticillata essential oil which could be used as alternative, complementary and/or adjuvant therapy for the treatment of various diseases.

551 (666) **CROSS TALK BETWEEN TRAPPIN-2 AND IL-9: CLINICAL RELEVANCE IN ALLERGIC DISEASES.**

Fiorella Caro[1], Nella Ambrosi[1], Nancy Tateosian[2], Marcela Lanfranconi[3], Antonio Thwaites[4], Marcelo Picollo[5], Ximena Villalonga[1], Diego Guerrieri[1], Verónica García[2], Eduardo Chuluyan[1].

1. Centro de Estudios Farmacológicos y Botánicos (CEFYBO) UBA-CONICET, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina. 2. Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN) UBA-CONICET. Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales. Universidad de Buenos Aires. Buenos Aires, Argentina. 3. Laboratorio Central, área Inmunología. Hospital Muñiz 4. Servicio de Alergia e Inmunología. Hospital Muñiz. [5]Servicio de Alergia pediátrica. Hospital Muñiz.

Mucosal homeostasis is maintained by the secretion of several factors. Among them, IL-9 and Trappin-2 has been described to be produced in steady state and under inflammatory condition. The aim of the present study was to determine a putative link between IL-9 and Trappin-2 in allergic female patients. For this, a sandwich ELISA was performed for IL-9 and Trappin-2 in the plasma of 19 patients (15-49 years). First we found an indirect correlation between IL-9 and the age of the patients ($r = - 0.39$, $p = 0.008$). Furthermore, a direct correlation was observed between IL-9 and Trappin-2 in the plasma of the patients ($r = 0.43$, $p = 0.03$). In order to verify that IL-9 is able to stimulate Trappin-2, the alveolar epithelial cell line A549 was treated in vitro with different concentration of IL-9 (0.1-10 ng/ml). After 24 and 48 hours, A549 cell culture supernatant was harvested and Trappin-2 was measured in it. We observed that IL-9 (1 and 10 ng/ml) was able to increase Trappin-2 in a dose and time dependent manner ($p < 0.03$ and $p < 0.04$, respectively). Following, the effect of Trappin-2 on the production of IL-9 was assessed by treating peripheral blood mononuclear cells with 5 ng/ml of Trappin-2 for 24 and 48 hs of culture. Then, cells were recovered and IL-9 was measured by intracellular staining on CD4+, CD4-, CD14+ and CD19+ cells with a flow cytometer. The analysis showed that Trappin-2 increased the staining of IL-9 on CD4+, CD4- and CD19+ but not on CD14+ cells by at least 50%. Overall these results confirm a positive feedback between Trappin-2 and IL-9 and suggest that could be functional in female allergic patients.

552 (896) **EFFECT OF PANAX GINSENG EXTRACT ON STAPHYLOCOCCUS AUREUS INVASION INTO BOVINE MAMMARY EPITHELIAL CELLS AND CYTOKINES PRODUCTION.**

Camila Beccaria[1], Paula Silvestrini[1], Sofía Clara Sacco[1], Melisa Lovato[1], María Sol Renna[1], Luis Fernando Calvinho[2], Celina Baravalle[1], Bibiana Dallard[1].

1. Laboratorio de Biología Celular y Molecular Aplicada. Instituto de Ciencias Veterinarias del Litoral (ICIVET-Litoral) (UNL-CONICET). 2. EEA Rafaela, INTA.

Panax ginseng extract (PGe) has been widely used as an herbal remedy for various disorders in humans and animals. The aim of this study was to evaluate the effect of PGe on S. aureus invasion into bovine mammary epithelial cells (MAC-T) and quantify the inflammatory response. The MAC-T cells viability treated with different concentrations of PGe (0, 0.5, 1 and 3 mg/ml) was measured by XTT assay. Then, supernatants from MAC-T cultured in 24-well plates with or without different concentrations of PGe were employed for cytokines ELISA test. For S. aureus invasion assays, MAC-T were cultured with PGe (0, 0.5, 1 and 3 mg/ml) before infecting with a S. aureus strain isolated from bovine mastitis, with a multiplicity of infection (MOI) 100:1 bacteria/cell. After challenged with S. aureus for 2 h the MAC-T were washed and treated with gentamicin for 2 h to eliminate extracellular bacteria. Supernatants were then collected and cultured to verify killing by gentamicin. Data were expressed as $\log_{10}$ of colony forming units (CFU)/ml of internalized bacteria. No cytotoxic effects of PGe on MAC-T viability were observed. The highest concentration of PGe (3 mg/ml) induced an increase in the IL-4 production compared with other concentrations and control after 24 h of treatment ($p<0.01$). The IL-1β and IL-6 levels were below the lowest detectable test limit in all treatments at 24 h. According to CFU/ml recovered, PGe inhibited S. aureus invasion into MAC-T cells in a dose-dependent manner (0.5 to 3 mg/ml) ranging from 5,82 to 5,28 $\log_{10}$ CFU/ml respectively compared with control cells (6,08 $\log_{10}$) ($p<0.01$). Results demonstrated that PGe significantly inhibited S. aureus invasion into MAC-T cells and promoted IL-4 production (anti-inflammatory cytokine). In this way, PGe could modify the early S. aureus-host interaction in bovine mammary gland. The properties of PGe reported here may open new way for the development of novel prevention or treatment strategies for bovine mastitis.

553 (1087) **A PRELIMINAR ANALYSIS OF THE INFLUENCE OF RESISTIN ON IMMUNOLOGICAL CELLS IN NONALCOHOLIC FATTY LIVER DISEASE.**

Cecilia Claudia García[1], Nadia Soledad Alegre[1], Plácida Baz[1], Javier Benavides[2], Luis Colombato[2], Daniel Poncino[3], Daniel García[3], Juan Manuel Romeo[4], Beatriz Ameigeiras[4], Alejandra Claudia Cherñavsky[1].

1. Instituto de Inmunología, Genética y Metabolismo, Hospital de Clínicas "José de San Martín" 2. Sección Hepatología, Servicio de Gastroenterología, Hospital Británico de Buenos Aires 3. Sección Hepatología, Sanatorio Méndez ObSBA 4. Unidad de Gastroenterología, Hospital General de Agudos "JM Ramos Mejia".

Background: Resistin (RES) is a cytokine highly expressed in plasma of nonalcoholic fatty liver disease (NAFLD) patients which promotes insulin resistance. It has a pro-inflammatory role through simulation of liver/adipose infiltrating monocytes/macrophages. As these cells are the main source of RES, a pro-inflammatory loop is created due to its role as chemokine for T cells. RES binding to adenylyl cyclase-associated protein 1 (CAP1), which expression was only reported in monocytes, activates nuclear factor kappa B (NF-kB). NF-kB is also activated via TCR to modulate CD69 expression in immunological cells. Aims: to evaluate CAP1 expression in peripheral blood mononuclear cells (PBMC) from patients and controls (Co), and RES-mediated modulation of CD69. Methods: PBMC were obtained by density gradient from whole blood of NAFLD patients (n= 8) and Co (n= 10). All patients provided written informed consent. CAP1 expression was studied by flow cytometry (FC) in PBMC stained with anti-CD3, -CD4, -CD14 and -CD56 antibodies. For functional approach, PBMC from Co were stimulated with coated anti-CD3 (3mg/ml) +/- RES (500 ng/ml) for 24 h, stained with anti-CD3, -CD4, -CD8 and -CD56 and evaluated for CD69 expression by FC. Mann-Whitney test was used. Results: CAP1 expression is decreased in monocytes (p=0.018), CD3+CD4+ (p=0.016) and CD3+CD4- (p=0.018) cells in patients with NAFLD (vs. Co). RES alone did not activate PBMC. Costimulation with anti-CD3+RES decreased CD69 mean fluorescence intensity (MFI) in CD3+CD4+ and CD3+CD4-, natural killer T (NKT) and NK $CD56^{bright}$ cells (p=0.0043, p=0.021, p=0.044 and p=0.035 respectively; anti-CD3+RES vs. anti-CD3). Conclusion: CAP1 is a functional receptor in healthy donors able to regulate RES influence on immunological cells. Thus, an overall decrease in CAP1 expression might modulate the stimulus induced by RES in the context of NAFLD. This potential regulatory circuit deserves further investigation.

554 (690) **STUDY OF AUTOPHAGY RESPONSE IN NEOPLASTIC CLL CELLS.**

Daniela Soledad Arroyo[1,2], María Cecilia Rodriguez[1,2], Claudio Bussi[2], Javier María Peralta Ramos[2], Pablo Iribarren[2].
1. Laboratorio de Biología Molecular y Citometría de Flujo del servicio de Oncología y Hematología, del Hospital Nacional de Clínicas, Fac. de Medicina, UNC. 2. Dpto de Bioquímica Clínica, Fac. de Ciencias Químicas, UNC. CIBICI-CONICET.

Chronic Lymphocytic Leukemia (CLL) is a disease characterized by the clonal proliferation and accumulation of mature, typically CD5-positive B-cells within the blood, bone marrow, lymph nodes, and spleen. Leukemic transformation is initiated by alterations that impair apoptosis of clonal B-cells. The pathway engaged in programmed cell death involves several Bcl-2 family proteins. Alternatively, Bcl-2–family proteins have antiautophagic function. In this regard, the autophagy response has dual phenotype in cancer depending of the type of tumor cells. This mechanism can be used for prolonging survival, or for cell death, as well. However, little is known the role of this degradative process in CLL-B cells. In previously studies, we observed that Rapamycin (mTOR inhibitor) modulates B cell death induced by Fludarabin. We therefore, performed an analysis of LC3B expression to evaluate autophagy induction, in peripheral blood mononuclear cells (PBMC) isolated from CLL-patients. We stimulated PBMC with Rapamycin and observed an increased expression of LC3B II, which correlated with the level of cell death in a co-culture with Rapamycin and Fludarabin. Moreover, when we used another mTOR inhibitor (PP242) to stimulate PBMC, we observed higher levels of LC3B II compared to Rapamycin. These preliminary experiments are being assayed in others CLL patient samples and also we plan to evaluate cell death using PP242 plus Fludarabin. These preliminary results may provide important clues to define new strategies for leukemia therapy.

555 (571) **STUDY OF SPLENIC MYELOID-DERIVED SUPPRESSOR CELLS IN CPG-ODN+IFA-TREATED TUMOR BEARING MICE.**

María Florencia Harman[1], Sofía Daiana Castell[1], Belkys Angelica Maletto[1], Victor Gabriel Morón[1], María Cristina Pistoresi[1].
1. CIBICI-CONICET- Facultad de Ciencias Químicas- UNC-Córdoba- Argentina.

Alike the aging microenvironment, tumor can be viewed as a chronic inflammatory condition characterized by increased levels of pro-inflammatory cytokines as well as an overall immunosuppressive state. Previously we demonstrated that aged CpG-ODN+IFA treated mice present an expansion of myeloid derived suppressor cells (MDSC) capable of suppressing T-cell proliferative response. To characterize the effect of CpG-ODN+IFA in splenic MDSC in a tumor model, BALB/c young mice were injected with 4T1 mammary carcinoma into the mammary fat pads. CpG-ODN+IFA was injected (s.c) weekly, starting on day 7. We observed delayed tumor progression in CpG-ODN+IFA-treated tumor bearing mice ($p \leq 0.05$). After 24 days of tumor growth there were no significant differences in $CD11b^{+}Gr1^{+}$ MDSC levels, although a lower frequency of granulocytic MDSC ($CD11b^{+}Ly6G^{+}Ly6C^{low}$) was observed ($p \leq 0.05$) while monocytic MDSC ($CD11b^{+}Ly6G^{-}Ly6C^{high}$) levels were augmented compared to non-treated tumor bearing mice ($p \leq 0.05$). In line with this, we also found reduced levels of reactive oxygen species (ROS) in total MDSC ($p \leq 0.05$) and mainly in the granulocytic subset ($p \leq 0.01$) of CpG-ODN+IFA-treated tumor bearing mice, but no significant differences were observed in nitric oxide (NO) production. In conclusion, systemic treatment of 4T1 tumor bearing young mice with CpG-ODN+IFA leads to a change in the composition of splenic MDSC subsets and these changes could be playing a role in the tumor progression.

556 (346) **VPAC1 AND VPAC2 RECEPTOR KNOCK-DOWN PRIME MACROPHAGES AND NEUTROPHILS TO PRO-INFLAMMATORY RESPONSES DURING EARLY PREGNANCY.**

Guillermina Calo[1], Christina Van[2], Vanesa Hauk[1], M. Sue O'Dorisio[3], Rosanna Ramhorst[1], James Waschek[2], Claudia Pérez Leirós[1].
1. Immunopharmacology laboratory, School of Sciences, IQUIBICEN, UBA-CONICET. 2. Department of Psychiatry, David Geffen School of Medicine, University of California, Los Angeles, USA. 3. Department of Pediatrics and Holden Comprehensive Cancer Center, RJ and LA Carver College of Medicine, University of Iowa, Iowa City, USA.

The maintenance of immune homeostasis at the maternal-placental interface is a dynamic process that involves different populations of leucocytes controlled by trophoblast cells. Macrophages express a predominant M2 alternative activation profile during normal pregnancy whereas neutrophil activation is observed in normal pregnancies but it is higher in pregnancies complicated by preeclampsia. Vasoactive intestinal peptide (VIP) is produced by trophoblast cells and is proposed to contribute to immune homeostasis at early pregnancy. It displays anti-inflammatory effects through binding to high affinity VPAC1 and VPAC2 receptors on macrophages and neutrophils. Our goal was to explore whether macrophages and neutrophils from VPAC1 and VPAC2 KO pregnant mice fail to control pro-inflammatory responses at early pregnancy. Macrophages were isolated from the peritoneum of pregnant C57, either WT or VPAC1ko or VPAC2ko mice at day 7.5 or 8.5 of pregnancy and then surface markers or cytokine production was assessed. Neutrophils were isolated from bone marrow, using Ficoll gradient centrifugation and ROS production by PMA was analyzed. VPAC2ko mice present a higher expression of the co-stimulatory molecules MHCII and CD86 (MFI MHCII X±SE: WT:12068±2788; VPAC2ko:77550±10617 $P<0.05$; MFI CD86 X±SE: WT:2711±1027; VPAC2ko:13136±2184 $P<0.05$)

and VPAC1ko mice presents the same tendency of increase. Upon phagocytosis of apoptotic cells, macrophages inhibit IL-12 synthesis in WT, and a loss of this pattern was observed in VPAC1ko macrophages. PMA-induced ROS synthesis was inhibited by VIP in WT and VPAC2ko mice, but not in VPAC1ko ($P<0.05$). Altogether these results indicate that macrophages and neutrophils of VPAC1 and VPAC2 KO pregnant mice present a different functional phenotype compared to WT that would prime them to develop pro-inflammatory responses at early pregnancy.

557 (846) **CORRELATION BETWEEN T CELL EXHAUSTION AND PROGRESSION, ACTIVITY AND RESPONSE TO TREATMENT IN PATIENTS WITH RHEUMATOID ARTHRITIS.**

Luisina I. Onofrio[1], Estefanía R. Zacca[1], Paola Ferrero[1], Cristina Acosta[1], Sergio M. Alonso[1], M. Cecilia Ramello[2], Carolina Montes[2], Jimena Tosello Boari[2], Eduardo Mussano[1], Laura Onetti[1], Isaac Cadile[1], M. Victoria Gazzoni[1], Raúl Jurado[1], Adriana Gruppi[2], Eva Acosta Rodríguez[2].
1. Hospital Nacional de Clínicas (HNC), Universidad Nacional de Córdoba, Córdoba, Argentina. 2. Bioquímica Clínica, Facultad Ciencias Químicas. Universidad Nacional de Córdoba, Córdoba, Argentina.

Rheumatoid arthritis (RA) is a progressive inflammatory autoimmune disease with articular and systemic effects. RA pathophysiology involves B and T cells (Tc) and detrimental interactions of proinflammatory cytokines. Treatment (Tx) includes antirheumatic drugs and biologic and synthetic agents. T cell exhaustion (Tex), a dysfunctional state characterized by loss of effector functions and expression of inhibitory receptors (IRs), is harmful during infections and cancer. As its role remains unexplored in autoimmunity, we aimed to study Tex during RA and its relationship to disease progression and activity and response to Tx. Fourteen healthy donors (HD) and 35 RA patients (RApt) (15 untreated and 20 with different Tx) were recruited in the Rheumatology Service (Hospital Nacional de Clínicas) and evaluated at different times post-Tx. Phenotype and function of different immune subpopulations from peripheral blood were evaluated by flow cytometry. Although the expression of the IRs CD160 and BTLA was barely modified by different Tx, there was a significant negative correlation ($p<0.05$) between the expression of CD160 on CD4 and CD8 Tc and the disease activity score (DAS28). Also, the% of CD8 Tc coexpressing the IRs BTLA, CD160, PD1 and TIM3 showed a negative correlation with DAS28 ($p <0.02$). Moreover, the increase in CD160 expression between months 0 and 3 post-Tx correlated to the response to Tx according to the EULAR criteria: being high, low and negative in RApt with good, moderate/low and poor responses, respectively. In vitro studies showed that the effector function of activated Tc from HD can be inhibited by CD160 and BTLA triggering. Whether the inhibitory pathway HVEM-CD160/BTLA is operative in RApt remains to be assessed. Altogether, our findings suggest that Tex correlates with RA disease activity and response to Tx and could be used as biomarker. Accordingly, IR triggering could emerge as a new RA Tx to inhibit exacerbated Tc effector function.

INMUNIDAD INNATA E INFLAMACIÓN / INNATE IMMUNE RESPONSE AND INFLAMMATION

558 (864) **CHARACTERIZATION OF URATE MONOSODIUM CRYSTALS EFFECT ON GAMMA/DELTA T CELLS.**

Nadia Yasmín Towstyka[1], Florencia Sabbione[1], Irene Keitelman[1], Jorge Geffner[2,3], Analía Trevani[1,3], Carolina Jancic[1,3].
1. Instituto de Medicina Experimental (IMEX) - CONICET - Academia Nacional de Medicina. 2. Instituto de Investigaciones Biomédicas en Retrovirus y SIDA (INBIRS) - CONICET - Universidad de Buenos Aires. 3. Departamento de Microbiología e inmunología. Universidad de Buenos Aires. Facultad de Medicina.

Uric acid is released from dying cells and functions as an adjuvant that promotes the generation of adaptive immune responses. The inflammatory effect of uric acid depends on its precipitation into monosodium urate crystals (MSUC). MSUC activate different intracellular pathways, i.e. Syk kinase and PI3K. Gamma/delta T cells act as sensors of cellular stress and infection. They recognize danger and pathogen-associated molecular patters, such as phosphoantigens. We have previously reported that MSUC activate gamma/delta T cells. Now, we aim to investigate whether MSUC modulate gamma/delta T cell activation triggered by phosphoantigens, and the mechanism involved in gamma/delta T cell activation by MSUC. Gamma/delta T cells were purified from human peripheral blood by using an anti-TCR gamma/delta MicroBead isolation kit. After purification, gamma/delta T cells were incubated or not with MSUC (200 ug/ml, 48 h). Then, we analyzed CD69 expression by flow cytometry, and IFN-gamma and TNF-alpha production by ELISA. To investigate the co-stimulatory effect of MSUC, we treated gamma/delta T cells with the phosphoantigen HMBPP (10 uM) and then we incubated cells with MSUC. Finally, to study the mechanism involved in gamma/delta T cell activation by MSUC, we treated cells with or without a protein kinase Syk inhibitor, GS-9973 (0.1 uM Gamma/delta T cells co-stimulated with MSUC and HMBPP produced higher levels of IFN-gamma and TNF-alpha compared to gamma/delta T cells activated by MSUC or HMBPP alone ($p<0.05$, $n=13$). On the other hand, treatment of gamma/delta T cells with Syk inhibitor GS-9973, completely abrogated the increase in CD69 expression induced by MSUC ($p<0.05$, $n=7$). Our results indicate that gamma/delta T cells activated with HMBPP are able to recognize MSUC efficiently, suggesting a possibly role in sensing cell death molecular patterns released during an infection. Also, we show that the protein kinase Syk is involved in gamma/delta T cells activation by MSUC.

559 (916) **MODULATION OF GAMMA/DELTA T CELL ACTIVATION BY NEUTROPHIL ELASTASE**

Nadia Yasmín Towstyka[1], Florencia Sabbione[1], Irene Keitelman[1], Jorge Geffner[2,3], Analía Trevani[1,3], Carolina Jancic[1,3].
1. Instituto de Medicina Experimental (IMEX) - CONICET - Academia Nacional de Medicina, Argentina. 2. Instituto de Investigaciones Biomédicas en Retrovirus y SIDA (INBIRS) - CONICET - Universidad de Buenos Aires. Argentina. 3. Departamento de Microbiología e inmunología. Facultad de Medicina. Universidad de Buenos Aires. Argentina.

Gamma/delta T cells play a critical role in the recruitment and activation of neutrophils at the sites of infection. In addition, neutrophils can regulate gamma/delta T cell activity. We have previously reported that elastase secreted by neutrophils enhances gamma/delta T cell activation triggered by anti-CD3 antibodies. Now, we aim to investigate the mechanism by which elastase modulates gamma/delta T cell activation. Gamma/delta T cells were purified from human peripheral blood mononuclear cells, by using an anti-TCR gamma/delta MicroBead isolation kit. Neutrophils were isolated by dextran sedimentation. After purification, gamma/delta T cells were stimulated with anti-CD3 antibodies (250 ng/ml), and cultured with or without neutrophils. After 24 h, we analyzed in gamma/delta T cells, the CD69 expression by flow cytometry; and the IFN-gamma and TNF-alpha production by ELISA. We evaluated cell conjugates formation and elastase expression by confocal microscopy. Finally, we analyzed the expression of the proteases activated receptor 1 (PAR1) by flow cytometry and the functionality of PAR1 by using the agonist thrombin (10 U/ml), and the antagonist RWJ56110 (10 uM). Gamma/delta T cells and neutrophils formed cell conjugates, 69±1% of these conjugates recruited elastase at the cell-to-cell contact ($p<0.05$, $n=4$). Gamma/delta T cells expressed PAR1 on cell surface ($p<0.001$, $n=12$). PAR1 was activated by thrombin treatment, measured as an increased in CD69 expression ($p<0.05$, $n=13$) and IFN-gamma ($p<0.05$, $n=7$) and TNF-alpha ($p<0.05$, $n=7$) production. Moreover, in presence of PAR1 antagonist, RWJ56110, neutrophils did not potentiate the activation of gamma/delta T cells induced by anti-CD3 antibodies, measured through the expression of CD69 ($p<0.05$, $n=6$) and the production of IFN-gamma ($p<0.05$, $n=4$) and TNF-alpha ($p<0.05$, $n=8$). Our results indicate that PAR1 mediates the effect of neutrophils elastase on gamma/delta T cells activated through CD3.

560 (69) **RECOGNITION OF TLR LIGANDS DISSIPATES A STRESS-LIKE PHENOTYPE IN DENDRITIC CELLS.**

Christian Rodriguez Rodrigues[1,2], Maria Laura García Castrañon[2], Celeste Nicolao[2], Julia Loos[2], Valeria Dávila[2], Andrea Cumino[2], Evelina Gatti[1], Philippe Pierre[1].

1. Centre d'Immunologie de Marseille-Luminy, Francia. 2. Departamento de Química, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata.

BACKGROUND: Dendritic cells (DCs) are professional antigen-presenting cells specialized in capturing antigens, processing and present to naive T cells, a process that leads tolerance or immunity. There is no data in literature about global translation status of these cells before and after microbial encounter. In this study we focused on the phosphorylation levels of eIF2 and eEF2 in different DC subpopulations before and after TLR stimulation, and how it relates to protein synthesis. METHODS: Spleen and bone marrow were isolated from WT or GADD34$^{-/-}$ FVB mice. GADD34 is a PP1 cofactor and functions in a negative feedback loop to reverse P-eIF-2a. Splenic DCs were purified by positive selection using CD11c^{+} beads and BMDCs were differentiated in presence of Flt3-L during 6 days. Analysis of phosphorylation and translation status, kinases, chaperones, in DCs were performed by WB, FACS, Immunohistology, Confocal Microscopy and Fluidigm. RESULTS: CD11b^{+} DCs and CD8a^{+} DCs express higher levels of P-eIF2a compared to lymphocytes ($p<0.0001$, n=10) or to pDC ($p<0.005$, n=10). In concordance, DCs at steady stage are arrested or present low levels of translation based in Puromycin labeling (n=3). Reversible situation have shown after PI:C, LPS, PAM2CSK4 and CpG stimulation (n=3). DC progenitors, highly active in translation, acquires phosphorylation status during Flt3-L differentiation and arrest their protein synthesis as have been observed previously in spleen (n=4). Gene expression differences in cytokines, chaperones, translation factors have shown in sorted Flt3-L BMDC after TLR stimulation (n=2). We have not shown any different in eIF2a phosphorylation between PKR$^{-/-}$ and WT BMDCs, but using the PERK inhibitor (GSK2606414) we have observed statistically differences ($p<0.05$, n=3). CONCLUSION: These data suggest that resting DCs induce low levels of protein synthesis, perhaps acting as sentinels, waiting of PAMPs recognition to exert their function in the immune response.

561 (344) **KLEBSIELLA PNEUMONIAE ISOLATES STIMULATED POORLY PMN BACTERICIDAL RESPONSES COMPARED TO OTHER OPPORTUNISTIC BACTERIA.**

Luis Castillo[1], Nahuel Rodriguez-Rodrigues[1], Daiana Martire-Greco[1], Verónica I. Landoni[1], Sonia A. Gómez[2], Gabriela C. Fernández[1].

1. Laboratorio de Fisiología de los Procesos Inflamatorios, Instituto de Medicina Experimental (IMEX)-CONICET/Academia Nacional de Medicina, Buenos Aires, Argentina. 2. Servicio Antimicrobianos, INEI-ANLIS Dr Carlos Malbrán, Buenos Aires, Argentina.

Klebsiella pneumoniae (KP) has become a relevant nosocomial pathogen attributed to the production of KP-Carbapanemase (KPC), an enzyme that mediates resistance to carbapenems, the last option of antibiotic treatment. KPC production is associated with higher mortality rates in immunocompromised hospitalized patients. To determine if KPC+ differentially modulates neutrophil (PMN) responses compared to KPC- or other opportunistic pathogens, we evaluated different parameters of PMN activation using a clinical isolates of KPC+ and KPC-, Escherichia coli ATCC 47055 (ECO) and Enterococcus faecalis ATCC 29212 (EFA). We measured CD11b expression and reactive oxygen species (ROS) generation using dihydrorhodamine 123 (DHR) by FACS, NETs formation by confocal microscopy and quimiotaxis. The ratio PMN: Bacteria was 1:20. We did not observe any differences between KPC+ vs KPC- in any of the parameters tested. However, KP species induced lower NETs area and ROS generation compared to ECO and EFA (NETs area mm^2 x10^{-4}: KP=0.22±0.06; ECO=2.17±0.22*; EFA=1.59±0.44*;%DHR+ PMN: KP=5.2±0.1; ECO= 39.8±11.3*; EFA=39.3±5.6*; *$p<0.05$ vs KP). To elucidate whether bacterial integrity modulates ROS levels, we used KP extracts (KP-E) and found that KP-E were able to inhibit ROS triggered by FMLP (%DHR+ PMN: FMLP=70.2±2.1; KP-E+FMLP= 21.9±3.1, $p<0.01$). To study the bacterial component that mediated this effect, catalase inhibition by sodium azide, LPS neutralization by polymyxin B or heat inactivation of KP-E were performed. None of these treatments were able to reverse KP-E's inhibitory activity. Moreover, KP-E-precipitated proteins did not induce per se any ROS inhibition, indicating that no protein component is involved. Although KPC+ or KPC- showed no differences in PMN responses, our results revealed that KP species stimulate poorly PMN bactericidal responses (NETs and ROS), suggesting that this mechanism could be a potential advantage for the establishment/persistence of KP infections.

562 (574) **CLINICAL CANDIDA ALBICANS STRAIN DOWN-REGULATES HUMAN ANTI MICROBIAL PEPTIDE BETA DEFENSIN 1 IN VITRO AND IN PATIENTS WITH RECURRENT VULVOVAGINAL CANDIDIASIS.**

María Soledad Miró[1], Emilse Rodríguez[1], Cecilia Vigezzi[1], Lara Vargas[2], Paula Alejandra Icely[1], Fernando Riera[2], Juan Pablo Caeiro[3], Claudia Elena Sotomayor[1].

1. CIBICI-CONICET, Facultad de Ciencias Químicas-Universidad Nacional de Córdoba. 2. Sanatorio Allende, Córdoba. [3]Hospital Privado, Córdoba.

Vulvovaginal candidiasis (VVC) and recurrent VVC (RVVC) are two forms of a disease that affects a large number of otherwise healthy women. Innate immune responses by the epithelium, including antimicrobial peptides (AMP) are critical for protection against Candida albicans (Ca) overgrowth. â-Defensin 1 (BD1) is important for control of early mucosal Ca infection. The aim of this study was to evaluate BD1 expression and regulation in patients with acute (AVVC) and the recurrent form of this mycosis. Inclusion criteria were: otherwise healthy women of at least 18 years of age, diagnosed with at least three microbiologically tested episodes of VVC within one year (RVVC group) or with a single episode (AVVC group). Healthy women were included as Control group. Cervicovaginal lavage (CVL) was obtained from all participants by instilling 3 ml of sterile saline into the posterior vagina. The cells recovered from CVL were used for RNA extraction or fixed for immunofluorescence (IF) staining against BD1. For In Vitro assays a cervical human epithelial cell line (HeLa) was incubated with: Ca recovered from a RVVC patient (Ca-RVVC), Ca SC5314 collection strain at different fungus/cell ratios (1:1, 5:1), heat killed Ca (HKC) and Zymozan (Zym) for 4 hr, and BD1 expression (Flow Cytometry) was evaluated. BD1 transcripts from CVL recovered cells were diminished in RVVC patients when compared to Controls. Also, BD1 protein expression (IF) was downregulated in this group of patients compared to Control and AVVC groups. HeLa cells incubated with Ca-RVVC showed a diminished expression of the protein (5:1 ratio) ($p<0,05$), meanwhile Ca SC5314, HKC and Zym didn't promote changes, indicating that pathogenic Ca strain is capable of downregulate BD1 expression in human epithelial cells. This is the first report that demonstrates that BD1 is downregulated in epithelial cells from RVVC patients, but not in AVVC patients, indicating an important role for this AMP in the disease.

563 (876) **EFFECT OF IL-10 ON RECRUITMENT AND FUNCTION OF NEUTROPHILS-LIKE MYELOID CELLS DURING THE COURSE OF IMMUNOSUPPRESSION INDUCED BY BACTERIAL ENDOTOXINS.**

Marlina Olyissa Córdoba Moreno[1], Adriana Fontanals[2], Gonzalo Pineda[3], Roberto Meiss[4], Martín A. Isturiz[1], M. Victoria Ramos[3], Bárbara Rearte[1].

1. Laboratorio de Fisiología de los procesos Inflamatorios, IMEX-Academia Nacional de Medicina. 2. Fundación Instituto Leloir, Buenos Aires, Argentina. [3]Laboratorio de Patogénesis e Inmunología de Procesos Infecciosos, IMEX-Academia Nacional de Medicina. [4]Departamento de Patología, Instituto de Estudios Oncológicos, Academia Nacional de Medicina.

In sepsis caused by Gram negatives, lipopolysaccharides (LPS) are associated to the immunosuppression (IS), the main cause of death. Numerous agents such as the IL-10 have been proposed as immunosuppressant. We have shown that even in IL-10 deficient BALB/c mice (KO) it was possible to establish a humoral IS similar to the wild type (WT). The aim of this work was to evaluate the role of IL-10 on the innate immunity alterations observed in LPS induced IS, done through cytometric and morphologic studies. First we determined the leukocyte number in peritoneum and peripheral blood (PB) after IS. In PB the leukocyte numbers was increased mainly associated with an increase in neutrophils (PMN) numbers in ISKO mice (Leukocytes (10^6/ml): ISWT:4±0.7; ISKO:13.7 ± 0.4*; *p<0.05; PMN (10^6/ml): ISWT:1.5±0.2; ISKO:10.4 ±0.9#; #p<0.05). Also, in this group, an influx of leucocytes into the peritoneum, mainly PMN like myeloid cells, was observed (Peritoneal cells (10^6/ml): ISWT:31±5; ISKO:52 ±10*; *p<0.05; PMN: (10^6/ml): ISWT:6.3±2.4; ISKO:39.4±9#; #p<0.05). These data correlated with a markedly decrease of bone marrow cells (BMC) in both IS groups (Total BMC (10^6/femur): WT:17±2; KO: 19±3; ISWT:7.4±1*; ISKO:5 ±1.5#; *#p<0.05).When E. coli phagocytosis in peritoneal myeloid cells was evaluated an elevated phagocytic index (PI) in both IS groups was observed being higher in the ISKO (PI: WT:1±0.3; KO:2.9±1.5; ISWT:123±31*; ISKO:290 ±5#$; *#$; p<0.05). However the oxidative burst was significantly higher only in peritoneal myeloid cells from ISKO mice (MIF DHR): ISWT:2.6±0.3; ISKO:9.3±2*; *p<0.05).The ISKO mice showed an augmented recruitment of PMN-like myeloid cells into PB and peritoneum associated with an increased phagocytosis of E coli and an elevated oxidative burst. These results suggest that the IS in absence of IL10 could improve innate immune response mainly mediated by PMN recruitment. These facts should be further developed in the future.

564 (963) **ASSOCIATION OF IL-6 AND AUTOANTIBODY LEVELS WITH THE NUMBER AND PHENOTYPE OF INFILTRATING NEUTROPHILS IN INFLAMED JOINTS OF PATIENTS WITH RHEUMATOID ARTHRITIS.**

Carolina Virginia Gorlino[1], Rodrigo Blas[2], María Soledad Díaz-Gabutti[1], Alicia Munarriz[3], Héctor Tamashiro[4], Rodolfo Pardo-Hidalgo[5], María Cristina Pistoresi-Palencia[6], María Silvia Di Genaro[1].

1. IMIBIO-CONICET, Facultad de Química, Bioquímica y Farmacia - Universidad Nacional de San Luis (UNSL), Argentina . 2. Medici, San Luis, Argentina. 3. Instituto Privado Cenyr, San Luis, Argentina. 4. Clínica Bolívar, San Luis, Argentina. 5. Centro de Rehabilitación Médica CER, San Juan, Argentina. 6. CIBICI-CONICET, Facultad de Ciencias Químicas - Universidad Nacional de Córdoba (UNC), Argentina.

Background/Purpose. In Rheumatoid arthritis (RA), neutrophils are recruited into inflamed joints enhancing tissue injure. Accumulated evidence has demonstrated that interleukin (IL)-6 modulates neutrophil influx at sites of inflammation and promotes humoral immunity. Among the numerous autoantibodies associated with RA, anti-cyclic citrullinated peptide antibodies (ACPA) are recognized the most disease-specific. The aim of this study was to investigate the association among ACPA, neutrophil influx and IL-6 in the inflamed joints of RA patients. Methods. Synovial fluid (SF) samples were obtained from 60 RA patients. All patients gave informed consent and the study was approved by the ethic board from IBYME. Cytokine and ACPA levels were measured by ELISA and the expression of CD16, CD62L, CXCR1, and CD66b were assessed by flow cytometry. Results. We showed that in SF of ACPA-positive patients, IL-6 levels positively correlated with neutrophil counts (p=0.009) and with IL-8 (p=0.04). Moreover, in SF of patients with high ACPA levels (ACPA-high SF: >200 U/ml) IL-6 was significantly increased (p<0.05) and IL-8 levels and disease activity showed positive correlation (p=0.03). To determine the effect of IL-6 on neutrophil phenotype, neutrophils from healthy donors were incubated with ACPA-high SF in the presence or absence of tocilizumab (TCZ), a monoclonal antibody which blocks the IL-6 receptor. Upon stimulation, a subset of $CD16^{high}CD62L^{low}$ neutrophils increased (p=0.004) and showed a decreased expression of CXCR1 (p=0.03) and an upregulation of CD66b (p=0.002). However, treatment with TCZ reduced the percentage of $CD16^{high}CD62L^{low}$ neutrophils (p=0.008), which showed an upregulation of CXCR1 (p=0.01) and a downregulation of CD66b (p=0.02). Conclusion. We demonstrate that IL-6 levels showed a relationship between neutrophil numbers and ACPA levels in SF of RA patients. We suggest that neutrophil activity in affected joints may be modulated by blocking IL-6 signaling.

565 (984) **LSP1 DEFICIENT DENDRITIC CELLS ARE RESPONSIBLE FOR GENERATING AN INADEQUATE CYTOTOXIC IMMUNE RESPONSE.**

Rachel Paola Acland Strack[1], María Mercedes Pascual[1], Belkys Maletto[1], María Cristina Pistoresi[1], Gabriel Morón[1].

1. CIBICI–CONICET, Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina.

Background: Leukocyte-specific protein 1 (LSP1), is a 52-kDa cytoplasmic F-actin binding phosphoprotein expressed in all human and murine leukocytes as well as in endothelial cells. LSP1 is an important regulator of actin cytoskeleton remodeling, modulating leukocytes motility. We previously showed that $Lsp1^{-/-}$ mice have an impaired CTL response after antigen exposure. We have also reported that $Lsp1^{-/-}$ dendritic cells (DCs) fail to induce a strong CTL response in vivo, with minimal alterations in T cell compartment. In order to study the role of LSP1 in the ability of DCs to induce immunity, we phenotypically and functionally characterized $Lsp1^{-/-}$ DCs. Methods: Murine bone marrow DCs (BMDCs) from $Lsp1^{-/-}$ and wild-type mice (B6 background) were differentiated with Flt3L during 10 days and then stimulated for 18h with CpG-ODN. After stimulus, phenotypic maturation was determined by flow cytometry, and MHC I-restricted antigen presentation, was analyzed by incubating DCs with latex beads conjugated to OVA and further incubation with the H2-K^b-restricted $OVA_{257-264}$-specific $CD8^+$ T cell hybridoma (B3Z). Results: BMDCs differentiate at similar rates from $Lsp1^{-/-}$ and wt mice, reaching in both cases 85-90% of $CD11c^+$ cells. After CpG-ODN stimulus, the frequency of $CD8^+$ DCs and pDCs equally raise up to 20% in $Lsp1^{-/-}$ and wt BMDCs. $Lsp1^{-/-}$ BMDCs show similar expression of CD40, CD86 and PDL2 but lower of I-A^b than wt BMDCs (p<0,01). Additionally, $Lsp1^{-/-}$ BMDCs produce less IL-6 and IL-12 after CpG stimulus than wt BMDCs (p<0,05), Finally, although $Lsp1^{-/-}$ and wt BMDCs capture a similar amount of beads, $Lsp1^{-/-}$ BMDCs have difficulty to activate the B3Z hybridoma after OVA-bead uptake. Conclusions: $Lsp1^{-/-}$ BMDCs have an impaired IL-12 production and a reduced antigen cross-presentation which could be involved in the diminished capacity of $Lsp1^{-/-}$DCs to generate an efficient CTL response.

566 (1026) **NEUTROPHIL EXTRACELLULAR TRAPS RELEASED IN RESPONSE OF CIGARETTE SMOKE EXTRACT INCREASE THE SECRETION OF PROINFLAMMATORY CYTOKINES BY HUMAN ALVEOLAR EPITHELIAL CELLS.**

Florencia Sabbione[1], Irene Keitelman[1], Mauricio Guzmán[2], Jeremías Galletti[2], Mariana Ferrero[4], Pablo Baldi[4], Nadia Towstyka[1], Mirta Giordano[2,3], Carolina Jancic[1,3], Analía Trevani[1,3].

1. Laboratorio de Inmunidad Innata, Instituto de Medicina Experimental (IMEX)-CONICET, Academia Nacional de Medicina; Buenos Aires, Argentina. 2. Laboratorio de Inmunidad Oncológica, Instituto de Medicina Experimental (IMEX)-CONICET, Academia Nacional de Medicina; Buenos Aires, Argentina. 3. Universidad de Buenos Aires. Facultad de Medicina. Departamento de Microbiología e inmunología. Buenos Aires. Argentina. 4. Instituto de Estudios de la Inmunidad Humoral (IDEHU, CONICET-UBA), Cátedra de Inmunología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina.

Cigarette smoking is a major cause of chronic obstructive pulmonary disease. Gas phase of cigarette smoke (CS) can reach

the alveolar epithelium inducing the secretion of chemokines and cytokines that contribute to recruit neutrophils (PMN) into the airway lumen. In response to diverse stimuli, PMN release neutrophil extracellular traps (NET) through a process called NETosis. Previous studies determined that CS and uric acid (UA), a major DAMP found at high concentrations in lungs of patients with acute lung injury, can induce NETosis. The aim of this study was to determine if a short exposure to CS is able to induce NETosis, and if these NET induce proinflammatory cytokine secretion by alveolar epithelial cells, comparing their effects with those produced by NET released in response to UA (8 mg/dl). CS extract (CSE) prepared according to a standard method from cigarettes containing an equivalent of 13.6 mg/ml tar was used at 10%. PMN were incubated with or without stimuli for 1h, then washed and cultured for 3 more hours at 37°C. NET were identified by confocal microscopy by colocalization of DNA with myeloperoxidase. Short exposure to both stimuli induced NETosis. NET released by 10^6 PMN were isolated and added to A549 epithelial cell monolayers. Both CSE- and UA-induced NET significantly increased the secretion of IL-8 and IL-6 ($p<0.05$; n=4), and IL-1b (n=2) by A549 as compared to that induced by supernatants from unstimulated PMN and basal conditions. These effects were not observed with supernatants from CS-stimulated PMN pretreated with the NETosis inhibitor CL-amidine (200 μM; n=2). These findings suggest that CSE might also promote alveolar inflammation by triggering NETosis. Together with our previous findings indicating similar properties of NET induced by monosodium urate crystals, these results alsosuggest that NET exert proinflammatory effects on epithelial cells independently of the sterile stimulus that induced their release.

567 (1067) **ABSCENCE OF CASPASE1 FAVOURS A T HELPER 2-LIKE RESPONSE AND PROTECTS MICE FROM LIVER DAMAGE DURING ACUTE TRYPANOSOMA CRUZI INFECTION.**

Augusto Paroli[1], Patricia Gonzalez[1], Sabrina Rizzo[1], Roxana Cano[1], Susana Gea[1].
1. Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI).

Background: Caspase-1 activated through formation of an inflammasome cleaves the pro-cytokines IL1β and IL18 and triggers pyroptosis. Previously, we demonstrated an increase of IL1β+F4/80+ hepatic leukocytes associated with an up-regulation of TLR9 and NLRP3 innate immune receptors of T. cruzi infected WT mice. NLRP3 inflammasome receptor modulates the expansion of different lymphocyte subpopulations. Here, we study the role of Caspase-1/11 on plasmatic GPT activity, inflammatory cytokine levels, expansion of CD4+ lymphocytes and production of reactive oxygen species (ROS) and nitric oxide (NO) by F4/80 hepatic macrophages during acute T. cruzi infection. Methods: Male C57BL/6 WT, nlrp3-/- and casp1/11-/- mice were infected with T. cruzi (1000 i.p. injected-Tulahuen strain). IL1R and IL18R expression on and production of intracellular cytokines (IL4, IL10, IL17 and IFNγ) in hepatic CD4+ T lymphocytes, as well as the detection of ROS and NO in hepatic F4/80+ cells were evaluated by flow cytometry at 14 and 21 days post infection (dpi). Results: During acute infection, Casp1/11-/- mice showed higher parasitemia but lower hepatic leukocyte infiltration compared to WT mice. Although F4/80+ hepatic cells were augmented, they showed a decreased TLR9 expression and intracellular IL10. Also, these macrophages exhibited an increased ROS and NO production. As expected, the number of IL1R+ and IL18R+CD4+ T lymphocytes was strongly diminished. Interestingly, IL4+CD4+ T cells were markedly increased while IL17+ or IL10+CD4+ cells were reduced vs. WT. Moreover, plasmatic levels of IL1β, IFNγ, IL6 and IL10 were diminished and GPT activity was slightly increased in Casp1KO mice.Conclusion: The absence of Casp1 during acute infection showed a diminished inflammatory response in infiltrating hepatic leukocytes associated with a Th2-like profile. Accordingly, the lower plasmatic GPT activity and inflammatory cytokine levels suggest a lesser hepatic damage compared to infected WT mice.

568 (444) **PURINERGIC SIGNALING PARTICIPATES IN PHENOTYPIC MODULATION OF CARDIAC MACROPHAGES DURING EXPERIMENTAL TRYPANOSOMA CRUZI INFECTION.**

Natalia Eberhardt[1], Nicolás E. Ponce[1,2], Liliana M. Sanmarco[1], Pilar Aoki[1].
1. CIBICI-CONICET- Departamento de Bioquímica Clínica-Facultad de Ciencias Químicas- UNC, Córdoba, Argentina. [2]INIMEC-CONICET- Instituto de Investigación Médica Mercedes y Martín Ferreyra, Córdoba, Argentina.

Extracellular ATP and adenosine are increasingly recognized as key mediators of the immune response to several pathogens. ATP can act as a danger signal initiating an innate immune response, while adenosine generally serves as a regulatory feedback mechanism to limit inflammation. Macrophages (Ma) are highly plastic cells and depending on the micro-environmental stimulation they exhibit a pro-inflammatory (classical/M1) or an anti-inflammatory (reparatory/M2) phenotype. Considering that ATP is converted to ADP/AMP and then to adenosine by CD39 and CD73 enzymes, respectively, we have reported that transient pharmacological inhibition of CD73 ectoenzyme during the early acute phase of murine T. cruzi infection induces microbicidal mechanisms, reduction in cardiac parasite load and improves the outcome of chronic cardiomyopathy. The aim of this study was to characterize the role of purinergic system in regulatory mechanisms triggered in cardiac tissue during T. cruzi infection. The kinetic of cardiac Ma subsets showed that M1 predominated over M2 profile throughout the infection in CD73KO mice ($p<0.001$). In contrast, C57BL/6 (WT) mice presented diminished M1 subset and significantly increased M2, which remained sustained since 7 dpi. Strikingly, CD73KO mice had higher parasitemia than WT mice (14 and 21dpi, $p<0.05$) and this correlated with diminished nitric oxide (NO) serum levels in deficient mice (21 dpi, $p<0.001$). Notably, CD73KO mice had diminished cardiac parasite load compared to WT (21 and 28 dpi, $p<0.05$) but augmented liver parasite load (21 dpi, $p<0.05$). The decrease in cardiac parasitism correlated with the M1 Ma phenotype prevalence and augmented tissue NO levels (21 dpi, $p<0.05$) compared to WT mice. In conclusion, ectonucleotidase pathway participates in cardiac adaptation of Ma subsets in order to interrupt macrophage-mediated inflammation. This study provides new insight on the role of purinergic signaling in the phenotypic modulation of immune cells.

569 (114) **THE PRESENCE OF PROKARYOTIC RNA IN LIVE BACTERIA IS NECESSARY TO GENERATE BACTERICIDAL RESPONSES IN NEUTROPHILS.**

Nahuel Rodriguez-Rodrigues[1], Luis Castillo[1], Daiana Martire-Greco[1], Verónica I. Landoni[1], M. Ayelén Milollo[1], Pablo Schierloh[1], Paula Barrionuevo[1], Gabriela C. Fernández[1].
1. Instituto de Medicina Experimental (IMEX)-CONICET, Academia Nacional de Medicina, Buenos Aires, Argentina.

While PAMPs are present in live and dead bacteria, vaccines developed from live microorganisms generate much vigorous immune responses, suggesting the existence of certain determinants related to bacterial viability that enhance immunity. Because neutrophils (PMN) are the first line of defense against infections, our aim was to determine if PMN have the ability to recognize bacterial viability. Different functions of PMN isolated from healthy donors were evaluated against live E. coli (EC) and heat-killed E. coli (HKEC) (ratio PMN: EC=1:20). We noted that the responses of PMN against EC were higher compared to HKEC in all evaluated functions: generation of ROS by FACS (%DHR PMN: EC=32.8±3.9; HKEC=9.9±3.8, $p<0.05$); NETs formation by confocal microscopy (area in μm^2: EC=21750±2289; HKEC=1424±436, $p<0.01$); Cell size by FACS (% of high FSC: EC=65.9±4.9; HKEC=37.6±4.7, $p<0.01$). CD11b expression by FACS (MFI= EC=173.9±9.8; HKEC=109.6±8.7, $p<0.05$). The EC was a better chemotactic stimulus (N° of PMN migrated through chemotaxis chamber: EC=28.7±2.3; HKEC=7.7±1.6, $p<0.01$). Similar results were obtained when bacteria were killed by PFA fixation or irradiation ($p<0.05$). These results demonstrate that the response trig-

gered by PMN is dependent on bacterial viability and independent of thekilling method. To explore which factor of bacterial viability generates the observed responses in PMN, prokaryotic RNA was purified from EC and used as stimulus. We observed that RNA triggers similar responses to those observed with live EC bacteria: generation of ROS (%DHR: Ctrl=7.9±1.7; RNA=31.7±6.9, p<0.01), NETs (area in μm^2: Ctrl=1258±780; RNA=15360±2520, p<0.01), PMN size (% of high FSC: EC=65.8±3.8; RNA=17.8±3.5, p<0.01), CD11b expression (MFI: Ctrl=100.4±0.3; RNA=154.8±13.7, p<0.01). No RNA could be isolated form HKEC, explaining the lack of effects with dead bacteria. During an infection, PMN can sense bacterial viability by the presence of prokaryotic RNA and trigger bactericidal responses.

570 (366) **HUMAN NK CELL ACTIVATION TRIGGERS GALECTIN 1 SECRETION WHICH REGULATES THE MAGNITUDE OF IFN-GAMMA SECRETION IN A CD45-DEPENDENT MANNER.**

Carolina Ines Domaica[1], Andrea Ziblat[1], Florencia Secchiari[1], Sol Yanel Nuñez[1], Nicolas Torres[1], Jessica Sierra[1], Ximena Raffo Iraolagoitia[1], Raúl Germán Spallanzani[2], Juan Carlos Stupirsky[3], Diego Omar Croci[3], Juan Manuel Perez Saez[3], Mercedes Beatriz Fuertes[1], Gabriel Adrián Rabinovich[3], Norberto Walter Zwirner[1].

1. Laboratorio de Fisiopatología de la Inmunidad Innata. Instituto de Biología y Medicina Experimental (IBYME-CONICET). 2. Harvard Medical School, Department of Microbiology and Immunobiology, Boston Cancer Research, Immunology. Cambridge, United States. 3. Laboratorio de Inmunopatología. Instituto de Biología y Medicina Experimental (IBYME-CONICET).

Galectin-1 (Gal-1) is an endogenous glycan-binding protein widely expressed at sites of inflammation and by tumor cells that controls a diversity of immune cell processes through binding to specific cell surface glycan structures or through intracellular ill-defined pathways. Gal-1 binds specifically to the cell surface glycoproteins CD45, CD43 and CD7. Natural killer (NK) cells trigger cytotoxicity and interferon (IFN)-gamma secretion upon engagement of activating receptors by ligands expressed on tumor cells. Previously we demonstrated that Gal-1 negatively regulates NK cell effector functions. The objective of this study was to elucidate the mechanisms involved in the regulation of NK cell effector functions by Gal-1. To further investigate this susceptibility, we analyzed the binding of biotinylated Gal-1 by flow cytometry to NK cells and observed that Gal-1 binds to resting and activated NK cells in a carbohydrate-dependent manner, as the addition of lactose inhibited such binding. NK cells activated with cytokines in the presence of Gal-1 and the PTP CD45 inhibitor exhibited an equivalent production of IFN-gamma than cytokine-activated NK cells. Also, by ELISA we observed that NK cells, independently of their activation status secreted Gal-1, but that activated NK cells exhibited a higher production of Gal-1 than resting NK cells (p<0,005). When isolated NK cells were pre-activated for 5 days with cytokines and re-stimulated with IL-2 in the absence or in the presence of Gal-1 we observed that Gal-1 didn´t inhibit IFN-gamma secretion. Collectively, our results suggest that at early stages of activation NK cells secretion of Gal-1 could contribute in a CD45-dependent manner to the regulation of IFN-gamma secretion as a mechanism to control excessive activation.

571 (481) **DUAL ROLE OF MONOCYTE-DERIVED INFLAMMATORY CELLS IN T. CRUZI INFECTION**

Carolina Veronica Poncini[1], Stella Maris González Cappa[1].

1. IMPaM, UBA-CONICET. Facultad de Medicina, Universidad de Buenos Aires.

Pathogens can cause inflammation when inoculated into the skin. The vector-transmitted protozoan parasite Trypanosoma cruzi induces poor cell-infiltration at the beginning of the infection and disseminates causing high mortality in experimental models. Here, we characterized the inflammatory foci at the parasite inoculation site and secondary lymphoid organs. While no macrophages, neutrophils or monocytes (Mo) recruited into the skin at 3 days post-infection, a population of $Ly6C^+$ Mo infiltrated first draining lymph nodes and then the spleen. Over time, the infiltrate became enriched in $CD11b^+CD11c^+Ly6C^+$ cells, resembling inflammatory dendritic cells (iDCs) previously reported in other infections. Adoptive transfer of Mo purified from bone marrow of CD11cGFP transgenic mice confirmed that iDC-like cells found in the spleen of infected mice were from monocytic origin. Freshly isolated iDC-like cells not only produced TNF and nitric oxide, but also IL-10 and displayed impaired capacity to induce lymphoproliferation. To explore their myeloid-suppressor nature, infected mice were treated with 5-fluorouracil. The treatment reduced $CD11b^+CD11c^+Ly6C^+$ cell number and confirmed its dual role, firstly by controlling parasite dissemination by iNOS-related mechanisms and then by the negative regulation of T cell-dependent anti-parasite response.

572 (725) **ALTERED NEUTROPHIL ACTIVATION IN ABSENCE OF IL-10 IN A MURINE MODEL OF HEMOLYTIC UREMIC SYNDROME.**

Gonzalo Ezequiel Pineda[1], Barbara Rearte[2], Marlina Córdoba Moreno[2], Andrea Bruballa[1], Romina Fernandez-Brando[1], Roberto Meiss[3], Martin Isturiz[2], Catalina Alba-Soto[4], Marina Palermo[1], María Victoria Ramos[1].

1. Laboratorio de Patogénesis e Inmunología de Procesos Infecciosos, IMEX-Academia Nacional de Medicina. 2. Laboratorio de Fisiología de los procesos Inflamatorios, IMEX-Academia Nacional de Medicina. 3. Departamento de Patología, Instituto de Estudios Oncológicos, Academia Nacional de Medicina 4. Instituto de Investigaciones en Microbiología y Parasitología Médicas (IMPaM). Facultad de Medicina. Universidad de Buenos Aires.

Hemolytic Uremic Syndrome (HUS), is a systemic disease caused by circulating Shiga-toxin (Stx) which mainly damages kidney. Besides Stx2, inflammatory response mediated by neutrophils (PMN) is essential to HUS evolution. We have demostrated that mice lacking IL-10 ($IL\text{-}10^{-/-}$) had a higher survival rate to Stx2 than controls. The aim of the work was to determine the role of PMN in HUS evolution in absence of IL-10. Before and 24h, 48h and 72h after of $1LD_{100}$ of Stx2 e.v., $IL\text{-}10^{-/-}$ and control mice were sacrificed. Total leukocytes were counted and PMN were identified as $Ly6G^+CD11b^+$ cells in blood and bone marrow. PMN production of reactive oxygen species (ROS) was evaluated without stimulus (basal) or after PMA stimulation, with DHR by FACS. In basal condition both strains showed similar absolute number of PMN in blood, although the percentage of PMN in bone marrow was increased in $IL\text{-}10^{-/-}$ mice (%PMN:$IL\text{-}10^{-/-}$= 52.8±4.1, control=39.9±1.9*,*p<0.05). Circulating PMN increased at 48h post-Stx2 in controls, meanwhile it was delayed at 72h in $IL\text{-}10^{-/-}$ mice (PMN(10^5/ml): Basal/Stx2:$IL\text{-}10^{-/-}$ (72h)=10.9±1.6/31.7±5.0*,control(48h)=8.5±1.2/16.4±3.3*,*p<0.05 vs basal). Peripheral PMN upregulated CD11b expression 48h after Stx2 in control mice but no changes were observed in $IL\text{-}10^{-/-}$ mice at any time, (MFI Basal/Stx (48h):$IL\text{-}10^{-/-}$ 906±106/662±241; control=408±84/1780±100*, *p<0.05). In contrast as it was reported for control mice, unstimulated PMN from $IL\text{-}10^{-/-}$ mice showed similar ROS production before (0h) and 72h after Stx2 (MFI of DHR:0h=7.0±0.5;72h=5.4±0.8), and in vitro PMA activation was conserved (0h=23.3±5.7*;72h=31.0±6.2*,*p<0.05 vs respective time). $IL10^{-/-}$ mice had a delayed neutrophilia in response to Stx2. Moreover, even though these PMN did not showed in vivo activation (upregulation of CD11b and ROS production), they are capable to produce oxidative burst upon in vitro PMA-stimulation. These alterations could be responsible for the increased survival after Stx2.

573 (223) **UROPATHOGENIC ESCHERICHIA COLI INFECTION OF THE MALE UROGENITAL TRACT IN WILD TYPE AND TLR4-KO MICE.**

Carolina Olivera[1], Gloria Janet Godoy[1], Florencia Celeste Salazar[1], Leonardo Rodolfo Sánchez[1], Rubén Darío Motrich[1], Virginia Elena Rivero[1].

1. CIBICI-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina.

Urinary tract infections are among the most common infections in men being Escherichia coli the most frequently isolated pathogen. Urine reflux into the intra-prostatic ducts is a frequent route of access for the uropathogenic bacteria that causes lower and upper male urogenital infections. In the present study, we analyzed the development of pain, bacterial spread and inflammation after transurethral instillation of saline solution or a suspension containing 1x 108 CFU of E. coli 1677 into adult C57BL/6 (wild type) and TLR4-KO mice. Pelvic pain was assayed as tactile allodynia using Von Frey filaments at baseline and at different time points after infection. At day 7, animals were euthanized, urethral and prostatic tissues were excised, and the presence of bacteria and leukocyte infiltration was analyzed by microbiological culture and immune staining followed by flow cytometry. Acute pelvic pain was evidenced by similarly increased allodynia responses in both, E coli-infected C57BL/6 and TLR4-KO mice when compared with control mice. Besides, bacterial inoculation resulted in an ascending infection of the male urogenital tract in both, C57BL/6 and TLR4-KO mice. Bacterial burden in urethra was similar between both mice strains. However, higher loads were detected in prostate tissue from infected TLR4-KO mice when compared to infected C57BL/6 mice ($p<0.05$). The analysis of tissue leukocyte infiltration revealed the presence of increased amounts of CD45+ cells in urethra and prostate from both mice strains being GR1+, F480+ and CD11b+ cells the most abundant populations detected, with only small proportions of CD3+ and CD19+ cells. Our results show that transurethral inoculation of uropathogenic E. coli 1677 causes and infection of the lower and upper male urogenital tract showing an ascendant pattern that is accompanied by local inflammation and pelvic pain development. In addition, our results suggest that immune effector mechanisms triggered by TLR4 are important for an efficient bacterial clearance.

574 (628) **EFFECTS OF SHORT-TERM FEVER-RANGE HYPERTHERMIA ON NEUTROPHIL RESPONSES TO PSEUDOMONAS AERUGINOSA.**

Irene Keitelman[1], Florencia Sabbione[1], Constanza Giai[3], Nadia Towstyka[1], Mauricio Guzmán[1], Jeremías Galletti[1], Carolina Jancic[1], Marisa I. Gómez[3], Analía S. Trevani[1,2].
1. Instituto de Medicina Experimental (IMEX) - CONICET, Academia Nacional de Medicina, Buenos Aires, Argentina. 2. Universidad de Buenos Aires. Facultad de Medicina. Departamento de Microbiología e inmunología. Buenos Aires. Argentina 3. Universidad de Buenos Aires. CONICET. Instituto de investigaciones en microbiología y parasitología médica (IMPAM). Buenos Aires. Argentina.

Fever response is a hallmark of infection and inflammatory disease, working as an alert system that promotes immune surveillance during challenge by invading pathogens. Previously we determined that 1h of hyperthermic conditions (39.5°C) significantly increased NETosis induced by PMA. However, cytokine release in response to LPS was significantly inhibited. In this study, we aim to determine if short term hyperthermia is able to modulate NET release and other neutrophil (PMN) functions in response to the opportunist pathogen Pseudomonas aeruginosa (Pa). Human PMN were incubated with or without Pa PAO-1 strain (MOI: 1:1) at 37°C or 39.5°C for 1h, and then cultured for different periods at 37°C according to the evaluated function. The secretion of IL-8 was determined by ELISA and elastase secretion was assayed by degradation of a specific substrate. NETosis was evaluated by determining co-localization of elastase and DNA by confocal microscopy (CM), and DNA released by fluorometry. Autophagy levels were determined by quantification of vesicular LC3B/cell by CM. Results indicated that 1h of exposure to 39.5°C and additional culture at 37°C did not modulate the capacity of bacteria to induce DNA release at 1, 2, 3 or 4h (n=4). Hyperthermia did not affect NET release induced by Pa evaluated by CM at 2, 3 and 4h post-thermic treatment (n=2). However, elastase levels detected in Pa-stimulated-PMN supernatants were reduced in cells exposed to hyperthermia (n=4; $p<0.05$) suggesting that part of it could have been released not only by NETosis but also during bacterial phagocytosis. Similar findings were obtained when autophagy levels were evaluated (n=4). As it was observed using LPS as stimulus, hyperthermia inhibited IL-8 secretion induced by Pa evaluated at 2, 3, or 4h post-stimulation (n=3). In summary, these results suggest that short-term hyperthermia differentially affects not only NETosis in response to PMA and Pa, but also distinct neutrophils responses induced by Pa.

575 (408) **OSTEOCYTES AND IL-6 CONTRIBUTE TO BONE PATHOLOGY IN GAUCHER DISEASE.**

Andrea N. Crivaro[1], Juan M Mucci[1], Malena Ferreyra[1], Constanza Bondar[1], Maximiliano Ormazabal[1], Victoria Delpino[2], Paula Rozenfeld[1].
1. Instituto de Estudios Inmunológicos y Fisiopatológicos (IIFP). [2]Instituto de Inmunología, Genética y Metabolismo (INIGEM).

Gaucher Disease (GD) is caused by deficiency of the lysosomal enzyme glucocerebrosidase leading to the accumulation of glucosylceramide. In spite of treatment, bone alterations in Gaucher patients persist. This could rely on the increment of the number of osteoclasts (induced by RANKL and/or citoquines) or the augmented apoptosis of osteocytes. Connexin43 (Cx43) is expressed in bone cells and its function seems to be essential for survival. The aim of our work was to study the involvement of osteocytes in bone pathology of GD. The study was performed using the MLO-Y4 osteocyte cell line treated with CBE (an inhibitor of glyucocerebrosidase) at different time points. The osteoclastogenic potential of conditioned media (CM) from CBE treated osteocytes was evaluated by osteoclast differentiation assays; and RANKL levels in osteocytes were evaluated by immunofluorescence. Cx43 expression was measured by qPCR and apoptosis was studied by Annexin-V and TUNEL staining. On the other hand, osteoclastogenesis assays were performed with the apoptotic body fraction of CM and the supernatant fraction of the CM both in the presence (or absence) of OPG. The CM from CBE treated osteocytes induced higher levels of osteoclast differentiation compared to control CM and higher surface RANKL levels were observed in treated cells. Cx43 expression diminished with CBE treatment and osteocyte apoptosis was increased. The induction of osteoclast differentiation by apoptotic bodies and supernatant was higher in CBE treated CM by both fractions and OPG treatment reduced osteoclast levels in both cases. Levels of IL-6, TNF-á and IL-1â were evaluated by ELISA in supernatant. Only IL-6 levels presented an increment in CBE supernatant. In conclusion, we have shown the possible involvement of osteocytes in bone pathology of GD through an induction of osteoclast differentiation. This induction would be related to a higher apoptotic state of osteocytes that could involve Cx43 as well as RANKL and IL-6.

576 (585) **LONG-TERM EXPOSURE TO PROSTAGLANDIN E2 ENHANCES LPS-INDUCED PRODUCTION OF INFLAMMATORY CYTOKINES BY MONOCYTES.**

Federico Remes Lenicov[1], Melina González Prinz[1], Augusto Varese[1], José Oddi[1], Juan Sabatté[1], Natalia Fernández[2], Jorge Geffner[1], Ana Ceballos[1].
1. Instituto de Investigaciones Biomédicas en Retrovirus y SIDA (INBIRS) - UBA – CONICET. 2. Instituto de Investigaciones Farmacológicas (ININFA) - UBA – CONICET.

Prostaglandin E2 (PGE2) is a pleiotropic agent produced during acute and chronic inflammation. It mediates either inflammatory or anti-inflammatory effects on different cells and experimental systems. It is well known that activation of monocytes by LPS results in the production of inflammatory cytokines and that the presence of PGE2 during LPS-stimulation results in the inhibition of this response. Here, we analyzed whether long-term exposure of monocytes to PGE2 also resulted in the inhibition of their proinflammatory profile. Monocytes were purified from peripheral blood mononuclear cells by gradient centrifugation. TNFalpha production was evaluated by ELISA and intracytoplasmic staining followed by flow cytometry, while IL-1beta, IL-6 and IL-10 were measured by ELISA. Phenotype was analyzed by flow cytometry. Monocytes were preincubated for 15 hs with PGE2 (10-8M), and

then stimulated by LPS (25 ng/ml). After 24 hs of culture, the production of TNFalpha, IL-1beta and IL-6 was analyzed. We found that long-term exposure to PGE2 results in a marked increase in the production of all inflammatory cytokines analyzed: 75 ± 8 vs 56 ± 7% of TNFalpha + cells (n=9), 2115 ± 615 vs 251 ± 49 pg/ml for IL-1beta (n=4), and 6950 ± 145 vs 3059 ± 110 pg/ml for IL-6 (n=4), for monocytes incubated in the presence vs absence of PGE2, respectively ($p<0.05$). No differences were found for IL-10. Similar results regarding cytokine production were observed in monocyte-derived macrophages. Treatment of monocytes with PGE2 did not change the basal expression of TLR4 and HLA-DR, nor the LPS-induced expression of CD83. We found that long-term exposure of human monocytes to PGE2 results in an increased ability to produce inflammatory cytokines triggered by LPS.

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577 (194) **IMPROVEMENT OF OVARIAN RESPONSE BY ADMINISTRATION OF BIOACTIVE SPHINGOLIPID CERAMIDE-1-PHOSPHATE (C1P) IN AGED FEMALE MICE.**

Leopoldina Scotti[1], Natalia Pascuali[1], Mariana Di Pietro[1], Marta Tesone[1,2], Griselda Irusta[1], Antonio Gómez Muñoz[3], Dalhia Abramovich[1], Fernanda Parborell[1],

1 Instituto de Biología y Medicina Experimental (IByME-CONICET). 2 Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires. 3 Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias y Tecnología, Universidad del País Vasco (UPV/EHU), Bilbao, España.

For a variety of reasons, many women postpone childbearing over 38 years, and a considerable proportion of aged female become infertile. Ovarian aging is dominated by a progressive loss of primordial follicles and decline in the quality of oocytes. The activation of ovarian angiogenesis has emerged as a new strategy for the improvement of age-related decline of ovarian response. Since the bioactive sphingolipid C1P is an important proangiogenic and anti-apoptotic factor, our aim was to study whether C1P can improve the ovarian response and angiogenesis in aged female mice. Age female mice (26-31 weeks) received C1P (10µl/ovary; 50µM) under the bursa of one ovary and vehicle on the other ovary, and were sacrificed 48h post-surgery. Young mice (6-9 weeks) were used as control. Ovaries were isolated for histological and immunohistochemical analysis (VW and SMA; endothelial and periendothelial cell markers, respectively), radioimmunoassay (E2 and P4 concentrations) and western blot (pFoxo3a/Foxo3a and AMH; ovarian reserve markers). The% of primary (PF), preantral (PrF) and antral follicles (AF) in age mice were lower than in young mice ($p<0,05$). C1P treatment increased the% of PrF and AF in age mice ($p<0,05$). The% of atretic follicles (AtrF) in age mice was higher than in young mice ($p<0,01$) and C1P decreased the% of AtrF ($p<0,05$) in age mice. C1P increased the concentration of ovarian E2 and P4 in age mice ($p<0,05$). C1P decreased pFoxo3a/Foxo3a ratio and increased AMH levels in age mice ($p<0,05$). The endothelial and periendothelial cell area increased in ovaries from age mice treated with C1P ($p<0,05$). In conclusion, C1P administration in aged mice increased the ovarian response, possibly by preserving the ovarian reserve and increasing the ovarian angiogenesis. These results may have potential clinical implications in the treatment of age-related infertility.

578 (311) **NOVEL IMMUNOREGULATORY ROLES FOR CRISP PROTEINS WITHIN THE EPIDIDYMIS**

Guillermo Carvajal[1], Nicolás Brukman[1], Mariana Weigel Muñoz[1], Agustina Battistone[3], Sylvie Breton[3], Livia Lustig[2], Patricia Cuasnicú[1],

1 Instituto de Biología y Medicina Experimental, CONICET, Argentina. 2 Inbiomed, UBA-CONICET, Facultad de Medicina, Argentina. 3 Massachusetts General Hospital, Boston, United State of America.

Epididymal CRISP1 and CRISP4 (Cysteine-Rich Secretory Proteins) associate with sperm during maturation and participate in different stages of fertilization. Whereas single knockout (KO) males for these molecules exhibit in vitro sperm fertilizing defects and normal fertility, the absence of both proteins (double KO, DKO) significantly affects both in vitro fertilization and male fertility, suggesting the existence of compensatory mechanisms between these proteins. Based on this, in the present work we investigated the mechanisms underlining the subfertility of DKO. Examination of the reproductive organs showed that 15 out of 45 DKO males had bigger caput epididymides accompanied by bigger testes. Histological studies of the epididymis of these males revealed the presence of desquamation of the epithelium, cytoplasmic vacuolation and abnormal presence of immune cells (i.e. macrophages, lymphocytes) in the interstitium and lumen mainly in the proximal regions, and damaged or no sperm in the cauda. Immunofluorescence experiments using specific markers for different epididymal epithelial cells revealed the damage and even absence of principal cells in the proximal regions of these mice. No signs of inflammation were observed in those animals with normal epididymal caput and testicular size, indicating the existence of two subgroups among the DKO mice. Interestingly, whereas the presence of the epididymal immune response positively correlated with lower sperm viability and fertility levels ($p^*<0.05$), fertility was also significantly ($p^*<0.01$) lower than controls in the group of males that did not show inflammation, suggesting that the subfertility of DKO males is due not only to the immunological effects on sperm viability but also to defects in the fertilizing ability of viable sperm. Together, these results support the relevance of CRISP proteins for animal fertility and revealed novel immunoregulatory roles for these proteins within the epididymis.

579 (352) **A HIGHLY ORGANIZED SIGNALING DISTRIBUTION COMPLEX IS CRITICAL FOR THE DEVELOPMENT OF MAMMALIAN SPERM HYPERACTIVATION.**

Guillermina María Luque[1], Ana Romarowski[1], Tomás D´alotto [1], Lis del Carmen Puga Molina [1], Belén Ugo[1], Nicolás Gilio[1], Dario Krapf[2], Mariano Gabriel Buffone[1],

1 Instituto de Biología y Medicina Experimental (IBYME, CONICET), Buenos Aires, Argentina. 2 Instituto de Biología Molecular y Celular de Rosario (IBR, CONICET), Rosario, Argentina.

Sperm acquire the ability to fertilize in the female genital tract in a process called capacitation. From a molecular point of view, bicarbonate and calcium (Ca2+) stimulation of the soluble adenylyl cyclase leads to the activation of the cAMP/PKA pathway. During capacitation sperm undergo a change in the motility pattern called hyperactivation (HA) which is critical to fertilization. Ca2+ is the primary second messenger that triggers this motility and it depends on CatSper channels. It has been described that CatSper1 proteins form a unique pattern of four linear ‘‘stripes’’ running down the principal piece of the flagellum. Catsper Ca2+ domains orchestrate the timing and extent of complex phosphorylation cascades because it colocalizes with Ca2+ signaling molecules. Our central hypothesis is that Cdc42 is an essential component of the highly organized signaling complex that controls intracellular Ca2+ through CatSper channels during mammalian sperm capacitation. This spatial distribution is critical for the development of HA. Using super-resolution microscopy, we observed that Cdc42 localized in the sperm tail and form a pattern of four linear ‘‘stripes’’ running down the principal piece, which resembles the localization of CatSper. By using a specific inhibitor of Cdc42, we detected that PKA-dependent phosphorylation were completely abrogated. This inhibition was bypassed by using membrane permeable analogs of cAMP. The rise in intracellular Ca2+ that occurs during capacitation as a result of CatSper activation, was abrogated with Cdc42 inhibition. When sperm where incubated in the presence of Cdc42 inhibitors, we observed a strong decrease in percentage of HA. This is consistent with the low levels of

intracellular Ca2+ observed. All together, these results suggest that Cdc42 is participating in a molecular complex with CatSper channels modulating the levels of intracellular Ca2+ and ultimately, the development of HA.

580 (491) **EFFECTS OF PREPUBERTAL EXPOSURE TO ANDROGENS ON HISTOFUNCTIONAL DEVELOPMENT OF THE RAT UTERUS**

Gisela Soledad Bracho[2], Carla Waigandt[2], Gabriela Anahí Altamirano[2], Laura Kass[2], Mónica Muñoz-de-Toro [2], Enrique Hugo Luque[2], Verónica Lis Bosquiazzo[1,2],
1 Departamento de Bioquímica Clínica y Cuantitativa, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina. 2 Instituto de Salud y Ambiente del Litoral (UNL- CONICET, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina.

The polycystic ovary syndrome (PCOS) is a disease that can affect women in the reproductive age. It is characterized for anovulation, hyperandrogenism and polycystic ovaries. PCOS is associated with an increased risk of endometrial cancer and fertility problems. This study was performed to evaluate the effects of hyperandrogenism on the histofunctional development of the rat uterus. To induce PCOS, Wistar rats were injected sc with sesame oil (control group) or Dehydroepiandrosterone (DHEA, 6 mg/100 g body weight) from postnatal day 21 (PND21) to PND41. Before sacrifice, the animals received an ip injection of Bromodeoxyuridine (BrdU, 6mg/100 g body weight). The uterine horns were dissected and included in paraffin. The thickness of the subepithelial and myometrial stroma, as well as, the density of the uterine glands and the nuclei density of the subepithelial and stromal compartments were evaluated in the histological sections. BrdU incorporation and androgen receptor (AR) expression were also quantified in all uterine compartments. The DHEA group showed an increment in the subepithelial (control: 153.3±10.4um vs DHEA: 238.3±24.7um; $p<0.05$) and myometrial (control: 149.6±10.4um vs DHEA: 292.3±17.9um; $p < 0.05$) thickness, and in the uterine gland density (control: 2.0±0.2vs DHEA: 2.6±0.2; $p<0.05$). On the other hand, the nuclei density in both compartments was decreased in DHEA animals compared to control rats. The proliferation of the subepithelial stroma was higher in the DHEA than in control animals (control: 1.56±0.5% vs DHEA: 7.3±2.1%; $p<0.05$). Also, DHEA induces the expression of AR in the cytoplasm of luminal and glandular epithelial cells and in the nuclei of the subepithelial and myometrial stroma. The present study provides evidence that DHEA treatment induce changes in uterine histomorphology and steroid receptor expression that could have long-time consequences on fertility and/or uterine anomalies.

581 (660) **TROPHOBLAST GIANT CELLS PROFILE AND IMMUNE MICROENVIRONMENT ARE MODULATED BY ENDOGENOUS VIP IN MURINE IMPLANTATION SITES**

Vanesa Hauk[1], Guillermina Calo[1], Lucila Gallino[1], Daiana Vota[1], Roberto Meiss[2], Rosanna Ramhorst[1], James Waschek[3], Claudia Pérez Leirós[1],
1 Immunopharmacology Laboratory, School of Sciences, IQUIBICEN, CONICET-UBA, Buenos Aires. 2Pathology Department, IMEX-CONICET, National Academy of Medicine, Buenos Aires. [3]The David Geffen School of Medicine, University of California, Los Angeles, USA.

Adequate trophoblast differentiation in an invasive phenotype is vital to ensure proper placentation. Among the factors that may facilitate trophoblast invasion are matrix metalloproteinases (MMP). Besides, trophoblast cells modulate leukocyte recruitment and function to maintain an anti-inflammatory and immunosuppressant microenvironment. Vasoactive intestinal peptide (VIP) is a pleiotropic peptide with immunomodulatory effects through its action on VPAC1 and VPAC2 receptors. Using different murine models we have shown that VIP favors a suitable anti-inflammatory and immunosuppressant profile for placentation and fetal growth. Moreover, we have found that VIP deficient pregnant mice have reduced litter size, increase the period inter-gestations and present an asymmetric distribution of implantation sites. Here we studied the relevance of endogenous VIP at the early maternal-placental interface and its impact on the local expression of immune and trophoblast functional markers in VIP KO mice. We isolated implantation sites from C57BL/6 WT mated to VIP-/- or WT males at day 8,5 of pregnancy and analyzed immune markers by RTqPCR, the percentage of Treg cells by flow cytometry or used laser capture microdissection (LCM) to isolate trophoblast giant cells (TGC) for the analysis of VPACs expression, MMP9 and TGC markers by RTqPCR. Implantation site explants from WT mothers crossed with VIP -/- males presented a reduced percentage of Foxp3+ Treg cells within CD4+ cells (X±SEM: 10,3±1,7% WTxWT vs 5,2 ±1,3%WTxKO) and decreased IL-10 expression compared with WTxWT matings ($P<0.05$). VIP-deficient TGC isolated by LCM showed a two-fold increased expression of VPAC2 and three-fold decrease of MMP9 ($P<0.05$), a marker of trophoblast invasiveness. These results demonstrate that VIP deficiency in TGC might affect their invasive phenotype and support the role of endogenous VIP for maintaining an immunosuppressant microenvironment at early pregnancy.

582 (726) **INVOLVEMENT OF THE WNT/β-CATENIN PATHWAY IN THE OVULATION PROCESS**

Paula Accialini[1], Griselda Irusta[1], Diana Bas[1], Fernanda Parborell[1], Dalhia Abramovich[1], Marta Tesone[1,2],
1 Instituto de Biología y Medicina Experimental (IByME-CONICET). 2 Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires.

The evolutionarily conserved Wnt/β-catenin signal transduction pathway controls many biological processes. Previous results from our lab showed that the canonical Wnt pathway is involved in ovulation and luteinization. The objective of this study was to deepen in the role of the Wnt/β-catenin pathway in the ovulation process. To this purpose prepubertal eCG-treated rats were injected with a Wnt inhibitor (XAV939) or vehicle (DMSO, Control group) into the bursa of both ovaries the day of hCG administration. Two days after hCG and Wnt inhibitor or vehicle injection, ovaries were collected and fixed in Bouin solution for histological analysis. We found a significant decrease in the percentage of Corpora Lutea ($p<0.05$) and the appearance of cystic structures ($p<0.001$) in the ovaries injected with XAV939, reflecting a failure in the ovulation process. As angiogenesis is essential in the follicular-luteal dynamics, we first measured the endothelial and periendothelial cell area in ovarian sections stained with Lectin and Alpha-Actin, respectively. We found that the endothelial cell area is significantly decreased in ovaries injected with the Wnt inhibitor ($p<0.05$), with no changes in the periendothelial cell area. Our result shows that the ovarian vascular development is regulated by the canonical Wnt pathway. This suggests that impairment in the formation of a vascular bed after Wnt inhibitor treatment may influence the transportation of hormones and factors to the growing follicles, affecting the ovulation.

