ABSTRACT BOOK

# ASOCHIN

## 6TH ANNUAL MEETING

SANTA CRUZ, COLCHAGUA/NOVEMBER 15-17/2023

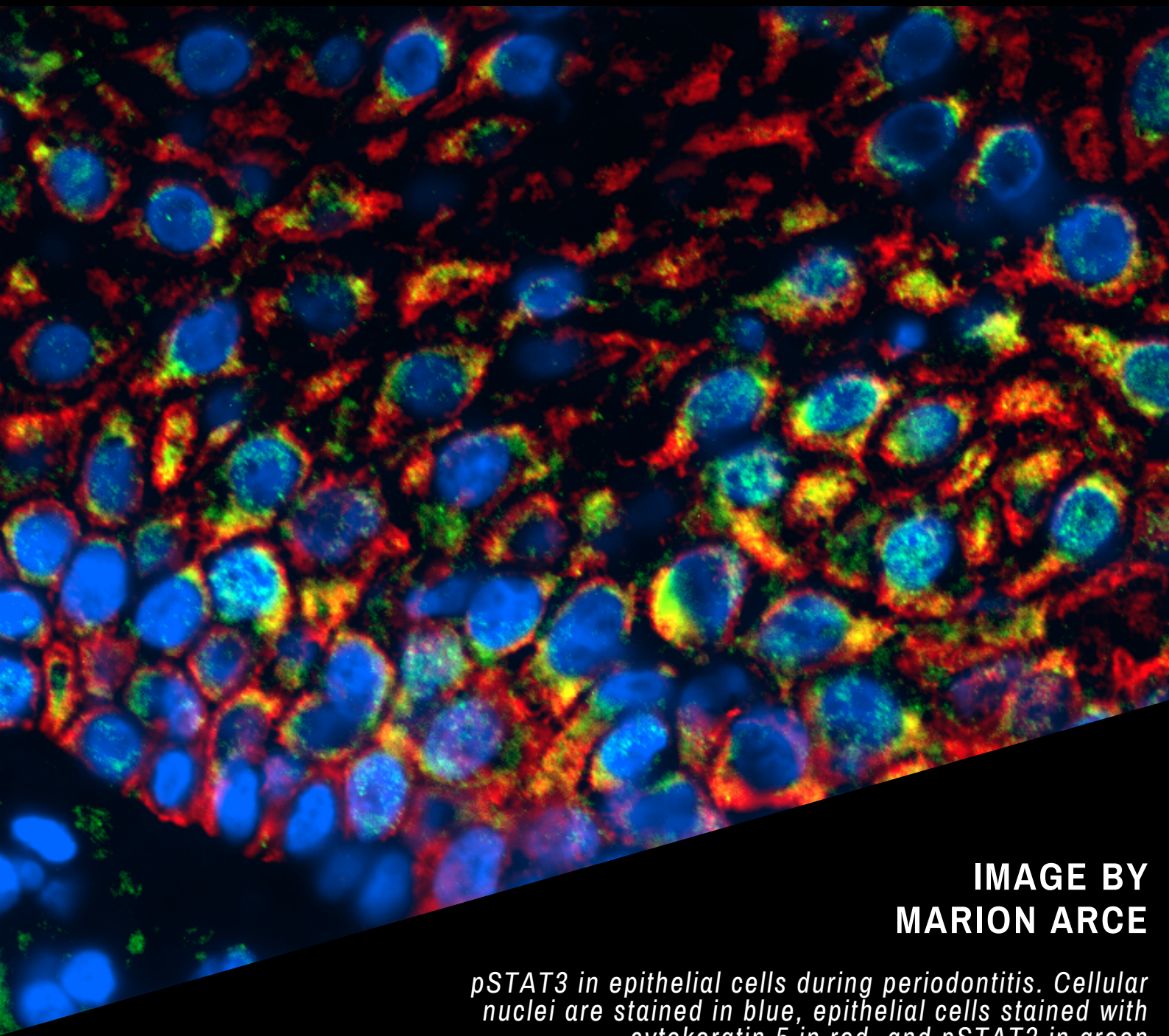

**IMAGE BY**
**MARION ARCE**

*pSTAT3 in epithelial cells during periodontitis. Cellular nuclei are stained in blue, epithelial cells stained with cytokeratin 5 in red, and pSTAT3 in green*

*Estimados miembros de la comunidad de Inmunología en Chile,*

*Queremos expresar nuestro profundo agradecimiento a todos aquellos que contribuyeron al rotundo éxito de nuestra Sexta Reunión Anual de la Asociación Chilena de Inmunología - ASOCHIN. Este evento no solo fue una ocasión de reencuentro, camaradería y ciencia dedicada a la Inmunología, sino que también marcó un hito significativo en nuestra misión de unir a la comunidad de Inmunólogos en Chile.*

*En primer lugar, extendemos nuestro reconocimiento especial a nuestros auspiciadores, cuyo apoyo desempeñó un papel crucial en la realización de este congreso. Su compromiso permitió que nos reunamos para compartir y disfrutar de los avances y descubrimientos en nuestra querida disciplina, la Inmunología.*

*La magnitud del éxito de nuestra reunión se reflejó en la participación activa de 120 asistentes, 3 charlas plenarias, 8 simposios, 22 expositores, 67 pósters y 10 short talks. Todo esto tuvo lugar en el marco del encantador escenario proporcionado por el Hotel Santa Cruz Plaza en Colchagua, creando un ambiente propicio para el intercambio de conocimientos y experiencias.*

*Nos enorgullece informar que reconocimos el talento de ocho estudiantes en la categoría de presentación de póster y dos estudiantes en la categoría de short talks. Además, este año nuevamente adjudicamos dos becas de asistencia para estudiantes. Una mención especial a Marion Arce, cuya extraordinaria fotografía embellece las páginas de nuestro libro de Resúmenes.*

*Como parte de nuestro compromiso con el reconocimiento a la excelencia en la Inmunología, establecimos el "Premio a la Trayectoria en Inmunología". Este galardón fue otorgado al Dr. Flavio Salazar Onfray en reconocimiento a su dedicación a la formación de estudiantes y su constante promoción de la Inmunología en Chile.*

*Esperamos sinceramente que esta experiencia haya sido memorable para todos ustedes y que podamos repetir este éxito en futuras instancias. Agradecemos nuevamente a cada uno de ustedes por su contribución y participación en hacer de este congreso un evento tan extraordinario.*

*Directiva ASOCHIN*

# 6th Annual Meeting ASOCHIN

## Hotel Santa Cruz, Colchagua

Asociación Chilena de Inmunología

# November 15th

| | |
|---|---|
| **10:15 - 10:30** | Welcome - **Dr. Daniela Sauma** |
| **10:30 - 11:30** | *"Harnessing the healing power of macrophages, a journey aided by epigenomic and high-dimensional biology approaches."*<br>**Dr. Laszlo Nagy**, Johns Hopkins University School of Medicine. |
| **11:30 - 13:00** | Symposium: New ASOCHIN Members |
| **11:30-12:00** | "*Role of meningeal lymphatic vasculature in homeostasis and disease of the central nervous system".*<br>**Dr. Noelia Escobedo**, Universidad Autónoma, Talca. |
| **12:00-12:30** | *"The relationship between periodontitis and type 2 diabetes mellitus: the role of extracellular vesicles".*<br>**Dr. María Luisa Mizgier**, Universidad de los Andes. |
| **12:30-13:00** | *"Nanoparticles and immunomodulators as therapeutical strategies to prevent Respiratory Syncytial Virus and Bovine Respiratory Disease Complex".*<br>**Dr. Fabián Díaz**, Iowa State University. |
| **13:00 - 13:30** | Sponsor visits and poster mounting |
| **13:30 - 15:00** | Lunch Break |
| **15:00 - 16:00** | Symposium: Counter-attacking self-attack: therapeutical strategies in autoimmunity |
| **15:00-15:30** | *"Immunomodulatory effect of ilex paraguariensis over immune cells and its impact in inflammatory diseases".*<br>**Dr. Andrés Herrada**, Universidad Autónoma, Talca. |
| **15:30-16:00** | *"The Yin and Yang of Interferon-gamma in Experimental and Human Multiple Sclerosis".*<br>**Dr. Rodrigo Naves**, Universidad de Chile. |
| **16:00 - 17:00** | Coffee Break |
| **17:00 - 17:20** | Sponsor Session BD |
| **17:30 - 18:30** | Symposium: The bad, the ugly and the good in the inflammatory response |
| **17:30-18:00** | *"IL-22 modulates microbiota-dependent gut motility"*<br>**Dr. Pedro Hernández**, Institut Curie. |
| **18:00-18:30** | *"D-lactate: A Potential Danger-Associated Metabolic Pattern"*<br>**Dr. Rafael Burgos,** Universidad Austral de Chile. |
| **18:30 - 20:00** | Poster Session - Beer and Science! |
| **19:30 - 21:00** | REUNION SOCIOS ASOCHIN: new board elections. |

# 6th Annual Meeting ASOCHIN

## Hotel Santa Cruz, Colchagua

Asociación Chilena de Inmunología

# November 16th

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|---|---|
| **08:40 - 09:00** | Sponsor Session MERCK - Optimización de inmunoensayos y su impacto en el análisis de biomarcadores |
| **09:00 - 10:00** | *"The oral microbiome as a diagnostic tool for periodontitis: an advance in clinical metagenomics".*<br>**Dr. Inmaculada Tomás Carmona**, Universidad de Santiago de Compostela. |
| **10:00 - 10:40** | Symposium: The oral-gut-brain axis |
| **10:00-10:20** | *"Looking for the association between oral health and the brain".*<br>**Dr. Jaime Díaz**, Universidad de Chile. |
| **10:20-10:40** | *"Mast Cells at the Crossroads of Enteric Nervous System and Intestinal Permeability, in the Gut-Brain axis."*<br>**Dr. Caroll Beltrán**, Universidad de Chile. |
| **10:40 - 11:20** | Coffee Break |
| **11:20 - 13:15** | Selected Short Talks |
| **11:20 - 11:30** | "Tissue-resident memory B cells in the kidney of a mouse model of Systemic Lupus Erythematosus". **Justine Castañeda** |
| **11:30 - 11:40** | "Regulation of microRNA degradation by the unfolded protein response sensor IRE1 in dendritic cells". **Alonso Lira** |
| **11:40 - 11:50** | "Deciphering the role of gut dysbiosis in the progression of Degenerative Cervical Myelopathy". **Nicole Ossandon** |
| **11:50 - 12:00** | "Mitochondria Transfer, a potential mechanism of CD4+ T cell exhaustion in oral cancer". **Bárbara Antilef** |
| **12:00 - 12:10** | "Ten-Eleven Translocation Expression is Associated with Monocytes Inflammatory Profile in Apical Periodontitis". **Maria Jose Bordagaray** |
| **12:10 - 12:20** | "Humoral immunity against SARS-CoV-2 evoked by heterologous vaccination schemes using Coronavac and BNT162b2 vaccines in adult population Chile". **Pablo Díaz** |
| **12:20 - 12:30** | "The non-damaged tissue of the Atlantic salmon gills affected by complex gills disease shows a network of interaction between the immune system and the tissue regeneration to counteract the infection". **Merari Goldstein** |
| **12:30 - 12:40** | "Functional and proteomic analysis of small extracellular vesicles from different subsets of T regulatory cells reveal distinct mechanisms of suppression".<br>**Javiera de Solminihac** |
| **12:40 - 12:50** | "Characterization and humanization analysis of Immunoglobulin New Antigen Receptor Variable Domains (VNARs) Derived from Cartilaginous Fishes Inhabiting the Chilean Coast to be Used in Biomedical Applications." **Richard Olivares** |
| **12:50 - 13:00** | "PD-L1 expression is associated with FTO expression and activity in human in-vitro models and tissues from Chilean patients of colorectal cancer."<br>**Diego Irribarra** |

# 6th Annual Meeting ASOCHIN

## Hotel Santa Cruz, Colchagua

Asociación Chilena de Inmunología

# November 16th

| | |
|---|---|
| **13:15 - 14:40** | Lunch Break |
| **14:40 - 15:00** | Sponsor Session Genexpress |
| **15:00 - 16:00** | Symposium: Looking at inborn errors of immunity! |
| **15:00-15:30** | *"Molecular defects in inborn errors of immunity"*<br>**Dr. Cecilia Poli**, Universidad del Desarrollo. |
| **15:30-16:30** | *"Molecular mechanism of C terminal COPA mutations"*<br>**Dr. Isabelle Meyts**, University of KU Leuven. |
| **16:30-17:00** | *"Rising of secondary immunodeficiency: cause and effect."*<br>**Dr. Nicole Le Corre**, Pontificia Universidad Católica de Chile. |
| **17:30 - 19:30** | Poster Session - Beer and Science! |
| **20:00 - 22:30** | Dinner |
| **23:00** | Party and cocktails!<br>Sponsored by Genexpress<br>Salón Inés de Suárez |

# 6th Annual Meeting ASOCHIN

## Hotel Santa Cruz, Colchagua

Asociación Chilena de Inmunología

# November 17th

| | |
|---|---|
| **09:00 - 10:30** | Symposium: Cellular architecture in tumor immunology |
| **09:00-09:30** | *"Cellular Archetypes in the Microenvironment of Human Metastases: unraveling insights through Single Cell Genomics and in vitro models."*<br>**Dr. Hugo González**, Fundación Ciencia y Vida. |
| **09:30-10:00** | *"MHC class I polypeptide-related sequence A (MICA) - NKG2D receptor axis in immuno-oncology."*<br>**Dr. María Carmen Molina**, Universidad de Chile. |
| **10:00-10:30** | *"Long-Term Survival and Immune Response Dynamics in Melanoma Patients Undergoing TAPCells-Based Vaccination Therapy"*<br>**Dr. Fermín González**, Universidad de Chile. |
| **10:30 - 11:30** | Coffee Break and Networking |
| **11:30 - 13:00** | Symposium: Metabolites from microbiota -and other sources- on homeostasis and disease |
| **11:30-12:00** | *"The role of FTO protein as key factor in the interaction between obesity and the immune response against colon cancer".*<br>**Dr. Glauben Landskron**, Universidad Finis Terrae. |
| **12:00-12:30** | *"Host microbiome on lung cancer".*<br>**Dr. Erick Riquelme**, Pontificia Universidad Católica de Chile. |
| **12:30-13:00** | *"IL-33 as modulator of the intestinal metabolite´s profile".*<br>**Dr. Karina Pino-Lagos**, Universidad de los Andes. |
| **13:00 - 14:30** | Lunch Break |
| **14:30 - 15:00** | Symposium: Stromal-Immune cell interactions in health and disease |
| **14:30-15:00** | *"Location and function of tissue-resident lymphocytes during inflammation and fibrosis".*<br>**Dr. Kelly Cautivo**, Gilead Sciences. |
| **15:30 - 16:00** | Remarkable Career in Immunology Award |
| **16:00 - 17:00** | Poster/Short-Talk Awards, presentation of the new Board and Closing Remarks |

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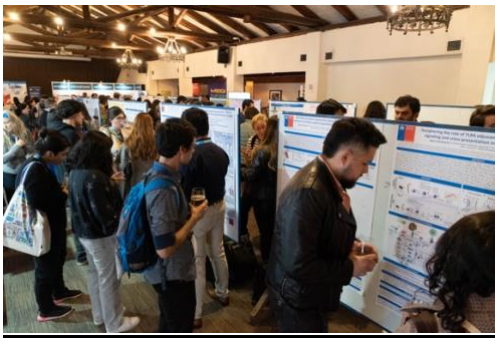
## TALKS AND SHORT TALKS









# TALKS

**Long-Term Survival and Immune Response Dynamics in Melanoma Patients Undergoing TAPCells-Based Vaccination Therapy**

**Fermín E. González**[1], Andrés Tittarelli[2], Cristian Pereda[3], María A. Gleisner[3,4], Mercedes N. López[3], Iván Flores[3], Fabián Tempio[3], Álvaro Lladser[5,6], Adnane Achour[7,8], Claudia Durán-Aniotz[9], Juan P. Miranda[10], Milton Larrondo[11], Flavio Salazar-Onfray[3,4,7,8]

(1) Universidad de Chile, Laboratory of Experimental Immunology & Cancer, Faculty of Dentistry, Santiago, Chile
(2) Universidad Tecnológica Metropolitana, Programa Institucional de Fomento a la Investigación, Desarrollo e Innovación, Santiago, Chile
(3) Universidad de Chile, Disciplinary Program of Immunology, Institute of Biomedical Sciences,, Faculty of Medicine, Santiago, Chile
(4) Millennium Institute on Immunology and Immunotherapy, Institute of Biomedical Sciences, Faculty of Medicine, Santiago, Chile
(5) Fundación Ciencia & Vida, Centro Científico y Tecnológico de Excelencia Ciencia & Vida, Santiago, Chile
(6) Universidad San Sebastián, Facultad de Medicina y Ciencia, Santiago, Chile
(7) Karolinska Institute, Science for Life Laboratory, Department of Medicine Solna, Stockholm, Sweden
(8) Karolinska University Hospital, Division of Infectious Diseases, Stockholm, Sweden
(9) Universidad Adolfo Ibañez, Latin American Brain Health Institute (BrainLat), Center for Social and Cognitive Neuroscience (CSCN), School of Psychology, Santiago, Chile
(10) Instituto Nacional del Cáncer, Santiago, Chile
(11) Hospital Clínico de la Universidad de Chile, Banco de Sangre, Santiago, Chile

Cancer vaccines are a promising treatment in refractory patients to checkpoint blockers because their ability in breaking tolerance. A phase I/II clinical trial with TAPCells vaccine, based on autologous APCs loaded with heat-shocked allogeneic melanoma cell lysates, was previously reported. TAPCells induced cellular immune responses, characterized by lysate-specific delayed-type hypersensitivity (DTH) reactions in nearly 60% of 86 melanoma patients, associated with prolonged survival.
Here, we provide a comprehensive update on this cohort, with a median follow-up >15 years. Patients were stratified into short-term (<36 months) and long-term (≥36 months) survivors and associations between clinical outcomes and demographic, medical, genetic, and immunologic parameters were explored.
DTH positive ($DTH^{pos}$) patients maintained a three-year overall survival rate of 53.1% vs. 16.1% for $DTH^{neg}$. Extended remissions for long-term survivors were observed in patients with $DTH^{pos}$ and lacking distant metastases ($M1c^{neg}$). $DTH^{pos}$, stage III disease, moderate adverse events, and younger age at treatment initiation yielded survival benefits also for short-term survivors. These outcomes were corroborated in a cohort of 24 patients who underwent compassionate TAPCells treatment. TAPCells therapy is linked to stable long remissions in 35.2% of patients and an even more striking 54.9% of $M1c^{neg}/DTH^{pos}$ melanoma patients. Augmented expression of CLEC2D on $CD4^+$ T cells and increased serum levels of IL-17A are observed in long-term survivors. Conversely, the TLR4 Asp299Gly polymorphism and decreased CD32 expression on B cells were associated with reduced survival in short-term survivors.
This study underscores the potential for TAPCells to arrest disease progression and highlights identifying biomarkers helpful in predicting long-term remission.