583 (839) **IMMATURITY CELL MARKERS IN SERTOLI CELLS: REGULATION OF CYP26B1 (RETINOIC ACID (RA)-DEGRADING ENZYME) AND ANTI-MüLLERIAN HORMONE (AMH) EXPRESSION BY ANDROGENS**

Nadia Yasmín Edelsztein[1, 2], Helena Fedora Schteingart[1], Rodolfo Alberto Rey[1, 3],
1Centro de Investigaciones Endocrinológicas "Dr. César Bergadá" (CEDIE) - CONICET - FEI, División de Endocrinología, Hospital de Niños Ricardo Gutiérrez. Buenos Aires, Argentina. 2 Dpto. de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. Buenos Aires, Argentina. 3Dpto. de Biología Celular, Histología, Embriología y Genética, Facultad de Medicina, Universidad de Buenos Aires. Buenos Aires, Argentina

RA-signaling responsable for meiosis initiation is temporarily blocked in fetal testis by CYP26B1. AMH expression in Sertoli

cells is inhibited by androgens at the onset of puberty. Little is known about these two phenomena. We sought to test if androgens have a direct effect on AMH and Cyp26b1 promoter activity through luciferase assays in prepubertal Sertoli cells (SMAT1) co-transfected with the androgen receptor (AR) and mutants of AMH or length variants of Cyp26b1 promoter. Results, expressed as percentage (mean±SEM), are compared against basal activity (theoretical value: 100%) using a one sample t-test. We have already shown the role of the proximal AMH promoter and SF1 recognition sequences in the inhibition caused by DHT (10-7M) (Edelsztein et al., SAIC 2015). Here, we show that DHT inhibition is reversed by treatment with the anti-androgen enzalutamide/ MDV3100 (10-5M) (90.7±5.3%;P=0.178, n=4), reaffirming the role of the AR in this inhibition. We further analyzed the inhibitory effect on AMH promoter activity by mutating the SF1 sites independently. Inhibition persisted, whether the site at -92b (49.3±5.3%;P=0.001, n=5) or -218b (54.8±6.4%;P=0.019, n=3) was mutated, indicating that annulment of both SF1 sites present in the AMH promoter is necessary to abrogate the inhibitory effect of androgens. Regarding Cyp26b1, a significant increase in promoter activity was observed only when cells were co-transfected with the AR and a Cyp26b1 construct containing ~9kb 5'-upstream of the first ATG (806.2±141.5;P=0.016, n=3) or 3kb (258.0±19.0;P=0.004, n=3), 2.2kb (356.8±66.7;P=0.031, n=3), 1.3kb (164.0±14.54;P=0.022, n=3) or 0.6kb (135.5±5.0;P=0.019, n=3) regions 5'-upstream of Cyp26b1 promoter; unveiling a regulation of Cyp26b1 by androgens. DHT treatment is able to alter Cyp26b1 and inhibit AMH promoter activity through the AR in SMAT1 cells. The latter requires at least one intact SF1 recognition sequence present in the proximal region of the AMH promoter to occur.

584 (866) **EXPRESSION OF MATRIX METALLOPROTEINASE 2 (MMP2) IN THE OVARY IN A BOVINE STRESS MODEL.**

Eduardo Matías Belotti[1], Antonela Stassi[1], María Melisa Velázquez[1], Valentina Matiller[1], Florencia Rey[1], Hugo Héctor Ortega[1], Natalia Raquel Salvetti[1], Leandro Perren[2],
1 Laboratorio de Biología Molecular y Celular Aplicada, Instituto de Ciencias Veterinarias del Litoral (ICiVet-Litoral), Universidad Nacional del Litoral (UNL) / Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Esperanza, Santa Fe, Argentina. 2 Médico Veterinario - Actividad Privada.

Dairy cattle are daily subjected to stressful situations some of which can be associated to reproductive problems. Under these conditions, the microenvironment of the ovary is modified, affecting directly normal follicular remodeling, necessary to ovulation. The aim of this study was to evaluate the matrix metalloproteinase 2 expression, enzyme that is involved in follicular wall tissue remodeling during ovulation process. MMP2 expression was evaluated by immunohistochemistry on ovarian samples of cows treated with adreconcorticotropin (ACTH) every 12 hours, for four days before ovulation time. Primary, small preantral, large preantral, antral, atresic and preovulatory follicles of treated animals (n=5) and control group (n=4) were evaluated. We detected that granulosa cells of preovulatory follicles of ACTH treated group presented higher expression than those of antral follicles of control group ($p<0.05$). Moreover, the theca interna cells of these preovulatory follicles showed a tendency to higher expression compared to the theca interna cells from antral follicles of control group ($p<0.09$). In large preantral and atretic follicles from treated group, we detected a tendency to increase the MMP2 expression in relation to control animals ($p=0.09$). These results might indicate that exist an increase of MMP2 expression in preovulatory follicles of ACTH treated group that could affect the follicular remodeling changes of preovulatory phase. Follicular wall degradation process (one of most important MMP2 roles), raised as a focal acute inflammatory response, depends on an equilibrated and complex mechanism, that could be affected by tissue metalloproteinases and their inhibitors.

585 (2053) **ANDROSTENEDIONE IN SUPERIOR MESENTERIC GANGLION INCREASE THE RELEASE AND METABOLISM OF PROGESTERONE IN ESTRUS. PARTICIPATION OF NITRIC OXIDE AND NOREPINEPHRINE.**

Agustina Leonela Orozco Reina[1], Mauco Gil Rosas[1], Adriana Soledad Vega Orozco[1], Marilina Casais[1],
1 Laboratorio de Biología de la Reproducción (LABIR). FQBF-UNSL, IMIBIO-SL, CONICET. San Luis. Argentina.

Corpus Luteum (CL) is an endocrine secretory gland whose main product is progesterone (P4), which is essential for maintenance during the estrous cycle. Previous works have shown that peripheral sympathetic ganglia have steroid receptors which are able to cause changes in ovarian steroidogenesis. However, at present little is known about the role of androstenedione (A2) in Superior Mesenteric Ganglion (SMG) on the formation and maintenance of CL in estrus. For this reason, the objectives were to demonstrate whether A2 in SMG modifies in ovary: 1) the P4 release 2) the gene expression of 3 β-HSD (RNAm and protein) and 20α-HSD (P4 synthesis and degradation respectively); and 3) whether such modifications are related with changes on NO, norepinephrine (NE) and on the expression levels of apoptosis markers such as Bax/Bcl-2, Fas/Fas-L and eNOS/ iNOS. The ex vivo SMG-Ovarian Nervous Plexus(ONP)-Ovary system was incubated with A2 added in ganglion. For this, we used a cuvette with two compartments with Krebs Ringer solution, pH 7.4, in a metabolic bath at 37°C. P4 (RIA), Nitrite (Griess method) and NE (HPLC) were determined at 15, 30, 60 and 120 min. The gene expression by RT-PCR at 120 min and protein levels by Western Blot. ANOVA-1 and Tukey test ($p<0.05$) was used. A2 in ganglion increased the ovarian P4 release in all times ($p<0.001$) according to an increase in the expression of 3 β-HSD and its protein ($p<0.05$); and a decreased 20α-HSD ($p<0.01$) was observed. Besides, increased the ovarian NO release ($p<0.001$) and expression of iNOS but NA decreased at 30, 60 and 120 min. No changes were observed in the expression of eNOS, Fas/Fas-L and in Bax/ Bcl2 ratio. These results demonstrate that A2, through of ONP, is able to increase the ovarian P4 synthesis and release, favoring the luteization due to an increase in NO levels and to a decrease of NE in ovary. These results may help to elucidate the role of A2 in hormone-dependent women pathologies as polycystic ovary.

586 (2054) **INVOLVEMENT OF MATRIX METALLOPROTEINASES AND THEIR INHIBITORS IN BOVINE CYSTIC OVARIAN DISEASE.**

María Belén Peralta[1], María Eugenia Baravalle[1], Eduardo Matías Belotti[1], Antonela Florencia Stassi[1], Natalia Raquel Salvetti[1], Hugo Héctor Ortega[1], Florencia Rey[1], Melisa María del Luján Velázquez[1],
1Laboratorio de Biología Celular y Molecular Aplicada. Instituto de Ciencias Veterinarias del Litoral (ICIVET-Litoral). Universidad Nacional del Litoral (UNL). Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET).

The most important regulators of tissue remodelling during ovarian follicular growth, development, ovulation and atresia are gonadotropins, steroid hormones, growth factors and different proteolytic enzymes. Matrix metalloproteinases such as collagenase or gelatinase (MMP-1, -8, -2, -9) and associated tissue inhibitors of metalloproteinases (TIMP-1, -2, -3 and -4) control connective tissue remodelling during follicular rupture. In this study, we hypothesized that an imbalance in the MMPs/TIMPs system may be an intraovarian component that contributes to the pathogenesis of cystic ovarian disease (COD) in cows. Taking into account that the control of MMP activity by TIMPs could determine their effects in both physiological and pathological conditions, MMP mRNA and protein expression was examined by real time PCR and immunohistochemistry, respectively, in ovaries from control cows and cows with COD. mRNA levels of MMP-2, TIMP-1 and TIMP-2 were lower in follicular cysts than in control preovulatory follicles ($p < 0,05$), while the results by immunohistochemistry showed this imbalance only for TIMP-2 protein expression ($p < 0,05$). Additional analysis by zymography to evaluate the gelatinase activity of MMP-2 and MMP-9 demonstrated higher MMP-2 activity in follicular fluid (FF) of cysts than in FF of preovulatory follicles ($p < 0,05$). On the other

hand, MMP-9 activity was increased in follicular cysts and absent in FF of preovulatory follicles. These findings suggest that the altered mRNA and protein expression of the MMP/TIMP system and enzymatic activity for MMP-9 may be related to the failure in ovulation and follicular development observed in COD.

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587 (786) **HOCL SCAVENGING STRUCTURES IN LIBRARIES OF NITRONES AND DITERPENES**

ER Verni[1], M Simirgiotis[2], J Borquez[2], L Thompson[3], E Aguilera[3], G Álvarez[3], L Celano[3], N Cataldo[3], M Gonzalez[3], H Cerecetto[3], AG Dias[4], D Costa[4], P Costa[4], DC Ramirez[1], SE Gomez Mejiba[1]

1Laboratory of Experimental Therapeutics, CONICET, San Luis, Argentina. 2Laboratory of Organic Chemistry, University of Antofagasta, Antofagasta, Chile. 3Laboratory of Chemistry, UFRJ, Rio de Janeiro, Brazil. 4Laboratory of Chemistry, UNLaR, Montevideo, Uruguay

Neutrophilic inflammation results from activation of neutrophils at sites of chronic or acute inflammation. Activated neutrophils release myeloperoxidase (MPO), the unique enzyme that uses H_2O_2 to oxidize chloride anions to the powerful oxidant HOCl. HOCl damaged proteins are seen and involved in a number of inflammatory diseases. Thus the search for inhibitors of MPO and/or scavengers of HOCl is of vital importance for the treatment of neutrophilic inflammation. The aim of this work is the search for highly characterized synthetic or natural structures that react with HClO/ClO^- (hypochlorous acid/hypochlorite, Ka = 7.58). We searched HOCl scavenging activity in 55 synthetic and natural compounds: 47 nitrones and 8 natural diterpenes. For this purpose, we used a screening assay to evaluate the ability to reduce luminescence caused by reaction of HClO with luminol. The variables were optimized and the method was developed for a concentration of 10 µM luminol and 50 µM HClO. Compound dilutions were prepared (0, 0.1, 1, 5, 10 and 25 µM, corresponding "0" to a HClO solution only) and then HClO and luminol solutions were added. Results showed that from the 8 diterpenes compounds, only one lessened the signal below 1 µM concentration. The rest of them probed to have this activity just above that value. Whereas, nitrones showed interesting properties: 13 showed inhibitory activity in the range of 5-10 µM, 28 compounds in 1-5 µM, 4 compounds between 0.1 and 1 µM, and finally two nitrones showed an interesting activity below 100 nM. Scavenging HOCl at sites of neutrophilic inflammation may be an interesting bioactivity forfinding new leader structures in drug discovery. Further studies aimed at determining toxicity, cell permeability, mechanism of action and in vivo activity are guaranteed for leading compounds. Supported by PROICO 2-3214 & PICT-2014-3369 (to DCR), PROICO 10-0414 (To SEGM) and PIP2015-2017 112215-0100603CO (To DCR, SEA & SEGM).

588. (429) **RENAL EXPRESSION AND URINARY EXCRETION OF THE ORGANIC ANION TRANSPORTER 5 (OAT5) IN RATS WITH OBSTRUCTIVE NEPHROPATHY**

Romina Valeria Campagno[1], Evangelina Cecilia Nosetto[1], María Julia Severin[1], Anabel Brandoni[1], Adriana Mónica Torres[1]

[1]Area Farmacología. Facultad De Ciencias Bioquímicas Y Farmacéuticas. Universidad Nacional De Rosario. CONICET.

The Organic Anion Transporter 5 (Oat5) is an apical protein located in the proximal tubule cells. Oat5 recognizes a wide spectrum of substrates, and mediates the transport of estrone/dehydroepiandrosterone sulfate, toxins (ochratoxin A) and therapeutic drugs such as nonsteroidal antiinflammatory, diuretics, and penicillin G. Little is known about the regulation of Oat5 in pathological situations. The purpose of this study was to evaluate the renal expression and the urinary excretion of Oat5 in male Wistar rats with obstructive nephropathy. Bilateral ureteral occlusion (B) was induced by ligation of both ureters for 5 h (B5, n=6). The studies were performed after 24 h of ureteral releasing. In parallel a Sham group (Sh, n=11) was processed. The creatinine clearance (Cl_{Cr}) was determined by conventional clearance techniques. Renal expression in homogenates (H), brush border membranes (BBM), and urinary abundance of Oat5 (U) were evaluated by electrophoresis and Western blotting (* $p<0.05$). Results: Mean ± SEM. Cl_{Cr} (mL/min.100g): Sh= 0.47 ± 0.03, B5=0.37 ± 0.03*; $Oat5_H$ (%): Sh= 100 ± 10, B5= 72 ± 7*; $Oat5_{BBM}$ (%): Sh= 100 ± 4, B5= 50 ± 7*; $Oat5_U$ (%): Sh= 100 ± 3, B5= 359 ± 13*. The treated animals showed a significant decrease in Cl_{Cr} confirming the establishment of the pathology. An increase in urinary abundance of Oat5 was observed. Moreover, the expression in both homogenates and BBM were significantly decreased in B5 animals. A significant decrease in renal expression of Oat5 might be explained by its exocytosis into the tubular lumen, as it is corroborated by its increase in urinary excretion. The alteration in the renal expression of this transporter in rats with obstructive nephropathy could lead to modifications in the renal handling and consequently in the pharmacokinetics of drugs carried by this protein.

589 (134) **THE 5-LIPOXYGENASE (5-LO) INHIBITOR ZILEUTON (ZI) AFFECTS THE EXPRESSION OF EARLY GENES INVOLVED IN RAT LIVER REGENERATION.**

Florencia Lorenzetti[1], Marina Cecilia Vera[1], Juan Alberto Monti[1], María Paula Ceballos[1], Juan Pablo Parody[1], Gerardo Bruno Pisani[2], María Teresa Ronco[1], María Cristina Carrillo[1,2], Ariel Darío Quiroga[1,2], María de Luján Álvarez[1,2]

[1]instituto De Fisiología Experimental (IFISE-CONICET). [2]área Morfología, Facultad De Ciencias Bioquímicas Y Farmacéuticas, (UNR)

Leukotrienes (LTs) are inflammatory eicosanoids synthesized via the 5-LO pathway. The main LTs -LTB4 and LTC4- are involved in cell proliferation and the liver plays a central role in their synthesis and metabolism. Previous studies from our lab showed that Zi treatment (a 5-LO inhibitor) caused a decrease in LTs content and a reduction in liver proliferation after partial hepatectomy (PH) in rats. Objectives: 1) to study the impact of Zi treatment on the expression of liver regeneration-associated early genes in rats, and 2) to analyze the effect of Zi in cell proliferation using human hepatoma cell lines. Methods and Results: In vivo studies: Adult male Wistar rats were subjected to sham surgery or PH (70% liver removal). Two hours before surgery, animals received Zi 40 mg/kg BW or vehicle. Rats were sacrificed 1 and 5 hours post-PH. Lipid peroxides levels-determined by TBARS- were 43%* lower in Zi treated PH group than in vehicle-treated PH group. NF-kB activity was 80%* lower in Zi-treated PH compared to vehicle-treated PH rats. Immunoblot analysis of nitric oxide synthase-2 (NOS-2), a critical player for tissue permeabilization and vascularization during liver regeneration, showed a 52%* reduction in Zi-treated PH rats compared to the vehicle-treated PH animals. In vitro studies:HepG2 and HuH7 human hepatoma cell lines were treated with Zi 30 and 50 µg/mL for 48 hours. MTT assays showed that Zi at a dose 50 µg/mL reduced the proliferation of HepG2 and HuH7 cells in 16%* and 37%* respectively. Furthermore, the presence of LTB4 increased the viability of HuH7 cells treated with Zi (*$p<0.05$). Conclusion: Altogether, these results demonstrate that Zi reduces rat liver regeneration, probably by diminishing oxidative stress production and, consequently, altering the NF-kB pathway. The reduction of NF-kB activity, in turn, can ultimately affect the expression of NOS-2. Our preliminary studies in human hepatoma cells show that Zi is able to reduce cellproliferation.

590 (502) **GERANIOL (GO) PREVENTS INTESTINAL MRP2 DOWN-REGULATION IN RATS WITH FRUCTOSE-INDUCED METABOLIC SYNDROME (MS)**

Felipe Zecchinati[1], Ana Sofía Londero[1], Maite Rocío Arana[1], Guillermo Nicolás Tocchetti[1], Virginia Gabriela Perdomo[2], María Manuela Barranco[3], Anahí Cilenzo[3], Aldo Domingo Mottino[1], Fabiana García[3], Silvina Stella Maris Villanueva[1]

[1]Instituto De Fisiología Experimental (IFISE-CONICET)-Facultad De Ciencias Bioquímicas Y Farmacéuticas-Universidad Nacional De Rosario. Rosario, Santa Fe,

Argentina. [2]Instituto De Biología Molecular Y Celular De Rosario (IBR-CONICET)-Facultad De Ciencias Bioquímicas Y Farmacéuticas-Universidad Nacional De Rosario. Rosario, Santa Fe, Argentina. [3]Instituto De Inmunología (CONICET)- Facultad de Ciencias Médicas-Universidad Nacional de Rosario. Rosario, Santa Fe, Argentina

Intestinal Mrp2 is an ABC transporter that limits the absorption of xenobiotics orally ingested, thus acting as a biochemical barrier. MS is a pathological condition characterized by insulin resistance, hyperinsulinemia and dyslipidemia as well as by chronic inflammation and oxidative stress (OS). In previous studies we observed that MS-like conditions induced by fructose in drinking water (10% v/v, during 3 weeks: FRU), reduced the expression and activity of intestinal Mrp2 in rats. We here evaluated the effect of GO (250 mg/kg/day; p.o), a monoterpene with antioxidant and anti-inflammatory properties, in preventing fructose-induced Mrp2 alterations. After 2 weeks of co-treatment, GO prevented down-regulation of Mrp2 protein evoked by MS, as detected by western blotting (C: 100%, FRU: 23%, FRU-GO: 107%, P<0.01). Mrp2 activity was evaluated using the in vitro model of everted intestinal sacs. Efflux of the Mrp2 substrate DNP-SG was decreased in FRU rats (-43%) respect to C (P<0.05), whereas Mrp2 activity in the co-treated group remained unchanged. Additionally, fructose generated OS in intestinal tissue as indicated by increasing lipid peroxidation products (+50%, P<0.01) and activity of the antioxidant enzyme SOD (+40%, P<0.01), respect to C, and by decreasing GSH/GSSG ratio (-42%) respect to C (P<0.05). Additionally, fructose increased the intestinal level of the pro-inflammatory cytokines IL-1β (+58%, P<0.05) and IL-6 (+59%, P<0.01) respect to C. Interestingly, GO not only reversed the parameters of SM studied, but also normalized the intestinal index of oxidative stress and IL-1 β and IL-6 levels in the FRU group. Administration of GO alone did not affect the parameters studied. Conclusion: the data indicate that OS and inflammation could be important mediators of Mrp2 down-regulation under MS-like conditions, and that GO could be a potential therapeutic tool to prevent the impairment in the intestinal barrier by counteracting the effect of such mediators.

591 (355) **ANTIHISTAMINES AND CORTICOIDS CO-TREATMENT RATIONALE. POTENTIATION OF GLUCOCORTICOIDS ANTI-INFLAMMATORY EFFECTS**

C. Daniel Zappia[1], Soto Ariadna[2], Gina Granja-Galeano [1], Natalia Fernandez[1], Carina Shayo[3], Alejandra Goldman [2], Carlos Fitzsimons [4], Federico Monczor [1.]

[1]instituto De Investigaciones Farmacológicas (ININFA), FFYB, UBA. CONICET. Argentina. [2]CESYMA, ECYT, Universidad Nacional De San Martín. [3]lab De Patología Y Farmacología Molecular, IBYME-CONICET. ARGENTINA. [4]swammerdam Institute For Life Sciences, University Of Amsterdam. The Netherlands.

Antihistamines and glucocorticoids are used to treat many inflammatory conditions, such as allergic rhinitis and asthma, and in many cases they are co-administered. However, this association has no clear rationale and has arisen from clinical practice. We have previously reported the potentiating effects of histamine H1 receptor signaling on glucocorticoid receptor (GR) activity in vitro. Here we evaluated the effect of the H1 antihistamine azelastine (Aze) on GR transcriptional activity induced by Dexamethasone (Dex). HEK293T cells were co-transfected with plasmids coding for GR, H1R and a GR-responsive luciferase reporter plasmids to study transactivation (TAT3-Luc) or transrepression (IL6-Luc) of responsive genes. In both cases, preincubation with 10 μM Aze induced a three-fold increment in the response elicited by 0.1 nM Dex (p<0.01). To further understand this modulation we studied genes involved in inflammatory processes in pathophysiologically relevant cell lines. In lung A549 cells and in promonocytic U937 cells, 10 μM Aze increased tenfold Dex-induced MKP1 expression and IL8, COX2 and GMCSF repression, respectively (p<0.05). Finally, we tested the clinical relevance of this modulation on a murine model of asthma. Balb/c mice were ip sensitized and airway challenged with ovoalbumin, and then treated with Dex alone (1 mg/kg optimal dose or 0.1 mg/kg suboptimal dose) or in combination with 0.5 mg/kg Aze. Vehicle and Aze alone-treated mice were used as experimental controls. We found that levels of allergen-specific immunoglobulin IgE and bronchoalveolar lavage eosinophilia were reduced only in animals treated with 1 mg/kg Dex or co-treated with 0.1 mg/kg Dex + Aze compared to allergic control mice (p<0.05). From a therapeutic point of view, our results suggest that the potentiating effect of Aze on Dex response could result in the reduction of the Dex doses needed to reach anti-inflammatory effects, particularly in asthma.

592 (719) **POTENTIATION OF HISTAMINE H1 RECEPTOR CALCIUM RESPONSE BY RATIONALLY DESIGNED GRK2 INHIBITORS**

Emiliana Echeverría[1], Maia Cabrera[1], Ezequiel Juritz[2], Federico Monczor[1], Carlos Davio[1], Carina Shayo[3], Pablo Lorenzano-Menna[4], Natalia Fernandez[1,5.]

[1]Instituto De Investigaciones Farmacológicas, Facultad De Farmacia Y Bioquímica, UBA, ININFA-CONICET-UBA. [2] Centro De Bioinformatica Y Biologia Integrativa, Facultad De Biologia, Universidad Andres Bello, Santiago, Chile. [3]instituto De Biología Y Medicina Experimental-CONICET. [4]Laboratorio De Oncología Molecular, UNQUI. 5 Cátedra De Química Medicinal, FFYB, UBA.

G protein-coupled receptors (GPCRs) are the largest family of cell-surface receptors and regulate most biological processes. Because approximately 30% of therapeutic drugs directly target GPCRs, it is of paramount importance to understand the mechanisms that regulate their function. GPCR kinase 2 (GRK2) plays a major role mediating desensitization through phosphorylation dependent and independent mechanisms and has been associated to the progression of several pathologies. Our aim was to obtain GRK2 inhibitors based on a rational drug design approach. Using the crystallographic image of GRK2, 13 compounds were chosen by virtual screening (VS) according to their docking energy and purchased to the supplier. Considering that histamine H1 receptor (H1R) desensitization depends on GRK2 activity, we evaluated the ability of the selected compounds to block H1R desensitization. Histamine-stimulated intracellular calcium release was tested in A549 cells endogenously expressing H1R using Fura2AM dye. 40 minutes incubation with 100nM of 3 of the selected compounds potentiated calcium response. Moreover, the incubation with these compounds reverted H1R desensitization induced by 10 minutes histamine pretreatment. To mitigate the risk of a future failure of the compounds due to a potential toxic effect in late phases of drug development, cytotoxicity was evaluated by trypan blue exclusion on hepatic HEPG2 and hematopoietic derived U937 cells after 48hs of treatment. Concentration response assays showed that the compounds that displayed cytotoxicity presented a CC50 value greater that 10uM. These results indicate that at concentrations used for calcium assay the compounds display no cytotoxicity though they inhibited GRK2 mediated H1R desensitization. Based on these observations, 3 of the candidates obtained by VS are promissory inhibitors for the treatment of pathologies where GRK2 mediated desensitization of GPCRs takes place.

ENDROCRINOLOGÍA I/ ENDOCRINOLOGY I

593 (271) **EFFECTS OF PROLONGED EXPOSURE TO GROWTH HORMONE (GH) AND ITS ONCOGENIC POTENTIAL IN MAMMARY GLAND**

Mariana A. Bojorge[1], María Eugenia Díaz[2,3], Johanna G. Miquet[1,3], Verónica Piazza[1,3], Ana I. Sotelo[1,3], Daniel Turyn[1,3], Lorena González

[1] Departamento de Química Biológica Facultad de Farmacia y Bioquímica Universidad de Buenos Aires, Junín 956 C1113AAD, Ciudad Autónoma de Buenos Aires, Argentina [2] Área Química Biológica, Departamento de Ciencias Básicas, Universidad Nacional de Luján, Buenos Aires, Argentina. [3] Instituto de Química y Fisicoquímica Biológica (IQUIFIB)- CONICET

Growth hormone (GH) is produced by the pituitary gland and it is involved in longitudinal growth promotion and metabolic processes. GH exerts many of its physiological functions directly and indirectly through the synthesis and release of insulin-like growth factor I (IGF-I). GH and IGF-I have an essential role in promoting normal growth and breast development. However, the GH/IGF-I axis has also been widely associated with mammary tumorigenesis. The aim of this work was to study the cellular and molecular effects of high GH levels in mammary gland that could be linked to the mitogenic effects of the hormone. Two animal models were used, transgenic mice that overexpress GH and normal mice treated with injections of the hormone in supraphysiological doses during one week. In order to study the molecular effects, the protein content of receptors involved in cell proliferation such as the GH receptor (GHR), the epidermal growth factor receptor (EGFR), the receptor for insulin like growth factor (IGF-IR) and estrogen receptor alpha (ERalpha) were assed. Additionally, the protein expression of transcription factors that induce cell proliferation like c-Myc, c-Fos and c-Jun and the mRNA levels of cyclin D1 were studied. The cellular effects of GH were analyzed by histological analysis. The results showed a significant decrease in protein content of GHR in transgenic mice and a marked increase in IGF-IR and EGFR levels in both animal models. No significant differences in the protein content of the estrogen receptor were verified. The levels of c-Fos were increased in transgenic mice and in animals treated with GH during one week, but c-Myc and c-Jun were not modified by high GH levels. Regarding cyclin D1 mRNA amount, transgenic mice showed an increased compared with normal mice. The histological analysis showed breast epithelial hyperplasia in the transgenic mice but no histological effects of exogenous GH administration were evidenced.

594 (319) **THE NATURAL ANTIOXIDANT NARINGIN IMPROVES BONE METABOLISM IN EXPERIMENTAL TYPE I DIABETES MELLITUS**

Gabriela Picotto[1], Valeria Rodriguez[1], Maria Angelica Rivoira[1], Ricardo Battaglino[2], Nori Tolosa de Talamoni[1],
[1] Biochemistry and Molecular Biology, INICSA (CONICET-UNC), Argentina, [2]Forsyth Institute, Boston (MA), USA

Diabetes mellitus (D.m.) is usually related to a reduced bone mineral density (BMD) and low bone remodeling that cannot be improved by insulin administration. As D.m. also produces oxidative stress, our hypothesis is that bone alterations may be associated with redox changes and if so, this could be avoided by an antioxidant therapy like naringin. Adult male Wistar rats were used: 1) controls, 2) diabetic rats treated with 60 mg/kg/bw of streptozotocin (STZ), 3-4) STZ rats treated with 40 or 80 mg/kg/bw/day of naringin for 30 days. Histomorphometry, BMD and content (BMC) and microcomputarized tomography were analyzed (μCT). We also determined vitamin D status and other systemic parameters of calcium metabolism. Bone marrow was studied for gluthatione content (GSH), catalase activity (CAT), while adipocyte and osteoclast (OC) numbers were counted from histological sections. Calcitriol and osteocalcin levels were reduced by STZ. Naringin returned osteocalcin values to control ones. STZ rats presented low BMD and BMC in distal femur and proximal tibiae, and the highest dose of naringin avoided this effect. STZ group presented reduced bone volume, thickness, trabecular number and intertrabecular spaces. All these changes were overcome with naringin-80. Diabetic rats had increased adipocytes and OC numbers and low GSH concentration and high CAT activitity. All these changes were prevented with naringin. In summary, our results suggest that naringin, a low cost antioxidant, protects the bone osteolytic effects triggered by insulin deficiency. Osteocalcin and redox status normalization and the reduction in the number of adipocytes and OC suggest that naringin is acting as a possible bone protector for experimental type 1 D.m.

595 (448) **MODULATORY EFFECT OF TESTOSTERONE ON THE THERMOGENIC ACTIVITY OF BEIGE ADIPOCYTES FROM RETROPERITONEAL ADIPOSE TISSUE**

Alejandro Ezequiel Harnichar[1], María Guillermina Zubiría[1], María Amanda Rey[1], Eduardo Spinedi[2], Andrés Giovambattista[1].
[1] Instituto Multidisciplinario de Biología Celular (IMBICE) CICPBA-CONICET-UNLP. [2]Centro de Endocrinología Experimental y Aplicada (CENEXA) CONICET-UNLP

It is well known that androgens modulate adipose tissue (AT) distribution and function. We earlier showed the effect of the lack of testosterone (T) on retroperitoneal AT (RPAT) function from orchidectomized rats and the inhibitory action of T on the adipogenic potential of adipocyte precursor cells (APCs) in vitro. We now studied the effect of T, in vivo and in vitro, on the thermogenic activity of beige adipocytes from RPAT. For this aim, RPAT pads were dissected from adult male control (CTR), pre-pubertally orchidectomized (ODX) and pair-fed control (CTR-PF) rats. Pads were processed for histology analysis and UCP-1 quantification (RT-PCR). In addition, APCs from adult male rats were isolated and cultured up to confluence, then cells were induced to differentiate (4 days) with a pro-browning cocktail in the absence or presence of 0.1 μM T (basal (B) or T, respectively); thereafter cells remained in culture medium without or with T. On differentiation day 8, we added 10 μM forskolin (FSK) for 4 hs to a subset of B or T cells: B without FSK (B-B), B with FSK (B-FSK), T without FSK (T-B), T with FSK (T-FSK). Cells were then processed to quantify UCP-1. We found that T decreased UCP-1 expression in in vitro differentiated adipocytes ($p<0.01$, T-B vs B-B). As expected, FSK increases UCP-1 gene expression in RPAT adipocytes (B-B vs B-FSK and T-B vs T-FSK, $p<0.01$). However, FSK-induced UCP-1 expression was low in T-treated cells ($p<0.01$, T-FSK vs B-FSK). On the other hand, UCP-1 gene expression in RPAT from ODX rats was high ($p<0.05$, ODX vs CTR and CTR-PF). Histological analysis indicated the presence of small adipocytes in RPAT from ODX rats ($p<0.05$, ODX vs CTR and CTR-PF) and the appearance of multivacuolar, beige-like adipocytes. We conclude that T could be modulating the thermogenic program of beige adipocytes from RPAT by regulating UCP-1 gene expression. (PIP 0198; FPREDM052015).

596 (462) **REGULATION AND ACTION OF THYROID HORMONES DURING EARLY PREGNANCY IN RATS**

Estefanía Rinaldini[1], Fiorella Campoverde[1], Verónica Penas[2], Carlos Gamarra Luques[1,3], María Belén Hapon[1,2]
[1]IMBECU CCT Mendoza CONICET. [2]Facultad de Ciencias Exactas y Naturales. UNCuyo [3]Facultad de Ciencias Médicas. UNCuyo.

One limiting processes of reproductive success is implantation. During this period steroid hormones (E2 and P4) stimulate the synthesis of vascular endothelial growth factor (VEGF-A), the main modulator of angiogenesis during peri-implantation period. Our recent data show that hypothyroidism affects the process of implantation in the rat. Therefore, the goal of this investigation was to elucidate the mechanisms by which thyroid hormones (THs) act at uterine level during implantation. For this purpose, were used adult female rats (Wistar). Hypothyroidism was induced by administration of propylthiouracil in the drinking water. Factors related to the regulation of THs transport into the cell interior, intracellular metabolism and differential expression of their receptors were determined by RT-qPCR. The interaction between THs and the mechanism of action of E2, P4 and angiogenesis, were studied during implantation in utero by Western blot. The results of this study indicated that the mRNA expression of the deiodinase enzyme 2 and thyroid hormone receptors (Thra and Thrb) decreased significantly ($p <0.05$); while Slc7a8, SLC7A5 and Slc16a10 transporters and deiodinase enzyme 3 showed no difference in hypothyroid rats. Inversely, protein expression of steroid receptors PGRA and ESR2 and VEGFA increased significantly ($p <0.05$) while ESR1 and PGRB showed no differences in hypothyroid rats. In conclusion, this study allowed us to establish that hypothyroidism alters components of THs signaling, and on the other hand affects the response to E2 and P4 and regulation of angiogenesis in uterine tissue during implantation. Therefore,

the presence of adequate levels of THs might be essential for the implantation process and subsequent fetal development.

597 (525) **EFFECT OF OLIGONUCLEOTIDE IMT504 IN MALE AND FEMALE NON-OBESE DIABETIC (NOD) MICE, A MODEL OF SPONTANEOUS DEVELOPMENT OF AUTO-IMMUNE INSULIN DEPENDENT DIABETES MELLITUS**

Stefania Bianchi[1], Alejandro Montaner[2], Norma Alejandra Chasseing[1], Milena Massimino[1], Dan Perez[1], Carlos Libertun[1,3], Victoria Adela Lux-Lantos[1], Maria Silvia Bianchi[1],
[1] Instituto de Biología y Medicina Experimental, Buenos Aires, Argentina. [2] Fundación Pablo Cassará (ICT Milstein-CONICET), Buenos Aires, Argentina. [3]Facultad de Medicina, Universidad de Buenos Aires. Buenos Aires, Argentina.

We have shown that the immunomodulatory oligonucleotide IMT504 induces marked recovery of single-dose streptozotocin (STZ)-induced toxic diabetes in rats, correlating with early expression of progenitor cell markers (1), without altering immune parameters (2). IMT504 also improves diabetes in an immunodependent model induced by multiple low doses of STZ in mice, diminishing glycemia (Gly) and reducing leukocyte islet infiltration (3). Here, we evaluated the effect of IMT504 on spontaneous autoimmune diabetes using NOD mice. Mice were considered diabetic after two consecutive non-fasted Gly levels ≥ 230 mg/dl. Diabetics were treated with 4 series of 5 IMT504 doses (20mg/kg/day, sc) (IMT) or saline as control (DC) with 2 resting days between series. After one week, glucose tolerance tests (GTT) were performed (2g/kg BW glucose ip). Five days later mice were sacrificed, blood samples and pancreases collected for hormonal determinations and histological studies. Spontaneous reversion of the diabetic condition was observed in 33% of male (M) and female (F) mice whereas 70% of M and 78% of F improved their Gly after IMT treatment. In M, Gly increase was lower in IMT-treated mice vs. DC [repeated measures ANOVA; interaction ns, treatment and time effects, $p<0.05$]. In F, Gly increase was only observed in DC [repeated measure ANOVA: interaction, $p<0.001$, DC Gly (mg/dl): day 1: 313± 23 vs day 35: 421± 74, $p<0.05$ vs IMT treated: day 1: 287± 20 vs day 35: 171± 16, $p<0.05$]. GTTs showed a partial recovery in glucose clearance in the IMT groups in both sexes [M (AUC): IMT: 2126± 96 vs DC: 2502± 75 Test t: $P<0.05$. F (AUC): IMT: 1898± 106 vs DC: 2921±148 Test t: $P<0.01$]. We demonstrate that IMT504 treatment promotes an improvement in the diabetic condition in male and female NOD mice warranting further investigation of its mechanism of action.

598 (665) **HIGH AROMATASE (ARO) TRANSCRIPT VARIANT EXPRESSION IN HUMAN PLACENTAL TISSUES FROM PRETERM DELIVERIES AND TERM DELIVERIES OF LARGE FOR GESTATIONAL AGE (LGA) NEWBORNS.**

Alan Carballo[1], Dolores Polop[1], Andrea Servian[1], Cristina Patricia Nemer[3], Claudia Cannizzaro[3], Paula Aliberti[2], Romina Sainz[1], Marco Aurelio Rivarola[2], Alicia Belgorosky[2], Nora Saraco[2]
[1] Servicio de Endocrinología, Hospital de pediatría "JP Garrahan", Buenos Aires. [2] Servicio de Endocrinología, Hospital de pediatría "JP Garrahan", Buenos Aires, CONICET. [3]Programa de Diagnóstico y Tratamiento Fetal, Hospital de pediatría "JP Garrahan" y Hospital Materno Infantil Ramón Sardá, Buenos Aires.

Aro is the key enzyme for estrogen biosynthesis from androgens and in human placenta (Pl) is expressed exclusively in syncytiotrophoblast. It has been reported that small newborns and large newborns as well as patients with Aro deficiency tend to increase the prevalence of metabolic syndrome in adulthood. We previously described a splicing variant of Aro mRNA (Intron9) that translates into inactive Aro protein. Our aim was to analyze Aro mRNA variants expression in Pl from preterm (PT) (<35 weeks) and term LGA compared to term adequate for gestational age (AGA) newborns. We proposed that Aro mRNA variants expression is involved in Aro activity regulation and hence in intrauterine estrogen-androgen balance. Total RNA was isolated from Pl of PT (GA: 30-35,n=4), LGA (GA: 39-41,n=8) and AGA (GA: 37-38 and 39-41,n=8 and n=11). Aro mRNA variants were analyzed by Real-time RT-PCR with primers for total (TotAro, Ex2-Ex3), intron 9 (IN9, Ex8-In9) and active (ActAro, Ex9-Ex10) Aro, and Cyclophilin (PPIA) as housekeeping gene. Statistics (Student test) were performed on ΔCt data. TotAro was higher in PT vs AGA (8.91±3.35 vs 1.58±0.40 AU,mean ± SE),while was lower in LGA vs AGA (0.81±0.36 vs 2.12±0.64), $p<0.05$. Analysis of each transcript variant related to total Aro showed that ActAro/TotAro ratio was higher in PT (2.26±0.26 vs AGA: 0.66±0.20) and in LGA (2.42±0.31 vs AGA: 1.37 ± 0.28), $p<0.05$. Not significant difference was found for IN9/TotAro in LGA compare to AGA. The high Active Aro mRNA expression, in preterm placentas agrees with reports of maternal salivary estriol and plasma estradiol increments in preterm parturition suggesting a role of Pl Aro modulating Pl estrogen production associated to prematurity. In addition, the higher ActAro/TotAro ratio observed in LGA vs AGA, suggest that the variation of the estrogen-androgen balance in Pl tissue might be involved not only in prematurity but also in fetal programming determining disorders later on the postnatal life.

599 (647) **HUNTING MUTATIONS, TARGETING DISEASE**

Maria Ines Perez Millan[1], Debora Braslavsky[2], Ana Keselman[2], Ignacio Bergada[2], Jacob Kitzman[3], Sally Camper[3], Adriana Seilicovich[1],
[1]INBIOMED, UBA-CONICET, Buenos Aires, Argentina. [2]CEDIE, CONICET, Hospital de Niños Ricardo Gutiérrez, Buenos Aires, Argentina. [3]Department of Human Genetics, University of Michigan, Ann Arbor, US.

Congenital multiple pituitary hormone deficiency (MPHD) arises from defects in pituitary development and is sometimes associated with craniofacial abnormalities. Our objectives are to understand this disease pathophysiology and to improve molecular diagnosis and treatment of MPHD. Mutations in at least eight genes have been found to cause hypopituitarism and up to 20 genes have been implicated but there is not yet enough evidence to prove pathogenicity. The overwhelming majority of patients do not have identified mutations in any of these genes. Our hypothesis is that the genetic factors that cause hormone deficiency are oligogenic and represent a collection of genes expressed in the developing embryonic pituitary, midline, and hypothalamus. We set up a new approach based on Molecular inversion probe (MIP) capture. We developed a refined version of the single-molecule molecular inversion probe (smMIP) capture assay. In this assay, a mixed pool of MIP capture probes is added to individual genomic DNA samples in a single-well reaction. Each probe is designed with two arms flanking a targeted region, such that when a probe anneals to its targeted genomic fragment, a polymerase copies the sequence between the flanking arms, and a ligase joins this copied sequence to the probe backbone. We established a panel of 67 genes associated with MPHD in humans and mice. This panel targets 693 coding exons. In our MIP experiments, we obtained deep coverage over targeted regions, with approximately 2.1 million reads per individual, 97.6% of targeted bases reach ≥8X read depth coverage and 95.1% of bases reach ≥40X, such that up to 125 individuals could be readily pooled for sequencing on a single HiSeq lane. We believe that identifying these potential variants will make it feasible to predict clinical outcomes from genetic data, which is necessary for patient diagnosis and prognosis, and for assessing the risk of future affected individuals.

600 (948) **L-3,4-DIHYDROXYPHENYLALANINE (L-DOPA) MODULATES HYPOTHALAMUS-PITUITARY-ADRENAL (HPA) AXIS RESPONSE AT PITUITARY LEVEL**

Santiago Jordi Orrillo[1], Nataly de Dios[1], Mariela Moreno Ayala[1], Sandra Zárate[1], Florencia Gottardo[1], Jimena Ferraris[1], Daniel Pisera[1],
[1]INBIOMED - Instituto de Investigaciones Biomédicas, UBA - CONICET, Facultad de Medicina, UBA Paraguay 2155, Ciudad Autónoma de Buenos Aires, Argentina

L-Dopa is the leading treatment of Parkinson's disease. This drug crosses the blood brain barrier and is converted to dopamine

(DA) by the aromatic L-amino acid decarboxylase (AADC) in the central nervous system (CNS) and peripheral tissues. Thus, L-Dopa is co-administrated with peripheral AADC inhibitors in order to enhance its availability in the CNS. Evidences suggest that neuroendocrine stress response is altered in Parkinson's disease. In addition, L-Dopa treatment increases the ACTH and cortisol secretion probably due to CRH neurons activation by dopaminergic receptors D1 and D2. The aim of the present research was to determine the effects of the co-treatment with L-Dopa and an inhibitor of AADC (NSD 1015) in the renewal of corticotropic cells. First, the expression of AADC was studied by immunofluorescence. We observed in primary culture of rat anterior pituitary cells the enzyme is expressed in corticotropes. Also, pituitary melanotropes from intermediate lobe producing POMC derived peptides express AADC. Likewise, AtT20 cells (a mouse corticotropic cell line) were immunoreactive for AADC. To study the effects of L-Dopa on cell apoptosis, AtT20 cells were incubated with L-Dopa (1mM, 8 h) in the presence of NSD (1mM). Apoptosis was determined by TUNEL assay. L-Dopa increased the percentage of TUNEL-positive cells, but the concomitant inhibition of AADC revealed an antiapoptotic effect of L-Dopa (C: 3.78; L-Dopa: 5.96; NSD: 3.69; L-Dopa+NSD: 2.24, $p<0.01$, c2). Our results suggest that L-Dopa per se has an antiapoptotic effect on AtT20 cells. Given that these cells express AADC, the actions of L-Dopa observed in the absence of NSD may be due to its local DA conversion. These pituitary actions may be involved in the altered response of HPA axis observed in patients with Parkinson´s disease treated with L-DOPA.

601 (1058) **TEMOZOLOMIDE MODULATES WNT/BETA CATENIN SIGNALING IN PROLACTINOMA CELLS**

Gianina Demarchi[1], Sofía Valla[1], Nadia Bonadeo[1], Daiana Vitale[1], Laura Alaniz[1], Silvia Berner[2], Carolina Cristina[1],
[1]centro De Investigaciones Básicas Y Aplicadas CIBA CITNOBA CONICET-UNNOBA. [2] Servicio De Neurocirugía, Clínica Santa Isabel

Prolactinomas are the most frequent pituitary adenomas. Some of them become refractory to conventional therapies turning into aggressive tumors. Wnt signaling plays a role in cell renewal, tumorigenesis and chemoresistance. Previous results of our group demonstrate expression of βCATENIN in a cohort of human pituitary adenomas including prolactinomas and activation of the Wnt pathway in experimental lactotroph hyperplasia. The use of the alkylating agent Temozolomide to treat aggressive prolactinomas is recent with successful results in some cases. Here we aimed to study the activation state of Wnt pathway in basal conditions and under Temozolomide treatment in the MMQ prolactinoma cell line. Cells were treated with Wnt3a ligand (1ng/ml) and/or Temozolomide (200uM) along 48 hs. Cell proliferation was evaluated by MTT and mRNA and protein levels by real time PCR and Western Blot respectively. Basal expression of Frizzled receptors, Lrp correceptor and βCATENIN, CYCLIND1 and C-MYC components were determined. We observed an increased cell proliferation under Wnt3a treatment indicating a possible role of Wnt pathway in the development of these tumors. In our experimental model Temozolomide decreased cell proliferation and Prolactin and Vegf synthesis. The angiogenic capability of Temozolomide treated or untreated cells was evaluated in a scratch assay with endothelial cells which showed reduced migration compared to controls. Temozolomide reduced mRNA expression of the Wnt pathway components βcatenin, the target gene CyclinD1, and the activated βCATENIN ($p<0,05$), then Temozolomide could be interfering with canonical Wnt signaling in prolactinomas. Wnt and Temozolomide combined treatment was not able to increase the MMQ proliferation as Wnt3a alone did, indicating an effect of the chemotherapeutic drug on the Wnt3a proliferative capability. Our data suggest an antitumoral effect of Temozolomide associated with a reversion of the Wnt proliferative effect in prolactinomas.

602 (1059) **NON-CLASSICAL TESTOSTERONE SIGNALING IMPLICATIONS IN PROSTATE SMOOTH MUSCLE CELL PROLIFERATION AND MUSCLE CELL PHENOTYPE.**

Nahuel Peinetti[1], Carolina Leimgruber[1], Mariana Cuello Rubio[1], Maria Victoria Scalerandi[1], Juan Pablo Nicola[2], Amado Alfredo Quintar[1], Cristina Alicia Maldonado[1],
[1] Centro de Microscopía Electrónica, Instituto de Investigaciones en Ciencias de la Salud (INICSA-CONICET), Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Ciudad Universitaria, Córdoba, Argentina. [2]Centro de Investigaciones en Bioquímica Clínica e Inmunología, Centro de Investigaciones en Bioquímica Clínica e Inmunología-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Ciudad Universitaria, Córdoba, Argentina.

Testosterone (T) effects are mediated by a classical pathway that takes place through the binding of T to the classical androgen receptor (AR) and migration to the nucleus, and by non-classical signaling pathways which are mediated by cell surface receptors capable of activating signaling cascades. The differentiation and proliferation of pSMC as well as the stroma/epithelium interaction are essential in the development of prostatic pathologies. Our objectives were to determine the presence of plasma membrane AR receptors in pSMC and their participation in cell differentiation, proliferation and growth factors expression. pSMC were obtained from Wistar rat prostate and stimulated in vitro with T 10-7M or the plasma membrane impermeable T-bovine serum albumin conjugate (T-BSA) 10-7M. Smooth muscle markers and the expression of FGF7 and TGFβ were evaluated by qPCR, and cell proliferation was assessed by immunocytochemistry of Ki67, and confirmed by cell counter; statistical analysis was performed by ANOVA-Tukey. The AR plasma membrane localization was demonstrated by immunofluorescence and confocal microscopy, and by co-localization with the membrane marker concanavalin-A. This assay was verified by flow cytometry, which showed a population of membrane AR positive cells of 18.87 ± 2.43%. After T-BSA stimuli, an increased expression of smooth markers calponin and α-actin was observed ($p<0.01$ vs ctrl), while vimentin RNAm was decreased ($p<0.01$ vs ctrl). T-BSA also increased cell proliferation after 24 or 48 hours stimulation ($p<0.05$ vs ctrl and T). Meanwhile, T increased FGF7 and TGFβ, implicated in proliferation and apoptosis of epithelial cells respectively, at higher levels than T-BSA ($p<0.01$). These results demonstrated the presence of membrane receptors and the participation of non-classical signaling mediating T-induced pSMC proliferation and differentiation; genomic effects were stronger modulators of growth factors implicated prostate cell communication.

603 (399) **CHARACTERIZATION OF MEMBRANE PROGESTERONE RECEPTORS (MPRS) IN THE PITUITARY. NOVEL ROLE OF mPRα IN THE FUNCTION OF LACTOTROPH POPULATION.**

Maria Andrea Camilletti[1]; Erika Yanil Faraoni[1]; Alejandra Inés Abeledo-Machado[1]; Maria Cecilia Bottino[1]; Jimena Ferraris[2]; Daniel Pisera[1]; Graciela Susana Díaz-Torga1
[1]IByME-CONICET, [2]INBIOMED

Progesterone (P4) function on lactotroph population is controversial, being found proliferative and anti-proliferative effects. Our main objective is to study the involvement of P4 membrane receptors (mPRs) in lactotroph physiology and pathology. We first characterized the relative expression of P4 receptors (PRs) by qRT-PCR in pituitary glands of Sprague Dawley female rats in diestrus. Among all PRs, the PR-A represent the 62,4%, PR-B 23,3% and the mPRs represent the 14,3%. mPRα was the most abundant mPR isoform in the pituitary gland (40,8% of total mPRs). Then we conducted double immunofluorescence studies (mPRα; PRL) and flow cytometry analysis for characterization of this receptor in lactotroph population. We found that 54% of total pituitary cells are mPRα+. The 65% of lactotroph population is mPRα+. We next conducted a functional study with a mPR agonist: Org OD 02-0, Axon Medchem (OD). In an ex vivo experiment we incubated female mouse pituitaries in medium without (control) or with OD 100nM during 30 min. We measured the secreted PRL and the pituitary PRL content by RIA assay. We measured

by specific ELISA, the content of active TGFβ1, potent inhibitor of lactotroph function. OD induced strong increase in the pituitary levels of active TGFβ1. In accordance, OD induced a decrease in PRL secretion with concomitant increase in PRL content, reflecting the inhibition of PRL secretion. Finally, we measured the mPRβ expression in two experimental models of prolactinoma: rats with chronic estradiol treatment and 8-month female mice with disruption of dopamine-type-2 receptor. In both models of prolactinoma the mPRβ expression (q-RT-PCR) was found reduced compared with their respective controls. We postulate that the inhibitory effects of P4 on lactotrophs are mediated, at least in part, by mPRs, inducing TGFβ1 activation. mPRβ expression was found decreased in experimental models of prolactinomas, a fact that could be involved in tumor development

604 (838) **ESTROGEN RECEPTOR β REGULATION BY ESTRADIOL AND ITS IMPACT ON PITUITARY CELL PROLIFERATION**

Pablo Aníbal Pérez[1], Florencia Picech[1], Liliana Del Valle Sosa[1], Juan Pablo Petiti[1], Ana Lucía De Paul[1], Alicia Inés Torres[1], Silvina Gutiérrez[1]

[1] Centro de Microscopía Electrónica. FCM-UNC. INICSA-CONICET

Estrogens are involved in the modulation of multiple cellular processes, exerting their effects through specific estrogen receptors (ER) α and β. ER expression levels strongly influence the estrogenic activity in a tissue or cell. The aim of this study was to determine the pituitary ERβ expression, analyze its role and the molecular mechanisms involved in the pituitary cell proliferation. For this purpose the following models were used: 1)in-vivo: anterior pituitary glands from Wistar rats (females at different estrous cycle stages, ovariectomized (OVX) and chronically stimulated with estradiol (E2) for 20, 40 and 60 days and male rats) and 2)in vitro: GH3 cells transfected to ERβ overexpression. E2 and ERβ (PPT) and ERβ (DPN) agonists were employed. Proliferation (BrdU technique) and DNA content (Propidium Iodide) were quantified. ERβ and β, cyclin D1 (CD1), Akt (t and p) and PTEN expression were determined by Western blot and quantified by flow cytometry. Subcellular localization was analyzed by immunofluorescence and electron microscopy. ANOVA-Tukey was used ($p<0.05$).

In vivo assays: The ERβ expression decreased significantly in estrus compared to the other cycle stages. A total inhibition of ERβwas observed with chronic E2 stimulus, while in OVX, increased reaching levels similar to those found in male. The ERα/β index increased in estrus and after chronic E2. In OVX, this index decreased significantly to a level similar to male. PTEN showed the same expression pattern that ERβ.

In vitro assays: ERβ overexpression in GH3 significantly inhibited cell proliferation and the S+G2/M population, reduced the expression of endogenous ERβ, CD1 and Akt phosphorylation, while increased PTEN levels, inducing its subcellular redistribution and increasing its nuclear expression.

These results suggest that E2 downregulates ERβ expression, which inhibit pituitary cell proliferation by decreasing ERβ and CD1 expression and inducing PTEN expression with consequent Akt inhibition

605 (1062) **INITIAL EXPERIENCE OF MOLECULAR STUDIES IN SPORADIC AND FAMILIAL MEDULLARY THYROID CANCER**

Agustín Saus[1], María Natalia Gonza[2], Norma Noemí Tolaba[3], Paola Bazzoni[4], Marcelo Monteros Alvi[3,4], Leopoldo Van Cauwlaert[5], Marcelo Nallar[5], Valeria Cerioni[1], Macarena Galindez[1], Christian Martín Moya[3]

[1]Programa de Endocrinología. [2]Programa de Medicina Nuclear. [3]Sector de Biología Molecular, [4]Sector de Anatomía Patológica, Programa de Diagnóstico y Tratamiento. [5]Programa de Cirugía. Hospital de Endocrinología y Metabolismo, Dr. Arturo Oñativia. Salta

Medullary thyroid cancer (MTC) is a neoplasm derived from C-cells and is responsible for a high proportion of deaths in thyroid cancer. MTC can occur sporadically or as part of multiple endocrine neoplasia (MEN2) syndrome. Hereditary forms account for 25% of cases and are due to activating mutations in the RET proto-oncogene (95-98%). Frequent mutations in HRAS and KRAS genes in sporadic MTC with normal RET has been reported. RET gene has no hotspot mutation sites, for that reason there is not commercial kits. So, when a mutational screening technique is not available, it must be sequenced the most exons of the gene.

Aims: To implement a molecular screening technique that helps the identification of pathogenic mutations, variants of uncertain significance (VUS) and single nucleotide polymorphisms (SNP). To use this molecular technique for study patients with both sporadic, familial MTC and/or MEN2.

Different molecular techniques were tested, High Resolution Melting (HRM) was developed for the identification of point mutations in RET (exon:3,5,8,9,10,11,13,14,15,16), HRAS and KRAS (exon:2,3). So far, 4 patients with sporadic MTC, 1 patient with MTC and Hirschsprung disease and his parents, and 2 families with familial MTC (FMTC) and MEN2A, one of 14 and another of 11 members were studied.

DNA from 32 patients obtained from peripheral blood (in some cases paraffin) was analyzed by HRM. Three pathogenic mutations, 1 VUS and 5 SNP all in RET, and 1 SNP in HRAS were identified. The 3 mutations were found in familial cases and in patients with Hirschsprung. No pathogenic mutations were identified in any of the sporadic MTC in RET, HRAS or KRAS genes.

HRM technique demonstrated to be a rapid, sensitive, specific and reliable method for detecting genetic variants. The molecular studies in MTC and MEN2 are important because they allow stratification diagnosis based on mutation carriers, early prevention and even avoid the onset of cancer by performing prophylactic thyroidectomy

ONCOLOGÍA III / ONCOLOGY III

606 (797) **IN VITRO ANTITUMOR PROPERTIES OF A TRIAZOLYL AMINOACYL(PEPTIDYL) PENICILLIN IN MURINE MELANOMA CELLS**

Yanina Bellizzi[1], Leonor Roguin[1], Viviana Blank[1], Patricia G. Cornier[2], Carina M. L. Delpiccolo[2], Dora B. Boggián[2], Ernesto G. Mata[2], Osvaldo Rey[3]

[1]IQUIFIB (UBA-CONICET) [2]Instituto de Química Rosario (CONICET-UNR) [3]INIGEM, CONICET

The triazolyl aminoacyl(peptidyl) penicillins (TAP) are novel hybrids compounds having in their structure a penicillanic core linked to a peptide portion via a triazole group. In a previous study, we showed that the derivative containing the dipeptide Leu-Phe (TAP6) exerts an antiproliferative potency around 30 times higher in HeLa (human cervix adenocarcinoma) and B16-F0 (murine melanoma) cells with respect to non-malignant cells. In this work, after exploring a wider panel of human and murine tumor cell lines, we showed that IC50 values obtained in human cells varied between 3-12 μM, while IC50 values ≥ 20 μM were determined in various murine cells except B16-F0, that showed an IC50= 3,5±0,3 μM. B16-F0 cells were then selected to investigate the mechanism of antitumor action of TAP6. By flow cytometry, we showed that TAP6 slowed down the cell cycle at the S phase and increase the percentage of early (10±3%) and late (45±4%) apoptotic cells. A significant enhancement (1,5-3 fold) of the expression levels of some hallmark proteins of the endoplasmic reticulum (ER) stress response was detected by Western blot after 6h of incubation. In addition, we found a reduction of the expression levels of Bcl-XL (0.40±0.05) and Bcl-2 (0.7±0.2), and an increase in the amount of the pro-apoptotic Bax protein (1.8±0.5). A time-dependent increment in the activity of caspase-3, -8 and -9 was also observed. The loss of the mitochondrial inner transmembrane potential was determined by flow cytometry, being the percentage of cells with reduced DiOC63 incorporation ~30% after 3h of TAP6 exposure. Taken together, our results indicated that TAP6 is a potent and selective antitumor agent in different tumor cell lines. TAP6 activates an ER stress response that contributes together with the

mitochondrial pathway to the induction of an apoptotic response. Even though we showed an increment of caspase-8 activity, the involvement of the death receptor pathway remained to be studied.

607 (780) **ROLE OF SURVIVIN IN MODULATING AUTOPHAGY IN RESPONSE TO U0126, A MAPK INHIBITOR, IN PANCREATIC TUMOR CELLS**

Martín Levermann[1], Cintia Yamila Mihalez[1,2], Mariángeles Diaz[2], Susana Costantino[1,2], Matías Pibuel[1,2], Silvina Laura Lompardía[1,2], Tomás Lombardo[1,2], Elida Alvarez[1,2], Daniela Laura Papademetrio[1,2]

[1]Cátedra de Inmunología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires [2]IDEHU, CONICET

Pancreatic cancer is one of the most difficult neoplastic pathologies to treat, with high resistance to both radio and chemotherapy. The lack of an effective protocol makes extremely important any approach to the understanding of these diseases. Because of that, we propose to study the relationship between autophagy, a mechanism launched by pancreatic tumors to evade the pro-apoptotic effects of a wide range of compounds, and survivin, one of the IAPs proteins, expressed on an elevated rate in these tumors. We studied the capability of U0126, an inhibitor of the survival pathway MAPK, to modulate autophagy in MIAPaCa-2WT and MIAPaCa-2surv-/-, a derivate cell line knock down for surivivin. First, we demonstrated by western blot that U0126 increased survivin levels in MIAPaCa-2WT cells ($p<0.001$). This increment is returned by the pre-treatment with 3-MA, an inhibitor of autophagy. Then, to evaluate the interaction of survivin and autophagy process, we obtained a derivate cell line knock down for survivin by employing an appropriated shRNA. With both cell lines, MIAPaCa-2WT and MIAPaCa-2surv-/- we performed TUNEL assays to evaluate the response to U0126. We demonstrated that the inhibition of autophagy sensitized MIAPaCa-2WT cells to the pro-apoptotic effect of 5-10uM of U0126 ($p<0.001$). Interestingly, this sensitizing effect in absent in MIAPaCa-2surv-/-, where the percentages of tunel+ cells are not different between those obtained by treatment with U0126 alone or after incubation with 3-MA ($p>0.05$). Moreover, these values are not different from those obtained by treatment of MIAPaCa-2WT cells with 3MA + U0126 ($p>0.05$). In addition, we observed decreased levels of LC3-IIB in MIAPaCa-2surv-/- vs MIAPaCa-2WT by western blot ($p<0.001$) and a reduction in the autophagosoms number in cells transfected with RFP-LC3 in knock down cell line vs WT. Our results suggest that survivin could mediate the capability of autophagy to offer chemoresistance to pancreatic MIAPaCa-2 cells.

608 (796) **PHOTOTOXIC ACTION OF A ZINC(II) CATIONIC PHTHALOCYANINE ON MURINE MELANOMA B16F0 CELLS**

Federico Valli[1], María Cecilia García Vior[2], Nicolás Chiarante[1], Josefina Awruch[2], Leonor Patricia Roguin[2], Julieta Verónica Marino[1]

[1]IQUIFIB (UBA-CONICET) [2] Departamento de Química Orgánica, Facultad de Farmacia y Bioquímica-UBA

Malignant melanoma is the most aggressive form of skin carcinoma, which possesses fast proliferation rate and highly invasive characteristics. Phthalocyanines (Pcs) are synthetic photosensitizers with potential application in photodynamic therapy. In order to find an efficient photosensitizer to be used in melanoma treatment, we study the effect of a sulfur-linked cationic zinc(II) phthalocyanine named Pc13 on murine melanoma B16F0 cells. While no citotoxicity was observed by MTT assay when cells were incubated with Pc13 in the dark, cell viability diminished in a concentration-dependent manner upon exposure to a light dose of 9.2 Jcm^{-2}, being the IC_{50} value of 0.19 ± 0.09 μM. The production of ROS in Pc13-loaded cells was demonstrated immediately after irradiation with the probe DCFH-DA. When cells were pretreated with 5 mM of the antioxidant trolox, cell viability was completely recovered. A cytosolic localization of Pc13, that emits a red fluorescence after exciting at 633 nm, was revealed by confocal microscopy. The presence of picnotic nuclei in Pc13-treated cells was also detected after Hoechst staining. In order to elucidate the induction of the mitochondrial pathway, Pc13-treated cells were irradiated and incubated with DiOC6 probe. Mitochondrial depolarization was observed 1 h post irradiation by fluorescence microscopy and flow cytometry. We also detected lower levels of poly-ADPribose-polymerase (PARP), a caspase-3 substrate involved in DNA repair. In addition, a rapid phosphorylation of p38, JNK and Erk1/2 MAPK, and a decrease of p-Akt levels were observed after irradiation of Pc13-loaded cells. Taken together, these results indicate that the phototoxic effect exerted by Pc13 on melanoma B16F0 cells is mediated by the formation of ROS and the induction of an apoptotic response characterized by mitochondrial depolarization, PARP cleavage and nuclear shrinkage. The role of kinases in the phototoxic action will be further investigated.

609 (501) **CTBP1 EXPRESSION DIMINUTION ON PRIMARY TUMOR IMPAIRS DEVELOPMENT OF SPONTANEOUS LUNG METASTASES ON A PROSTATE CANCER AND METABOLIC SYNDROME MODEL**

Guillermo Nicolás Dalton[1], Cintia Massillo[1], Juliana Porretti[1], Georgina Scalise[1], Paula Lucía Farré[1], Paola De Luca[1], Adriana De Siervi[1].

[1]Laboratorio de Oncología Molecular y Nuevos Blancos Terapéuticos - IByME– CONICET

Metabolic syndrome (MeS) increases prostate cancer (PCa) risk. C-Terminal Binding Protein (CtBP1) is a transcriptional corepressor that is activated by NADH binding. Previously our group established a MeS and PCa mice model that identified to CtBP1 as a novel link associating both diseases. We found that CtBP1 diminished the capability of PCa cell lines to adhere to a collagen matrix, repressing the epithelial marker CDH1 and inducing the mesenchymal marker VIM expressions. Death from PCa is not caused by the primary tumor, but rather the formation of metastasis; therefore, early diagnosis and prevention of metastasis in patients is of high relevance. Our aim was to investigate MeS/CtBP1 impact over PCa progression using an in vivo model of spontaneous PCa metastasis. NSG mice were fed with control or high fat diets during 12 weeks to induce MeS. Then PC3-CtBP1 depleted expression (PC3.shCtBP1) or -control (PC3.PGIPZ) cells were injected s.c. on MeS and control animals. Body weight and tumor size were measured 1 and 3 times a week, respectively. Thirty days after cell inoculation, tumors were around 1 cm^3, with no significant differences between treatments; however mice showed around 20% weight loss. Hence, mice were sacrificed and tumors, lungs and livers were collected for RNA isolation and histopathological analysis. Using human GAPDH specific primers in RT-qPCR from lungs, we found that CtBP1 depletion led to a dramatic decrease of lung metastases regardless of diet. In addition, H&E from lungs identified to PC3.shCtBP1/MeS group as the mice with the lowest metastatic lesions. Gene expression comparison between primary tumors and metastases showed that CtBP1 and cadherins mRNA levels were decreased in metastases. Our study uncovers for the first time the role of CtBP1 in PCa progression and its molecular targets in MeS mice.

610 (377) **CTBP1 AND METABOLIC SYNDROME INDUCE BREAST CANCER TUMOR PROGRESSION AND METASTASIS**

Paula Lucía Farré[1], Guillermo Nicolás Dalton[1], Rocío Belén Duca[1], Georgina Scalise[1], Cintia Massillo[1], Juliana Porretti[1], Adriana De Siervi[1], Paola De Luca[1]

[1]Laboratorio de Oncología Molecular y Nuevos Blancos Terapéuticos - IBYME – CONICET

Breast cancer (BrCa) is a main worldwide public health problem. Metabolic syndrome (MeS) increases the incidence and aggressiveness of BrCa. C-terminal binding protein 1 (CtBP1) is a co-repressor of tumor suppressor genes that is activated by low NAD+/NADH ratio. Recently, we found that CtBP1 and MeS induced breast carcinogenesis and tumor growth using a MeS-like mice model. We also showed that CtBP1 and MeS decreased BrCa cell adhesion, a crucial process in the beginning of metastasis.

The aim of this work was to explore CtBP1 and MeS role in BrCa cell migration and metastasis. By wound healing assay, we found that CtBP1 increased cell migration of MDA-MB-231 and 4T1 BrCa cells. MeS nude mice induced by chronically feeding animals with high fat diet and control diet, were injected with CtBP1-depleted expression or control MDA-MB-231 cells. Six weeks post-injection primary tumors were excised by surgery and, 2 weeks later, mice were sacrificed. Consistently with the onset of metastasis, MeS increased the number of mice that developed ascites (50% in MeS vs 20% in control). Tumor cells (TC) in ascites, lung and liver were detected by RT-qPCR using specific primers for human GAPDH. We found that MeS increased TC in liver. In addition, CtBP1 hyperactivation by MeS significantly increased lung metastasis. Interestingly, human Vimentin mRNA was induced in TC from ascites compared to primary TC; while it was diminished in lung. Finally, we analyzed expression of cell adhesion and EMT-related genes in primary tumor tissue by RT-qPCR. We found that CtBP1 and MeS modulated cell adhesion and EMT expression genes: Vimentin, Slug, ITGB4, ITGB6, Col17A, FABP4 and PRSS2. Altogether, these results suggest a key role for MeS and CtBP1 inducing BrCa EMT and metastasis.

611 (339) **MIRNAS EXPRESSION PROFILE INDUCED BY CTBP1 PROTEIN IS CRITICAL FOR TUMOR GROWTH AND PROGRESSION OF BREAST CANCER ASSOCIATED TO METABOLIC SYNDROME**

Rocío Belén Duca[1], Paula Lucía Farré[1], Guillermo Nicolás Dalton[1], Juliana Porretti[1], Georgina Scalise[1], Cintia Massillo[1], Bruno Berardino[2], Adriana De Siervi[1], Paola De Luca[1]
[1]Laboratorio de Oncología Molecular y Nuevos Blancos Terapéuticos - IByME - CONICET [2]Laboratorio de Neuroepigenética - Departamento de Química Biológica - FCEyN – UBA

Breast cancer (BrCa) is the leading cause of cancer death in women and metabolic syndrome (MeS) constitutes a risk factor for this disease. C-terminal binding protein 1 (CtBP1) is a co-repressor of tumor suppressors activated by low NAD+/NADH ratio. Recently, we generated a MeS-like experimental model by chronically feeding mice with high fat diet. We found that CtBP1 and MeS induced breast carcinogenesis and tumor growth. Since miRNAs function as master regulators of cellular processes, the aim of this work was to identify the miRNA expression profile associated to BrCa in MeS mice. GeneChip miRNA 4.0 (Affymetrix) was hybridized to RNA from CtBP1-depleted or control MDA-MB-231 xenografts generated in MeS mice. After data normalization, we identified 42 CtBP1 regulated miRNAs. Gene ontology analysis of miRNAs predicted target genes determined using the bioinformatic tool ChemiRs, revealed enrichment in several biological processes, such as fatty acid biosynthetic process, cell cycle, cell migration and epithelial to mesenchymal transition. Furthermore using miRSystem tool we identified several CtBP1-regulated miRNAs involved in tumor growth (miR-381-3p, miR-146a-5p and miR-378a-3p) and tumor progression (miR-223-3p, miR-146a-5p, miR-494-3p, miR-381-3p, miR-433-3p, miR-522-3p, miR-940, miR-378a-3p). To validate these results we assessed miRNAs expression levels in CtBP1-depleted or control MDA-MB-231 xenografts by miRNA-RT-qPCR. CtBP1 induced miR-146a-5p and let7e-3p expression while repressed the expression of miR-378a-3p. Finally, we also generated 4T1-derived allografts in Balb/c mice with MeS or control, and miRNAs expression in tumor samples analyzed by miRNA-RT-qPCR showed that MeS induced miR-378a-3p, miR-146a-5p and let-7e-3p. These results show that both, CtBP1 and MeS, drives miRNAs expression which might be helpful as biomarkers for diagnosis, management and therapy of BrCa patients.

612 (612) **NUCLEAR INTERACTION OF ER-PR AFTER LIGAND-INDEPENDENT ACTIVATION BY FGF2**

Sebastian Giulianelli[1,2], María Alicia Gorostiaga[2], Ana Catarina Guerreiro[3], Francisco Amado[3], Luisa Helguero[4], Claudia Lanari[2].
[1]IBIOMAR - Centro Nacional Patagónico – CONICET [2]Instituto de Biología y Medicina Experimental – CONICET [3]QOPNA - Universidad de Aveiro, Portugal. [4]iBiMED - Universidade de Aveiro, Portugal

Ligand-independent activation of estrogen receptors alpha (ERα) and progesterone receptors (PR) in breast cancer is one of the mechanisms triggering hormone independent tumor growth. We have demonstrated that fibroblast growth factor 2 (FGF2) induces cell proliferation and tumor growth in experimental models of breast cancer, involving ERα and PR activation.The interaction of both receptors at the genomic level at the c-Myc promoter has been described after activation with their natural ligands. The aim of this study was to investigate the interaction of both receptors after incubation with FGF2. Nuclear co-localization between ERα and PR, assessed by confocal microscopy, was observed in human breast cancer T47D cells treated with FGF2. We next analyzed the interaction of the protein interactome to a well-defined progestin and estrogen sensitive region of c-Myc promoter. After biotin-DNA pull-down assays of nuclear extracts from FGF2-stimulated and non-stimulated cells, proteins were identified by liquid chromatography (LC)-tandem mass spectrometry (MS/MS) approach. Using a label-free relative quantitation (emPAI), we identified several proteins including PR, ERα, FOXA1, DDX17, DDX5, NCL and HLTF grouped in gene ontology (GO) biological process termed “positive regulation of transcription from RNA polymerase II promoter”. Finally, specific binding of PR/ERα/FOXA1 was detected at the same FGF2-sensitive regions at the c-Myc gene promoter after chromatin immunoprecipitation analysis ($p<0.001$). Our findings indicate that PR and ERα interact, after ligand-independent activation, in a complex that binds chromatin regulating gene transcription. This event may have important implications for therapeutic interventions.

613 (811) **ROLE OF THE IMMUNE SYSTEM IN ANTIPROGESTIN-INDUCED MAMMARY TUMOR REGRESSION**

Gonzalo Ricardo Sequeira[1], Ana Sahores[1], Tomas Dalotto-Moreno[1], Silvia Vanzulli[2], Laura Polo[1], Virginia Novaro[1], Caroline Lamb[1], Mariana Salatino[1], Claudia Lanari[1].
[1]Instituto de Biología y Medicina Experimental, [2]Academia Nacional de Medicina

The role of the immune system in the regression of mammary carcinomas (MC) using endocrine therapies has been poorly investigated. We have shown that mifepristone (MFP, antiprogestin) induces the regression of murine MC that express progesterone receptors regardless of the immune system. Characterization of the immune cells in regressing tumors may provide clues related to their role in the prevention of tumor regrowth. Our aim was to characterize the infiltrating cells in MFP-treated MC and to evaluate whether MFP was able to protect against tumor growth in a tumor re-challenge assay after complete tumor resection. Bone marrow (BM) cells from BALB/c-GFP+ mice were iv inoculated into immunodeficient NSG mice to establish the NSG/BM-GFP+ mouse model. 59-2-HI tumors, originated in BALB/c mice, were inoculated into NSG or NSG/BM-GFP+ female mice. MFP pellets (0.2 mg) were implanted sc when the tumors reached 50 mm^2. Tumors were excised after 3 or 6 days and isolated cells analyzed by flow cytometry. An increase in T lymphocytes (CD8+) and in macrophages (CD11b+ F480+; $p<0.01$), and a decrease in the T_{reg} subpopulation (CD4+ CD25+ Foxp3+; $p<0.05$), was observed in MFP-treated tumors. In the re-challenge assay, tumors were excised and pellets removed 6 days after MFP treatment. Animals were orthotopically re-inoculated with 59-2-HI tumors in the opposite flank 5 days after surgery. Sham operated animals were used as controls. All secondary transplants growing in untreated mice reached 200 mm^2 before day 47 while only 50% of those mice previously treated with MFP reached this size, $p<0.05$. The reduced intratumor T_{reg}/CD8+ ratio observed in regressing tumors might be used as a predictive marker of treatment response. In sum, the data presented herein agree with the hypothesis that regressing tumors expose intracellular antigens that generate a protective immune memory response, which could be associated with the long free relapse survival induced by endocrine therapy.

614 (320) **ANALYSIS OF APOPTOSIS RELATED GENES IN THE DEVELOPMENT AND PROGRESSION OF PROSTATE CANCER**

Conrado Marco Olivieri[1], Federico Pablo Cantor[1], Elba Vázquez[1], Geraldine Gueron[1], Javier Cotignola[1].
[1]Laboratorio de Inflamación y Cáncer, IQUIBICEN/Dpto. de Química Biológica, CONICET/FCEN-UBA

Evasion of apoptosis is considered one of the hallmarks of cancer cells. However, it is now known that apoptotic cells can release factors that influence the proliferation, survival, movement and morphogenesis of neighboring cells and tissues. Prostate Cancer (PCa) is one of the most studied tumors in the last decade, and there is a growing interest in understanding the role of apoptosis during PCa development and progression. We aimed to analyze the expression of genes involved in apoptosis using gene expression microarrays and RNAseq data from public repositories. We included different prostate tissues in the analysis (healthy normal, adjacent non-tumoral, primary tumor and metastasis). We found that several genes coding for proteasome subunits (e.g. PSMA3, PSMB5, PSMD4) were significantly under-expressed in the adjacent non-tumoral and primary tumor tissues compared to the healthy normal tissue. Statistical significant differences in PSM proteins were not observed between primary tumor and adjacent non-tumoral tissues. These results suggest that PSM proteins might be an early event during PCa development. RNAseq experiments allowed us to compare the expression of apoptosis-related gene between different clinico-pathological features (tumor stage, biochemical relapse (BCR) and Gleason score). We found 35 genes with differential expression between patients with and without BCR. The top 3 deregulated genes were: BAD (fold-change (FC)=1.65, $p=2.3x10^{-5}$), GZMB (FC=0.14, p=6.3 x10-5) and TRADD (FC=1.43, $p=7.1x10^{-5}$). Finally, we performed ROC (Receiver Operating Characteristics) analysis to determine whether the inclusion of expression profiles improves BCR prognosis. The Area Under Curve (AUC) was 0.815 when the pre-surgical PSA and Gleason score were considered. When we added the gene expression in the models, the AUCs were 0.92 (BAD) and 0.99 (BAD+GZMB). This results suggest the analysis of apoptosis-related genes expression could improve the prognostic of PCa relapse.

615 (416) **CLINICAL RELEVANCE OF ANNEXIN 2 IN PROSTATE CANCER BONE METASTASIS AND ITS ASSOCIATION WITH HEME-OXYGENASE 1**

Nicolás Anselmino[1], Alejandra Páez[1], Daiana Leonardi[1], Emiliano Ortiz [1], Javier Cotignola[1], Geraldine Gueron[1], Elba Vazquez[1].
[1]Laboratorio de Inflamación y Cáncer, IQUIBICEN/Dpto. Química Biológica, CONICET/FCEN-UBA

HO-HO-1 is a critical mediator of cellular homeostasis. In prostate cancer (PCa) HO-1 may have a regulatory role beyond its enzymatic activity. We screened for HO-1 interacting proteins using a proteomics approach.PC3 cells were transiently transfected with FLAGHO-1, treated with H2O2 and the immunoprecipitated protein complexes were subjected to LC-ESI MS/MS. Proteomics analysis revealed the presence of annexin2 (ANXA2) among HO-1 interacting proteins. Interestingly ANXA2 was significantly downregulated across the Oncomine database in prostate adenocarcinoma vs. normal prostate gland, lying within the 13 lowest ranked genes, with a P-value of 0.003. We also screened for HO-1 and ANXA2 expression in PCa tissue micro arrays (TMAs). When assessing HO-1 across the different TNM staging system, the rate of positive cases was higher in T2-T3 N0M0 compared to T2-T3 with spreading to regional lymph nodes or the presence of distant metastasis. A reduction in ANXA2 positive staining was detected between normal prostate and adenocarcinoma. Given that bone is the most common homing organ in PCa metastasis, we assessed ANXA2 expression levels using a co-culture transwell system of PC3 and the pre-osteoclastic Raw264.7 cell lines. When cells were co-cultured, ANXA2 mRNA levels were detected significantly upregulated in PC3 cells and diminished in Raw264.7. Immunofluorescence analysis has shown there was a clear re-localization of ANXA2 in Raw264.7 towards the cell cytosolic compartment under co-culture conditions, with a concomitant reduction in cell membrane immunostaining. Interestingly, hemin pre-treatment of tumoral cells prevented these effects. Given the tight association between ANXA2 and cell adhesiveness, HO-1 induction in PCa cells may force bone cells to keep ANXA2 in the cell membrane, preventing the attachment of tumoral cells to the bone niche, impairing bone metastasis.