Keywords: Immunotherapy, Melanoma, Dendritic cell, Clinical trials, Biomarkers

Funding: This work was financially supported by grants FONDECYT 1090238, 1171213 and 1231853; FONDEF D02I1088, D04T2026, and D05I10366); and Millennium Science Initiative, National Agency for Research and Development (ICN09_016/ICN 2021_045).

**D-lactate: A Potential Danger-Associated Metabolic Pattern**

**Rafael A. Burgos**[1], John Quiroga[1], Pablo Alarcón[1], Carolina Manosalva[2]

(1) Universidad Austral de Chile, Laboratory of Inflammation Pharmacology and Immunometabolism, Institute of Pharmacology and Morphophysiology, Faculty of Veterinary Sciences, Valdivia, Chile
(2) Universidad Austral de Chile, Institute of Pharmacy, Faculty of Sciences, Valdivia, Chile

D-lactate is an enantiomer of lactate originating from carbohydrate metabolism via the conversion of methylglyoxal (MG) or bacterial metabolic pathways. Physiologically, D-lactate concentrations remain low, with elevated levels typically observed only in cases of digestive metabolic disorders, where they can reach millimolar ranges. For instance, ruminants subjected to diets rich in highly fermentable carbohydrates ofte
n experience D-lactoacidemia, a condition characterized by the development of neutrophilic aseptic polysynovitis. Our research has demonstrated that D-lactate has the potential to induce metabolic reprogramming in both neutrophils and fibroblast-like synoviocytes. This reprogramming is marked by a notable increase in glycolytic activity. Mechanistically, we have established that this effect is governed by NF-κB and HIF-1α, two transcription factors known to play pivotal roles in the upregulation of IL-6 and COX-2 expression. Additionally, our investigations have revealed that D-lactate can stimulate the release of extracellular DNA traps (NETs) in polymorphonuclear leukocytes. This phenomenon is mediated through the GSK3β pathway and the generation of mitochondrial reactive oxygen species (mtROS). Notably, the release of mtROS and NETs induced by D-lactate can be mitigated through the use of rotenone, suggesting an association with cellular metabolic disturbances involving complex I of the electron transport chain. Furthermore, recent research indicates that D-lactate could potentially function as an agonist for TLR2 and TLR9 receptors, resulting in an upsurge in NF-κB activity within macrophages. Considering that elevated levels of D-lactate are typically associated with metabolic disruption, we postulate that this enantiomer may serve as a damage-associated molecular pattern (DAMP).

Keywords: D-lactate, Synoviocyte, polymorphonuclear leucocytes, Immunometabolism

Acknowledgments: FONDECYT 1210754FONDECYT POSTDOCTORADO 3230482

# SHORT TALKS

**Tissue-resident memory B cells in the kidney of a mouse model of Systemic Lupus Erythematosus**

**Justine Castañeda**[1], Nicolás Valdivieso[2,3], Valeska Simon[3], Daniela Sauma[1,3], Mario Rosemblatt[1,2,3], María Rosa Bono[1,3], Sarah Nuñez[1,2]

(1) Centro Ciencia & Vida, Santiago, Chile
(2) Facultad de Medicina y Ciencia, Universidad San Sebastián, Santiago, Chile
(3) Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile

Systemic autoimmune diseases, such as Systemic lupus erythematosus (SLE), are generally characterized by the activation of self-reactive B cells leading to the generation of self-reactive memory B cell (Bmem) and plasma cells. Consequently, SLE patients have increased numbers of circulating memory B cells which often correlate with disease activity. It has been recently demonstrated that in the context of infection, Bmem are not only maintained in the circulation but also acquire tissue residency in affected organs such as the lungs. Whether tissue-resident Bmem can also arise in autoimmune disease an play a local role in tissue damage has not been explored. In this work, we used the mouse model BWF1 that spontaneously develops a lupus-like disease, including severe glomerulonephritis. By combining intravascular labeling and immune phenotyping to distinguish circulating vs non-circulating cells, we show that in BWF1 mice that develop glomerulonephritis there is a remarkable accumulation of non-circulating B cells in the kidneys. A significant fraction of non-circulating kidney B cells are class-switched and $IgM^+$ memory B cells that express CD73 and PD-L2. Interestingly, class-switched memory B cells in the kidney express signature markers of tissue residency CXCR3 and CD69. We are currently exploring if kidney-resident B cells can contribute to local inflammation and damage through the production of cytokines and self-reactive antibodies. Moreover, we are addressing whether this tissue-resident subset is resistant to B cell-depletion therapy, which may be a relevant limitation to the current use of Rituximab in SLE patients.

Keywords: Autoimmunity, Lupus, Memory B cell, Tissue-resident lymphocyte

Funding: ANID 11221128, ANID 1230183, ANID 1220196, ANID FB210008, ANID EQM 220027

**Regulation of microRNA degradation by the unfolded protein response sensor IRE1 in dendritic cells.**

**Alonso Lira**[1], Fabiola Osorio[1], María Julieta Gonzalez[2]

(1) Universidad de Chile, Programa de Inmunología, Facultad de Medicina, Av. Independencia 1027, Santiago, Chile
(2) Universidad de Chile, Programa de Biología Molecular y Celular, Facultad de Medicina, Av. Independecia 1027, Santiago, Chile

The Unfolded Protein Response (UPR) is a vital cellular stress pathway that maintains proteostasis of the endoplasmic reticulum (ER). IRE1 and XBP1s, a main arm of the UPR, are well-studied components which in addition to their canonical roles in protein folding, they also regulate the development and function of conventional type 1 dendritic cells (cDC1). cDC1s play pivotal roles in antitumor and antiviral responses and interestingly, in steady state, these cells display constitutive IRE1 activation.
IRE1 contains an endoribonuclease (RNase) domain which perform two important functions: it mediates the unconventional splicing of *Xbp1* mRNA, prompting the translation of the transcription factor XBP1s (XBP1 spliced), master regulator of ER homeostasis. And second, the IRE1 RNase domain also degrades various mRNAs and microRNAs containing the consensus sequence *CUGCAG*, in a process known as RIDD (*Regulated IRE1 Dependent Decay*). Although it is described that RIDD is important for cDC1 function, recent evidence indicates that this process could also influence the levels of microRNAs (miRNAs) that are critical for development, survival and inflammation. However, to date, no evidence connecting IRE1 activity and miRNA levels has been made in the cDC1 lineage. Thus, **a key question to ask is whether IRE1 through RIDD, can degrade miRNAs associated with cDC1 function. In this study, we explore the interplay between RIDD activation and miRNAs degradation in cDC1, with a specific focus on miRNAs linked to their pro-inflammatory function (miR-155 5p) and survival (miR-125a 5p).** We carried out bioinformatic analyses using the RNAfold platform which revealed that both miR-125a and miR-155 possess recognition sequences for IRE1 RNase activity within the required secondary structure context (stem-loop). Then, as cDC1 source, we studied a bone marrow DC culture system termed 'OP9-DL1 DCs' which recapitulates the characteristics of cDC1 present in lymphoid tissues and organs. To model the function of the RNase domain of IRE1, we used different colonies of conditional knock-out mice including CD11c-Cre XBP1 fl/fl (XBP1ΔDC) mice, which lacks XBP1s in DCs and exhibits constitutive activation of RIDD in cDC1 and the CD11c-Cre x IRE1fl/fl mouse (IRE1TRUNCΔDC) which lack the RNase domain of IRE1 in DCs, thereby inhibiting RIDD.

Studies assessing expression of miR-155, a pro-inflammatory miRNA that indirectly induces the production of the pro-inflammatory cytokines IL-12, IL-6 and TNF-a, showed that miR-155 levels are reduced in cDC1s lacking XBP1 (which spontaneously activate RIDD) in resting and poly I:C-activated state. Concomitantly, these cells exhibit a reduction in the expression of IL-12, IL-6 and TNF-a in a RIDD-dependent manner (previous reported findings from our lab, Medel, B *et al* (2023). The Unfolded Protein Response Sensor IRE1 Regulates Activation of In Vitro Differentiated Type 1 Conventional DCs with Viral Stimuli. *International Journal of Molecular Sciences*). Future experiments will include the validation of direct miR-155 targets by western blot and inhibition of the RNase activity of IRE1 through pharmacological / genetic tools. Additionally, we will use molecular mimetics of miR-155 in cDC1 from XBP1ΔDC mice to determine whether there is a recovery in the pro-inflammatory cytokine production upon miR-155 overexpression. Following the same approach, we will employ miR-155 inhibitors in cDC1s from WT mice to assess whether a similar decrease in the production of pro-inflammatory cytokines occurs, as observed in cDC1 with active RIDD.

Studies aiming to assess cDC1 survival showed that XBP1 deficient cDC1s display increased viability compared to WT counterparts in resting and poly I:C activated conditions. In this context, expression of the pro-apoptotic microRNA miR-125a in in XBP1-deficient cDC1 showed a tendency towards decrease and a tendency towards increase in IRE1-deficient cDC1s. To obtain a clearer picture, we will determine expression of the 3p variant of miR-125a , which is also described to be targeted by RIDD. Therefore, future experiments will be to meausure the levels of miR-125a 3p expression in cDC1 in contexts of RIDD activation/inhibition.

Future studies include RNA sequencing (RNAseq) of miRNAs in RIDD-activated and non-activated sorted cDC1 cells and *in vitro* cleavage assays will explore IRE1 ability to cleave miRNAs with consensus cleavage sequences. This project will highlight novel insights on the regulation of miRNA homeostasis in cDC1s through RIDD, establishing a new regulatory axis that can be further explored for therapeutic purposes.

Keywords: IRE1, UPR, microRNAs, cDC1, Innate Immunity

**Deciphering the role of gut dysbiosis in the progression of Degenerative Cervical Myelopathy**

**Nicole Ossandon**[1], Jose Miguel Pacheco[1], Francisca Espinoza[2], Pia M. Vidal[1]

(1) Universidad Católica de la Santísima Concepción, Basic Sciences, Neuroimmunology and Regeneration of the Central Nervous System Unit, Medicine, Alonso de Ribera 2850, Concepción, Chile
(2) Universidad Católica de la Santísima Concepción, Basic Sciences, Developmental Neurobiology Unit, Medicine, Alonso de Ribera 2850, Concepcion, Chile

Degenerative Cervical Myelopathy (DCM) is the commonest cause of spinal cord impairment in the elderly population. It is mainly characterized by local and systemic neuroinflammation, spinal cord ischemia, apoptosis, and astrogliosis, that contributes to locomotor deterioration. Gastrointestinal dysfunction is common in human patients. We have recently demonstrated the presence of gut dysbiosis, imbalance in the gut microbiota composition, in the DCM mouse model, that can be associated with locomotor deterioration. Herein, we investigated the role of a probiotic as a therapeutic strategy to halt disease progression.
DCM was induced in C57BL/6 mice by implanting an aromatic polyether material underneath the C5-C6 laminae to cause progressive compression of the spinal cord. Animals received probiotic or vehicle treatment to restore gut microbiota composition. Locomotor outcomes were measured using the Open field, von Frey, and the ladder walk tests. Neuroinflammation was analyzed by immunohistochemistry within the spinal cord. Gut microbiota composition was assessed by 16S rRNA sequencing of fecal samples.
Locomotor assessments with the open field and ladder walk tests did not show a significant effect of probiotic treatment attenuating disease progression. However, a significantly (* $p<0.05$) decreased mechanical withdrawal threshold was observed following probiotic treatment as compared to the vehicle treatment group.

Keywords: Gut dysbiosis, Neuroinflammation, Degenerative Cervical Myelopathy

**Mitochondria transfer, a potential mechanism of CD4+ T cell exhaustion in oral cancer**

**Bárbara Antilef Cáceres**[1], Solange Cisterna[1], Romina Quiroga[1], Camilo Cabrera[1], Sergio Sanhueza Novoa[1], Camila Muñoz Grez[1], Felipe Zuñiga[1], Luciano Ferrada[2], Wilfredo González[3,4], Andrés Caicedo[6], Patricia Luz-Crawford[5], Liliana Lamperti[1], Mauricio Hernández[7], Estefanía Nova-Lamperti[1]

(1) Molecular and Translational Immunology Laboratory, Clinical Biochemistry and Immunology Department, Pharmacy Faculty, Universidad de Concepción, Concepción, Chile
(2) CMA BIO BIO, Faculty of Biological Sciences, Universidad de Concepción, Concepción, Chile
(3) Universidad de los Andes, Dentistry Faculty, Santiago, Chile
(4) Center for Research and Innovation in Biomedicine, Universidad de los Andes, Santiago, Chile
(5) Laboratorio de Inmunología Celular y Molecular, Centro de Investigación Biomédica, Universidad de Los Andes, Santiago, Chile
(6) Universidad San Francisco de Quito USFQ, Colegio de Ciencias de la Salud, Escuela de Medicina, Quito, Ecuador
(7) MELISA institute, Compañía de biotecnología, San pedro de la paz, Chile

The tumor microenvironment in oral squamous cell carcinoma (OSCC) promotes an exhausted phenotype in $CD4^+$T-cells, which has been associated with changes in cell metabolism. The main organelle of cell metabolism is the mitochondria and in recent years it has been indicated that several cells have the capacity to transfer mitochondria. However, it has not been evaluated whether mitochondria transfer from cancer cells to $CD4^+$T-cells induces an exhausted phenotype. The aim of this investigation was to analyze exhaustion and changes in cell metabolism in $CD4^+$T-cells after mitochondria transfer from OSCC-cells. $MitoTrackerGreen^+$-mitochondria from OSCC-cells were MitoCepted to $CD4^+$T-cells. Then, surface molecule expression, proliferation, cytokine secretion, mitochondrial oxidative stress and glucose metabolism after mitochondria transfer were analyzed by flow cytometry. In addition, a metabolomic analysis was perfomed on MitoCepted T-cells to evaluate changes in T-cell metabolites. We observed that $CD4^+$T-cells that acquired mitochondria had reduced cell proliferation and increased expression of two inhibitory proteins (TIGIT; CTLA4), and proteins associated with exhausted phenotype (PD-1; PDL-1; LAG3). Regarding cytokines, a significant decrease was observed in the mitocepted group for IFN-gamma, TNF-alpha, IL-10 and IL-4, when compared with the control. In addition, it was observed that after tumor-mitochondria transfer, $CD4^+$T-cells exhibited a greater production of mitochondrial superoxide, a reduction of the pyruvate dehydrogenase cofactor Vitamin B1 and significanly higher glucose uptake and lactate production, in comparions with the control sample. Overall, the acquisition of isolated mitochondria from oral cancer cells by $CD4^+$T-cells promotes an exhausted phenotype and a dysfunctional $CD4^+$T-cell, affecting the anti-tumor response in OSCC.

Keywords: mitochondrial transfer, immunoregulation, oral cancer oscc, exhausted phenotype

Funding: REGULAR FONDECYT PROJECT 1211418

Acknowledgments: Molecular and Translational Immunology Laboratory, Clinical Biochemistry and Immunology Department, Pharmacy Faculty, Universidad de Concepción, Concepción, Chile

**Ten-Eleven Translocation Expression is Associated with Monocytes Inflammatory Profile in Apical Periodontitis**

**María José Bordagaray**[1,4], Alejandra Fernández[3], Elizabeth Pellegrini[4], Mauricio Garrido[1], Marcela Hernández[2,4]

(1) Universidad de Chile, Department of Conservative Dentistry, Faculty of Dentistry, 943 Olivos, Santiago, Chile
(2) Universidad de Chile, Department of Pathology and Oral Medicine, Faculty of Dentistry, 943 Olivos, Santiago, Chile
(3) Universidad Andrés Bello, Faculty of Dentistry, 237 Echaurren, Santiago, Chile
(4) Universidad de Chile, Laboratory of Periodontal Biology, Faculty of Dentistry, 943 Olivos, Santiago, Chile

**Introduction:** Apical periodontitis (AP) is the destruction of peri-radicular tissues and is associated with low-grade systemic inflammation. Their mechanistic link is unknown, but monocytes are proposed to carry DNA-methylation profiles that might contribute to systemic inflammation. DNA-methylation status depends on the DNA-methylase (DMNT) and Ten-eleven-translocation (TET) enzymes. The DMNT-TET expression, global secretory profile, and DNA-methylation of TNF-α in AP individuals remains elusive. **Objectives**: Determine DNMT-TET expression and its association with the global secretory profile and DNA-methylation of TNF-αin monocytes of individuals with AP and controls. **Methods**: Cross-sectional. AP (n=25) and controls (n=29) individuals consulting at Universidad de Chile were included. Demographic and clinical data were recorded. Monocytes were isolated and cultured for 24h. RNA/DNA were extracted. mRNA expression of DMNT3a and TET1 was determined by qPCR. The secretory profile was assessed by Multiplex. DNA was bisulfite-treated, TNF-α gene was amplified and sequenced. The results were analyzed with STATA V.16 ($p<0.05$). **Results:** DMNT3a and TET1 were downregulated in AP versus controls ($p<0.05$), while the secretory levels of TNF-α and IL-1β were higher in AP compared to controls ($p<0.05$). AP TNF-α promoter was hypomethylated in comparison with controls ($p=0.026$), but DNA-methylation of TNF-α was not correlated with DMNT3a or TET1 expression ($p>0.05$). TET1 was negatively correlated with the levels of the proinflammatory cytokines IL-17, IL-1β, IL-6, and TNF-α ($p<0.05$). **Conclusions:** Monocytes in AP demonstrated a proinflammatory profile and lower expression of enzymes involved in methylation pattern modifications. Thus, DMNT and TET expression in monocytes possibly regulates low-grade systemic inflammation in AP.