616 (991) **MICRORNAS REGULATING EMT TRANSCRIPTION FACTORS ARE DIFFERENTIALLY EXPRESSED IN BREAST CANCER TUMORS WITH LYMPH NODE METASTASIS**

Elisa Pérez-Moreno[1], Gabriela Valarezo[1], Valentina Zavala[1], Wanda Fernández[2], Pilar Carvallo[1].
[1]Departamento de Biología celular y molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica De Chile. Santiago, Chile. [2]Unidad de Anatomía Patológica, Hospital San Borja Arriarán. Santiago, Chile

Breast cancer is the leading cause of cancer-associated deaths in women. Lymph nodes near to the primary breast tumor have a high chance of developing a secondary tumor, representing one of the first signs of metastasis. Metastasis is promoted by Epithelial-Mesenchymal transition (EMT), process leaded by the transcription factors SNAIL, SLUG, ZEB and TWIST. MicroRNAs are small non-coding RNAs, whose expression has been demonstrated to be altered in different cancer types. Because their ability to regulate large sets of genes involved in cancer growth and metastasis, microRNAs have emerged as candidate molecular biomarkers and novel therapeutic targets. The aim of this study was to identify differentially expressed microRNAs in breast tumors with lymph node metastasis, and that were involved in epithelial-mesenchymal transition. For this, we used microRNA microarray data from 50 fresh frozen breast tumors with different tumor grades (1 to 3), 28 from patients with lymph node metastasis. Microarray data was analyzed using RankProd R package, and in silico analysis were performed to identify predicted targets for microRNAs. Also, transcription factors expression was evaluated by immunohistochemistry. Microarray analysis revealed 17 microRNAs differentially expressed between tumors with different lymph node status ($p<0.05$), and 10 of them are predicted to regulate the expression of the EMT transcription factors. Five microRNAs (miR-197, miR-574, miR-181c, miR-409 and miR-663) have an inverse correlation with the transcription factors expression, supporting their predicted regulation. Our results suggest that deregulation of specific microRNAs, and therefore their targets, could induce a metastatic behavior of tumor cells of the primary breast tumors, promoting invasion and colonization of the lymph nodes. In this sense, the changes in the expression of microRNAs may serve as biomarkers and/or prognosis in breast cancer patients.

617 (985) **DIFFERENTIAL EXPRESSION OF EPITHELIAL TO MESENCHYMAL TRANSITION TRANSCRIPTION FACTORS IN BREAST CANCER TUMORS IN RELATION TO LYMPH NODE STATUS**

Victoria Ortega-Hernández[1], Patricia Gajardo-Meneses [1], Wanda Fernandez [2], Pilar Carvallo[1].
[1]Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile [2]Unidad de Anatomía Patológica, Hospital San Borja Arriarán, Santiago, Chile

Breast cancer is the leading cause of cancer death among women worldwide, being distant metastases the main cause of death. Epithelial to mesenchymal transition (EMT) has been implicated in promoting cancer invasion and metastasis, and can be induced by TGF-β through the expression of transcription factors TWIST, SNAIL, SLUG and ZEB1. The expression of these transcription factors has been previously analyzed in several cell lines, although no studies have been performed in breast cancer tumors with different prognosis. The aim of this study was to analyze the differential expression of TWIST, SNAIL, SLUG

and ZEB1 in breast cancer cell lines and tumors with different subtype and lymph node status. For this purpose, we evaluated the expression of each transcription factor in 60 breast cancer tumors by immunohistochemistry. Additionally, these proteins were evaluated after TGF-β treatment in HCC1937 (basal-like) and T47D (luminal) breast cancer lines, by immunocytochemistry. Our results showed that 13 of 33 luminal tumors showed expression of at least one of the transcription factors analyzed, being ZEB1 expressed in 7/13. Six of these tumors derived into lymph node metastases. In addition all basal-like tumors (n=14) expressed at least one transcription factor, being ZEB1 expressed in 5 tumors, 4 of these derived into lymph node metastases, as well as 4 tumors expressing TWIST and SNAIL. For breast cancer cell lines, our results showed that after TGF-β treatment, HCC1937 cells increased expression of TWIST, SLUG and ZEB1 (H-score= 60, 200 and 210) compared to control (H-score= 27, 25 and 20). T47D cells showed expression of SNAIL and SLUG (H-score 150 and 130), compared to control (H-score= 0 and 0). In conclusion we found differential expression of EMT transcription factors in relation to tumor subtype and lymph node status. Additionally, luminal and basal-like derived cell lines showed differential expression of these proteins after TGF-β treatment.

618 (625) **TUMOR MICROENVIRONMENT TRIGGERS PHOTODYNAMIC RESISTANCE THROUGH FIBROBLAST POPULATION BY HIF-1 PATHWAY MODULATION**

María Julia Lamberti[1], Jeroen Krijgsveld[2], Natalia Belén Rumie Vittar[1], Viviana Alicia Rivarola[1].
[1]Laboratorio 14, Departamento de Biología Molecular, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, Argentina [2]European Molecular Biology Laboratory (EMBL), Heidelberg, Alemania

The study of the tumor microenvironment (TME) has given rise to the concept that tumor progression is dependent on a network of interactions among cancer cells and the surrounding stroma. One of the most important aspects of the TME crosstalk is the ability of cancer cells to modulate stroma behavior, and vice versa, through the collective action of a variety of soluble mediators. In this sense, we aimed to identify soluble factors present in the TME putatively linked to cancer-relevant pathways by performing a high-throughput secretome profiling. To mimic TME heterogeneity and architecture, we co-cultured colorectal (CRC) tumor cells (SW480, TC) with stromal fibroblasts cells (MRC-5, FC) as 3D-spheroids. The characterization of the homotypic (TC) and heterotypic (TC+FC) spheroids´ secretomes was performed using label-free LC–MS. Bioinformatic analysis using PID database revealed that HIF-1 signaling pathway was the most highly enriched within the proteins whose secretion was enhanced in heterotypic spheroids. In a previous report, we demonstrated that HIF-1 was strongly associated with CRC-resistance to photodynamic therapy (PDT), an antitumor therapeutic that combines photosensitizing agents, O_2 and light to create a harmful photochemical reaction. Consistently, the presence of FC considerably diminished TC sensitivity to photodynamic activity (MTT). In addition, despite the biological significance of the HIF-1 pathway of secretomes was reduced after photosensitization, this decreased was partially reversed in heterotypic 3D-cultures. HIF-1 pathway modulation by both PDT and FC was further confirmed through the evaluation of the expression of the HIF-target gene VEGF (RT-qPCR). Collectively, these results delineate a mechanism by which FC enhance TC survival and treatment resistance, which can potentially guide translational research specifically aimed at effective clinical interventions for fibroblast-enriched and consequently HIF-1 pathway overexpressing tumors.

619 (659) **ROLE OF HYALURONAN (HA) AND ITS RECEPTOR RHAMM IN PROLIFERATION, MIGRATION AND DIFFERENTIATION USING JEG-3 CHORIOCARCINOMA CELL LINE. CHARACTERIZATION OF ENZYMES RELATED WHIT HA METABOLISMS**

Matías Pibuel[1,2], Marilina Mascaró[1,2], Mariángeles Díaz[2], Silvina Lompardía[1,2], Cintia Yamila Mihalez[1,2], Daniela Papademetrio[1,2], Martin Levermann[1,2], Élida Álvarez[1,2], Silvia Hajos[1,2]
[1]Cátedra de Inmunología, Facultad de Farmacia y Bioquímica [2]IDEHU (CONICET)

Choriocarcinoma (CC) is an aggressive neoplasia that affects women in fertile age. Although molecular aspects of this pathology have been studied those related with extracellular matrix have not been totally clarified. The aim of this work was to evaluate the expression and involvement of HA and RHAMM in CC biology. Human JAR, JEG-3, BeWo and rat Rcho CC cell lines were used. HA secretion was measured by ELISA-like assay. RHAMM expression was determined by western blot and IFI. RHAMM, HA synthases (HAS) and hyaluronidases (Hyals) mRNA expression were evaluated by RT-PCR. The effect of several molecular weight HA (high, low, and oligomers) in migration, proliferation and differentiation (HLA-G and Syncitin-2 mRNA expression) was determined by wound healing and transwell assays, [3H]-thymidine incorporation and RT-PCR, respectively. Ly294002 (PI3K inhibitor) and UO126 (MEK1/2 inhibitor) were used to evaluate signalling pathways involved using JEG-3 cell line. All cell lines studied showed HAS1-2 and Hyals1-2 expression while only BeWo showed higher basal secretion of HA respect to its cell-free medium ($p<0,001$). JEG-3 expressed RHAMM and migrated towards 500 ug/ml LMW-HA significantly higher than controls ($p<0,01$), cell proliferation was not affected. Addition of anti-RHAMM into the upper chamber reduced migration index towards LMW-HA ($p<0,001$). Co-treatment of LMW-HA with Ly294002 but not whit UO126 reduced migration index ($p<0,001$) compared with the addition of LMW-HA alone. Besides, both LMW- and HMW-HA induced an increase of HLA-G while decrease of Syncitin-2 mRNA expression. In conclusion LMW-HA induced migration through RHAMM mostly through PI3K signalling pathway. Both LMW- and HMW-HA would be involved in inducing cell differentiation to an extravellous phenotype. Similar profile of RHAMM, HAS and Hyals found in RCho and JEG-3 cells suggests that these cells may be good models to study the role of HA metabolism in CC.

TOXICOLOGÍA / TOXICOLOGY

620 (229) **CHANGES IN OVARIAN GENE EXPRESSION INDUCED BY 3-METHYLCHOLATHRENE AND PREVENTED BY ALPHA-NAPHTHOFLAVONE**

Eric Alejandro Rhon-Calderón[1,2], Rocío Alejandra Galarza[1,3], Alejandro Lomniczi[2], Alicia Graciela Faletti[1,3]
[1]Universidad de Buenos Aires, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Centro de Estudios Farmacológicos y Botánicos (CEFYBO), Facultad de Medicina, Buenos Aires, Argentina [2]Neuroscience Division, Oregon National Primate Research Center, Oregon Health And Science University, Portland, United States of America [3]Universidad de Buenos Aires, Facultad de Medicina, Dto. de Toxicología y Farmacología, Buenos Aires, Argentina.

3-Methylcholanthrene (3MC), a polyaromatic hydrocarbon, is an environmental pollutant that causes reproductive toxicity. Previous studies showed that 3MC alters the ovarian function by affecting the follicle integrity in rodents. The aim of the present work was to study the effect of daily exposure to 3MC on ovarian gene expression involved in folliculogenesis, cell cycle and xenobiotic metabolism. Ovarian tissue from immature female rats, daily injected with 3MC (0.1 and 1 mg/kg) and/or α-naphthoflavone (αNF, 80 mg/kg) for 20 days, were used. Both doses of 3MC increased the mRNA expressionof Akt1, Cdk2, Dnajb6, Igf2, Calr, Hspa8, Icam1, Ddr2, Dll1, Hes1, Notch2, Jag1, Cyp1a1, Cyp1b1, Ddx5, Foxn3 and Sp1 ($p<0.01$); decreased the expression of Esr1, Cd9 and Itgb8 ($p<0.001$); and showed no changes in Foxo3, Gdf9, Bmp15 and Itga4; all compared with controls. To assess whether the aryl hydrocarbon receptor (AhR) is involved in the 3MC-induced changes, chromatin immunoprecipitation assay was used. We found an increase in the promotersof Cyp1a1 (290%***), Cyp1b1 (168%***), Hes1 (78%***), Jag1 (35%*), Igf2

(77%***), Dnajb6 (95%***), Cdk2 (49%***), Sp1 (61%***) and Icam1 (175%***), after AhR recruitment, compared with controls (*p<0.05, ***p<0.001). By studying trimethylation and acetylation of histone 3 from these genes, we found an increase in 4 (Jag1, Cyp1a1, Dnajb6, Igf2, p<0.01) and 7 (Jag1, Cyp1a1, Dnajb6, Cdk2, Igf2, Icam1, Sp1, p<0.001), respectively, and a decrease in Cdk2 (p<0.01) trimethylation, all compared with controls (p<0.001), respectively. All these changes were prevented by daily treatment with αNF. In conclusion i) daily exposure to 3MC alters the expression of different genes involved in the ovarian function by acting on different transcriptional processes, ii) the 3MC action seems to be mediated by AhR; and iii) αNF prevents the toxic effect of 3MC in the ovary.

621 (343) **PERINATAL EXPOSURE TO A GLYPHOSATE BASED HERBICIDE CAUSES IMPLANTATION FAILURES AND TRANSGENERATIONAL INDUCTION OF CONGENITAL ANOMALIES IN RATS**

Guillermina Pacini[1], Jorgelina Varayoud[1], Ramiro Alarcón[1], Enrique Luque[1], María Mercedes Milesi [1]
[1]Instituto de Salud y Ambiente del Litoral, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral -Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) - Santa Fe, Argentina

Glyphosate based herbicides (GBH) are extensively used for agricultural purposes all over the world, which is closely associated with a constant increase in the use of transgenic glyphosate-resistant soybean single-cropping. Recently, we showed that a brief exposure to a GBH, administered subcutaneously during the first postnatal week alters uterine development in prepubertal rats, and induces post-implantation embryo loss at adulthood. In the present study we evaluate if an oral administration of GBH during the perinatal period (gestation and lactation) affects female fertility and/or induces transgenerational effects on prenatal development of their progeny. Pregnant rats (F0) were orally exposed to 200 mg GBH/Kg/day through food, from gestational day (GD) 9 until weaning (postnatal day 21, PND21). The body weight gain and the vaginal canal-opening of the F1 females were evaluated. On PND90, F1 females were submitted to a fertility test to evaluate the pregnancy rates, and on GD19, the number of corpora lutea (CLs) and the implantation and resorption sites. To determine transgenerational effects on the F2 offspring development, we evaluate the fetal weight, length and morphology, and the placental weight. GBH exposure did not alter the body weight gain of the F1 females with age, but led to early onset of vaginal opening, indicating early puberty. Although all GBH-treated F1 females resulted pregnant, a decreased number of implanted embryos were detected. Moreover, F2 offspring exhibited a delayed growth, evidenced by lower fetal weight and length. A higher placental weight was detected in the GBH group. Surprisingly, structural congenital anomalies, such as, conjoined fetuses and abnormally developed limbs were detected in the F2 offspring. We concluded that perinatal exposure to a GBH induced female subfertility by decreasing the number of implanted embryos, and caused transgenerational induction of congenital anomalies.

622 (360) **PERINATAL EXPOSURE TO A GLYPHOSATE-BASED HERBICIDE MODIFIES THE ABUNDANCE OF ESTROGEN RECEPTOR ALPHA TRANSCRIPTS WITH ALTERNATIVE 5'-UNTRANSLATED REGIONS IN THE PRE-IMPLANTATION RAT UTERUS**

Virginia Lorenz[1], Jorgelina Varayoud[1], Guillermina Pacini[1], Enrique Hugo Luque[1], María Mercedes Milesi[1]
[1]Instituto de Salud y Ambiente del Litoral (ISAL), Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral - Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)

Usage of glyphosate-based herbicides (GBHs)has been expanded over the lastdecade. Recently, we found that oral exposure to a GBH in rats during perinatal period (pregnancy and lactation) produced subfertility as a consequence of implantation failures. Implantation is a complex process regulated by endocrine signaling pathways primary mediated via steroid receptors, such as estrogen receptor alpha (ERa). It has been identified five promoters called E1, OT, O, ON, and OS which control ERa transcription initiation yielding differenttranscripts with alternative 5´ untranslated regions. The purpose of this workwas to investigate the effects of in utero and lactational exposure to a GBH over the control of ERa expression in the pre-implantation uterus. Pregnant rats (F0) were orally exposed to200 mg GBH/Kg/day through food from gestational day (GD) 9 until weaning (postnatal day 21, PND21). When F1 females reached the sexual maturity (PND90) were mated with males of proven fertility, and pregnancy was confirmed by vaginal smears. Pregnant F1 females were sacrificed on GD5 (pre-implantation period), and uterine samples were collected and store at -80°C until analysis. Relative expression of total ERa mRNA and ERa transcripts containing alternative 5'-untranslated regions E1, OT, O, ON, and OS was evaluated by real time RT-PCR. First we found that during the pre-implantation period, ERa gene transcription was regulated by OS, O, OT and E1 promoters,regardless of treatment group. When we analyzed GBH effects, we detected an increase of total ERa mRNA expression. Moreover, the increase in total ERa mRNA in GBH group was mediated by an increased expression of ERa-O variant. These findings showed that perinatal exposure to a GBH alters ERa expression during the pre-implantation period, by affecting therelative abundance of alternative 5'-untranslated region of ERa transcripts in the uterus. These alterations could explain, at least in part, the GBH-mediated subfertility.

623 (782) **ACUTE EXPOSURE TO NICKEL-DOPED NANOPARTICLES: MECHANISMS UNDERLYING ITS EFFECTS**

Mariana Garcés[1], Natalia Magnani[1], Marchini Timoteo[1], Lourdes Caceres[1], Guaglianone Alejandro[1], Andrea Mebert[2], Fiorella Tesan[3], Maria Jimena Salgueiro[3], Marcela Zubillaga[3], Martin Desimone[2], Pablo Evelson[1]
[1]Universidad de Buenos Aires. CONICET. Instituto de Bioquímica y Medicina Molecular (IBIMOL). Facultad de Farmacia y Bioquímica. Buenos Aires, Argentina [2]IQUIMEFA-UBA-CONICET.Facultad de Farmacia y Bioquímica. Buenos Aires, Argentina [3]Cátedra de Física. Facultad de Farmacia Y Bioquímica. Buenos Aires, Argentina

Nanotechnology involves the manipulation and application of particles having at least one dimension smaller than 100 nm long. In vitro and in vivo toxicological studies suggest that nanoparticles are able to generate reactive oxygen species (ROS), leading to the release of proinflammatory mediators and oxidative stress. Transition metals, such as Ni (II), can be associate to nanoparticles (NP) and might be involved in the toxicological processes triggered by particulate matterinhalation. The aim of this work was to synthesize and characterize NP dopedwith Ni (II), and to study its biodistribution and mitochondrial alterations. Femaleswiss mice (25 g) were intranasally instilled with a Ni-NP suspension (1 mgNi/kg body weight), delivered in a single dose (control group: Si-NP). Sampleswere collected after 1 h. NP share comparable physicochemical properties with air pollution PM in size (NP-Si: 170 ± 2 nm; Ni-NP: 200 ± 20 nm) and shape, asassayed by TEM and SEM. Ni released from Ni-NP was less than 1% at 3 h. NPsredox potential was evaluated as the inhibition of H_2O_2production by the glucose/glucose oxidase system and EPR. Ni-NP was able tosignificantly inhibit H_2O_2 production by 43% at 45 µg Ni/mL and 24%, 22.5 µg Ni/mL. A signal corresponding to $OH^{\cdot}$. generated from NP, NP-Ni and Ni (II) was observed in the EPR spectrum. NP were labeled with ^{99m}Tc; biodistribution studies showed that NPs mostly remain in lung (79%), while 18% were in stomach and 1% remained in the injection point. Scintigraphy imaging confirmed these results. Ni content in lung, heart, and plasma showed that Ni accumulates mostly in the lung (p<0.01 vs Ni in heart and plasma). An increased in H_2O_2 production (41%, p<0.05 in lung and 39%, p<0.05 in heart); and an impaired cardiac mitochondrial function (RCR_{NP}: 5.0 and $RCR_{NP\text{-}Ni}$: 2.1) were observed in lung and heart mitochondria. These findings contribute to the understanding of nanoparticle toxicity present in air pollution.

624 (824) **EFFECTS OF DIESEL EXHAUST PARTICLES (DEP) ON THE REDOX BALANCE OF HUMAN CONJUNCTIVAL EPITHELIAL CELLS**

Romina Mayra Lasagni Vitar[1, 2], Julia Tau[3], Natasha Stephanie Janezic[1], Ailen Gala Hvozda Arana[1], Agustina Inés Tesone[3], Agustina Peverini[1], Claudia Gabriela Reides[1,2], Alejandro Berra[3], Sandra María Ferreira[1,2], Susana Francisca Llesuy[1, 2]

[1]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Química General e Inorgánica [2]IBIMOL UBA-CONICET. [3]Universidad de Buenos Aires, Facultad de Medicina, Departamento de Patología, Laboratorio de Investigaciones Oculares.

Air pollution causes adverse effects on human health and the eyes are particularly vulnerable because they are constantly exposed to the environment. Diesel exhaust particles (DEP) are one of the mayor forms of particulate matter of urban air pollution. The aim of the present study was to evaluate the redoxbalance in human conjunctival epithelial cells (IOBA-NHC) after the incubationwith DEP for 24 hours. IOBA-NHC were incubated with DEP at differentconcentrations (10, 50, and 100 µg/mL) for 24 hours. The following parameters were evaluated: the activities of enzymes glutathione reductase (GR), glutathione S-transferase (GST), glutathione peroxidase (GPx), thioredoxin reductase (TRxR) and glucose 6P-deshidrogenase (G6PDH); and reduced (GSH) and oxidized (GSSG) glutathione levels. One-way ANOVA test and Dunnett's test as post hoc test were used for statistical analysis. The cells exposed to DEP at 50 and 100 µg/mL showed a significant decrease in GR (17% and 37%, $p<0.05$) andG6PDH (49% and 70%, $p<0.05$). TRxR was found decreased in DEP100 group (27%, $p<0.05$), but not in DEP50 group. GSH levels were diminished in both groups (41% and 41%, $p<0.01$), meanwhile there was no significant difference in GSSG levels among all groups. GST activity displayed an increase in both groups (42% and 45%, $p<0.01$), as well as GPx activity (41% and 50%, $p<0.05$). There was nosignificant difference between DEP10 and control groups in all themeasurements. The increase in GST and GPX could lead to anincreasing GSH consumption. Furthermore, the decay in GR activity compromised the GSH/GSSG recycling, and this situation could be aggravated by the reduced availability of NADPH due to a decrease in G6PDH activity. As TRxR activity is diminished, the recycling of thioredoxin, another important source of sulfhydryl group, is also affected. These results suggest that the alteration of redox balance could be a possible mechanism of damage in the human conjunctival epithelial cells exposed to DEP.

625 (827) **SOLUBLE GUANYLYL CYCLASE ALPHA 1 SUBUNIT AS A POTENTIAL BIOMARKER TO EVALUATE ESTROGEN-LIKE EFFECTS OF ENDOCRINE DISRUPTORS**

Sonia Alejandra Ronchetti[1], Agustina Gurruchaga[1], Georgina Cordeiro[1], Analía Gabriela Ricci[2], Beatriz Haydée Duvilanski[1], Jimena Paula Cabilla[1]

[1]INBIOMED (UBA-CONICET), Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155, Piso 10, Ciudad Autónoma de Buenos Aires, Argentina. [2]IBYME-CONICET, Vuelta de Obligado 2490, Ciudad Autónoma de Buenos Aires, Argentina.

Endocrine disruptors (EDs) are compounds that interfere in the action of endogenous hormones. A particular class of EDs called xenoestrogens (XEs), mimic cell responses normally induced by estrogen (E2). The main nitric oxide receptor soluble guanylyl cyclase (sGC) is a cytosolic heterodimercomposed by two subunits, alpha and beta, and catalyzes cGMP formation. It isubiquitously present throughout all the zoological scale. E2 differentiallyaffects sGC subunits by increasing alpha1 (a1) and decreasing beta1 (b1) expression. Previously we have shown that a1 expression is particularly sensitive to E2 levels in vivo and in vitro. The aim of the present work was to investigate the expression of a1 as an indicator of EDs exposition in some estrogen-responsive cell lines. Lactosomatotroph-derived pituitary cell line GH3 and endometrial tumor cell line ECC-1 were incubated with several XEs which binds estrogen receptor for 48 h. a1 and b1 expression was determined by western blot. 1 nM cadmium (Cd) and 1 nM arsenic (As) treatments increased a1 expression in GH3 cells (a1 relative units (RU) as% of control; Cd: 152±10*; As: 145.6±13*, E2: 236.5±21.2***, *$p<0.05$, ***$p<0.001$), while b1 levels were unaffected. Similar results were observed in ECC-1 cells. These effects showed to be specific for E2 or E2-like compounds since it was not reproduced after incubation with other proliferation inducers (a1 RU as% of control; 10 mM forskolin: 135±23; 100 mM 3-isobutyl-1-methylxanthine (IBMX): 104.3±14.2) or prolactin, a classic downstream E2-induced gene (a1 RU as% of control; 1 ng/mL PRL: 120±25). Moreover, ethynylestradiol (EE2) and diethylstilbestrol (DES), were shown to increase a1 expression (a1 RU as% of control; EE2=159.94%**, DES=172.1%**, $p<0.01$) without affecting b1 levels. Altogether, these results support soluble guanylyl cyclase alpha1 subunit as a novel potential biomarker to rapidly assess estrogen receptor-dependent EDs exposure in vitro.

626 (941) **CADMIUM ALTERS MORPHOLOGY, APOPTOSIS AND INFLAMMATION MARKERS IN LUNG. EFFECT OF DIFFERENT DIETS**

Gabriel Boldrini[1], Silvina Alvarez[1], Glenda Martin[1], Veronica Biaggio[1], Nidia Gomez[1], Sofía Gimenez[1]

[1]Instituto Multidisciplinario de Investigaciones Biológicas San Luis IMIBIO-SL.

Cadmium (Cd) is a toxic metal and an important environmental contaminant. We studied its effects on apoptosis and inflammation markers, leucocytes inbronchoalveolar lavages (BAL) and the histoarchitecture of rat lung underdifferent diets. 4 lots of female Wistar rats were used: 2 lots received casein(Cas) and 2 lots soybean (Soy) as protein sources. Within each group, 1 lotreceived regular water (control-Co) and the other, 15 ppm of Cd in the drinkingwater for 60 days. BAL was performed and cells obtained were counted. Lungswere fixed, sectioned, stained, and examined for evidence of injury. Total RNAwas isolated with Trizol and cDNA was obtained. Cyclooxygenase-2 (COX-2), transforming growth factor beta (TGF-β), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), vascular cell adhesion molecule-1(VCAM-1), p53, BAX and Bcl-2 were determined by PCR. S28 was used as control. Alveolar macrophages decreased in Soy-Cd vs Soy-Co ($p<0,05$); neutrophils in BAL augmented in Soy-Cd vs Cas-Cd and So-Co ($p<0,01$); lymphocytes increased in Cas-Cd vs Cas-Co ($p<0,01$) and in Soy-Cd vs Soy-Co ($p<0,05$). NF-κB showed a significant decreased in Soy groups vs Cas groups ($p<0,001$) and also decreased in Soy-Cd vs its control ($p<0,01$). COX-2 increased in Cas-Cd vs Cas-Co ($p<0,05$) and decreased in Soy groups vs Cas groups ($p<0,05$). TGF-β expression decreased in Soy-Co and Soy-Cd groups vs Cas groups ($p<0,001$). VCAM-1 showed increased levels in Soy-Cd vs its control ($p<0,05$) and also in Soy-Co vs Cas-Co ($p<0,005$). p53 levels showed a decreased in Soy-Cd vs Cas-Cd ($p<0,001$). Bax/Bcl-2 ratio increased in Cas-Cd vs Cas-Co ($p<0,05$). Significant morphological changes in lung parenchyma were observed in intoxicated rats when compared to the control group. Morphological changes were less severe in Soy-Cd group. This shows lung histoarchitecture is altered by Cd, which is consistent with the change in apoptotic and inflammation markers, and Soy might protect lung against the metal.

PRESENTACION DE POSTERS SAFE III / SAFE POSTER PRESENTATION III

AGENTES ANTI-INFECCIOSOS / /ANTI-INFECTIOUS AGENTS

627 (931) **CAENORHABDITIS ELEGANS AS A MODEL FOR TESTING NOVEL ANTHELMINTIC COMPOUNDS ISOLATED FROM PLANT EXTRACTS**

María Julia Castro[1,2], Ignacio Bergé[1], María Belén Faraoni[2,3], Cecilia Bouzat[1],

[1]INIBBIB-CONICET, Universidad Nacional del Sur, Bahía Blanca, 8000, Argentina. [2]INQUISUR-Departamento de

Química, Universidad Nacional del Sur, Bahía Blanca. (3) Miembro de CIC.

Parasitic nematodes affect human health as well as livestock and crops. Resistance to current anthelmintic drugs has prompted the search for new compounds. The use of parasitic worms for drug testing is costly and difficult. In contrast, the free-living nematode Caenorhabditiselegans has emerged as a valuable platform for the study of anthelmintic drugs. Considering that natural products provide a diverse and unique source of bioactive lead compounds for drug discovery, extracts of Diplotaxistenuifolia and Thelespermamegapotamicum, and compounds isolated from their flowers and leaves were investigated for anthelmintic activity against C. elegans. From D. tenuifolia we isolated and identified by NMR a flavonol glycoside, isorhamnetin-3-O-b-D-glucoside (1), which is one of the main compounds of the ethyl acetate subfraction. By GC-MS analysis of the essential oil obtained from T. megapotamicum we revealed the qualitative and quantitative composition of the volatile compounds. The most abundant is limonene (2). Short- and long-term effects of these two compounds on anthelmintic activity were determined by evaluating the changes in locomotion, measured by the number of thrashes/min after a 10-min exposure, and the changes in larval growth. Neither compound affected the number of thrashes/min with respect to the control (~200/min). When compounds were added to the growth media, worms developed to the larval 4 stage with slightly reduced body size. However, larval development was severely impaired when the F1 generation was continuously treated with compound 1, whereas with compound 2 no significant changes were observed. Quercetin, an unglycosylated commercial flavonoid whose action at C. eleganshas been tested previously, did not affect significantly larval growth. Our results propose that flavonol glycosides might provide a new class of anthelmintic drugs.

628 (1029) **NEW BACTERIOCIN OF E. FAECALIS CECT7121: SYNERGY STUDIES AGAINST MULTIDRUG-RESISTANT ENTEROCOCCI ISOLATED FROM BOVINE MASTITIS**

Gastón Delpech(1), Mónica Ceci(2), Mariana Bistoletti(3), Sergio Sánchez Bruni(3), Mónica Sparo(1).
[1]Microbiología Clínica, Medicina, UNCPBA. Olavarría, Argentina. [2]Centro de Estudios Bioquímicos. Tandil, Argentina. [3]CIVETAN (CONICET-UNCPBA). Tandil, Argentina.

Bovine mastitis impacts negatively on animal production and on the quality of milk. AP-CECT7121, a new bacteriocin produced by *Enterococcus faecalis* CECT7121, shows a variety of interesting biological properties with potential use in clinical Veterinary practice, without undesirable side effects. It is a peptide with a wide spectrum of bactericidal activity against Gram positive bacteria and bacteriostatic activity against some Gram negative bacteria. The aim of this study was to evaluate the synergism of AP-CECT7121 associated with gentamicin against multidrug-resistant enterococci isolated from bovine mastitis. *N*: 4 multidrug-resistant *E. faecium*strains isolated from different mastitic dairy cows were tested. Animals from dairy farms, located in the Province of Buenos Aires-Argentina, during the period 2014-2015 were included. AP-CECT7121 potency was assessed by time-kill curves alone or with sub-inhibitory concentrations of gentamicin. High level gentamicin resistance was not observed in any enterococcal isolate. Viable counts, after 0, 2, 4, 8 and 24 h of incubation, were carried out. The AP-CECT7121exhibited bactericidal activity alone against all tested enterococci. The association with gentamicin slightly enhanced bactericidal activity against all isolates of *E .faecium*. Bactericidal activity of AP-CECT7121 alone and associated with subinhibitory concentrations of gentamicin against enterococci proved that this peptide constitute an attractive candidate for its use as a natural therapeutic tool for control and prevention of bovine mastitis produced by multi-resistant bacteria such as vancomycin-resistant *E. faecium*.

629 (1071) **ANTIMICROBIAL SUSCEPTIBILITY OF STAPHYLOCOCCUS SPP. ISOLATED FROM MASTITIS IN DAIRY GOAT FROM CORDOBA, ARGENTINA.**

Nicolás Javier Litterio(1), Ma. Soledad Aguilar (1) Ma. Pilar Zarazaga (1) MartínHimelfarb (1), MATÍAS LORENZUTTI (1)
1Universidad Católica de Córdoba, Facultad de Cs. Agropecuarias

Surveillance of bacterial resistance to antimicrobials (ATM) is recommended by WHO and OIE to assess their progress and make rational decisions for their control, against the severity of this phenomenon worldwide. The aim of this study was to analyze the quantitative profile of antimicrobial susceptibility in staphylococci isolated from mastitic milk of goats from the province of Cordoba, Argentina. *S. aureus* (*n*=52) and coagulase-negative staphylococci (CNS) (*n*=34) strains wereisolated; minimum inhibitory concentration (MIC) of benzylpenicillin, cloxacillin, gentamicin and oxytetracycline against these strains was determined by broth microdilution test (CLSI, 2013). Considering the clinical breakpoints (CLSI, 2013), a susceptibility of 71%, 100% and 50% for benzylpenicillin, gentamicin and oxytetracycline registered respectively against *S. aureus*, whereas the corresponding against CNS were 68%, 94%, 50%. Moreover, taking into account the epidemiological cutoff values (ECOFF; EUCAST, 2016), a highest percentage of resistant strainsto oxytetracycline for both *S. aureus*and CNS (67% and 56%, respectively) was observed, with MIC values two and five times higher than those reported by the reference entity. On the other hand, theproportions of wild type and resistant strains for cloxacillin and gentamicin are similar to the report by EUCAST. The emergence of resistance for oxytetracycline in the region is consistent with data from surveys conducted during the sampling, where treatments with ATM for both mastitis and otherdiseases do not follow rational therapeutic regimens, and this is aggravated by poor veterinary care in the area. It is emphasized that the data reported in this study are the basis for monitoring of resistance at a regional level, as they are also useful for the determination of rational dose regimens for the treatment of goat mastitis.

FARMACOQUÍMICA / PHARMACOCHEMISTRY

630 (78) **CATIVIC ACID-CAFFEIC ACID HYBRID TRIGGERS APOPTOSIS AND INHIBITS PROLIFERATION OF HUMAN NEUROBLASTOMA CELLS**

Natalia Paola Alza[1,2], Ana Paula Murray[2], Gabriela Alejandra Salvador[1],
1) Instituto de Investigaciones Bioquímicas de Bahía Blanca. Universidad Nacional del Sur y CONICET, 8000 Bahía Blanca, Argentina. (2) Instituto de Química del Sur. Universidad Nacional del Sur y CONICET, 8000 Bahía Blanca, Argentina.

A new approach in the field of drug discovery, including anticancer drugs, is the design and development of hybrids from natural products. Specifically, the search of anticancer agents that can induce apoptosis and the investigation of the signaling pathways involved in their effect constitute an emerging field of interest. The aim of this study was to evaluate the effect on cell growth and induction of apoptosis of a semisynthetic hybrid of 17-hydroxicativic acid and caffeic acid (**1**) on human neuroblastoma IMR-32 cells.

In addition, we investigated the involvement of MAPK signaling pathways in the biological effect of **1**. Hybrid **1** showed to inhibit cell growth with an IC_{50} value of 18.0 ± 1.3 μM ($p < 0.05$) and increased ($p < 0.05$) caspase-3 activity significantly in **1**-treated cells. Additionaly, BrDU positive cells diminished after exposure to **1** (10 μM) compared to control condition (36.6 ± 0.7% and 58.1 ±0.9% respectively, $p < 0.001$). For further characterizing the biological effect of **1**, we analyzed cell cycle distribution by flow cytometry. Cell population in S phase significantly increased after exposure to **1** (10 μM) with a reciprocal decrease in the G2/M phase compared to the control group ($p < 0.001$).

We next investigated the state of MAPK, ERK1/2 and JNK. Levels of ERK1/2 phosphorylation were increased in IMR-32 cellsexposed to **1** (10 - 25 μM) for 12 and 24 h. This was accompanied by ERK1/2 and c-Jun nuclear translocation. For determining, the role of ERK1/2 and JNK in the bioactivity of **1** we used pharmacological

manipulation. Pretreatment with ERK inhibitor, U0126, and JNK inhibitor, SP600125, reduced cell viability compared with conditions treated with **1** alone ($p<0.001$).

In conclusion, hybrid **1** is able to trigger antiproliferative signals and apoptosis in IMR-32 cells and trough the inhibition of MAPK activity its biological effect could be enhanced.

FARMACOTECNIA / /PHARMACY TECHNOLOGY

631 (1081) **SAFETY CHARACTERISTICS OF BENEFICIAL VAGINAL LACTIC ACID BACTERIA (LAB) TO BE INCLUDED IN A PROBIOTIC PRODUCT**

Antonella Marchesi, Jessica Silva, Cecilia Aristimuño Ficoseco, María Elena Fátima Nader,
CERELA-CONICET. Centro de Referencia para Lactobacilos

The women vaginal tract infections are one the most frequent gynecologist attendance, being usually treated with antibiotics, which can contribute to the emergence and spread of resistance in humans. The application of probiotics as preventive or therapeutic agents is being used for women´s healthand constitutes a novel alternative to replace the excessive use of antibiotic. Our research group is working on the design of probiotic formulations with autochthonous lactic acid bacteria (LAB) isolated from the female human vagina, and their beneficial characteristic were previously evaluated. In this study, safety characteristics such as antibiotic resistance (by genetic and microbiological assays) andvirulence factors (hyaluronidase, gelatinase and hemolysis capability) of LAB strains selected by their beneficial properties were evaluated. The antibiotic resistance was studied by phenotypic and genetic assays using the following antimicrobials: Ampicillin, Tetracycline, Chloramphenicol, Gentamicin, Streptomycin, Kanamycin, Vancomycin and Erythromycin. The minimum inhibitory concentration (MIC) assay was performed by the plate diffusion technique with lactobacilli in LSM media agar and antibiotic in the wells, later incubated in microaerophylic conditions at 37 °C. The beneficial strains studied were *Lactobacillus reuteri, L. gasseri, L. rhamnosus, L. salivarius,* applying the European Food Safety Authority (EFSA) cutoff values. The presence of antibiotic resistance genes (*van*A, *van*B and *van*X *erm*(C), *bla, cat*, *erm*(B), *aac*(6´)-*aph*(2´´), *aph*(3´´)-III, *str*A, *str*B, *aad*A, *aad*E, *ant*(6), *tet*(M), *tet*(K), *tet*(L), *tet*(S)) were evaluated by PCR using specific primers. Phenotypic assays in agar solid media with specific substrates were used for the detection of virulence factors. There is a wide degree of coincidence between the results obtained in the phenotypic and genotypic resistance pattern of the strain to the different antibiotic. Furthermore, the results indicate the resistance to ERY (80% of coincidence between phenotypic and genotypic studies) for all the strains assayed. *L.rhamnosus CRL1508* and *L.rhamnosus CRL1511* showed coding genes for TET/VAN. In addition, *L.gasseri CRL1267* and *L.reuteri CRL1327* were resistant to CLIN (50%) and KAN (50%) in phenotypic assay. Most of the strainswere sensitive to CHLOR/STRE/GEN, which are the antibiotic most frequently applied to the therapy of urogenital infections. All of the beneficial vaginal *Lactobacillus* strains studied were free of enzymes related with virulence factors. The results of this work indicate that the LAB are safe and can be included in the design of a pharmabiotic product to restore the vaginal microbiota and prevent infections.

632 (2060) **SOLID DISPERSIONS. A TOOL TO IMPROVE THE DISSOLUTION RATE OF OXFENDAZOLE.**

Marina Silvana Ardusso[1], Daniel Allemandi[1], Sergio Sánchez Bruni[2], Santiago Palma[1].
[1]Departamento de Farmacia, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba - Unidad de Investigación y Desarrollo en Tecnología Farmacéutica, UNITEFA (CONICET), Córdoba, Argentina; [2]Laboratorio de Farmacología. Facultad de Ciencias Veterinarias, Universidad del Centro de la Provincia de Buenos Aires, Campus Universitario, CIVETAN (CONICET), Tandil, Buenos Aires, Argentina.

Introduction: Parasitism is a disease that may affect men and pets as well. Treating this disease, Anthelminticsbenzimidazoles (BZD) have achieved a therapeutic significance due to theircharacteristics of broad spectrum, low toxicity and low cost. The formulation, bioavailability and efficacy of BZD anthelminticsare limited by the low gastrointestinal absorption and lack of aqueous solubility. To confront this, several technological methods have been reported for the improvement of drug solubility and dissolution rate. Such an approach, with significant advantages, is the formulation of hydrophobic drugs in high-energy amorphous forms, such as solid dispersions (SDs). Our hypothesis is to increase the rate of dissolution oxfendazole (OFZ) incorporating it in SDs as a strategy to improve bioavailability. OBJECTIVE: Prepare and evaluate physicochemical and biopharmaceutical properties of SDs using poloxamer 188 (PXM) as carrier of OFZ. Methods: SDs were prepared by melting of OFZ and PXM. The systems were characterized by XRP Diffraction, Scanning Electron Microscopy (SEM), solubility studies, and dissolution tests. Results: Nointeractions between the components of SDs were observed in XRD diffractograms. The SEM of SD observed irregular particles where it is not possible to differentiate the OFZ from PXM. While in physical mixture, the OFZ can be seen spread over the carrier surface. The solubility indicates that if PXM presence is up to 1% w/v then OFZ solubility in HCl 0.1 N is not altered. In addition, 50 and 200 mg (OFZ) SDs dissolution profiles at time = 15min showed an increasedrate of 12.4 and 12.8 respectively, compared to the same dose of OFZ. Whereas applying the F1 difference factor to the corresponding 10 and 50 mg SDs profiles, it determined a curve overlapping. This increase in dissolution rate may be due to the presence of PXM that improves the particles wettability. Conclusions: In every evaluated dose, a remarkable increase in OFZ dissolution rate in SDs is achieved in comparison to OFZ.

FITOFARMACOLOGÍA / PHYTOPHARMACOLOGY

633 (60) **CARDIOPROTECTIVE ACTIONS OF CURCUMIN ON THE PATHOGENIC NFAT/CYCLOOXYGENASE TYPE 2/PROSTAGLANDIN E2 PATHWAY INDUCED DURING TRYPANOSOMA CRUZI INFECTION**

Ricardo S. Corral[1], Susana Wicz[1], Matías Hernández[1],
[1]Servicio de Parasitología-Chagas, Hospital de Niños "Dr. Ricardo Gutiérrez", Ciudad de Buenos Aires, Argentina

Diverse cardiovascular signaling routes have been considered critical for Chagas cardiomyopathy. Along this line, *Trypanosomacruzi* infection and endothelin-1 (ET-1) have been shown to cooperatively activate the Ca2+/nuclear factor of activated T cells (NFAT) cascade in cardiomyocytes, leading to cyclooxygenase type 2 (COX-2) induction and increased release of prostanoids and prohypertrophic peptides. Our study aimed to determine whether the cardioprotective and anti-inflammatory effects of curcumin (Cur) could be helpful to interfere with this key machinery for pathogenesis of Chagas myocarditis. Cur therapy was evaluated *in vivo* using a murine model of *T. cruzi* infection and *in vitro* using ET-1-stimulated and parasite-infected mouse cardiomyocytes. Cur administered orally to acute Chagas mice enhanced survival postinfection ($P<0.05$) and hindered local inflamematory processes [leukocyte recruitment, activation of the eicosanoid pathway and b-type natriuretic peptide (BNP) overexpression, $P<0.05$], without modifying parasite burden in the heart. Molecular analysis of myocardial targets revealed that Cur is capable of blocking Ca2+-dependent NFATc1 transcriptional activity ($P<0.01$), COX-2 ($P<0.05$) and microsomal prostaglandin E synthase-1 (mPGES-1, $P<0.01$) induction, and subsequent production of PGE2 ($P<0.01$) in cardiomyocytes exposed to ET-1 and parasites. Furthermore, the decline of cardiac cell-derived PG levels achieved upon Cur treatment impaired effective PGE2/EP4 receptor interaction, resulting in attenuated expression of BNP ($P<0.01$) in both infected and uninfected cells. Our current findings show a putative mechanism of action of Cur involving inhibition of the Ca2+/NFAT-dependent, pathogenic COX-2/mPGES-1/PGE2 pathway in *T. cruzi*-infected myofibers,

underpinning cardioprotection achieved in treated infected mice. With a view to the limited therapeutic possibilities available, Cur represents a promising approach for the treatment of Chagas heart disease.

634 (155) **EVIDENCE OF GASTROPROTECTIVE EFFECT OF ACACIA AROMA GILL EX HOOK&ARN (TUSCA) LEAVES INFUSION**

Félix Facundo Taboada[2], Natalia Habib[1], Susana Genta[1,2], *[1]Facultad de Bioquímica, Química y Farmacia, UNT. [2]INSIBIO (CONICET-UNT)*

Acacia aroma Gill ex Hook&Arn., Tusca, is an autochthonous herb of Northwest Argentina. The peptic ulcer is a pathology resulting of the imbalance between offensive and defensive factors in gastric mucosa and the search for new treatments is a current challenge. The aim of the present study was to evaluate the potential gastroprotective and antisecretory effects of 5% infusion of Tusca leaves. This extract was lyophilized and subjected to a phytochemical screening, and its antioxidant activity was determined. *In vivo* studies were developed in two models of induced gastric ulcers in male Wistar rats (ethanol and pylorus ligation). The experimental groups (n=6 animals/group) were: 1-Control group; 2-Positive control group treated with Sucralfate (100mg/Kg) or Omeprazole (40mg/Kg) orally; 3-Aqueous extract treated group received orally 150 mg dry extract /kg. Mucus content was evaluated by Alcian Blue method and ulceration parameters (number of ulcers, severity and ulcerated area percentage) were determined. Chemical, microscopic studies of gastric content and a histological evaluation were performed. Also, a gastric emptying assay was carried out. Tusca extract showed a significant free radical scavenging effect, compared with the controls quercetin and BHT. The extract caused a significant ($p < 0.05$) decrease of the ulcerated area, being the severity and number of ulcers lower, compared with untreated control group, the mucus content was significantly higher than control group. The rats treated with the extract showed a decrease in the gastric juice volume and free acidity compared with control group. The gastric emptying was no modified with the treatment. In conclusion, Tusca extract has a significant gastroprotective effect likely related to free radical scavenging activity and through the strengthening of the mucus layer. Additional studies are needed to determine the active compounds and specific mechanisms involved.

635 (173) **TARAXACUM OFFICINALE: POTENTIAL ANTITUMOR AGENT AGAINST CERVICAL CANCER**

Raúl Fernando Venezuela(1), Laura Mugas(2), Ana Ximena Kiguen(1), Jessica Paola Mosmann(1), Marina Soledad Monetti(1), Brenda Konigheim(1), Susana Nuñez Montoya(2), Cecilia Cuffini(1),
[1]nstituto de Virologia "Dr. J. M. Vanella" Fac. Cs. Medicas. Universidad Nacional de Córdoba, [2]MBIV-CONICET, Dpto. Farmacia, Fac. Cs. Químicas, Universidad Nacional de Córdoba

Natural compounds are the foundation of pharmacological treatments and more than 50% of all anticancer drugs are of natural origins or at least derived from compounds present in Nature. *Taraxacumofficinale* G. Weber ex F.H. Wigg. (Familia), popular known as "dandelion" has been shown to exert diverse biological activities, including apoptosis in leukemia and melanoma cell lines. In this study, we investigated the *in vitro* cytotoxicity and antiproliferative actions of *T. officinale*extracts in cervical cancer cell lines, with the aim of evaluating their possible use as alternative or complementary cancer treatments.

Ethanolic extracts were prepared from of aerial parts and roots: Ap-EtOH and R-EtHO. The *T. officinale* latex (L) was obtained from the roots directly. The extracts were concentrated to dryness. Both extracts and latex were dissolved in DMSO (100 µg/mL). The study was conducted on cervical cancer cell lines (Caski and Hela) and normal keratinocyte HaCaT. All cell lines were incubated with 15 different concentrations (10-1000 µg/ml)for triplicate, of each extract and latex. After 72 h incubation, cytotoxicity was assessed using the MTT dye. Cell morphology was observed using an inverted fluorescence microscope and apoptotic morphological changes were detected by Hoechst staining. Doxorubicin 5µM was use as a positive control.

Both extracts and latex showed a dose-dependent cytotoxic effect in all cell lines. The order of cytotoxicity was R-EtOH> L>Ap_EtOH.

R-EtOH and L were more cytotoxic in Hela and Caski that in HaCaT. The viability reduction was dependent on exposure time for R-EtOH and L. Fluorescence microscopy showed apoptotic morphological changes, such as fragmentation and chromatin condensation, in treated Hela and Caski cells.

Our results suggest that *T. officinale* contains components that could induce apoptosis in Hela and Caski cells, thus, it should be explored for its antitumor potential in cervical cancer.

636 (401) **NATIVE MEDICINAL PLANTS EFFECT ON CENTRAL NERVOUS SYSTEM PATHOLOGIES**

Valentina Pastore, Carolina Marcucci Natalia Colettis, Beatriz Graciela Varela, Hernán Gerónimo Bach, Rafael Alejandro Ricco, Marcelo Luis Wagner, Mariel Marder
Universidad de Buenos Aires. Consejo Nacional de Investigaciones Científicas y Técnicas. Instituto de Química y Fisicoquímica Biológicas Prof. Dr. Alejandro C. Paladini (IQUIFIB). Facultad de Farmacia y Bioquímica. Buenos Aires, Argentina. Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Departamento de Farmacología. Cátedra de Farmacobotánica. Buenos Aires, Argentina. Instituto Nacional de Tecnología Agropecuaria (INTA). Instituto de Recursos Biológicos. Buenos Aires, Argentina.

Neurodegenerative, neurological and psychiatric diseases are pathologies with huge social and economic impacts. Their treatments are based on drugs that alleviate symptoms and their efficacy is diminished by side effects, so, there is a clinical need to find alternative treatments, which is the aim of our research. Our country harbors several thousands of plant species, which lack scientific information although many of them are used in folk medicine.

We have wide experience studying the effect on CNS of natural and synthetic compounds. Our hypothesis is that native plants have unexplored compounds with multiple biological activities on CNS.

Herein we present a preliminary screening of the CNS effect, related to their traditional use; and chemical composition, of hydroalcoholic extracts of 5 Argentinian plants: aerial parts of *Salvia guaranítica* (Labiatae) and *Ligariacuneifolia* (Loranthaceae); and underground parts of *Valerianacarnosa*, *macrorhiza*and *clarionifolia* (Valerianaceae).

These extracts were evaluated *in vitro* for the presence of acetyl and butyrilcholinesterase inhibitors (AChE/BChE, mice brain homogenate/plasma); and *in vivo* for their capacity to inhibit pentylenetetrazol (PTZ) seizures, on locomotion and exploratory behavior (holeboard assay) in Swiss male mice (CICUAL EXP-FyB N° 0058084/2015).

Valeriana extracts showed AChE inhibition. *V. clarionifolia*was the most active one with an IC_{50} 2.6 ± 0.8 mg/ml. Meanwhile, *Ligariacuneifolia* (100 mg/kg i.p. and 300 mg/kg p.o.) protected mice for tonic phase seizures induced by PTZ. Moreover, *V. macrorhiza* increased mice locomotor activity and all valerianas showed an anxiolytic profile in the hole board assay (300-600 mg/kg p.o.).

Our study is an important contribution for the discovery of unkwown native herbal products with CNS effects to develop novel therapeutical agents and strategies.

637 (479) **STABILITY OF AN AQUEOUS EXTRACT OF LARREA DIVARICATA CAV. DURING A SIMULATED DIGESTION PROCESS**

Ignacio Nahuel Peralta[1], Demian Monti[1], Renzo Martino[1], Maria Rosario Alonso[1,3], Claudia Alejandra Anesini[1,2],
[1]IQUIMEFA (Instituto de Química y Metabolismo del Fármaco) [2]Cátedra de Farmacognosia. FFYB.UBA [3]Cátedra de Farmacología. FFYB.UBA

Medicinal plants are used as therapeutic agents but little is known about their pharmacokinetic properties especially the stability in gastric and intestinal fluids, phenomenon that affects their bioavailability. Solubility and stability in gastrointestinal fluids is normally a prerequisite for the potential in vivo beneficial role of an extract given by an oral route.

Larreadivaricata Cav. is a South American plant widely distributed in Argentina, which aqueous extract exerts antioxidant, antitumoral and antimicrobial activities. Nevertheless, nothing is known about its stability in simulated digestion fluids. So the aim of this work was to study the stability but also the solubility of a lyophilized aqueous extract of this plant compressed as a pill. In order to do this, quantification of its majority polyphenol compound nor-dihydroguaiaretic acid (NDGA), of the total polyphenols and flavonoids as well as the antioxidant activity parameters such as DPPH scavenger activity and reducing power were assayed after submitting the extract to different incubations time in simulated digestive fluids.

In all cases, the percentage of recovery of NDGA was high, in the order of 90%. These values suggested not only that NDGA did not change its solubility depending on pH but also it didn't suffer any metabolism in presence of enzymes or pH variance which is important because of NDGA's wellknown antioxidant activities. On the other hand, total polyphenols were not modified in simulated gastrointestinal fluids, but total flavonoids slightly decreased after incubation in gastric fluid (60 min and 120 min) in the range from 12 to 18% respectively. On the contrary incubations in intestinal fluid during 120 and 240 min provoked a higher decrease than in gastric fluids reaching a value of 45% and 40% respectively. The DPPH scavenger activity decreased in intestinal fluids in concordance to the decrease of flavonoids level in the same fluid, nevertheless, the percentage of decrease of the antioxidant activity was lower, near 12%. These results suggested that others polyphenols, such as NDGA, could balance out the activity in view of flavonoids drop. The same was observed withthe reducing power in the intestinal fluid during 240 min of incubation, in which the activity decreased only 14% suggesting that other polyphenols could buffer the decrease of flavonoids in that fluid.

We concluded that the extract was stable in gastrointestinal simulated fluids maintaining its antioxidant activities which could support its use by an oral route.

638 (929) **IN VITRO ANTIOXIDANT AND ANTIPROLIFERATIVE ACTIVITIES OF POTATO CULTIVARS FROM THE ARGENTINIAN NORTHWEST POLYPHENOLIC EXTRACTS**

María Julia Martinez[1], Luciana Barbini[2], Adriana Balbina Andreu[1],
[1]Instituto Investigaciones Biológicas, Universidad Nacional de Mar del Plata, Deán Funes 3350, Mar del Plata, Argentina. [2]Cátedra de Microbiología Clínica, Departamento de Química, Universidad Nacional de Mar del Plata, Deán Funes 3350, Mar del Plata, Argentina.

Plant polyphenols are secondary metabolites, studied for their beneficial effects on human health. Potato is an important source of antioxidants for human diet. The anticancer activity of polyphenolic extracts has been largely described. Hepatocellular carcinoma (HCC), one of the most frequent tumors worldwide, is associated to high mortality. Although the availability of treatments, HCC patients remain with poor prognosis. Natural compounds appear as a potential source of new anti-HCC drugs. The objective of this study was to study the polyphenolic composition and the *invitro* antioxidant and cytotoxic activities of potato polyphenolic extracts (PPE). Six potato varieties were selected: 5 Andean (CCS1283, CCS1307, CS1418, CL658 and CCS1385) and one industrial variety. PPE characterization was made by spectrophotometric assays and HPLC-DAD analysis and the antioxidant activity was studied by DPPH assay. The cytotoxic analysis was performed on a human hepatocellular carcinoma cell line, by the MTT assay. The results showed that phenolic acids are the main group of compounds in all 6 PPE, showing the pigmented varieties higher levels. The characterization of the PPE composition by HPLC-DAD showed that chlorogenic acid is the main phenolic acid in all the varieties, followed by caffeic acid. Anthocyanidin HPLC-DAD profile resulted completely different in the pigmented varieties. Also, pigmented potatoes presented higher antioxidant activities, compared to non-pigmented ones. Only, 4 of the studied PPE reducedcell viability [50% cytotoxic concentrations (CC50)]: CCS1385 37,28 µg CGA equiv/mL; CS1418 54,55 µg CGA equiv/mL; CCS1307 66,71 µg CGA equiv/mL; and 71 µg CGA equiv/mL for CL658. A positive significant correlation between antioxidant activity and total phenolic content was found, and an inverse correlation between antioxidant, total phenolic content and cytotoxic activity, suggesting that PPE of different polyphenolic compositions can exert anti-HCC activity.

639 (932) **EFFECT OF LUTEOLIN ON ANTIOXIDANT ACTIVITY OF SUPEROXIDE DISMUTASE AND CATALASE ALTERED BY GENTAMICIN IN HUMAN LEUKOCYTES**

Pamela Soledad Bustos[1], Paulina Laura Páez[2], José Luis Caberar[1], María Gabriela Ortega[1],
[1]IMBIV-CONICET – Dpto. de Farmacia - Fac. de Cs. Químicas, UNC. [2]UNITEFA-CONICET - Dpto. de Farmacia - Fac. de Cs. Químicas, UNC.

Superoxide dismutase (SOD) and catalase (CAT) are important endogenous antioxidant defenses against attack by reactive oxygen species (ROS). SOD, dismutes to superoxide anion in oxygen and hydrogen peroxide, while CAT catalyzes the decomposition of hidrogen peroxide into oxygen and water. Gentamicin (GEN) is an antibiotic whose clinical usefulness is limited by the development of side effects that would be associated with increased oxidative stress in human cells. Previous studies in our group demonstrated that GEN is able to induce ROS production and alter the activity of these enzymes in mononuclear (MN) and polymorphonuclear (PMN) human leukocytes. In order to find natural compounds that neutralize the leukotoxicity of GEN, we evaluated the effect of luteolin (L), a flavonoid isolated from fruits of *Prosopisstrombulifera* var. *strombulifera* with antioxidant activity,as potential protective agent against oxidative stress induced by GEN. The SOD activity was evaluated by assaying Methionine, Riboflavin and NBT, and the CAT activity, using potassium dichromate in acetic acid, both assays by spectrophotometry.GEN, in MN induced an increase of the activity of both enzymes SOD and CAT at 0.25 and 8 µg/mL, as a response that counteracts the ROS induced by the antibiotic, while at 128 and 256 µg/mL occurs a decrease of enzyme activity, possibly due to exhaustion produced by the exacerbated generation of ROS. On the other hand, in PMN, GEN caused a decrease of enzyme activity at all concentrations tested. As for the effect of L it was observed that luteolin (0.08, 0.25 and 0.8 µM in MN and 0.4, 1.7 and 6.8 µM in PMN) tends to level the antioxidant activity of SOD and CAT at similar values to basal level, especially at higher concentrations.

Therefore, L shown ROS scavenger effect and contribute to the activity of endogenous antioxidant defenses, demonstrating a marked protective activity of L against oxidative stress generated by GEN in human leukocytes

640 (949) **EFFECT OF IRRADIATION FREQUENCY IN PHOTODYNAMIC INACTIVATION OF CANDIDA TROPICALIS BIOFILMS BY NATURAL ANTHRAQUINONES IN COMBINATION WITH ANTIFUNGALS**

Juliana Marioni[1], José Luis Cabrera[1], María Gabriela Paraje[2], Susana Carolina Núñez Montoya[1],
[1]IMBIV, UNC, CONICET, FCQ, Córdoba, Argentina. [2]IMBIV, UNC, CONICET, FCEFyN, Córdoba, Argentina.

A new strategy to improve the effect of antifungal agents against biofilms is their combination with natural compounds. We proved that rubiadin (1) and rubiadin 1-methyl ether (2), two natural photosensitizing anthraquinones (AQs), reduce the biofilm formation of *C. tropicalis* due to a photodynamic action. Antibiofilm effect of each AQ was enhanced by applying an irradiation sequence. Thus, the bioactive concentration of each AQ was halved, achieving also an increase in the reducing effect only in the case of 2 (63 ± 7%R).

The aim of this work was to expand the antibiofilm activity of these AQs against C. tropicalis, applyingthe same irradiation sequence but using each AQ combined each other or with amphotericin B (AmB).

AQs were identified by their NMR spectral data (94% purity). Compounds Combination was performed by the checkerboard microdilution method, using three concentrations (SubMIC, MIC y SupraMIC). Irradiation sequence comprises light exposure for 15 min at 0, 3, 6, 24, 27 and 30 h of incubation, with an actinic lamp placed 20 cm above the microplate (Vmax 420 nm). Similar microplateswere kept in darkness for the total incubation time (48 h). Biofilm quantification was performed by O'Toole method.

An enhancing effect by the light action was observed for the combination of 1 SubMIC and AmB SubMIC (74 ± 9%R), and for the combination of 2 SubMIC and AmB SubMIC (82 ± 2%R). Combination of both AQs reached only a 50%R at SubMIC concentrations.

The increase in the irradiation frequency not only allowed halving the bioactive concentrations of each compound, using SubCIM concentrations, but also potentiated significantly the photo-inactivation of *C. tropicalis* biofilms when each AQ was combined with AmB. Our results support that the combination of drugs with different action mechanism improves antibiofilm effect. Therefore, each AQ combined with AmB, have potential application in Antimicrobial Photodynamic Therapy for the treatment of *C. tropicalis* biofilms.

641 (973) **MODULATORY EFFECT OF URERA AURANTIACA EXTRACT ON IMMUNE AND TUMORAL CELLS DURING INFLAMMATION**

Carla Marrassini[1,2], Claudia Anesini[1,2],
[1]IQUIMEFA-CONICET (Instituto de Química y Metabolismo del Fármaco) [2]Cátedra de Farmacognosia, Facultad de Farmacia y Bioquímica, UBA

There is a known link between inflammation and cancer. *Ureraaurantiaca* (Urticaceae) is a medicinal plant used in inflammation.

The effect of a methanol extract (UA) upon the viability (MTT method) and proliferation (thymidine tritiated uptake) of normal and tumoral lymphocytes under the effect of prostaglandin E_2 (PGE_2), and on nitric oxide (NO) production by LPS-stimulated macrophages (Griess method) was analyzed. PGE_2 mechanism of action was studied in presence of inhibitors: staurosporine (ST) 10^{-9}M (PKC inhibitor), SB 203580 10mM (MAPK P38 inhibitor), PD 98059 5µM (ERK1/2 inhibitor), sodium azide (SA) 2mM (peroxidase inhibitor). Results are expressed as fold of stimulation respect to basal (Mean±SEM) of 3 experiments made by triplicate (*p<0.05, **p<0.01 significantly ANOVA+Turkey test).

Normal lymphocytes: Basal: 1; PGE_2 (1nM): 1.78±0.1*; UA (1µg/ml): 2.84±0.2*; PGE_2 (1nM) + ST: 0.97±0.08; PGE_2 (1nM) + SA: 0.85±0.07; UA (1µg/ml) + PGE_2 (1nM): 0.78±0.07.

Macrophages: Basal: 1; LPS (1µg/ml): 1.49±0.1*; UA (100µg/ml): 0.71±0.07**; UA + LPS: 0.84±0.08.

Tumoral lymphocytes: Basal: 1; PGE_2 (0.1nM): 1.53±0.1*; UA (10µg/ml): 1.43±0.14*; UA (100µg/ml): 0.61±0.05**; PGE_2 (0.1nM) + SB: 0.94±0.09; PGE_2 (0.1nM) + PD: 1.06±0.09; PGE_2 (0.1nM) + ST: 1.05±0.09; UA (10µg/ml) + PGE_2 (0.1nM): 0.89±0.08.

UA stimulated normal lymphocytes but reversed PGE_2 effect. Their proliferation induced by PGE_2 is related to PKC and H_2O_2. In macrophages, UA did not modify cell viability and reversed LPS-induced NO. In tumoral lymphocytes, UA exerted a biphasic effect: at low concentrations increased cell proliferation and at higher it exerted antiproliferative activity. UA was capable to reverse PGE_2 proliferative action. Their proliferation induced by PGE_2 is related to PKC, ERK1/2 and MAP Kinase P38 pathways.

The observed effects may be related to polyphenols, flavonoids, and tannins. UA showed modulatory effects during inflammatory conditions reinforcing its anti-inflammatory action.

642 (2055) **INHIBITION OF BOTHROPS DIPORUS VENOM ACTIVITY BY DERMAL APPLICATION OF A CISSAMPELOS PAREIRA EXTRACTS FORMULATION**

Bárbara Verónica Ricciardi Verrastro[1], Gabriela Ricciardi[1], Pamela Teibler[2], Laura Lozina[2], Silvana Maruñak[2], Eduardo Dellacassa[3], Ana María Torres[2],
1Laboratorio de Productos Naturales Prof. Armando Ricciardi - FaCENA-UNNE - Av. Libertad 5470 - CorrientesM 2Laboratorio de Toxicología - Cátedra de Farmacología y Toxicología - FCV-UNNE. Sargento Cabral 2139 - Corrientes 3Facultad de Química - Departamento de Química Orgánica - Universidad de la República - Uruguay.

Cissampelospareira L., ka'apeva, is an herbal species possessing ethnobotanical uses as snakebite antivenom, either as an infusion or poultice. In Argentina, ofidic accidents by yarara represent a serious public health problem, being *B. diporus*, known as "yararáchica", responsible for the 80% of them. In a previous study we described its *in vitro* antivenom activity, so in this work we intended to verify the *in vivo* activity by using a heat sensitive gel formulation for local application.

The most active *C. pareira* extract found (ethanolic leaves) was fractionated by flash column chromatography, tracking its antivenom activity by SDS-PAGE. The polar fraction (F6) was selected as the most active and formulation of dermal application (1% plant extract, 10% ethanol 96º, 10% DMS, 80% poloxamer) was prepared. BALB/c mice were used (each group n=4, protocol approved by ethics committee of the FCV-UNNE). V group, was subcutaneously injected with a 200ug dose of venom in 0.1 ml PBS; V+C group was injected with the same dose and after 15 minutes, the heat sensitive gel was applied every 12 hours for 3 days; C group was applied gel only to check safety. Once the protocol was complied, mice were killed by anesthesia with xylazine hydrochloride-ketamine and a fragment of each lesion fixing in 10% formalin were retired for histological evaluation. The tissues were processed and stained with hematoxylin-eosin in order to be observed by optical microscopy. Histopathological analysis showed differences between the groups: **V+C group** had areas with absence of epidermis with mild inflammatory infiltrate and neutrophilic absence of hemorrhage; **V group** evidenced dermonecrosis and intense bleeding with inflammatory infiltrate.

After 3 days of gel application it was possible to obtain a remarkable improvement in the tissue renovation, so demonstrating the activity and usefulness of the preparation. Even when it is essential improving the permeation of the bioactive compounds; the formulation seems to be a valid option for local treatment.

643 (422) **CHEMOPROPHYLACTIC EFFICACY OF Î²-MYRCENE AGAINST ECHINOCOCCUS GRANULOSUS METACESTODES**

Julia Fabbri[(1,2)], Patricia Eugenia Pensel[(1,2)], Clara María Albani[(1,2)], Guillermo María Denegri[(1,2)], María Celina Elissondo[(1,2)],
[1]Laboratorio de Zoonosis Parasitarias, FCEyN, UNMdP, [2]CONICET

Human cystic echinococcosis is a zoonosis caused by the larval stage of *Echinococcus granulosus*. Due to the difficulties in achieving treatment success and toxicity of the used compounds, the search of new therapeutic alternatives such as the use of traditional medicinal plants has increased. Beta-myrcene is one of the main components of the essential oil of *Rosmarinus officinalis*. The *in vitro* effects against protoescoleces and cysts of *E. granulosus* were previously demonstrated. The aim of this work was to determine the chemoprophylactic efficacy of β-myrcene against *E. granulosus*. Animal procedures and management protocols were approved by the Institutional Animal Care and Use Committee (RD 148/15) of the FCEyN, UNMdP. Forty CF-1 female mice were allocated into 4 groups: a) water control; b) oil control; (c) 25 mg/kg ABZ suspension; (d) 250 mg/kg β-myrcene. Treatments were performed daily during 30 days by intragastric inoculation. Five months after infection, mice were euthanized, cysts were removed from the peritoneal cavity and the treatment efficacy was evaluated by the mean cysts weight, and ultrastructural changes of cysts. The mean weight of cysts recovered from mice treated with ABZ suspension and β-myrcenewere lower than that obtained from control mice. Nevertheless, no statistical differences were

found between groups (P=0.0667). No structural and ultrastructural changes were observed in cysts obtained from control mice. In contrast, the ultrastructural study of cysts developed in mice treated with ABZ suspension and β-myrcene revealed loss of the characteristic multicellular structure, showing areas with loss of cells and the presence of altered cells. The data obtained demonstrated that the chemoprophylactic efficacy of β-myrcene against *E. granulosus*is similar to the effects produced by ABZ suspension. *In vivo* studies to test the clinical efficacy of β-myrceneare currently being undertaken on the murine model of cystic echinococcosis

INMUNOFARMACOLOGÍA / IMMUNOPHARMACOLOGY

644 (566) **EFFECT OF SELECTIVE SEROTONIN REUPTAKE INHIBITORS ON CHRONIC STRESS-INDUCED IMMUNOMODULATION AND MOLECULAR ALTERATIONS RELATED TO EL-4 LYMPHOMA INVASION IN C57BL/6J MICE.**

María Emilia Di Rosso[1], María Rosa González Murano[1], Ana María Genaro[1,2],
[1]Instituto de Investigaciones Biomédicas (BIOMED-UCA-CONICET), Ciudad de Buenos Aires, Argentina. [2]1era Cátedra de Farmacología, Facultad de Medicina, UBA, Ciudad de Buenos Aires, Argentina.

Chronic stress is involved in the onset of specific psychiatric diseases such as major depression. Fluoxetine (F) and sertraline (S), two selective serotonin reuptake inhibitors, are widely used for the treatment of depressive symptoms of cancer patients although there are contradictory evidences about its effects on immunity and neoplastic processes. We have previously reported that both F or S are able to revert chronic stress enhancement of EL-4 lymphoma growth and of spontaneous metastasis. In the present work we studied the effect of F or S on chronic stress-induced reduction in T cell proliferation and molecular alterations related to cell invasion. Female C57BL/6J mice were subjected (E) or not (C) to a heterotrophic chronic stress model for five weeks. Chronic administration of F or S reverted chronic stress-induced decrease in T cell proliferation to the selective mitogen Con A evaluated by [3H]-thymidine incorporation (Interaction stress x drug, $p<0.05$, n=4). Moreover, F or S were able to enhance T cell proliferation compared to C animals ($p<0.05$, n=4). After five weeks of chronic stress exposure and chronic administration of F or S, micewere subcutaneously injected with EL-4 T lymphoma cells to generate a solid tumor. Chronic administration of F or S reverted chronic stress-induced increase in MMP-2 and MMP-9 mRNA levels in the solid tumors evaluated by qRT-PCR (Interaction stress x drug, $p<0.05$ and $p<0.001$ respectively, n=4). Furthermore the reduction in MMPs inhibitors TIMP-1 and TIMP-2 mRNA levels observed in E mice, was reverted by both F or S (Interaction stress x drug, $p<0.05$ and $p<0.01$ respectively, n=4). These results suggest that chronic antidepressant treatment prevents enhanced tumor evolution by reversing T-cell impairment and tumor cell invasion capacity. Our growing understanding of novel pharmacological actions of these drugs provides new perspectives in cancer therapy.

MICROBIOLOGÍA / MICROBIOLOGY

645 (1052) **SURFACE PROPERTIES OF LACTIC ACID BACTERIA FOR THE DESIGN OF A PROBIOTIC FORMULA FOR BOVINE FEED-LOT**

Flavia Ivana Mansilla, Natalia Cecilia Maldonado, María Cecilia Aristimuño Ficoseco, Constanza Victoria Melian, Graciela Margarita Vignolo, Maria Elena Fatima Nader
CERELA-CONICET. Centro de Referencia para Lactobacilos.

The use of Probiotics in Intensive Steers Systems (ISS) has emerged as an alternative to decrease antibiotic application. New Probiotic Formulas are being designed in Pharmaceutical and Food fields for human and animal health, by using bacteria isolated from the indigenous microbiota of thespecific host. The screening of the bacterial surface properties to promote the adhesion, biofilm formation and colonization to the host must be evaluated for the selection of probiotic strains. Our research group is working on the design of a probiotic formula for bovine feedlot, then around 500 strains were previously isolated, identifying 72 lactic acid bacteria (LAB) by genetic tools. The aimof this work was to perform the screening of the surface properties of the LAB, identified into the *Enterococcus*, *Lactobacillus, Pediococcus* and *Weissella* genera. Autoaggregation, surfacehydrophobicity and Biofilm Formation (BF) were evaluated. The hydrophobic nature of the cell surface was determined by the MATH (microbial adhesion to hydrocarbons: toluene and xylene) method. The extent of aggregation was studied by the decrease of optical density of cell suspensions. BF was assayed by the crystal violet-stained method in MRS media with and without Tween 80 (MRS-T). Theresults obtained indicate that most of the strains (85% and 82%) show a low degree of hydrophobicity (0-30%) in toluene and xylene, respectively. High aggregation patters were observed in 5% of the LABs. There was a correlation between the two surface properties in *L mucosae* CRL2069 and CRL2115. The strains showing BF in MRS were: *L mucosae* CRL2083 and CRL2063; and in MRS-T: *E faecium* CRL2141, *E hirae*CRL2062, *L mucosae* CRL2101 and CRL2112, and *L fermentum* CRL2085. From the results obtained, the strains selected were: CRL2141 (high BF andhydrophobicity) and CRL2069 (high aggregation and hydrophobicity). The screening performed allowed to select strains expressing surface properties to be further evaluated for the design of a probiotic formulation for ISS.

646 (1077) **SENSITIVITY PROFILES OF ANTIMICROBIAL PEPTIDES PRODUCED BY LACTIC-ACID BACTERIA AGAINST REGIONAL S. AUREUS STRAINS ISOLATED FROM BOVINE MASTITIS.**

Gabriela Edith Aguirre[1], Matias Lorenzutti[1,2], Pilar Zarazaga[1,2], Ingrid Capello[1], Aldana Gramaglia[1], Nicolas Litterio[1,2],
[1]Universidad Nacional de Villa María. Instituto de Cs. Básicas y Aplicadas Carrera de Veterinaria. Obispo Ferreyra 411. CP: 5963 Villa del Rosario, Córdoba. [2]Universidad Católica de Córdoba. Facultad de Cs Agropecuarias, carrera de Veterinaria. Cátedra de Farmacología y Toxicología. Av. Armada Argentina 3555. CP: X5016DHK, Córdoba.

In response to the growing development of bacterial resistance to antimicrobial agents (ATM), the use of antimicrobial peptides (AP) produced by lactic-acid bacteria (LAB) could be an alternative. There is limited evidence about antimicrobial efficacy of APs against bacteria that cause bovine mastitis. The objective of this study was to assess the antimicrobial activity of APs against *S. aureus* strains isolated from bovine mastitis in Cordoba, Argentina. *S. aureus* (*n*=46) and LAB (*n*=54) strains were isolated from milk samples. The antimicrobial activity of substances in cell-free extracts of LAB against *S. aureus* strains was evaluated by agar diffusion technique. After treatment with proteinase K, four APs were recovered, which were concentrated and purified by adsorption-desorption, dialysis, lyophilization and SDS-PAGE electrophoresis. The APs concentrations were estimated by ImageJ™ software. The minimum inhibitory concentration (MIC) for APs (3 produced by *Lactobacillus* spp and1 by *Enterococcus* spp) against *S. aureus* were determined. APs concentrations obtained from purification were unable to reach MIC_{50} and MIC_{90} against *S. aureus,* but MIC_{35} were calculated for all peptides. Only one of the APs was identified by mass spectrometry, pending the results of the remaining. The amino acid sequence of the identified peptide 50S-L32, corresponds to ribosome-constitutive *Lactobacillus brevis*. There was no reports of its antimicrobial activity, and its amino acidic content is characteristic of cationic APs of bacterial origin. A 35.42% inhibition of *S. aureus* strains was achieved only with a relative high concentration of the peptide (0.115 µg/ml). Understanding thestructure-activity relationship of APs is essential for development of new therapeutic antimicrobial alternatives.

NANOMEDICINA / NANOMEDICINE

647 (763) **EVALUATION OF RADIOLABELED TPGS-BASED NANOMICELLES AS IMAGING PROBES. IN VIVO CHARACTERIZATION IN HEALTHY AND TUMOR BEARING ANIMALS.**

Fiorella Carla Tesan[3], Diego Giaquinta[3], Melisa Nicoud[2, 3], Marcela Moretton[1, 4], Vanina Medina[2, 3], Diego Chiapetta[1, 4], Marcela Beatriz Zubillaga[3], María Jimena Salgueiro[3],
[1]Consejo Nacional de Investigaciones Científicas y Técnicas. Buenos Aires, Argentina. [2]Consejo Nacional de Investigaciones Científicas y Técnicas. Instituto de Investigaciones Biomédicas. Pontificia Universidad Católica Argentina. Buenos Aires, Argentina. [3]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Fisicomatemática, Cátedra de Física. Buenos Aires, Argentina. [4]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Tecnología Farmacéutica, Cátedra de Tecnología Farmacéutica I. Buenos Aires, Argentina.

TPGS-based nanomicelles (TBN) is a system that has been used for delivery of therapeutic drugs. The aim of this work is to label TBN with a gamma emitting nuclide as ^{99m}Tc and evaluate its potential use as an imaging probe for cancer diagnosis. Radiolabelling of TBN with ^{99m}Tc was performed by the direct method. Radiochemical impurities (free ^{99m}Tc and radiocolloids) were assessed by thin layer chromatography and filtration. Size and morphology of radiolabeled nanomicelleswere characterized by dynamic light scattering and by transmission electronic microscopy respectively. Pharmacokinetic studies were performed in healthy animals by two different assays: a 24h plasmatic concentration curve and biodistribution (BD) by means of small animal imaging (static and dynamic studies) after intravenous administration (1mCi/animal) of the radiolabeled probe. Regions of interest were drawn over organs of interest in the static images acquired in order to get semi quantitative results of the radioactive micelles BD. To evaluate the tumor uptake, two animal models of breast cáncerwere used: N-nitroso-N-methyl-urea induced mammary adenocarcinoma in Sprague Dawley rats and a syngeneic subcutaneous tumor (4T1 breast cell line) in balb-c mice. The uptake ratio (Tumor/Background) was calculated. Plasmatic concentration over time in healthy animals resulted in a two phase decay curve (R^2=0.85;%ID_0=41.46; Pl=1.984; K_{fast}=2.052h^{-1}; K_{slow}=0.3379h^{-1}).%Total activity in BD studies for 1 and 12 hs post injection were: 6.1±0.5; 3.9±0.1 soft tissue, 1.2±0.2; 1±0.1 bone, 1.5±0.6; 0.7±0.3 heart, 16.6±1.3; 26.5±1.7 kidneys, 8.6±1.1; 11.1±0.1 liver. T/B was between 30 and 80 in the images performed in the chemically induced tumors. However no uptake was appreciated in images of the 4T1 tumors in mice. Further studies must be conducted to establish the quantity of TBN to be administered for in vivo protocols. Animal procedures were approved by the CICUAL of the FFYB, UBA (Res CD 3761/13)

TOXICOLOGÍA / TOXICOLOGY

648 (1041) **CAPACITY OF MAYORITARY COMPOUNDS OF ACHYROCLINE SATUREIOIDES TO PROTECT FROM THE DAMAGE INDUCED BY AFLATOXIN B1 IN WISTAR RATS**

María Carola Sabini[1], Franco Matías Escobar[1], Laura Noelia Cariddi[1], Guillermo Bagnis[2], Laura Comini[3], Florencia Menis Candela[1], Alejandra Magnoli[1,2], Carla Barberis[1], Liliana Inés Sabini[1], Ana María Dalcero[1],
[1]Dpto. Microbiología e Inmunología, Facultad de Ciencias Exactas Físico- Químicas y Naturales, Universidad Nacional de Río Cuarto. [2]Facultad de Agronomía y Veterinaria. Universidad Nacional de Río Cuarto. [3]Farmacognosia, Departamento de Farmacia, Universidad Nacional de Córdoba Ciudad Universitaria.