Keywords: Apical Periodontitis, Monocytes, DNA-Methylation, DNMT, TET

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**Humoral immunity against SARS-CoV-2 evoked by heterologous vaccination schemes using Coronavac and BNT162b2 vaccines in adult population Chile**

**Pablo Díaz**[1], Diego Díaz Dinamarca[1], Gisselle Barra[1], Rodrigo Puentes[1], Loredana Arata Reyes[1], Jonnathan Grossolli Galvez[1], Boris Riveros Rodriguez[1], Luis Ardiles[1], Julio Santelises[1], Valeria Vasques-Saez[1], Daniel Escobar[1], Daniel Soto[1], Cecilia Canales[1], Janepsy Díaz Tito[1], Liliana Lamperti[2], Daniela Castillo[2], Mychel Urra[2], Felipe Zuñiga[2], Valeska Ormazabal[2], Estefanía Nova-Lamperti[2], Rosana Benitez[3], Alejandra Rivera[3], Claudia Cortes[6,7,8], Maria Teresa Valenzuela[4], Heriberto Garcia-Escorza[1], Abel Vásquez[1,5,9]

(1) Insitituto de Salud Pública de Chile, Marathon 1000, Santiago, Chile
(2) Universidad de Concepción, Faculatd de Química y Farmacia, Concepción, Chile
(3) Clínica Dávila, Santiago, Chile
(4) Universidad de los Andes, Medicina, Santiago, Chile
(5) Universidad del Alba, Medicina, Santiago, Chile
(6) Universidad de Chile, Medicina, Santiago, Chile
(7) Instituto Milenio de Inmunología e Inmunoterapia, Santiago, Chile
(8) Clínica Santa María, Santiago, Chile
(9) Universidad del Desarrollo, Medicina, Santiago, Chile

**Introduction**: Severe acute respiratory syndrome virus 2 (SARS-CoV-2) caused over million deaths worldwide, and over 61,000 deaths in Chile. Chilean authorities implemented a vaccination scheme against SARS-CoV-2 using CoronaVac and BNT162b2. Given the global need for vaccine boosters to fight emerging virus variants, studying SARS-CoV-2 immune response is crucial. We characterized humoral immune response in inoculated adults chilean volunteers with CoronaVac(2x); CoronaVac(2x)+BNT162b2(1x); and BNT162b2(3x) schemes.**Methods**:469 participants recruited from Clínica Dávila and Health Family Center Víctor-Manuel Fernández from city of Concepción. We also included participants recovered from COVID-19 but not vaccinated (RCN). Antibodies were analyzed, including anti-N, anti-S1-RBD, and neutralizing antibodies against SARS-CoV-2. **Results:** We found that **antibodies against-N** of SARS-CoV-2 were significantly higher in CoronaVac(2x) and RCN groups compared to CoronaVac(2x)+BNT162b2(1x) or BNT162b2(3x) groups. However, CoronaVac(2x)+BNT162b2(1x) and BNT162b2(3x) groups exhibited a higher concentration of **S1-RBD antibodies** than CoronaVac(2x) group and RCN group. There were no significant differences in S1-RBD antibody concentration between CoronaVac(2x)+BNT162b2(1x) and BNT162b2(3x) groups. Finally, the group immunized with BNT162b2(3x) had a statistically significant higher levels of **neutralizing antibodies** compared to the other groups (~2-25 folds). Also, no differences were found when we analyze the demographic and comorbidity data in humoral response. **Discussion**:These findings suggest that vaccination **induces the secretion of antibodies against SARS-CoV-2, and a booster dose of BNT162b2 is necessary to generate a protective immune response.** These data support the Chilean Ministry of Health decision to promote heterologous vaccination as they indicate that a significant portion of the Chilean population has neutralizing antibodies against SARS-CoV-2.

Keywords: Heterologous vaccination, SARS-CoV-2, SARS-CoV-2 neutralizing antibodies, immunization schedules, chilean vaccination
Funding: This project was financed with the Institute of Public Health budget Acknowledgments: To Dr. Fernando Valiente and Clínica Santa María who kindly facilitate the serum samples from donnors with 2 doses of Coronavac.

**The non-damaged tissue of the Atlantic salmon gills affected by complex gills disease shows a network of interaction between the immune system and the tissue regeneration to counteract the infection.**

**Merari Goldstein**[1], Mabel Vidal[1,2], María J. Santillán-Araneda[1], Karina Reichel-Fuentes[1], Eva Vallejos Vidal[1,3], Felipe E. Reyes-López[1]

(1) Universidad de Santiago de Chile, Centro de Biotecnología Acuícola, Facultad de Química y Biología, Alameda 3363, Edificio Eduardo Morales Santos, Piso 3, 9170002 Estación Central, Santiago, Chile
(2) Universidad San Sebastián, Arquitectura y Diseño, Facultad de Ingeniería, Lientur 1457, 4080871, Concepción, Chile
(3) Universidad de Las Américas, Núcleo de Investigación Aplicada en Ciencias Veterinarias y Agronómicas, Facultad de Medicina Veterinaria y Agronomía, Walker Martínez 1360, Piso 3, 8250122 La Florida, Santiago, Chile

The complex gill disease/disorder (CGD) is a non-specific disease characterized by non-specific pathological changes and a primary etiology difficult to establish. CGD is considered one of the major challenges to gill health in marine-farmed Atlantic salmon. Contradictorily, there is no information regarding the immune response and the biological mechanisms to contain the disease progression in the non-damaged gill filament. In this study, we performed transcriptomic profiling on gills with CGD lesions in Atlantic salmon farmed in a seawater environment located in the fjords of the Aysén Region (Chile). For total RNA extraction, we selected Atlantic salmon affected by CGD. Then, we divided the same gill filament into two portions: damaged tissue (DT; pale area) and non-damaged tissue (NDT; reddish zone). The RNA-Seq transcriptomic analysis used a pooling strategy (n = 3 pools per condition; n = 5 fish per group). We found 332 downregulated and 364 upregulated differential expressed genes (DEGs). In the functional enrichment analysis, we identified several processes associated with the immune response at the innate level through the degranulation of neutrophils, adaptive through the expression of butyrophilins (BTN) genes, and cytokines such as interleukin 10 (IL-10). We also identified other biological processes rinvolved in cellular communication (cell-cell and cell-extracellular matrix) and the organization of the extracellular matrix, mainly represented by the balance between the formation and degradation of collagen. These results indicate a complex network of interaction between the immune system and the tissue regeneration in the NDT to counteract the infection by CGD.

Keywords: Complex gill disease, Salmo salar, immune system, environmental interaction, RNA-seq

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**Functional and proteomic analysis of small extracellular vesicles from different subsets of T regulatory cells reveal distinct mechanisms of suppression**

**Javiera de Solminihac**[1], María José Moya-Guzmán[1], Carolina Rivera[1], Camila Pinto[1], Thilo Kaehne[2], Karina Pino-Lagos[1]

(1) Universidad de los Andes, Centro de Investigación e Innovación Biomédica, facultad de medicina, Santiago, Chile
(2) Otto von Guericke University, Magdeburg, Germany, Institute of Experimental Medicine, Medical Faculty, Magdeburg, Germany

T regulatory cells (Tregs) act as modulators of immunity. One of their suppressive mechanisms is the release of small extracellular vesicles (sEV). Tregs can be classified based on their origin: thymic or natural (nTregs) and induced (iTregs) Tregs. However, it is unknown whether their origin determines their mechanism of action, including the properties of their sEV. nTregs and naïve CD4+ T cells were purified using magnetic beads. nTregs were cultured for 48h and iTregs were induced with IL-2 and TGF-b alone (iTregs) or with retinoic acid (RATregs). sEV were purified using IZON columns and characterized by size using the Nano-tracking analysis (NTA) equipment. Protein content was evaluated by tandem-mass spectrometry (MS/MS). Suppression assays were performed by polyclonally activating splenocytes for 72h in the presence of sEV obtained from the three types of Tregs. Cell phenotype and apoptosis were evaluated by flow cytometry and cytokine production by Elisa. sEV obtained from the three-types of Tregs did not show differences in particle´s number or size. Suppression of T cell proliferation was sEVs-dose-dependent, observing iTregs-derived sEV as less effective on inhibition. sEV isolation from RATreg favor the release of IFN-γ, IL-17 and apoptosis. Proteomic data indicates that three-types of sEV contain proteins involved on T cell activation and cell death. Tregs secrete sEV as part of their suppression mechanisms. Comparison of different sources of sEV indicates that RATregs differ from nTregs and iTregs as theis sEV trigger pro-inflammatory cytokine release and apoptosis on target cells.

Keywords: sEV, Tregs, suppression, proteomics

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**Characterization and humanization analysis of Immunoglobulin New Antigen Receptor Variable Domains (VNARs) Derived from Cartilaginous Fishes Inhabiting the Chilean Coast to be Used in Biomedical Applications.**

**Richard Olivares Olivares**[1,2,3], Angello Retamal Díaz[1,2,3], Angélica Bravo[3], Carlos Bustamante[4], Margarita Lay[1,2,3]

(1) Universidad de Antofagasta, Departamento de Biotecnología, Facultad de Ciencias del Mar y Recursos Biológicos, Av. Universidad de Antofagasta#02800, Antofagasta, Chile
(2) Universidad de Antofagasta, Instituto Milenio en Inmunología e Inmunoterapia, Av. Universidad de Antofagasta#02800, Antofagasta, Chile
(3) Universidad de Antofagasta, Centro de Investigación en Inmunología y Biotecnología Biomédica de Antofagasta (CIIBBA), Av. Universidad de Antofagasta#02800, Antofagasta, Chile
(4) Universidad de Antofagasta, Instituto de Ciencias Naturales Alexander von Humboldt, Laboratorio de Biología Pesquera, Facultad de Ciencias del Mar y Recursos Biológicos, Av. Universidad de Antofagasta#02800, Antofagasta, Chile

**Introduction:** Antibodies are powerful tools in research and diagnostics. Particularly, monoclonal antibodies have widely been used to detect various pathogenic diseases due to their higher purity and specificity which gives them multiple functions such as targeting protein-protein interactions and identifying single members of protein families.
The antibody is a structural complex composed of two heavy chains and two light chains. The heavy chain of a conventional antibody consists of three constant domains (CH1, 2 and 3) and one variable domain (VH), while the light chain consists of one constant domain (CL) and one variable domain (VL). VH is the responsible for antigen recognition. However, the efficacy of conventional antibodies could be limited by their large molecular size (≈150kDa) and complex structure, where their paratopes may not be able to access certain antigens, resulting to poor binding affinity. Degeneration by high temperatures and humidity are the main factors affecting sensitivity that could simply lead to false positive results. To overcome these problems, exploration of new antigen binders with natural heat-stability is extensively needed for improving the current diagnostic platform. In recent years, single domain antibodies have been in the spotlight of researchers due to their great therapeutic and diagnostic potential. These antibodies are found in camelids (HcAb or VHH) and cartilaginous fishes (IgNAR or VNAR). *Mustelus mento* as well as *Mustelus whitneyi* are cartilaginous fishes found on the Chilean coasts and share taxonomic similarities with *Mustelus canis* and *Triakis scyllium* where new antigen receptor immunoglobulin (IgNAR) has already been reported. Each chain in IgNAR consists of five constant domains followed by one variable domain. The variable domain of IgNAR, or referred to as VNAR, contains only two complementarity-determining regions (CDRs), as known as CDR1 and 3. Therefore, it allows VNAR diversity to be achieved by long variable CDR3 that protrude with additional diversity in CDR1 and connected through two hypervariable regions (HV), HV2 and 4, which confers VNRAs the formation of special paratopes that can access confined epitopes.
VNARs are so far the smallest natural immunoglobulin-based protein scaffolds, with a molecular mass of about 12kDa, in the animal kingdom. Due to their small size, high specificity for a cognate antigen, and high physiochemical stability, these antibody domains have been considered as promising candidates for biomedical development.
In this study, we propose the development of an innovative platform for the generation of variable single chain (VNAR) antibodies from a Chilean cartilaginous fish to be used in biomedical therapies.

**Aims: (1)** To identify and describe VNAR domain sequences in *M. mento* and *M. whitneyi* using conserved regions from previously described cartilaginous fishes. **(2)** To develop VNARs libraries and characterize the natural coding sequence for single-domain antibodies of *M. mento* and *M. whitneyi*. **(3)** To design and propose *In Silico* potential methods for humanization to be used in biomedical applications.

**Methods:** The design of degenerate primers and characterization of IgNAR expression in *Mustelus Mento* and *Mustelus whitneyi* was performed by bioinformatics assays. The obtainment of VNARs was done by using RT-PCR, bacterial transformation, and characterized by Sanger sequencing. Finally, the design of the humanization strategy for IgNAR variable domain sequences was performed through bioinformatic software's.

**Results:** Degenerate primers were design to amplify and characterize *M. Mento* and *M. whitneyi's* IgNAR variable domain sequences. VNARs library was done by clone VNARs PCR products, using three pairs of specific primers, and were successfully transformed into chemically competent cells (Figure I & II). The coding sequences for VNAR domains of *M. mento* and *M. whitneyi* displayed similarities with sequences from the variable light chain of the human immunoglobulin.

**Discussion:** The identification and characterization of VNARs in *M. mento* and *M. whitneyi* may open new opportunities for the generation of therapeutic and diagnostic agents. The ability of these VNARs to access confined epitopes, due to the uniqueness of their paratopes, suggests that they could be especially valuable in detecting molecular targets that are difficult for conventional antibodies to reach due to their size and complexity. In addition to VNARs characterization, we have highlighted the importance of humanizing these sequences for biomedical applications. The *In Silico* humanization strategy is a fundamental step to ensure compatibility with human biological systems and to minimize potential immunogenic responses. However, it is crucial to emphasize that the successful implementation of this strategy will require meticulous analysis of both the VNAR sequences and the factors that provide stability and efficacy.

**PD-L1 expression is associated with FTO expression and activity in human in-vitro models and tissues from Chilean patients of colorectal cancer.**

**Diego Irribarra**[1], Antonia Domínguez[1], Rocio Cofré[1], Veronica Fuentes[1], Consuelo Santibañez[1], Marjorie De la Fuente[1], Karen Dubois-Camacho[2,3], Victor Cortes[2,4], Antonella Sanguineti[2,4], Mario Abedrapo[4], Daniela Sauma[5], Marcela Hermoso[2,3], Glauben Landskron*[1]

(1) Laboratorio de Investigación en Biomedicina, Escuela de Medicina Universidad Finis Terrae, Santiago, Chile
(2) Laboratorio de Inmunidad innata, Programa de Inmunología, ICBM, Facultad de Medicina, Universidad de Chile, Santiago, Chile
(3) Department of Gastroenterology and Hepatology, University Medical Center Groningen, University of Groningen, Netherlands.
(4) Departamento de Cirugía, Hospital Clínico Universidad de Chile.
(5) Laboratorio de Inmunología, Departamento de Biología, Facultad de Ciencias, Universidad de Chile.

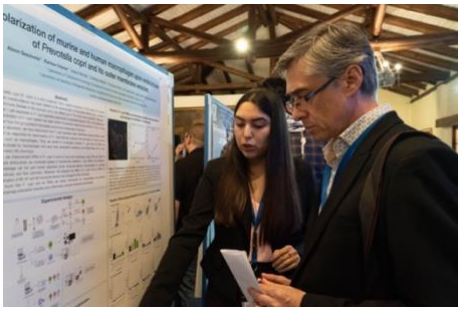
**Introduction:** Colorectal cancer (CRC) is one of the most frequent and deadly cancers worldwide, and a determining factor in cancer prognosis and immune evasion is the expression of the immune checkpoint markers PD-1 and PD-L1, often upregulated in many cancers. The mechanisms underlying PD-1/PD-L1 overexpression are not completely understood, but recent studies have shown that the N6-methyladenosine (m6A) modification could regulate its expression impacting the efficacy of ICI blockade treatment. The m6A-demethylase protein called FTO regulates PD-L1 expression in an IFN-independent manner in CRC cell lines. Preliminary work from our lab shows increased FTO levels in tumor cells and stroma compared to healthy tissue, particularly in T cells, macrophages and fibroblasts from the tumor microenvironment (TME). However, the association between FTO expression and PD1/PDL-1 has not been described in tissue from CRC patients and whether modulating FTO-demethylase activity could impact PDL-1 expression.
The aim of this study was to associate the expression of PD1 and PD-L1 with FTO in tissue samples from colorectal cancer patients and further assess PD-L1 expression mediated by FTO activity in-vitro.

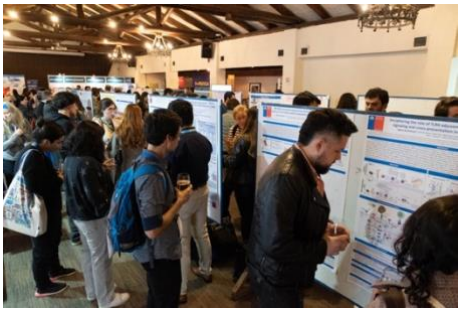
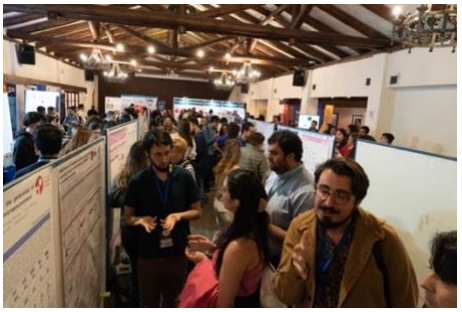
**Methods:** Eight patients were included with informed consent. FTO, PD-1, PDL-1 immunohistochemistry was performed in 4µm-thick histological sections fixed in formalin derived from tumor tissue and adjacent benign colon tissues from the surgical specimen of the same patient who underwent resective surgery.
Additionally, the colon adenocarcinoma cell line HCT116 was treated to 10µM of FB23-2 (FTO inhibitor), 10µM of dimethyl-2-oxoglutarate (DMKG, FTO substrate) and 2mM of butyrate for 6 hours, for further determination of PD-L1 by western blot. For western blot analysis, cultured cells were lysed in RIPA buffer supplemented with phosphatase and protease inhibitors. Lysates were subjected to SDS-PAGE, transferring the separated proteins to a PVDF membrane, then exposed to primary anti-PDL1 antibody (Cell Signaling, cat.13684) and GAPDH as loading control.
Results: The results of immunohistochemistry show a positive nuclear immunoreactivity for FTO in the tumor and stroma from all colorectal cancer tissues. In all benign adjacent tissues it was localized in the gut-associated lymphoid tissue (GALT) and epithelial cells.
Positive membranous immunoreactivity for PD-1 was observed in 4 CRC tissues located in the GALT and, notably, in the tumor stroma. In 6 benign adjacent tissues it was restricted to the GALT.
Positive membranous and cytoplasmic immunoreactivity for PDL-1 was observed in 7 CRC tissues localized in tumor cells and stroma. In 6 adjacent benign tissues it was only localized in epithelial cellsIn the cell line HCT116, western blot analysis showed an increase in PD-L1 protein levels in cells treated with DMKG compared to control cells (DMSO). On the other hand, a slight decrease of PD-L1 was observed in cells treated with butyrate and FB23 compared to the control.