Aflatoxin B1 (AFB1), produced by A. flavus and A. parasiticus, is carcinogenic, teratogenic, hepatotoxic, immunotoxic. It is the most important worldwide mycotoxin for its effect on public health and animal productivity. Macela has several scientifically proven medicinal properties, such as antioxidant, antimicrobial. The objective was to evaluate the ability of majority compounds (MC) of A. satureioides to protect of genotoxic and liver damage induced by AFB1 in rats.

Wistar rats (200 g) in lots of 4 (2 males and 2 females) were used. Animals were inoculated by intraperitoneal injection. Treatments were: 1-AFB1 (1 mg/kg body weight) + Luteolin (L) (2.5 mg/kg b.w.); 2-AFB1 (1 mg/kg) + Quercetin (Q) (2.5 mg/kg); 3-AFB1 (1 mg/kg) + Chlorogenic acid (CHLA) (5 mg/kg); 4-Negative control: saline solution; 5-Positive control: cyclophosphamide 30 mg/kg; 6-AFB1 Control: AFB1 (1 mg/kg) dissolved in methanol (5%) and saline solution. Animals were sacrificed by cervical dislocation at 24h post-injection. Experiments: 1-Micronuclei in mouse bone marrow: Schmidt W. (1975), number of micronuclei in 1000 polychromatic erythrocytes (PCE) and toxicity index were determined. 2- Hystopathology: Parts of the organs were pre-served in 10% buffered formaldehyde (pH 7.4). These samples were cut at 4 µm thickness and subjected to haematoxylin/eosin staining for microscopic histological. Photomicrographs were taken and analyzed.

The results of genotoxicity revealed that AFB1 at 1 mg/kg showed an index of genotoxicity of 35 MN/1000 PCE, showing statistically significant difference with the negative control ($p < 0.001$), and that genotoxic damage was reversed with treatment of L and CHLA. While, Q did not protect from damage. Hystopathological examination of the livers of AFB1-treated group revealed characteristic lesions like slight fat accumulation and swollen cells. The negative control group and the treatments showed a liver with normal appearance. In conclusion, L and CHLA protect genotoxic and hepatotoxic damage induced by AFB1.

MC: Majority compounds L: Luteolin Q: Quercetin CHLA: Chlorogenic acid

649 (1095) **NIFURTIMOX TRANSFER INTO BREASTMILK IN LACTATING WOMEN WITH CHAGAS DISEASE**

Maria Elena Marson[1,3,4], Facundo Garcia Bournissen[2,3], Samanta Moroni[2], Guillermo Moscatelli[2], Jaime Altcheh[2,3], Guido Mastrantonio[1,3,4],
[1]Facultad de Ciencias Exactas-Universidad Nacional de La Plata, [2]Hospital de Niños Ricardo Gutierrez, [3]CONICET, 4Comision de Investigaciones Cientificas de la provincia de buenos aires (CIC)

Background: Chagas disease is a serious public health problem in Latin America, and in other non-endemic regions. Nifurtimox (NFX) is safe and effective for the treatment of paediatricChagas disease but discouraged in breastfeeding women because no information on NFX transfer intobreast milk is available. However, this phenomenon was not evaluated until now, and measurement techniques for NFX in milk were not developed. Objectives and Methods: We present the development of a simple and fast method to quantify NFX in human plasma and breast milk using HPLC-UV analysis. We aimed to evaluate NFX concentrations in both matrices in order to estimate potential infant exposure through breast milk. The method was applied to a prospective cohort study of lactating women with Chagas disease treated with NFX for 30 days. The clinical protocol was approved by both ethical and research review committees of Children's Hospital Ricardo Gutierrez, Buenos Aires City.

Results: Methods developed for NFX extraction and quantification were satisfactory for clinical purposes attended. Bioanalytical parameters for plasma and breast milk provides a linear range of1.70 – 12.05 µg/mL and 2.80 – 11.25 µg/mL with average recoveries of 70.5 (variation coefficient (VC)% 0.99) and 69.5 (VC% 1.6) respectively. The analytical response within the linear range was reproducible, showing a%VC intra-day and between days for both matrices of less than 10%. Samples of human plasma and breast milk from 10 patients under treatment were dosed. Plasma and breast milk concentration range were < LOD – 9.5 µg/mL.

Discussion and Conclusion: This is the first report of a method to quantify NFX in breast milk. Observed concentrations were lower than those reported in the bibliography. These results

confirms previous theoretical estimates proposing that transfer of NFX into breast milk is limited andunlikely to lead to significant exposure of the breastfed infant.

650 (2009) **IPOMOEA CARNEA INDUCES APOPTOSIS IN LYMPH NODE CELLS IN A GUINEA PIG MODEL**

Enrique NicolasGarcia [1], María Victoria Aguirre[2], Juan Santiago Todaro[2], Luciana Andrea Cholich[1],
[1]Cátedra de Farmacología y Toxicología, Facultad de Ciencias Veterinarias, Universidad Nacional del Nordeste, Sargento Cabral 2139, Corrientes 3400, Argentina. [2]Cátedra de Bioquímica, Facultad de Medicina. Universidad Nacional del Nordeste, Mariano Moreno 1240 Corrientes 3400, Argentina

Ipomoea carnea causes poisoning of goats, sheep and cattle in many tropical and subtropical countries. Swainsonine (SW) an alkaloid isolated from this plant, it is a powerful inhibitor of lysosomalenzimes and the abnormal glycoprotein metabolism causes immunological dysfunction and neuropathy. Several studies have communicated that SW induces apoptosis *in vivo* and *in vitro*. Up to date, however, the effects of *I. carnea* on lymphoid tissues have not yet been examined. Thus, the aim of this study was to determine the frequency and the distribution of programmed cell death in mesenteric lymph nodes of guinea pig poisoned by *I. carnea* along 40 days of study. Experimental poisoning was achieved by feeding animals with pellets prepared with milled leaves of *I. carnea* mixed with commercial crushed pellets for rodents. For *in situ* detection of DNA fragmentation in paraffin-embedded lymph node sections, the TUNEL method was performed using *in situ* Cell Death Detection Kit. The apoptotic index was expressed as the percentage of TUNEL-positive cells out of the total number of cells counted. TUNEL reaction stained mainly apoptotic nuclei of mesenteric lymph node cells. The pattern of labeling distribution of dead cells was different in each experimental group. The 40 days treated group showed high immunoreactivity in the germinal center of follicles. The 40 days *I. carnea* fed group showed 15.57± 5.31% apoptotic cells. This value was statistically significant ($P<0.001$) compared to the other experimental groups (control group: 3.00 ± 1.58% and 20 days treated group: 5.80 ±1.10%, respectively). Our results evidence the enhancement of the programmed cell death of mesenteric lymph node cells induced by 40 days *I. carnea* ingestion. We conclude that this study provides additional knowledge about the effects of *I. carnea* poisoning on secondary lymphoid organs that might be useful as source of information to develop strategies for minimizing livestock production losses.

651 (2048) **CROSS-REACTIVITY OF BOTHROPS ANTIVENOMS WITH COLUBRIDAE SNAKE VENOMS FROM NORTHEASTERN ARGENTINA**

Matías Nicolás Sánchez[1,2], Carlos Ariel López[2], María Agustina Quintana[2], Gladys Pamela Teibler[3], María Elisa Peichoto[1,2],
[1]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Ministerio de Ciencia Tecnología e Innovación Productiva, Argentina. [2]Instituto Nacional de Medicina Tropical (INMeT), Ministerio de Salud de la Nación, Neuquén y Jujuy s/n, Puerto Iguazú (3370), Argentina. [3]Facultad de Ciencias Veterinarias, Universidad Nacional del Nordeste (UNNE), Sargento Cabral 2139, Corrientes (3400), Argentina.
It is currently known that some colubrid snakes (Colubridae) may cause human envenomation with mild to severe local and/or systemic effects, especially in children. These signs can be misinterpreted as mild envenomation by Bothropsspecies (Viperidae), and in this case it is common for physicians to prescribe Bothrops antivenoms; however, there is little information about their cross-reactivity with colubrid venoms.

The aim of this study was to evaluate the immunological profile and extent of cross-reactivity of two anti-*Bothrops*serums with some venoms of colubrid snakes commonly found in northeastern Argentina.

We performed SDS-PAGE (12% gel) of venoms from the following colubrid snakes: *Oxyrhopusguibei*, *Philodryasolfersii*, *Philodryaspatagoniensis* and *Leptophisahaetullamarginatus*, under reducing and non-reducing conditions. Then, we carried out western blotting analysis using bivalent and tetravalent anti-*Bothrops*serums.

SDS-PAGE gels from colubrid venoms showed distinct protein patterns, and it was possible to recognize protein bands ranging from 14.4 to 66.3 kDa when venom proteins were reduced with β-mercaptoethanol. Regarding the western blotting analysis, the order of cross-reactivity between bivalent/tetravalent antivenom and antigens present in colubrid venoms was the following: *O. guibei* and *P. patagoniensis* > *P. olfersii* >*L. ahaetullamarginatus*. In the latter the cross-reactivity was practically absent using both antivenoms.

These findings, although preliminary, constitute an evidence that proteins present in some colubrid venoms tested in this work contains similar epitopes than those present in *Bothrops* venoms and let us predict their toxicological complexity. They also constitute an effort towards the understanding of the protein composition of venoms from colubrid species and give insight into future directions for the isolation and characterization of key components present in these venoms.

PRESENTACIÓN DE POSTERS SAIC VI / SAIC POSTER PRESENTATION VI

REPRODUCCIÓN II/ REPRODUCTION II

652 (814) **ALTERATIONS OF ADIPONECTIN RECEPTORS AND 5-AMP KINASE EXPRESSION IN FOLLICULAR STRUCTURES OF COWS WITH CYSTIC OVARIAN DISEASE**

Natalia Carolina Gareis[1], Pablo Uriel Díaz[1], Natalia Raquel Salvetti[1], Carolina Guadalupe Panzani[1], Hugo Héctor Ortega[1], Gustavo Juan Hein[1], Florencia Rey[1]
[1]Laboratorio de Biología Celular y Molecular Aplicada, Instituto de Ciencias Veterinarias del Litoral (ICiVet-Litoral), Universidad Nacional del Litoral (UNL) / CONICET, Esperanza, Santa Fe, Argentina.

Adiponectin is an adipokine produced by adipose tissue which regulates several reproductive processes. Its role in steroidogenesis, considering the energy balance for the ovary functions, could be potentially associated to the development of reproductive disorders such as cystic ovarian disease (COD). The aim of this study was to determine the expression of components of the adiponectin system in ovarian follicles of cows with spontaneous COD and control cows. Thus, the receptors of adiponectin, AdipoR1 and AdipoR2, and the enzyme 5-AMP kinase (AMPK) were evaluated. Animals with spontaneous COD (n=8) and control cows (n=8) were ovariectomized and ovaries obtained were processed histologically to embedded in paraffin. Expression of target protein was assessed by indirect immunohistochemistry on ovarian tissue sections. Results showed similar expression of AdipoR1 in follicular structures evaluated in ovaries from control cows and cows with COD, showing a tendency to decrease the expression in granulosa cells of cysts regarding to antral follicles (as reference structure) of control cows ($p<0.1$). However, a higher expression of AdipoR2 was detected in theca cells of cysts compared to antral follicles of control cows ($p<0.05$) without differences in granulosa cells. Moreover, the expression of AMPK in theca cells of cysts was higher compared to control antral follicles ($p<0.05$) without differences in granulosa cells of the follicles analyzed. The results of the present study showed variations in proteins involved in response and signaling to adiponectin. Considering the association of this adipokine with several metabolic functions, such as improving the insulin sensitivity, and also its essential role for follicular growth and maturation, the alterations here detected could indicate a potential contribution of this cytokine in ovarian function related to reproductive disorders.

653 (924) **SPINK3 MODULATES A STORE OPERATED CALCIUM CHANNEL DURING CAPACITATION**

Lucia Zalazar[1], Gerardo De Blas[2], Daniela Villamonte[1], Andreina Cesari[1]
[1]Instituto de Investigaciones biológicas (CONICET-UNMdP), [2]Instituto de Histología y Embriología de Mendoza (IHEM/CONICET-UNCuyo)

Capacitation is a key process in the acquisition of sperm fertilization competence. It involves changes in the membrane structure including an increment in Ca2+ influx that triggers signal transduction pathways inside the sperm.

SPINK3 is a mouse secretory protein from seminal vesicles that binds to sperm surface during their transit along the male duct. Our previous works demonstrated that SPINK3 reduces intracellular calcium upon capacitation and is able to modulate the signaling produced downstream this event. To gain insight into the mechanism by which SPINK3 reduces the calcium influx into sperm during capacitation, we designed real time imaging assays. Sperm were charged with Fluo3-AM, incubated with or without SPINK3. Then, a capacitating stimulus was applied and intracellular Ca2+ was monitored by confocal microscopy throughout time.

Our findings demonstrated that a BSA stimulus triggers a Ca2+ increase during the first seconds of capacitation in the 60% of the cells analyzed. SPINK3 was able to reduce this effect in the 80% of the cases. Moreover, the level of Ca2+ signal in population of cells treated showing a positive response to BSA was lower in the presence of SPINK3 than in the control. To explore which membrane calcium channel is regulated by SPINK3, pharmacological trials were performed by using calcium channels antagonists. In this sense, when the cells were treated with two different store operated Ca2+ channels antagonists (YM58483 and SKF96365) a similar response than SPINK3 were observed. Contrary, a voltage-operated Ca2+ channels antagonist has no inhibitory effect on the calcium increment caused by BSA, showing percentages of response similar to control.

In this work, we demonstrated that SPINK3 modulates the Ca2+ influx produced during capacitation probably by regulating a store operated Ca2+ channel. We proposed that SPINK3 reduces the number of sperm that undergo a premature capacitation and consequently an acrosome reaction.

654 (1010) **SHIGA TOXIN TYPE 2 IMPAIRS TROPHOBLAST CELL MIGRATION BUT NOT CELL VIABILITY**

M.Lujan Scalise[1], Flavia Sacerdoti[1], Cristina Ibarra[1]
[1]Laboratorio de Fisiopatogenia, Departamento de Fisiología, Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO Houssay-CONICET), Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina.

Shiga toxin type 2 (Stx2) is the main virulence factor of Shiga toxin producing Escherichia coli (STEC). STEC are pathogens involved in food-borne diseases and are responsible for Hemolytic Uremic Syndrome (HUS) development. We have previously demonstrated that Stx2 induce miscarriage and premature delivery in rats. We propose that STEC infections during early pregnancy may cause damage in the development of placenta mediated by Stx2. The aim of this study was to evaluate the effects of Stx2, alone or in combination with lipopolysaccharide (LPS), on human first trimester trophoblast cells. In order to analyze if viability or cell migration could be affected by Stx2, citotoxicity and wound-healing assays were performed. HTR-8 and Swan 71 first trimester trophoblast cells were exposed to different concentrations of pure Stx2 (1 ng to 1 µg/ml), with or without LPS (5 µg/ml). Cell viability was analyzed by neutral red uptake at 72 h after treatment. On the other hand, HTR-8 cells were seed in 24 well plates and pre-incubated for 24 h in arrested conditions with the different concentrations of Stx2 (1-10 ng/ml) with or without LPS to estimate the effect of Stx2 on extent of cell migration. After that cells were washed with PBS and a vertical scratch was made in the center of each well. Photos of the scratch were taken at regular times (0, 5, 24 h). Images were analyzed by TScratch software version 1.0 to estimate the percentage of bound closure. Stx2 did not affect HTR-8 or Swan 71 cell viability even in combination with LPS. However Stx2 impaired HTR-8 cell migration after 24 h of treatment with Stx2 alone or in combination with LPS. These results suggest that Stx2 can alter in vitro cell trofoblast migration. These data suggest that Stx2 may affect trophoblast invasion and early placentation. Although nowadays there are not reports indicating that Stx2 may affect early pregnancy in humans this data suggest a possible direct effect of Stx2 in human trophoblast migration

655 (1038) **TESTIS GENE EXPRESSION ALTERATIONS ASSOCIATED WITH THE FIRST 48 HOURS OF EXPERIMENTALLY INDUCED PUBERTY IN THE RHESUS MONKEY (MACACA MULATTA)**

Paula Aliberti[1,2,3], Rahil Sethi[4], Uma Chandran[4], Gary Marshall[5,6], Seyedmehdi Nourashrafeddin[5,6], Esperanza Berensztein[1], Alicia Belgorosky[1,2], Suresh Ramaswamy[5,6], William Walker[5,6], Tony Plant[5,6]
[1]Servicio de Endocrinología, Hospital de Pediatría Garrahan, Buenos Aires, [2]CONICET, [3]Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, [4]Department of Biomedical Informatics, University of Pittsburgh Cancer Institute, [5]Department of Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh School of Medicine, [6]Magee Womens Research Institute, Pittsburgh, PA, Estados Unidos.

In primates, spermatogenesis is initiated many years after birth by re-activation of gonadotropin secretion occurring with puberty onset. Global changes in testis gene expression associated with initiation of primate puberty have not been characterized. To address this question, 3 juvenile rhesus monkeys (14-24 mo of age) were treated with a pulsatile iv infusion of recombinant monkey LH and FSH for 48h and 3 with vehicle. Puberty was initiated in the LH+FSH treated monkeys as shown by testosterone production, elevated BrdU labeling of Ap spermatogonia and Sertoli cells, and appearance of differentiating B spermatogonia. Total RNA was isolated from the LH+FSH and VEH treated juvenile testes and subjected to RNA-Seq. Comparison of expression profiles identified 594 genes that were differentially expressed.

As expected, LH-regulated RNAs associated with steroid production (LHCGR, STAR, CYP11a, CYP17, HSD3B2) were induced. However, FSH-inducible mRNAs (INHA, CYP19A1) were not. There was a reduction in GFRA1 and ZBTB16 expression, genes associated with maintaining undifferentiated spermatogonia. Genes encoding cytokines and regulators of cell adhesion and extracellular matrix formation were differentially regulated. Pathway analysis software identified categories consistent with increased steroid production and energy demands associated with cell biosynthetic activity and proliferation.

We have provided the first description of the testicular transcriptome of a representative higher primate during juvenile development and identified changes in gene expression that occur at the earliest stages of puberty. We propose that altered expression of gonadotropin responsive genes that 1) maintain the undifferentiated state of spermatogonia and 2) underlie cell-cell interactions resulting in initial formation of the blood-testis barrier and the post-pubertal stem cell niche, represent the first steps in the differentiation program that initiate spermatogenesis in the primate testis.

656 (2029) **LDL AND SOLUBLE FRACTION OF EGG YOLK: EFFECTS ON BOAR SPERM AFTER TEMPERATURE DECREASE**

Manuel Tomás Orrego[1], Sofía Inés Melián[1], María Margarita Martínez Sarrasague[1], Alejandra Noemí Cimato[1], Lidia Leonor Piehl[1]
[1]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Física.

Cryocapacitation is the main problem in cryopreserved sperm of susceptible species, in which tyrosine phosphorylation (P-Tyr), cell death and low fertility were reported. Cryopreservation extenders are usually supplemented with egg yolk (EY) as membrane stabilizer. Low density lipoproteins from EY (EY-LDL) have been proposed as responsible for EY cryoprotective action.

The aim of this work was to evaluate EY-LDL and soluble EY (S-EY) interactions with sperm, as well as their effects on P-Tyr.

Semen samples in diluent AndrostarÒplus (D) were stabilized 2 h at 17 °C, centrifuged and resuspended (cc = 1.5x109 cells/ml) in D with: 1) no additive, 2) EY-LDL, 3) S-EY, 4) EY-LDL with the spin probe 3b-doxyl-5a-cholestane (3-DC) incorporated, 5) S-EY added with 3-DC. Temperature was decreased from 17 to 5 °C (0.1 °C/min), additives were separated and cells were washed three times. Before and after treatments, cells were evaluated for: P-Tyr by Western blot, protein pattern (PP) by SDS-PAGE, and presence of 3-DC by Electron Spin Resonance (ESR).

Before any treatments, we observed several sperm P-Tyr proteins and frequently a weak band of 32 kD among them. This band sometimes appeared or increased its intensity after temperature decrease in D; but, we have not observed any changes with EY-LDL or S-EY treatments in most cases. However, blots of sperm treated with S-EY, unlike those of EY-LDL sperm, presented some S-EY P-Tyr proteins. This protein transfer was also observed in sperm PP and confirmed by controls. After treatments, sperm cells incorporated the probe 3-DC, a cholesterol analogue. Sperm maintained their motility in the presence of EY-LDL or S-EY, but not in D. Results would indicate that P-Tyr was dependent on the animal and the sample; both EY-LDL and S-EY could prevent changes in P-Tyr; the use of EY-LDL would avoid the interactions of sperm with EY proteins, and the transference of cholesterol from additives to sperm could be a possible stabilization mechanism.

657 (2039) **STUDY OF S100A9 EFFECT ON HUMAN GAMETE INTERACTION AND SPERM CAPACITATION**

Estefanía Massa[1], Gloria García[1], Carlos Zumoffen[1], Carlos Morente[2], Sergio Ghersevich[1]

[1]Area de Bioquímica Clínica-Facultad de Ciencias Bioquímicas y Farmacéuticas-UNR, [2]Programa de Reproducción Asistida de Rosario (PROAR)

Our previous results indicate the presence of S100A9 protein in human oviduct secretion. We showed that S100A9 could bind to human spermatozoa and the acrosome status might affect this binding. S100A9 caused a significant increase in induced acrosome reaction. Also, S100A9 was able to bind to human zona pellucida (ZP). Our hypothesis was to consider that S100A9 can modulate the reproductive process, affecting gamete physiology. The aim of the study was to evaluate the effect of S100A9 on sperm-ZP interaction and on sperm protein tyrosine phosphorylation (PTP). Human motile sperm were obtained from normozoospermic donors. Human oocytes were donated from patients from a fertility clinic. To evaluate sperm-ZP interaction, 3 to 5 oocytes were placed in a medium droplet with 5mg/ml BSA and 0, 0.1, 1.0 and 10.0µg/ml of human recombinant S100A9 (hrS100A9) and inseminated with 105 motile sperm/ml, at 37ºC, 5% pCO_2. After 4 h, oocytes were placed in 5% Y Eosine drops to stain dead sperm. Mean number of live sperm attached to ZP was calculated. To assess the effect of S100A9 on PTP, motile sperm were incubated in capacitating conditions and in the presence of hrS100A9 (0, 0.1, 1.0 and 10.0µg/ml) during 6 h at 37ºC, 5% pCO_2. After incubation, sperm viability and motility were evaluated and sperm proteins were extracted. Samples were analyzed by Western blot. Bands were revealed with chemiluminescence and densitometric analysis was carried out.%PTP was calculated in each treatment, considering control values as 100%. Statistical analysis was performed with ANOVA. Our results show that the presence of S100A9 did not have any significant effect on number of sperm bound to ZP ($p>0.05$, n=8). The higher dose of S100A9 caused a significant augment on%PTP compared to control ($p<0.05$, n=5). In conclusion, S100A9 modulates sperm signaling pathways involved in sperm capacitation, suggesting that it could be involved in the regulation of the reproductive process.

658 (292) **MODIFIED EXPRESSION OF BMP RECEPTOR 1B (BMPR1B) DURING BOVINE CYSTIC OVARIAN DISEASE DEVELOPMENT**

Pablo Uriel Díaz[1], Natalia Carolina Gareis[1], Cristian Jesús Manuel Leiva[1], Florencia Rey[1], José Gabriel Bertoli[2], Luciano Cattaneo[3], Natalia Raquel Salvetti[1], Hugo Hector Ortega[1]

[1]Laboratorio de Biología Celular y Molecular, Instituto de Ciencias Veterinarias del Litoral (ICiVet-Litoral), Universidad Nacional del Litoral (UNL) / CONICET. [2]Cátedra de Producción de Bovinos de Leche, Facultad de Ciencias Veterinarias, UNL. [3]Cátedra de Teriogenología, Facultad de Ciencias Veterinarias, UNL. Esperanza, Santa Fe, Argentina.

Follicular persistence and COD are causes of reproductive alterations in dairy cattle. In the ovary the bone morphogenetic proteins (BMPs) are expressed in granulosa cells (GC) and theca cells (TC), and have important actions during follicular development through binding to a high affinity heterotetrameric complex of type I and type II receptors. Our objective was to study the immunolocalization of BMP receptor 1B (BMPR1B) in the ovaries of healthy cows and animals with spontaneous or ACTH induced COD. In addition, we evaluated the expression of BMPR1B in an experimental model of follicular persistence induced by low levels of progesterone. In all groups we determined the expression of BMPR1B by indirect immunohistochemistry and digital image analysis in different follicular categories. We observed differences in the CG of small preantral follicles, with higher expression in the ACTH-induced and spontaneous COD groups than in the control group ($p<0.05$). Comparison of cysts with reference structures (small and large antral follicles) from the control group showed that ACTH-induced and spontaneous cysts presented higher BMPR1B expression than small antral follicles from the control group ($p<0.05$). In theca cells, BMPR1B expression was higher in atretic follicles from the ACTH-induced group than in those from the spontaneous COD group ($p<0.05$), without differences with the control group ($p<0.05$). Comparison between control and progesterone-induced persistence groups for each follicular category showed higher BMPR1B expression in granulosa cells of atretic follicles of groups with 5 and 15 days of follicular persistence than in those of the control group ($p<0.05$). These results suggest that changes in the expression of BMPR1B can lead to an alteration in ovarian response to BMPs and thus contribute to the pathogenesis of ovarian alterations as follicular persistence and COD.

659 (294) **HYPOPHYSEAL PROGESTERONE RECEPTOR AND OVARIAN PROLACTIN RECEPTOR EXPRESSION ARE TIGHTLY RELATED TO THE REPRODUCTIVE STAGE OF ADULT SOUTH-AMERICAN PLAINS VIZCACHA (LAGOSTOMUS MAXIMUS)**

Sofía Proietto[1,2], Verónica Berta Dorfman[1,2], María Clara Corso[1,2], Pablo Ignacio Felipe Inserra[1,2], Santiago Elías Charif[1,2], Alejandro Raul Schmidt[1,2], Santiago Andrés Cortasa[1,2], Alfredo Daniel Vitullo[1,2], Julia Halperin[1,2]

[1]Centro de Estudios Biomedicos, Biotecnológicos, Ambientales y Diagnóstico (CEBBAD), Universidad Maimónides. [2]CONICET.

The South American plains vizcacha, Lagostomus maximus, shows differential reproductive features like ovulation up to 800 oocytes per reproductive cycle and pre-ovulatory follicle formation and ovulation during gestation. The aim of this work was to study progesterone (Pg) and prolactin (PRL) involvement in ovulation during pregnancy in the vizcacha. Hypophysis and ovaries of non-pregnant non-ovulating (NPNO), non-pregnant ovulating (NPO), early-pregnant (EP), mid-pregnant (MP), term-pregnant (TP) and lactating (Lac) female vizcachas (n=6 per group) were used to study Pg receptor (PR), PRL, PRL receptor (PRLR) and 20αHSD by immunohistochemistry (IHC) and PCR, and Pg serum levels by ELISA. Cytoplasmic immunoreactivity of PR was detected in adenohypophysis of all animals but MP showed PR nuclear localization. In addition, MP showed a significant increment ($p<0.05$) in the PR immunoreactive cellular area vs the other groups. Hypophyseal PRL expression showed an expected increment ($p<0.05$) in TP and Lac related to other groups. Interestingly, although ovarian PR and PRLR expression increase along EP and MP, PRLR markedly diminishes in TP (by IHC and PCR). Moreover, PCR studies showed that 20αHSD expression, an enzyme that catabolizes Pg

to its inactive form and whose expression is repressed by PRL, is almost absent during the entire pregnancy except at the end (TP) when its expression rises drastically ($p<0.05$). 20αHSD data are in agreement with the marked drop in the circulating Pg measured at the end of gestation which is a known sign to initiate parturition. Our results suggest that, at the hypophysis level, cytoplasmatic-nuclear translocation of PR in the mid pregnancy enables activation of the hypophyseal-ovary axis and luteinization. At the ovarian level, suppression of PRLR expression at the end of pregnancy allows de-repression of 20αHSD in order to induce a necessary fall in Pg that will trigger parturition (PICT2014-1281 and FCFF).

660 (336) **MODIFIED EXPRESSION OF CYTOKINES IN PERSISTENT FOLLICLES INDUCED BY PROLONGED TREATMENT WITH PROGESTERONE IN COWS**

Antonela F. Stassi[1,4], E. Matías Belotti[1,3], María E. Baravalle[1,2], Natalia R. Salvetti[1,2], Florencia Rey[1,2], Hugo H. Ortega[1,2]

[1]Laboratorio de Biología Celular y Molecular Aplicada, Instituto de Ciencias Veterinarias del Litoral (ICiVet-Litoral), Universidad Nacional del Litoral (UNL) / CONICET. [2]Cátedra de Biología Celular, Facultad de Ciencias Veterinarias del Litoral, UNL. [3]Cátedra de Diagnóstico por Imágenes, Facultad de Ciencias Veterinarias del Litoral, UNL. [4]Cátedra de Análisis Clínicos, Facultad de Ciencias Veterinarias del Litoral, UNL, Esperanza, Santa Fe, Argentina.

The ovulatory process shares many similarities with an acute inflammatory reaction and cytokines may be implicated in the control of several ovarian functions. Cystic ovarian disease (COD) is an important cause of reproductive failure in cattle and the study of the processes that lead to ovulatory failure and persistence of the dominant follicle is the key to understanding the pathogenesis of COD. The aim of this study was to examine the expression of interleukin IL-8, IL-1β, IL-1 receptor I (IL-1 RI), IL-1 receptor II (IL-1 RII), IL-1 receptor antagonist (IL-1 Ra) and IL-4 in ovaries of animals with induced follicular persistence. An experimental model of follicular persistence was performed, with an intravaginal progesterone device to get subluteal concentrations of progesterone, obtaining dominant follicles around ovulation (n = 5; P0) and follicles that persist for 5 (n = 5; P 5), 10 (n = 5; P10) or 15 days (n = 5; P15) after the expected time of ovulation.

Controls were ovariectomized in proestrus (n = 5; C). The expression of IL-8, IL-1β, IL-1 RI, IL-1 RII, IL-1 Ra and IL-4 was evaluated by immunohistochemistry in follicular structures. The IL-8 and IL-1β showed increased expression in granulosa and theca of persistent follicles from P0 group regarding to antral follicles from the control group (as reference structure). IL-1 RI showed lower expression in the granulosa of antral follicle of P0 relative to P5, P10 and P15 groups. Immunoexpression of IL-1 RII was lower in the granulosa of antral follicles from the control group regarding persistent follicles of all groups, except in P0. The IL-1 Ra and IL-4 showed increased expression in theca of persistent follicle from P0 and P15 groups than antral follicles from the control group. This, suggest that in follicular persistence exist an alteration in inflammatory mechanisms associated with ovulation and interleukins could contribute to follicular persistence associated with COD.

661 (363) **METFORMIN AS A TRANSCRIPTIONAL REGULATOR OF INSULIN MEDIATORS IN A PRENATAL HYPERANDROGENIZED RAT MODEL**

Maria Florencia Heber[1], Giselle Adriana Abruzzese[1], Silvana Rocio Ferreira[1], Alicia Beatriz Motta[1]

[1]Laboratorio de Fisiopatología Ovárica; Centro de Estudios Farmacológicos y Botánicos (CEFYBO), CONICET, Facultad de Medicina, Universidad de Buenos Aires (UBA), Buenos Aires, Argentina.

Around 70% of women with Polycystic ovary syndrome (PCOS) develop insulin resistance (InR), a state that affects tissues differentially. Insulin responsiveness of target tissues in women with PCOS has been studied at a functional level, but little is known about transcriptional regulation. Insulin sensitizer drugs such as metformin (Met) improve InR as well as reproductive abnormalities in PCOS. Here, we tested the effects of androgen excess on the gene expression of key molecules of the insulin signaling pathway and the effects of Met on the deregulations found. A prenatal hyperandrogenization (PH) model was used. Control (C) and PH rats were euthanized at 90 days of age (adulthood, N=80). From day 70 to 90, 20 rats of each group were treated orally daily with 50 mg/kg of Met (PHM). We evaluated serum insulin and glucose levels and the HOMA-IR index. Gene expression of the insulin receptor (IR), insulin substrate 1 and 2 (IRS-1, IRS-2) and glucose transporters (GLUT2 and GLUT4) was measured by qPCR in hepatic and ovarian tissue. Serum insulin and glucose levels and the HOMA-IR index were higher in PH group vs C, and Met restored the levels to those of the C group ($p<0.01$). mRNA levels of IR in both the liver and ovary were lower in PH group vs C, only in the liver Met increased levels of IR (PHM group, $p<0.01$). Gene expression of hepatic GLUT2 and IRS-1 (in both tissues) was lower in PH group vs C, and Met restored levels to C values ($p<0.01$). In the ovary, the mRNA levels of GLUT4 in PH and PHM groups were lower than in C group ($p<0.01$). IRS-2 expression was lower in PH and PHM groups vs C in both hepatic and ovarian tissue ($p<0.01$). PH induced an InR state, which was reversed with Met. Hepatic and ovarian tissue showed decreased gene expression of molecules involved in insulin signaling caused by PH. The liver was more sensitive to the treatment with Met than the ovary, restoring almost totally the gene expression of insulin pathway mediators.

662 (403) **UTERINE PROLIFERATIVE CHANGES IN RATS EXPOSED TO A CAFETERIA DIET AFTER WEANING**

María Paula Gastiazoro[1], Marlise Luciana Guerrero Schimpf[1], Gisela Lazzarino[1], María Florencia Andreoli[2], Jorge Guillermo Ramos[2], Milena Durando[1], Enrique Hugo Luque[1], Jorgelina Varayoud[1]

[1]Instituto de Salud y Ambiente del Litoral (ISAL), Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral - CONICET, [2]Departamento de Bioquímica Clínica y Cuantitativa, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina.

The development of endometrial cancer is associated with obesity. A model of obesity that more closely reflects western diet habits is the cafeteria (CAF) diet. The CAF diet effects on uterine proliferative response is unknown. The aim of this study was to evaluate the CAF diet effects onuterine morphology, proliferation and estrogen-related proteins on adult rats.

Twenty-one-day-old female Wistar rats were fed after weaning with: a standard rodent chow diet (control group, C) or cafeteria diet (CAF group), with highly palatable energy dense foods that are prevalent in western society. The animals were weekly weighted and sacrificed on the first day of diestrous-stage, 20 weeks after CAF treatment. Blood was collected to determine metabolic parameters and estradiol levels and the uterus was removed and included in paraffin for histological and immunohistochemical studies. Estrogen Receptor alpha (ERa), Vimentin (Vim) and Ki67 (proliferation marker) were determined using immunohistochemistry. The CAF group showed obesity after 14 weeks of treatment ($p<0.01$). The CAF diet did not alter serum levels of glucose, cholesterol, triglycerides, insulin and estradiol, but leptin levels were increased (C 1.8±0.2 vs CAF 6.8±1.2 ng/ml, $p<0.001$). The uterus of CAF group showed morphological changes with an increase of glandular and stromal areas.

In addition, CAF group showed higher Vim expression in the subepithelial and periglandular stroma. An up regulation of ERa was detected in all uterine compartments: luminal epithelium (C 2.2±0.3 vs CAF 4.7±0.5); glandular epithelium (C 3.6±0.2 vs CAF 8.7±1.0); subepithelial stroma (C 0.4±0.1 vs CAF 0.9±0.1 ($p<0.05$). Similar to ERa, Ki67 expression was increased in luminal epithelium (C 40.6±5.1 vs CAF 68.1±2.2) and glandular epithelium (C 63.5±5.1 vs CAF 76.1±1.4) ($p<0.01$).

These results suggest that CAF diet induced uterine proliferative changes and could predispose to endometrial cancer.

663 (437) **INTRAGESTATIONAL ROLE OF GHRELIN ON OFFSPRING´S POSNATAL DEVELOPMENT AND REPRODUCTIVE FUNCTION.**

Pedro Javier Torres[1,2], Eugenia Mercedes Luque[1,2], Noelia Di Giorgio[3], Marcela Diez[1], Silvia Figueroa[1], Valeria Paola Carlini[1,2], Laura María Vincenti[1], Ana Carolina Martini[1,2]
[1]Instituto de Fisiología, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Córdoba. [2]Instituto de Investigaciones en Ciencias de la Salud (INICSA), CONICET-FCM, Córdoba. [3]Laboratorio de Neuroendocrinología, Instituto de Biología y Medicina Experimental (IBYME), CONICET, CABA.

It has been proposed that ghrelin (Ghr) exerts an important role in pregnancy success and offspring´s postnatal development. Therefore, the objective of this study was to evaluate the effects of intragestational exogenous Ghr administration or endogenous Ghr inhibition (using an antagonist) on mice posnatal development. Dams were injected (s.c.) through pregnancy with: Ghr (4 nmol/animal/day), an antagonist (Ant: (D-Lys3)GHRP-6; 6 nmol/animal/day) or vehicle (C: isotonic solution). Their litters were evaluated for posnatal growth, neurobiologic development and sexual maturation and, at adulthood, for reproductive success. Results were analyzed using ANOVA. Neither daily intake

nor dam´s weight was modified by the treatments. Litter growth or physical/neurobiological maturation were not altered either. Male offspring under

intragestational treatment with Ant showed a faster sexual development (testicular descent day 19: Ant=64±5.9% vs Ghr=50.1±6.9% and C=44.9±7.9%, n=7-13 litters/treatment; $p<0.05$) and an increased peripuberal relative testicular weight ($p<0.05$). Female pups intragestationally exposed to Ant exhibited, at posnatal day 23, a higher ovarian volume (Ant=1085.7±64.0mm3 vs Ghr=663.3±102.8mm3 and C=512.3±116.4mm3; n=4-6 ovaries/treatment, $p<0.01$) and a higher number of follicles >200µ/ovary ($p<0.05$). No differences between groups or gender were detected on IGF-1 concentrations (140.0±15.3ng/ml, n=44). When adulthood was reached, male intragestationally exposed to Ant exhibited a smaller relative testicular weight and an increase in the percentage of immotile spermatozoa (Ant=35.5±3.3% vs Ghr=23.3±3.3% and C=25.4±3.5%, n=13-15 animals/treatment; $p<0.05$). No differences were detected in the pregnancy success of the female offspring. These results suggest that intragestational modifications on Ghr concentrations may exert long lasting effects on the litter sexual maturation and fertility.

664 (442) **FETAL PROGRAMMING CAUSED BY ANDROGEN EXCESS ALTERS OVARIAN FUNCTIONS AND ADIPOKINE SECRETION**

Giselle Adriana Abruzzese[1], Maria Florencia Heber[1], Alicia Beatriz Motta[1],
[1]Laboratorio de Fisiopatología Ovárica; Centro de Estudios Farmacológicos y Botánicos (CEFYBO), CONICET, Facultad de Medicina, Universidad de Buenos Aires (UBA), Buenos Aires, Argentina.

Fetal programming caused by prenatal hyperandrogenism is hypothesized as one of the main factors contributing to polycystic ovary syndrome (PCOS). PCOS is an endocrine-metabolic disorder. Adipokines are involved in metabolic disturbances and also in fertility issues. We aimed to characterize in a PCOS murine model the ovarian secretion pattern of the adipokines Leptin (Lp), Adiponectin (Ad) and Chemerin (Ch) and the consequences on ovarian functions. Pregnants rats were hyperandrogenized with testosterone and a control group was obtained by the injection of vehicle. The prenatally hyperandrogenized (PH) female offspring (N= 150) and control offspring (C, N= 96) were characterized according to the estrous cycle as ovulatory (PHo) and anovulatory (PHa) phenotypes at pubertal age. We evaluate ovarian histology. The adipokine secretion pattern and StAR (the limiting enzyme of steroidogenesis) gene expression were quantified by qPCR. All the groups did not display body weight differences ($p > 0.05$). In the three independent repetitions of the animal procedure,C rats showed (100%) regular estrous cycle. Within the PH group, 43–51% showed irregular estrous cycles and were considered as PHo, whereas 27–39% presented anovulatory cycles and were considered PHa. Histological examination of ovaries from the PH offspring revealed the presence of cysts and an excess of small antral follicles. The adipokine secretion pattern was altered in the PH groups. Lp levels were lower in PHa group than in C and PHo ($p<0.01$). Ad levels were higher in the PHo than in C and PHa ($p<0.01$). Ch levels were increased in the PHo vs C and PHa ($p<0.05$). StAR secretion was only altered in the PHa group being higher than in both, C and PHo groups ($p<0.05$). In conclusion, fetal programming causes alterations in ovarian adipokines without the presence of overweight. The differentially deregulation of these adipokines is involved in the ovarian alterations displayed in PCOS phenotypes.

665 (550) **AN OVERLOAD OF SATURATED FAT IN MATERNAL DIET PROGRAMS INTESTINE IMPAIRMENTS IN INCRETIN EXPRESSION IN THE RAT OFFSPRING**

María Belén Mazzucco[1], Martina Radice[1], Evangelina Capobianco[1], Alicia Jawerbaum[1], Verónica White[1]
[1]Centro de Estudios Farmacológicos y Botánicos- CONICET.

We have previously found that fetuses from rats fed with a saturated-fat rich diet show high circulating levels of lipids and leptin, and resistance to leptin action in the liver. We have also found alterations in mRNA levels of 2 incretins: gastric inhibitory peptide (gip) and glucagon-like peptide (gcg) in the intestine from these fetuses. Leptin and nutrients are well-known stimulators of incretin production.

We aimed to analyze whether an overload of saturated fat in the maternal diet induces impairments in leptin-induced intestine gip and gcg mRNA levels in the fetus and whether the anomalies persist in the offspring.

Methods: Female Wistar rats were fed with either a standard (5% fat) (controls) or a saturated fat-rich diet (28% fat) from 6 weeks of age (SFD rats). After 8 weeks of diet, they were mated with control males. Control and SFD pregnant rats were euthanized at 21 days of gestation, fetal intestines obtained and either preserved or cultured (3 h) with or without leptin (100 ng/ml). Another group of control and SFD rats were allowed to deliver, their offspring euthanized at 140 days of age, and the proximal portion of their intestine obtained. gip and gcg mRNA levels were assessed by PCR.

Results: Leptin addition to the culture induced an increase in gcg and gip mRNA levels ($p<0.05$) in both female and male fetal intestines from the control group. Differently, no changes were observed when culturing the intestines from the DGS group in the presence or absence of leptin. Female offspring intestines from SFD rats showed lower gip mRNA levels (23%, $p<0.05$). Male offspring intestines from the SFD group showed lower gcg mRNA levels (18%, $p<0.05$) than those from controls.

Conclusions: Saturated fat in the maternal diet induces anomalies in the intestine mRNA levels of incretins in the offspring. Absence of fetal intestine responses to leptin might be a first step in the development of the impairments observed in the offspring's later life.

666 (565) **FIBROBLAST GROWTH FACTOR 2 (FGF2) IS PRESENT IN HUMAN SPERMATOZOA AND IS INVOLVED IN SPERM MOTILITY**

Darío José Garbarino Azúa[1], Lucía Saucedo[1], Santiago Giordana[2], Fernando Neuspiller[2], Mónica Hebe Vazquez Levin[1], Clara Isabel Marín Briggiler[1]
[1]Instituto de Biología y Medicina Experimental (IBYME), [2]Instituto Valenciano de Infertilidad (IVI), Buenos Aires.

Fibroblast growth factors (FGFs) and their receptors (FGFRs) have been described in several processes in somatic cells, but there is scarce information of their relevance in sperm function.

Previous studies from our group have shown the presence of FGFRs in the head and flagellum of human spermatozoa and their participation in motility regulation. The objectives of this work were to determine: 1) the presence and localization of FGF2 in the male gamete, 2) the relationship between sperm FGF2 levels and routine semen parameters, 3) the effect of recombinant FGF2 (rFGF2) on the recovery of motile spermatozoa in a selection procedure (swim-up). Semen samples were obtained from donors or patients attending an infertility clinic. Spermatozoa were subjected to Western immunoblotting, immunocytochemistry or flow cytometry using anti FGF2 antibody. Semen parameters were analyzed according World Heath Organization guidelines (2010). A FGF2 form of 18 kDa was found in human sperm extracts, and the protein was localized in the sperm head (acrosomal region) and flagellum. Sperm FGF2 levels showed a significant positive correlation ($P<0.01$) with sperm concentration ($r=0.47$, $n=30$) and sperm motility ($r=0.46$), but were not associated with semen volume ($r=0.24$), sperm vitality ($r=0.02$) or normal sperm morphology ($r=0.32$). Moreover, FGF2 levels in semen samples with abnormal motility ($\leq$ 40 % motile cells) were significantly lower ($P<0.05$) than those of samples with normal motility (477±50 vs. 737±62). The addition of rFGF2 (10 ng/mL) to semen samples before the swim-up procedure resulted in a significant increase ($P<0.05$) in the recovery of motile cells ($n=9$). In conclusion, the results demonstrated the presence of FGF2 in the human sperm head and flagellum, and that FGF2 levels are related with sperm concentration and motility. Supplementation with rFGF2 would be useful in the handling of human sperm samples in vitro, especially in cases of reduced sperm motility.

667 (591) **EXPRESSION OF RECEPTORS FOR TGF-B SUPERFAMILY IN COWS WITH SPONTANEOUS AND ACTH-INDUCED CYSTIC OVARIAN DISEASE**

Valentina Matiller[1,2], Gustavo Hein[1], Ayelén Amweg[1], Fernanda Rodríguez[1], José Bertoli[2], Ernesto Quercia[3], Florencia Rey[1], Natalia Salvetti[1]

[1]Laboratorio de Biología Celular y Molecular Aplicada, Instituto de Ciencias Veterinarias del Litoral (ICiVet-Litoral), Universidad Nacional del Litoral (UNL) / CONICET, [2]Cátedra de Producción de Bovinos de Leche, Facultad de Ciencias Veterinarias del Litoral, UNL, Esperanza, Santa Fe, Argentina. [3]Médico Veterinario. actividad privada.

Preliminary results shows significant changes in the expression of components of the TGF-B isoforms TGF-B1, 2 and 3, and inhibin / activin / follistatin system in ovarian structures of dairy cattle with spontaneous and ACTH-induced cystic ovarian disease (COD). Given the evidence of its role as an important molecules in paracrine and autocrine signaling pathways that regulate the growth of ovarian follicle, and knowing that this components carry out their actions through binding to two types of membrane receptors, the objective of the present work was examine the expression of TGF-B receptor (TGF-BR)I, TGF-BRII, TGF-BRIII, Activin Receptor (ACVR)IB and ACVRIIB in ovaries of cows with COD previously evaluated for their related ligands. We worked with sections obtained from bovine ovaries with spontaneous COD (n=10), ACTH-induced COD (n=10), and controls ovaries (n=10). In 4 um thick sections, the presence and expression of receptors was determined by indirect immunohistochemistry in granulosa and theca cells. Type I receptors expressed higher staining ($p<0.05$) in tertiary follicles of spontaneous COD group than controls follicles, and type II receptor ACVRIIB, showed higher immunostaining in theca cells of spontaneous COD group than control. Here we detected changes in the expression of the receptors necessary for the response to the growth factors of TGF-B family in the COD cow's ovaries in relation to the controls. The high expression of all receptors analyzed in follicular cysts could be linked to the persistence over time of these structures. We can conclude that alterations found at ligands level materialize a change in signalling favouring these actions at the cellular level. Thus, to unravel intraovarian signaling pathways, we postulate data that may contribute to the knowledge of the pathogenesis of COD and that can be applicable to the design of protocols and therapeutic measures.

668 (598) **VEGF SYSTEM EXPRESSION AS IMPORTANT INTRAOVARIAN COMPONENT IN THE FOLLICULAR PERSISTENCE ASSOCIATED WITH BOVINE COD**

María Eugenia Baravalle[1], Antonela Stassi[1], Eduardo Matías Belotti[1], Melisa Velázquez[1], Belén Peralta[1], Natalia Raquel Salvetti[1], Hugo Héctor Ortega[1]

[1]ICIVET Litoral UNL-CONICET.

Cystic ovarian disease (COD) is one of the most important causes of reproductive failure in cattle. Cause anovulation and continuous growth of follicles to excessive diameters that fail to ovulate, persist and then interfere with normal ovarian function. Angiogenesis is a complex process regulated by angiogenic factors. Vascular Endothelial Growth Factor (VEGF) is one of the most important pro-angiogenic factors and VEGF Receptor-2 (VEGFR2) is the major mediator of the angiogenic effects of VEGF. The VEGFA protein expression in both the theca (TC) and granulosa cells (GC) increases significantly as follicles grew and matured. Thus we hypothesized that the alteration of these factors may contribute to the follicular persistence and pathogenesis of COD. The aim of this study was to examine VEGFA and VEGFR2 expression by immunohistochemistry in ovarian follicular structures during the development of follicular persistence induced in cows by long time progesterone administration. A low dose of progesterone was administered ($n=5$) for 0 (ovulation time) and 5, 10, and 15 days after the expected day of ovulation: groups P0, P5, P10, and P15, using an intravaginal progesterone-releasing device. Control cows (group C) received no additional hormonal treatment. Results showed changes in VEGFA and VEGFR2 expression along folliculogenesis, in GC and TC in the different stages of follicular persistence ($p<0.05$), in P0 and P15 for VEGFA and in all groups for VEGFR2. When groups were compared, increased expression of VEGFA and VEGFR2 in TC was detected in persistent follicles from P0 in relation to the antral follicles from C group (reference structure) ($p<0.05$). The results of this study suggest that excessive growth of persistent follicles depends on developed vasculature in the ovary, showing its participation throughout the ovarian folliculogenesis and considering the experimental model, its relationship with the pathogenesis of bovine COD.

669 (631) **INTERRELATIONSHIP BETWEEN PPARGAMMA AND MTOR PATHWAYS IN RAT DECIDUA DURING EARLY ORGANOGENESIS**

Sabrina Lorena Roberti[1], Romina Higa[1], Verónica White[1], Evangelina Capobianco[1], Alicia Jawerbaum[1]

[1]Laboratorio de Reproducción y Metabolismo, CEFyBO-CONICET-UBA. Facultad de Medicina.

Peroxisome proliferator activated receptor gamma (PPARgamma) is a ligand activated transcription factor that regulates metabolic processes and has an essential role in embryonic and placental development. Mammalian target of rapamycine (mTOR) signaling is also essential for embryo growth and regulates nutrient transfer through the placenta. The decidua serves for the embryonic histotrophic nutrition before the establishment of a mature placenta. We hypothesized that these two signaling pathways are interrelated in the rat decidua during early organogenesis and thus studied the effect of in vivo inhibition of mTOR and PPARgamma in the decidua during early organogenesis. Methods: Wistar rats were mated and during days 7, 8 and 9 of pregnancy the females received sc injections of rapamycin (mTOR inhibitor), T0070907 (PPARgamma inhibitor) or vehicle. On day 9 of pregnancy, the decidua was explanted and the levels of PPARgamma, phosphorylated and total ribosomal protein S6 (RPS6, phosphorylated through the mTORC1 pathway) and phosphorylated and total serum and glucocorticoid-inducible kinase 1 (SGK1, phosphorylated through the mTORC2 pathway) were evaluated by Western blot. Results: Maternal administration of rapamycin (0.75 mg/kg sc) inhibited 68% of RPS6 phosphorylation. In turn, this inhibition increased PPARgamma levels (36%, $p< 0.01$). On the other hand, administration of the PPARgamma inhibitor T0070907 (0.001 mg/kg sc) inhibited mTOR signaling, as shown by the reduced levels

of phosphorylated RPS6 (25%, p<0.05) and SGK1 (51%, p<0.01) and no changes in the levels of total RPS6 and SGK1.

Conclusions: There is a positive regulation of PPARgamma levels when mTOR is inhibited, and a negative regulation of mTOR signaling when PPARgamma is inhibited, interrelationships that may be relevant in the regulation of nutrients availability during early organogenesis.

670 (664) **VIP REGULATES GLUCOSE TRANSPORT BY HUMAN TROPHOBLAST-DERIVED CELLS**

Daiana Vota[1], Fátima Merech[1], Vanesa Hauk[1], Daniel Paparini[1], Rosanna Ramhorst[1], Claudia Pérez Leirós[1]
[1]Immunopharmacology Laboratory, School of Sciences, IQUIBICEN, CONICET-UBA.

The transport of nutrients across the placenta is strictly regulated and deficiencies in metabolism and transport of several factors like glucose, aminoacids and lipids are associated to intrauterine growth restriction (IUGR), large for gestational age newborns, among other complications. Glucose is the major energy substrate for the placenta and the fetus. Its transfer to the fetus is regulated by maternal levels, placental glucose metabolism and facilitated transport through Glut carrier proteins. Glut 1 appears to be the primary transporter and is found at both the maternal and the fetal-facing trophoblast membrane. The vasoactive intestinal peptide (VIP) is a pleiotropic neuropeptide that was shown to inhibit glucose oxidation in rat enterocytes. On the other hand, we have previously demonstrated that VIP is synthesized by human first and thirst trimester trophoblast (Tb) cells and favors Tb cell migration and invasion, two main processes required for placentation. Based on these evidences, the aim of the present work was to study the role of VIP and its receptors in the regulation of glucose transport bv Tb cells. We used two human Tb derived cell lines, Swan71 and BeWo cultured with/without VIP (10-100nM) for different times. We evaluated the expression of GLUT1 by qRT-PCR, glucose uptake by flow cytometry using the glucose fluorescent analog 2-NBDG and glucose transcellular transport on monolayer by means of transwell systems. Same assays were carried out in VIP knocked down Tb cells by VIP siRNA transfection.

Our results show that VIP induced GLUT 1 mRNA expression at 7h (BeWo: 50nM VIP showed an increment of 31± 3 % respect to basal expression, n=3, p<0.05) as well as an increment of glucose uptake and transcellular transport. An increased GLUT1 expression was also found in VIP siRNA vs. scramble transfected Tb cells (p<0.05).

Results of this in vitro model are consistent with a role of VIP as a local factor to modulate glucose transport across the placenta

671 (771) **RELEVANCE OF SPERM NUCLEAR EVALUATION AS A COMPLEMENT TO THE STANDARD TECHNIQUES FOR THE ESTIMATION OF BOVINE SEMEN QUALITY**

Liliana Olga González[1,2], María Susana Ghirardosi[1,2], María Laura Fischman[1,2], María Rosa Ferrari[1,2], Humberto Osvaldo Cisale[1,2]
[1]Universidad de Buenos Aires. Facultad de Ciencias Veterinarias. Cátedra de Física Biológica. [2]Universidad de Buenos Aires. Facultad de Ciencias Veterinarias. Instituto de Investigación y Tecnología en Reproducción Animal (INITRA). Buenos Aires, Argentina.

The quality of cryopreserved bovine semen samples used in artificial insemination (AI) should be guaranteed. Some researchers point out that parameters such as progressive motility and morphology are good estimators for bull fertility. Others suggest that semen evaluation by in vitro techniques should include the study of many sperm characteristics as possible. Normally, the routine quality evaluation does not include testing sperm nucleus. The aim of this work was to study the association of given fertility field with different sperm parameters, including the assessment of the quality of the nucleus in commercial samples used in AI made in Argentina over recent years.

Samples (n = 54) from different AI centers, classified into three categories of fertility (High, Medium and Low) based on field results were evaluated. The assessments included, at a general level, concentration, progressive motility, membrane functionality (HOSt-est), acrosome integrity (phase contrast microscopy), viability (CFDA-PI) and sperm morphology (Bengal Rose). At nuclear level were evaluated: morphology (Feulgen reaction), maturation (Aniline Blue) and chromatin packing (Toluidine Blue), and response to decondensant agents (NCD). These parameters were related with fertility of bulls by cluster analysis (InfoStat/E 2014). Analysis of dendrograms revealed that nuclear characters, progressive motility and morphology, discriminated clearly males with high fertility but not with media and low fertility. The results indicate that none of the parameters evaluated alone would include the three categories of fertility in different clusters and confirm the importance of analyzing the greatest possible number of parameters including sperm quality nuclear testing. This inclusion would discard samples that have excellent results in quality assurance standards, but which are carriers of not compensable defects, which lead to low performance in the application of that semen on field.

HEMATOLOGÍA / HEMATOLOGY

672 (181) **ERYTHROPOIETIN-INDUCED ENDOTHELIAL CELL MIGRATION RELIES ON AN INTRACELLULAR CALCIUM INCREASE**

Romina Eugenia Maltaneri[1,2], María Eugenia Chamorro[1,2], Agustina Schiappacasse[1,2], Alcira Beatriz Nesse[1,2], Daniela Cecilia Vittori[1,2].
[1]Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Química Biológica. [2]CONICET-IQUIBICEN.

Apart from its effect on the hematopoietic system, erythropoietin (Epo), a glycosylated cytokine produced by the adult kidney, has also been involved in angiogenesis, a process with important physiological and pathological implications. Since the mechanisms underlying such proangiogenic behavior are little understood, it was our purpose to study possible effectors of Epo in cultures of the endothelial cell line EA.hy926, with special interest in the comparison with its non-hematopoietic carbamylated derivative (cEpo).

Calcium (Ca) plays a key role in cell migration, through regulation of focal adhesions and cytoskeleton dynamics. In wound-healing assays, Epo-induced migration (200 ng/mL, 15 h) was inhibited by incubation with the Ca^{2+} chelator EGTA (1 mM) (Control: 27±2.0%; *Epo: 35±0.9%; Epo+EGTA: 27±2.1%; *P<0.05, Kruskal Wallis-Dunn, n=7). Epo also generated a transient $[Ca^{2+}]_i$ rise (flow cytometry, Fluo4-AM) in EA.hy926 cells within the first 30 seconds of stimulation, peaking at 7 sec (39±7.1% Epo *vs.* unstimulated cells, P<0.05, Kruskal Wallis-Dunn; n=4), as demonstrated for other angiogenic factors. The carbamylated derivative of Epo (cEpo), which has no proangiogenic effect, failed to produce such $[Ca^{2+}]_i$ rise. The absence of Ca^{2+} in the resuspension buffer abrogated the Epo-induced transient increase in $[Ca^{2+}]_i$, suggesting an extracellular source of the cation. Accordingly, a 15 h-incubation with Epo significantly increased the expression levels of the TRPC3 calcium channel (flow cytometry: *Epo: 125±12.9; cEpo: 103±8.0 expressed as % of control; *P<0.05, Kruskal Wallis-Dunn, n=5). Moreover, calpain activity appears to be involved in Epo-stimulated migration.

Our results demonstrate that Epo induces endothelial migration by affecting Ca homeostasis through an increase in Ca influx via modulation of Ca channels. These findings highlight the importance of intracellular Ca dynamics in angiogenesis, a prime target in the treatment of different pathologies.

673 (182) **N-HOMOCYSTEINYLATION ALTERS ERYTHROPOIETIN FUNCTIONS**

Agustina Schiappacasse[1,2], Romina Eugenia Maltaneri[1,2], María Eugenia Chamorro[1,2], Diana Wetzler[1,2], Alcira Beatriz Nesse[1,2], Daniela Cecilia Vittori[1,2].
[1]Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Química Biológica. [2]CONICET-IQUIBICEN.

Erythropoietin (Epo) is a cytokine known by its hematopoietic properties. Despite the successful introduction of recombinant human Epo to overcome the anemia associated with different pathologies, a significant number of patients fails to respond. High levels of homocysteine –a risk factor for cardiovascular disease– have been linked with altered protein structure due to N-homocysteinylation of protein lysine residues by reaction with the highly reactive homocysteine thiolactone (HTL). Therefore, it was interesting to study whether the erythropoietic and antiapoptotic properties of Epo changed after N-homocysteinylation. Epo was incubated with HTL at 37 °C for 24 h (Epo:HTL 1:2000 M) and residual HTL was removed by washing. Changes undergone by the molecule were evaluated by polyacrylamide gel electrophoresis, zone capillary electrophoresis, the Ellman reaction and fluorescence and circular dichroism measurements. Regarding erythropoietic action, Epo and Epo-HTL (8 ng/mL) were compared in 48 h-growth of the Epo-dependent UT-7 cells capable of erythroid differentiation. Cell proliferation assay (trypan blue): Epo 45.2 ± 8.8 $x10^4$ cell/mL; *EpoHTL 29.6 ± 2.7 $x10^4$ cell/ml; *P<0.05 (*P<0.05), n=8; MTT assay: Epo 0.892 ± 0.071 O.D.; **EpoHTL 0.502 ± 0.061 O.D.; **P<0.01; n=8. Phosphatidylserine translocation was used to evaluate apoptosis (Flow cytometry, annexin V-positive cells): Epo 21.7 ± 2.6%; *EpoHTL 46.7 ± 1.5%; *P<0.05; n=4.

The analysis of molecule alterations shows structural changes of Epo due to HTL action.

The results indicating reduced erythropoietic and antiapoptotic effects of Epo-HTL on the UT-7 cell line, suggest a new possible form of erythropoietin resistance, especially important in patients with cardio-renal syndrome.

674 (464) **DETERMINATION OF ALLELE BURDEN OF JAK2V617F MUTATION IN GENOMIC AND PLASMA CELL FREE DNA IN HEALTHY INDIVIDUALS**

María Alejandra Cardozo[1], Luisa Gaydou[1], Luisina Riera[1], Adriana Mabel Follonier[1], Verónica Lis Bosquiazzo[1,2], Jorge Guillermo Ramos[1,2].

[1]Departamento de Bioquímica Clínica y Cuantitativa, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina. [2]Instituto de Salud y Ambiente del Litoral (UNL- CONICET), Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina.

Mutations in JAK_2 gene are common in chronic BCR-ABL (-) negative myeloproliferative neoplasms. The most frequent mutation is observed in exon 14 G1849T resulting in the substitution of a valine for a phenylalanine in the 617 position (JAK_2 V617F) leading to a protein with constitutive tyrosine kinase activity. Some studies have shown low percentages (1-3%) of the mutation in healthy individuals, suggesting that it may be present before the onset of the disease. It is necessary not only to know the cut-off value of the JAK_2 mutation allele burden in healthy individuals but also to develop quantitative methods with robust responses with low limits of detection (0.1-1%). We designed an allele specific real time PCR assays (qPCR) for the determination of allele burden of JAK_2V617F (percentage of alleles JAK_2V617F / total alleles JAK_2) in samples of genomic DNA (gDNA) and plasma cell free DNA (cfDNA).The calibration curve was made with a mixture of gDNA from healthy individuals and gDNA derived from a homozygous JAK_2V617F-Human-erythroleukemia cell line. gDNA was obtained from peripheral blood of 100 healthy individuals using a modified Miller and Dykes technique, while cfDNA was obtained from the plasma of 32 healthy patients using the QIAmp DNA Blood Mini kit. In order to discard subclinic haematological diseases a blood count for each individual was performed. The JAK_2V617F mutation was detected both the gDNA and cfDNA of healthy people. Allele burden in gDNA was 0.066% [95% CI: 0.047-0.093%], while in cfDNA was 0.054% [95% CI: 0.034-0.087%]. Statistical differences were not detected between genomic and plasmatic compartments (p=0.23). There was no correlation between the allele burden of JAK_2V617F and the patient's age, gender or the different hematimetric parameters. These results demonstrate that the developed methodology allowed the quantification of low allele burden in healthy individual samples.

675 (790) **CONSTITUTIVE HIGH BCL2 AND BCLXL LEVELS IMPAIR AUTOPHAGIC HODGKIN LYMPHOMA CELL DEATH**

Claudia Alejandra Franco Cortes[1], Francisco Javier Oliver Martos[1], Stella Maris Ranuncolo[1]

[1]Instituto de Ciencias Básicas y Medicina Experimental (ICBME), Instituto Universitario del Hospital Italiano de Buenos Aires (IUHIBA).

Hodgkin lymphoma (HL) is a lymphatic system malignancy derived from germinal center B cells. Despite chemosensitive HL patients can be cured, 40% are refractory to current chemotherapy regimens and relapse within 12 months from diagnosis. Even more, following second or third chemo schemes, 55% of those patients remain unresponsive. This and the young age of diagnosis highlight the need to better understand HL molecular biology.

We have previously reported that HL relies on the alternative NFkB pathway, mediated by reB/NIK, to survive. Its constitutive activativation is the result of stable NIK protein expression both in human HL cell lines and patient biopsies. Depletion of either relB or NIK by shRNAs or pharmacological NIK inhibitors induce HL cell death.

To further explain the relB depletion induced phenotype in L1236, U-H01 and KM-H2 HL cell lines, we investigated the target genes that might account for the HL cell death. Chromatin immunoprecipitation showed relB/p52 bound to Bcl2 and $Bclx_L$ promoters, among other cell cycle control genes. We detected a significant downregulation of both Bcl2 and $Bclx_L$ mRNA and protein levels, following relB or NIK knockdown, indicating relB direct regulation. Interestingly a Bcl2 cDNA, with the shrelB target sequence mutated, was capable of partially rescue the toxicity induced by relB depletion in L1236, U-H01 and KM-H2 HL cells. Furthermore, co-immunoprecipitation assays revealed that Bcl2 and $Bclx_L$ interact with the autophagy executing protein Beclin1, inhibiting cell death. Despite the well known Bcl2 and $Bclx_L$ antiapoptotic activity, dead HL cells, showed autophagic features upon relB depletion. The analysis of the apoptosis activation markers, PARP1 and caspase3, did not change in shRNA relB knockdown HL cells as compared to controls.

These results shed light on new targetable signalling pathways that play key roles in HL cell survival. High Bcl2 and $Bclx_L$ levels, sequester Beclin1, inhibiting autophagic HL cell death.

676 (815) **IN VITRO COMPARATIVE STUDY OF THE PHOSPHORYLATION INHIBITION AND APOPTOTIC ACTIVITY INDUCED BY THE ORIGINAL AND A BIOEQUIVALENT PHARMACOLOGICAL IMATINIB**

Leandro Germán Gutiérrez[1], Micaela Palmitelli[1], Marcelo de Campos Nebel [1], Marcela González Cid [1], Irene Larripa[1]

[1]IMEX, CONICET-Academia Nacional de Medicina.

The development of tyrosine kinase inhibitors (TKI) marked the biggest advance in the treatment of chronic myeloid leukemia (CML). The TKIs block the ATP binding site of the onco-protein BCR/ABL1 with high affinity and specificity. Thereby, the tumor cells lose their proliferative advantage and the apoptotic process is induced.

The aim of this study was to compare the biological effectiveness of generic TKI (Imatinova®, Celnova Pharma) versus the original inhibitor (Glivec®, Novartis) *in vitro,* through an induction of apoptosis and inhibition of phosphorylation of BCR/ABL1 in K562, MEG01 (*BCR/ABL1* p210) and SUB15 (*BCR/ABL1* p190) cell lines.

Early apoptosis was assessed by flow cytometry with Annexin V-FITC in K562 and MEG01 cells subjected to 2.5 µM of each TKIs and evaluated at 24, 48 and 72 hs. This analysis showed a maximum level of apoptosis at 72 hs, with values higher than 90% in both cell lines. Late apoptosis was evaluated by acridine orange/ethidium bromide staining. K562 and MEG01 cells were treated with increasing concentrations of each TKIs (0.25-5.0 uM) and analyzed by fluorescence microscopy at 72hs. Both TKIs showed values of apoptosis higher than 83% for both cell lines. Therefore no significant differences were observed between both TKIs regarding apoptosis.

We also investigate the inhibition on BCR/ABL1 tyrosine kinase activity through the effect on phosphorylation status of CRKL (Crk-like protein, sustrate of BCR/ABL1) in K562, MEG01 and SUB15 cell lines by western blot (WB) using the antibody p-CRKL. Our results showed that both drugs induced similar reduction in p-CRKL with the doses tested (5-20μM). Higher doses than 20μM induced elevated levels of apoptosis preventing the WB analysis.

These studies in cell lines *BCR/ABL1* positive (p210 and p190) showed that induced apoptosis and the reduction of levels p-CRKL were similar, indicating that, in vitro, Imatinova® and Glivec® show the same behavior which would indicate an analog biological response.

677 (391) **IDENTIFICATION OF DRIVER AND SUBCLONAL MUTATIONS IN MYELOFIBROSIS AND ACUTE MYELOID LEUKEMIA POST CLASSIC MYELOPROLIFERATIVE NEOPLASMS (MPN)**

Karen Scheps, Carolina Meyer, Leandro Gutierrez, Alicia Enrico, Paula Heller, Mariana Rodríguez Zubieta, Carlos De Brasi, Irene Larripa[1].
[1] Lab. Genética, IMEX, CONICET-Academia Nacional de Medicina. [2]Hospital Italiano La Plata. [3]UE IDIM-CONICET. [4]Hospital Universitario Austral, Servicio de Anatomía Patológica.

Introduction: ClassicMPNs include polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). Evolution to secondary myelofibrosis is part of the natural history of the first two syndromes and, at any stage, acute myeloid leukemia (AML) can be acquired.

MPNs are characterized by the presence of driver oncogenic mutations in *JAK2*, *CALR* and *MPL* genes, being mutually exclusive. Also secondary subclonal mutations have been reported in ASXL1, IDH1, IDH2 genes, among others, associated with disease progression.

Aims: Analyze the presence of drivers and subclonal mutations in patients with MF and AML post MNP and determine its prognostic impact.

Patients and Methods: DNA of 35 patients with MF or AML post MPN were analyzed. $JAK2^{V617F}$ mutation was studied by DARMs PCR. The Allele burden of this mutation was assessed by qPCR. Type 1 and 2 *CALR* mutations (exon 9) were investigated by PCR and electrophoresis. *MPL* (exon 10) and *ASXL1* (exon 13) mutations were analyzed by PCR and direct sequencing. The mutations IDH1/2 were screened by CSGE.

Results: Mutations $JAK2^{V617F}$, *CALR* and *MPL* were detected in 24 (68.5%), 3 (8.5%) and 2 (5.7%) cases respectively, 4 (11.4%) were triple negative and in 2 cases the studies remain incomplete. The mean of the allele burden of the $JAK2^{V617F}$ mutation was 62.7%, splitting the patients into 2 groups with high and low levels. The analysis of exon 13 of *ASXL1* revealed 4 indels (3 not previously reported) and 3 rare missense variants. Five of these patients had disease progression or AML evolution. No mutations in the *IDH1/2* genes have been detected yet.

Discussion: Distribution of driver mutations differs from the literature, with a higher prevalence of $JAK2^{V617F}$ at expense of *CALR*. The highest allele burden was detected in patients with secondary MF post PV. *ASXL1* indel mutations allowed identifying patients at increased risk, reinforcing the importance of these studies to choose the best risk-adapted therapy.

678 (485) **IRON OVERLOAD INDUCES CELLULAR REDISTRIBUTION OF HEPATIC AND DUODENAL IRON TRANSPORTERS IN MICE**

Gisela Giorgi[1], María Florencia Fernández Delias [1], Norma María Giusto[2], Marta E Roque [1].
[1]Fisiología Humana. Biología, Bioquímica y Farmacia. INBIOSUR- CONICET, [2]INIBIBB-CONICET.

The liver and duodenum are particularly susceptible to iron-related disorders. These tissues take up plasma iron from transferrin or non-transferrin-bound iron, which appears during iron overload. We assessed the effect of iron status on the levels of the mammalian ZIP14 (Zrt-Irt-like Protein14), divalent metal transporter1 (DMT1) and transferrin receptor1 (TfR1). Objective: The aim of the present study was to determine the effect of iron overload on the expression of iron transporters in the liver and in the duodenum. M*aterials and Methods:* CF1 female mice (25±5g;three months old) were bred at the animal facility of the UNS. After acclimation, mice were divided in two groups (n=6/group; paired design): *1)Iron adequate* (FeA); *2)Iron overload* (FeO) Fe-Saccharate (5d/20d.ip; 1,3g/kg). The procedures followed the Guide for the Care and Use of Laboratory Animals of NIH. The protocol was approved by the Committee on Experimental Animal Use and Care of the UNS. Immunohistochemical studies were assessed to determine DMT1, ZIP14, TfR1 localization. Results: *Duodenum:* In FeA mice, ZIP14 was found mainly in apical membrane of enterocytes. However, slight cytoplasmic ZIP14 expression was seen in FeO. DMT1 expression was detected in the cytoplasm of enterocyte in FeA, however in FeO was manly perinuclear. TfR1 expression detected in perinuclear and basolateral zone of enterocytes was strong in FeA and was slight in FeO. *Liver:* ZIP14 and DMT1 expression observed in the cytoplasm of hepatocyte was weak in FeA and was intense in FeO. Evident TfR1 expression was detected in cellular membrane and in cytoplasm of hepatocytes in FeA, while weak expression was observed in FeO. We detected a significant hemosiderin in Kupffer cells in FeO respect to FeA. Conclusions: The cellular redistribution of duodenal ZIP14, DMT1 y TfR1 and hepatic DMT1 and ZIP14 by iron overload could be explained as a physiological response to decrease the dietary iron uptake and stimulating its hepatic storage.

679 (516) **IRON OVERLOAD INDUCES CHANGES OF ZRT-IRT-LIKE PROTEIN 14 AND TRANSFERRIN RECEPTOR 1 TRANSPORTERS IN PANCREATIC CELLS IN MICE.**

María Florencia Fernández Delias[1], Gisela Giorgi[1], Norma María Giusto[2], Marta E Roque[1].
[1]Fisiología Humana. Biología, Bioquímica y Farmacia. INBIOSUR- CONICET. Universidad Nacional del Sur. [2]INIBIBB-CONICET

We demonstrated that iron excess induce a coordinated expression of hepcidin and of the divalent metal transporter1 (DMT1) iron importer in pancreatic tissue. However, the functions of other iron importers are not entirely clear in this tissue. Objective: The aim of this study was to clarify the effects of iron excess on pancreas, in terms of regulation of iron proteins such as transferrin receptor1 (TfR1) and ZIP14 (Zrt-Irt-like Protein 14). Materials and Methods: CF1 female mice (25±5g; 3 months old) were bred at the animal facility of the UNS. After acclimation, mice were divided in two groups (n=6/group; paired design): 1)Iron adequate (FeA); 2)Iron *overload* (FeO) Fe-Saccharate (5d/20d.ip; 1,3g/kg). The procedures followed the Guide for the Care and Use of Laboratory Animals of NIH. The protocol was approved by the Committee on Experimental Animal Use and Care of the UNS. Immunohistochemical studies were assessed to determine TfR1, ZIP14 and Ferritin localization. Perl´s stain. Results: Slight TfR1 expression was observed in Langerhans islets in FeO, while abundant expression was observed in FeA. In acinar cells, we not detected TfR1 expression in both FeO and FeA mice. ZIP14 expression in acinar cells was intense in FeO and was weak in FeA. However, in Langerhans islets, ZIP14 expression was similar for the two groups. The expression of ferritin detected in acinar and connective tissue cells was intense in FeO and it was slight in FeA. In Langerhans islets we observed weak ferritin expression in FeO, while it expression was intense in FeA. Abundant hemosiderin was observed in the pancreatic interacinar connective tissues in FeO, however it was absent in FeA. Conclusions: In iron overload, pancreatic ZIP14 might be the major transporter for iron excess uptake in acinar cells, while TfR1 seems not to be involved. Finally, we propose that pancreas possess an adaptive role in iron excess, which the exocrine area could protect the endocrine zone against iron.

680 (597) **STUDY AND IDENTIFICATION OF CLONAL T-LYMPHOCYTE EXPANSIONS**

Leonardo Dionisio[1], Federico Torreguitart[1], Paula Iommi[1], Lorena Zanella[1], Nehuen Gasparini[1], Natalia Santillan[1], Cecilia Lang[1], Evangelina Agriello[1, 2]
[1]LEB Laboratorios. [2]Cátedra de Hematología Clínica, UNS.

T-cell expansions may be due to polyclonal or monoclonal proliferations. Unlike B-cell, does not exist a specific clonality marker, so TCR repertoire is evaluated by Flow Cytometry (FC). Aberrant expression of some markers can help in clonality assessment. The aim is to determine the scope of FC in the study of clonal T-cell expansions. For that, 64 samples obtained from patients with lymphocytosis were evaluated. T-cell markers were studied using a FACSCanto II cytometer and data analysis performed with Infinicyt software and APS tool. Clonality assessment was performed by FC studying TCR Vβ repertoire and by PCR studying TCRγ gene. FISH was used to evaluate rearrangement of ALK gene. 11% of samples showed polyclonal expansions. From clonal ones, 7% resulted γδ+. From αβ+, 50% were CD8+, 44% CD4+ and 6% CD4+CD8+. Most CD8+ clonal cells expressed CD45++, CD3++, CD2+d, CD5+d, CD7+d, CD38+/++, CD56++, associated to LLGG. Patients showed neutropenia and autoimmunity with good prognosis. Even so, we found 2 cases with CD30++, CD5-, CD26+, HLA-DR++, one of them ALK+ and both with poor prognosis, associated to Anaplastic Lymphoma. CD4+CD8+ and γδ samples showed a variable phenotype with clinical features similar to CD8+ group. On the other hand, CD4+ clonal cells showed different phenotypes: 1) CD45++, CD3+d, CD2+, CD5++, CD7+d, CD25-, CD28+, TCL-1++ with hyperleucocytosis and lymphadenophaties, associated to T-PLL, 2) CD3+d, CD25++, CD28++, CD7-, CD5++, CD2+, TCL-1-, with HTLV+, associated with ALLT and 3) CD45++, CD3+d, CD7+d, CD5++, CD2+d, CD25-, CD28+, TCL-1-, skin involvement, associated with Sézary Syndrome. Clonal CD4+ showed poor prognosis, aggressive pharmacological therapy and high mortality. We concluded that FC allowed the identification of clonal T-cells rapidly: TCR repertoire established clonality and phenotype analysis allowed identify clonal entities with different prognosis, evidencing that clonality not always implies malignancy.

681 (599) **EFFECT OF THYROID HORMONE MEMBRANE RECEPTOR INHIBITION IN THE TREATMENT WITH REXINOIDS OF CUTANEOUS T CELL LYMPHOMA**

Maria Florencia Cayrol[1], Maria Victoria Revuelta[2], Mercedes Debernardi[1], Maria Celeste Diaz Flaqué[1], Alejandra Paulazo[1], Helena Sterle[1], Leandro Cerchietti[2], Graciela Cremaschi[1].
[1]Laboratorio de Neuroinmunomodulación y Oncología molecular, Instituto de Investigaciones Biomédicas (BIOMED)- Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)- Universidad Católica Argentina (UCA), Buenos Aires, Argentina. [2]Department of Medicine, Hematology-Oncology Division, Weill Cornell Medical College, New York, NY.