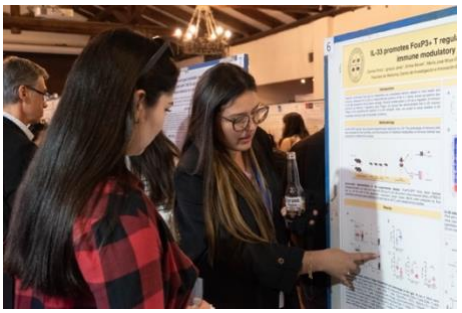
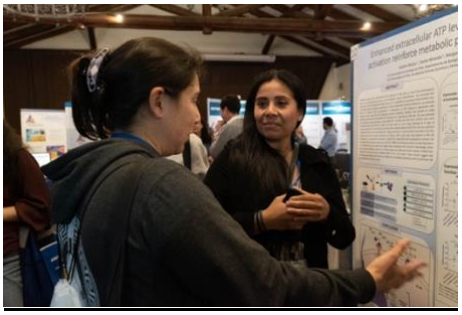
**Discussion:** DMKG is a substrate of the FTO enzyme, whose demethylase activity on mRNA has been associated with positive regulation of PD-L1 in cancer models. Moreover, FB23-2 is a reported inhibitor of FTO catalytic activity. These findings could indicate that modulating substrate availability of FTO enzyme regulates its demethylase activity on PD-L1 mRNA, increasing the levels of this protein. This effect could not be limited to tumor cells but also occur in the TME, as PD-L1 was also observed in the tumor and stroma from our samples, suggesting the association between FTO demethylase activity and increased PD-L1 expression in the TME of colorectal cancer.
Taken together, our results support a regulatory role of FTO activity on PD-L1 expression in colorectal cancer cells, with implications for immune evasion. These preliminary findings suggest that FTO may have potential as a therapeutic target, however, further research is needed to confirm this hypothesis.

## POSTERS






# APPLIED IMMUNOLOGY AND VACCINES

**The Role of Neutrophils in the Acute Local Response Induced by TRIMELVax Vaccine and Their Effect on Dendritic Cell Migration to the Draining Lymph Node and Implications in Tumor Rejection.**

**Amarilis Perez**[1,3], María A. Gleisner[1,2], Andrés Tittarelli[4], Romina Falcón[1,2], Constanza Zurita[4], Fermín E. González[5], Cristian Pereda[1,2], Iván Flores[1,2], Flavio Salazar-Onfray[1,2]

(1) Institute of Biomedical Science, Faculty of Medicine, Universidad de Chile.
(2) Millennium Institute of Immunology and Immunotherapy, Universidad de Chile.
(3) Faculty of Chemical and Pharmaceutical Sciences, Universidad de Chile.
(4) Universidad Tecnológica Metropolitana.
(5) Universidad de Chile, Laboratory of Experimental Immunology & Cancer, Faculty of Dentistry, Santiago, Chile.

TRIMELVax is an innovative immunotherapeutic approach for malignant melanoma, combining heat shock-conditioned human melanoma cell lysates with CCH as adjuvant. In preclinical studies it has demonstrated significant effectiveness in reducing tumor growth and improving survival in mice with melanoma. However, the precise immunological mechanisms behind its anti-tumor effects have remained unclear. This study focused on understanding how TRIMELVax induces inflammation at the injection site, recruits innate immune cells and triggers the DCs migration to the draining lymph node. To achieve this, C57BL/6 mice, were injected in the footpad with TRIMELVax, PBS, or GVax and the skin and pLN were harvest for qPCR or FACS analysis. Results showed that TRIMELVax rapidly induced a specific pattern of proinflammatory cytokines and chemokines, leading to an acute innate immune response at the administration site. Neutrophils, type 1 macrophages, monocytes, cDC1, Langerhans cells, and mo-DCs were recruited to the footpad, while type 2 macrophages decreased. This early inflammation facilitated a superior migration of cDC1 to the lymph node compared to controls. Crucially, neutrophils play a fundamental role in TRIMELVax's mechanism of action. Depleting neutrophils reduced DC migration, especially cDC1, to the drinig lymph node and eliminated TRIMELVax's ability to control tumor growth. In summary, TRIMELVax triggers a rapid and potent activation of the innate immune system, facilitating a more effective adaptive immune response mediated by cDC1 against malignant melanoma tumors. These results underscore the promise of TRIMELVax as a potential immunotherapeutic approach for melanoma treatment.

Keywords: TRIMEL, Neutrophils, Malignant melanoma, Immunotherapy

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**BNT162b2 vaccination improves the cellular and humoral immune response in the Chilean pediatric population immunized with CoronaVac**

**Diego Díaz Dinamarca**[1], Pablo Díaz[1], Gisselle Barra[1], Daniela Cárdenas[2], Natalia Santis[3], Rodrigo Puentes[3], Daniel Escobar[1], Nicolas Muena[2], Janepsy Díaz[3], Nicole D Tischler[2], Abel Vásquez[1,4]

(1) Instituto de Salud Pública de Chile, ANDID, Av. Marathon 1000, Santiago, Chile.
(2) Fundación Ciencia y Vida, Laboratorio de Virología Molecular, Santiago,Chile.
(3) Departamento Agencia Nacional de Dispositivos Médicos, Innovación y Desarrollo, Instituto de Salud Pública de Chile, Santiago, Chile.
(4) Facultad de Ciencias de la Salud, Escuela de Medicina, Universidad del Alba, Santiago, Chile.

The SARS-CoV-2 Omicron variant continues to cause millions of COVID-19—associated pediatric hospitalizations, severe disease, and death globally. BNT162b2 and CoronaVac are among the topmost widely used COVID-19 vaccines across the world. Research in the pediatric population is scarce, and given the worldwide need for booster vaccines to combat the impact of Omicron variants, this SARS-CoV-2 immune response study is considered crucial. In this study, the humoral and cellular immune response against Omicron were characterized for children (10-16 years old) who were administered different regimens: (a) CoronaVac(2x), (b) CoronaVac(2x) + BNT162b2(1x), (c) CoronaVac(2x) + BNT162b2(2x), and (d) BNT162b2(3x). We observed that participants with CoronaVac(2x) + BNT162b2(2x) promoted higher Anti-S1-RBD antibody titer, greater neutralization of antibodies and activation of CD4+ and CD8+ T lymphocytes specific to Omicron compared to CoronaVac(2x). Furthermore, from all groups tested, immunogenicity against SARS-CoV-2 was highest in the BNT162b2 (3x). We conclude that BNT162b2(3x) and CoronaVac(2x) + BNT162b2(2x) promotes greater immunogenicity against Omicron in the pediatric population compared to CoronaVac(2x), CoronaVac(2x) + BNT162b2(1x). Our findings indicate that both the BNT162b2 (3x) and the combined CoronaVac(2x) + BNT162b2(2x) regimens provide more potent immunogenicity against Omicron in children than the other combinations. These findings suggest that vaccination induces the secretion of antibodies and T cells against SARS-CoV-2, and a booster dose of BNT162b2 is necessary to generate a protective immune response against Omicron. In the current state of the pandemic, these data support the Ministry of Health of the Government of Chile's decision to promote heterologous vaccination.

Keywords: SARS-CoV-2, Pediatric Vaccination, COVID-19, BNT162b2 (Pfizer-BioNTech), CoronaVac vaccine

**Deciphering the role of TLR4 adjuvants protein agonists in cell signaling and cross-presentation in murine dendritic cells.**

**Diego Díaz Dinamarca**[1], Michelle Salazar[2], Pablo Díaz[1], Daniel Escobar[1], Fabian Salazar[2,3], Mayarling F Troncoso[5], Augusto Manubens[4], Sergio Lavandero[5,6], Janepsy Díaz[7], Maria Ines Becker[2], Abel Vásquez[8]

(1) Sección de Biotecnología, ANDID, Instituto de Salud Pública de Chile, Santiago, Chile
(2) Laboratorio de Inmunología, Fundación Ciencia y Tecnología para el Desarrollo (FUCITED), Santiago, Chile
(3) Medical Research Council Centre for Medical Mycology, University of Exeter, Exeter, United Kingdom.
(4) Investigación y Desarrollo, BIOSONDA S.A., Santiago, Chile.
(5) Advanced Center for Chronic Diseases (ACCDiS), Facultad Ciencias Químicas y Farmacéuticas and Facultad de Medicina,, Universidad de Chile, Santiago, Chile
(6) Department of Internal Medicine (Cardiology Division), University of Texas Southwestern Medical Center, Dallas, TX, United States.
(7) Departamento Agencia Nacional de Dispositivos Médicos, Innovación y Desarrollo, Instituto de Salud Pública de Chile, Santiago, Chile.
(8) Facultad de Ciencias de la Salud, Escuela de Medicina, Universidad del Alba, Santiago, Chile.