Cutaneous T Cell Lymphomas (CTCL) are neoplasms of mature T cells with clinical presentations from discrete skin lesions to visceral disease. CTCL are exposed to a complex paracrine and endocrine environment that influence their development. Recently we found that both nuclear (TR) and membrane (mTR, integrin aVb3) TH receptors regulate transcriptional programs required for the survival and proliferation of TCL, including CTCL. For CTCL treatment the most used rexinoid, bexarotene (BEXA), is associated with hypothyroidism being patients candidate for TH replacement therapy. The consequences of TH administration on the activity of BEXA in CTCL cells are unknown. Our aim was to study the effect of TH and their genetic programs on the antilymphoma activity of rexinoids. To accomplish this, we perform functional assays andnext generation sequencing studies in CTLC BEXA-treated cells (HuT78 and MJ) inthe presence or absence of TR or mTR. Results show, that BEXA activity decreaseon both, conventional or 3D CTCL cell cultures, in the presence ofphysiological concentrations of TH. Onthe other hand, in vivo assays performed on C57BL/6 mice show that CD3+ population, mainly the CD8+ cells, decrease due to BEXA-induced hypothyroidism, as TH replacement revert this effect. Considering this, we evaluated the effect in vitro of mTR inhibition in combination with BEXA treatment. We found that either, using silencing RNA or pharmacological inhibitors of mTR, the anti-lymphoma activity of BEXA increases. The improvement of BEXA activity could be explained by the increase of cell apoptosis and the inhibition of cell cycle markers. RNAseq results support the latest, showing that inhibition of mTR in cells treated with BEXA, increases the regulation of genes involved in cell cycle, apoptosis and tumorigénesis (REL, ID2, LGALS1, ZC3H12, among others). Based on our results, we proposed that mTR inhibition could be a new strategy to improve rexinoid treatment in CTCL patients.

682 (1039) **ASCORBIC ACID ON PLATELET FUNCTION. NEW FUNCTIONS FOR AN OLD DRUG.**

Paola Carla Ivani[1,2] , Sebastián Suarez[2], Marcelo Adrian Martí[2], Mirta Ana Schattner [1], Roberto Gabriel Pozner[1,2]
[1]Instituto de Medicina Experimental (IMEX), Academia Nacional de Medicina-CONICET. [2]Depto. de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires.

Early studies have shown that ascorbic acid (Asc), the reduced form of vitamin C, inhibits platelet (Plt) aggregation partly by reducing the levels of reactive oxygen species. However, recent studies revealed that excessive vitamin C consumption may aggravate cardiovascular diseases in susceptible populations. To further characterize the mechanisms of Asc on Plt activation, in this study we evaluated some activation responses of Plt at normal or low concentration of the classical agonist PAR-1-agonist peptide (AP) or arachidonic acid (AA) in the presence of Asc, and the possible implication of HNO, a new reactive nitrogen species of biological interest.

In line of previous reports, Plt aggregation induced by AA 0.9mM or PAR1-AP 6μM were significantly inhibited when Asc 10mM was added to the cuvette of a light transmission aggregometer (49±10 or 24±9% of inhibition for AA 0.9mM or PAR1-AP 6μM, respectively, $p<0.05$, n=15). This effect was dependent on GPIIbIIIa activation, revealed by a similar inhibition of fibrinogen binding (evaluated by flow cytometry) obtained in the presence of Asc. Moreover, p-selectin externalization (flow cytometry) was also abrogated by Asc, indicating that alpha-granule release is also sensitive to the inhibition of Asc. All the inhibitory effects on the Plt activation responses tested were dependent on the concentration of Asc.

Surprisingly, when Plt were stimulated with sub-threshold (non-aggregating) concentrations of AA or PAR1- AP, the presence of Asc 10 mM resulted in an aggregatory response (60±5 or 45±6% of aggregation for AA 0.2mM or PAR1-AP 3.2μM, respectively, $p<0,0001$, n=15). This priming effect on the Plt aggregation responses were dependent on the concentration of Asc.

To determine the level of an interesting reactive nitrogen specie, HNO produced by Plt was determined by detecting the spectral changes obtained by nitrosylation of a manganese(III) prophyrin (specific for HNO) and also by an HNO-selective electrode (based on a cobalt(II) porphyrin), which allows for time-resolved quantitation of HNO.

HNO generation was not detected when Plt were stimulated by sub-threshold concentrations of agonists (in the presence or absence of Asc), but it was produced as a consequence of Plt activation with AA 0.9mM or PAR1-AP 6μM (260±50 or 170±35nM, respectively, the value of HNO was estimated by adding a solution of Angeli's salt at a final concentration of 200μM). Interestingly, the HNO production was higher in the presence of Asc (430±51 or 190±32nM when stimulated by AA or PAR1-AP, respectively), but the difference was significantly only for AA-stimulated Plt.

Altogether, these results present Asc as a dual modulator for Plt aggregation and present evidence of HNO production in primary cell as a new endogenous negative regulator for Plt function.

683 (2024) **HOMOCYSTEINE THIOLACTONE EFFECTS ON TISULAR PLASMINOGEN ACTIVATOR**

Heliana de Lourdes Hernández Herrera[1], Valeria Genoud[1], Silvana Gionco[1], Ana María Lauricella[1].
[1]*Facultad de Ciencias Exactas y Naturales.*

Background: Elevated level of homocysteinemia proved to be a risk factor for vascular disease. Homocysteine thiolactone (HTL) is a highly reactive form that mediates N-binding to ε-amino group of lysine residues resulting in modified proteins. Since impair fibrinolysis is associated to thrombosis, changes on N-homocysteinylated tissue plasminogen activator (tPAHTL) properties may be involved in this dysfunction.

Aims: To evaluate HTL in-vitro effects on tPA molecule and enzimatic activity.

Methods: Recombinant tPA treated with HTL (molar ratio tPA:HTL=1:760; 3h, 37°C) or saline buffer as control, were dialyzated to remove unreacted HTL. tPAHTL molecule were evaluated by Capillary Zone Electrophoresis (CZE). tPAHTL activity was studied by: a) amydolytic and b) fibrinolytic methods. a) Plasminogen (Plg 0.3 µmol/L) activation by tPAHTL (360 U/mL) were studied through chromogenic substrate S-2251 (0.5 mmol/L). b) Lysis of purified fibrinogen (2.5 g/L) clotted with thrombin (1 U/mL) in the presence of Plg and tPAHTL were evaluated. In both kinetic assays, OD405 nm were recorded versus time and Maximum velocity (Vmax) between tPAHTL vs control, were compared from the sigmoid curves. Assays were performed in sextuplicate

Results: CZE: electroferograms of tPAHTL showed diminished migration time vs control (tPAHTL 7.056±0.009 vs 7.49±0.08 min). a) Amidolytic assays showed that Plg activation with tPAHTL resulted slower than control (Vmax HTL 0.0055±0.0002 versus 0.0104±0.0001 1/min); b) Fibrinolysis activity was also diminished by tPAHTL (Vmax HTL 0.004±0.001 vs 0.011±0.002 1/min, Lysis timeHTL 44.0±2.5 vs 13.2±0.4 min). Significant differences ($p<0.05$) between treated samples and controls were observed in all assays.

Conclusion: HTL induced changes in tPA molecule decreasing its activity. Hyperhomocysteinemic patients would have altered tPA with minor lytic activity, contributing to prothrombotic state.

GASTROENTEROLOGÍA / GASTROENTEROLOGY

684 (2034) **HELICOBACTER PYLORI INFECTION: REGARDING THE ISSUE IN OUR REGION**

Pamela Bucci[1], María Rosa Baroni[1], Rita Giani[1], Antonela Giusti[1], Hilaria Grieve[2], Yanina Barbaglia[4], Félix Jiménez[4], Brian Braccio[3], Fabián Tedeschi[1], Fabián Zalazar[1].
[1]*Facultad de Bioquímica y Ciencias Biológicas (Universidad Nacional del Litoral (Santa Fe).* [2]*Alumna de la Maestría en Biología Molecular Médica (Universidad de Buenos Aires).* [3]*Residente Bioquímico, Ministerio de Salud de la Provincia de Santa Fe.* [4]*Servicio de Gastroenterología, Hospital "Dr. Jose M. Cullen" (Santa Fe).*

Helicobacter pylori (*H. pylori*) is responsible for chronic gastritis and is a major etiologic factor in the sequence leading to gastric carcinoma, which could result from the presence of specific virulence factors as well as host factors such as interleukins gene polymorphisms.The eradication rate is variable, mainly due to an increasing resistance to clarithromycin (Cla).Our goals were: a) to identify virulence factors of *H. pylori* and the SNP -174 G>C in the IL-6 gene promoter, b)to correlate genotypes and the SNP with the changes in gastric mucosa and c)to detect mutations conferring resistance to Cla in the 23S RNA gene.Gastric biopsies from adult patients from Santa Fe province (n=169) were used as starting material. *H. pylori* was identified by nested PCR. CagA, vacA and babA2 genes were detected by Multiplex PCR. Both SNP -174 G>C and point mutations in the 23S rRNA gene were analyzed by PCR-RFLP.Fisher's exact test was used ($p<0.05$ was considered significant) and Odds ratio was calculated. *H. pylori*(+) patients were classified as: a)Chronic Gastritis (GC), b)Chronic Active Gastritis without intestinal metaplasia(GCAMI-) and c) Chronic Active Gastritis with Intestinal metaplasia(GCAMI+).The GCAMI+ group showed the highest proportion of cagA(+), vacAs1m1(+) and cagA(+)/vacAs1m1 strains. There were no differences between the GC and GCAMI- groups.The number of cagA(+), vacAs1m1(+) and cagA(+)/ vacAs1m1 strains was higher in GCAMI+ than in GC (Odds ratio:5.8, IC:1.3 to 25.3). No association between the cagA(+)/ vacAs1m1(+)/babA2(+) genotype and the different histopathological diagnoses was found.The frequence of GG haplotype in the promoter of IL-6 was greater in GCAMI+ than in GCAMI-; moreover, all patients cagA(+)/vacAs1m1(+) in CGAMI+ had the GGhaplotype (Odds ratio = 6.4, CI =1.4-27.4).We found a 17% of strains resistant to Cla. These data could contribute to the managment of the disease in our region as well as to understand the pathogenesis of *H.pylori* infection

685 (187) **ROLE OF USP9X IN VMP1-MEDIATED AUTOPHAGY**

Felipe Javier Renna[1], Tamara Orquera[1], Daniel Grasso[1], Maria Ines Vaccaro[1].
[1]*IBIMOL (UBA-CONICET), Cátedra Fisiopatología, Facultad de Farmacia y Bioquímica, UBA. Junín 956, 1113AAD, Ciudad de Buenos Aires.*

The intracellular activation of zymogen granules triggers the acute pancreatitis and the consequence gland self-digestion. Most of acute pancreatitis are self-limited suggesting the importance of the stress response mechanism of pancreas acinar cells. VMP1 is an autophagy protein which is able to induce autophagy even in non-starved condition. Moreover, VMP1 is induced by acute pancreatitis and mediates the zymophagy, a selective autophagy-mediated degradation of zymogen granules. We demonstrated that the deubiquitinase USP9x interacts with VMP1 during zymophagy in acute pancreatitis. The objective of this work is to know the role of USP9x in autophagy process. In HeLa cells, we demonstrate by immunofluorescence (IF) that USP9x responds to starvation as early as 15 minutes with a relocation pattern without changing its expression by western blot. At the basal condition, USP9x is distributed over the whole cytoplasm meanwhile upon starvation it quickly moves to a region rich in lysosomes, marked with Lamp1 fluorescence. USP9x is necessary for autophagosome formation since shRNA-mediated depletion of USP9x inhibits the autophagy, evaluated by LC3 recruitment. USP9x interacts with VMP1 and colocalizes with VMP1 at the reticulum in an area near to the nucleus suggesting its implication in the initiation mechanism of autophagosome formation. USP9x deubiquitinates BECN1, which is an important autophagy protein. By IF, we show the ubiquitination of BECN1 during starvation and its accumulation when it is co-expressed with a ubiquitin molecule without chain formation capability. Consequently, shRNA-mediated depletion of USP9x increases the general ubiquitin signal in the cytoplasm. Our data suggest that USP9x is a novel component of the set of proteins implicated in autophagy, necessary for autphagosome formation and involved in a mechanism where VMP1 and BECN1 are implicated.

686 (398) **THE INSULIN-LIKE GROWTH FACTOR-I RECEPTOR (IGFR) IS INVOLVED IN ESTRADIOL-17BETA-D-GLUCURONIDE (E17G) INDUCED CHOLESTASIS IN ISOLATED PERFUSED RAT LIVER (IPRL).**

Ismael Ricardo Barosso[1,2], Romina Andermatten[1,2], Nadia Ciriaci[1,2], Gisel Miszczuk[1,2], Paula Maidagán[1,2], Enrique Sánchez Pózzi[1,2],
[1]*Instituto de Fisiología Experimental IFISE CONICET,* [2]*Facultad de ciencias Bioquímicas y Farmaceúticas UNR*

E17G internalizes canalicular transporters such as Mrp2 and Bsep (model of intrahepatic cholestasis of pregnancy), by activating two different pathways each one involving an estrogen receptor: ERα or GPR30. GPR30 activates adenylyl cyclase-PKA and PI3K/Akt, being the latter pathway responsible for keeping transporters in a subapical space, restraining reinsertion. Previous works demonstrated that IGFR participates downstream of GPR30 in culture of rat hepatocyte couplets, so the role of IGFR was evaluated in a physiological model as IPRL.

Livers were perfused in situ via the portal vein in a non-recirculating single-pass design. Taurocholate (5µM, Bsep sustrate) and

1-chloro-2,4-dinitrobenzene (0.5 µM, precursor of DNP-glutathione, Mrp2 substrate) were added to the perfusion medium to estimate Bsep and Mrp2 activities. After a 20-min. equilibration period, Tyrphostin AG1024 (AG,100 nM), IGFR inhibitor, or its solvent (DMSO, 370µl/L) was added to the reservoir. Ten minutes later, a 5 min basal bile sample was collected, followed by administration of E17G (3 µmol/liver, intraportal) or its solvent (DMSO/10% BSA in saline), and bile collected at 5-min intervals for 40 min.: bile flow was determined gravimetrically (only if initial bile flow was greater than 15 µL/ min/kg). Biliary excretion of DNP-glutathione was determined by HPLC and that of BS by 3a-hydroxysteroid method.

AG did not prevent the drop induced by E17G (40 %) in the three parameters measured, but accelerated the recovery of both bile flow (significant from 15 min of E17G administration on) and biliary excretion of DNP-glutathione and BS (significant from 25 min of E17G administration on). ($p<0.05$, n=3). A similar behavior was previously observed with inhibitors of PI3K, a protein that allegedly participates in the same pathway of IGFR.

Conclusion: these results confirm the role of IGFR in estrogen cholestasis, which expands the potential therapeutic targets for its treatment.

687 (369) **INVOLVEMENT OF TUMOR NECROSIS FACTOR ALPHA (TNF)-INDUCED ROS SIGNALING IN THE IMPAIRMENT OF MRP2 FUNCTION**

Nadia Ciriaci[1], Romina Andermatten[1], Ismael Barosso[1], María Laura Ruiz[1], Enrique Sanchez Pozzi[1].
[1]Instituto de Fisiología Experimental - IFISE. Facultad de Ciencias Bioquímicas y Farmacéuticas (CONICET-UNR).

Previous studies gave evidences that TNF could modulate Mrp2 through endocytic internalization via activation of two pathways: one involving PI3K/Akt and the other MEK/ERK.

Given that TNF-induced reactive oxygen species (ROS) production activates PI3K and that oxidative stress induces internalization of the bile salt export pump (Bsep), we decided to elucidate whether TNF-induced ROS signaling participates in the impairment of Mrp2 function.

Methodology: Isolated rat hepatocyte couplets were cotreated with TNF (6,25 pg/ml) and antioxidants mannitol (Man 60 mM) and vitamin C (VitC 100 µM) with or without preincubation with inhibitors of PI3K (wortmannin, W 100 nM) or MEK1/2 (PD 5 µM, upstream of ERK1/2). Then, functional changes in Mrp2 activity were evaluated by assessing the canalicular vacuolar accumulation (cVA) of glutathione methylfluorescein (GMF), a fluorescent Mrp2 substrate derived from 5-chloromethylfluorescein diacetate (2,5 µM).

Primary-cultured rat hepatocytes were exposed to VitC and TNF. Then, activation of ERK1/2 and Akt (PI3K effector) was confirmed evaluating their phosphorylation status via western blotting.

Results (% of Control, n:3-5): Treatment with Man and VitC partially prevented TNF-induced impairment of canalicular accumulation of GMF: TNF (70±2a); TNF+Man (80±4a,b); TNF+VitC (89±2a,b). Protection with W (85±2a,b) showed addition with antioxidant (TNF+W+VitC: 92±2a,b,c). In contrast, preincubation of PD (86±1a,b) did not add up with VitC (TNF+PD+VitC: 85±1a,b), suggesting that MEK/ERK and ROS are in the same pathway. Consistently, TNF-induced phosphorylation of ERK decreased in presence VitC: TNF (137±4a); TNF+VitC (116±1b) indicating that ROS production precedes ERK activation. a $p<0.05$ vs Control, b $p<0.05$ vs TNF, c $p<0.05$ vs TNF+VitC and TNF+W.

Conclusion: ROS are possible modulators of TNF-α-induced impairment of Mrp2 function through a MEK/ERK-dependent pathway.

688 (478) **MITOCHONDRIAL FUNCTION AND DYNAMICS DURING SELECTIVE AUTOPHAGY IN ACUTE PANCREATITIS**

Virginia Vanasco[1,2], Alejandro Ropolo[1,2], Daniel Grasso[1,2], Silvia Alvarez[1,2], María Ines Vaccaro[1,2].
[1]Instituto de Bioquímica y Medicina Molecular (UBA-CONICET), [2]Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires.

The selective autophagic pathway, zymophagy, is an early protective mechanism in acute pancreatitis (AP). Mitochondria, as ATP source and other biological molecules, are necessary for autophagy in order to maintain mitochondrial bioenergetics for sustaining an adequate autophagic flux during disease response. The aim was to analyze mitochondrial dynamics and function during selective autophagy induced by AP in animal and cellular models. Female Sprague-Dawley rats (45 days old) were ip injected with 50mg/kg caerulein (CAE) during 1h intervals. Zymophagy induced by AP was analysed through VMP1, P62 and LC3 expression. For mitochondrial dynamics, OPA1 and DRP1 expressions were determined. OPA1 expression (mitochondrial fusion protein) was significantly decreased after 1 h of pancreatitis, but a time-dependent increase was observed up to 48 h afterwards. Moreover, no expression of DRP1 (mitochondrial fission protein) was observed during the first 24 h of PA. Mitochondrial function was assessed by determining respiration and mitochondrial ATP synthesis. Mitochondrial O_2 consumption and ATP production decreased by 35% and 70% respectively (CG: 40±5 ng-atO/min. mg protein; 140±18 nmol ATP/ min.mg.protein, $P<0.01$) between 1 and 24 h of AP, with a consequent decrease in ATP/O ratio. At 48 h control values were observed for both parameters. In the cell model, AR42J pancreatic acinar-cells were treated with 7.4uM CAE. Mitochondrial degradation by mitophagy was evaluated by transfecting cells with dual pMITO vector. Early mitophagy induction was observed, being maximal after 30 min of treatment. We confirm mitochondrial elongation and its relationship with mitophagy in cells stained with Mitotraker and transfected with LC3. Our results show that mitochondrial dysfunction occurs early during experimental AP. Also, dysfunctional mitochondria are initially degraded by mitophagy, while functional mitochondria may avoid degradation by mitochondrial fusion and elongation.

689 (370) **SPHINGOSINE 1-PHOSPHATE RECEPTOR 2 (S1PR2) IS POTENTIALLY INVOLVED IN TAUROLITHOCHOLATE (TLC)-INDUCED IMPAIRMENT OF MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN 2 (MRP2) ACTIVITY**

Romina Belén Andermatten[1], Nadia Ciriaci[1], Ma. Valeria Razori[1], Ismael R. Barosso[1], Enrique J. Sanchez Pozzi[1].
[1]Instituto de Fisiología Experimental (IFISE) - Facultad de Cs. Bioquímicas y Farmacéuticas (CONICET-UNR)

TLC is a bile salt that induces internalization of the canalicular transporter Mrp2 through adenylyl cyclase (AC)/PKA and PI3K-dependent pathways. There are a few G-protein coupled receptors that interact with bile salts. Among them, S1PR2 in the only one, described up to now, that is present in hepatocytes and potentially activates adenylyl cyclase. In consequence our aim was to evaluate the role of this receptor in the impairment of Mrp2 transport activity induced by TLC.

Methodology: Isolated rat hepatocyte couplets were preincubated with the antagonist of S1PR2, JTE-013 (JTE, 10 µM) and then exposed to TLC (2.5 µM). Studies of additivity in the protection of inhibitors were carried out by coadministration of JTE with PI3K inhibitor wortmannina (W, 100 nM) before exposure to TLC. Functional changes in Mrp2 under the treatments described above were evaluated by assessing the canalicular vacuolar accumulation (cVA) of glutathione methylfluorescein (GMF), a fluorescent Mrp2 substrate derived from the addition of chloromethylfluorescein diacetate (CMFDA, 2.5 µM). Moreover, isolated hepatocytes were pretreated with JTE and exposed to TLC. Then, cells were lysed and western blot was performed for analysis of AKT phosphorylation, an indicator of AKT activation.

Results: (% of control ± SEM; n=3-9). Treatment with JTE partially prevented TLC-induced impairment in Mrp2 activity: TLC (55±2a), TLC+JTE (79±2a,b). The preventive effects of W and JTE were not additive: TLC (55±2a), TLC+JTE (79±2a,b), TLC+W (77±6a,b), TLC+W+JTE (79±6a,b) suggesting that S1PR2 and PI3K share the same signaling pathway. Activation of AKT induced by TLC decreased in presence of JTE: TLC (584±23a), TLC+JTE (161±27a,b). a $p<0.05$ vs control, b $p<0.05$ vs TLC.

Conclusion: The interaction of TLC with S1PR2 would be one of the first events in the signaling pathway that leads to Mrp2 activity impairment, followed by a cascade activation of several proteins including PI3K/AKT.

690 (498) **ANTICHOLESTATIC MECHANISMS OF URSODESOXYCHOLIC ACID IN INFLAMATORY CHOLESTASIS INDUCED BY LIPOPOLYSACCHARIDE (LPS)**

María Valeria Razori(1), Paula María Maidágan(1), Romina Andermatten(1), María Laura Ruiz(1), Marcelo Gabriel Roma(1)

[1]Instituto de Fisiología Experimental (CONICET – UNR). Argentina.

Backgrouds and aims: Sepsis-induced cholestasis is causally associated with the release of LPS from Gram (-) bacteria; LPS stimulates the production of proinflammatory cytokines which, in turn, impair expression/localization of key transporters involved in bile formation, such as Bsep and Mrp2. Ursodeoxycholic acid (UDCA) is the first choice therapy for cholestatic diseases, but its therapeutic efficacy in LPS-induced cholestasis is unknown. Therefore, we will study its therapeutic mechanisms here.

Methodology: Male Wistar rats were randomized in 4 experimental groups: Control, UDCA (25 mg/Kg/day, i.p., 5 days), LPS (total dose of 10 mg/Kg, i.p., over the last 2 days), and UDCA+LPS. On the 6th day, serum alkaline phosphatase (ALP, an hepatic enzyme removed by detergent bile salts (BS) accumulated in cholestasis), bile flow (BF), bile salt output (BSO), total glutathione output (GSHtO), and total/plasma membrane liver protein expression of canalicular transporters were assessed.

Results (% of control): Pretreatment of LPS-treated rats with UDCA reduced serum ALP as compared with LPS alone (193±14*# vs. 254±15*), and increased BSO (85±20*# vs. 57±10*), a finding in line with the increased proportion of Bsep in plasma membrane (94±38*# vs. 39±11*). LPS reduced BF (79±3*), GSHtO (47±7*), and the protein levels of Bsep (41±11*) and Mrp2 (39±20*) in whole liver homogenate, but UDCA failed to improve these parameters (*p<0.05 vs. control; #p<0.05 vs. LPS).

Conclusion: Our results suggest that UDCA improves BSO and reduces hepatic retention of BS by increasing canalicular membrane localization of Bsep, an effect likely due to its capability to stimulate trafficking of this carrier into the apical pole. Although partial in its therapeutic mechanisms, UDCA may be ideal to complement therapies with high-affinity nuclear receptor ligands that improve canalicular transporter expression, by helping to afford proper localization of newly synthesized carriers

691 (537) **PROMISING THERAPEUTIC EFFECT OF COENZYME Q10 IN ETHINYL ESTRADIOL-INDUCED CHOLESTASIS**

Manuela Martinefski[1], Myriam Rodriguez[2,3], Fabian Buontempo[1], Silvia Lucangioli[1,3], Liliana Bianciotti[2,3], Valeria Tripodi[1,3],

[1]Departamento de Tecnología Farmacéutica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina, [2]Fisiopatología-INIGEM-CONICET, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina, [3]Consejo Nacional de Investigaciones Científicas y Tecnológicas, CONICET, Buenos Aires, Argentina

Intrahepatic cholestasis of pregnancy (ICP) is a high risk liver disease given the eventual deleterious consequences that may occur in the fetous. ICP is characterized by the accumulation of bile acids, particularly hydrophobic bile acids such as litocholic acid (LCA), which induces oxidative stress and apoptosis. We previously reported that women with IPC show decreased coenzyme Q10 (CoQ10) and enhanced bile acids in plasma. These findings were also observed in ethinyl estradiol (EE)-induced cholestasis in rats. The aim of this work was to evaluate the effect of CoQ10 supplementation in EE-induced cholestasis in rats. Cholestasis was induced in Sprague-Dawley rats by a daily intraperitoneal injection of 5 mg/kg EE for 5 days (EE). A group of these rats also received daily oral 250 mg/kg CoQ10 supplementation for 5 days (EE+CoQ10). Another group of rats received only CoQ10 supplementation for the same period of time (CoQ10). Serum, bile acids (total and LCA), coenzyme Q9 (CoQ9) and CoQ10, aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase were assessed. Bile flow was also measured. No differences were observed between CoQ10 and control groups except for an increase in plasma CoQ10 ($p<0.001$). However, CoQ10 supplementation in cholestatic rats (EE+CoQ10) increased both CoQ10 and CoQ9 plasma levels ($p<0.05$), and enhanced bile flow($p<0.05$) as compared with EE. Furthermore, it also decreased serum alkaline phosphatase and bile acids, particularly LCA levels as compared with EE ($p<0.05$). Present findings show that CoQ10 administration improved cholestasis and further suggest that CoQ10 supplementation may be a potential therapeutic strategy in estrogen-induced cholestasis in humans as ICP.

692 (682) **ATRIAL NATRIURETIC FACTOR (ANF) ALLEVIATES ENDOPLASMIC RETICULUM STRESS IN RAT ACUTE PANCREATITIS**

Ana Paula Courreges[1], Ana Clara Najenson[1], Marcelo Sergio Vatta[2], Liliana Graciela Bianciotti[1,3],

[1]Instituto de Inmunología, Genética y Metabolismo (INIGEM-CONICET), Facultad de Farmacia y Bioquímica, UBA. [2]Cátedra de Fisiologìa-IQUIMEFA-CONICET, Facultad de Farmacia y Bioquímica, UBA. [3]Cátedra de Fisiopatologìa, Facultad de Farmacia y Bioquímica, UBA

The endoplasmic-reticulum (ER) stress response constitutes a cellular process that is triggered by a variety of conditions that disturb proteins folding in the ER. Eukaryotic cells have developed an evolutionarily conserved adaptive mechanism, the unfolded protein response (UPR), which aims to clear unfolded proteins and restore ER homeostasis. Recent studies support that the ER stress is one of the earliest events triggering acute pancreatitis (AP). Based on previous findings from our laboratory showing that ANF significantly attenuates the severity of AP by reducing trypsinogen activation and the inflammatory response, we sought to establish whether ANF affected ER stress in a validated AP animal model. AP was induced in Sprague Dawley strain rats (200-220 g) by four repetitive cerulein injections (40 µg/kg). Thirty minutes before the first cerulein injection animals were infused with either saline (control) or ANF (1 µg/kg/h) for 60 min. Following euthanasia pancreatic samples were harvested for western blot analysis and transmission electronic microscopy (TEM) studies. Chaperone BiP, the primary controller of UPR, was overexpressed in cerulein induced AP ($p < 0,001$) but pretreatment with ANF prevented it ($p < 0,05$). Preliminary results show that the pro-apoptotic transcription factor CHOP was also increased in animals with AP and decreased by ANF. Acinar cells from animals with AP showed ER swelling as well as gap junction disruption, cytoplasm vacuolization, inter e intra-edema, reduced zymogen granules, nuclear interdigitation, and necrotic areas as revealed by TEM. Animals pre-treated with ANF showed significantly reduced ultraestructural damage, including ER swelling. Present results show that ANF reduced ER swelling and the major pancreatic ultrastructural features of AP as well as UPR response suggesting that the atrial peptide alleviates ER stress in early AP in the rat.

693 (594) **TAUROURSODEOXYCHOLATE PREVENTS ESTRADIOL 17β-D-GLUCURONIDE-MEDIATED CHOLESTASIS BY INHIBITING THE PHOSPHORYLATIVE ACTIVATION OF PRO-CHOLESTATIC PROTEIN KINASES, INDEPENDENTLY OF PROTEIN PHOSPHATASES.**

Paula M. Maidágan[1], Andrea C. Boaglio[1], María V. Razori[1], Nadia Ciriaci[1], Gisel S. Miszczuk[1], Fernando A. Crocenzi[1], Marcelo G. Roma[1],

[1]Instituto de Fisiología Experimental (IFISE-CONICET) – Facultad de Ciencias Bioquímicas y Farmacéuticas (CONICET – UNR)

We have previously shown (*Hepatology* 52:1465-76, 2010) that estradiol 17β-D-glucuronide ($E_2$17G), etiologic agent of pregnancy-

induced cholestasis, alters function and location of both Mrp2 and Bsep, two key canalicular transporters involved in bile formation, by enhancing phosphorylative activation of "classical" protein kinase C (PKCc) and phosphatidylinositol 3 kinase (PI3K)/Akt-dependent signaling pathways. Pregnancy-induced cholestasis is treated with ursodeoxycholate, but its therapeutic mechanisms are unknown. We therefore evaluated whether its active metabolite, tauroursodeoxycholate (TUDC), prevents $E_2$17G-induced canalicular secretory failure by inhibiting the phosphorylative activation of PKCc and PI3K/Akt, and whether protein phosphatases (PPs) are involved. For this purpose, we studied by Western Blot, in isolated rat hepatocytes, the effect of TUDC on activation of PKCc (% of membrane translocation) and Akt (% of phosphorylated form). Pretreatment with TUDC (100 µM), followed by exposure to $E_2$17G (100 µM), prevented activation of PKCc (-34±4%) and Akt (-37±2%); $p<0.05$ *vs.* $E_2$17G. Canalicular transport function was assessed in rat hepatocyte couplets by quantifying the % of couplets displaying canalicular vacuolar accumulation (CVA) of the fluorescent Bsep and Mrp2 substrates cholyl-lysilfluorescein (CLF) and GSH-*S*-methylfluorescein (G*S*-MF), respectively. $E_2$17G decreased CVA of CLF by 41±3% ($p<0.05$ vs. control), while TUDC partially prevented this alteration by 79±7% ($p<0.05$ *vs.* $E_2$17G). $E_2$17G-induced impairment in CVA of G*S*-MF was also prevented by TUDC. Neither the PP1/PP2A inhibitors, tautomycin (1 nM) and okadaic acid (5 nM), nor the PP2B inhibitor tacrolimus (1 µM) affected the preventive effect of TUDC. We conclude that TUDC preserves function and location of Bsep and Mrp2 in $E_2$17G-induced cholestasis by preventing activation of upstream signaling pathways that activate PKCc and PI3K/Akt rather than by increasing dephosphorylation via PPs.

694 (959) **GLUCAGON-LIKE PEPTIDE 2 (GLP-2) PREVENTS DOWNREGULATION OF EXPRESSION AND ACTIVITY OF INTESTINAL P-GLYCOPROTEIN (P-GP) IN ENDOTOXEMIC RATS.**

Maite Rocío Arana[1], Guillermo Nicolás Tocchetti[1], Felipe Zecchinati[1], Camila Dominguez[1], Ana Sofía Londero[1], Silvina Stellamaris Villanueva[1], Aldo Domingo Mottino[1],
[1]Instituto de Fisiología Experimental (IFISE-CONICET)-Facultad de Ciencias Bioquímicas y Farmacéuticas-Universidad Nacional de Rosario, Rosario, Santa Fe, Argentina

The export pump P-gp is expressed on the surface of the enterocyte and prevents absorption of many toxins and drugs. Endotoxemia induced by lipopolysaccharide (LPS) administration to rats affects the intestinal barrier function by decreasing the expression and activity of P-gp. Aim: to evaluate the protective effect of GLP-2 on LPS-induced downregulation of P-gp. Methods: Female Wistar rats were treated with LPS (5 mg/kg b.w.) by a single i.p. injection and/or with GLP-2 (125 µg/kg b.w.) by s.c. injections every 12 h for a 72-h period, starting 60 hs before LPS administration. Control rats were treated with the respective vehicle. Studies were performed in the 40-cm most distal segment of the small intestine (distal jejunum/ileum), 24 h after LPS injection. Results: LPS decreased the expression (Westernblotting) of P-gp (-65%) respect to C ($p<0.05$), which was prevented by co-administration of GLP-2 (86% vs 100% for C; $p>0.05$). GLP-2 alone did not change statistically P-gp expression (153% vs 100% for C; $p>0.05$). RT-PCR studies demonstrated similar variations among groups in mRNA levels for Mdr1a, one of the two genes encoding P-gp in rodents. Serosal to mucosal transport of Rhodamine 123 in everted intestinal sacs (P-gp activity) was decreased by LPS (-59%) respect to C ($p<0.05$) and was also prevented by GLP-2 treatment (110% vs 100% for C; $p>0.05$), with no change for GLP-2 given alone (84% vs 100% for C; $p>0.05$). Additional determinations using the specific inhibitor PSC-833 confirm involvement of P-gp in Rhodamine transport for all groups. Conclusion: The data suggest that GLP-2 could be of potential therapeutic value in situations that involve alterations in intestinal barrier function associated with downregulation of P-gp under endotoxemic conditions. Further studies are necessary to elucidate the mechanism of protection, probably indirect, considering that GLP-2 itself does not regulate P-gp expression.

695 (406) **ESTRADIOL 17 BETA-D-GLUCURONIDE (E17G) INDUCES SWITCH OF THE CANALICULAR TRANSPORTERS BSEP AND MRP2 FROM RAFT TO NON-RAFT MICRODOMAINS, FOLLOWED BY CLATHRIN-DEPENDENT ENDOCYTOSIS.**

Gisel Sabrina Miszczuk[(1)], Ismael Ricardo Barosso[(1)], Andrea Carolina Boaglio[(1)], Maria Cecilia Larocca[(1)], Enrique Juan Sánchez Pozzi[(1)], Marcelo Gabriel Roma[(1)], Fernando Ariel Crocenzi[(1)].
(1) Instituto de Fisiología Experimental (IFISE) - CONICET - UNR

Endocytosis of canalicular transporters is a key pathomechanism of E17G-induced cholestasis. In preliminary experiments in rat hepatocyte couplets, we reported that this process is prevented by specific inhibitors of the clathrin-dependent endocytosis (Medicina 74 Supl. III, 106, 2014). In physiological conditions, canalicular transporters are mostly presented in raft (high-cholesterol, caveolin-enriched) microdomains. Clathrin-dependent endocytosis requires both the presence of the cargo in non-raft (low-cholesterol, clathrin-enriched) microdomains and the recruitment of the adaptor protein AP2 to these domains. First, we evaluated whether E17G induces a shift of Bsep and Mrp2 from raft to non-raft microdomains, in order to allow for clathrin-dependent endocytosis from the latter domain. E17G (11 µmol/g b.w.) was administered to whole rats, and liver samples were taken after 20 min, when the maximum decrease of bile flow is reached. Highly purified plasma membrane fractions composed of raft or non-raft microdomains were obtained by ultracentrifugation from liver homogenates; enrichment of these fractions was assessed by western blot of the specific markers caveolin and clathrin, respectively. Western blot of Bsep and Mrp2 showed that E17G induced a switch between microdomains, leading to a high enrichment of these transporters in the non-raft fraction (Bsep: ~80% of total vs ~40% in controls; Mrp2: ~80% of total vs ~25% in controls). To evaluate the dependency of AP2 for E17G-induced canalicular transporter endocytosis, we performed siRNA knockdown of AP2 in sandwich-cultured rat hepatocytes. AP2 knockdown completely prevented E17G-induced endocytosis of Bsep and Mrp2, as shown by confocal microscopy analysis. Thus, we proposed that E17G-induced endocytosis of Bsep and Mrp2 in rat livers involves a switch of these transporters from raft to non-raft canalicular microdomains, from where they are endocytosed by a clathrin-dependent mechanism.

NEFROLOGÍA / NEPHROLOGY

696 (148) **FINDING OF A TESTICULAR PROTEIN IN KIDNEY DUCTS**

María Eugenia Cabrillana[1,2], María de los Ángeles Monclus[1,2], Layla Simón Abi Funes[1], Regina Colombo[1], Tania Estefania Saez Lancellotti[1,2], Amanda Vincenti[1,2], Miguel Fornes[1,2].
[1]Instituto de Histología y Embriología de Mendoza (IHEM), CONICET, Mendoza [2]Consejo de Investigación de La Universidad del Aconcagua (CIUDA).

During sperm maturation, tail structures are stabilized, through oxidation of thiol groups. It was proposed that sperm thiol oxidation is necessary for sperm motility. This oxidation occurs in many sperm tail protein, as in ODF1. ODF1 is a cytoskeleton protein which function in the sperm has not been clarified at all. ODF1 was described as a testi and sperm protein exclusively, and was involved in the development of the flagellum. The aim of this work was to evaluate the presence of this protein in other tissues. We applied western blott and inmunofluorescence techniques using an antibody against ODF1. Surprisingly, the strongest signal was found in marrow kidney extracts and cells from collecting duct. Also some cortical ducts presented positive mark. Extracts from brain, liver, skin, and lung did not show positive signal to ODF1 antibody. This was the first time that ODF1 was described in other tissue. Now we are evaluating the presence of the mARN of this protein in the kidney. In the future we will analyze if ODF1 participate in the development of the primary cilium in collecting ducts.

697 (212) **ENDOTHELIN INHIBITION DURING RENAL POSTNATAL DEVELOPMENT: EFFECTS ON SODIUM AND WATER TRANSPORTERS AND THE IMPACT OF A SALT OVERLOAD DURING ADULTHOOD**

Maria Florencia Albertoni Borghese[1], Camila Kessler[1], Maria del Carmen Ortiz[1], Celeste Sanz[1], Rocio Moreira Szokalo[1], Monica Majowicz[1].
[1]Cátedra de Biología Celular y Molecular, Facultad de Farmacia y Bioquímica, Universidad De Buenos Aires

Renal development in rodents continues after birth. The fact that the expression of ET receptors (ET_A and ET_B) increase at the end of the embryonic period and very early after birth supports the importance of ET system in the rat renal postnatal development. We aimed to evaluate the expression of epithelial sodium channel, α subunit (α-ENaC) and Aquaporin 2 (AQP2) in the renal medulla of male adult Sprague-Dawley rats treated with bosentan ("B", dual antagonist of both ET_A and ET_B; dose= 20mg/Kg/day) during their first 20 days of life. Control rats (C) received an equivalent volume of distilled water instead of B. The results were obtained after feeding the animals with a normosodic diet (NS) or with a hypersodic diet (HS; 8% NaCl) during 8 days in order to evaluate their salt sensitivity. Four experimental groups were obtained: CNS, CHS, BNS and BHS. The percentage difference between arterial pressure (AP) before and after HS was higher in BHS group vs BNS and CHS; $p<0.01$. Both α-ENaC and AQP2 expression measured by western blot and real time PCR (qPCR) decreased in CHS vs CNS ($p<0.05$ and $p<0.01$ respectively), but did not change in BHS nor BNS. These results are in accordance with the results of renal function that showed a decreased diuresis and natriuresis in BHS vs CHS. Considering that both transporters are regulated by vasopressin through V_2 receptors we measured V_2 expression by qPCR and found a significant increase in V_2 mRNA only in BHS group. In BHS, the expression of both ENaC and AQP2 does not decrease as in CHS, so these animals have a diminished ability to excrete sodium and water (BHS showed decreased diuresis and fractional sodium excretion; $p<0.05$ vs CHS), which leads to a significant increase in AP. These results suggest that the pharmacological inhibition of ET system in the postnatal period alters the ability of male adult rats to eliminate a salt overload, increasing the salt sensitivity of the animals and predisposing to hypertension.

698 (270) **THE ROLE OF SODIUM CHLORIDE COTRANSPORTER AND EPHITELIAL SODIUM CHANNEL IN THE GONAD-DEPENDENT REGULATION OF KALLIKREIN KININ SYSTEM AFTER ROMK INHIBITION**

Alejandro F. Celía[1], Luis A Di Ciano[1], Dario Guevara[1], Sandra Vlachovsky[1], Fernando R. Ibarra[1,2], Elvira E. Arrizurieta[1,3], Elisabet M. Oddo[1], Pablo J. Azurmendi[1].
[1]Lab. Riñón Exp. y Bioquímica Molecular, IIM A. Lanari, UBA [2]Departamento de Ciencias Fisiológicas, Facultad de Medicina, UBA [3]CONICET

Our previous reports have shown that high K^+ intake and prepuberal gonadectomy (Gx) diminish blood pressure with a concomitant increase in urine kallikrein activity (UKa) and plasma aldosterone levels, revealing a link between those systems. Since kallikrein co-localize in the same distal nephron segments of aldosterone effectors, we explored the rectifying outer medulla K^+ (ROMK) channel blockade by glibenclamide (Gli) in different gonad contexts. The aim of this study was to elucidate the behavior of Na^+/Cl^- cotransporter (NCC) and epithelial Na^+ channel (ENaC) after ROMK blockade upon kallikrein-kinin system (KKS) and its interaction with sexual hormones. Spontaneously hypertensive rats of both sexes, half of them Gx at weaning, were studied at 12 weeks of life. Glucose solution (4%) with or without Gli (10 mg/kg bwt) was orally administered in the last 3 days of the experiment. Protein abundance of NCC and α-ENaC and Slc12a3 (NCC), Scnna (α-ENaC) and Scnng (α-ENaC) mRNA levels were tested in renal cortex homogenates by western blot and real time PCR, respectively. Renal cortex kallikrein activity (RKa) was measured by colorimetric assay. After Gli treatment, the protein abundance of NCC in male animals increased in intact and diminished post-Gx (from 1.2±0.2 and 1.6±0.3 to 1.9±0.2 and 0.8±0.2, $p<0.05$; respectively), whereas α-ENaC diminished from 0.9±0.1 to 0.6±0.1 ($p<0.05$) in intact female group. Slc12a3 and Scnna mRNA levels were not different across the groups whereas Scnng diminished from 1.1±0.2 to 0.6±0.1 in intact female group. The RKa increased post-Gli (from 14±1 to 19±2 nKat/gKW, $p<0.05$) in intact male rats, a similar pattern previously found in klk1 mRNA levels (from 0.8±0.3 to 2.3±0.5) without changes in UKa. The ROMK blockade showed a gonad-dependent release impairment of kallikrein into urine. The changes in NCC and ENaC would suggest a role in this phenomenon, involving the coupled K^+ recycling for distal Na^+ reabsorption in the KKS regulation.

699 (273)**SALT-SENSITIVE HYPERTENSION RESPONSE TO HORMONE REPLACEMENT IN ADULT OVARIECTOMIZED WISTAR RATS**

Luis A Di Ciano[1], Pablo J Azurmendi[1], Sandra G Vlachovsky[1], Gisele A Moirón[1], Alejandro F Celía[1], Elisabet M Oddo[1], Elvira E Arrizurieta[1,3], Claudia M Silberstein[1,2], Fernando R Ibarra[1,2].
[1]Instituto de Investigaciones Médicas A Lanari, Universidad de Buenos Aires, Buenos Aires, Argentina, [2]Universidad de Buenos Aires, Facultad de Medicina. Consejo Nacional de Investigaciones Científicas y Tecnológicas, Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO Houssay-Conicet), Departamento De Ciencias Fisiológicas. Buenos Aires, Argentina [3]Consejo Nacional de Investigaciones Científicas Y Técnicas

Ovariectomy in adult Wistar rats induces a decrease in the expression of D1-like dopamine receptors (D1DR), in Cyp450 4A (Cyp4A) expression and activity and an increase in the dephosphorylation of Na^+, K^+-ATPase (NKA Ser-23) in renal tissue. As a consequence, oVx rats, that otherwise have a normal mean arterial pressure (MAP), are unable to handle a sodium load (NaCL 1%) and develop sodium-sensitive hypertension. In this work we have studied whether estrogen or progesterone replacement can modify the response to HS intake in oVx rats. Accordingly, oVx rats were supplemented with pellets designed to be released over a 14-day period and received normal (NS) or HS intake. Under estrogen replacement rats were able to increase sodium excretion and to maintain a normal MAP ($p<0.01$ vs oVx rats). An increase in D1DR expression and in Cyp4A expression was observed in oVxE2 rats accompanied by a recovered phosphorylation of NKA-Ser 23 compared to oVxHS rats ($p<0.003$). Progesterone supplementation, on the other hand, did not restore sodium excretion or MAPregulation upon HS intake and it induced a strong phosphorylation in NKA-Ser 23 under both NS and HS intake. However, it did not change the expression of D1DR and Cyp4A, which remained low as in oVx rats, while a significant increase in NaCl cotransporter NCC was observed in oVxP4 rats. Neither estrogen/progesterone or NS/HS intake modified NKA mRNA expression. Findings suggest that besides a phosphorylated NKA Ser-23, an estrogenic environment is required to maintain an adequate renal circuit D1DR-Cyp4A-NKA Ser-23 which allows sodium excretion and normal regulation of MAP.

700 (334) **ALTERED RENAL EXPRESSION OF CAVEOLIN-2 IN RATS WITH OBSTRUCTIVE CHOLESTASIS.**

Evangelina Cecilia Nosetto[1], Romina Valeria Campagno[1], Adriana Mónica Torres[1], Anabel Brandoni[1].
[1]Area Farmacología. Facultad de Ciencias Bioquímicas Y Farmacéuticas. Universidad Nacional de Rosario. CONICET.

Caveolin 2 (Cav2) is a constitutive protein of caveolae, cholesterol and glycosphingolipid-rich invaginations of the plasma membrane. It could also participate as a vesicular transporter and a signalling regulator. Renal expression of Cav2 in pathological conditions, such as obstructive cholestasis remains poorly understood. The aim of this work was to evaluate the renal expression of Cav2 in rats with obstructive cholestasis by means of in *vivo*

and *in vitro* experimental models. Bile duct ligation of 21h (BDL n=4) was performed in Wistar rats. Sham-operated rats served as controls (S n=4). Renal homogenates (H) and basolateral membranes (M) were obtained. Moreover, isolated renal cells from control animals were incubated with S (n=5) or BDL (n=8) serum for 3h. All incubations were performed at 37°C with constant agitation and exposition to 95% O_2–5% CO_2. Cell homogenates (CH) and cell membranes (CM) were obtained from respective incubations. Cell viability was tested by Trypan Blue exclusion. Approximately 95% of the freshly isolated cells excluded the dye. Cell viability was maintained during 3h-period independently of the incubation achieved. Cav2 expression (%) was determined by immunoblotting. Mean±SEM. H: S=100±3 BDL=106±4; M: S=100±5 BDL=200±9*; CH: S=100±4 BDL=82±8; CM: S=100±5 BDL=145±11* (*p<0.05). Renal expression of Cav2 was higher in M from rats with obstructive cholestasis. An increase in CM was also observed in renal cells incubated with BDL serum. Some components present in serum from rat with obstructive cholestasis might be regulating the expression of Cav2 in M of renal cells. Redistribution towards M might be occurring as no difference was observed in Cav2 expression in H. Many cellular processes such as vesicular trafficking and signalling pathways among others are governed by caveolins. Then, changes observed in this work in the expression of Cav2 could be influencing various mechanisms in renal cells in the presence of obstructive cholestasis.

701 (455) **URINARY EXCRETION OF SODIUM-DICARBOXYLATE COTRANSPORTER 1 (NADC1) IN A PRECLINICAL MODEL OF OBSTRUCTIVE NEPHROPATHY.**

Romina Valeria Campagno[1], María Julia Severin[1], Anabel Brandoni[1], Adriana Mónica Torres[1].
[1]Area Farmacología. Facultad de Ciencias Bioquímicas y Farmacéuticas. Universidad Nacional de Rosario. CONICET.

Sodium-Dicarboxylate Cotransporter 1 (NaDC1) is expressed in the brush border membrane of proximal tubular cells. Its primary function is to reabsorb filtered Krebs Cycle intermediates, particularly citrate. Our laboratory has been pioneer in the urinary detection of NaDC1 in different models of renal dysfunction. The aim of this work was to assess the urinary excretion of NaDC1 ($NaDC1_U$) in male Wistar rats with obstructive nephropathy. Bilateral ureteral occlusion (B) was induced by ligation of both ureters for 1 h (B1, n=5), 2 h (B2, n=7), 5 h (B5, n=6) and 24 h (B24, n=6). The studies were performed after 24 h of ureteral releasing. Parallel to each group a Sham one (Sh, n=11) was processed. Urea (U_p) and creatinine (Cr_p) plasma levels were determined. The fractional excretion of water (FEH_2O) was calculated according to the classical formula. $NaDC1_U$ was evaluated by electrophoresis and Western blotting. Results: Mean ± SEM. Data were analyzed with ANOVA plus Newman Keuls P<0,05: (a) vs Sh, (b) vs B1, (c) vs B2, (d) vs B5, (e) vs B24. U_p(g/L): Sh=0.43±0.03, B1=0.56±0.02, B2=0.67±0.05, B5=0.77±0.08, B24=4.32±0.32^{a,b,c,d}; Cr_p(mg/L): Sh=5.73±0.16, B1=5.73±0.19, B2=7.47±0.46, B5=7.95±0.56, B24=41.54±3.54^{a,b,c,d}; FEH_2O(%): Sh=0.48±0.04, B1=0.72±0.06, B2=0.79±0.06, B5=1.15±0.08, B24=11.70±2.88^{a,b,c,d};$NaDC1$(%): Sh=100±4, B1=294±6^{a}, B2=439±30^{a,b}, B5=603±25^{a,b,c}, B24=769±28^{a,b,c,d}. B24 animals displayed an increase in U_p and Cr_p accompanied by a remarkable increase in FE H_2O. $NaDC1_U$ increased in all experimental groups. Moreover, the transporter showed a significant urinary increase in B1, B2 and B5 groups compared to Sh group, in the absence of modifications of traditional parameters to evaluate the renal function. These preliminary results allow us to propose the urinary excretion of NaDC1 as a potential early biomarker of obstructive nephropathy.

702 **(703) RENAL DOPAMINERGIC SYSTEM DYSFUNCTION AND ITS ASSOCIATION WITH ARTERIAL HYPERTENSION AND RENAL INFLAMMATION IN AN EXPERIMENTAL MODEL OF METABOLIC SYNDROME INDUCED BY FRUCTOSE OVERLOAD**

Natalia Lucia Rukavina Mikusic[1,3,4], Nicolas Martin Kouyoumdzian[1,3,4], Maria Cecilia Kravetz[1], Edgardo Rouvier[1], Gabriel Robbesaul[1], Maria Alvarez Primo[1], Julieta Del Mauro[1], Hyun Jin Lee[1], Ana Maria Puyo[1], Jorge Toblli[2,4], Belisario Enrique Fernandez[1,3,4], Marcelo Roberto Choi[1,3,4].
[1]FFYB, UBA [2]Hospital Aleman [3]INFIBIOC [4]CONICET

Introduction: Renal dopaminergic system (RDS) promotes sodium excretion and anti-inflammatory actions. A high fructose diet induces metabolic and hemodynamic changes that could be associated with an impairment of the RDS, leading to renal inflammation, sodium retention and arterial hypertension. Aim: To evaluate RDS state and its association with the development of hypertension and overexpression of renal inflammatory markers in fructose overloaded (FO) rats. Methods: Male Sprague Dawley rats were assigned to Control (C, tap water) or FO (10% w/v of fructose solution) groups, during 4, 8 and 12 weeks (n=8/group/period). Urinary L-dopa and dopamine (DA), diuresis and albuminuria were determined. Systolic blood pressure (SBP) and metabolic parameters were measured. Western blot analysis of renal expression of D1R, NFκβ, IL-6, TNF-α, TGF-β1 and nephrin were performed. Results: FO increased SBP (mmHg, C4: 121±8 vs. F4: 145±1*; C8: 130±4 vs. F8: 161±10#; C12: 133±5 vs. F12: 163±4#), which positively correlated (R2=0.78; p<0.002) to urinary L-dopa/DA index (C4: 0.49±0.05 vs. F4: 1.9±0.09#; C8: 0.53±0.06 vs. F8: 2.35±0.1#; C12: 0.54±0.07 vs. F12: 2.57±0.2#). A significant decrease of D1R expression was accompanied by a significant increase in nFκβ, IL-6, TNF-α, TGF-β1 expression since week 4. Microalbuminuria (C12:13.11±1.4 vs F12:57.6±2.5#) and a significant decrease in nephrin expression (C12: 1.00±0.10 vs. F12: 0.73±0.05#) were only observed at week 12. (*p<0.05, #p<0.01 vs. C). Conclusion: FO was associated with an increased L-dopa/DA index and decreased D1R expression since week 4 of treatment. The RDS dysfunction was accompanied by an increase in blood pressure levels and renal expression of inflammatory markers in all experimental periods. Alteration of L-dopa/DA index could be postulated as an earlier marker of renal dysfunction, easy to detect before structural damage was evidenced by other markers as microalbuminuria and decreased nephrin expression

703 (572) **PLASMA COPEPTIN (CPP), A SURROGATE MARKER OF ARGININE VASOPRESSIN HORMONE (AVP), CORRELATES WITH TOTAL KIDNEY VOLUME (TKV) GROWTH AND GFR DECLINE IN ADPKD. A PRELIMINARY REPORT.**

Pablo Javier Azurmendi[1], Adriana R. Fraga[1,2], Alejandro F. Celía[1], María F. Martínez[1], Rodolfo S. Martin[1,2], Elisabet M. Oddo[1].
[1]Lab. Riñón Exp. y Bioquímica Molecular, IIM A Lanari, UBA [2]CONICET

ADPKD progression has been related with renal AVP-V2 receptor axis, since V2 receptor antagonists have proven to ameliorate TKV growth, and several clinical trials with specific treatment are already ongoing. However, the natural course of the AVP system in ADPKD has not been fully elucidated. Plasma levels of CPP - a product of cleavage of pre-AVP protein in pituitary gland - are more stable than AVP, hence is accepted as surrogate marker. In order to know the plasma and urine AVP (measured as CPP) behavior and their effect on ADPKD progression, we studied TKV, estimated GFR(eGFR), urine albumin/creatinine (UACR), urine MCP-1 and total protein (UTP),in a cohort of 14 (9 women/5 men) patients followed for 4 years from 29 ± 2 years old with normal renal function at baseline. The CPP and MCP-1 were measured by ELISA, UACR by immune-nephelometry and UTP by pirogalol red. TKV and eGFR were calculated byellipsoid and MDRD formulas, respectively. Plasma CPP was fully detectable and quantified (baseline: 1.35±0.16 ng/ml; final: 1.33±0.20). Female doubled male levels at both times (from 1.6±0.2 and 0.8±0.2 to 1.6±0.3 and0.9±0.1 ng/ml, p < 0.05; respectively) but a similar annual change (-0.04±0.05vs -0.02±0.04) was found. Urine CPP could not be evaluated, since 15/28 samples were detectable and 5 of them were below of quantification limit. During follow-up, individual annual changes in plasma CPP correlates directly with TKV (r= 0.60, p < 0.05) and inversely with eGFR (r= -0.57, p < 0.05),

whereas no correlation with urine MCP-1, UACR, UTP and age were found. The results showed that AVP increases while kidney grows and GFR fall in our cohort of young patients. CPP measurement would be useful to determine optimal time to start and stop treatment with V2 receptor antagonists. A sexual dimorphism in plasma CPP was also found, it meaning and impact in disease progression must be elucidated.

704 (883) **INTRARENAL RENIN ANGIOTENSIN SYSTEM COMPONENTS IN RESPONSE TO TEMPOL IN RATS FED WITH A HIGH SALT DIET**

Nicolas Martin Kouyoumdzian[1], Natalia Lucia Rukavina Mikusic[1], Gabriel Cao[1], Silvana Lorena Della Penna[1], Marcelo Roberto Choi[1], Susana Gorzalczany[2], Belisario Enrique Fernandez[1], Jorge Eduardo Toblli[1], Maria Ines Roson[1].
[1]Instituto de Investigaciones Cardiológicas ININCA, UBA-CONICET [2]Cátedra de Farmacología, FFYB, UBA

An imbalance between components of the renal renin-angiotensin system (RAS) has been suggested to be involved in the pathogenesis of hypertension, which in turn, is associated with renal oxidative stress. The aim of this study was to determine the antioxidant Tempol (T) effect on arterial pressure and renal RAS in rats fed with high-salt (HS) diet. Sprague-Dawley rats were divided in four groups (n=6/group): normal-salt (NS, 0.4% NaCl); HS (8% NaCl); NS-T; and HS-T. T was administered in the drinking water (1 mM). Mean arterial pressure (MAP), glomerular filtration rate (GFR), and urinary sodium excretion (UV_{Na}) were measured. Angiotensin II (Ang II), Angiotensin 1-7 (Ang 1-7), Angiotensin Converting Enzyme 2 (ACE2), Mas Receptor (MasR), Angiotensin Type 1 and 2 Receptors (AT1R and AT2R) expression were evaluated in renal tissues by immunohistochemistry. Immunostaining was evaluated by Image Pro Plus software analysis and expressed as integrated optical density (IOD) ± SEM in renal tissue. In HS group, MAP values (NS: 92±3 vs HS: 108±3, p<0.05) and expression of Ang II (NS: 1404±197 vs HS: 3424±146, p<0.01) and AT1R (p<0.01) increased, and ACE2 expression decreased (NS: 642±49 vs HS: 179±23, p<0.01). Antioxidant supplementation with T in HS group, increased natriuresis and GFR, prevented changes in blood pressure and the imbalance of renal RAS components: expression of Ang II (HS: 3424±146 vs HS-T: 1889±187, p<0.01) and AT1R (p<0.01) decreased, and expressions of AT2 (HS: 236±7 vs HS-T: 331±13, p<0.01), ACE2 (HS: 179±23 vs HS-T: 567±29, p<0.01), Ang 1-7 (HS: 686±41 vs HS-T: 5313±475, p<0.01) and MasR (HS: 435±78 vs HS-T: 1160±86, p<0.01) increased. These findings suggest that a HS diet alters the physiological balance between hypertensive and antihypertensive components of the renal RAS, favouring the renal expression of Ang II and the down-regulation of ACE2. Inhibition of oxidative stress by T prevented this imbalance and decreased blood pressure levels.

ENDOCRINOLOGÍA II/ ENDOCRINOLOGY II

705 (371) **LIVER PROLIFERATION IN MICE WITH DIABETES MELLITUS TYPE 1 (DM 1) STUDIED TEN WEEKS AFTER ADMINISTRATION OF CARCINOGEN DIETHYLNITROSAMINE (DEN)**

Ainelén Soledad Arboatti[1], Flavia Lambertucci[1,2], María Guillermina Sedlmeier[2], Gerardo Bruno Pisani[3], Juan Alberto Monti[1,2], Daniel Eleazar Antonio Francés[1,2], María Teresa Ronco[1,2], Cristina Ester Carnovale[1,2].
[1]Instituto de Fisiología Experimental (IFISE)-CONICET. [2]Cátedra de Fisiología, Facultad de Ciencias Bioquímicas y Farmacéuticas-UNR. [3]Cátedra de Morfología, Facultad de Ciencias Bioquímicas y Farmacéuticas-UNR.

DM1 is an autoimmune disease caused by selective destruction of b-pancreatic cells. Diabetes is associated with increased cancer risk. Previously our study showed that one injection of DEN (75 mg/kg bw, ip) promoted cell cycle without increasing the proliferation index (PI) after 4 weeks in diabetic mice treated with insulin. To analyze which proteins are altered in the early stage of initiation of hepatocarcinoma, in this study we analyze the proliferative process after 10 weeks of DEN. Four-week-old mice C57BL/6 were randomly divided into 4 experimental groups: Control (C, vehicle), C treated with DEN (C+DEN), DBT with insulin (DBT+I, diabetogenic agent Streptozotocin (STZ), 200 mg/kg bw, ip and I, 2 UI/day subcutaneously) and DBT+I+DEN animals that received STZ, DEN and I (n=4). Mice were euthanized at 10 week after DEN-injection. In liver sections we performed histological studies and quantification of hepatocytes in the different phases of the cell cycle (immunohistochemistry of PCNA). DBT+I+DEN showed an increase in hepatocytes in phases G1, S and M as well as PI compared to C (p<0.05). DBT+I+DEN showed an increase in hepatocytes in phase G1 (+155%), in phase S (+138%), in phase M (+78%), PI (hepatocytes in phase G1, S, G2 y M, +142%) (p<0.05 vs DBT+I) which would indicate a promotion in the cell cycle with an increase in phase M. We evaluated by Western blot, markers of cell cycle progression: cyclin (Cyc) D1 and E1. DBT+I+DEN showed an increase in Cyc E (+258%) showing highest expression in phase S and Cyc D1 (+128%). These results showed that the dose of DEN used induces cell cycle progression toward phase M. Vascular endothelial growth factor (VEGF), signaling protein produced by cells that stimulates angiogenesis, showed an increase in DBT+I+DEN (+199%).These results suggest that at 10 weeks after DEN administration, cell environment is modified and allowed as to analyze proteins that increase sensitivity cancer develop in the diabetic state..

706 (372) **GLUCOCORTICOID OR MINERALOCORTICOID RECEPTORS: ARE BOTH RECEPTORS INVOLVED IN THE GLUCOCORTICOID EFFECTS ON ADIPOCYTE PRECURSOR CELLS?**

Alejandra Paula Giordano[1], María Guillermina Zubiría[1], Andrea Estefanía Portales[1], Eduardo Spinedi[2], Andrés Giovambattista[1].
[1]Instituto Multidisciplinario de Biología Celular (IMBICE) CICPBA-CONICET-UNLP. [2]Centro de Endocrinología Experimental y Aplicada (CENEXA) CONICET-UNLP.

We previously demonstrated that dexamethasone (DXM) increases adipogenic capacity and number of adipocyte precursor cells (APCs) from retroperitoneal adipose tissue (RPAT). We now evaluated whether mineralocorticoid (MR) and/or glucocorticoid receptors (GR) mediate/s these DXM effects. For this aim, stromal vascular fraction cells isolated from RPAT from adult male rats, were cultured up to confluence and maintained in basal culture medium (CTR) or with DXM either alone (0.25 µM, DXM) or combined with a GR inhibitor (RU486 1µM, DXM-RU), or a MR inhibitor (Spironolactone 10µM, DXM-SP) or both (DXM-RU-SP) for 48 h. The expression of APCs competency markers (PPAR-γ2 and Zfp423), MR and GR were quantified (qPCR). Additionally, cell differentiation was induced and on differentiation day (Dd) 4, we determined the mRNA levels of PPAR-γ2 and C/EBPα (qPCR), and the percentage of PPAR-γ positive cells and its cell nuclear signal intensity (immunofluorescence). Cell lipid content (Oil-Red O) was measured on Dd 10. We found an increase in PPAR-γ2 expression in DXM treated APCs (P<0.05) that was only reverted by blocking both receptors (DXM-RU-SP, P<0.05 vs DXM). Zfp423, MR and GR gene expression did not change. Differentiated cells treated with DXM showed increased mRNA levels of C/EBPα (P<0.05 vs CTR), an effect suppressed by the DXM-RU-SP combination (P<0.05 vs DXM). No changes were found in PPAR-γ2 mRNA levels. Interestingly, the percentage of PPAR-γ positive cells and PPAR-γ nuclear intensity were higher in DXM cells (P<0.05 vs CTR), and both parameters decreased in DXM-RU-SP cells (P<0.05 vs DXM). Finally, differentiated DXM treated cells showed high adipocyte lipid content, which was reverted only in presence of both inhibitors simultaneously (P<0.05 vs DXM). We conclude that DXM effects on APCs may be driven through MR and GR, and the inhibition of both receptors is necessary for the complete abolishment of these effects.

707 (375) **PI3K IS INVOLVED IN THE 2-IODOHEXADECANAL INHIBITION OF SODIUM/IODIDE SYMPORTER GENE (NIS) EXPRESSION**

Leonardo Salvarredi[1], Romina Oglio[1], Luciano Rossich[1], Mario Pisarev[2,3], Lisa Thomasz[1, 2], Guillermo Juvenal[1,2].
[1]Comisión Nacional de Energía Atómica. [2]CONICET. [3]UBA.

Although thyroid gland function is mainly under the control of pituitary TSH, other factors, such as iodine, play a role in this process. The thyroid is capable of producing different iodolipids such as 2-iodohexadecanal (2-IHDA). It was shown that this iodolipid has an inhibitory effect over Na+/I- symporter (NIS) gene expression. On the other hand the activation of the insulin/phosphoinositide-3-kinase (PI3K) signaling pathway in TSH-stimulated thyroid cells reduces NIS expression at the transcriptional level. Objective: To evaluate if the activation of phosphoinositide-3-kinase (PI3K) signaling pathway is involved in 2-IHDA repression of NIS. Methodology and results: FRTL-5 cell lines were treated with 2-IHDA 10uM and PI3K activity was examined at different times by Western Blot, using a specific antibody that recognizes phosphorylated PKB/Akt. 2-IHDA increased PI3K activity 0.1, 0.5 and 1h later ($p<0.05$). When we determined Nis mRNA levels in cells treated with 2-IHDA for 24h an inhibitory effect over Nis expression was observed ($p<0.05$). If previously cell cultures were treated with the PI3K inhibitor LY294002, inhibition of PI3K blocked 2-IHDA repression of NIS mRNA. This effect takes place at the transcriptional level, as Ly inhibited 2-IHDA transcription repression of a luciferase reporter construct containing a 2.8-kb DNA fragment of the rat NIS promoter. These results demonstrate a central role for PI3K in the repression of Nis gene transcription by 2-IHDA and suggest the existence of putative PI3K-responsive elements

708 (392) **ONTOGENIC STUDY OF THE PITUITARY TGFB1 SYSTEM**

Alejandra Inés Abeledo Machado[1], María Andrea Camilletti[1], Erika Faraoni[1], Jimena Ferraris[2], Graciela Díaz-Torga[1].
[1]Instituto de Biología y Medicina Experimental, CONICET. [2]Instituto de Investigaciones Biomédicas, CONICET.

TGFβ1 is a known inhibitor of lactotroph cell proliferation and prolactin secretion. TGFβ1 bioavailability is tightly regulated by different components of the "TGFβ1 system" including latent binding proteins (LTBPs), local activators, and TGFβ receptors. Pituitary TGFβ1 activity is regulated by dopamine and estradiol. We previously demonstrated that estradiol inhibits most of the component of the pituitary TGFb1 system. The aim of the present work was to study the ontogeny of the pituitary TGFb1 system in males and females Sprague Dawley rats at different ages: 11, 23 and 45 days. Adult females were used in diestrus. ELISAs were performed to quantify total and active TGFβ1 content in pituitary homogenates. We evaluated the expression of the components of TGFβ1 system by qPCRs. Serum prolactin was assayed by RIA. We found increased expression of total TGFβ1, TβRII, ALK1, SMAD7 and the latent proteins LTBP1 and LTBP3 in 11d pups, compared to adults, without gender differences. On the other hand, active cytokine levels were found increased in 11d females compared to 11d males and to adults. It was concomitant with elevated TGFβ1 mRNA and increased cytokine biological activity, reflected in higher expression of TGFβ1 target genes, in this group. In parallel, serum prolactin levels were low during the first month of life and gradually increased toward puberty, in both male and female rats. It was concomitant with an age-related increase in the expression of estradiol receptor alpha. We postulated that the high expression and activity of the pituitary TGFb1 system found in 11d pups could be related to the immaturity of the negative control exercised on the pituitary TGFb1 by estradiol. As TGFb1 plays essential roles in morphogenesis, and in fully developed organisms is required for tissue homeostasis, the importance of the increased expression and activity of the pituitary TGFb1 system found in infantile rats, deserves more in-depth studies.

709 (452) **ESTRADIOL PLAYS A MAJOR ROLE ON MAMMARY GLAND STEM CELL HIERARCHIZATION TOGETHER WITH PROGESTERONE RECEPTOR ISOFORMS RATIO**

Maria Sol Recouvreux[1], Laura Todaro[1], Marina Simian[2].
[1]Instituto de Oncología Angel H. Roffo. [2]Instituto de Nanosistemas, Universidad Nacional de San Martín.

The ovarian steroid hormones estrogen (E) and progesterone (P) play vital roles in the development of the normal mammary gland (MG) and are likewise linked to mammary carcinogenesis via their receptors. Progesterone receptor (PR) is expressed as two isoforms, PRA and PRB. In human breast tumors the expression ratio of PRA/PRB has been found to be altered, and the overexpression of either isoform is suggested to have distinct clinical implications. PR signaling plays a major role in the maintenance of the stem cell population in the MG. However, the role of each isoform on the dynamics of this stem/progenitor cell hierarchy has not been unraveled. We propose that the altered PRA/B ratio affects the expansion, self-renewal, hierarchy and differentiation of MG stem cells. We previously showed that MGs derived from PRB mice present a higher percentage of stem cells ($p=0.003$), and increased mammosphere-forming capacity ($p=0.0016$) than those derived from WT and PRA. Also, we described that upon ovariectomy (OVX), there is an increment in stem cells and mammosphere forming capacity only in MGs of WT and PRA mice. Surprisingly, we found that the morphology of mammospheres derived from WT, PRA and PRB MGs were different. PRA mammospheres are mostly solid, while PRB are mostly hollow, and WT show both types. Upon OVX, WT and PRA mammospheres become hollow, while there are no changes in PRB. This result indicates that ovarian hormones have a major role in the hierarchy of stem cell and their differentiation. Moreover, antiestrogen treatment (ICI 182780) led to an increase in mammosphere forming capacity ($p<0,001$) and these mammospheres were hollow both in WT and PRA compared to controls, while E treatment led to solid mammospheres. Our results suggest that the PRA/PR B ratio regulates stem cell self-renewal, expansion, hierarchization and differentation upon exposure to E and P. We propose, also, that overexpression of PRB leads to resistance to hormonal treatments.

710 (459) **REGULATION OF NADPH OXIDASE NOX4 BY IL-DELTA IN THYROID TUMOR CANCER CELL LINES.**

Lisa Thomasz[1, 2], Romina Oglio[1], Leonardo Salvarredi[1], Luciano Rossich[1], Marina Perona[1], Mario Pisarev[1, 2], Guillermo Juvenal[1, 2].
[1]Comisión Nacional de Energía Atómica (CNEA). [2]CONICET

Iodine is not used only by the thyroid to synthesize thyroid hormones but also directly influences a number of thyroid parameters such as thyroid proliferation and function. Several iodinated lipids, biosynthesized by the thyroid, were postulated as intermediaries in the action of iodide. Among these, iodolactone (the IL-δ) and 2-iodohexadecanal (2-IHDA) have shown to inhibit several thyroid parameters. The antiproliferative effect of the IL-δ is not restricted to the thyroid gland. Since IL-δ has anti-tumor properties in breast cancer, neuroblastoma, glioblastoma, melanoma, lung carcinoma cells and colon cancer cell line. IL-δ induced apoptosis in a colon cancer cell line (HT-29) mediated by ROS generation. The aim of the present work was to study the contribution of ROS induced by IL-δ on proliferation and apoptosis in thyroid tumor cancer cell lines, and to analyze the sources of reactive oxygen species leading to apoptosis. Methodology and results: Cancer thyroid follicular (WRO) and papilar (TPC1) cells lines were treated with IL-δ. Proliferation and apoptosis was analyzed. IL-δ caused a significant loss of cell viability on WRO and TPC1 cells in a concentration dependent manner and induced apoptosis after 3 h of treatment. Furthermore, IL-δ (10 μM) increased ROS production (140% WRO and 130% TPC). To analyze the contribution of ROS on IL-δ induced apoptosis, cells were treated with IL-δ plus Trolox 100 μM and NAC 2.5 mM (radical scavengers). Trolox and NAC impeded IL-δ induced caspase-3 activity. Only in the WRO cell line IL-δ upregulates NADPH oxidase Nox4 (1.7 after 1 h; 2.3 after 3 h of treatment). It correlates with its pro-apoptotic effect. siRNA targeted knock-down of Nox4 attenuates ROS production and apoptosis ($p<0.05$) in WRO treated cells. Conclusions: These re-

sults show that the up-regulation of Nox4 by IL-δ is required for its pro-apoptotic effect in the thyroid cancer follicular cell line, WRO.

711 (487) **ANDROGEN-DRIVEN BONE MORPHOGENETIC PROTEINS (BMPS) DOWNREGULATION IN DERMAL PAPILLA CELLS DISRUPTS HAIR FOLLICLE STEM CELLS DIFFERENTIATION.**

Julieta María Ceruti[1], Valeria Yael Krum[1], Gabriela Kusinsky[1], Gustavo Leirós[1], María Eugenia Balañá[1].
[1]Instituto de Ciencia y Tecnología Dr. César Milstein – CONICET.

During androgenetic alopecia (AGA) androgens cause hair follicle (HF) miniaturization and baldness through mechanisms which remain unclear. HF formation begins when signals from the mesenchyme-derived dermal papilla cells (DPC) reach multipotent epidermal stem cells in the bulge region (HFSC). The HF bulb microenvironment is rich in bone morphogenetic proteins (BMPs) that act on DPC to maintain key signature features in vitro and hair-inducing activity in vivo. DPC cultured as monolayer (ML) loss their hair inductive capacity with passages, however, when DPC are cultured as spheroids (DPC-Sph), show an appropriate cellular phenotype and restore inductive properties. We previously reported that androgens abrogate DPC-induced hair follicle differentiation suggesting that androgens deregulate DPC-secreted factors involved in normal HFSC differentiation. The aim of this work was to evaluate if BMPs are involved in DPC-induced HFSC differentiation. BMP-2 and BMP-4 mRNA expression was evaluated in androgen responsive DPC cultured as ML or as DPC-Sph. In DPC-Sph, dihydrotestosterone (DHT) significantly downregulated the expression of both BMP-2 and BMP-4 mRNA. However, only BMP-2 was downregulated in ML. When comparing their basal expression level, a significant higher amount of both BMPs was detected in DPC-Sph. We then used conditioned media from DPC-Sph to induce HFSC hair-linage differentiation. When these media was conditioned in presence of DHT, HFSC-differentiation was impaired. When BMP-2 or BMP-4 was added to conditioned media from DPC-Sph cultured in presence of DHT, these media recovered the ability to differentiate HFSC. These results suggest that BMPs can overcome the effect of DHT on the inhibition of HFSC differentiation. We conclude that BMPs are critical factors of the complex epithelial–mesenchymal cross-talk deregulated by androgens during AGA and are necessary to induce HFSC differentiation

712 (510) **CASE-CONTROL STUDY ON LIFETIME HORMONE EXPOSURE IN AMYOTROPHIC LATERAL SCLEROSIS WOMEN**

Gisella Gargiulo Monachelli[1,2], Mariela Bettini[3], Ignacio Mele[2], Mirtha Amores[2], Agustina Lara[1], Alejandro Federico De Nicola[1], Maria Claudia Gonzalez Deniselle[1, 4].
[1]Instituto de Biología y Medicina Experimental. [2]Servicio de Neurología, Hospital JA Fernandez. [3]Servicio de Neurología, Hospital Italiano de Buenos Aires. 4Dpto de Ciencias Fisiologicas, FMED, UBA.

Numerous epidemiological studies have shown a higher incidence of ALS in men than in women, which could denote a possible protective effect of female hormones, or a negative effect of androgens. We studied this through a questionnaire to ALS female patients and healthy female controls. Female ALS patients (18-90 years) and age-matched healthy controls without neurological conditions were selected. We questioned age of menarche and menopause, use of oral contraceptives (OC) and its duration, number of pregnancies, spontaneous abortions, whether or not they breastfed, and the presence of acne, hirsutism and/or ovarian cysts. Body mass index (BMI) was calculated in women prior to the onset of symptoms/diagnosis and in controls at the time of the questionnaire. Only in postmenopausal women of both groups we calculated the reproductive time-span (RT=menopause-menarche) and the lifetime estrogen exposure (EE=RT-[postovulatory time-(pregnancies+abortions+OC time)] in years. Fifty ALS and 50 controls with a mean age of 59±1.7 and 60±1.5 years, respectively, were analyzed. The age of menarche in ALS was higher than controls ($p<0.001$), with no difference in the age of menopause. Forty-six percent of ALS women used OC, versus 28% of controls ($p=0.06$). BMI was slightly lower in ALS women (p=NS). There was a higher incidence of hirsutism (11.4% vs 0%, $p<0.001$) and ovarian cysts (16.3% vs 10%, p=NS) in ALS women. No significant difference was found between the number of pregnancies, abortions or the possibility of breastfeeding between the two groups. The RT was lower in ALS patients than controls ($p=0.001$), whereas the EE was without change. The data analysis revealed: a later age of menarche, greater exposure to OC (possibly containing progestins with partial androgenic effect) shorter RT and higher incidence of hirsutism/ovarian cysts in ALS women compared to controls. These findings suggest that a greater exposure to androgenic steroids could be a risk factor for ALS.

713 (564) **IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF FOUR NOVEL MUTATIONS IN THE THYROID HORMONE RECEPTOR BETA GENE RESPONSIBLE FOR RESISTANCE TO THYROID HORMONE**

María Cecilia Olcese[1,2], Ezequiela Adrover[1,2], Agustina Aguinaga[1,2], Maricel Molina[1,2], Ana Chiesa[3], Mirta B. Miras[4], Rocio Bracamonte[5], Mónica Bre[6], Héctor M. Targovnik[1,2], Carina M. Rivolta[1,2].
[1]Instituto de Inmunología, Genética y Metabolismo (INIGEM), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) y Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina. [2]Cátedra de Genética, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina. [3]Centro de Investigaciones Endocrinológicas, CEDIE-CONICET, División Endocrinología, Hospital de Niños "Ricardo Gutiérrez", Buenos Aires, Argentina. [4]Servicio de Endocrinología, Hospital de Niños "Santísima Trinidad", Córdoba, Argentina. [5]Servicio de Endocrinología, Hospital de Clínicas, Córdoba, Argentina. 6División Endocrinología, Hospital General de Niños "Pedro de Elizalde", Buenos Aires, Argentina.

Resistance to thyroid hormone (RTH), usually due to heterozygous mutations in thyroid hormone receptor beta (TRbeta) gene, is characterized by raised T4 and T3 levels, nonsuppressed TSH, and a variable phenotype encompassing both hyperthyroid and hypothyroid features. Since 1969 when RTH was first reported, more than 3000 cases and over 125 mutations have been identified. The incidence is estimated to be 1 in 40,000. 6 unrelated argentinian families with clinical evidences of RTH were studied. In order to identify mutations causing this pathology, genomic DNA was isolated from blood cells and the exons 7-10 of the TRbeta gene, including the flanking intronic regions were amplified by PCR. DNA sequences from each amplified fragment were performed with the Taq polymerase-based chain terminator method and using the specific forward and reverse TRbeta primers. Direct sequence analysis revealed 2 novel missense mutations in exon 9: c.917A>C transvertion that results in a p.K306T substitution and c.1012C>G transition causing a p.R338L change and two known missense mutations: c.959G>A; p.R320H and c.1378G>A; p.E460K. A novel mutation in exon 10: c.1304A>C transvertion that results in a p.H435P substitution was identified too. In silico studies were performed to elucidate a correlation between structural disturbances and putative functional commitment, achieving a possible explanation of the pathogenic mechanism of the novel missense mutations analysed. All new substitutions are located in positions evolutionarily conserved and modify the structure of the TRbeta. Due to errors in diagnosis, patients may be inappropriately treated with anti-thyroid drugs for a long period of time or suffer thyroid ablation. Molecular analysis is essential for the diagnosis and treatment of this patients group as well as to increase our understanding of the pathophysiology of the thyroid gland.

714 (663) **THE THYROID STATUS MODULATES THE 4T1 BREAST CANCER DEVELOPMENT IN VIVO THROUGH THE REGULATION OF CELL PROLIFERATION, THE**

COMPOSITION OF TUMOR MICROENVIRONMENT AND THE ANTITUMOR IMMUNE RESPONSE

Helena Andrea Sterle[1], Alejandra Paulazo[1], Mariana Amorós[2], Ximena Hildebrandt[1], Mariana García[3], Marcela Bolontrade[2], Graciela Alicia Cremaschi[1,4].

[1]Instituto de Investigaciones Biomédicas (BIOMED) - UCA – CONICET. [2]Instituto de Biología y Medicina Experimental (IBYME). [3]Laboratorio de Terapia Génica, Facultad de Ciencias Biomédicas, Universidad Austral. [4]Laboratorio de Radioisótopos, Facultad de Farmacia y Bioquímica, UBA.

The tumor development is regulated by a wide range of factors, including hormones produced by the endocrine system. However, very little is known about the influence of thyroid hormone (TH) levels on tumor growth. Previously we showed that THs are able to regulate the antitumor immune response. Our aim was to evaluate the effect of thyroid status on the development of 4T1 breast carcinoma in syngeneic mice. For this, Balb/c mice were treated with thyroxine (12mg/l) for 30 days or propylthiouracil (500mg/l) for 15 days in the drinking water to obtain hyperthyroid (hyper) or hypothyroid (hypo) mice, respectively. Mice were then inoculated s.c with 4T1 cells.Hyper mice showed an increased tumor growth rate ($p<0.05$) compared to controls, while hypo mice bared tumors with reduced volume ($p<0.05$). The number of infiltrating immune cells was similar in all groups. However, hyper tumors showed a higher percentage of CD3+ T cells ($p<0.05$) and decreased MDSC ($p<0.05$). Additionally, tumor draining lymph nodes of hypo mice presented increased CD4+ ($p<0.05$) and CD8+ ($p<0.05$) T cells percentages and decreased CD19+ cells ($p<0.05$). On the other hand, spleens of hyper mice showed increased percentages of MDSC ($p<0.05$) and NK cells ($p<0.05$) even though the NK activity was decreased ($p<0.05$) in this group.The composition of the tumor microenvironment is also crucial to determine de tumor progression. To evaluate the effect of thyroid status on the migration of mesenchymal stem cells (MSC) cells to 4T1 tumors, MSC were obtained from murine bone marrow, stained with DiR, inoculated in the tail vein of tumor-bearing mice and analyzed by in vivo imaging.The results showed a decreased presence of MSC in hyper tumors compared to control and hypo mice ($p<0.05$).Our results suggest that the thyroid status modulates not only the proliferation of 4T1 cells but also the migration of MSC to the tumors and the antitumor immune responses thus modifying tumor growth.

715 (665) **HIGH AROMATASE (ARO) TRANSCRIPT VARIANT EXPRESSION IN HUMAN PLACENTAL TISSUES FROM PRETERM DELIVERIES AND TERM DELIVERIES OF LARGE FOR GESTATIONAL AGE (LGA) NEWBORNS**

Alan Carballo[1], Dolores Polop[1], Andrea Servian[1], Cristina Patricia Nemer[3], Claudia Cannizzaro[3], Paula Aliberti[2], Romina Sainz[1], Marco Aurelio Rivarola[2], Alicia Belgorosky[2], Nora Saraco[2].

[1]Servicio de Endocrinología, Hospital de pediatría "JP Garrahan", Buenos Aires. [2]Servicio de Endocrinología, Hospital de pediatría "JP Garrahan", Buenos Aires, CONICET. [3]Programa de Diagnóstico y Tratamiento Fetal, Hospital de pediatría "JP Garrahan" y Hospital Materno Infantil Ramón Sardá, Buenos Aires.

Aro is the key enzyme for estrogen biosynthesis from androgens and in human placenta (Pl) is expressed exclusively in syncytiotrophoblast. It has been reported that small newborns and large newborns as well as patients with Aro deficiency tend to increase the prevalence of metabolic syndrome in adulthood. We previously described a splicing variant of Aro mRNA (Intron9) that translates into inactive Aro protein. Our aim was to analyze Aro mRNA variants expression in Pl from preterm (PT) (<35 weeks) and term LGA compared to term adequate for gestational age (AGA) newborns. We proposed that Aro mRNA variants expression is involved in Aro activity regulation and hence in intrauterine estrogen-androgen balance. Total RNA was isolated from Pl of PT (GA: 30-35,n=4), LGA (GA: 39-41,n=8) and AGA (GA: 37-38 and 39-41,n=8 and n=11). Aro mRNA variants were analyzed by Real-time RT-PCR with primers for total (TotAro, Ex2-Ex3), intron 9 (IN9, Ex8-In9) and active (ActAro, Ex9-Ex10) Aro, and Cyclophilin (PPIA) as housekeeping gene. Statistics (Student test) were performed on ΔCt data.TotAro was higher in PT vs AGA (8.91±3.35 vs 1.58±0.40 AU,mean ± SE),while was lower in LGA vs AGA (0.81±0.36 vs 2.12±0.64), $p<0.05$. Analysis of each transcript variant related to total Aro showed that ActAro/TotAro ratio was higher in PT (2.26±0.26 vs AGA: 0.66±0.20) and in LGA (2.42±0.31 vs AGA: 1.37 ± 0.28), $p<0.05$. Not significant difference was found for IN9/TotAro in LGA compare to AGA. The high Active Aro mRNA expression, in preterm placentas agrees with reports of maternal salivary estriol and plasma estradiol increments in preterm parturition suggesting a role of Pl Aro modulating Pl estrogen production associated to prematurity. In addition, the higher ActAro/TotAro ratio observed in LGA vs AGA, suggest that the variation of the estrogen-androgen balance in Pl tissue might be involved not only in prematurity but also in fetal programming determining disorders later on the postnatal life.

716 (706) **ZINC DEFICIENCY ACCOMPANIES HYPOTHYROIDISM IN MICE ALTERING PROLIFERATION AND INTRACELLULAR SIGNALING. ZINC SUPPLEMENTATION REVERSES IMMUNOSUPPRESSION IN HYPOTHYROID MICE.**

Maria Alejandra Paulazo[1], Helena Andrea Sterle[1], Maria Laura Barreiro Arcos[1], Maria Celeste Diaz Flaque[1], Maria Florencia Cayrol[1], Graciela Alicia Cremaschi[1,2], Alicia Juana Klecha[1],[2].

[1]Instituto de Investigaciones Biomédicas BIOMED-UCA-CONICET.[2]Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires.

Zinc (Zn) is essential for all highly proliferating cells, especially those of the immune system. Zn deficiency (ZnD) affects the functioning of many proteins crucial for intracellular signals in immunocompetent cells. Furthermore, it is required for optimal activity of many hormones, including thyroid hormones (TH). Also, it was demonstrated that hypothyroidism leads to immunosuppression, but alterations of Zn levels were not studied. Our aim was to evaluate the effect of ZnD on lymphocyte function both in vitro and in vivo, and its impact in hypothyroid status. Reversion of these effects by Zn supplementation was studied as well. For in vitro assays, cells were cultured in the absence or presence of specific intra-(TPEN) or extracellular (DTPA) Zn chelators. ZnD in vivo was evaluated in lymphocytes from mice fed a reduced Zn diet and hypothyroidism by propylthiouracil treatment. Both Zn chelators significantly inhibited proliferative responses of T cells, stimulated with the T cell-mitogen ConA. To ascertain the induction of apoptosis, caspase-3 activity, cell binding of annexin V / propidium iodide, nuclear condensation and DNA fragmentation were evaluated. In all cases, a remarkable induction of apoptosis was observed in cells treated with chelators ($p<0.05$). Also, Zn chelators inhibited ERK phosphorylation induced with ConA ($p<0.05$). All these effects were reverted with the addition of Zn ($p<0.01$). Similar effects were observed on T cells from ZnD mice. Hypothyroid mice showed decreased T lymphocyte activity, together with low levels of circulating TH and decreased lymph node and femur Zn levels. Zn supplementation was not able to reverse the hormonal levels, although it reversed T cell function impairment. Our results showed that ZnD lead to altered lymphoid intracellular signaling thus affecting cell proliferation and contributing to the immunosuppression of hypothyroid conditions. Zn supplementation would be a complementary treatment for hypothyroid patients.

717 (791) **INTERACTION BETWEEN THE NUCLEAR FACTOR KAPPA B (NF-KB) AND THE ESTROGEN RECEPTOR ALPHA (ERA) IN THE CELLULAR SENESCENCE PROCESS DURING THE DEVELOPMENT OF ESTROGEN-INDUCED PITUITARY TUMORS**

Bethania Mongi-Bragato[1], Ezequiel Grondona[1], Liliana del Valle Sosa[1], Lucía Carreño[1], Juan Pablo Petiti[1], Silvina Gutiérrez[1], Alicia Torres[1], Ana Lucía De Paul[1].

[1]Centro de Microscopía Electrónica, Instituto de Investigaciones en Ciencias de la Salud (INICSA-CONICET)

Facultad de Ciencias Médicas, Universidad Nacional de Córdoba.

Evidences from our group demonstrated the emergence of cellular senescence process as a growth control mechanism during the progression of estrogen-induced pituitary tumors. Also, estrogen exerts a modulatory action on pituitary cells proliferation. Considering the preponderant role of NF-kB (p65) in cellular senescence and points of convergence between ERa and NF-kB signaling pathways in cell cycle control, we evaluate the contribution of the interaction between these two proteins in the pituitary senescence in experimental pituitary tumors. Wistar adult male rats were implanted subcutaneously with silastic capsules containing estradiol benzoate (30mg) for 10, 20, 40 and 60 days (E10-60). The control group was implanted with empty capsules. Subsequently, ERa:NF-kB immunoprecipitation was performed at the different stages of tumoral development. NF-kB and IkBa levels were also determined from nuclear and cytosolic fractions by Western blot. The ERa:NF-kB co-localization was analyzed by immunofluorescence (IF) and transmission electron microscopy (TEM). Statistical analysis: ANOVA-Fischer test ($p<0.05$). During the course of estrogen-induced pituitary tumoral development, a significant ERa:NF-kB association was detected, with a marked interaction at E10 and E60, result that was corroborated by IF and TEM. Also, a significant increase in NF-kB and IkBa protein levels in the cytosolic compartment was detected. Interestingly, a substantial increased in the NF-kB nuclear levels was evidenced at E20 and E40 compared to those observed at E10 and E60. Probably, ERa recruits NF-kB at the cytoplasmic compartment in order to inhibit their function as a transcription factor and thereby modulate cell senescence-associated molecular mechanisms during the progression of the experimental pituitary tumor. These results suggest a cross-talk between NF-kB and ERa signaling pathway that may lead to the emergence of cellular senescence, thus contributing to the control of the cell growth.

718 (813) **SIGNS OF ALTERATIONS IN THE MITOCHONDRIAL DYNAMIC AND OXIDATIVE STRESS IN THE SENESCENCE PROCESS DURING THE DEVELOPMENT OF ESTROGEN-INDUCED PITUITARY TUMORS**

Ezequiel Grondona[1], María Eugenia Sabatino[1], Bethania Mongi Bragato[1], Lucía Carreño[1], Liliana Sosa[1], Juan Pablo Petiti[1], Silvina Gutiérrez[1], Alicia Torres[1], Alexandra Latini[2], Ana Lucía De Paul[1].

[1]Centro de Microscopía Electrónica, Instituto de Investigaciones en Ciencias de la Salud (INICSA-CONISET), Facultad de Ciencias Médicas, Universidad Nacional de Córdoba. [2]Laboratorio de Bioenergética y Estrés Oxidativo, Centro de Ciencias Biológicas, Universidad Federal de Santa Catarina, Florianópolis, Brasil.

Evidence of cellular senescence process during in vivo estrogen-induced pituitary tumor development was recently described in our laboratory. Since mitochondrial metabolism and dynamic are targets of estrogen action and senescence is considered a stress response triggered by different factors including oxidative stress; we evaluate the effects of estrogen in vivo on mitochondrial function and dynamics in experimentally

induced proliferative lesions. To induce pituitary tumoral development, Wistar male rats were exposed to estradiol benzoate (30mg) implanted subcutaneously in silastic capsules for 10, 20, 40 and 60 days (E10-60). Control group: animals treated with empty capsules. The morphological and morphometric mitochondrial analysis was evaluated by transmission electron microscopy; ROS production and mitochondrial membrane potential was determined by flow cytometry. Mfn1, Mfn2, OPA-1, Drp1; 8OHdG and Nrf2 protein expression were assessed by immunohistochemistry and western blot. Statistical analysis: ANOVA-Fischer test ($p<0.05$). Increases in mitochondrial number accompanied by a circular and less elongated morphology was observed at E10. The gradual increase of mitochondrial fusion proteins expression: Mfn1, Mfn2 and OPA-1 and the reduction of Drp1 fission protein levels, suggested the prevalence toward the mitochondrial fusion. A significant increase in ROS production and changes in mitochondrial membrane polarity, were signs of oxidative stress. The increase of nuclear 8OHdG expression at the beginning of tumoral development and increases in Nrf2 levels revealed the activation of defense mechanisms against the estrogen-induced proliferative injury. These data suggest that alterations in the mitochondrial dynamic and oxidative stress detected in early stages of estrogen-induced pituitary tumor development could be responsible for the emergence of senescence as a regulatory mechanism of cellular growth.

719 (881) **DEHIDROEPIANDROSTENEDIONE MODULATES CELLULAR EVENTS INVOLVED IN VASCULAR REPAIR**

Adrián Esteban Campelo[1], Virginia Massheimer[1].

[1]Instituto de Ciencias Biológicas y Biomédicas del Sur (INIBIOSUR), Dpto BByF Universidad Nacional del Sur.

In recent years dehidroepiandrostenedione (DHEA) has emerged as a promising alternative for hormone replacement therapy due to its ability to act as a precursor for local formation of active steroids. Maintenance of vascular health depends mainly on the prevention of vascular injury and the promotion of vessel remodeling (angiogenesis). Endothelial cells (EC) migration and proliferation, and the expression of endothelial factors that enhance EC adhesion to subendothelium (uPA and tPA) are crucial events in new vessel formation. In this study we evaluated the effects of DHEA on processes involved in the initiation of vascular lesions (platelet adhesion and aggregation) and in angiogenesis. We demonstrated that EC treatment with 20nM DHEA produces an inhibition on platelet adhesion to endothelium (24h - 25%below Cont $p<0.05$), and decreases endothelium dependent platelet aggregation (60min - 15%below cont $p<0.05$) in a nitric oxide dependent manner, since preincubation with NAME annulated this effect ($p<0.01$). EC proliferation studies (MTT assay) showed that 24h treatment with DHEA stimulates cell growth (32, 22 and 12% above Cont 2, 20 and 200nM DHEA $p<0.05$). Indeed, using wound healing assays, we found that the steroid also promotes cell motility (9±2, 25±8 Cont, 20nM DHEA migrating cells/field $p<0.01$). The expression of uPA, tPA and androgen receptor (AR) was measured by inmunoblot. To that end, EC were treated for 12 to 48h with 20 or 200nM DHEA. The steroid enhances the expression of both factors (30-80% above control $p<0.05$). The androgen receptor expression was also increased, suggesting that DHEA mechanism of action could involve AR. Finally, in rat aortic ring angiogenesis assays, we observed that DHEA treatment promotes EC sprouting and capillary like tube formation (30% above control, $p<0.05$). The presented results show that DHEA exerts a direct action on EC, contributing to the prevention of vascular injury and promoting angiogenesis

720 (897) **IMPORTANCE OF HORMONAL OVARIAN FOLLICULAR FLUID LEVELS IN AN ASSISTED FERTILIZATION PROGRAM: ROLE OF THYROID HORMONES AND ESTRADIOL**

Monica Rosales[1], Andrea Abdala[2], Lucio Ratto[2], Darío Jacobsen[1], Mariel Cano[1], Patricia Maidana[1], Myriam Nuñez[3], Diego Lange[2], Javier Singla[2], Ernesto Gomez Passanante[2], Sergio Provenzano[2], Viviana Mesch[1], Gabriela Mendeluk[1],

[1]Dpto. Bioquímica Clínica–INFIBIOC, Facultad de Farmacia y Bioquímica-UBA. [2] Div.Ginecología, Hospital de Clínicas-UBA. [3]Cátedra de Matemática, Facultad de Farmacia y Bioquímica-UBA.

Follicular fluid (FF) is the microenvironment in which the follicles develop and the oocytes mature. Hormonal composition influences oocyte quality and maturity, main parameters for assisted fertilization outcome. Thyroid hormones in the FF would have a positive role during folliculogenesis and ovulation.

Aim: to analyze the role of thyroid hormone and estradiol in the FF in relation to oocyte maturation rate (OMR) in women recruited for assisted fertilization procedure.

Subjects and methods: 51 women (29 to 42 years) without autoimmunity or medication affecting thyroid function were evaluated after a controlled ovarian stimulation protocol. In the remnant FF

after oocyte search estradiol (E2f) was determined by electrochemiluminesce; T3 (T3f), T4 (T4f), TSH (TSHf) and free T4 (T4Lf) by chemiluminescence. All oocytes retrieved were evaluated to analyze the complex clusters-coronary expansion degree and the oocytes maturational stage. The OMR was calculated as: Number of metaphase II oocytes / Number of oocytes retrieved x100. A logistic regression model was applied to determine whether a relationship exists between OMR and independent variables: T3f, T4f, TSHf, T4Lf and E2f. The response variable was coded considering an OMR cut-off value $\geq$ 60.

Results: hormone levels in FF were: T3f: 99.1 ± 29.7 ng/dl, T4f 6.9 ± 2.42 ug/dl, TSHf 1.4 ± 0 6 mIU/ml, T4Lf: 1.2 ± 0.2 ng/dl and E2f: 344.8 (43.1 to 832.2) pg/ml. The OMR was 66 (57-74) %. The results are expressed as mean ± SD or median (range) according to data distribution. No relationship between OMR and T4f, TSHf and T4Lf was found, while the OR (95 % CI) for T3f and E2f were: T3f : 0.977 (0.954 to 1.001), p = 0.057; E2f : 1.002 (1.000 to 1.005), p = 0.091.

Conclusion: we found that T3f and E2f should influence the OMR, supporting the facilitating role of thyroid hormones in oocyte maturation. Prospective studies with larger number of patients should be conducted to verify these results.

ONCOLOGÍA IV / ONCOLOGY IV

721 (548) **CONDITIONED MEDIA FROM NORMAL MAMMARY CELLS INDUCE MESENCHYMAL CHANGES IN BREAST CANCER CELLS. EFFECT OF IONIZING RADIATION**

Guadalupe Vedoya[1], Nora Mohamad[1], Tamara Galarza[1], Mónica Táquez Delgado[1], Graciela Cricco[1], Gabriela Martín[1,2].

[1]Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Laboratorio de radioisótopos. [2]CONICET

Conventional radiation therapy for breast cancer is delivered in fractionated doses of 2 Gy post-mastectomy or post conservative surgery. Experimental evidence suggests that ionizing radiation may promote migration and invasion of tumor surviving cells by intricate implications in the microenvironment and induction of epithelial mesenchymal transition (EMT). We hypothesize that communication between benign epithelial and neoplastic cells included in the irradiated target volume may affect radiotherapy efficacy affecting local recurrences and metastasis. MCF-10A cells were used as a model of benign breast cells, while MDA-MB-231 and MCF-7 as breast cancer cells. MCF-10A cells were 2 Gy irradiated or not, incubated for 24 hours and then conditioned media were collected (2GyCM or CM). MDA-MB-231 and MCF-7 cells irradiated (I) or not (NI) were incubated with 2GyCM or CM to evaluate clonogenicity, migration, gelatinolytic activity and EMT molecular markers. Results showed that CM and 2GyCM increased clonogenic growth in I and NI MDA-MB-231 (p< 00.1) and MCF-7 cells (p<0.05). A significant rise in the expression of mesenchymal markers vimentin and slug was determined by western blot in both cell lines, I and NI, when incubated with CM (p<0.05 vs I and NI) and 2GyCM (p<0.01 vs I and NI). A drop in the expression of the epithelial marker E-cadherin by immunoblot was also observed in MCF-7 cells in the same experimental conditions. MMP9 gelatinolytic activity evaluated by zymography was also enhanced by CM and 2GyCM in both tumor cells lines, I and NI. The most significant increase was observed when 2GyCM was employed (p <0.01 vs I and NI controls).The same results were evidenced in migration studies. Our findings show that breast tumor cells (I and NI) acquire increased mesenchymal features related to invasion when exposed to CM and 2GyCM from normal cells signaling the relevance of tumor-host interaction in tumor behavior and in the response to radiotherapy.

722 (563) **KLF4 EXPRESSION PATTERN IN RELATION TO PROLIFERATION AND DIFFERENTIATION MARKERS IN TONGUE CARCINOMAS**

María L. Paparella[1], M. Eugenia Cozzarin[2], Omar A. Coso[2], Ana R. Raimondi[2]

[1]Cátedra de Anatomía Patológica, Facultad de Odontología, Universidad de Buenos Aires [2]Instituto de Fisiología, Biología Molecular y Neurociencias, IFIBYNE UBA, CONICET.

Oral squamous cell carcinoma (OSCC) is among the most prevalent cancers in the world and is characterized by high morbidity and few therapeutic options. Phenotypic alterations of carcinoma cells progress OSCC to the more advanced stages, and the lack of differentiation is a main features of poorly differentiated OSCC. Krüppel-like factor 4 (KLF4) is highly expressed in differentiated postmitotic epithelial cells. Previously, we have reported expression of Klf4 in murine tongue epithelium as well as epithelial dysplastic changes upon loss of Klf4. The aim of the study was to analyze the expression of KLF4 in OSCC and the involvement in proliferation and differentiation of OSCC cells. We examined the expression of KLF4, p53, Ki-67 and Keratin 14 (K14) in biopsies of tongue SCC (SCC, n= 15) and normal human oral mucosa (NHOM, n=4) by immunohistochemistry. We evaluated percentage and intensity of staining. We found expression of KLF4 restricted to intermediate and upper layers of the epithelium of the NHOM, however the supra-adjacent epithelium (SAE) to OSCC cells presented a generalized expression throughout the epithelium. The SCC presented a decrease in the labeling intensity compared with NHOM and SAE. The decrease was significant when compared SCC and SAE (2.0+/-0.2, 2.9+/-0.1 p<0.05 Wilcoxon Test). 60% (9/15) of the SCC presented Ki-67 expression (> 50% of positives cells) and 100% of the SAE showed Ki-67 labeling in the basal and upper layer. K14 was expressed in the basal layer of the epithelium of the NHOM. We observed changes in the pattern of K14 expression from localized (NHOM) to a generalized expression thorough epithelium in SAE and SCC. Finally, only 20 % of SCC showed p53 immunostaining. These results may reflect oral carcinoma cells progression through the dedifferentiation process and warrant further studies. The changes in KLF-4 expression between the SAE and SCC may represent different stages in oral carcinogenesis.

723 (583) **EFFECT OF DOXORUBICIN AND PACLITAXEL IN COMBINATION WITH THE BETA2 ADRENERGIC AGONIST SALBUTAMOL ON BREAST CANCER MDA-MB-231 CELL PROLIFERATION**

Martina Jabloñski[1], Ezequiel Rivero[1], Lucía Gargiulo[1], Ariana Bruzzone[2], Isabel Lüthy[1].

[1]Instituto de Biología y Medicina Experimental, [2]Instituto de Investigaciones Bioquímica Bahía Blanca INIBIBB–CONICET

We have previously described (SAIC 2015, abstract 008) that the beta2 adrenergic agonist salbutamol (Salb) inhibited cell migration, invasion and experimental metastases in MDA-MB-231 cells inoculated in NSG mice. Also (SAIC 2015, abstract 538) that beta2-adrenergic receptors are overexpressed in claudin-low tumors. The goal of the present investigation was to evaluate if Salb, which toxicity is low, could be repurposed for the treatment of breast cancer. For this, we evaluated the effect of co-incubation of salbutamol with chemotherapy agents to diminish their effective concentrations. Human breast cancer MDA-MB-231 cells were incubated for 2 days with DMEM-F12 medium with 2% Fetal Calf Serum in the presence or absence (control) of doxorubicin (Doxo, 100 pM, 1, 100 nM) or paclitaxel (Pacli, 10, 100 pM, or 1 nM) with or without Salb (10, 100 nM or 1 uM). Cell proliferation was performed by [3H]-thymidine incorporation and statistical analysis by two-way ANOVA. The effect of Doxo +/- Salb on cell proliferation was analyzed. As an example, 100 nM Salb alone inhibited 50.9 +/- 1.23 % cell proliferation. When incubated with Doxo 100 pM, the inhibition was 37.1 +/- 5.10 %, representing a 42% reduction with respect to Doxo alone. When analyzed by two-way ANOVA, the effect of both Doxo and Salb was highly significant (p<0.0001). Moreover, the interaction (indicating synergism) was also highly significant (p<0.0001). Similar results were obtained with paclitaxel. Salb 10 nM when co-incubated with Pacli 1 nM inhibited cell proliferation by 48 % with regard to Pacli alone. Again, by two ways ANOVA, the effect of Pacli and Salb was highly significant (p<0.0001) as well as their interaction (p<0.0001). These

results suggest that the concentration of chemotherapeutic drugs in claudin-low tumors could be reduced in the presence of Salb, diminishing their toxicity. Even if more experiments are needed to assess the in vivo effect, these are encouraging results.

724 (584) **RHBDD2: A CANCER 5FU RESPONSIVE GENE, AS A POTENTIAL PROGNOSTIC AND THERAPEUTIC MARKER IN COLORECTAL CANCER TREATED WITH NEOADJUVANT CHEMOTHERAPY**

Sabina Palma[1], Ariel O. Zwenger[2], Graciela Gigola[1], María V. Crocce[1], Martín C. Abba[1], Ezequiel Lacunza[1]
[1]CINIBA (Centro de Investigaciones Inmunológicas Básicas y Aplicadas) Fac. Cs Médicas, UNLP, La Plata, Argentina [2]Servicio de Oncología, Hospital Provincial Neuquén, Neuquén Argentina

Colorectal cancer (CRC) is a molecular heterogeneous disease. Sequencing technologies have characterized this heterogeneity to define molecular subtypes that allow stratify patients so that they receive adequate and specific treatment. RHBDD2 gene was found overexpressed in the advanced stages of breast and CR cancers. Previous studies also demonstrated that RHBDD2 expression is induced under stress caused by agents such as DTT or 5Fu as an adaptive response. This led to its association with ER stress and the UPR pathway. In this study RHBDD2 expression was evaluated in CRC exposed to chemotherapeutic agents, in order to understand its role in tumor biology and establish its potential utility as a marker of chemoresistance or as a therapeutic target. RHBDD2 expression was analyzed in paired samples (before and after treatment) of advanced rectal cancer. In most of them an abrupt decrease in protein expression after the treatment was observed. However, in those where there was no reduction in the expression, a significant association with metastasis was found. Similar results were obtained in rat induced CR tumors. Furthermore, colon cancer cell lines were employed to evaluate the effect of 5Fu on the expression of RHBDD2 and the UPR genes. Results confirmed that 5Fu induced the transient expression of all of them, but with variation according to the molecular subtype of the cell line. Taken together, results allow us to infer that RHBDD2 is a protein related to the UPR that responds to stress caused by neoadjuvant treatment, establishing two groups of post-treatment response: with decreased expression and without decline, being the latter associated with a worse prognosis. This differential response could be associated to the intrinsic tumor subtype. Establish to which group are the tumors that do not show variation of RHBDD2 after treatment would give an important value to the protein as a marker for monitoring response to therapy and as a therapeutic target.

725 (619) **GLUCOCORTICOID RECEPTOR FUNCTION IS MODULATED BY HO-1 IN PROSTATE CANCER**

Daiana B. Leonardi[1], Javier Brandani[1], Felipe M. Jaworski[1], Alejandra Paez[1], Adalí Pecci[2], Myriam Nuñez[3], Geraldine Gueron[1], Javier Cotignola[1], Elba S. Vazquez[1].
[1]Laboratorio de Inflamación y Cáncer, IQUIBICEN/Dpto. Química Biológica, CONICET/FCEN-UBA [2] IFYBINE, CONICET [3]Departamento de Matemática, Facultad de Farmacia y Bioquímica, UBA

Glucocorticoids are used in the treatment of prostate cancer (PCa) to control disease progression, and to reduce cancer-related pain and side effects of chemo and hormonal therapy. However, they may also have the potential to drive castration-resistant prostate cancer growth via a mutated androgen receptor or glucocorticoid receptor (GR). Given that inflammation is critical for the development and progression of PCa, we first studied whether heme oxygenase 1 (HO-1), an anti-oxidant and anti-inflammatory protein, regulates GR. Dexamethasone (Dex, 10 nM for 6 h) induced GR mRNA levels in PC3 (GR+/AR-) and C4-2B (GR+/AR+). GR transcriptional activity was analysed in PCa cell lines transfected with a reporter plasmid containing glucocorticoid response elements. Cells were treated either with hemin (a potent specific inducer of HO-1, 80 µM for 24 h), Dex or both drugs. In all cases cells were cultured in medium with charcoal-dextran treated serum. Hemin significantly reduced (45%; p=0.03) basal and Dex-induced (35%; p=0.01) GR activity in PC3 cells but not in C4-2B. By confocal microscopy the GR nuclear translocation was detected after Dex treatment, while hemin+Dex co-treatment resulted in a partial GR cytoplasmic retention. Co-immunoprecipitation assays showed physical interaction between GR and HO-1 in nuclear and cytoplasmic compartments. PC3 xenografts were generated in nude mice by s.c. inoculation (3.6x106 cells). Animals received 6 i.p. dosis of Dex (0.2 mg/kg), hemin (25 mg/kg) or both drugs for 12 days. Dex reduced the tumour growth (p<0,001) while the co-treatment with hemin reverted this effect. Tumours were surgically removed and HO-1 and GR expression were determined. These results demonstrate that hemin-induced HO-1 modulates GR expression and activity in PCa cells. Furthermore, in vivo experiments suggest that GR/HO-1 interaction might impact on tumour growth.

726 (637) **CHEMORESISTANCE IN PANCREAS TUMOR MEDIATED BY SURVIVIN IN GEMCITABINE TREATMENT**

Cintia Yamila Mihalez[1,2], Martin Levermann[1,2], Susana Costantino[1,2], Mariángeles Díaz[2], Silvina Laura Lompardía[1,2], Tomás Lombardo[1,2], Matías Pibuel[1,2], Daniela Laura Papademetrio[1,2], Elida Alvarez[1,2].
[1]Cátedra de Inmunología - Facultad de Farmacia y Bioquímica [2] IDEHU-CONICET

Pancreatic adenocarcinomas are highly resistant to treatment. Gemcitabine (Gem) is the drug of choice with cytostatic effect on the tumor. Previous results indicate that during Gem treatment, levels of survivin raise, effect accompanied by an increase in basal autophagy. Previously, we saw that pharmacological inhibition of survivin was able to modulate the level of apoptosis after the treatment with Gem. From that, during the present study, we obtained a knock down cell line for survivin (MiaPaCa-2Surv-/-), in order to determine the effect of negative regulation on the sensitivity to Gem treatment in the presence or absence of 3-MA. We found that the cell line MiaPaCa-2Surv-/- established, showed low levels of survivin expression, without modifying the basal levels of apoptosis even after treatment with 3-MA (p>0.05). These results correlated with decreased levels of LC3-II in MiaPaCa-2Surv-/- vs MiaPaca-2WT determined by Western blot (p <0.001), with a lower number of autophagosomes in cells transfected with RFP-LC3 line knock down vs WT (p<0.05). This provides a potential role for survivin as positive modulator of autophagy, in this manner it would confer an active role in chemoresistance against the tumor treatment. To answer this question, cells were treated with Gem (10, 100 and 1000 µg/ml) for 48 hours and the percentage of apoptotic cells were determinated by the TUNEL assay. Surprisingly, after down regulation of survivin, no differences were found in the percentage of TUNEL + cells with or without 3-MA, neither increase in the number of apoptotic cells was found (p>0.05), while in the WT line, inhibition of autophagy produces sensitization of cells to the pro-apoptotic action of Gem (p<0.001). Together, these results indicate that the lack of survivin results in changes in cell autophagy levels; this fact finally avoid the enhancer effect of 3-MA during the sensitization of tumor cells in the development of new therapeutic strategies for tumor treatment.

727 (638) **4-METHYLUMBELLIFERONE INDUCES APOPTOSIS IN HUMAN ACUTE LEUKEMIA CELL LINES**

Mariángeles Díaz[1], Matías Pibuel[1,2], Tomás Lombardo[1,2], Cintia Yamila Mihalez[1,2], Martín Levermann[1,2], Daniela Laura Papademetrio[1,2], Elida Alvarez[1,2], Silvia Elvira Hajos[1,2], Silvina Laura Lompardía[1,2].
[1]IDEHU-CONICET, [2]Cátedra de Inmunología, Facultad de Farmacia y Bioquímica, UBA.

4-methylumbelliferone (4MU) is a derivative of cumarine that has been approved in Europe and Asia to treat biliary spasms. During the last years, it has been proposed as a potential new drug for the treatment of solid tumors due to its capacity to inhibit hyaluronan synthesis, but yet little is known about its effect on hematological malignancies. Previous work of our lab showed

that 4MU induced senescence in human chronic myeloid leukemia cell lines. Considering this, we hypothesize that 4MU would be a potential new drug for the treatment of leukemia. Therefore, the aim of this work was to evaluate the effect of 4MU on acute myelomonocytic leukemia cell line (U937) and on acute T-lymphoblastic leukemia cell line (Jurkat). Cells were exposed to 0.1, 0.5, 1 and 2 mM 4MU and the following parameters were analyzed: cell proliferation by 3H-T uptake, cell death by flow cytometry using FDA/IP stain, apoptosis by fluorescence microscopy using acridine orange/ethidium bromide and DAPI stain and Bcl-2 modulation by western blot. The results showed that 4MU abrogated both cells growth in a dose-dependent manner ($p<0.01$) and increased IP+ cells percentage ($p<0.01$). 4MU treatment augmented the number of cells with nuclear morphology and fluorescence pattern characteristic of apoptotic cells ($p<0.01$). Moreover, 4MU increased the percentage of cells with DNA fragmentation ($p<0.01$) and down-regulated Bcl-2 in both cell lines ($p<0.05$). We conclude that 4MU inhibits cell proliferation inducing apoptosis however another tumor suppression mechanism could be involved. These findings highlight the potential use of 4MU for acute leukemia treatment.

728 (642) **ANALYSIS OF BIOLOGICAL AND PHYSICAL MARKERS AS PROSPECTIVE INDICATORS OF TUMOR RESPONSE FOR THE INDIVIDUALIZED BNCT TREATMENT IN A MELANOMA ANIMAL MODEL**

Marina Carpano[1], Carla Rodriguez[1], Susana Nievas[2], Gustavo Santa Cruz[2], Juan Longhino[3], Rómulo Cabrini[1], Guillermo Juvenal[1;4], Mario Pisarev[1;4], Alejandra Dagrosa[1;4]
[1]Departamento de radiobiología, CAC , Comisión Nacional de Energía Atómica (CNEA) [2]Departamento de Coordinación BNCT, CAC, Comisión Nacional de Energía Atómica (CNEA) [3]Reactor Nuclear RA-6, CAB, Comisión Nacional de Energía Atómica (CNEA) [4]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)

Melanoma is characterized by therapeutic resistance, aggressive clinical behavior, and predisposition to develop metastasis. Application of Boron Neutron Capture Therapy (BNCT) could be an option for the treatment of melanoma. BNCT is a kind of radiation therapy that offers a suitable way to kill tumor cells without significantly harming surrounding normal tissues. We performed different studies in our laboratory in order to optimize the individual application of BNCT for cutaneous melanoma treatments. The aim of the present study was to optimize the application of BNCT in order to improve melanoma BNCT treatments by seeking a correlation between biological and physical markers of boron uptake and tumor response. Methods: 30 male NIH nude mice were implanted subcutaneously (s.c.) in the right flank with 3 x106 Mel-J cells. The animals were divided into 2 groups: 1) Sham irradiated (control group); 2) BNCT (neutron beam plus BPA, 350 mg/kg b.w.). Each mouse was labeled and irradiated in the hyperthermal neutron beam of the Argentine RA-6 research nuclear reactor (in-air thermal neutron flux of about 4.96 108 n/cm2s), shielding their bodies and exposing only the tumor. Animals were anesthetized s.c. with diazepam and ketamine (200 mg/kg b.w) and irradiated in groups of 8 for about 55 minutes. Tumor growth and histology were evaluated for 30 days post treatment. Body and tumor temperatures of each mouse were measured by infrared thermography, pre and post treatment, as a non-invasive indicator of boron uptake and, consequently, of tumor response. Results: Total physical dose received by the tumors was 6.88 Gy. 27.03% of the animals showed complete tumor regression (complete response) and a 36.4% tumor control was observed (partial response) after 40 days post irradiation. During the first 20 days post irradiation a significant tumor control was obtained ($p<0.001$), after which tumors began to re-grow. Infrared thermography showed that tumors with higher temperatures (and according to our previous studies, higher boron uptake relative to blood), exhibited better tumor control in terms of relative volume reduction. Conclusion: Temperature measurements by infrared termography appear to be a prognostic indicator of therapeutic success for optimizing the application of BNCT on an individual basis.

729 (648) **EFFECT OF DNA REPAIR INHIBITION DURING THE TREATMENT OF THYROID CARCINOMA BY BNCT**

Carla Rodriguez[1], Marina Carpano[1], Silvia Thorp[2], Emiliano Pozzi[3], Paula Curotto[3], Guillermo Juvenal[1,4], Mario Pisarev[1,4], María Alejandra Dagrosa[1,4]
[1]Dpt of Radiobiology (CAC, CNEA) [2]Dept. of Instrumentation and Control (CAE, CNEA) [3]RA-3-Investigation and Production Reactors (CAE, CNEA) [4]Research Council of Argentina (CONICET)

Introduction: Boron Neutron Capture Therapy (BNCT) has been proposed as an alternative treatment for poorly differentiated thyroid carcinoma which do not respond to conventional therapies as it does not uptake radioactive iodine. Previously we determined that after BNCT, DNA double strand break repair pathway by Homologous Recombination (HR), is activated while Non Homologous End Joining (NHEJ) pathway is not. These results indicate that there is a specific response to BNCT and rescue mechanisms are activated decreasing the efficacy of the therapy. In order to reduce non desirable effects, we used the specific Rad51 inhibitor B02 [(E)-3-benzyl-2-(2-(pyridin-3-yl) vinyl) quinazolin-4(3H)-one] during the treatment. It has been demonstrated that it specifically inhibited Rad51 repairing activity and in consequence decreases tumor cell survival after different kind of treatments. The aim of this work was to evaluate the efficacy of BNCT in vitro using B02 during the treatment. Materials and Methods: cells from a human follicular thyroid carcinoma (WRO) were incubated with or without BPA (0,14 M) and with or without B02 (5 mM) and after 16 hours were irradiated with a thermal neutron flux in the RA-3 Nuclear Reactor Facility. After 72 hours cell survival was determined by the MTT method and DNA damage by immunofluorescence of gH2AX. Results: when B02 (5 mM) was incorporated into the culture medium before the irradiation with a neutron beam only or neutron beam plus BPA, the cell survival decrease significantly respect to the groups without the addition of B02 (BNCT+B02 and NCT+B02 at 5 Gy vs C, $p<0,05$). The gH2AX foci showed to be larger 30 minutes after the BNCT+B02 treatment indicating severe DNA damage ($p<0.01$). Conclusion: B02 would increase the effect of neutron irradiation and could be used as a complement of BNCT for the treatment of follicular thyroid carcinoma.

730 (649) **THE AVAILABLY OF 20-HYDROXYEICOSATETRAENOIC ACID (20-HETE) IS REQUIRED FOR THE METASTATIC FEATURES OF HUMAN CASTRATION-RESISTANT PROSTATE CANCER PC3 CELLS**

Cecilia Colombero[1], Laura C. Panelo[2], Paula Sacca[3], Monica A. Costas[2], Susana Nowicki[1]
[1]Centro de Investigaciones Endocrinológicas "Dr. Cesar Bergada" (CEDIE) CONICET-FEI-División de Endocrinología, Hospital de Niños "Ricardo Gutierrez". [2]Laboratorio de Biología Molecular y Apoptosis, IDIM-CONICET. [3]Instituto de Biología y Medicina Experimental (IBYME)

The 20-hydroxylation of arachidonic acid renders 20-HETE. Previous results from our group have shown that the inhibition of 20-HETE synthesis reduces androgen-sensitive prostate cancer (PCa) cells viability. The aim of this study was to evaluate the role of 20-HETE on the in vitro characterization of metastatic processes in PCa cells. Two human PCa cell lines were used: LNCaP (androgen-sensitive) and PC-3 (androgen-insensitive); both were incubated with either 20-HETE or HET0016 (a selective inhibitor of 20-HETE synthesis). Scratch wound assay (cell migration), and colony growth in soft agar (anoikis resistance) were performed. Also, E-cadherin and vimentin protein expression (epithelial-mesenchymal transition, EMT) were assessed by WB. Cell localization and structure of actin, tubulin and vimentin were determined by IF. PC-3 cells migration in control conditions was 38%, this was reduced to 30%* and 25%*** by HET0016 1 and 10uM, respectively; also, incubation with 20-HETE 100nM increased cell migration to 54%***. The analysis of cytoskeletal proteins distribution revealed that HET0016 disorganized actin filaments throughout PC-3 cells, while tubulin filaments remained unchanged. Furthermore, HET0016 reduced by 45%** the ability

of PC-3 cells to form colonies in soft agar. Interestingly, the analysis of proteins involved in EMT showed that HET0016 increased the expression of E-cadherin in PC-3, as well as, reduced the expression of vimentin, without modifying its intracellular distribution. Conversely, 20-HETE decreased the expression of E-cadherin in PC-3 cells and increased the expression of vimentin. As for LNCaP cells, the incubation with HET0016 or 20-HETE did not affect cell migration or the expression of EMT-related proteins. Our results suggest that 20-HETE availability is necessary in the steps involved in metastasis of the highly aggressive prostate cancer cell line PC-3. *p<0.05,**p<0.01,***p<0.001 vs control in ANOVA followed by Dunnet's post-hoc analysis.

731 (570) **THE ANTITUMORAL EFFECTS OF HO-1 IN COLORECTAL CANCER ARE DEPENDENT ON THE PRESENCE OF A WILD TYPE P53**

Eliana Noelia Alonso[1], Norberto Ariel Gandini[1], María Julia Ferronato[1], María Eugenia Fermento[1], Mariquena Natali Marquestaut[1], Nancy Carolina Andrés[1], Martín Carlos Abba[2], Cintia Lorena Massillo[3], Alejandro Carlos Curino[1], María Marta Facchinetti[1].

[1]Laboratorio de Biología del Cáncer, Instituto de Investigaciones Bioquímicas Bahía Blanca (INIBIBB), Centro Científico Tecnológico Bahía Blanca (CONICET-UNS), Bahía Blanca, Argentina. [2]CINIBA, Facultad de Ciencias Médicas, Universidad Nacional de La Plata, La Plata, Argentina. [3]Laboratorio de Oncología Molecular y Nuevos Blancos Terapéuticos, IBYME-CONICET, Buenos Aires, Argentina

Previously, we reported that heme oxygenase-1 (HO-1) is associated with increased overall survival time of patients with invasive colorectal cancer (CRC) and that HO-1 activation decreases the viability of human CRC cell lines that carry a wild-type p53 although it has no effect on cells with absent/mutated p53. The hypothesis of this work is that p53 might be involved in the antitumoral effect of HO-1 in CRC. By using a xenograft of p53 wild type-containing HCT116, we first demonstrated that genetic overexpression of HO-1 delayed tumor growth ($p<0.05$) and decreased tumor volume and weight ($p<0.01$) compared to controls. Also, p53 was upregulated in HO-1-overexpressing cells ($p<0.05$). We then evaluated the effects of pharmacologic activation of HO-1 (hemin) in HCT116 p53wt or HCT116 p53-/- xenograft tumors. Hemin (H) treatment delayed tumor growth ($p<0.01$) and decreased tumor volume (H=740.6 vs C=3082 mm3; $p<0.01$) and weight (H=0.8 vs C=3.2 g; $p<0.05$) only in HCT116 p53wt tumors, although it increased HO-1 levels in both tumor types ($p<0.01$). In support of these results, in silico studies showed a trend towards higher overall survival in CRC patients with high HO-1 and p53wt compared to those with low HO-1 or mutated p53. To start investigating p53 regulation by HO-1, we performed HO-1 chromatin immunoprecipitation assays and found that HO-1 does not bind to p53 promoter. In addition to affecting cell viability, genetic and pharmacological modulation of HO-1 decreased cell migration ($p<0.05$) and invasion ($p<0.001$) only in HCT116 cells, not affecting these processes in HCT116 p53-/- cells. These effects were accompanied by E-cadherin upregulation ($p<0.05$). In concordance, preliminary results obtained in human CRC biopsies by immunohistochemistry, showed a positive correlation between HO-1 and E-cadherin ($p<0.01$). In conclusion, our data demonstrate an antitumoral role for HO-1 in CRC and show evidence of the importance of a wild type p53 for this role.

732 (577) **ANTITUMORAL PROPERTIES OF PROSOPIS STROMBULIFERA (LAM) BENTH. AQUEOUS EXTRACT AGAINST COLORECTAL CANCER AND ITS ACTIVE CONSTITUENTS DETERMINATION**

Fabio Andrés Persia[1], Ana Karen García[2], María Belén Hapon[1,3], Carlos Gamarra-Luques[1,2].

[1]Instituto de Medicina y Biología Experimental de Cuyo (IMBECU) – CONICET [2]Facultad de Ciencias Médicas - Universidad Nacional de Cuyo [3]Facultad de Ciencias Exactas y Naturales - Universidad Nacional de Cuyo

Many drugs used in oncology have been provided by nature. Contribution of plants to cancer treatment is evidenced by the success of drugs like vinblastine, topotecan, paclitaxel and others. Prosopis strombulifera (Ps) is a native plant from Mendoza popularly used as astringent, anti-inflammatory, odontalgic and anti-diarrheic agent. Our recent data show that Ps aqueous extract is cytotoxic against tumoral cell lines. The aim of the present study was to demonstrate the capability of aqueous extract to interfere with colorectal tumor progression in vivo and to identify its chemical main components with cytotoxic activity. Tumor induction was performed in 3 groups (n=8) of BALB/c males, 6 weeks old, by 1,2-dimethylhydrazine (DMH, 21mg/kg/week, S.C., 22 weeks) administration. In vivo treatments were 5-fluorouracil (5FU, 30mg/kg /week, I.P) or Ps (150 mg/day in drinking water); both started 8 weeks after tumor induction; a third group did not receive any treatment. The phytochemical analysis was performed by specific reactions. Chemical constituents were fractioned by solid-liquid extraction and its cytotoxicity measured by MTT tetrazolium assay. Ps administration significantly increases animal survival from 24 weeks after tumor induction in non-treated group to 34.5 weeks (Mantel Cox, p=0.0188), while 5FU group survive 27 weeks. A qualitative phytochemical screening of the aqueous extract revealed a positive reaction for flavonoids, carbohydrates, sterols, terpenes and tannins; whereas, alkaloids, proteins and resins were are not detected. Between isolated fractions, only terpenes were able to induce cytotoxicity on HCT-116 human colorectal cells; while aqueous extract shows an IC50 of 502µg/ml, the isolated terpenes IC50 were 528µg/ml (5FU positive control was 8.86µg/ml). In conclusion, the present work makes Ps a promising natural product for cancer research and treatment, being terpenes the main candidates for further chemical-analytical studies.

733 (2017) **ERBB-DEPENDENT P-REX1/RAC ACTIVATION LEADS TO AN INCREASE IN TGFβ2 EXPRESSION IN BREAST CANCER.**

Angela Lara[1], Agustin Gonzalez[1], Silvia Vornetti[2], Graciela Horton[2], Karina Gon[2], Claudia Arias[2], Evangelina Bonavía2, Carlos Garbovesky[2], Fernando Correa[1], Lourdes Pérez[3], Martin Abba[4], Edith Kordon[3], Nicasio Cuneo[2], Diego Flaks[2], Eva Wertheimer[1],

(1) Centro de Estudios Farmacológicos y Botánicos (CEFYBO), CONICET, UBA. (2) Hospital Oncologico Marie Curie. (3) Instituto de Fisiología Molecular y Neurociencia (IFIBYNE), CONICET, UBA (4) Centro de Investigaciones Inmunologicas, Básicas y Aplicadas (CINIBA), Universidad Nacional de la Plata

Due to the importance of breast cancer for Argentinian public health, it is essential to identify biomarkers of the local population which could improve early diagnosis, predict disease progression and provide guidelines for treatment with targeted therapies. P-Rex1, a Rac-GEF essential for ErbB-induced Rac activation and migration, is overexpressed in breast cancer. The activation of ErbB receptors modifies the expression of a large number of genes involved in breast cancer. Some of those genes are also regulated by P-Rex1. The aim of this work was to study the effect of HRG-triggered Rac activation on the expression of TGFβ2, which is also involved in cell migration, and to determine its dependence on P-Rex1. A time-course analysis of Rac1 activation induced with 10 ng/ml HRG in T47D breast cancer cells (analyzed by Rac-activated pull down assays) revealed that the amount of activated Rac increases rapidly with a peak at 5 minutes and is maintained for at least one hour, followed by a gradual decrease at 6 h post-stimulation. Rac activation by HRG leads to an increase in TGFβ2 mRNA levels, which is inhibited by P-Rex1 silencing (data obtained by cDNA microarray and quantitative PCR). Since breast cancer is a multifactorial disease and current therapies often fail to find the main focus that drives disease progression, our working hypothesis is that there is an interaction between different signaling pathways, which share a convergence node in P-Rex1/ Rac, and interact and synergize during breast cancer progression. Future experiments will determine whether activation of Rac depends on the convergence of the ErbB and TGFβ2 pathways. In addition,

we seek to determine the relevance of P-Rex1 in an Argentinean population of breast cancer patients. We observed that P-Rex1 mRNA levels in tumor samples are higher than in its concomitant adjacent tissue. These results confirm that these patients could be the focus of new therapies targeting P-Rex1 or its effectors.

734 (2018) **ALLELE BURDEN OF JAK2V617F MUTATION IN PLASMA CELL FREE DNA OF HEALTHY INDIVIDUALS SMOKERS AND NON-SMOKERS**

Luisina A. Riera[1,2], María A. Cardozo[1], Luisa Gaydou[1], Adriana Follonier[1], Verónica L. Bosquiazzo[1,3], Jorge G. Ramos[1,3]

[1]Departamento de Bioquímica Clínica y Cuantitativa, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina. [2]Maestría Biología Molecular Médica - Universidad de Buenos Aires, Argentina. [3]Instituto de Salud y Ambiente del Litoral (ISAL) - CONICET, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina.

Smoking is one of the most studied risk factors associated with the development of cancer and heart disease. Several authors have shown that components of cigarette smoke promote the development of a variety of mutations affecting different genes among which is the V617F mutation in the JAK2 gene. JAK2 is a member of the family of tyrosine kinases that are linked to cytokine receptors constitutively. Its activation stimulates the proliferation, differentiation, migration and cell apoptosis. The V617F mutation corresponds to an autoinhibitory domain, resulting in constitutive activation of the signaling pathway and is one of the major diagnostic criteria of Chronic BCR-ABL negative Myeloproliferative Neoplasms. Since this mutation may be present in healthy individuals; that smoking is a risk factor for its appearance; and that the cell free DNA (cfDNA) sample is representative of various body compartments, then the mutation detection could be a prognostic marker for the development of clonal pathology. Therefore we hypothesized that the allelic burden of JAK2 mutated gene in cfDNA of healthy smokers is significantly higher than in non-smokers. For this purpose samples were collected from healthy individuals: 16 smokers and 14 non-smokers. cfDNA was obtained from plasma using the QIAmp DNA Blood Mini kit. The determination of the allele burden of JAK2V617F (% alleles JAK2V617F/total alleles JAK2) in cfDNA was determined by an allele specific real time PCR assay (qPCR). In order to discard subclinic haematological diseases a blood count for each individual was performed. The JAK2V617F mutation was detected both the cfDNA of smokers and non-smokers. The allele burden in smokers was 0.030% [95% CI: 0.017-0.055%], while in non-smokers was 0.111% [95% CI: 0.061-0.203%] with statistical significance (p=0.003). These results demonstrate that the mutation could be present either in healthy smokers and non-smokers, and it prognostic value as a tumoral marker must to be investigated.

735 (362) **ANTITUMORAL EFFECTS OF THE VITAMIN D ANALOGUE EM1 ON GLIOBLASTOMA MULTIFORME CELL LINES**

María Julia Ferronato[1], Eliana Noelia Alonso[1], María Eugenia Fermento[1], Diego Javier Obiol[1], Norberto Ariel Gandini[1], Mario Alfredo Quevedo[2], Alejandro López Romero[3]. Evangelina Mascaró[4], Cristian Vitale [4], María Marta Facchinetti[1], Alejandro Carlos Curino[1]

[1]Laboratorio de Biología del Cáncer, Instituto de Investigaciones Bioquímicas Bahía Blanca (INIBIBB), Centro Científico Tecnológico Bahía Blanca (CONICET-UNS), Bahía Blanca, Argentina. [2] Unidad de Investigación y Desarrollo en Tecnología Farmacéutica (UNITEFA-CONICET), Facultad de Ciencias Químicas, Ciudad Universitaria, Universidad Nacional de Córdoba, Córdoba, Argentina. [3] Departamento de Hematología, Laboratorios IACA, Bahía Blanca, Argentina. [4] Laboratorio de Química Orgánica, Departamento de Química, Universidad Nacional del Sur (INQUISUR), Bahía Blanca, Argentina

Currently, first-line adjuvant treatment for glioblastoma is based on the combination of radiotherapy and temozolomide. Although the combination treatment has slightly improved patient survival, novel strategies aimed at prolonging the survival and ensuring a better quality of life are necessary. EM1 is a novel calcitriol analogue with antitumoral properties and, as a steroid compound, has the potential to cross the blood-brain barrier. The aim of the present study was to evaluate the potential of EM1 as a chemosensitizing agent in glioblastoma treatment by investigating its antitumoral effects on human T98G and U251 cells, and modeling EM1 binding to its receptor VDR by in silico assays. In culture results showed that the analogue exerts a significant decrease in T98G cell viability through cell cycle arrest in G0/G1 phase ($p < 0.01$). This result was accompanied by an increase in the expression of p21, p27 and VDR and a decrease in cyclin D1 and p-AKT, assayed by western blot. Moreover, the treatment with EM1 did not affect cellular viability (WST-1) of human primary astrocytes, thus demonstrating a differential effect on non-malignant cells. EM1 was also able to retard U251 and T98G migration ($p < 0.001$) in wound healing assays and to inhibit T98G cell invasion ($p < 0.001$) through Matrigel. The binding of EM1 to VDR was explored by means of molecular docking and molecular dynamics. Per-residue interaction analyses showed that EM1 is able to bind to VDR establishing intermolecular contacts with most of the residues that interact with calcitriol. Energetic analysis showed a higher affinity of EM1 for VDR than calcitriol (ΔG= -80.4 and -73.5 Kcal/mol, respectively). There were significant electrostatic interactions between the phosphate group present in this ligand and His397 of VDR and additional Van der Waals interactions that further enhanced the affinity of EM1 for VDR. Altogether, these results suggest the potential use of this analogue in glioblastoma treatment.

736 (439) **EFFECT OF PHARMACOLOGIC AND GENETIC P300 MODULATION IN THE PROGRESSION OF HORMONE-INDEPENDENT BREAST CANCER**

María Eugenia Fermento[1], María Emilia Villegas[1], María Julia Ferronato[1], Norberto Ariel Gandini[1], Eliana Noelia Alonso[1], María Marta Facchinetti[1], Alejandro Carlos Curino[1].

Bahía Blanca (INIBIBB), Centro Científico Tecnológico Bahía Blanca (CONICET-UNS), Bahía Blanca, Argentina

Breast cancer (BC) is a heterogeneous disease consisting of many subtypes that have different responses to treatment and different clinical outcomes, suggesting the need to find new molecular markers. Elsewhere we demonstrated that pharmacological inhibition of p300 acetylase function has an antitumoral role in murine hormone-independent (HI) BC LM3 cell line, and decreases tumor progression in a syngeneic murine model of LM3. In this work, we investigated the role p300 has on BC by performing pharmacological inhibition of p300 acetyl-transferase function and analyzing the effects on viability, migration, invasion and adhesion in human triple negative BC (TNBC) MDA-MB-231 cell line and on tumor progression in a xenograft murine model of MDA-MB-231. We observed that inhibition of p300 reduced viability (WST and cell count), migration (wound healing assay), invasion (Matrigel) and adhesion (cell count) in MDA-MB-231 cells ($p<0.05$). Furthermore, significant reduction in the number of lung metastases ($p<0.05$) was observed in a xenograft tumor model of MDA-MB-231 following treatment with the p300 inhibitor. As these results were obtained by pharmacological modulation of p300, we decided to investigate the genetic modulation of p300 in a TNBC cell line and on tumor progression in an HI syngeneic murine model. We obtained MDA-MB-231 stably overexpressing a shRNA for p300 (MDA-MB-231-p300NEG) and control cells (MDA-MB-231-CTRL). The reduction in p300 levels was confirmed by RT-qPCR. We observed reduced viability, migration, invasion and adhesion in cultured MDA-MB-231-p300NEG cells compared to MDA-MB-231-CTRL cells ($p<0.05$). In the murine model, we observed significant reduction in the tumor burden and in the number of lung metastases in mice injected with LM3 overexpressing a shRNA for p300 (LM3-p300NEG), compared to mice injected with LM3-CTRL ($p<0.05$). Altogether, these results show a protumoral role of p300 in HI and/or TN BC.

737 (2026) **COMPARISON OF MOLECULAR SUBTYPES OF BREAST CANCER, INCLUDING METASTASIS AND PRIMARY TUMOR AXILLARY LYMPH NODE.**

Rodrigo Zuñiga[1], Carlo Lozano[1], Claudio Córdova Lepe[2], Pablo Olivero[2], Paola Ochova[1]
1.-Hospital Carlos Van Buren; 2.- Universidad de Valparaiso.

Introduction. Currently much of the prognostic information, along with the development of treatment strategy (cytotoxic, endocrine therapy or anti HER2 antibody) and classification of molecular subtype of breast cancer, is from the immunohistochemical phenotype of the primary tumor. However, the intrinsic heterogeneity of neoplastic cells in general appreciated in breast cancer does consider that expression of the tumor markers currently used in the clinic, could be modified during tumor development and spread. Materials and methods. Sixty-six samples of patients diagnosed with breast cancer Carlos Van Buren Valparaiso Hospital were studied with hetatoxilina and eosin (see Appendix B), immunohistochemistry for the expression of HER2, ER (estrogen receptor), RP (Receiver progesterone) and Ki67 (see Appendix C, D and E) thus primary tumor and axillary lymph nodes metastases. Both samples were classified into subtypes: Luminal A, Luminal B, Luminal B / Her2 and Her2 negative Triple and compared. Results. No significant differences between the expression of ER, PR, HER2 and Ki67 primary tumor and lymph node metastasis ($p > 0.05$) was found. 21 cases (31.8%) changed their molecular classification between the primary tumor and their lymph node metastases (see Appendix F). Triple Negative subtype changed molecular betterprognosis in lymph nodes in 6 of 10 cases, but not in the opposite direction ($p = 0.03$). In all cases where there was a change, 16 (24.2%) they would be undertreated according treatment protocols for different molecular subtypes of breast cancer. Conclusion. The observed changes in molecular subtypes between primary tumors and lymph nodes in this study to involve changes to other tumor subtypes and treatment characteristics. This last point should be amended, if it considers this additional information.

738 (2056) **USE OF ULTRASONOGRAPHY IN PRECLINICAL STUDIES: TUMOR MEASUREMENTS USING B-MODE ULTRASOUND REAL TIME, AND VASCULAR CHARACTERIZATION BY POWER DOPPLER ULTRASOUND OF TUMORS IN IMMUNODEFICIENT MICE.**

Eduardo Matías Belotti[1, 2], Pablo Uriel Díaz[1, 2], Antonela Stassi[1, 2], Facundo Salinas[2], Natalia R. Salvetti[1, 2], Belkis Marelli[1, 2], Ernesto Podestá[3], Hugo Hector Ortega[1, 2]
(1) Laboratorio de Biología Celular y Molecular Aplicada, Instituto de Ciencias Veterinarias del Litoral (ICiVet-Litoral), Universidad Nacional del Litoral (UNL) / Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Esperanza, Santa Fe, Argentina. (2) Centro de Medicina Comparada, Instituto de Ciencias Veterinarias del Litoral (ICiVet-Litoral), Universidad Nacional del Litoral (UNL) / Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Esperanza, Santa Fe, Argentina. (3) INBIOMED, Universidad de Buenos Aires / Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Buenos Aires, Argentina.

The standard method for measurement of subcutaneous tumors in immunodeficient mice is through the use of calipers. Nevertheless, it introduces a lot of bias. In the last decades, the use of ultrasonography as a diagnosis method for tumors has advanced, including the possibility of getting better assessment to gauge those neoplasms of different ways. Ultrasound (US) power Doppler image is widely applied for observing the perfusion of vasculature because it provides noninvasive and reproducible information and helps in assessing longitudinally the angiogenesis-related disease as well as monitoring the therapeutic response. The objective of this study is to report a different option to evaluate tumors in preclinical procedures. Immunodeficient mice (Foxn1 nu/nu Balb/c athymic nude mice) injected with neoplastic cells (MDA-MB-231), on the right flank, were evaluated every week during 2 months, beginning at 14 days of the injection of neoplastic cells; they were assessed by US with a color Doppler equipment (Z6 Vet, Mindray, China), coupled to a 5.0-10 MHz linear probe (75L50EAV, Mindray) . B-mode scanning in real time was used, for different mensuration (conjugated diameter and volume) of the tumors. The use of US equipment equipped with Doppler allows us to add data to the morphometric usually acquired with standard ultrasound equipment, like blood flow intensity of the tumor at different moments in the protocol. These parameters allow us to get more measures than caliper mensuration, in less time of manipulation of the animals (the acquisition duration of each power Doppler image was about 8–10 seconds), with a non-invasive method, providing more objective data. The use of tools to provide as many data as possible with the least handling of animals is a breakthrough that involves the refinement of animal procedures and contributes to the welfare of the experimental animals.

739 (2057) **NATURALLY PHOTOSENSITIZERS IN PHOTODYNAMIC THERAPY APPLIED COLORECTAL CARCINOMA CELLS USING A THREE DIMENSIONAL MODEL**

Pamela Gilardi[1], Ingrid Sol Cogno[1], Laura Comini[2], José Luis Cabrera[2], Susana Nuñez Montoya[2], Viviana Rivarola[1]
(1)Departamento Biología Molecular, Facultad de Ciencias Exactas Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, Córdoba, Argentina. (2)Farmacognosia, Departamento de Farmacia, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba (IMBIV-CONICET), Ciudad Universitaria, Córdoba, Argentina.

The history of ethnobotanical studies shows that several genera of plants belonging to Argentina can be selected according to their use in traditional medicine as sources of cancer drugs. In our laboratory we have studied photodynamic capacity of different plant compounds called anthraquinones (AQs) in breast cancer cells. These compounds were isolated from a plant called Heterophyllaeapustulata Hook f. (Rubiaceae) growing northwest of Argentina. Photodynamic therapy (PDT) against cancer is the cytotoxic effect induced by the combined action of light and photosensitizing compounds (Fs). Cases have been reported eradication of colorectal cancer (CRC) using PDT as a treatment, but other data show an incomplete response to treatment, presenting long-term recurrence. The aim of this work was to study the effect of photodynamic AQs, such as Rubiadina and Soranjidiol in colorectal carcinoma cells in a three dimensional model. To do this, first the cytotoxic effect (MTT) of these molecules in a dimensional model cell cancer SW480 colon was standardized and then nuclear morphology was evaluated using Hoescht staining (fluorescence microscopy). Both molecules with 50um and 15 minutes incubation did not result in decreased cell survival in dark conditions, but induced 80% cell death under irradiation (5J / cm2). At 16h post-treatment, a high number of apoptotic nuclei AQs evaluated for both observed. Research conducted in three-dimensional cultures currently represents a good option to emulate the situation of in vivo tissues. In our study we develop a three dimensional model of spheroids, which showed que100uM and 1h incubation with 60% Rubiadina death occurred with a light dose of 20J / cm^2. However 60% of death 200um, 1h incubation and 30J / cm^2 was observed for soranjidiol. The results encouraged us to further investigate in more detail the mechanism of action of these molecules for use as photosensitizers future to improve the efficacy of PDT for application in CCR.

740 (2066) **EFFECT OF THE COMBINED TREATMENT WITH MIFEPRISTONE AND CHEMOTHERAPY ON BREAST CANCER BRAIN METASTASES**

Ayelen Rubin[1], Veronica De la Fuente[2], Edgardo Salvatierra[3], Silvia Vanzulli[4], Osvaldo Podhajcer[3], Claudia Lanari[1], Paola Rojas[1]
(1) Instituto de Biología y Medicina Experimental (2) Facultad de Ciencias Exactas y Naturales - UBA (3) Instituto Leloir (4) Academia de Medicina

The treatment of brain metastasis is limited due to the drug's adversity to cross the blood brain barrier, because of the pres-

ence of endothelial cells' multidrug resistance efflux transporters, such as the P-glycoprotein (P-gp). The antiprogestin Mifepristone (MFP) is known to inhibit the P-gp activity. We hypothesize that a combination of MFP and Pegylated doxorubicin liposomes (doxo) improves the drug efficacy on tumors that do not express progesterone receptors. Using the metastatic triple negative MDA-231-BrM2 breast cancer cell line, transfected with GFP and luciferase, we explored different protocols to generate tumors growing in the brain of NSG mice. Spontaneous metastases were obtained by subcutaneous injection (sc) of 2 x 106 cells, and experimental metastases by intracardiac injection (ic) of 1 x 106 cells or by intracranial injection of 2 x 105 cells. In the ic and sc models small and scattered brain metastases as well as liver, lung and kidney metastases were observed. The intracranial model enabled us to obtain measurable brain tumors in a short time. Thus we selected this method to evaluate the effect of MFP (6 mg pellets, sc) and / or doxo (4,5 mg/kg, iv) on tumors growing in the brain. Treatment was initiated 10 days after cell inoculation. The amount of tumor cells after brain excision was measured by flow cytometry (GFP+ cells) and by luminometry (amount of luciferase). A decreased luminescence signal was observed in brains from mice treated with both agents as compared with the control mice ($p<0.05$), whereas no effects were observed between the single treatment groups and the controls. The results obtained with flow cytometry yielded a similar trend. Our data suggest that MFP may be a promising agent to treat brain metastasis of cancers, which do not necessarily express progesterone receptors, to increase the effectiveness of chemotherapeutic agents. The participation of the P-gp mediating this effect needs to be confirmed

741 (718) **GLYPICAN-3 (GPC3) MODULATES EPITHELIAL-MESENCHYMAL TRANSITION (EMT) OF HUMAN BREAST CANCER CELLS BY DOWNREGULATING ZEB1**

Gisela Vanina Novack[1], Lilian Fedra Castillo[1], María Amparo Lago Huvelle[2], María Candelaria Llorens de los Ríos[3], Ana María Cabanillas[3], Elisa Bal de Kier Joffé[1], María Giselle Peters[1]

[1]Universidad de Buenos Aires, Instituto de Oncología "Ángel H. Roffo", Área Investigación, Buenos Aires, Argentina [2]Universidad de Buenos Aires, CONICET, Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN), Facultad de Ciencias Exactas y Naturales, Buenos Aires, Argentina [3]Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba and Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI-CONICET), Córdoba, Argentina

Glypican-3 (GPC3) is a heparan sulfate proteoglycan related to cancer. Previously, we overexpressed GPC3 in MDA-MB231 human breast cancer cells (metastatic and GPC3-). We showed that GPC3 inhibits clonogenic and migratory capacities, while increases homotypic adhesion and apoptotic susceptibility. Moreover, GPC3 induced the reexpression of the epithelial marker E-Cadherin. Our results suggest that GPC3 would act as a metastatic suppressor by regulating epithelial-mesenchymal transition (EMT). In this work we observed that GPC3 overexpression induces a change in the MDA-MB231 cell morphology, from a fibroblastic to a more epithelial phenotype, as well as prevents the F-actin stress fibers formation. In vivo assays showed that GPC3 overexpression inhibits tumorigenicity (64% -vector vs 20% -GPC3; $p<0.05$), local invasion (75% -vector vs 0% -GPC3; $p<0.05$) and spontaneous metastasis incidence (50% -vector vs 0% -GPC3; $p<0.001$). To elucidate the molecular mechanisms involved in these effects, we evaluated the canonical Wnt pathway activity. Although GPC3 induced an inhibition of this pathway, no changes in the E-Cadherin expression levels were observed when we reverse the effect of GPC3 on Wnt signaling. So, we studied the expression of the E-Cadherin transcriptional suppressors: SNAIL, SLUG and ZEB1. GPC3 leaded a decrease in the ZEB1 mRNA and protein levels. When we overexpress ZEB1 in MDA-MB231-GPC3 cells, the increase of E-Cadherin expression induced by GPC3 was reversed. To test this in vivo, we performed IHC for E-Cadherin and ZEB1, in MDA-MB231-GPC3 tumors. ZEB1 staining was detected in 73±15% of -vector tumor cells vs 33±3% of -GPC3 tumor ones ($p<0.01$). While the most of control tumor cells was negative for E-Cadherin, this signal was positive in the 95±5% of -GPC3 tumor cells ($p<0.001$). Our results show that GPC3 expression reverted EMT by downregulating ZEB1 expression in human breast cancer cells. This effect would be independent of the canonical Wnt pathway.

INFLAMACIÓN E INMUNOLOGÍA / INFLAMMATION AND IMMUNOLOGY

742 (388) **ADVANCES IN THE DEVELOPMENT OF AN IN VITRO TEST FOR DETECTION OF TUBERCULOSIS INFECTION**

Nicolas Oscar Amiano[1], María Paula Morelli[1], Florencia Castello[1], Nancy Tateosian[1], Joaquin Pellegrini[1], Agustin Rolandelli[1], Rodrigo Hernandez del Pino[1], Eduardo Chuluyan[2], Juan Iovanna[3], Alberto Levi[4], Domingo Palmero[4], Veronica García[1]

[1]Instituto de Química Biológica, Facultad de Ciencias Exactas y Naturales (IQUIBICEN), UBA-CONICET, [2]Centro de Estudios Farmacológicos y Botánicos (Cefybo), Facultad de Medicina, UBA,. [3]Centre De Recherche En Cancérologie de Marseille (CRCM), INSERM U1068, Cnrs Umr 7258, Aix-Marseille Université Institut Paoli-Calmettes, Parc Scientifique Et Technologique De Luminy, Marseille, France. [4]División Tisioneumonología Hospital F.J. Muñiz

Current IFNγ release assays (IGRAs) cannot discriminate active and latent infection with *Mycobacterium tuberculosis (Mtb).* Moreover, no gold standard to diagnosis latent tuberculosis (LTBI) is available. Previously, we demonstrated that the dormancy antigen Rv2626c and six peptide-pools derived from its sequence discriminated by IFN-γ secretion LTBI subjects from healthy donors (HD) and tuberculosis (TB) patients. Stimulation of peripheral blood mononuclear cells (PBMCs) or whole blood with peptides or Rv2626c induced differential amounts of IFN-γ among the groups. The aim of this work was to advance in the development of a diagnostic kit to detect tuberculosis infection. For this, we focused on two main objectives: i) To develop new immunogenic pools with less than six peptides to diminish costs and ii) To use new pools to evaluate their immunogenicity in whole blood incubated in polystyrene tubes (as used by QuantiFERON-TB - QFT). IFNγrelease was measured by ELISA. To design new pools we first stimulated LTBI PBMCs with all the individual peptides (13-15 mers, overlapped by 11 aa; 36 in total, covering the complete Rv2626c sequence). Peptides 1, 2, 3, 5, 12, 22, 23, 30 and 36 were the more immunogenic peptides. Then, we generated the new pools a, BD, IM1, IM2, IM3 and PBMCs were stimulated with them (37º, 5d). We found that treatment with these pools significantly differentiate among the groups. Furthermore, when we stimulated whole blood with these pools (37º, 16h) we observed that pool IM2 clearly differentiated LTBI individuals from HD and TB (0.473±0.020 IU/mL of IFNγ (LTBI), 0.095±0.030 (HD), 0.008±0.008 (TB); $p<0.001$). Overall these results demonstrated the immunogenicity of new pools from Rv2626c antigen using the same tubes provided by QFT kit. Together, our findings indicate that pools composed by less than 6 peptides from Rv2626c antigen are strongly immunogenic which will allow reducing costs for the manufacturing of the kit to detect *Mtb* infection

743 (379) **PLATELETS-NEUTROPHIL EXTRACELLULAR TRAPS (NETS)-ENDOTHELIUM INTERACTION: IMPLICANCE IN THE DIFFERENT ORGAN DAMAGE BETWEEN SEPTIC (S) AND BURNED (B) PATIENTS.**

Tomás Kaufman[1], Débora Magosevich[2], Carolina Moreno[3], Alejandra Guzman[4], Paola D´Atri[1], Agostina Carestia[1], Fernando Pálizas[3], Carlos Fondevila[3], Mirta Schattner[1]

[1]Laboratorio de Trombosis Experimental, Instituto Dde Medicina Experimental, CONICET - Academia Nacional de Medicina, Buenos Aires, Argentina. [2]Sanatorio Sagrado

Corazón, Buenos Aires, Argentina. [3]Clínica Bazterrica, Buenos Aires, Argentina. [4]Hospital de Quemados, Buenos Aires, Argentina.

We studied NET formation (NETosis), the role of platelets and endothelium in two acute inflammatory pathologies, an infectious (S) and a sterile one (B).

Objective: to shed light on the differential organ damage in S and B.

Methods: we studied 22 S and 19 B patients 1 and 4 days post admission (dpa) to the intensive care unit and 30 healthy donors (H) as control. We evaluated NETosis, plasmatic nucleosome (nuc) levels, von Willebrand Factor (vWF), interleukin (IL)6 and 10, platelet P-selectin and Toll-like receptors (TLRs) expression.

Results: shown as ±SEM, *p<0.05 vs H, †p<0.05 vs unstimulated, #p<0.05 vs S, &p<0.05 vs 1dpa, Student T-test. Spontaneous NETosis (μg/ml DNA, fluorometry) was higher in S and B 1dpa (H:0.23±0.03; S:0.64±0,15*, B:0.51±0.14*) and 4dpa (S:0.51±0.07*, B:0.43,±0.08*). Stimulation with platelets+LPS or TNF-α induced NETosis on H neutrophils (platelets+LPS: 0.57±0.15†, TNF-a: 0.51±0.05†) but not in S or B. Nuc levels (μg/ml, ELISA), an indirect measure of NETosis, were elevated in S and B 1dpa (H:0.01±0,01, S:0.36±0,11*, B:0.16±0,04*) and 4dpa (S:0.26±0.07*, B:0.20±0.04*). Patients with nuc >0.75 1dpa did not survive. vWF levels (ELISA) were elevated in S vs B 1dpa and 4dpa. Neither S nor B showed P-selectin expression. Levels of pro- and anti-inflammatory cytokines (IL6 and IL10, respectively) were augmented in patients 1dpa, being IL6 higher in S. IL6 decreased in S and increased in B 4dpa; IL10 remained high in S and B. Platelet TLR4 expression (cytometry) was increased in S 1dpa and 4dpa. In B, this increment was seen at 4dpa. We found no correlation between the analyzed parameters and capability of neutrophils to form NETs or with nuc levels.

Conclusion: S and B showed increased NETs, nuc, inflammation markers and endothelial activation. S had increased inflammatory response. Nuc>0.75 μg/ml correlated with mortality. Different organ damage between S and B would not be explained by differences in NETosis or platelet/endothelial activation

744 (438) **TRIOLEIN REDUCES MMP-1 UPREGULATION BY DERMAL FIBROBLASTS GENERATED BY ROS PRODUCTION BY UVB-IRRADIATED KERATINOCYTES.**

Karin Hagelin[1]
[1]ICT- MIlstein, Fundación Pablo Cassará

Cytokine production and oxidative stress generated by ultraviolet radiation B (UVB) skin exposure are main factors of skin photoaging. Interleukin-6 (IL-6) produced by irradiated keratinocytes is proposed to have a role in metalloproteinases (MMPs) expression activation in dermal fibroblasts. We examined the effect of triolein treatment of UVB-irradiated keratinocytes on MMP1 (interstitial collagenase) expression response of dermal fibroblasts. We assayed UVB-irradiated keratinocytes soluble signals, mainly IL-6 and reactive oxygen species (ROS). IL-6 expression and ROS generation were analyzed in UVB-irradiated keratinocytes. MMP1 mRNA expression response was examined in fibroblasts grown in keratinocytes conditioned medium. We evaluated the effect of treating keratinocytes with triolein on IL-6 expression and ROS generation in keratinocytes, and MMP1 expression in fibroblasts. The irradiation of epidermal cells with sublethal UVB doses increased IL-6 expression and ROS generation. Conditioned culture medium collected from keratinocytes was used to culture dermal fibroblasts. MMP1 mRNA upregulation was observed in fibroblasts cultured in conditioned medium by UVB-irradiated keratinocytes. Triolein treatment reduced the IL-6 expression and ROS generation in keratinocytes and this effect was reflected in downregulation of MMP1 expression in fibroblasts. Triolein reduces both the expression of IL-6 and ROS generation in irradiated keratinocytes. It seems to exert an anti-inflammatory and anti-oxidative stress effect on irradiated keratinocytes that in turn reduces MMP1 expression in dermal fibroblasts. Collectively, these results indicate that triolein acts as a photoprotective agent.

745 (854) **PULMONARY MYELOPEROXIDASE ACTIVITY WORSENS INSULIN RESISTANCE IN OBESE MICE.**

Maria Cecilia Della Vedova[1], Marcos David Muñoz[1], Verni Ernesto Ricardo [2], Lucas Damian Santillan[2], Nidia Noemi Gomez[3], Sandra Ester Gomez Mejiba[2], Dario Ceferino Ramirez[1]
[1]Laboratory of Experimental and Translational Medicine-IMIBIO-SL-CONICET-UNSL. [2]Laboratory of Experimental Therapeutics-IMIBIO-SL-CONICET-UNSL. [3]Laboratory of Morphophysiology -IMIBIO-SL-CONICET-UNSL

Neutrophilic inflammation (NI) is a poorly known process occurring in the obese lung that may determine many obesity-associated metabolic abnormalities, including insulin resistance (IR). At sites of NI myeloperoxidase (MPO) oxidizes chloride anions to hypochlorous acid or hypochlorite (HOCl/OCl$^-$) which can damage the lung, increase systemic oxidative stress/inflammation and thus worsen IR. Bacterial lipopolisaccharide (LPS) is a stressor that when administrated by intratracheal instillation (ITI) causes NI in the lung. Hydrazide 4-aminobenzoic acid (ABAH) is a fairly-specific inhibitor of MPO. Taurine is a non-cell permeable scavenger of HOCl. Herein we hypothesized that inhibition of oxidative processes mediated by MPO in the obese lung can reduce IR in obesity. To test this hypothesis we used male B6 mice which were fed a high-fat diet and fructose (obese) and a low-fat diet and tap-water (control). During the last week of diet mice both group of mice were ITI once a day with either PBS (vehicle), ABAH (10 uM /mouse) or taurine (5nM/mouse). The last day of diet animals were ITI with 2.5 ug LPS/mouse or PBS alone; and 6 h later a glucose tolerance test was performed. A total of 6 groups for control and obese mice were compared (PBS, LPS, ABAH, ABAH+LPS, Tau and Tau+LPS). Compared to control, LPS treatment to obese mice increases more lung MPO activity, chlorotyrosine, nitrotyrosine, TNF-a and reduce insulin sensitivity. These differences between control and obese mice upon treatment with LPS were abrogated when animals were pre-treated with either ABAH or taurine. We conclude that MPO-driven oxidative modification in the lung of obese animals are responsible for worsening IR and may provide a therapeutic target to reduce IR in obese subjects. Supported by PROICO 2-3214 & PICT-2014-3369 (to DCR), PROICO 10-0414 (To SEGM) and PIP2015-2017-112215-0100603CO (To DCR, SEA & SEGM).

746 (480) **THE AQUEOUS EXTRACT OF RHIZOMES OF AN ARGENTINIAN MEDICINAL PLANT (SMILAX CAMPESTRIS) DECREASES PROINFLAMMATORY CYTOKINES PRODUCTION IN HUMAN LPS-ACTIVATED MACROPHAGES.**

Luciana Soledad Salaverry[1], Andrea Cecilia Parrado[1, 2], Franco Mangone[2], Ana Rugna[1], Guillermo Blanco[2], Teresa Gentile[1, 2], Andrea Canellada[1, 2], Estela Rey-Roldan[1, 2]
[1]Cátedra de Inmunología- Facultad de Farmacia y Bioquímica- UBA. [2]IDEHU- CONICET

Folk medicine has used different plant extracts for their anti-inflammatory and antioxidant effects. *Smilax campestris* (*S. campestris*) or "sarsaparilla" is a medicinal plant wildly distributed in northern Argentina. Previously we showed an antiinflammatory effect of aqueous extract of rhizomes of *S. campestris* on metalloproteinase-9 activity (MMP-9), an enzyme involved in the extracellular matrix remodeling process. *S. campestris* significantly decreased the activity of MMP-9 in LPS-activated macrophage cells. In the current study, we evaluated the effect of this extract on proinflammatory cytokines (IL-1beta, IL-6), the chemokine IL-8 levels (ELISA) and cytotoxicity (lactate dehydrogenase release assay, LDH) in human monocyte-macrophage cells, THP-1. Cells were stimulated under two conditions: the absence (basal state) or presence (activated state) of the inflammatory stimulus, lipopolysaccharide (LPS). Cells were incubated with different concentrations of *S. campestris* extract for 24/48 hs (10, 100, 1000, 10.000 ng of tannic acid/ml extract); LPS-activated macrophage cells were aditionally incubated with LPS (1 ug/ml) to induce an inflammatory context. The aqueous extract of rhizomes of *S. campestris* did not

alter IL-1beta, IL-6 and IL-8 levels in basal conditions (absence of LPS). But interestingly, the extract significantly decreased all cytokines meassured levels in human activated macrophage cells ($p < 0.01$), returning to basal levels. In these experimental conditions, the extract did not show a cytotoxic effect. In conclusion, we suggest an anti-inflammatory effect of aqueous extract of rhizomes of *S. campestris,* by the inhibition of proinflammatory cytokines, in human LPS activated macrophage cells. Our results provide evidence for the use of *S. campestris* aqueous extract as a therapeutic agent against several inflammatory diseases.

747 (872) **LONG-TERM EFFECTS OF DMPO: SWITCHING OF MACROPHAGE'S PHENOTYPES TO REDUCE ADIPOSE TISSUE INFLAMMATION IN OBESITY**

Marcos David Muñoz[1], Maria Cecilia Della Vedova[1], Sergio Eduardo Alvarez[2], Sandra Esther Gomez Mejiba[3], Dario Ceferino Ramirez [1]

[1]Laboratory Of Experimental And Translational Medicine;-IMIBIO-SL-CONICET-UNSL. [2]Laboratory Of Signal Transduction -IMIBIO-SL-CONICET-UNSL. [3]Laboratory Of Experimental Therapeutics-IMIBIO-SL-CONICET-UNSL

Macrophages are tissue cells from the innate-immune system where they play a number of homeostatic and defense functions. Inside the tissues and under tissue-specific microenvironmental pressures monocytes are recruited and differentiated to specific phenotypes. This phenotype is a consequence of the expression ofspecific genes that are under the control of one or more transcription factors.In this context, inflammatory phenotype of adipose tissue (AT) macrophages (ATM-M1) is responsible for adipose tissue oxidative stress and inflammation mediators that reduce whole-body insulin sensitivity and cause a number of metabolic abnormalities-associated to obesity. Intratracheal instillation of the nitrone spin trap 5,5-dimethyl-1-pirroline *N*-oxide (DMPO) to diet-induced obese-mice reduced markers of AT oxidative stress and inflammation, reduced serum concentration of inflammatory cytokines and improved insulin sensitivity. Thus we hypothesized that DMPO may produce transcripcional effects in macrophages at the AT and maybe other tissues. To approach this hypothesis we determined the transcripcional effects of DMPO in RAW264.7 cells after 6h incubation and with or without lipopolysaccharide (LPS) to model transcriptional profile of ATM. Microarray data showed that LPS caused an M1-transcriptional pattern, whereas DMPO inhibited these changes. Remarkable effects were observed in the expression of IRF-7 and PPAR-d, master regulators of genes that determine M1 and M2 macrophage phenotype. LPS induced IRF-7, but reduced PPAR-d expression; whereas DMPO reduced IRF-7, but induced PPAR-d expression. Taking together our data suggest that DMPO may serve as a structural platform for the design of novel compounds to reduce AT inflammation and, thus other inflammatory abnormalities-associated to obesity, such as insulin resistance and metabolic syndrome. Supported by PROICO 2-3214 & PICT-2014-3369 (to DCR), PROICO 10-0414 (To SEGM) and PIP2015-2017-112215-0100603CO (To DCR, SEA & SEGM)

748 (499) **ROLE OF ALPHA-GLUCANS IN THE RESPIRATORY BURST IN MONOCYTE-DERIVED DENDRITIC CELLS**

Alejandra Duarte[1], María Mercedes Romero[1], Laura Correa Feo[1], Isabel Piazzon[1], Mercedes Alemán[1]

[1]IMEX-CONICET-ANM

Tuberculosis remains the single largest infectious disease with two million deaths estimated to occur yearly, more than any time in history. The spread of *Mycobacterium tuberculosis (Mtb)* from the lungs to other sites occur before the development of adaptive immune responses. *Mtb* has evolved successful strategies to invade and persist within host cells. The major carbohydrate constituents from *Mtb* surface are α-glucans, a sugar which has been implicated in fungal virulence, and represent up to 80% of the extracellular polysaccharides in Mycobacteria. Previous results demonstrated that reactive oxygen species (ROS) in neutrophils are generated by non-opsonized mycobacteria through α-glucans. Here we evaluated the role of α-glucans in the endogenous production of ROS and their impact on the maturation of dendritic cells (DC) and *Mtb*-specific lymphocytes expansion. Results Monocyte-derived DC were incubated 24 hours with *Mtb* (H37Rv) subjected or not to a-amyloglucosidase treatment (*Mtb-e*) and thereafter maturation markers were evaluated by cytometry. Expression of CD86 were induced by *Mtb* ($p<0.001$) but significantly less induced by *Mtb-e* ($p<0.001$). HLA-DR as well as IFNg were induced by *Mtb* ($p<0.001$) but not by *Mtb-e*. In addition, LPS primed DC were incubated with 123Dihidrorhodamine (123DHR) before *Mtb* challenge and thereafter emission of oxidized DHR was evaluated by flow cytometry. Whereas *Mtb* induced significant ROS production ($p<0.002$), *Mtb-e* was unable to induce ROS, which was coherent with their incapability to induce lymphoproliferation (MLR) ($p<0.002$). Conclusion Here we describe that lack of a-glucans in *Mtb* leads to the loss of their ability to induce ROS and also DC maturation and antigen presentation. Thus, the role of *Mtb* α-glucans could be essential for the onset of the protective immune response because maturation and function of DC are critical against tuberculosis disease.

749 (877) **A B-CELL SUPERANTIGEN INDUCES THE APOPTOSIS OF MURINE AND HUMAN MALIGNANT B CELLS.**

Alejandra Duarte[1], Daniela Lorenzo[1], Juliana Mundiñano[1], Paula Berguer[2], Alejandro Sielecki[1], Mercedes Alemán[1], Irene Nepomnaschy[1], Isabel Piazzon[1]

[1]IMEX-CONICET, Academia Nacional de Medicina de Buenos Aires, CABA, Argentina. [2]Instituto Leloir, IIBBA, CONICET, Buenos Aires, Argentina.

B-cell superantigens (Sags) are bacterial and viral proteins which affect immune responsiveness binding conserved sites of the VH or VL regions of immunoglobulin molecules outside their complementarity-determining regions inducing apoptosis of normal cognate B cells. We had demonstrated that T-cell Sags induce the apoptosis of cognate malignant T cells.Exposure to bacterial and retroviral T Sags *in vivo* increases lymphoma-bearing mice survival. In the present study we investigated whether protein L (PpL), a B-cell Sag that interacts with κ+ bearing cells are able to induce the apoptosis in spontaneous murine lymphoma B cells and human Daudi cells. Results: PpL induced apoptosis (measured by Annexin V-FITC) in murine κ+lymphoma B both *in vitro* ($p<0.01$) and *in vivo* ($p<0.01$).Besides, PpL induced apoptosis in human Daudi cells ($p<0.001$). Apoptosis was not modified by caspase-8 inhibitor, and no differences in Bid, Fas and Fas-L (measured by western and PCR) were found suggesting that the extrinsic pathway of apoptosis was not activated by PpL. The involvement of the intrinsic pathway was clearly indicated by: i) alteration in the mitochondrial membrane potential ($\Delta\Psi m$) both in murine ($p<0.001$) and human ($p<0.001$) lymphoma B cells exposed to PpL; ii) decreases of apoptosis by caspase-9 inhibitor; iii) significant increases of Bim and Bax proteins ($p<0.001$) and down-regulation of Bcl-2 ($p<0.001$); iv) the translocation from the cytoplasm to the mitochondria of Bax and Bim pro-apoptotic proteins ($p<0.0001$) and its inhibition by caspase-9 but not by caspase-8 inhibitor and v) the translocation of Bcl-2 protein from the mitochondria to the cytosol ($p<0.001$) and its inhibition by caspase-9 inhibitor but not by caspase-8 inhibitor. In conclusion our data demonstrate that the mitochondrial but not extrinsic pathway is involved in Sag-induced apoptosis in B-cell malignancies. Our results suggest that B-cell Sags could be envisaged as a therapeutic tool for these malignancies.

750 (549) **DISRUPTION OF TUMOR NECROSIS FACTOR-ALFA RECEPTOR 1 (TNFR1) SIGNALING PATHWAY INCREASES INTERLEUKIN-1 BETA LEVELS, EXACERBATES HEPATIC INFLAMMATORY RESPONSE AND APOPTOSIS DURING A HIGH FAT DIET (HFD)**

Flavia Lambertucci[1], María Guillermina Sedlmeier[1], Ainelén Arboatti[1], Silvina Villar[2], María Paula Ceballos[1], Eduardo Roggero[2], María De Lujan Alvarez[1], Ariel Dario Quiroga[1], Cristina Ester Carnovale[1], Daniel Eleazar Francés[1], María Teresa Ronco[1]

[1]Institución IFISE-CONICET. [2]Institución IDICER-CONICET

One hallmark of obesity is chronic low grade inflammation and increased pro-inflammatory circulating cytokines, which play an important role in the development of insulin resistance (IR). Obesity-associated hepatic inflammation is due to increased Kupffer cell activation and recruited hepatic macrophages. Sustained inflammation may increase the susceptibility of hepatocytes to apoptotic cell death and therefore exacerbate liver damage. In this work, we aim to study hepatic TNFR1 receptor association with inflammation and apoptosis in a model of IR and altered energy homeostasis induced by HFD. C57BL/6 wild type (WT) and TNFR1 knock-out (KO) mice (n=6) were fed with regular chow diet (CHOW-WT, CHOW-KO) or a 40% high-fat diet for 16 weeks (HFD-WT, HFD-KO). Here, we report that after 16 weeks of HFD, the plasmatic levels of interleukin(IL)-1β were increased by HFD especially in KO mice (HFD-KO: 189 pg/ml; HFD-WT: 1078 pg/ml; $p<0.05$). Chemokines play pivotal roles in the recruitment of immune cells at sites of inflammation. To gain insight into the process, we isolated non-parenchymal cells (NPCs) and parenchymal cells (hepatocytes) from liver and analyzed the macrophages content (number of $F4/80^+$ cells in HFD WT: 2.9% and HFD-KO: 11.5%) and hepatocyte apoptosis (percentage of hypodiploid cells, HFD WT: 8.5% and HFD-KO: 14.5%) by flow cytometry ($p<0.05$). To correlate these findings with the histological damage, liver sections were stained with hematoxylin/eosin (H&E), showing moderate to severe lobular inflammation in HFD-KO mice. Concordantly, liver extracts enriched in plasma membrane presented an increase in IL-1 receptor and TLR4 expression in HFD-KO (+40% and +60%, respectively) compared to HFD-WT mice ($p<0.05$). Based on these results and according to previous analysis of hepatic apoptosis in this model, we suggest a deleterious role of IL-1β associated to HFD-hepatic inflammation and apoptosis that is exacerbated in mice lacking TNFR1 receptor

751 (895) **DIFFERENTIAL EFFECTS OF ESCHERICHIA COLI SHIGA TYPE 2 AND SUBTILASE TOXINS ON PRO-INFLAMMATORY CYTOKINES AND CHEMOKINES RELEASED BY HUMAN RENAL CELLS**

Romina Soledad Alvarez[1], Nadia Yasmín Towstyka[2], Carolina Jancic[2], Cristina Ibarra[1], María Marta Amaral[1]
[1]Laboratorio De Fisiopatogenia, IFIBIO-UBA-CONICET, Facultad De Medicina, Universidad De Buenos Aires. [2]Laboratorio De Inmunidad INNATA, IMEX-CONICET, Academia Nacional De Medicina, Buenos Aires.

Post diarrhea hemolytic uremic syndrome (HUS), a complication of Shiga toxin (Stx)-producing *E. coli* (STEC) infection, is the most common cause of acute renal failure in children in Argentina. Renal damage is mainly attributed to Stx, being Stx2 epidemiologically more relevant than Stx1. Subtilase (SubAB) is a cytotoxin produced by STEC isolated from cases of childhood diarrhea. Therefore, it is proposed that SubAB may contribute to HUS pathogenesis. Previously, we demonstrated that Stx2 and SubAB caused cell viability decrease on primary cultures of human glomerular endothelial cells (HGEC) and human tubular epithelial cell line (HK-2) after 24 h of treatment, only at high toxins concentrations (Stx2: 1-10 ng/ml and SubAB: 100-1000 ng/ml). In this work, we have evaluated the response of selected pro-inflammatory mediators, after treatment of HGEC and HK-2 with Stx2 or SubAB. Cells were incubated with Stx2 (0.001 - 10 ng/ml) or SubAB (0.1 - 1000 ng/ml) for 24 h. Cultures supernatant were collected and IL-6, IL-8 and TNF-α were quantified by ELISA. TNF-α biological activity was evaluated by a cytotoxicity assay on L929 cell line. SubAB caused a decrease in IL-6, IL-8 and TNF-α released by both, HGEC and HK-2 and in a dose-dependent manner. On the contrary, Stx2 (0.01 and 0.1 ng/ml) increased these soluble mediators released by HGEC. TNF-α α, IL-8 and IL-6 were up regulated 3.3-fold, 0.4-fold and 0.3-fold, respectively, relative to those in untreated control cells. TNF-α biologically active was found by the decrease of L929 cell viability with supernatants of HGEC treated with 0.01 and 0.1 ng/ml Stx2 ($p< 0.05$, n=6). Stx2 did not cause a significant modulation on the release of IL-6, IL-8 and TNF-α by HK-2. These results suggest that toxins could have different effects on the inflammatory responses triggered in the kidney. Such interplay may have important consequences for the pathogenesis of disease during infection with STEC strains that produce both toxins.

752 (633) **CARDIAC BIOENERGETICS AND MITOCHONDRIAL PATHWAYS IN A RAT MODEL OF LOW AND SEVERE ENDOTOXEMIA.**

Tamara Antonela Vico[1,2], Virginia Vanasco[1,2], Timoteo Marchini[1,2], Juan S. Adán Areán[2], Pablo Evelson[1,2], Silvia Alvarez[1,2]
[1]Instituto de Bioquímica y Medicina Molecular (UBA - CONICET). [2]Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires

Despite being the major source of energy in the cell, mitochondria participate in multiple cell signaling pathways. Besides, mitochondria dysfunction is suggested to play a central role in inflammatory pathologies like metabolic, cardiovascular and neurological diseases, and also in acute endotoxemia and sepsis. The aim of this study was to analize cardiac bioenergetics and mitochondrial function in low and severe endotoxemia. Female Sprague-Dawley rats (45 days old) were separated in three experimental groups and i.p. injected with a single dose of LPS (0.5 or 8 mg/kg) or vehicle. After 6 h of treatment, mitochondria were isolated from left ventricle. O_2 uptake in state 4 (rest respiration state) and state 3 (active state) was measured and a 15% and 24% decrease in state 3 was observed in LPS 0.5 and LPS 8 mg/kg groups, respectively (Control: 74.8±22 ng-atO/min.mg protein). Complex I-III activity showed a 15% decrease at the highest dose of LPS (Control: 92.1±14 nmol/min.mg protein). Complex II-III and IV activities showed no statistical differences. ATP production was observed decreased by 33% at the highest dose (Control: 186.2±55 nmol ATP/min.mg protein, $p<0.05$). The calculated P/O ratios were 2.8; 2.2 and 2 for control, LPS 0.5 and 8 mg/kg; respectively. Mitochondrial membrane potential was measured by flow cytometry and showed a 17% decrease in LPS 8 mg/kg dose (Control IFM: 234±25). To evaluate NO role in this inflammatory processes, iNOS and eNOS expression were measured by Western Blott in left ventricle tissue, showing an increase in both enzyme expressions for LPS 8 mg/kg treatment. These results correlated with nitrite/nitrate levels in plasma. These results suggest that mitochondrial dysfunction occurs in different levels of endotoxemia, setting the mitochondria as a potential therapeutic target to treat inflammatory diseases.

753 (700) **SIRTUIN MODULATORS: LIMITING THE SENESCENCE ASSOCIATED SECRETORY PHENOTYPE IN RETINAL PIGMENT EPITHELIUM (RPE) CELLS**

Mariela C. Marazita[1], Pablo Tate[1], Melisa Marquioni-Ramella[1], Angela M. Suburo[1]
[1]Laboratorio de Medicina Molecular y Celular, IIMT-CONICET, Universidad Austral.

Cellular senescence triggers the expression of a wide variety of inflammatory factors named the senescence associated secretory phenotype (SASP). The SASP may contribute to diseases of aging by disrupting tissue structure and function. Age-related macular degeneration (AMD) is a progressive disease which leads to irreversible loss of vision. Cell senescence of the retinal RPE is suggested to play a central role in the etiology of AMD. We have previously showed that oxidative-stress induced senescence in RPE cells dysregulates the expression of factors linked to AMD progression, like IL-8, VEGF and CFH. We now hypothesized that sirtuins, NAD-dependent deacetylases and key regulators of cellular stress response and aging, may modulate the SASP in RPE. Aims: To evaluate the effect of nicotinic acid (NA), a NAD+ precursor, and caffeic acid (CAF) a polyphenol with anti-inflammatory properties, on Sirtuins and SASP. Methods: Human derived RPE cells (ARPE-19 line) were incubated with 200 μM H2O2 for 90 minutes during 3 consecutive days and then maintained in fresh culture medium for seven days to establish senescent cultures. These cultures were exposed to 1 mM NA, or 6 μg/ml CAF for 8 days. Cells were collected and RNAm levels for IL-8, Sirtuin 1

(SIRT1) and Sirtuin 3 (SIRT3) analyzed by quantitative real time PCR. Senescence was determined by positive staining for Senescence Associated β-gal activity and increased expression of p21 and p16 by western blot. Results: NA and CAF upregulated SIRT3 and SIRT1 respectively, compared to senescent cultures without treatment ($p < 0,05$). Both, NA and CAF, significantly decreased IL-8 mRNA levels, a hallmark of the SASP ($p < 0,05$). Neither of them reverted other senescent markers (SA-β-gal+, p21, p16). Conclusions: NA and CAF negatively regulate a key component of the SASP without altering the tumor suppresive activity of senescence. Targeting SIRT1/3 might be a novel approach to control the SASP in RPE cells

754 (758) ALTERED NEUTROPHIL CHEMOTAXIS IN THE PROGENY OF DIABETIC DAMS

Rocío Nahimé Magrini-Huaman[1,2], Juan Francisco Delgado[2], Bibiana Ruiz[2], Erica Ramis Pelegrina[2], Adriana Cañelas[2], Claudio Marcelo Larrea[2], Alejandro Tapia[1], Gabriela Egly Feresin[1], Héctor Coirini[2,3,4]

[1]Instituto de Biotecnología, Universidad Nacional de San Juan. [2]Facultad de Ciencias Médicas, Universidad Católica de Cuyo. [3]Instituto de Biología y Medicina Experimental. [4]Departamento de Bioquímica Humana, Facultad de Medicina UBA.

The uncontrolled hyperglycemia during pregnancy, increases oxidative stress on the progeny however, early treatment could reduce or reverse the produced effects of the reactive species generated. The inclusion in the diet of monounsaturated fatty acids has shown a positive effect in this regards. The aim of this study was to analyze the action of extra virgin olive (OL) and pistachio oil (PS) using corn oil (MZ) as control, on the inflammatory response at the peripheral level in adult offspring from mothers with experimental diabetes (DO) or from control mothers (CO). Rats were supplemented with oil from day 2 to 62 (8µl/15g body weight). At 4 months old, neutrophil chemotaxis in agarose and the expression of CD44 (an adhesion molecule expressed by these cells) by flow cytometry, were evaluated. Chemotaxis studies showed significant changes between CO/DO, indicating that the altered intrauterine conditions affect neutrophil migration regardless of the treatment or gender. The DO showed 35% higher migration than the CO ($p<0.05$). Sexual differences was also observed ($p<0.05$), female neutrophils showed 23% greater mobility than those from male rats. However treatment with oils showed no significant differences among groups. Regarding the expression of CD44, no significant changes between CO/DO groups or by gender were observed, but PS-treated animals had a 22% reduction ($p<0.05$) compared to MZ. Studies indicated that the experimental diabetes during pregnancy altered the migratory ability of neutrophils but not the CD44 expression. The increase in chemotaxis may be probably related to increased insulin production because DO become glucose intolerant. On the other hand the decrease in CD44 by PZ is reflecting a greater protective action of this oil than OL or MZ. (08CM13 UCCuyo, PIP0243 CONICET, Fund. Rene Barón).

755 (869) **SHORT-TERM ANTI-INFLAMMATORY EFFECTS OF 5,5-DIMETHYL-1-PYRROLINE N-OXIDE (DMPO)**

Marcos David Muñoz[1], Lucas J. Gutierrez[2], Daniel Ricardo Enriz[2], Sergio Eduardo Alvarez[3], Sandra Ester Gomez Mejiba[4], Dario Ceferino Ramirez[1]

[1]Laboratory of Experimental and Translational Medicine; IMIBIO-SL-CONICET-UNSL. [2]Laboratory of Medicinal Chemistry;I MIBIO-SL-CONICET-UNSL. [3]Laboratory of Signal Transduction IMIBIO-SL-CONICET-UNSL. [4]Laboratory of Experimental Therapeutics-IMIBIO-SL-CONICET-UNSL

5,5-dimethyl-1-pirroline *N*-oxide (DMPO) is a nitrone spin trap originally synthesized as a nitrone spin trap to study free radicals by electron spin resonance spectroscopy and recently by immuno-spin trapping. Herein we envisioned at studying what are the mechanisms involved in these anti-inflammatory effects of this old drug with new properties. To accomplish this goal we used a well known model of macrophage-like cells (RAW264.6) primed with LPS; which induce a well known MAPK signaling cascade that ends in activation of NF-kB—the master regulator of inflammation, inducible nitric oxide (iNOS) expression and nitric oxide synthesis. DMPO blocked NO synthesis, iNOS induction and MAPK signaling; but it did not affect LPS binding to LPS to membrane receptors. Thus we hypothesized that DMPO, and likewise other nitrones, may somehow affect very early LPS triggered signaling downstream of LPS-receptor binding. *In silico* data showed that DMPO binds to a very narrow sequence of aminoacids inside the TIR domain of TLR-2. TIR domains are conserved throughout TLRs (TLR-4; 6; 10) and species, particularly in a región called BB-loop which is responsible for downstream signal transduction. Molecular dynamics data shows that DMPO binds almost exclusively to these residues located at the BB-loop. Taking together, our data indicate that DMPO anti-inflammatory effect, is at least in part due to its binding to specific residues in the cytoplasmic portion of TLRs, thus further signaling is damped. Supported by PROICO 2-3214 & PICT-2014-3369 (to DCR), PROICO 10-0414 (To SEGM) and PIP2015-2017-112215-0100603CO (To DCR, SEA & SEGM).

756 (888) **IMMUNOGENICITY OF BOTHROPS ALTERNATUS VENOM IN HORSES AND LAYING HENS**

Mariano Gabriel Farace[1,2], Carlos Leonidas Leiva[1,2], Pablo Anibal Chacana[1], Mariano Fernández Miyakawa[1,2]

[1]Instituto de Patobiología, CICVYA, INTA. [2]Consejo Nacional Investigaciones Científicas Y TécnicaS (CONICET)

Bothrops alternatus (yarará) venom contains over 100 proteins of varying toxicity and snake bites are usually treated by the administration of antivenoms. These antisera are mainly produced from horses or sheep and, in the last years, also egg yolk antibodies (IgY) have been explored as an alternative due to its advantages regarding animal welfare and lower costs of production. Although this therapy is being used for more than 100 years, no relevant innovations have been made to optimize the quality of the antivenoms regarding their efficacy and safety. In fact, current immunization protocols are carried out by using whole venoms and do not consider differences in the immunogenicity of the components. Several studies revealed that most toxic venom components are not necessarily the most immunogenic, particularly those of low-molecular weight. The aim of this work was to compare the neutralizing activity and differential recognition of the venom proteins of antisera obtained from horses and hens. In order to determine differences in the efficacy between IgG and IgY-based antivenoms, *in vitro* neutralization of proteolytic, procoagulant and indirect haemolytic activity of *B. alternatus* venom was assayed. In all cases, horse antivenom showed higher neutralizing activity than IgY. Western blot analysis was done to assess if decreased neutralizing activity of IgY-antivenom was due to differential immune response of hens and horses to the components of the venom. Although most of the proteins were recognized by both antisera, some toxins from the venom were differentially detected by either IgY or horse IgG. Further identification of these proteins would allow optimizing the production of IgY or IgG-based antivenoms by using a rational mixture of antigens to immunize the animals. In this way, use of venom fractions or proteins as immunogens could improve antibody therapy by producing more protective antisera with less adverse effects.

757 (955) **IN VIVO NEUTRALIZATION OF BOTHROPS ALTERNATUS VENOM BY EGG YOLK ANTIBODIES**

Carlos Leónidas Leiva[1, 3], Mariano Farace[1, 3], Adriana Cangelosi[2], Virginia Mariconda[2], Natalia Casanova[1], Patricia Geoghegan[2], Luisa Brero[2], Mariano Fernández-Miyakawa[1, 3], Pablo Chacana[1]

[1]Instituto de Patobiología, CICVYA, INTA. [2]Centro Nacional de Control de Calidad de Biológicos, ANLIS-MALBRÁN. [3]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)

Therapy of snakebite envenoming is based on the use of antisera mainly produced in horses. An alternative to mammal poly-

clonal sera is the use of egg yolk antibodies due to its advantages regarding animal welfare and lower costs of production. In this work the efficacy of two immunization schedules was evaluated in order to optimize the production of IgY-based antivenoms against *Bothrops alternatus* (yarará). Two groups of laying hens were immunized via intramusuclar with *B. alternatus* whole venom three times every 15 days. The first group (A) received low doses of antigen (40 µg, 80 µg, 120 µg) while the second group (B) received higher doses (400 µg , 400 µg , 2400 µg). For the first immunization, the venom was emulsified with Freund`s Complete adjuvant and Freund`s Incomplete adjuvant was used for the subsequent boosters. Eggs were collected during 10 days after the last immunization. IgY antivenoms were obtained by ammonium sulphate double precipitation method and preserved using 0.01% (w/v) thimerosal. Protein concentration of the egg extracts, determined by the Bradford reagent method, was of 45 mg/mL for Group A and 25 mg/mL for Group B. *In vivo* neutralization efficacy assessed on a ED50-mouse model by mixing 1 LD50 with increasing volumes of IgY antivenom. The quantity of *B. alternatus* venom neutralized by 1 mL from both IgY antivenoms (A and B) was 0.158 mg. In conclusion, immunization of hens with either low or high sub-lethal doses of *B. alternatus* venom for a one-month period produced antivenoms with similar neutralizing potencies. Thus, lower doses of venoms could be used for immunization without diminishing the potency of the antivenom produced. In this way, IgY technology taken together with the optimization of immunization schedules may lead to improve conventional antivenom production methods.

758 (1094) **O-GLCNAC PARTICIPATION DURING INFLAMMATORY RESPONSE IN THP-1-LIKE MACROPHAGES**

Alexis Paulina Jiménez Uribe[1, 2], Lourdes Andrea Arriaga Pizano[2], Armando Isibasi[1, 2]
[1]Facultad de Química, Universidad Nacional Autónoma de México. [2]Unidad de Investigación Médica en Inmunoquímica, Hospital de Especialidades, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social.

Aim: To determinate the role of the postranslational modification O-GlcNAc during inflammatory response in THP-1-like macrophages.

Methods: THP-1 cell cultivated in RPMI medium suplemented with Bovine Fetal Serum and antibiotics were differentiated to macrophages with PMA 10ng/mL for 48 hours, once washed and differentiated, cells were stimulated with TLR ligands LPS (1mg/mL), PGN (1mg/mL), loxoribin (68mg/mL) or E. Coli DNA (1mcg/ml) for 1 hour to evaluate intracellular O-GlcNAc levels by flow cytometry. For intracellular distribution of O-GlcNAc and OGT evaluation by confocal microscopy, cells were activated with LPS (1mcg/mL) for 0, 5, 15, 30 and 60 minutes. To evaluate the O-GlcNAc participation in cytokine production, cell were preincubated with alloxan, wich decrease intracellular O-GlcNAc levels, and then stimulated with LPS (1mcg/mL) for 12 hours for cytokine determination by a bead-based immunoassay by flow cytometry.

Results: In response to TLR ligands there is no changes in intracellular levels of O-GlcNac, however, this postranslational modification chages their intracellular distribution in response to LPS, at 15 minutes O-GlcNAc appears in nucleus and the enzyme OGT, wich adds this sugar, seems to be in a perinuclear distribution. When intracellular O-GlcNAc levels are decrease due addition of alloxan, cells can not respond efficiently, and decrease IL-6, IL-8, TNF and MCP-1 production.

Conclusion: The postranslation modification O-GlcNAc modified their intracelular distribution in response to LPS, and is important in the induction of cytokine production.

759 (2010) **THE MICROENCAPSULATION OF THE FLAVONOID GENISTEIN WITH SOLUBLE CHITOSAN ALLOWS EFFICIENT RELEASE IN COLON AND ATTENUATION OF OXIDATIVE STRESS DURING EXPERIMENTAL COLITIS.**

Noelia Luciana Vanden Braber[1], Ivanna Novotny Núñez [2], Luciana Paola Bohl[1], Carina Porporatto[1], Mariana Angélica Montenegro[1], Silvia Graciela Correa[2]
[1]Centro de Investigaciones y Transferencia de Villa María (CIT VM-CONICET), Universidad Nacional de Villa María, Argentina. [2]Centro De Investigaciones en Bioquímica Clínica e Inmunología (CIBICI- CONICET), Universidad Nacional de Córdoba, Argentina.

Genistein (G) is a flavonoid with antioxidant, anti-inflammatory and anti-tumoral properties whose absorption occurs in the stomach and small intestine with significant loss of the bioactivity. The hypothesis of the present work was that upon microencapsulation, G could modulate the inflammatory process in the classic colitis model. Colitis was induced by administration of 3% (w / v) dextran sulfate sodium in drinking water for 5 days. G was microencapsulated (MCG) in water soluble chitosan (WSCh) by spray-drying. Female C57BL/6 mice were divided into 5 groups: Control, Colitis, Colitis+G, Colitis+MCG and Colitis+WSCh. G (0.08 mg per day), MCG (3.15 mg per day) and WSCh (3.07 mg per day) were administered orally for 7 days starting at the beginning of colitis induction and animals were clinically evaluated during 10 days. The disease activity index (DAI) was calculated through weight loss, consistency and bleeding stool. Colon was isolated and processed for pro and anti-inflammatory cytokine analysis and superoxide dismutase (SOD), catalase and myeloperoxidase (MPO) activities. Statistical analyses were performed by ANOVA/ Tukey. In MCG treated animals, the DAI was 70% lower than in Colitis group ($p<0.05$) while Colitis+G did not show significant improvements. Levels of IL-6, TNF-α and MCP-1 decreased with MCG administration ($p<0.05$) while a significant increment of IL-10 was found. Colitis+WSCh group showed no remarkable clinical recovery although a marked decrease in pro-inflammatory cytokines and oxidative stress was observed. SOD, catalase and MPO activities in Colitis+MCG group were comparable to Control. Together, the microencapsulation strategy allowed the delivery of the bioactive flavonoid to the colon with an effective attenuation of the inflammation as well as the recovery of the oxidative balance.

760 (2035) **GLYOXYLATE, A NEW MARKER OF TYPE-2 DIABETES, INDUCES NEUTROPHIL ACTIVATION**

Nahuel Rodriguez-Rodrigues[1], Luis Castillo[1], Daiana Martire-Greco[1], Veronica I. Landoni[1], Gabriela C. Fernandez[1]
[1]Instituto de Medicina Experimental (IMEX)-CONICET, Academia Nacional de Medicina, Buenos Aires, Argentina.

It has been shown that glyoxylate (Glx) levels become significantly elevated in a mouse model of diabetes and in type-2 diabetic patients, placing this new metabolic marker in the context of diabetes pathology. As diabetes has been associated with inflammation inducing micro- and macro vascular damage, we hypothesize that Glx may be involved in diabetic-derived inflammation. Moreover, PMN activation has been associated with tissue injury in different pathophysiological conditions. Therefore, our aim was to investigate the direct role of Glx in neutrophil (PMN) responses. We evaluated activation of isolated human PMN by Glx (10 µg/ml) measuring the up-regulation of CD11b and ROS generation with Dihydrorhodamine by flow cytometry, NETs formation by confocal microscopy and chemokinesis using a Boyden chamber. We also evaluated the adhesion of Glx-treated PMN to endothelial cells (HMEC-1) by measuring the activity alkaline phosphatase (AP) with the substrate p-nitrophenylphosphate (NPP) in the adhered PMN after extensive washing (Enzymatic Unit definition: 1 EU of AP=1 nmol of NPP/ min). We found an up-regulation of the adhesion marker CD11b after Glx treatment, associated with an increased chemokinesis and higher adhesion to endothelial cells (mean fluorescence intensity CD11b: Control= 293.7±9.0, Glx=469.1±76.0*; Nº of PMN migrated/field: Ctrol=10.1±1.6, Glx=22.4±2.2*; AP EU: Control=32.4±3.0, Glx=51.4±3.3*, *$p<0.05$). Additionally, incubation with Glx caused and increased in ROS and NETs production (% of DHR+ PMN: Ctrol=11.7±1.8, Glx=27.7±5.3*; NETs area (μm^2): Ctrol=1528±780.9, Glx=30761 ± 3362*, *$p<0.05$). Altogether, our results indicate that the increased levels of Glx observed in diabetes contribute to the inflammatory state present in this disease, and possibly to tissue injury, by causing PMN activation.

SESIÓN MULTIDISCIPLINARIA PARA ESTUDIANTES DE GRADO / UNGRADUETED STUDENTS MULTIDISIPLINARY SESSION

804 (351) **NOVEL LIGANDS OF THE HSP90-ATPASE DOMAIN AND THEIR EFECTS IN A PROSTATE CANCER CELL MODEL**

Fernando Federicci[1], María Fernanda Camisay[1,2], Sonia Alejandra De Leo[1,2], Mario Daniel Galigniana[1,3], Gisela Ileana Mazaira[1,2]

[1]Departamento de Química Biológica, FCEN, UBA. [2]IQUIBICEN – UBA. [3]IByME – UBA.

The activity and stability of several oncoproteins depend on the molecular chaperone Hsp90. Tumor cells are thought to be 'addicted' to Hsp90, such that most of the client proteins of the chaperone evade degradation and, therefore, cells avoid the cell death program. The inhibition of Hsp90 ATPase activity shows strong antitumor effects, and Hsp90 inhibitors seem to be the only chemotherapeutic agents capable to affect all cancer hallmarks. However, side effects are still an important concern. Previous studies showed that both resorcinol derivatives and Schiff bases of 2,4-dihydroxy-benzaldehyde or 5-chloro-2,4-dihydroxy-benzaldehyde, show good binding capacity to the ATPase domain of Hsp90, and reduced toxic effects. Here, we analyzed novel drugs based on those chemical frames as potential therapeutic agents. First, drugs were designed and analyzed by *in silico* molecular docking simulations. Then, the effect on Hsp90 ATPase activity in vitro, the viability of prostate cancer cell models, and inhibitory action on GR and AR nuclear translocation were assessed. Geldanamycin (GA), a known Hsp90 inhibitor, was always used as a positive control in all tests. A total of 13 compounds (named as S49 to S61) were assayed, and most of them confirmed the *in silico* predictions on their ability to inhibit Hsp90 ATPase activity. While cell treatment with GA prevented steroid receptor nuclear import, the synthetic drugs were not active in this regard. Nonetheless, compounds S49, S50, S52, S56, S57, S58, S60 and S61 showed inhibitory action on the viability of PC3 cells. The most significant effects were observed for S57 and S58, whose potency on cell viability was equivalent to that measured for GA. These properties could have pharmacological relevance since the lack of side effects such as steroid receptor inhibition is desirable. Moreover, the study provides novel insights to design more active and less toxic drugs.

761 (174) **CHANGES IN THE BRAIN REDOX STATUS IN A GLUTAMATE EXCITOTOXICITY MODEL**

Ailen Gala Hvozda Arana[1], Natasha Stephanie Janezic[1], Agustina Peverini[1], Claudia Gabriela Reides[1,2], Romina Mayra Lasagni Vitar[1,2], Nathalie Weichsler[1], Susana Fransisca Llesuy[1,2], Sandra Maria Ferreira[1,2]

[1]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Catedra de Química General e Inorgánica [2]IBIMOL, UBA-CONICET

Glutamate is an excitatory neurotransmitter in central nervous system. Glutamate excitotoxicity is thought to play an important role in neuronal damage. The aim was to evaluate changes in redox homeostasis in brain of rats subject to an experimental model of glutamate excitotoxicity.The experimental model consisted of one group (n=6) injected ip with 1g glutamate/kg weight (GG) at days 1, 5 and 9 and control group (CG) injected with saline solution (n=6) at same days. The following markers were evaluated in brain homogenates: the activities of superoxide dismutase (SOD), catalase (CAT) thioredoxin reductase (TR), glutathione peroxidase (GPx), glutathione transferase (GT) glutathione reductase (GR), and glucose-6-phosphate dehydrogenase (G6P), protein oxidation (PO), lipid damage (TBARS), ascorbic acid concentration (AA), and glutathione (GSH) levels. SOD activity was 5.90±0.10 U/mg protein in GG (CG 4.63±0.17 U/mg protein $p<0.05$), CAT levels were 0.35±0.06 pmol/mg protein in GG (CG 0.18±0.02 pmol/mg prot $p<0.05$), TR was 9.4±1.5 nmol/min.mg protein in GG (CG 7.1±0.4 nmol/min.mg protein $p<0.05$). GT in GG was 0.038±0.004 U/mg protein (CG 0.028±0.001 U/mg protein $p<0.05$). GR in GG was 22.6±1.0 nmol/ min.mg protein (CG 19.6±1.2 nmol/min.mg protein $p<0.05$). G6PD was 0.017±0.001 U/min.mg protein for GG (CG 0.014±0.001 U/min.mg protein $p<0.05$). PO was 6.30±0.02 nmol/mg protein for GG (CG 5.07±0.05 nmol/mg protein $p<0.05$). AA was 64±18 mM for GG (CG 160±3 mM $p<0.01$). GSH concentration was 0.32±0.04 mmol/g organ for GG (CG 0.43±0.04 mmol/g organ $p<0.05$). No significant differences were found in TBARS levels and GPx activity. The brain is vulnerable to oxidative stress in glutamate excitotoxicity model. The decrease in non-enzymatic antioxidants and a compensatory up-regulation of antioxidant enzymes activities may be a consequence of an increase in oxidative process. The activities of enzymes associated with GSH metabolism were increased suggesting a response to GSH decay.

762 (177) **CHARACTERIZATION OF ANTIOXIDANT ACTIVITY OF AQUEOUS EXTRACTS FROM GINKGO BILOBA**

Agustina Peverini[(1)], Claudia Gabriela Reides[(1,2)], Ailen Gala Hvozda Arana[(1)], Natasha Stephanie Janezic[(1)], Romina Mayra Lasagni Vitar[(1,2)], Sandra Maria Ferreira[(1,2)], Susana Francisca Llesuy[(1,2)],

[1]Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Cátedra de Química General e Inorgánica. [2]IBIMOL. UBA-CONICET

Growing interest in natural antioxidants has developed due to a need for more effective, less toxic and cost effective antioxidants, and medicinal plants appear to have these desired advantages. *Ginkgo biloba extract* has been claimed effective in a variety of disorders associated with neurodegeneration like Parkinson, dementia, Alzheimer and glaucoma. The aims of this work were to evaluate the antioxidant capacity and the effect on lipid-peroxidation of aqueous extracts from *Ginkgo biloba*. Infusion and decoction of 5 % w/v were prepared and brain homogenates were used to determine the effect on lipid peroxidation. Antioxidant activity was assessed by 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), and 2,20-azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid radical (ABTS) scavenging activity. Decoction displayed significant higher values than infusion in terms of DPPH ($p<0.05$) and ABTS ($p<0.05$). The total polyphenol (TP) and total flavonoids (TF) contents were also evaluated. Decoction showed significant higher values in terms of TP and TF (TP(µmol gallic acid/g)= 49.3 ± 1.7 for infusion and 54.8 ± 2.2 for decoction, $p<0.05$; TF(µmol gallic acid/g)= 6.8 ± 1.3 for infusion and 7.9 ± 0.9 for decoction, $p<0.01$). In addition, infusion and decoction showed a strong inhibition of lipid-peroxidation of brain homogenates measured as thiobarbituric acid-reactive products of lipid-peroxidation (TBARS). The concentration required to decrease 50 % of TBARS levels in the absence of additives (IC50) was calculated. Decoction produced a higher percentage of inhibition than infusion (IC50= 109 ± 2 µg/mL for infusion and 59 ± 1 µg/mL for decoction, $p<0.01$). The results obtained in the present study indicate that aqueous extract of *Ginkgo biloba* exhibit antioxidant properties and a protective effect on lipid-peroxidation process. Therefore, they could be use as a source of natural antioxidants in the treatment of several diseases associated with oxidative stress damage.

763 (186) **ANGIOTENSIN II INDUCE EMT THROUGH AKT ACTIVATION. ANGIOTENSIN 1-7 INHIBIT ANGIOTENSINA II INDUCED TUMORIGENIC FEATURES IN BREAST CANCER CELLS**

Melisa Del Valle Suberbordes[1], Nadia Cambados[1], Thomas Walther[2], Carolina Schere Levy[1],

[1]Instituto de Fisiología, Biología Molecular y Neurociencias (IFIByNE)-CONICET, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires [2]Medizinische Fakultät Kinderchirurgie-Fetalzentrum, Universität Leipzig, Alemania.

Angiotensin II (AngII), the main effector peptide of the Renin Angiotensin System, has been implicated in multiple aspects of cancer progression. Angiotensin (1-7) [Ang (1-7)], is a biologically active heptapeptide, generated predominantly from AngII that counterbalances AngII actions in different pathophysiological

settings. The porpoise of this study was to analyse the impact of Ang-(1-7) on AngII-induced pro-tumorigenic features on normal mammary epithelial cells and breast cancer cells. We found that AngII promoted EMT on normal mammary epithelial cell NMuMG, epithelial markers such as E-cadherin were inhibited (Agni vs C, p<0.001) while mesenchymal markers such as fibronectin, N-cadherin and a-SMA were enhanced (AngII vs C, p<0.001). In contrast, Ang-(1-7) was unable to induce changes on EMT markers. Nevertheless, Ang-(1-7) completely abolished AngII induced EMT changes when both peptides were simultaneously added to the cell culture (AngII vs AngII+Ang-(1-7), p<0.001). We also found that in the presence of an AKT inhibitor the expression of the EMT biomarkers were partially restored to values near to control suggesting that AKT phosphorylation is at least in part necessary for AngII induced EMT (N-Cad and Fib, P<0.001, a-SMA *P*<0.01 and E-Cad *P*<0.05). In addition, Ang-(1-7) also abolished the invasion induced by AngII on breast cancer cells (AngII vs AngII+Ang-(1 7), P<0.01). Interestingly, cotreatment with Ang-(1-7) and AngII significantly reduced AngII induced VEGF and MM-9 expression levels in breast cancer cells (P <0.001). We conclude that Ang-(1-7) counteracts tumor aggressive signals triggered by AngII in breast cancer cells emerging as a potential therapy to prevent breast cancer progression

764 (503) **OXIDATIVE AND INFLAMMATORY RESPONSE OF RAW 264.7 MURINE MACROPHAGES EXPOSED TO AIR POLLUTION PARTICULATE MATTER**

Lourdes Cáceres[1], Mariela Paz[2], Mariana Garcés[1], Diego Ojeda[3], Deborah Tasat[4], Daniel González Maglio[2], Timoteo Marchini[1], Pablo Evelson[1]

[1]Universidad de Buenos Aires. CONICET. Instituto de Bioquímica y Medicina Molecular (IBIMOL). Facultad de Farmacia y Bioquímica. Buenos Aires, Argentina [2]Universidad de Buenos Aires. CONICET. Instituto de Estudios de la Inmunidad Humoral (IDEHU). Facultad de Farmacia y Bioquímica. Buenos Aires, Argentina [3]Universidad de Buenos Aires. CONICET. Instituto de Investigaciones Biomédicas en Retrovirus y SIDA (INBIRS). Facultad de Medicina. Buenos Aires, Argentina [4]Universidad Nacional de General San Martín. Escuela de Ciencia y Tecnología. Buenos Aires, Argentina.

The exposure to environmental particulate matter (PM) is associated with increased cardiorespiratory morbidity and mortality rates. Alveolar macrophages are suggested to play a central role in this scenario, triggering both inflammation and oxidative stress following PM inhalation. However, the pathways linking these responses remain uncertain. In order to address such mechanisms, we studied the murine macrophage cell line RAW 264.7 after an exposure to different PM samples, Residual Oil Fly Ash (ROFA) and Concentrated Ambient Particles (CAPs), at 0, 50, 100, and 200 µg/mL for 24 h. Intracellular redox status was assessed by dichlorofluorescein fluorescence, which was increased in a dose-dependent manner by up to 73% and 71% at 200 µg/mL of ROFA and CAPs, respectively (control: 16 ± 5 (x10^3) AU, p<0.05). Oxidative damage to lipids was evaluated as thiobarbituric acid reactive substances (TBARS) by a fluorometric assay, finding a dose-dependent increase by up to 356% and 144% at 200 µg/mL of ROFA and CAPs, respectively (control: 0.29 ± 0.03 mmol TBARS/mg protein, p<0.01). Nitric oxide production was indirectly determined as nitrite content in cell culture supernatants by the Griess assay, finding a dose-dependent increase by up to 37% at 200 µg/mL of ROFA (control: 3.9 ± 0.3 nmol NO_2^-/mg protein, p<0.01), while no significant differences were observed after CAPs incubation. Moreover, cell culture supernatants from both ROFA- and CAPs-exposed cells showed increased levels of TNF-α and IL-6. Given that ROFA and CAPs differ in elemental composition, PM chemistry might be determinant for the reported effects. These findings suggest that alveolar macrophages are in part responsible for the oxidative and inflammatory response observed after the exposure to environmental PM, and therefore contribute to the understanding of the cellular pathways triggered by PM inhalation.

765 (662) **AN EARLY ANTIOXIDANT RESPONSE IS GENERATED BY HUMAN CONJUNCTIVAL EPITHELIAL CELLS AFTER DIESEL EXHAUST PARTICLES (DEP) EXPOSURE**

Natasha Stephanie Janezic[1], Romina Mayra Lasagni Vitar[1, 2], Julia Tau[3], Ailen Gala Hvozda Arana[1], Agustina Inés Tesone[3], Agustina Peverini[1], Claudia Gabriela Reides[1, 2], Alejandro Berra[3], Sandra María Ferreira[1, 2], Susana Francisca Llesuy[1, 2]

[1]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Química General e Inorgánica. [2]IBIMOL, UBA-CONICET [3]Universidad de Buenos Aires, Facultad de Medicina, Departamento de Patología, Laboratorio de Investigaciones Oculares.

The exposure to airborne particulate matter shows adverse health effects in urban population. The aim of the present study was to evaluate oxidative stress markers in human conjunctival epithelial cells (IOBA-NHC) after the incubation with diesel exhaust particles (DEP) for 1 hour. The incubation was performed with DEP at concentrations of 50 and 100 µg/mL for 1 hour and the following parameters were evaluated: glutathione S-transferase (GST), glutathione reductase (GR), thioredoxin reductase (TrxR) and glucose 6P-deshidrogenase (G6PDH) activity; reduced glutathione (GSH); lipid and protein oxidation. One-way ANOVA test and Dunnett's test as post hoc test were used for statistical analysis. The cells exposed to 50 and 100 µg/mL of DEP showed a significant increase in the activity of GST (74% and 99%, respectively, p<0.01), GR (60% and 46 %, respectively, p<0.01) and TrxR (124% and 108%, respectively, p<0.01), compared to the control group. DEP100 group displayed an increase in G6PDH activity (67%, p<0.05), meanwhile DEP50 group tended to an increasing but it was not significant comparing to the control group. GSH levels exhibited an elevation in DEP100 group (39%, p<0.05). DEP 50 and DEP 100 groups also presented an increase in lipid oxidation (66% and 79%, respectively, p<0.05) meanwhile protein oxidation showed no significant difference among all groups. The increase in antioxidant enzymes activities as well as the incremented GSH levels could be an early adaptive response to an increasing pro-oxidant environment triggered by DEP exposure, previously reported. This antioxidant response generated by the cells seems to be enough to avoid oxidative damage to proteins but not to lipids, suggesting that the last ones are the earliest target of damage. Oxidative stress could play an important role in the development of DEP effects on human conjunctival epithelial cells.

766 (979) **ANGIOTENSIN II PROMOTES ACTIVATION OF MTOR PATHWAY COMPONENTS IN H295R ADRENOCORTICAL CELLS.**

Katia Estefanía Helfenberger[1], Ana Fernanda Castillo[1], Ana Fiore[1], Paola Finocchietto[2], Ernesto J. Podesta[1], Cecilia Poderoso[1]

[1]INBIOMED (UBA-CONICET). Departamento de Bioquímica Humana. Facultad de Medicina. Universidad de Buenos Aires [2]INIGEM (UBA-CONICET). Laboratorio del Metabolismo del Oxígeno. Hospital de Clínicas. Facultad de Medicina. Universidad de Buenos Aires

Primary aldosteronism (PA) is one of the most common causes of endocrine hypertension, accounting for 5 to 10% of hypertensive patients. It is characterized by autonomous excessive aldosterone secretion by the adrenal gland. In particular, studies have focused on the PI3K/AKT/mTOR pathway, which has been described to be overactive in adrenal carcinoma and other tumor types. While the activation and participation of this pathway in hypersecretion of aldosterone by zona glomerulosa has been described, a possible hormonal regulation of this pathway is not known in adrenal gland up to date. It has been described that the enzyme ACSL4 (acyl-CoA synthetase 4) modulates mTOR pathway, promoting phosphorylation of its components (AKT, p70 kinase, S6 ribosomal protein, mTOR complex) in breast cancer cells. ACSL4 is also involved in physiological steroid production, including aldosterone synthesis. Then, the aim of this work is to study the mechanisms

involved in the modulation of the mTOR pathway in human adrenocortical cells and a possible role of ACSL4 in this process. We used human adrenocarcinoma H295R cells described as a model of PA, capable to respond to Angiotensin II (Ang II) stimulation. We observed by immunoblot that Ang II promotes AKT phosphorylation (pAKT) in serine 308, which occurs upstream mTOR complex activation, in a time-dependent manner (two-fold increase after Ang II 1h). Ang II stimulation elicits a significant increase of phospho-S6 (Ang II 30 min: 1.4 **p<0.01; 1h: 2.4; 2h: 2.9; 4h: 2.6; ***p<0.001, relativized to control) as well as AKT phosphorylation in threonine 473 (one-fold increase after Ang II 30min), indicating downstream activation of mTOR. Inhibition of ACSL4 with Rosiglitazone decreased S6 phosphorylation by 80% even in the presence of Ang II. These results highlight the importance of hormonal regulation of the mTOR pathway in adrenocortical cells and a role for ACSL4 suggesting a possible relationship to the development of PA.

PRESENTACIÓN DE POSTERS SAI IV / SAI POSTERS PRESENTATION IV

HIPERSENSIBILIDAD/HYPERSENSITIVITY

767 (611) **IDENTIFICATION OF CROSS-REACTIVE B-CELL EPITOPES BETWEEN GLY M 5.0101 [GLYCINE MAX] AND á-CASEIN [BOS TAURUS] BY EPITOPE MAPPING - MASS SPECTROMETRY.**

Ángela María Candreva[1,2], Mario Ferrer-Navarro[3], Silvia Bronsoms[4], Alejandra Quiroga[1], Renata Curciarello[2], Silvana Petruccelli[1], Guillermo Horacio Docena[2], Sebastián Alejandro Trejo[4,5].

[1]Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina. [2]Instituto de Estudios Inmunológicos y Fisiopatológicos (IIFP)- CONICET, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina. [3]Institut de Biotecnologia i de Biomedicina, Universitat Autònoma de Barcelona (IBB-UAB), Barcelona, Spain. [4]Servei de Proteòmica I Biologia Estructural (sePBioEs) de la Universitat Autònoma de Barcelona, Barcelona, Spain.

Cow's milk proteins are one of the most common causes of food allergy and intolerance in a restricted proportion of IgE-mediated milk allergic patients treated with soy formulae, due to the cross-allergenicity previously described. We aimed to characterize cross-reactive epitopes between prevalent soy and milk allergens using mass spectrometry tools. A bovine casein-specific monoclonal antibody (1D5 mAb), purified milk caseins (Bos d 8) and the recombinant soy allergen Gly m 5.0101 (rGlym5) were employed to identify linear epitopes by MALDI-TOF MS. The 1D5 mAb was immobilized onto magnetic beads and incubated with the peptide mixture previously obtained by enzymatic digestion of both allergens. The MALDI-TOF MS analysis was performed to compare bound and unbound peptide profiles. Finally, mapped peptides were sequenced de novo by MALDI-TOF to identify dominant epitopes. We showed that casein-specific 1D5 mAb recognized 4 peptides on áS1-casein and 3 cross-reactive epitopes in rGlym5 of mass: 2260.2 Da (residues 125to142); 1993.2 Da (residues 367to385) and 1864.0 Da (residues 527to543).The alignment of the sequences of these cross-reactivity peptides and peptides of áS1-casein, using the study of occurrence frequency analysis of this epitopes residues (by WebLogo), enables us to identify 4 critical residues charged and neutral (polar and non-polar) for 1D5 mAb binding. In conclusion, this method identified common sequential B epitopes and critical aminoacidic between allergens with no phylogenetic relationship. These findings may be relevant for potential immunotherapies for patients and led us to understand the clinical intolerance of milk allergic patients that are exposed to soy formula as dairy substitute during treatment.

768 (504) **TOXOPLASMA GONDII CHRONIC INFECTION PREVENTS THE DEVELOPMENT OF ATOPIC DERMATITIS.**

Matías Damián Perrone Sibilia[1], María de los Ángeles Aldirico[1], Ariadna Soto[1], Mariano Sergio Picchio[1], Vanesa Roxana Sanchez[1], Nadia Arcon[1], Florencia Magalí Giorgio[1], Valentina Martin[1], Silvia Vanzulli[2], Ignacio Martín Fenoy[1], Alejandra Goldman[1].

[1]Laboratorio de Inmunología, Vacunas y Alergia - CESyMA - ECyT - UNSAM [2]IIHEMA - Academia Nacional de Medicina.

We previously showed that *T. gondii* infection diminishes the susceptibility to develop experimental asthma. The parasite immunomodulatory ability extends to systemic level. Hence, these results suggested that this protozoan infection could modulate other allergic disorders. The aim of the present work was to study whether *T. gondii* infection also modulates the development of atopic dermatitis. One month before allergic sensitization, adult BALB/c mice were orally infected with *T. Gondii* cysts. Mice were epicutaneous sensitized with OVA (TDA) or PBS (T). Treatment was repeated twice with a 2-week resting period between each sensitization. Controls include non-infected mice sensitized with OVA (DA) and non-infected mice treated with PBS (N). The analysis was performed one day after the last week of sensitization. Skin and blood samples were collected to evaluate histopathology, and OVA-specific IgE, IgG1 and IgG2a antibodies respectively. *Ex vivo* stimulation of splenocytes with OVA was performed and supernatant IL-4, IL-5 and IFN-gamma cytokine levels were evaluated. Allergic mice showed pathological changes characteristic of atopic dermatitis, compared with normal mice. These changes include epidermal hyperplasia, hyperkeratosis and infiltration of inflammatory cells in the dermis and epidermis. Mice infected with *T. gondii* before allergic sensitization showed a histopathology similar to normal mice, with no epidermal hyperplasia, orthokeratosis and poor inflammatory infiltrate in the dermis and epidermis. A trend to decreased OVA-specific IgE and significant diminished IgG1 levels in TDA compared to the DA group were detected ($p<0.05$). No differences were observed in IgG2a levels. *T. gondii* infection also induced a reduction in both OVA specific Th2 IL-4 ($p<0.001$) and IL-5 ($p<0.01$) and Th1 IFN-gamma levels ($p<0.01$). These results show that, in addition to lung airway inflammation, chronic *T. gondii* infection can suppress atopic dermatitis.

769 (727) **ACETYLCHOLINE REINFORCES THE POLARIZATION OF DENDRITIC CELLS TOWARD A TH2-PROMOTING PROFILE INDUCED BY TSLP THROUGH MUSCARINIC RECEPTORS.**

Maria Soledad Gori[1], Luciana Moverer[1], Florencia Sabbione[2], Carolina Jancic[2], Walter Scordo[4], Nadia Towstyka[2], Julieta Alcain[1], Jorge Geffner[3], Mónica Vermeulen[1], Gabriela Salamone[1].

[1]Laboratorio de Células Presentadoras de Antígeno y Respuesta Inflamatoria, IMEX CONICET – Academia Nacional de Medicina. [2]Laboratorio de Inmunidad Innata, IMEX CONICET – Academia Nacional de Medicina. [3]INBIRS, CONICET – UBA. [4]Servicio de Medicina Transfusional, Hospital Italiano de Buenos Aires.

Acetylcholine (ACh) is the most important parasympathetic neurotransmitter and increasing evidence indicates that it is able to modulate the immune response. We have previously shown that dendritic cells (DC) express muscarinic receptors, as well as the enzymes responsible for the synthesis and degradation of ACh. Then, we proved that ACh polarizes DC toward a Th2-promoting profile, increasing OX40L expression and moreover, reinforces the expression of OX40L and CD83 on DC, as well as IL-8 and TNF-α production induced by the Th2-promoting cytokine, thymic stromal lymphopoietin (TSLP). This molecule plays a key role in the induction and maintenance of allergic responses at the epithelial surfaces. In this work, we evaluated which cholinergic receptors (muscarinic or nicotinic) were involved in the promotion of TSLP-mediated effects induced by ACh on DC. For this, CD14+ cells were isolated from peripheral blood mononuclear cells of

healthy adult nonsmoker donors by positive selection (Miltenyi) and obtained monocytes were cultured for 5 days with GM-CSF+IL-4. DC were pre-incubated for 30 min with or without non-selective muscarinic (atropine, AT 10-7M) and nicotinic (mecamylamine, MM 10-7M) receptor antagonists. Then, DC were cultured for 18 h with ACh (10-8M), TSLP (15 ng/ml), or ACh plus TSLP. We showed that AT, but not MM, significantly inhibited the enhancing effect induced by ACh on the ability of TSLP to increase OX40L ($p<0.05$) and CD83 ($p<0.05$) expression, as well as the production of TNF-α ($p<0.01$) and IL-8 ($p<0.0001$). Surprisingly, AT inhibited the OX40L and CD83 expression induced by TSLP, suggesting the modulation of the DC cholinergic system by this cytokine. These results show the involvement of muscarinic receptors in the polarization of DC toward a Th2-promoting profile induced by both TSLP and ACh, suggesting that anticholinergics could also protect the airway in the course of inflammatory chronic diseases by the inhibition of this DC profile.

770 (317) **HISTAMINE RECEPTORS H3/H4 INVOLVEMENT IN A MOUSE MODEL OF DERMATITIS.**

Julieta Alcain[1, 2], Soledad Gori[1], Gabriela Salamone[1], Mónica Vermeulen[1].
[1]Laboratorio de Células Presentadoras de Antígeno y Respuesta Inflamatoria. IMEX-CONICET, Academia Nacional de Medicina (ANM). [2]Departamento de Fisiología y Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales (FCEN), UBA.

Dermatitis is an inflammatory disease characterized by an imbalance of the immune response toward a Th2 profile, whose main inflammatory mediator is histamine (HIS). HIS exerts its actions through four receptors (H1-H4). Previously, we demonstrated, using a model of pulmonary inflammation to OVA, that HIS is able to induce the recruitment of CD8+ T lymphocytes in the lung of mice. The purpose of the present work was to study the modulatory role of HIS in another inflammatory disease affecting the skin, contact dermatitis, and the role of H3 and H4 receptors. For this, we developed a mouse model of dermatitis using the irritating agent dinitrophenyl benzene (0.7% DNFB in acetone/water). For this, 20 ìl of DNFB (or vehicle) was administered for 4 consecutive days on both ears, along with 20 ìl of a H3/H4 receptor antagonist (thioperamide, Thio, 10-7M) on the right ear. Seven days after the first administration, DNFB (0.2% in PBS) was administered intraperitoneally in all mice. Topical treatment of the injured zone with Thio partially reversed the symptoms of the disease associated with inflammation in the application area (Ct, 0.267 mm ± 0.033 mm; DNFB, 0.667 mm ± 0.044 mm; DNFB+Thio, 0.416 mm ± 0.072 mm. $p<0.001$, n=6), IFN-ã and IL-5 production by T lymphocytes, withouth affecting T cell proliferation in a mixed allogenic reaction. Interestingly, the application of Thio at the site of injury stimulated CD11b+ CD11c- cell recruitment (Ct, 6.740 % ± 0.7153%; Thio, 4.167% ± 1.014%; DNFB, 7.200% ± 1.480%; DNFB+Thio 37.67% ± 11 95%. $p<0.001$, n=4) and inhibited CD11c+ CD11b- cell recruitment (Ct, 9.767% ± 1.071%; Thio, 11.50% ± 1.500%; DNFB, 13.73% ± 2.019%; DNFB+Thio, 8.233% ± 1.660%. $p<0.05$) in the zonal lymph node. In conclusion, these results could indicate that the use of an antagonist of H3/H4 receptors could reverse the effects induced in an inflammatory disease such as dermatitis through the inhibition of both Th2 response and epithelial swelling.

771 (951) **ALLERGENIC COMPONENTS AND CROSS-REACTIVITY BETWEEN BETA VULGARIS AND OTHER CHENOPODIACEAE POLLEN EXTRACTS.**

Marcelo Javier Gálvez[1], Andrea Bianchimano[1], Adriana Martínez[1], María Gabriela Murray[1], María Inés Prat[1].
[1]INBIOSUR (CONICET-UNS).

Chenopodiaceae pollen has been recognized as an important allergen source causing pollinosis. In this regard, *Chenopodium album* and *Salsola kali* have been widely studied. On the other hand, *Beta vulgaris* allergenic potential and its antigenic relationship with other species of the same family have not been well known. Moreover, this species is not included in the clinical diagnosis. The aim of this study was to determine the allergenicity of *B. vulgaris*, the main components involved and their relationship with other Chenopodiaceae species, which may lead to better diagnosis and immunotherapy. The IgE reactivity and allergen profile of *B. vulgaris* were studied by western blot. A pooled sera from individuals with allergic symptoms, positive skin prick tests and specific IgE against the three species determined by ELISA was used. ELISA and western blot inhibition test were employed to evaluate the IgE cross-reactivity between *B. vulgaris* and other Chenopodiaceae. In these cases, the pooled sera was pre-treated with each pollen extract. *B. vulgaris* allergen profile (15-76 kDa) presented an immunodominant component of 45 kDa. The inhibition curve obtained by ELISA showed that 50 % inhibition coefficient of pooled sera pre-treated with serial dilution of *B. vulgaris, S. kali or C. album* extract were 0.24 ìg/ml, 0.08 ìg/ml and 0.94 ìg/ml respectively. The western blot inhibition studies detected IgE cross-reactivity among the species. Particularly, the 45 kDa band was better inhibited by *S. kali* extract than *C. album*, despite the greater taxonomical relationship of this last. The presence of specific IgE against *B. vulgaris* in sensitized individuals demonstrates its allergenicity. Since the degree of cross-reactivity varies among Chenopodiaceae care should be taken when selecting individual extracts or mixtures for diagnosis and immunotherapy. Future studies on the characterization of *B. vulgaris* allergens allow justify their usefulness in laboratory and clinical routine.

INMUNOLOGÍA CLÍNICA, INMUNODEFICIENCIAS Y AUTOINMUNIDAD / CLINICAL IMMUNOLOGY, IMMUNE DEFICIENCY AND AUTOIMMUNITY

772 (132) **ANALYSIS OF CLINICAL AND IMMUNOLOGICAL FINDINGS IN CHILDREN WITH SYSTEMIC LUPUS ERYTHEMATOSUS.**

Georgina Roffé[1], Martin Alejandro Nazr Usandivaras[1], Mayra Etcheverry[2], Hector Quiroz[1], Jeannette Balbaryski[1], Eduardo Gaddi[1].
[1]División Inmunología, Hospital General de Niños "Pedro de Elizalde". Buenos Aires, Argentina [2]Servicio de Reumatología, Hospital General de Niños "Pedro de Elizalde". Buenos Aires, Argentina.

Systemic lupus erythematosus (SLE) is an autoimmune pathology characterized by periods of active or inactive disease, and an observed prevalence of 0.02% in pediatric population. During the evolution of the disease changes in humoral and cellular immune parameters, including lymphocytes subsets, are observed. Our aim was to study changes observed in different immune parameters during the follow-up of pediatric SLE. A group of 20 children with SLE, with different schemes of immunosuppressive therapy was studied. Serum immunoglobulin, C3, C4 complement components, and different autoantibodies were evaluated. Naïve (N), central memory (CM), regulatory (Tregs) T lymphocytes, and N, memory (M), no-swichted M (NSM), class-swichted M (CSM) and double negatives (DN) B lymphocytes percentage levels, were also evaluated by flow cytometry. Same parameters were studied in 20 healthy children (Co). A significant ($p<0.05$) increased of IgM and IgA serum levels, and decreased levels of C3 and C4, (119 mg/dL vs 50 mg/dL, 223 mg/dL vs 148 mg/dL), and (82 mg/dL vs 100 mg/dL, 13 mg/dL vs 25 mg/dL,) respectively, was recorded in children with SLE in comparison with Co group. Patients with SLE presented also a significant ($p<0.05$) lower percentage level of LTCD4 N (CD45RA+CD62L+) (42,6% vs 54,2%), with higher CM (CD45RA-CD62L+) (47,6% vs 29,3%) and Tregs (CD4+CD25++CD127-) (12,5% vs 5,2%), compared with Co group. A significant increase of M B cells (CD19+CD27+), and the subsets CSM (IgD-CD27+) and DN (IgD-CD27-), (35.1% vs 24.6%, 25,4% vs 14,3 and 9.4% vs 4.7%), respectively, was recorded in patients with SLE. Conversely, diminished percentage levels of N (IgD+CD27-) B cells, in comparison with Co was also observed: (55,4% vs 68,1%). Humoral and cellular changes present in SLE patients could be result of combined effect of the

continuous but variable immune activation and the action of immunosuppressive therapies.

773 (135) **EVALUATION OF THYROID FUNCTION IN HIV-PEDIATRIC PATIENTS INFECTED BY VERTICAL TRANSMISSION.**

Estefania Capecce[1], Georgina Roffé[1], Claudia Insua[2], Eduardo Gaddi[1], Graciela Barboni[1].
[1]División Inmunología, Hospital General de Niños "Pedro de Elizalde". Buenos Aires, Argentina. [2]División Endocrinología, Hospital General de Niños "Pedro de Elizalde". Buenos Aires, Argentina.

HIV infection is a chronic pathology in which many alterations of endocrine system were reported. Such abnormalities are consequences of direct viral action, opportunistic infections, and antiretroviral therapy. Several studies have attempted to establish an association between different study parameters of thyroid function and progression of HIV infection, while others indicate that frequency of thyroid disease in adult patients HIV (+) is the same as in the general population (4.3 to 9.5%). Our aim was to describe the prevalence and characteristics of thyroid abnormalities in a cohort of children HIV (+). A descriptive transversal study in 72 HIV-infected patients, by vertical transmission, under 18 year-old, with different antiretroviral treatment was performed. Serum values of TSH, T3, T4, free T4 (FT4) and anti-thyroid peroxidase antibodies (ATPO) (Chemiluminescence-IMMULITE 2000), the percentage of lymphocytes (L) CD4 + (Flow CytometryFACScan BD) and plasma viral load (CV) levels Nuclisens EasyQ) were also analyzed. A level of LTCD4 <25% as a marker of moderate or severe immunosuppression was considered. All children were also evaluated clinically. Six out of 72 (8.33%) evaluated patients (3 males, 3 females, age-range: 5-14 years), presented thyroid alterations. All showed subclinical hypothyroidism with increased TSH (range: 4.67- 7.13 mUI/mL), normal free thyroxine levels (range: 0.86-1.50 ng/dL) and negative ATPO. Detectable CV was present in 83% of patients (n: 5), and LTCD4<25% was observed in 66.6 % (n: 4). None of six children showed clinically evident hypothyroidism symptoms. In the entire study population not significant correlations between LTCD4 and CV with TSH levels were found. Subclinical hypothyroidism frequency was similar to others analyzed series in both adults and children with HIV-infection. Not enough clinical or immunological evidence to suggest periodic evaluation of thyroid function in asymptomatic HIV-infected children was found.

774 (156) **MONITORING ENGRAFTMENT STATUS OF CHRONIC GRANULOMATOUS DISEASE PATIENTS AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION BY DIHYDRORHODAMINE 123 ASSAY.**

María Paula Dieuzeide[1], Matías Oleastro[1], Laura Pérez[1].
[1]Hospital Nacional de Pediatría "Prof. Dr. Juan P. Garrahan". Servicio de Inmunología y Reumatología.

Chronic granulomatous disease (CGD) is a rare inherited disorder characterized by diminished or absent production of reactive oxygen intermediates resulting in an impaired ability of phagocytes to kill ingested infectious agents. It is caused by a mutation of any one of five components of the nicotinamide dinucleotide phosphate (NADPH) oxidase. This defect leads to recurrent, life-threatening bacterial and fungal infections and granulomatous inflammation. Allogeneic hematopoietic stem cell transplantation (HSCT) is currently the only curative treatment for CGD. The flow cytometry-based dihydrorhodamine 123 (DHR) oxidation assay is an effective test for CGD diagnosis. Measuring the fluorescent signal from each separate cell, DHR assay also appears to provide screening information suggestive of disease genotype and X-linked CGD carrier state, on the basis of the histogram pattern. We present here the usefulness of the DHR assay as a test for determining the engraftment status of two X-CGD patients (P) following HLA-identical related HSCT. The sibling donor for P1 was a female X-linked CGD carrier who had 38% of normal neutrophils. The donor for P2 was an unaffected sibling. DHR assay was performed loading leukocytes with DHR after red blood cell lysis and stimulating with phorbol myristate acetate (PMA) in the presence of catalase. P1 showed 0, 22, 10, 33, 36 and 35% of fully functional neutrophils at diagnosis and at day +2,+6,+20,+36 and +41 (day after stem cell infusion) respectively. P2 showed 0, 72, 93, 96 and 98% of them at diagnosis and at +22,+29,+57 and +141. Conclusion: Analysis of the chimerism status of the CGD patients after HSCT may be effectively monitored through DHR assay, provided that initial oxidative function of the donor and pre-transplant recipient is performed. DHR results a useful tool for simplicity and rapidity in evaluating engraftment and graft failure in order to select the opportune tailored *interventions.*

775 (226) **CYTOTOXIC CD8 T LYMPHOCYTES ARE DISPENSABLE FOR EXPERIMENTAL AUTOIMMUNE PROSTATITIS DEVELOPMENT.**

Florencia Celeste Salazar[1], Gloria Janet Godoy[1], Leonardo Rodolfo Sánchez[1], Virginia Elena Rivero[1], Rubén Darío Motrich[1].
[1]CIBICI-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina.

Chronic Pelvic Pain Syndrome (CPPS) is the most prevalent disease in the urologic clinic affecting young men. CPPS patients experience pelvic pain and prostate inflammation for at least 3 months in the absence of infection. The etiology of CPPS still remains unknown and autoimmunity has been proposed as a cause. Animal models of Experimental Autoimmune Prostatitis (EAP) have been largely used for the study of CPPS. They have revealed a major role of Th1 lymphocytes in the development of disease. Here, we analyzed the role of cytotoxic CD8 T lymphocytes and their contribution to the induction and development of EAP by studying the development of the prostate specific immune response and prostate tissue inflammation in CD8-KO and wild type (C57BL/6) mice immunized with prostate antigens (PA) or saline (C). Animals were immunized on days 0 and 15. Animals were euthanized on day 24 and the specific immune response, prostate histopathology and tissue infiltrating leukocytes were analyzed. Similarly, elevated PA-specific immune responses, with important frequencies of specific IFNg+CD4+ and IL17+CD4+ T cells in prostate draining lymph nodes, were shown by either PA-immunized CD8-KO or wild type animals ($p>0.05$) when compared with control animals ($p<0.05$). These peripheral immune responses were accompanied by prostate histological lesions, characterized by hemorrhage, epithelial cell desquamation and marked periglandular mononuclear cell infiltration, in both PA-immunized CD8-KO and wild type animals. Infiltrates were mainly composed of CD4 T cells and macrophages. As expected, control animals did not develop prostate histological lesions. Our results demonstrate that cytotoxic CD8 T cells do not play a major role in EAP induction and pathogenesis. Moreover, our results corroborate the previous notion that, after PA immunization, a Th1 associated immune response develops and drives the induction of prostate tissue inflammation and chronic pelvic pain.

776 (234) **DO GM-CSF PRODUCING CD4 T CELLS (TH-GM CELLS) PLAY A ROLE IN EXPERIMENTAL AUTOIMMUNE PROSTATITIS?**

Florencia Celeste Salazar[1], Gloria Janet Godoy[1], Virginia Elena Rivero[1], Rubén Darío Motrich[1].
[1]CIBICI-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina.

Chronic Pelvic Pain Syndrome (CPPS) is the most prevalent urologic disease. Its aetiology remains elusive and autoimmunity has been proposed as a cause. Experimental Autoimmune Prostatitis (EAP) models have been largely used for the study of CPPS. To date, they have revealed a major role of Th1 lymphocytes in the development of disease. Recently, GM-CSF produced by CD4 T helper cells (Th-GM cells) have been described and identified to serve a nonredundant function in the initiation of autoimmunity. Here, we studied the overall immune response and disease development in our model of EAP in NOD and C57BL/6 mice, with spe-

cial focus on the induction of Th-GM cells. Either NOD or C57BL/6 mice were immunized with prostate antigens (PA) or saline (C) plus complete Freund's adjuvants on days 0 and 15. Animals were euthanized on day 24 and the induction of PA-specific immune response (Th1 and Th-GM cells), prostate histopathology and tissue infiltrating leukocytes were analyzed. Significantly increased levels of GM-CSF+RORgt+ and RORgt+IL22+ CD4 T cells were detected in prostate draining lymph node cells from PA-immunized NOD mice when compared to controls ($p>0.05$). However, those levels were lower than the markedly higher levels of IFNg+ CD4 T cells detected, revealing the induction of a prominent Th1 immune response. These peripheral immune responses resulted in prostate tissue inflammatory lesions (hemorrhage, edema, epithelial cell desquamation) and periglandular leukocyte infiltration. Infiltrates were mainly composed of CD4 T cells, macrophages and Gr1+ cells. Similar results were found when analyzing C57BL/6 mice. As expected, control animals did not develop either PA-specific immune responses or prostate inflammation. Our results demonstrate that Th-GM cells are induced upon PA-immunization in EAP. However, Th1 cells are still the most prominently elicited immune response after immunization suggesting that Th-GM cells are playing a secondary/redundant role in EAP.

777 (999) **CHANGES IN PERIPHERAL BLOOD B CELL SUBSETS IN PATIENTS WITH RHEUMATOID ARTHRITIS.**

Estefanía R. Zacca[1], Luisina I. Onofrio[1], Cristina Acosta[1], Paola Ferrero[1], Sergio M. Alonso[1], María C. Ramello[2], Carolina Montes[2], Eduardo Mussano[1], Laura Onetti[1], Isaac Cadile[1], M. Inés Stancich[1], M. Carolina Taboada Bonfanti[1], Eva Acosta Roríguez[2], Adriana Gruppi[2].
[1]Hospital Nacional de Clínicas, UNC. Córdoba, Argentina. [2]Departamento de Bioquímica Clínica, Facultad Ciencias Químicas, UNC. Córdoba, Argentina.

Rheumatoid arthritis (RA) is the most frequent autoimmune disease that primarily affects joints, and, sometimes, is associated with extra-articular manifestations. Despite major progress in RA treatment, response to therapy is often suboptimal. Then, predictive biomarkers of response to treatment are needed. It has been reported that the absence and dysfunction of some B cell subsets is associated with autoimmunity. The aim of this study was to evaluate B cell subsets in RA patients and healthy individuals and analyze the effect of treatment in these populations. Peripheral blood samples were obtained from healthy controls (HC, n=14), untreated RA patients (uRA, n=15), RA patients treated with Metotrexate (RA-Mtx, n=18), TNF inhibitors (RA-anti-TNF, n=13) or a JAK inhibitor Tofacitinib (RA-Tofa, n=5). Samples were obtained at baseline and after 3 months of treatment (n=13). Disease activity was evaluated by 28-joint count Disease Activity Score (DAS-28) and the treatment response by the EULAR response criteria. By flow cytometry we observed that % of immature B cells $CD19^{+}CD24^{hi}CD38^{hi}$ in uRA and RA-Mtx were similar to HC, however a significantly diminished % was observed in RA- anti-TNF and RA-Tofa ($p<0,05$). The values of mature B cells $CD19^{+}CD24^{int}CD38^{int}$, memory B cells $CD19^{+}CD24^{hi}CD38^{-}$ and $CD19^{+}CD24^{-}CD38^{hi}$ B cells were similar between groups. Notably, a significant increase in the $CD19^{+}CD24^{hi}CD38^{-}$ subset ($p<0,05$) and a significant decreased in the $CD19^{+}CD24^{hi}CD38^{hi}$ subset ($p<0,05$), was observed in good responders RA-treated patients. Both subsets present equal % of the inhibitory molecule PDL1- and LAP-expressing cells, and the $CD19^{+}CD24^{hi}CD38^{-}$ had higher % of $CD39^{+}CD73^{+}$cells. These results suggest that changes in B cell subsets could be used as predictive biomarkers of response to therapy. Furthermore, TNF and the JAK-STAT pathway could be involved in the development/survival of CD19+CD24hiCD38hi, because when these pathways are inhibited that population decreases.

778 (552) **EVALUATION OF PLATELET ANTIGENS INVOLVED IN ALLOIMMUNE THROMBOCYTOPENIA.**

Stella Mattaloni[1,2], Ariana Migoni[2], Carolina Trucco Boggione[1,2], Melina Luján Brajovich[1,2], Nicolás Mufarrege[1,2], Liliana Racca[2], Claudia Biondi[2], Alejandra Ensink[2], Carlos Cotorruelo[1,2].
[1]IDICER-CONICET. [2]Facultad de Cs. Bioq. y Farm. UNR.

In Caucasians, fetal/neonatal alloimmune thrombocytopenia (FNAIT) is most frequently caused by maternal alloimmunization against the human platelet antigen HPA-1a. Previous observations in immunized pregnant women from our hospital have shown that anti-HPA-1a is not mainly involved in the FNAIT cases studied. Moreover, the specificity of the alloantibodies found could not be defined in most of the cases. Considering that our current population is the result of a complex process of hybridization between Caucasians, Native Americans and Africans and that the admixture degree varies among different social groups, we consider critical to evaluate the distribution of platelet phenotypes in our population. The aim of this study is to analyze phenotype and allele frequencies of the HPA-1 system in two different groups: patients from a public hospital [G1] and patients from a private laboratory [G2]. Blood samples were obtained from 179 individuals from G1 and 156 from G2. HPA typing was performed by molecular strategies based on PCR-SSP and PCR-RFLP. For G1, HPA-1 phenotype frequencies were: HPA-1(a+,b-)=84.9%, HPA-1(a+,b+)=15.1%, HPA-1(a-,b+)=0% while for G2 were: HPA-1(a+,b-)=74.4%, HPA-1(a+,b+)=23.1%, HPA-1(a-,b+)=2.6%. The allele frequency found for G1 was: *HPA-1A*=92.5%, *HPA-1B*=7.5% while for G2 was *HPA-1A*=85.9%, *HPA-1B*=14.1%. Statistically significant differences were found for the HPA-1 phenotype distribution between both groups (z-test; p=0.009) and for the allele frequency (chi-square test with Yates' correction, p=0.0059). These results are consistent with our previous studies in other blood group systems showing ethnic variability in the different social groups analyzed. These findings suggest that variants found in G2 are representative of the Caucasian population. The absence of HPA-1(a-,b+) phenotype in G1 explain why anti-HPA-1a is not responsible for most of the FNAIT in our hospital. We speculate that another, yet unreported HPA antigen could be involved.

779 (410) **ANALYSIS OF ERYTHROCYTE MEMBRANE-BOUND AUTOLOGOUS IGG IN ADULT AND NEWBORN BLOOD SAMPLES.**

Nicolás Daniel Mufarrege[1], María Alejandra Ensinck[2], Liliana Racca[2], Carolina Trucco Boggione[1], Melina Luján Brajovich[1], Stella Maris Mattaloni[1], Silvia García Borrás[2], Claudia Biondi[2], Carlos Cotorruelo[1,2].
[1]IdICER-CONICET. [2]Facultad de Ciencias Bioquímicas y Farmacéuticas - Universidad Nacional de Rosario.

While adult red blood cells (RBCs) survive in circulation for 120 days, newborn RBCs survive only 60-80 days. During ageing, senescent (Se) RBCs expose removal markers, as the putative senescence antigen, that account for their selective recognition by macrophages and clearance from circulation. There is evidence that adult RBC ageing leads to the binding of autologous IgG followed by recognition and phagocytosis. However, the senescence process in newborn RBCs is not well characterized. The purpose of this work was to investigate erythrocyte membrane-bound autologous IgG in SeRBCs and Young (Y) RBCs obtained from adult and cord blood samples. Direct fluorescent staining was done on adult (n=20) and cord blood (n=17) erythrocyte suspensions using an anti-human IgG labeled with Alexa 488. SeRBC and YRBC populations were separated by flow cytometry using forward scatter and side scatter parameters. The percentage of IgG positive RBCs was measured in these populations. The average percentage of IgG positive RBCs for adult samples was: YRBCs=0.4±0.25 vs SeRBCs=2.3±1.40 while for the cord ones was YRBCs=0.5±0.12 vs SeRBCs=5.2±2.86. A significant increase in membrane-bound IgG in SeRBC populations of both adult and cord RBCs ($p<0.0001$, paired samples t-test) was observed. The average percentage of IgG positive cord SeRBCs was significantly higher than that found for adults ($p<0.0005$, independent samples t-test). These findings reflect that autologous IgG is involved in the selective removal from circulation of the newborn SeRBCs. The increase in the percentage of IgG positive SeRBCs observed in cord when compared with adult samples suggests that the senescence antigen in the newborn SeRBC membrane could be more

accessible to the autologous IgG recognition. This could account for the shorter newborn RBCs life span. Altogether these results give some insight into the senescence red blood cell process.

780 (407) **PARTICIPATION OF THE RHD PROTEIN IN THE ERYTHROCYTE SENESCENCE PROCESS.**

Nicolás Daniel Mufarrege[1], María Alejandra Ensinck[2], Liliana Racca[2], Carolina Trucco Boggione[1], Melina Luján Brajovich[1], Stella Maris Mattaloni[1], Silvia García Borrás[2], Claudia Biondi[2], Carlos Cotorruelo[1,2].
[1]IdICER-CONICET. [2]Facultad de Ciencias Bioquímicas y Farmacéuticas - Universidad Nacional de Rosario.

Adult red blood cells (RBCs) undergo multiple changes while they age *in vivo*. Some of these involve modifications at membrane level and the loss of membrane with some proteins by vesiculation. These changes result in events that trigger removal of Senescent (Se) RBCs. In newborn RBCs, the senescence process is not well characterized. The aim of this study was to evaluate the participation of the RhD protein in the senescence process. Adult (n=22) and cord (n=20) RhD positive RBC suspensions were incubated with IgM anti-RhD and subsequently with APC labeled anti-µ. RhD negative samples were used as negative controls. SeRBC and YRBC populations were separated by flow cytometry using forward scatter and side scatter parameters. Indirect fluorescence intensity (IFI) was measured to detect anti-RhD bound to the RBC membrane surface. Paired samples t-test showed that the average of the median values of IFI associated with RhD protein expression both in adult and cord YRBCs was significantly higher when compared with SeRBCs (adult YRBC=706.0±213.79 vs SeRBC=557.5±178.88, p<0.0001; cord YRBC=846.6±241.99 vs SeRBC=728.8±180.58, p<0.0001). Independent samples t-test demonstrated that the average of the median values of IFI associated with RhD protein expression was significantly higher in cord YRBCs (p<0.03) as well as in cord SeRBCs (p<0.005) when compared with adults RBCs. These findings show that modifications at RBC membrane level during ageing involve the RhD protein. Our results suggest that microvesicles formed during the senescence process could contain the RhD protein explaining its loss of expression observed in SeRBCs. The increase in the RhD expression observed in cord RBCs (both in Y and Se) might indicate that the D antigen in newborn erythrocytes is more accessible to the anti-D recognition. These results may contribute to a better understanding of the RBC ageing process.

781 (486) **PROOSTEOCLASTOGENIC STATE IN PATIENTS WITH GAUCHER DISEASE: CORRELATION WITH BONE PATHOLOGY.**

Constanza Bondar[1], Beatriz Oliveri[2], Diana González[2], Andrea Crivaro[1], Romina Ceci[1], Maximiliano Ormazábal[1], Juan Marcos Mucci[1], Paula Rozenfeld[1].
[1]Instituto de Estudios Inmunológicos y Fisiopatológicos (IIFP). UNLP-CONICET. [2]Instituto de Inmunología, Genética y Metabolismo (INIGEM). UBA-CONICET.

Gaucher disease is the most frequent of lysosomal storage diseases.This autosomic recessive disease is caused by mutations on the gene encoding for the lysosomal enzyme glucocerebrosidase. Deficiency of this enzyme leads to the accumulation of its substrate glucosylceramide, mainly in macrophages. Clinical manifestations include anemia, hepatosplenomegaly and bone alterations. In spite of treatment, bone alterations in Gaucher patients persist. Chitotriosidase is the most reliable biomarker used in the follow up of the disease, although its correlation with bone status has not been studied. Previous results suggest that osteoclasts are involved in bone tissue damage during the disease. The aim of our work was to study the pro-osteoclastogenic potential in patients and to evaluate its correlation with chitotriosidase activity levels and clinical parameters. To evaluate the pro-osteoclastogenic state, PBMC from patients under Enzyme Replacement Therapy were obtained by ficoll gradient and cultured in the presence of M-CSF. Mature osteoclasts were defined as multinucleated TRAP positive cells. On the other hand, z-scores from bone densitometry (total and column) were analyzed in patients, as well as serum levels of CTX and chitotriosidase. As expected, serum chitotriosidase activity was higher in patients. We also found that osteoclast differentiation was increased in patients. However, no correlation between osteoclasts and serum chitotriosidase was observed. Osteoclast levels presented a negative correlation with z-score (both total and column) while a positive correlation with CTX was observed. In conclusion, our results show for the first time a correlation between osteoclast differentiation and bone clinical parameters in Gaucher disease, but not between osteoclasts and chitotriosidase. In addition, these results support the involvement of osteoclasts in the bone pathology of Gaucher disease.

782 (166) **EFFECTS OF UREA TREATMENT ON THE ANTI-NEUTROPHIL CYTOPLASMIC ANTIBODIES (ANCA).**

Laura Domínguez[1], Paola Rolandi[2], Marcelo Zamora[1], Estela Motta[1].
[1]Immunology Department, Hospital Dr. Oscar Alende, Mar del Plata, Argentina. [2]Laboratory Department, Hospital Materno Infantil, Mar del Plata, Argentina.

Cytoplasmic fluorescence (C-ANCA) usually occurs with proteinase 3 (PR3) specificity and was mostly observed in granulomatosis with polyangiitis (GPA), meanwhile perinuclear fluorescence (P-ANCA) occurs with mieloperoxidase (MPO) specificity and is observed in microscopic polyangiitis (MPA). Occasionally, patients with vasculitis may present C-ANCA pattern with MPO and P-ANCA with PR3. The study of the avidity in these cases could help to know the quality of these antibodies. So the objective of this study was to evaluate the avidity of PR3-ANCA and MPO-ANCA and its relation with clinical status. Materials and Methods: The avidity was studied in 9 patients who had positive results by ELISA, 7 of them with PR3-ANCA and 2 with MPO-ANCA. Low avidity antibodies were stripped by washing the ELISA wells with urea. Indirect immunofluorescence (IIF) was performed with urea in ethanol and formalin-fixed neutrophils in 2 patients. Results: 5/7 samples PR3-ANCA positive presented avidity less than 30% (1 ulcerative colitis, 1 GPA, 1 C-ANCA vasculitis, 1 lung disease and 1 unknown). The other 2 samples showed avidity higher than 80% (1 C-ANCA vasculitis and 1 GPA). The 2 samples with MPO-ANCA had similar avidities with 50% average (1 rapidly progressive glomerulonephritis and 1 unknown). One of the patients with PR3-ANCA low avidity (11.9%) had P-ANCA in ethanol-fixed slide and C-ANCA in formalin slide. After urea treatment, no pattern was observed in ethanol slides meanwhile in formalin the titer decreased. In contrast, in a patient with PR3-ANCA high avidity (86.3%) no change was observed in C-ANCA pattern with urea. Conclusions: The presence of P-ANCA with PR3 specificity could be associated with low avidity antibodies. In the patient studied, a different avidity of antibodies was observed in formalin slide compared with ethanol slide. Despite the low number of patients studied, it seems that the avidity of PR3-ANCA is not related to clinical status.

783 (368) **DEVELOPMENT OF A NEW IMMUNOANALYTICAL FLUORESCENCE-BASED TECHNOLOGY FOR AUTOANTIBODY DETECTION IN MYASTHENIA GRAVIS. COMPARISON OF ANTIGEN SOURCES AND CHARACTERIZATION OF REFERENCE SERA.**

Paula N. Manuelli[1], Daniel H. Gonzalez Maglio[1], Florencia Aguirre[2], Andres Villa[2], Juliana Leoni[1], Francisco J. Barrantes[3], Mariela L. Paz[1].
[1]Immunology Department, Pharmacy and Biochemistry School, IDEHU-CONICET, Universidad de Buenos Aires. [2]Neuroimmunology Division, Myasthenia Gravis Section, Hospital Ramos Mejía, Buenos Aires. [3]Laboratory of Molecular Neurobiology, Institute of Biomedical Research, UCA-CONICET, Buenos Aires, Argentina.

Myasthenia Gravis (MG) is an autoimmune disease mediated by pathogenic autoantibodies (ACRA) directed against the nicotinic acetylcholine receptor (nAChR). The radioimmunoassay (RIA), an expensive and environmentally harmful method, is the current

reference assay to detect ACRA, the serological markers of the disease. We aim to develop and validate a new methodology for ACRA measurement by flow cytometry, simple and eco-friendly, using polystyrene microbeads, nAChR, and fluorescent probes. Various sources of nAChR were compared: RD human muscle cells, bovine muscle (bm) and *Torpedo marmorata* electric organ (Tm). Immunofluorescence (IF) was not sufficiently sensitive to detect antigens in crude extracts. Antigen purified from bm extract by anion exchange chromatography and affinity chromatography-purified Tm nAChR were subsequently used for the IF detection. We also characterized different ACRA + sera from MG patients (n=5, A to E), confirming their reactivity by RIA. The new analytical technique to assay the antigen-antibody interaction relied on coating the surface of 4 µm polystyrene beads with purified nAChRs from Tm or bm (70 ìg/100 cm^2). Coating efficiency was verified by fluorescent microscopy. Flow cytometry was subsequently performed to measure the MFI (mean fluorescent intensity) of $1x10^5$ antigen-coated-beads incubated with 1 µM α-BTX-$AlexaFluor^{488}$ (BTX), human normal serum pool (HNS) or ACRA + serum (1:100) and FITC-labelled secondary antibodies (1:200). Fluorescence microscopy images showed that the beads were effectively coated with nAChR, as the receptor could be recognized by fluorescent-labelled α-BTX, a highly specific neurotoxin for the nAChR. Flow cytometry analyses showed positive results for both types of coated beads. For Tm, MFI: autofluorescence vs BTX ($p<0.05$), HNS vs serum C, D, E ($p<0.001$) A, B ($p<0.05$). For bm, MFI: AF vs BTX ($p<0.01$), HNS vs serum A, C, D ($p<0.001$) E ($p<0.01$) B ($p<0.05$). More sera need to be tested to validate the method.

VACUNAS E INMUNOTERAPIA / VACCINES AND IMMUNOTHERAPY

784 (461) **MURINE IMMUNE RESPONSE TO INTRANASAL IMMUNIZATION WITH VIRB7 PROTEIN FROM BRUCELLA ABORTUS.**

Florencia Muñoz González[1], Iván Alonso Paiva[1], Andrea Giselle Fernández[1], María Soledad Hielpos[1], Juliana Falivene[1], Mariana Cristina Ferrero[1], Pablo César Baldi[1].
[1]IDEHU-Facultad de Farmacia y Bioquímica-UBA-CONICET.

Brucellosis is frequently transmitted to humans through mucosae. Currently, there are no human vaccines against this disease. Using a murine model we evaluated VirB7, a virulence factor from Brucella, as a potential component of an acellular vaccine against brucellosis. Six week-old Balb/c mice were weekly immunized for 3 weeks with VirB7 (10ug) plus cholera toxin (CT, 2ug), CT alone or saline. One week after last immunization serum, saliva, feces, bronchoalveolar (BAL) and vaginal lavage fluids, lung homogenates and spleens were obtained. Levels of VirB7-specific antibodies were measured by indirect ELISA in all samples. *In vitro* production of gamma interferon (IFN-ã), interleukin 2 (IL-2) and IL-5 by spleen cells stimulated with VirB7 (1 or 10 µg), ConA or RPMI was determined. Other mice (VirB7 plus CT) were injected intradermally in opposite footpads with VirB7 or saline to evaluate DTH (24 to 72 h). Immunized mice showed increased serum levels of total antibodies against VirB7 ($p<0.0001$), and of specific IgA in saliva, feces and BAL ($p<0.01$; $p<0.05$; $p<0.001$) compared with controls. IgA titers in lungs and serum were 80 and 9600, respectively. Serum titers of IgG1 were higher than those of IgG2a (16000 vs. 3840). The IgG1/IgG2a ratio was 6. A specific DTH response was detected at all times evaluated. Splenocytes from immunized mice secreted high levels of IFN-ã (mean 34677 pg/ml and 50270 pg/ml for 1 ug and 10 µg VirB7, $p<0.01$ and $p<0.001$ vs RPMI), IL-2 (187 pg/ml and 395 pg/ml, $p<0.05$ and $p<0.001$) and IL-5 (504 pg/ml and 700 pg/ml, $p<0.05$ and $p<0.01$). No significant differences versus RPMI were found in the control group for any cytokine. Intranasal administration of VirB7 plus CT induced a systemic and mucosal (lung and gut) specific immune response, which may contribute to prevent the mucosal entry of Brucella and its dissemination. The activated cellular immune response, shown by DTH reactions and high levels of IFN-ã, may be crucial for this protective effect.

785 (668) **THE TLR AGONIST PROFILIN PROTEIN FROM TOXOPLASMA GONDII ENHANCE IMMUNITY AGAINST GRA7 PROTEIN IN MICE.**

Nadia Arcon[1], Mariano S. Picchio[1], Ignacio M. Fenoy[1], Matias D. Perrone Sibilia[1], Ariadna S. Soto[1], Vanesa R. Sánchez[1], Maria De Los Ángeles Aldirico[1], Alejandra Goldman[1], Valentina Martin[1].
[1]Laboratorio de Inmunología, Vacunas y Alergia. CESyMA. ECyT. Univ. Nac. de San Martín.

The development of vaccines against *T. gondii* infection is a high priority, given the high rate of infection close to 30% of the world population. The only commercial vaccine is based on live attenuated parasites used in sheep to prevent abortions induced by congenital toxoplasmosis. TLR ligands are attractive adjuvant candidates in vaccine development. *T. gondii* profiling (TgPF), is a protein involved in parasite motility and is recognized by TLR-5, -11 and -12 receptors of the innate immune system. Activation of these receptors results in the expression of IL-12, cytokine involved in the differentiation of *naive* T cells towards a Th1 phenotype, immune response profile associated with protection against infection by this parasite. The aim of the present work was to study the immunogenicity of a vaccine formulation containing a recombinant form of TgPF (rTgPF) in combination with *T. gondii* dense granule GRA7 recombinant protein (rGRA7). Mice were intradermaly immunized 4 times with a 2-week interval with rTgPF, rGRA7, rGRA7+ACF or rGRA7+rTgPF. rTgPF significantly enhanced anti-GRA7 IgG levels compared to rGRA7 without adjuvant (p<0,05), reaching values similar to the ACF adjuvanted group. The humoral response either in rGRA7+ACF or rGRA7+rTgPF showed a mixed Th1/Th2 profile. rGRA7 *in vitro* stimulation of splenocytes induced significant and similar proliferative responses in mice vaccinated with rGRA7+rTgPF and rGRA7+ACF (p<0,05 vs control), while rGRA7+rTgPF generated higher levels of IFN-ã and IL-10 secretion compared to the rGRA7+ACF (p<0,05). No specific humoral response against rTgPF could be detected in any experimental group. However, a significant proliferative response (p<0,05 vs control) with a Th1 profile (p<0,05 vs control) was generated after rTgPF *in vitro* stimulation of splenocytes from rTgPF and rGRA7+rTgPF immunized mice. Together these results show that TgPF is an attractive adjuvant candidate for the development of a vaccine against toxoplasmosis.

786 (884) **A NEW STRATEGY TO REACH IMMUNE PROTECTION AGAINST TRYPANOSOMA CRUZI: THE USE OF RECOMBINANT BCG AS DELIVERY SYSTEM.**

Iván Bontempi[1], Karen Leal[2], Miguel Vicco[1], Luz Rodeles[1], Gabriel Cabrera[1], Estefanía Prochetto[1], Ana Bortolotti[3], Héctor Ricardo Morbidoni[3], Odir Delagostin[2], Sibele Borsuk[2], Iván Marcipar[1].
[1]Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Argentina. [2]Centro de Biotecnologia - Universidade Federal de Pelotas, Brasil. [3]Departamento de Microbiología, Escuela de Ciencias Médica, Universidad Nacional de Rosario, Argentina.

The enzymes Trasnsialidase (TS) and Cruzipain (CZ) are the most promising vaccine candidates tested in animal models against *Trypanosoma cruzi* (*T. cruzi*) infection. An unevaluated vaccine platform for this parasite is the use of recombinant Bacillus Calmette-Guerin (rBCG). The extraordinary adjuvant properties of this vaccine make it an attractive vector for the development of recombinant vaccines. In this project, we designed rBCG with fractions of TS and CZ of *T. cruzi* and we evaluated it in a model of *T. cruzi* infection. Two fractions of the TS and one fraction of CZ were inserted respectively in two different expression plasmids of mycobacteria. Protein expression was confirmed for each construction. Then, we evaluated vaccine efficacy of clones in a model of *T. cruzi*infection. BALB/c mice were immunized every 30 days twice with the six immunogens (nTS-Pus2000, nTS-Pus977, cTS-Pus2000, cTS-Pus977, fCZ-Pus2000, fCZ-Pus977), and a control group using BCG. 30 days after the last immunization,

significant delayed-type hypersensitivity (DTH) reactivity of rBCG constructions was obtained compared to mice groups immunized only with BCG (p<0,05, immunized groups vs control group; Mann Withney test). At this time, nTS-Pus2000 group but not others increased the activity of Trypanolytic antibodies compared to the control group (p <0.05, nTS-Pus2000 vs BCG, Mann Whitney test). Afterward, mice groups were challenged with 1000 *T. cruzi* parasites. Only the nTS-Pus977 and nTS-Pus2000 groups had survivals of 80 and 100%, respectively, compared to 20% of the BCG control group (p <0.05; nTS-Pus2000 vs BCG; Mantel Cox test). Furthermore, these groups had lower parasitemia and weight loss at 14 days compared with the control group. These first results confirmed that recombinant BCG is a supported platform for designing vaccines against *T. cruzi* infections, and showed that the construction nTS-BCG is a very promising vaccine candidate to use in future assessments.

787 (86) **POTENTIATION OF HUMORAL IMMUNE RESPONSE ELICITED BY A COMMERCIAL VACCINE AGAINST BOVINE RESPIRATORY DISEASE IN A MURINE MODEL EMPLOYING AN IMMUNOMODULATORY BACTERIAL STRAIN.**

Ailén Díaz[1], Brenda Almozni[1], Franco Mangone[1], Monica Sparo[2], Marcela Manghi[1], Andrea Canellada[1], Marisa Castro[1].

[1]Instituto de Estudios de la Inmunidad Humoral "Prof. Dr. Ricardo A. Margni" (IDEHU, CONICET-UBA). Cátedra de Inmunología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires. C.A.B.A., Argentina. [2]Cátedra de Microbiología y Parasitología, Facultad de Ciencias Médicas, Universidad Nacional de La Plata, Buenos Aires, Argentina.

Previously, we demonstrated that the intragastric (ig) inoculation of an immunomodulatory strain (*Enterococcus faecalis* CECT7121 (*Ef*)), enhances the cellular immune response in mice after DTP immunization. Actually, we are studying the immune response induced by a commercial vaccine against the Respiratory Bovine Disease (RBD). RBD affects cattle causing big economic losses. The etiologic agent is complex and includes bacteria and viruses, highlighting *Pasteurella multocida* (PM) and *Mannheimia haemolytica* (MH) as the main bacterial agents. The RBD vaccines used in Argentine do not always provide an adequate control for the disease, reason why it is necessary to improve their potency. This work aimed to evaluate the adjuvanteffect of *Ef* on the humoral immune response elicited by a commercial RBD vaccine. *Ef* suspensions (0,2 ml; 3.10^8 CFU/ml) were administered daily by the ig route to BALB/c mice on days -3 to -1; 12 to 14 of the immunization schedule. The RBD immunization (0,25 ml) was performed on days 0, 15 and 27 by sc injection. Mice were grouped in: *Ef/Vac (inoculated with Ef ig and immunized with the vaccine sc); *Ef/PBS (Ef ig and PBS sc); *PBS/Vac (PBS ig and vaccine sc); *PBS/PBS (PBS ig and PBS sc). Blood samples were obtained on days 0, 10, 27 and day 37 when animals were sacrificed. Specific antibody levels (IgG, IgG1, and IgG2a anti-PM and anti-MH) in sera, and IL-2, IL-12 e IFN-γ in spleen supernatants were determined by ELISA. RBD immunization induced specific IgG titers in both immunized groups at day 37. Treatment with Ef increased levels of IgG (p<0,01 for PM; p<0,0001 for MH), IgG2a (p<0,001 for PM and MH) and also IgG1 (p<0,0001 for PM; p<0,01 for MH). The cellular response was weak; negligible amounts of IL-2 an IL-12 were measured for all groups, and no statistical differences were found for IFNã between groups. These results indicate that the administration of E. faecalis CECT7121 was able to enhance the humoral response elicited by a RBD vaccine and reinforce our hypothesis that this bacterium could be used as an adjuvant strategy to potentiate weak immune responses.

788 (115) **ADENOVIRAL VECTOR ENCODING THE CATALYTIC DOMAIN OF THE CYSTEINE PROTEASE CRUZIPAIN CONFERS IMMUNOPROTECTION AGAINST INFECTION BY THE PARASITE TRYPANOSOMA CRUZI.**

Antonella Lucía Bastone[1,2], Augusto Ernesto Bivona[1,2], Andrés Sánchez Alberti[1,2], Natacha Cerny[1,3], Silvia Inés Cazorla[2], Alejandro César Cardoso Landaburu[1,2], Emilio Luis Malchiodi[1,2].

[1]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Inmunología-IDEHU (UBA-CONICET), Buenos Aires, Argentina. [2]Universidad de Buenos Aires, Facultad de Medicina, IMPAM (UBA-CONICET), Buenos Aires, Argentina. [3]Universidad Nacional de Luján, INEDES, Lujan, Buenos Aires, Argentina.

Chagas Disease is caused by the protozoan *Trypanosoma cruzi* and it affects about 7 million people all over the world, particularly poor people in developing countries. Available drugs for treatment are only effective in the acute phase of the disease and have several adverse effects and high toxicity. The severity of the Chagas disease together with the lack of adequate chemotherapeutic treatment for the chronic infection justify the need to develop effective prophylactic and/or therapeutic vaccines. To this end, we evaluated in this work an immunization protocol using an adenoviral vector encoding the catalytic N-terminus domain of Cruzipain, *T. cruzi's* main cysteine protease (AdNtCz), delivered by different administration routes. We also evaluated *Salmonella enterica serovar Typhimurium aroA 7207* as another delivery system, encoding NtCz as well. Adenoviral vector encoding â-galactosidase from *Escherichia coli* (Adâgal) was employed as control group. After being challenged with the parasite, vaccinated mice showed reduced parasitemia (AUC control/AUC vaccinated = 3, p<0.001), increased survival rates and lower weight loss compared to control group. Most importantly, the Prime Boost protocol (1 dose AdNtCz + 1 dose of recombinant Cruzipain with CpG Oligodeoxynucleotide as adjuvant) showed 100% of survival against a lethal dose of parasites (p<0.05). These results suggest that AdNtCz conferred immunoprotection against infection.

789 (569) **HUMORAL IMMUNE RESPONSE TO RECOMBINANT STAPHYLOCOCCUS AUREUS ANTIGENS FORMULATED WITH A LIPOSOMAL FORMULATION AND ODN-CPG IN CALVES.**

Ivana Gabriela Reidel[1], Guillermo Alejandro Suarez Archilla[2], Luis Fernando Calvinho[2], Cecilia María Camussone[2], Carolina Melania Isabel Veaute[1].

[1]Laboratorio de Inmunología Básica, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral. [2]Estación Experimental Agropecuaria, Instituto Nacional de Tecnología Agropecuaria-Rafaela.

Bovine mastitis caused by *Staphylococcus aureus* is a major and costly problem of dairy cattle all over the world. To date, successful control is gained only through prevention of new infections and culling of infected animals. The aim of this study was to evaluate the efficacy of the combined administration of recombinant *S. aureus* antigens and a liposomal adjuvant in humoral immune response stimulation. We also assessed the addition of CpG oligonucleotides (ODN-CpG) as immunostimulant. Liposomes were prepared with dipalmitoyl- phosphatidylcholine, cholesterol and stearylamine (7:2:2 molar ratio) by Ethanol Injection method, with recombinant fibronectin-binding protein (FnBP) and clumping factor (Clf) as antigens. Six groups of 8 months old female calves were immunized by the subcutaneous route with 3 dosesof 200ìg of protein, at 0, 15 and 45 days. One year later, they received a booster. Antigens were formulated with liposomes (Lip), ODN-CpG, liposomes with ODN-CpG, or aluminum hydroxide (Al(OH)3). Control groups received Al(OH)3 or Lip+ODN-CpG without proteins. Anti-FnBP and anti-Clf, IgG, IgG1 and IgG2 were evaluated by indirect ELISA in sera diluted 1:2000. No calves included in this study had specific *S. aureus* antibodies previous to the immunization protocol. No specific humoral response was induced in control groups. Although there were no significant differences in IgG levels between Lip+FnBP/Clf+ODN-CpG and Al(OH)3+FnBP/Clf, they led to higher IgG levels than Lip+FnBP/Clf or ODN-CpG+FnBP/Clf (p<0,001, Bonferroni test). Moreover, after booster, both liposomal formulations induced a rapid increase in IgG levels, with IgG1 levels similar to Al(OH)3+FnBP/Clf. Only calves in Lip+FnBP/Clf+ODN-CpG group produced specific IgG2. Combined

administration of liposomes and ODN-CpG was efficient to induce high antibody levels against recombinant antigens in calves with production of IgG2 and induction of long term memory.

790 (730) **IL-17 AND IFN-γ IN THE ESTABLISHMENT OF THE LIVER PROTECTION AFTER VACCINATION WITH KUNITZ TYPE MOLECULE DURING FASCIOLA HEPATICA INFECTION.**

Leonardo Silvane[1], Daiana Celias[1], Belkys Maletto[1], María Fernanda Sánchez Vallecillo[1], Ana Chiodetti[1], Santiago Palma[2], Daniel Allemandi[2], Laura Cervi[1].
[1]Departamento de Bioquímica Clínica. Facultad Ciencias Químicas. Universidad Nacional Córdoba, CIBICI-CONICET. Córdoba, Argentina. [2]Departamento de Farmacia. Facultad de Ciencias Químicas. Universidad Nacional Córdoba, UNITEFA (CONICET), Córdoba, Argentina.

The liver fluke *Fasciola hepatica* infect livestock worldwide. Fascioliasis is also a food borne disease with up to 17 million humans infected. The prevailing control strategy based on anthelmintic drugs is unsustainable due to widespread resistance; hence vaccination appears as an attractive option to pursue. In our laboratory we tested the efficacy of a vaccine formulated with Kunitz type molecule (KTM), an inhibitor of serine proteases with a key role in survival of the parasite, and CpG-ODN/Coa-ASC16, an adjuvant with capacity to induce Th1 and Th17 responses. Our previous data showed that the immunization with KTM/CpG-ODN/Coa-ASC16 (KTM-adjuvant) reduces the liver damage caused by the infection in mice. In this study we are interested in studying the immune response developed after immunization with the vaccine in infected mice. BALB/c mice were subcutaneously immunized with KTM-adjuvant, CpG-ODN/Coa-ASC16 or saline buffer at day 0, 7 and 14. One week after the last vaccination, mice were challenged orally with 6 *F. hepatica* metacercariae and euthanized 25 days after infection. Splenocytes from all group of mice where restimulated with KTM for 3 days, and cytokines were measured in the supernatant of cultures by ELISA. The immunization of mice with KTM-adjuvant promoted a significant increase in IL-17 and IFN-γ production ($p<0,05$) by splenocytes or peritoneal cells (PC) of infected mice compared with those immunized with the adjuvant alone or just infected. Besides, this vaccination induced a decrease in IL-10 production ($p<0,05$) by splenocytes compared to the levels found in cells of mice only infected. Also, vaccination with KTM-adjuvant induced a substantial increase in the percentage of F4/80 high CD11b high (defined as macrophages) of the total live PC from infected mice. Our data suggest that the exacerbated Th1 and Th17 responses found in peritoneum and spleen of mice immunized with KTM-adjuvant may have a role in the protection observed against *F. hepatica*.

791 (947) **IMMUNE LIBRARIES OF SINGLE DOMAIN ANTIBODY-DISPLAYED IN PHAGES ARE ESPECIALLY SUITED FOR SELECTION OF ANTIBODIES AGAINST SURFACE TUMOR ANTIGENS.**

Mariela Urrutia[1], María Laura Lacreu[1].
[1]Fundación Instituto Leloir.

One of the most strategies for the development of cancer therapies relies on mAbs against surface antigens (Ags). MAb raised against recombinant membrane Ags, often fails to recognize the native Ag on the cell membrane. As tumor Ag presumably not expressed in high copy number, it is important to use immune libraries. Single domain Abs (sdAb) composed of variable domain of llama heavy-chain antibodies posse small size, low immunogenicity and good tissue penetration. Our goal is to obtain sdAbs against surface antigens using sdAb-phage display library enrichment-selection on whole cells. To develop the immune phage display library, we immunized llama with 3 metastatic human melanoma cell lines (MHML) membranes. SdAb sequences from llama periphery blood lymphocytes were cloned into a phage display vector. A sdAb phage display library of reasonable size (10^7 clones) and good diversity was obtained. For library enrichment in sdAbs that recognize melanoma Ags, we used two cell types: red blood cells (negative steps) to subtract Abs that recognize common Ags to other human cells, and melanoma cells (positive steps) to keep Abs against them. Two rounds of enrichment had negative plus positive step, and the last 3 rounds had just a positive step. Panning evaluation by ELISA shown that the reactivity of the Abs anti-MHML increased meanwhile the rounds advanced (0.19, 0.45, 0.73 and 1.25; for 1st, 3nr, 4th and 5th rounds respectively). In each round of panning, phage-sdAb preincubated with melanoma or control cells were eluted. The 3nr round of panning shown the highest amount of phage-sdAb eluted anti-melanoma respect control cells (3.5 fold). The 3nr round sdAb diversity was evaluated by DNA-digestion, identifying 9 patterns among 20 clones. To select sdAbs anti-melanoma surface Ags, 20 phage-sdAbs from 3nr round were studied in two whole cell assays: dry cell-ELISA and FACS. Selection of 4 phage-sdAbs anti-melanoma and mama tumoral surface Ags are presented.

803 (832) **USE OF A STAPHYLOCOCCAL SUPERANTIGEN AS A TOOL IN A NOVEL VACCINE DESIGN AGAINST TRYPANOSOMA CRUZI INFECTION.**

Antonoglou María Belén[1], Sanchez Alberti Andrés[1,2], Bivona Augusto E.[1,2], Fernández Lynch María Julieta[1], Noli Truant Sofía[1], Sarratea María Belén[1], Fernández Marisa Mariel[1], Malchiodi Emilio Luis[1,2].
[1]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Inmunología-IDEHU (UBA-CONICET), Buenos Aires, Argentina. [2]Universidad de Buenos Aires, Facultad de Medicina, Departamento de Microbiología, Parasitología e Inmunología-IMPAM (UBA-CONICET), Buenos Aires, Argentina.

Chagas disease is a neglected infection caused by *Trypanosoma cruzi* which affects millions of people worldwide. Despite several efforts, there is still no vaccine to treat or prevent the infection. SEG is a bacterial superantigen (SAgs) that promotes a cytokine storm after binding MCH-II molecules and TCRs. Due to their characteristics, mutant SAgs are proposed to be used as immune modulators. In the present work we evaluated the use of SEG mutant N24A in combination with the catalytic domain of the cystein proteinase of *T. cruzi*, cruzipain (Nt-Cz) in vaccination protocols to analyze the immune response. SEG-N24A and Nt-Cz were cloned in bacterial plasmids, produced as recombinant proteins in *E. coli* and purified by Ni^{++}/NTA column. C3H/HeN mice were subcutaneously immunized with five doses of 10µg of total protein as follows: I-PBS; II- N24A; III-Nt-Cz with CpG-ODN as adjuvant; IV- N24A and Nt-Cz; V- N24A and Nt-Cz with CpG-ODN. Seven days after the last immunization, Nt-Cz-specific serum IgG titers were determined by indirect ELISA. In addition, delayed-type hypersensitivity (DTH) test was performed by intradermal injection of Nt-Cz in the mice footpad and swelling responses were measured 48 h later. Specific antibody titers against Nt-Cz were detected in groups III, IV and V, showing significant differences against the control group. Despite no significant differences in DTH assay, there was a stronger cellular immune reaction in the groups containing Nt-Cz compared with the control group. These results suggest that SEG-N24A is as able as ODN-CpG to induce specific IgG and DTH against a vaccine candidate as Nt-Cz. Importantly, no collateral superantigen activity was detected in mice. These preliminary studies show a potential adjuvant activity of SEG-N24A.Its use in combination with parasite antigens represents a promising tool for vaccines against *T. cruzi*.

792 (915) **AN ALTERNATIVE INFLUENZA VACCINE BASED ON THE SURFACE BACULOVIRUS DISPLAY OF THE OUTER PORTION OF MATRIX 2 PROTEIN (M2E). IT´S EVALUATION IN THE ANIMAL MODEL.**

Juan Manuel Morales[1], Paula Molinari[2], Nora Mattion[1], Oscar Taboga[2], Paula Alvarez[1].
[1]Centro de Virología Animal, ICT-Milstein, CONICET, Buenos Aires, Argentina. [2]Instituto de Biotecnología, CICVyA, INTA, Castelar, Buenos Aires, Argentina.

Influenza is a contagious respiratory illness causing annual epidemics and occasional pandemics. The death toll of influenza

epidemics worldwide is in the range of 250,000 to 500,000 each year. Current vaccines are based primarily on antibody responses against the viral glycoprotein HA. However, due to the frequent antigenic drifts of the circulating virus, a universal influenza vaccine would represent a dramatic medical advance. The nucleoprotein (NP) and the Matrix 2 (M2) proteins are the major target antigens for this kind of vaccines. Particulary, the ectodomain of M2 (M2e) is highly conserved in many influenza virus strains circulating in the human population, and several studies have shown that immunization with this 23-mer peptide can protect mice against homologous and heterologous infection with influenza A. In this sense, in previous works, we shown the protection ability of a vaccine based on the display of 1 copy of M2e at the surface of Baculovirus (Bv) as fusion to gp64 protein. However, it is known that the immunization with more than 1 copy of M2e increase significantly the immune response, then here our objective was to develop a vaccine expressing 4 copies in tandem of M2e (4M2e) at the Bv surface. The construction was made fusing the peptide to the membrane anchor from the vesicular stomatitis virus (VSV) G glycoprotein. This allows, unlike gp64-fusion proteins, that the decoration of the virion particles was not only restricted to the poles of the virions. To generate the display system, we used a vector containing the gp64 signal peptide and the membrane anchor from VSV. To obtain the recombinant Bv (rBv) we used the "Bac to Bac" system (Invitrogen). Purified stocks of this rBv expressing the 4M2e peptide were produced and titulated and the immune response was evaluated in Balb/c mice. Significantly high specific antibody titers in sera of immunized mice confirmed the immunogenicity of developed vaccine, even in the absence of adjuvant.

793 (972) **DEVELOPEMENT OF AN ALTERNATIVE INFLUENZA VACCINE BASED ON THE SURFACE BACULOVIRUS DISPLAY OF THE CONSERVED STALK DOMAIN OF THE HEMAGGLUTININ (HA) PROTEIN.**

Juan Manuel Morales[1], Paula Molinari[2], Nora Mattion[1], Oscar Taboga[2], Paula Álvarez[1].
[1]Centro de Virología Animal, ICT-Milstein, CONICET, Buenos Aires, Argentina. [2]Instituto de Biotecnología, CICVyA, INTA, Castelar, Buenos Aires, Argentina.

Influenza is a contagious respiratory illness causing annual epidemics and occasional pandemics. The death toll of influenza epidemics worldwide is in the range of 250,000 to 500,000 each year. Current vaccines are based primarily on antibody responses against the viral glycoprotein HA. Although antibodies to HA provide potent virus strain-specific protection, due to the frequent antigenic drifts of the circulating virus, a universal influenza vaccine, able to confer cross protection against different influenza variants and subtypes, would represent a dramatic medical advance. The influenza A nucleoprotein (NP), the Matrix 2 (M2) proteins and more recently, the highly conserved stalk domain of the hemagglutinin (HA) protein are the major target antigens for this kind of cross-reactive vaccines. In this sense, it has been demonstrated that a truncated HA molecule, lacking a significant portion of the globular head confers protection to mice upon challenge with influenza virus. Herein, we describe a novel influenza vaccine construct which is based on the conserved stalk region of the HA protein. The objective was to develop a vaccine expressing a modified HA molecule, which lacks the globular head domain and maintains the integrity of the stalk region at the Baculovirus (Bv) surface. In the present work we have designed a synthetic gene according this criteria and the construction was made fusing this gene to the membrane anchor from the vesicular stomatitis virus (VSV) G glycoprotein. This allows, unlike gp64-fusion proteins, that the decoration of the virion particles was not only restricted to the poles of the virions. To generate the display system, we used a vector containing the gp64 signal peptide and the membrane anchor from VSV. To obtain the recombinant Bv (rBv) we used the "Bac to Bac" system (Invitrogen). Purified stocks of this rBv, expressing the headless HA molecule were produced and titulated. This stocks will be used to evaluate the protection ability of the vaccine in the animal model.

794 (304) **CHARACTERIZATION OF AN ANTI-INTERFERON-ALPHA2B CHIMERIC FUSION PROTEIN FOR THERAPEUTIC USE.**

Carolina Attallah[1], Eduardo Mufarrege[1], Marina Etcheverrigaray[1], Marcos Oggero[1].
[1]Laboratorio de Cultivos Celulares. Facultad de Bioquímica y Ciencias Biológicas. Universidad Nacional del Litoral. Santa Fe (S3000ZAA). Argentina.

Since the late 70's, the interferon alpha (IFN-alpha) has been associated with systemic lupus erythematosus (SLE). From that moment, an impressive number of studies have confirmed the importance of IFN-alpha in lupus, and have prompted investigation of anti-IFN-alpha therapeutic strategies. In this work, we have characterized an anti-IFN-alpha2b chimeric fusion protein in terms of its IFN-alpha affinity, in vitro biological activity and the ability to produce antibodies in transgenic animals inoculated with this protein produced from different types of cells. Therefore, CHO-K1 (Chinese hamster ovary), HEK293 (Human Embryonic Kidney) and NS0 (derived from murine myeloma) cells were used to produce the fusion protein. The IFN-alpha affinity is in the order of 10-8 M-1. The fusion proteins produced for different types of cells presented the ability to neutralize the IFN-alpha antiproliferative and antiviral activities. On the other hand, the proteins were stable until 10 min at 55°C, preserving the ability of binding the IFN-alpha2b. In addition, we evaluated the immunogenicity at humoral level by in vivo assays in HLA-DR1 transgenic mice. These animals were inoculated with fusion proteins and we evaluated the anti-anti-IFN-alpha2b antibodies production. The highest antibodies title was obtained with the protein derived from HEK293 cells. This characterization allows us take into account the abilities of an anti-IFN-alpha2b antibody as potential therapeutic agent for SLE and the cell types to produce it.

795 (1040) **DEVELOP AND RHEOLOGICAL STUDY OF A THERMORESPONSIVE AND MUCOADHESIVE IN SITU GEL BASED ON POLOXAMER 407-CHITOSAN AS VACCINE DELIVERY SYSTEM OF POLYMERIC ANTIGEN BLSOMP31.**

Alejandra Diaz[1], Daniela Quinteros[2], Silvina Gutiérrez[1], Santiago Palma[2], Daniel Allemandi[2], Vanesa Zylberman[3], Fernando Alberto Goldbaum[3], Silvia Marcela Estein[1].
[1]CIVETAN- CONICET, Facultad de Ciencias Veterinarias, UNCPBA. [2]UNITEFA- CONICET, Facultad de Ciencias Químicas, Univerdidad Nacional de Córdoba. [3]Inmunova S.A.

The polymeric antigen BLSOmp31 emulsified in Incomplete Freund Adjuvant by parenteral route conferred significant protection against brucellosis in lambs. Conjunctival immunization with this chimeric protein could generate effective immunity at the major portals of entry for *Brucella ovis.* To achieve this, the immunogen BLSOmp31 needs to be associated to an appropriate delivery system. The aim of this study was to develop and characterize a thermoresponsive and mucoadhesive *in situ* gel as vaccine delivery system for BLSOmp31. Methods: Poloxamer 407 (16% w/v) was added to an aqueous solution of BLSOmp31. To enhance the mucoadhesive capability of the formulation, chitosan (0.25 % w/v in 0.5% acetic acid solution) was added. All reagents were dispersed at 20ºC and then mixed on a magnetic stirrer (4ºC) until a homogeneous solution was obtained. Rheological studies were performed to determine the sol-gel transition temperature and to evaluate the viscosity at 37°C. Measurements were carried out using an Anton Paar MCR 301 rheometer (from 0 to 50°C). To evaluate the diffusion of BLSOmp31 out of the gel in aqueous media, the formulation was cooled at 37°C in a water bath. When the temperature inside the tube reached 37°C and gelation occurred, PBS (pH 6.85) was incorporated with mild magnetic stirring. At selected time intervals (0 to 270 min) samples were withdrawn. BLSOmp31 released from the gel was determined by a capture ELISA. Results and conclusions: This formulation showed a reverse thermogelation in which aqueous solution was free-flowing liquid at room temperature and formed a stable gel at 37°C. The profile of release BLSOmp31 from P407-Ch gel

was slow at the beginning and then it began to increase (50% of BLSOmp31 was released after 210 min). The results indicate that the mixture solution developed presents an appropriate behavior as a thermoresponsive delivery system, a good viscosity and could be able to deliver structurally intact immunogen BLSOmp31.

796 (657) ORAL CO-DELIVERY OF BRUCELLA ABORTUS U-OMP19 INCREASES DMLT MUCOSAL ANTIBODY RESPONSES AND IMPROVES PROTECTION AGAINST HEAT-LABILE ENTEROTOXIN ORAL CHALLENGE.

Franco Luis Martinez[1], Laura Bruno[1], Lorena Coria[1], Juliana Cassataro[1].
[1]Instituto de Investigaciones Biotecnológicas "Dr. Rodolfo A. Ugalde". IIB-INTECH/UNSAM-CONICET.

Enterotoxigenic *E. coli* (ETEC) is the most common cause of bacterial diarrhea both among children in developing countries and in travelers to these regions. ETEC causes disease by colonizing the small intestine and producing enterotoxins. Both naturally acquired infection and oral-mucosal vaccination against heat-labile toxin (LT) or colonization factors can induce protective immunity. LT is being used as oral adjuvant/antigen (Ag) in mice. Since its toxicity limits its practical use in humans, a double mutant of LT (dmLT) which is less toxic and retains its adjuvant properties is under clinical trial. We propose to use a bacterial protease inhibitor (U-Omp19 from *Brucella*) as platform to deliver antigens in oral formulations against infectious diseases. This protein can protect Ags delivered in oral vaccines from digestion and it can also trigger and direct the type of mucosal and systemic immune responses. Thus in this work our aim was to investigate the effect of U-Omp19 co-delivery on dmLT immunogenicity and protective efficacy. To this end CD1 mice were orally immunized at days 0, 28 and 42 with i) saline ii) dmLT or iii) dmLT+U-Omp19. Three doses of dmLT were studied alone or plus U-Omp19. Fecal and serum anti-LT antibodies (Abs) were evaluated by ELISA. Results obtained indicated that U-Omp19 co-delivery increased ($P<0.05$) the mucosal anti-LT Abs (IgA and IgG in fecal extracts) while anti-LT Abs elicited at sera were not changed. One month after last immunization mice were challenged orally with LT and 3h later each intestine and carcass was weighted (patent mouse gut assay). We have shown that U-Omp19 when co-delivered with dmLT induced significant protection ($P<0.05$) against oral challenge with LT, while dmLT alone did not. All together our results indicated that U-Omp19 can help to reduce the dose of dmLT and can increase dmLT efficacy to neutralize LT in vivo. So, U-Omp19 would be a good candidate to be included in dmLT vaccine formulations against ETEC.

RESPUESTA IMMUNE ADAPTATIVA / ADAPTATIVE IMMUNE RESPONSE

797 (1035) **GALECTIN-3 DEFICIENCY DRIVES LUPUS-LIKE AUTOIMMUNE DISEASE BY PROMOTING SPONTANEOUS GERMINAL CENTERS FORMATION THROUGH AN IFN-ã-DEPENDENT MECHANISM.**

Cristian Gabriel Beccaria[1], Facundo Fiocca Vernengo[1], Jimena Tosello Boari[1], M. Carolina Amezcua Vesely[1], M. Cecilia Ramello[1], Melisa Gorosito Serran[1], Juan Mucci[2], Oscar Campetella[2], Carolina Lucía Montes[1], Eva Virginia Acosta Rodriguez[1], Adriana Gruppi[1].
[1]CIBICI-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina. [2]Instituto de Investigaciones Biotecnológicas, Universidad Nacional de San Martín, San Martín, Argentina.

Autoimmune diseases are a major health problem, affecting ~10% of the developed world's population. Since there is no cure for autoimmunity it is extremely important to study the mechanisms that trigger these diseases. One of the hallmarks in the antibody-mediated autoimmune diseases is the spontaneous generation of Germinal Centers (GC). We have recently identified Galectin-3 (Gal-3) as a critical regulator of GC formation and autoantibody production. Mice lacking Gal-3 spontaneously develop a lupus-like disease, characterized by increased numbers of GC B cells, Tfh cells, Antibody-Secreting-Cells, hyperglobulinemia, autoantibody production, and mononuclear infiltration in kidneys ($p < 0.05$ in all cases). In the absence of Gal-3, B and T cells exhibited an activating phenotype that appears to be comprised to GC reaction. In Gal-3 KO mice we observed increased frequencies of CD80, CD86, CXCR4, IL-21R and CD69 positive B cells ($p < 0.05$ in all cases) and increased percentages of CD69 and CD44 positive CD4+T cells ($p < 0.05$ for both), comparably to WT counterparts. Furthermore, we found more proliferating B cells measured by Ki-67 expression in Gal-3 KO mice than in WT mice ($p < 0.05$). Interestingly, Gal-3 KO mice produced excessive quantities of IFN-γ, a major cytokine that has long been associated with lupus development. We observed that not only T cells could produce IFN-γ but also B cells, and these B cells expressed high levels of IFN-ãR and T-bet. IFN-γ blockade reduced GC B cells and Tfh cell frequencies ($p < 0.05$ for both), and immunoglobulin class switching, demonstrating that IFN-γ overproduction was required to sustain lupus-like disease. These studies demonstrate that absence of Gal-3 profoundly alter the immune homeostasis and suggest that Gal-3/IFN-γ axis is a novel potential pathway for therapeutic strategies in autoimmune diseases.

798 (561) **RELEVANCE OF GALECTIN-7 (GAL7) IN ALTERED SKIN PHYSIOLOGY.**

Nicolás Alejandro Pinto[1], Sebastián Matías Maller[1], Rosa María Morales[1], Sabrina Gisella Gatto[1], Tomás D´Alotto Moreno[1], Gabriel Adrián Rabinovich[1], Juan Pablo Cerliani[1].
[1]Instituto de Biología y Medicina Experimental.

Galectins are members of a family of b-galactoside binding proteins, which have been involved in a number of biological processes such as immune system homeostasis and cancer biology. In particular, Galectin-7 (Gal7) is differentially expressed in all stratified epithelia mainly in epidermis and in physiological conditions this lectin contributes to skin homeostasis, showing an augmented expression in inflamed skin. The aim of this work is to elucidate the relevance of Gal7 in the skin immune system in a chemically induced carcinogenesis model. First, we observed that Gal7 induces more suppressive Myeloid-derived Suppressor Cell (MDSC) compared to control differentiated cells in in vitro assays. After differentiation, we activated them with LPS and we performed suppression assays. We observed that MDSC induced with Gal7 suppressed spleen cells proliferation in a higher extent than control activated MDSC. Considering these results, we performed a two stage chemical induced carcinogenic protocol in wild type (WT), $Gal7^{-/-}$ (KO), and Gal7 transgenic (Tg) mice (Tg46, which overexpress Gal7) during 3 months with DMBA and TPA. Tg mice showed more and bigger skin papilomes and shorter extent of free papilome survival compared to WT and/or KO mice. Even though there was no difference in spleen or papilome MDSC percentage, we obtained spleen MDSC from WT, KO and Tg using cell sorting and performed suppression assays. Accordingly to in vitro results Tg mice derived MDSC were more immune-suppressive than WT and KO. In addition, Tg mice papilomes contain a larger percentage of CD4 and CD8 exhausted ($PD\text{-}1^{+}$) cells than KOand WT mice. Tg mice spleen and papilomes showed also a larger Treg/CD8 ratio, favoring an immune suppressive microenvironment in Tg papilomes. In conclusion, our results suggest that Gal7 promotes a tolerogenic microenvironment in the skin favoring keratinocyte transformation/proliferation disorders.

799 (860) **LOW TLR9 EXPRESSION IN CD4+ AND CD8+ HUMAN PERIPHERAL BLOOD CELLS MIGHT PLAY A ROLE IN NAFLD.**

Nadia Soledad Alegre[1], Cecilia Claudia García[1], Luis Ariel Billordo[1], Javier Benavides[2], Luis Colombato[2], Daniel Poncino[3], Daniel García[3], Silvia Frías[4], Beatriz Ameigeiras[4], Alejandra Claudia Cherñavsky[1].
[1]Instituto de Inmunología, Genética y Metabolismo, Hospital de Clínicas "José de San Martín" [2]Sección Hepatología, Servicio de Gastroenterología "Hospital Británico de

Buenos Aires". [3]Sección Hepatología, "Sanatorio Méndez ObSBA". [4]Unidad de Gastroenterología, Hospital General de Agudos "JM Ramos Mejia".

Background: Liver exposure to intestinal-derived bacterial products promotes a pro-inflammatory milieu that induces innate immunity and contributes to the pathogenesis of nonalcoholic fatty liver disease (NAFLD) via pattern-recognition receptors such as Toll-like receptors (TLRs). TLRs are expressed and co-stimulate T cell activation. Particularly TLR9 recognize bacterial DNA and is involved in Kupffer cell activation leading to nonalcoholic steatohepatitis (NASH). To study whether TLR9 modulates T cell activation in human NAFLD, we evaluated TLR9 expression in circulating and liver T cells, and TLR9 co-stimulatory role in peripheral blood (PB) T cell activation. Methods: Blood and liver samples came from 16 adult NAFLD patients (4 simple steatosis and 12 NASH) and 8 healthy controls (CO). PB mononuclear cells (PBMC) were obtained by Ficoll-Hypaque density gradient and liver cell suspensions by mechanical disruption. Cells were stained with conjugated anti-CD4, -CD8, -TLR9 and IgG2a isotype and analyzed by flow cytometry (FC). Functional assay were performed using $CD3^+$ cells obtained from PBMC by negative selection. The $CD3^+$ fraction was stimulated with soluble anti-CD3 [saCD3; 250 ng/ml] and/or ODN 2395 [2 mM] for 24 hs. Cells were harvested and stained with conjugated anti-CD4, -CD8, -CD69 and analyzed by FC. Mann-Whitney test was used. All patients provided written informed consent. Results: PB $CD4^+$ and $CD8^+$ cells from NAFLD showed lower expression of TLR9 (p=0,0490 and 0,0034; vs. CO, respectively) . $CD8^+$ but not $CD4^+$ T cells from NAFLD stimulated with saCD3 and co-stimulated with ODN 2395 showed less expression of CD69 than CO (p= 0,0043). T cells from CO and NAFLD were similarly stimulated with saCD3 (p=ns) but no stimulation was found with ODN 2395 alone. Conclusions: A diminished TLR9 expression together with a lower *in vitro* activation of $CD8^+$ T cells from NAFLD patients could contribute to counterbalance the pro-inflammatory environment.

800 (2004) **TRIIODOTHYRONINE (T3)-STIMULATED DENDRITIC CELLS (DCS) INDUCE A PRO-INFLAMMATORY ADAPTIVE RESPONSE IN VIVO.**

Vanina Alejandra Alamino[1], María del Mar Montesinos[1], María Florencia Soler[1], Lucila Giusiano[1], Nicolás Gigena[1], Gabriel Adrián Rabinovich[1], Claudia Gabriela Pellizas[1].
[1]Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI-CONICET). [2]Facultad de Ciencias Químicas, Universidad Nacional de Córdoba.

We previously reported that mice DCs, the main antigen-presenting cells, express thyroid hormone receptor β1 and that physiological levels of T3 stimulate the maturation of DCs and the ability to develop a Th1-type response *in vitro (FASEB J 2008,22:1032)*, as well as cytotoxic and antitumoral effects in an *in vivo* model of B16 melanoma (*Cancer Res 2015,420:105).* Furthermore, *in vitro*, T3 stimulated DC production of the Th17-skewing cytokines TGF-β, IL-6, IL-23 and IL-1β and reduced the expression of programmed death-ligand-1 (PD-L1). In addition, T3-matured DCs increased the production of IL-17 and decreased the frequency of Treg cells in allogenic splenocytes (*Thyroid 2015,25:S1*). The aim of this study was to further analyze the immune response induced by T3-stimulated DCs *in vivo*. For this purpose, mice bone marrow derived DCs treated with ovalbumin (OVA) and 5 nM T3 (OVA+T3-DCs) for 18 h, were injected i.v. into OTII transgenic mice. One week later, splenocytes were re-stimulated *ex vivo* with OVA_{323}, and proliferation, IL-17 and IFN-β releases, and $CD4^+CD25^+FoxP3^+$ (Tregs) and $PD1^+$ cells were determined 4 days later by MTT assay, ELISA and FACS, respectively. Results registered that OVA+T3-DCs treated mice increased splenocytes' proliferation and spleen cells secreted higher IL-17 and IFN-β levels than those OVA-DCs injected mice, indicating the generation of a specific immune response. Incontrast, splenocytes from OVA+T3-DCs group decreased Treg population and exhibited a tendency towards a reduction of PD-1 expression compared to those from OVA+DCs-treated mice. These results reinforce the critical role of T3 in the regulation and maintenance of immune homeostasis since T3-exposed DCs favor the promotion of adaptive immunity towards a pro-inflammatory profile. Our findings may be exploited to manipulate the immunogenic potential of DCs to positively regulate the development of protective immunity or negatively control the generation of autoimmune diseases.

801 (90) **CYTOKINES INVOLVED IN THE CHANGE OF ANTIBODY SPECIFICITY INDUCED BY LDV (LACTATE DESHYDROGENASE-ELEVATING VIRUS).**

Macarena A. Ottobre Saborido[1], Lilia A. Retegui[1], Jean-Paul Coutelier[2], José L. Aparicio[1].
[1]Instituto de Química y Fisicoquímica Biológicas (UBA-CONICET), Facultad de Farmacia y Bioquímica, Buenos Aires, Argentina. [2]Unit of Experimental Medicine, de Duve Institute, Université Catholique de Louvain, Brussels, Belgium.

Introduction: LDV is a persistent and non pathogenic RNA arterivirus that induces NK, macrophages and B- cell activation in mice. It was found that LDV- infection modified Ab specificity to different antigens, depending on the genetic background of the host. Objectives: The purpose of this work was to study, through anti-cytokine auto-vaccination, the cytokines involved in the LDV effects. Methods: BALB/c, CBA/Ht and C57BL/6 mice were infected with 2×10^7 50% infectious doses of LDV in saline. Subsequently, the animals were inoculated in the footpads, four times at 2-wk intervals, with 2ìg of IL-15/OVA, IL-23/OVA or IL-17/OVA complexes emulsified in Gerbu adjuvant. Ten days after the last inoculation the animals were bled. Serum lactate dehydrogenase (LD) was determined enzymatically, whereas IL-17 concentration was measured by ELISA. The proportion of Ab directed to native OVA epitopes was calculated by competition ELISA assays. Results: Vaccination with IL-15/OVA, IL-23/OVA or IL-17/OVA elicited similar titers of anti-OVA Ab in LDV-infected (1/160.000 to 1/300.000) and non-infected animals (1/145.000 to 1/300.000). The proportion of native anti-OVA Ab in BALB/c mice vaccinated with IL-15/OVA or IL-23/OVA was elevated and similar between non-infected (85 and 81%, respectively) and LDV-infected animals (80 and 84%). By contrast, control CBA/Ht mice vaccinated with IL-15/OVA or IL-17/OVA developed low titers of anti native OVA epitopes (56 and 38%, respectively) that increased to 81 and 71%, respectively, in LDV-infected animals. Furthermore, vaccination with IL-17/OVA augmented plasmatic IL-17 levels in LDV-infected animals in comparison with control (150±12 pg/ml and 23±4 pg/ml P<0.001, respectively) this effect was also detected when serum LD was measured (4475±80 U/l and 2455±20 U/l, respectively, P<0.001). Conclusions: This work indicated that IL-15 and IL-17 should be involved in LDV- infection effects on changes of Ab specificity to a given antigen.

802 (602) **DEVELOPMENT OF BIOBETTERS: IMMUNOGENICITY ANALYSIS OF DEIMMUNIZED AND FUNCTIONAL THERAPEUTIC (DEFT) VERSIONS OF A LONG LASTING RHIFN-A2B.**

Eduardo Mufarrege[1], Sofía Inés Giorgetti[1], Marina Etcheverrigaray[1], Frances Terry[2], William Martin[2], Anne S. DeGroot[2,3].
[1]Laboratorio de Cultivos Celulares – FBCB – UNL (Santa Fe, Argentina). [2]EpiVax, Inc., (Providence, RI, USA). [3]Institute for Immunology and Informatics, University of Rhode Island, RI, USA.

Alpha interferons (IFN-α) have proven clinical utility for the treatment of chronic hepatitis B and C virus infections. However, there is growing evidence that repeated dosing of IFN-α2b over several months induces neutralizing antibodies against the therapeutic in a significant number of patients. Associations between IFN immunogenicity and loss of efficacy have been described. In an attempt to improve the *in vivo* biological efficacy of IFN-α2b wild type (WT-IFN), a hyperglycosylated protein (4N-IFN) was developed. However, *in silico* analysis revealed that 4N-IFN had more predicted T cell epitopes than WT-IFN. In order to develop a safer and most efficient IFN therapy, we used the DeFT (Deimmunization of Functional Therapeutics) approach to produce functional, deimmunized versions of 4N-IFN. Using the Optimatrix

of the *in silico* toolkit ISPRI, 4N-IFN sequence was optimized to reduce MHC binding of clustered epitopes. Eight modifications were selected and integrated in three variants: 4N-IFN(VAR1) [5]; 4N-IFN(VAR2) [8] and 4N-IFN(VAR3) [3]; the number of modifications is identified by the number in the brackets. Preliminary experiments revealed 4N-IFN(VAR2) did not have any antiviral activity thus was not included in further assays. For 4N-IFN(VAR1) and 4N-IFN(VAR3) T-cell proliferation assay showed reduced immunogenicity and Th1 and Th2 cytokine profile, when compared to controls (commercial NG-IFN (non-glycosylated), PEG-IFN, WT-IFN and 4N-IFN). Immunogenicity ranking in decreasing order for Th1 profile was: WT-IFN > 4N-IFN > NG-IFN >PEG-IFN = 4N-IFN(VAR1) > 4N-IFN(VAR3). For Th2 responses the results were: NG-IFN > PEG-IFN > WT-IFN > 4N-IFN > 4N-IFN(VAR3) > 4N-IFN(VAR1). These results demonstrate that deimmunization of the long lasting IFN variant reduced its immunogenicity as measured in vitro using T cell assays and cytokine profiling. In conclusion, 4N-IFN(VAR1) and 4N-IFN-4N(VAR3) appear to be promising candidates for antiviral therapy of HCV and HBV.

503 (520) **EFFECT OF BLOCKING OF CTLA-4 IMMUNE CHECK- POINT ON THE GROWTH OF TWO MURINE TUMORS DISPLAYING DIFFERENT IMMUNOGENICITY.**

[1]Daniela Romina Montagna, [1]Ariel Ramiro Strazza, [1]Paula Chiarella, [1]Graciela I. Dran, [1]Raúl Alejandro Ruggiero.
[1]IMEX-CONICET- Academia Nacional de Medicina

CTLA-4 is an immune checkpoint expressed in T reg and activated T cells that transmits inhibitory signals to T cells and down-regulates immune responses. On this basis, there is a growing interest in the possible therapeutic benefits of blocking CTLA-4 as a means of enhancing immune responses against cancer. In fact *ipilimumab* – a monoclonal anti-CTLA-4 antibody – was recently approved for the treatment of human advanced melanoma. However, despite these promissory expectations, most experimental studies in this topic were performed in mice bearing strongly immunogenic chemically-induced tumors that are not the best models for clinical cancer. Herein, we have studied the effect of an antibody against CTLA-4 on the growth of two murine tumors displaying widely different degrees of immunogenicity: the strongly immunogenic methylcholanthrene-induced MC-C fibrosarcoma and the spontaneous LMM3 carcinoma displaying undetectable immunogenicity. Tumor-bearing mice (n = 6 mice per group) received 3 doses of 100 µg i.p. of anti-CTLA-4 antibody each 4 days starting at day 3, 10 or 17 after the s.c. inoculum with $5x10^5$ MC-C or LMM3 tumor cells, that is when the tumor was incipient (I), medium (M) or large (L). Results were expressed as tumor volume (mm^3) [media ± SE] at day 35 of tumor growth. MC-C: Control Group (without treatment): 2,027± 63; Treated Groups: I: 314 ± 74 ($p < 0.001$ vs. Control); M: 2,085 ± 273; G: 3119 ± 286 ($p < 0.05$). LMM3: Control Group: 2,142 ± 104; Treated Groups: I: 2797 ± 204 ($p < 0.05$); M: 1929 ± 129; G: 2071 ± 161. Our results revealed that CTLA-4 blocking was therapeutically efficient against small-sized strongly immunogenic tumors only. On large immunogenic and on small non-immunogenic tumors, a slight but significant tumor enhancement was observed. Presumably, the combination of CTLA-4 blocking with other approaches such as PDL-1 immune checkpoint blocking, could eventually expand the antitumor effects of these antitumor immunological strategies.

805 (805) **BREAST MILK INTAKE DETERMINED BY DOSE-TO-MOTHER DEUTERIUM-OXIDE TURNOVER TECHNIQUE IN A GROUP OF ARGENTINEAN INFANTS: PRELIMINARY STUDY.**

Silvina Mariela Vidueiros[1], Cristián Nápoli[1], Cristina Possidoni[2], Gabriel Tarducci[3], Sergio Giordanengo[2], Inés Fernandez[1], Anabel Pallaro[1]
[1]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Nutrición. [2]Hospital Sagrado Corazón de Jesús de Basavilbaso, Provincia de Entre Ríos. 3 Universidad Nacional de La Plata, Facultad de Humanidades y Ciencias de la Educación, Cátedra de Actividad Física para la Salud, IdHICS CONICET.

Dose- to-mother deuterium-oxide turnover technique (DMDOT) is the reference method to measure breast milk intake. The aims of this study were: 1) to measure and compare intake of breast milk (BM) and water from non-breast milk sources (W non-BM) in exclusive breastfeeding (EBF) and non-EBF infants, and 2) to estimate and compare adequacy of energy and protein requirements between these groups. Subjects and materials: This study was performed in 19 mother-infant pairs at 4 months to birth from Entre Ríos and Buenos Aires provinces. Mothers received an oral dose of deuterated water and 6 samples of saliva were collected from mother and baby during a period of 15 days. Deuterium enrichment was determined in a Shimadzu FTIR-spectrometer-Affinity to obtain the intake of BM and W non-BM. Infants were classified into EBF (n = 12) or non-EBF (n = 7) categories based on a 24-hour recall to the mother. Energy and protein requirements were calculated according FAO recommendations. Results: Variation of the deuterium enrichment in the saliva of the infant was observed, being higher in EBF infants. BM intake was 950,2 ± 196,5 mL/d and 634,4 ± 255,4 mL/d in EBF and non-EBF, respectively ($p<0,01$). W non-BM was significantly higher in non-EBF compare to EBF (258,6 ± 312.9 mL/d vs -4,8 ± 48,0 mL/d, $p<0,0001$). All EBF infants met their energy and protein requirements as well as 57% of non-EBF infants, taking into account only BM intake. Conclusion: DMDOT is an innovative method to breast milk measurement and can be used as an assessment tool for evaluating infant feeding pattern and requirements adequacy and to investigate the extent to which the breast milk is being replaced by the consumption of other foods in order to estimate exclusive breastfeeding. This preliminary study was not meant to describe the breast-feeding situation in Argentina.

806 (850) **VARIATION IN MITOCHONDRIAL GENOME AND TELOMERE LENGTH CORRELATES WITH DNA METHYLATION IN HUMAN BREAST CANCER**

Cané L[1*], Longarzo ML[1*], Debandi M[1], Cálcena E[1], Peltomäki P[2], Richard SM[1], Bolzán AD[1], Pavicic WH[1,2].
[1]Instituto Multidisciplinario de Biología Celular (IMBICE), CICPBA-UNLP-CONICET La Plata, La Plata, Argentina. [2]Department of Medical and Clinical Genetics, University of Helsinki, Helsinki, Finland. Equal contribution.*

Nuclear genes methylation plays an important role in human tumorigenesis. Many Tumor Suppressor Genes (TSG) and miRNA genes have associated CpG islands, suggesting epigenetic regulation of their expression. Mitochondrial DNA copy number variation (mtDNA-CN) and telomere length (TL) alteration could be involved in methylation changes on nuclear genes. We investigated 62 tumoral/non-tumoral adjacent (T/N) paired samples from Argentinean breast cancer patients. Methylation status at their promoters, was analyzed by MS-MLPA method on 24 TSGs, 10 miRNAs and the surrogate marker for global methylation LINE-1. The CIMP (CpG island methylator phenotype) condition was also evaluated. TL and mtDNA-CN variations were determined by qPCR method. We found a significant mtDNA-CN decrease in 63% of the samples by comparing T with the corresponding N tissue (average decrease = 24%, $p<0.01$). Moreover, significantly larger telomeres were found in 60% of the T compared with the N tissues (mean value of log[kb tel]: 2.86 and 2.79, respectively; $p<0.05$). Comparing TvsN we found hypermethylation in miRNAs 124a1/2/3, 148a and 152, hypomethylation in 208a, 373 and no changes in 18bI, 1-1,200a. TSGs *APC*, *CDH13* and *RASSF1*, and the CIMP markers *RUNX3*, *NEUROG1* and *IGF2* showed hypermethylation ($p<0.001$). miR-124a2/3 showed a significant correlation (p:0.04 and p:0.03) between mtDNA-CN and methylation levels. An inverse association between methylation and mtDNA copies was found for *RASSF1* gene in tumor tissue (p:0.03, r:-0.3). *IGF2* gene showed a significant correlation ($p<0.01$, rho:0.5) between TL and promoter methylation levels. For the LINE-1 marker, while no methylation differences were seen in TvsN tissues, when analyzing tumor samples a significant positive correlation (p:0.03, r:0.31) was found between TL and methylation level. Our results strongly suggest that variation in mtDNA, telomere length and nuclear DNA methylation are associated events in breast cancer.

ONCOLOGÍA IV / ONCOLOGY IV

807 (926) **GALLBLADDER CANCER IN SALTA: EPIDEMIOLOGY, RISK FACTORS AND PROFILE OF KRAS GENE ALTERATIONS**

Cinthia Natalia Saucedo[1,2]
[1]Institute of Experimental Pathology, [2]UNSa-CONICET Salta, Argentina

Gallbladder cancer (GBC) has unique characteristics in Argentina; large differences in terms of age-standardized-mortality rates (ASMR) due to this tumor type have been reported for the NW region of the country (8-9/10^5 women/ year) respect to the eastern Pampeana region (1.4/10^5 women/ year). Moreover, the profile of abnormalities of key genes such as *kRAS* and *TP53* in gallbladder cancer seems to differ worldwide.

We surveyed the clinical records of the San Bernardo hospital, the main health institutions in Salta city, looking for information about the epidemiology of GBC. A total of 8,135 patients were operated for different gallbladder pathologies during the period 2006-2015. Among them 83 (1.02%) were diagnosed with GBC, and the vast majority were women. The mean age of the patients at the time of diagnosis was 61 years.

Cholelithiasis constitutes the major risk factor for GBC, and the composition of the gallstones seems to be related to nutritional habits. Therefore, we collected 70 samples of gallstones and gallbladder tissue from patients with cholelithiasis and GBC operated during 2015, to analyze their chemical composition and the *kRAS* gene status, respectively. Fourier infrared spectroscopy analysis showed that apart from the frequent components detected in this type of concretions, a considerable amount of gallstones from these patients had high levels of heavy metals. The molecular genetic analysis to evaluate *kRAS* is still in course, and data will be presented.GBC particularly affects women in Salta, and represents a significant public health problem.

GASTROENTEROLOGÍA / GASTROENTEROLOGY

808 (2030) **A RATIONALE TO TARGET NRF2 TO REDUCE THE RISK OF METABOLIC SYNDROME: A STUDY IN OVERWEIGHT BOYS AND RATS FED A HYPERCALORIC DIET**

Lucas Damián Santillán[1], Sandra Esther Gómez Mejiba[2], María Sofía Giménez[3], Darío C. Ramírez[1]
[1]Laboratory of Experimental and Translational Medicine, IMIBIO-SL, CCT-San Luis, CONICET-UNSL, San Luis, Argentina, [2]Laboratory of Experimental Therapuetics, IMIBIO-SL, CCT-San Luis, CONICET-UNSL, San Luis, Argentina, [3]Laboratory of Nutrition, Metabolism and Environment, IMIBIO-SL, CCT-San Luis, CONICET-UNSL, San Luis, Argentina.

Excess of energy is metabolized to free fatty acids which should be stored as triglycerides (TG) otherwise they cause inflammation, and thus a high risk for obesity-associated abnormalities. Nrf2 controls the expression of phase II/III, antioxidant and adipogenic genes. Low Nrf2 expression may determine inflammation and a high metabolic risk in overweight/obesity. To test this hypothesis we performed a study in overweight children and in an experimental model of rats fed a hypercaloric diet (HCD). In a population of overweight boys (OW, n=22) and normal weight boys (NW, n=27) from San Luis City we measured clinical and biochemical parameters related to metabolic syndrome, including hypertension, insulin resistance, lipid metabolism, oxidative stress and inflammation. Compared to NW, OW boys had insulin resistance, higher atherogenic index, altered plasma lipid profile, increased markers of oxidative stress and inflammatory lipid profile. Interestingly, GPx activity and GSH/GSSG ratio and leukocyte Nrf2 expression were lower in those OW children at high metabolic risk. Nrf2 expression negatively correlated with metabolic risk in OW boys. Experimentally we fed male SD rats (n=19) for 16 weeks with a normocaloric (n=7) and HCD (n=12) and found that some rats fed the HCD were obesity sensitive (OS, n=7) whereas the others were obesity resistant (OR, n=5). Compared to OS and in perirenal adipose tissue, OR rats showed a pattern of oxidative stress (increased NOX-2, reduced antioxidant enzymes and increased oxidative stress markers), and inflammation (increased VCAM-1, TNF-a, and lipid profile); but reduced lipogenesis (low Nrf2, PPAR-γ, lipogenic enzyme gene expression, total lipids and TG). Low Nrf2 expression determines reduced adipogenesis, but increased metabolic syndrome's risk. Interventions aimed at increasing expression/activity of Nrf2 may provide a strategy to reduce metabolic risk in overweight/obese patients. PICT-2014-3369 (to DCR).

809 (517) **PROMISING THERAPEUTIC EFFECT OF COENZYME Q10 IN ETHINYL ESTRADIOL-INDUCED CHOLESTASIS**

Manuela Martinefski[1], Myriam Rodriguez [2,3], Fabian Buontempo[1], Silvia Lucangioli[1,3], Valeria Tripodi[1,3]
[1]Departamento de Tecnología Farmacéutica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina, [2]Fiosiopatología-INIGEM-CONICET, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina, [3]Consejo Nacional de Investigaciones Científicas y Tecnológicas, CONICET, Buenos Aires, Argentina

Intrahepatic cholestasis of pregnancy (ICP) is a high risk liver disease given the eventual deleterious consequences that may occur in the fetous. ICP is characterized by the accumulation of bile acids, particularly hydrophobic bile acids such as litocholic acid (LCA), which induces oxidative stress and apoptosis. We previously reported that women with IPC show decreased coenzyme Q10 (CoQ10) and enhanced bile acids in plasma. These findings were also observed in ethinyl estradiol (EE)-induced cholestasis in rats. The aim of this work was to evaluate the effect of CoQ10 supplementation in EE-induced cholestasis in rats. Cholestasis was induced in Sprague-Dawley rats by a daily intraperitoneal injection of 5 mg/kg EE for 5 days (EE). A group of these rats also received daily oral 250 mg/kg CoQ10 supplementation for 5 days (EE+CoQ10). Another group of rats received only CoQ10 supplementation for the same period of time (CoQ10). Serum, bile acids (total and LCA), coenzyme Q9 (CoQ9) and CoQ10, aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase were assessed. Bile flow was also measured. No differences were observed between CoQ10 and control groups except for an increase in plasma CoQ10 ($p<0.001$). However, CoQ10 supplementation in cholestatic rats (EE+CoQ10) increased both CoQ10 and CoQ9 plasma levels ($p<0.05$), and enhanced bile flow($p<0.05$) as compared with EE. Furthermore, it also decreased serum alkaline phosphatase and bile acids, particularly LCA levels as compared with EE ($p<0.05$). Present findings show that CoQ10 administration improved cholestasis and further suggest that CoQ10 supplementation may be a potential therapeutic strategy in estrogen-induced cholestasis in humans as ICP.