Toll-like receptor 4 (TLR-4) has been researched as a target of vaccine adjuvants because they modulate the innate immune system and activate adaptive immunity. TLR4 has unique dual signaling capabilities due to the recruitment of two adapter proteins, myeloid differentiation marker 88 (MyD88) and interferon-b adapter inducer containing the toll-interleukin-1 receptor (TIR) domain (TRIF). MyD88-mediated signaling triggers a proinflammatory innate immune response, while TRIF-mediated signaling leads to an adaptive immune response. Thus, in this context, TLR-based adjuvants induce cross-presentation of exogenous antigens to CD8+ T lymphocytes.
In this work, we characterized the effects of two protein-based adjuvants (PBAs) on TLR4 signaling and cross-presentation. As models of TLR4-PBAs, we used hemocyanin from *Fissurella latimarginata* (FLH) and a recombinant surface immunogenic protein (rSIP) from *Streptococcus agalactiae*. rSIP and FLH require MyD88 and TRIF to activate nuclear factor kappa beta (NF-kB) and interferon regulatory factor 3 (IRF3). However, rSIP and FLH have a specific pattern of cytokines (IL-6 and IP-10) and genes products (IL-6, CoX-2, CD80, IP-10, IFIT-1, CD86) associated with MyD88 and TRIF recruitment.
Functionally, rSIP and FLH promote antigen cross-presentation in a manner dependent on TLR4, MyD88, and TRIF signaling. However, FLH activates a specific TRIF-dependent signaling pathway associated with cytokine expression and a pathway dependent on MyD88 and TRIF recruitment for antigen cross-presentation. Our results explain how FLH and rSIP could act as TLR-4-PBAs clinically useful vaccine adjuvants that selectively activate TRIF- and MyD88-dependent signaling to drive Th1 adaptive immune responses.

Keywords: TLR4, Vaccine Adyuvant, MyD88, TRIF, Cross-presentation

**Characterization of cellular immune response of prototype vaccine based on Lactococcus lactis secreting an immunogenic factor against Group B Streptococcus**

**Michal Díaz Samirin**[1,2], Abel Vásquez[1], Diego Díaz Dinamarca[1], Daniel Soto[1]

(1) Instituto de Salud Pública de Chile, ANDID, Av. Marathon 1000, Santiago, Chile.
(2) Universidad de Santiago de Chile, Departamento de Ingeniería Química y Bioprocesos, Facultad de Ingeniería, Av. Libertador Bernardo O'Higgins 3363, Santiago, Chile.

Group B *Streptococcus* (GBS) is the primary etiological agent of sepsis and meningitis in newborns and is associated with premature birth and stillbirth. Its only current treatment for vertical transmission is the administration of intrapartum antibiotic prophylaxis (IAP), which can be challenging in low and middle-income countries and contributes to antibiotic resistance. Therefore, the development of a licensed vaccine against GBS is one of the pending challenges and a significant addition to the global road map to defeat meningitis by 2030, a goal of the World Health Organization.
We propose the use of *Lactococcus lactis*, a GRAS (generally recognized as safe) and transient organism in the digestive tract, as an antigen carrier able to generate humoral and cellular immune response. This work characterizes the cellular immune response through spleen T-lymphocyte CD4+ activation and proliferation, humoral immune response in antibody IgG titers, and analysis of protection against GBS infection.
Using BALB/c mice, we determined that those provided with the prototype vaccine an antigen-specific T-cell immune response antigen characterized by show a significant increase of T-lymphocyte CD4+ activation as CD69+ and CD4+ proliferation (Celltrace Violet). Despite that antibody titers where partial increased in our murine model, the prototype vaccine based on *L. lactis* promotes a decrease of GBS infection in murine model.
Our experimental observations support the notion that a vaccine based on *Lactococcus lactis* generates cellular immune response which could involve in prevent GBS colonization in an antigen-specific manner.

Keywords: Group B Streptococcus, Lactococcus Lactis, Mucosal Vaccine, T cell immunity

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**Effect of Mitochondrial Transfer derived from mesenchymal stem cells on postnatal immune cells.**

**Sebastián Valdés-Cabrera**[1,3], Pablo Castro-Córdova[1,3], Maroun Khoury[1,2,3,4]

(1) IMPACT, Center of Interventional Medicine for Precision and Advanced Cellular Therapy, Santiago, Chile
(2) Cells for Cells, Santiago, Chile
(3) Laboratory of Nano-Regenerative Medicine, Centro de Investigación Biomédica, Faculty of Medicine, Universidad de los Andes, Santiago, Chile
(4) Consorcio Regenero, Chilean Consortium for Regenerative Medicine, Santiago, Chile

Artificial Mitochondrial transfer (MitoT) from umbilical cord Mesenchymal Stem Cells (MSC) has the potential to rescue mitochondria-deficient cells in diseases such as cancer, lung and heart injuries, as well as immune and inflammatory diseases. Previously, we shown that MitoT has the potential to induce T naïves differentiation to Tregs, which inhibits the proliferation of target peripheral blood mononuclear cells (PBMC). Due to the advantages of using cord blood in immunotherapies, in the present work, we explore MitoT from umbilical cord MSCs (UC-MSC) to postnatal mononuclear cells derived from umbilical cord (UCB-MC).

UCB-MCs were isolated from fresh cord blood samples using Ficoll density gradient. $CD34_{neg}$ and T-naïves cells were isolated using an immunomagnetic negative selection kit. MSCs were stained with Mitotracker-Green, and subsequently, Mitochondria were isolated using a Mitochondria isolation kit. Subsequently, UCB-MC or T-naïves were incubated with increasing mitochondria concentrations, and MitoT was induced with a thermic shock and cultured for ~20h. Subsequently, cells were immunostained and analyzed for flow cytometry.

For postnatal immune cells, we observed a dose-dependent MitoT to CD3+ T cells, CD19+ B, CD56+ natural killer, CD11c+ dendritic, and T naïves cells. We observed the protective role of MitoT in T-lymphocytes after apoptosis induction by an immunotoxin, staurosporine. We observed that MitoT increases the T-naïve differentiation to Tregs, which has a comparable capacity to inhibit the proliferation of target PBMC than Tregs of PBMC. These results open new avenues for the development of therapies based on cells and organelles for immune-mediated diseases such as graft-versus-host disease.

Keywords: Cell-based therapy, Mitochondrial Transfer, Mitochondria

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# AUTOIMMUNITY AND INFLAMMAGING

**Immune response to subunits of cytolethal distending toxin from Aggregatibacter actinomycetemcomitans in patients with rheumatoid arthritis**

**Karissa Chavez Villacreses**[1], Isabel Méndez Pérez[1], Daniela Castillo[1], Chris Gabriela Quinteros[1], Belén Flores[1,2], Florencia Vicencio[1,2], Emilio Seguel[1,3], Mauricio Bittner[3], Alejandra Herrera[4], Nancy Vasquez[4], Sebastián Irarrázaval[5], Katina Schinnerling[1]

(1) Laboratory of Translational Immunology, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago, Chile.
(2) Departamento de Periodoncia, Facultad de Odontología, Universidad Andrés Bello, Santiago, Chile.
(3) Laboratorio de Microbiología y Biotecnología Oral, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago, Chile.
(4) Departmento de Reumatología, Pontificia Universidad Católica de Chile, Santiago, Chile.
(5) Departamento de Ortopedia y Traumatología, Pontificia Universidad Católica de Chile, Santiago, Chile.

Mucosal surfaces including the gingival mucosa of the oral cavity are considered as sites of disease initiation in rheumatoid arthritis (RA), a chronic joint-destroying autoimmune disorder. Periodontal disease is common in RA, indicating that oral pathobionts like *Aggregatibacter actinomycetemcomitans* (*Aa*) may promote the development of systemic autoimmune inflammation. *Aa* is a gram-negative coccobacillus which produces cytolethal distending toxin (*Aa*CDT), a protein complex composed of three subunits: CdtA (binding), CdtB (DNase I-like and PIP3 phosphatase activity) and CdtC (stability), inducing inflammasome activation and apoptosis in host cells.
Although *Aa* has been linked to RA, nothing is known about the role of *Aa*CDT in RA immunopathogenesis. Thus, our aim was to investigate the effect of *Aa*CDT subunits on human monocytes and the presence of *Aa*CDT-specific humoral response in RA.
We stimulated monocytes in whole blood to analyze the production of pro-inflammatory cytokines in response to recombinant CDT subunits by flow cytometry. To study antibody responses, we determined relative levels of IgG and IgA specific to *Aa*CdtA, *Aa*CdtB and *Aa*CdtC in sera of RA patients, osteoarthritis and healthy controls by indirect ELISA.
We found that *Aa*CdtA induces strong IL-8 and TNF (but no IL-6 and IL-1β) production in blood monocytes, while *Aa*CdtB and *Aa*CdtC were only weak inducers of pro-inflammatory responses. Characteristic IgA antibody responses to *Aa*CdtA and *Aa*CdtB (but not *Aa*CdtC) were present in 40% of RA patients.
The results highlight a role of *Aa*CDT in triggering pro-inflammatory immune responses at mucosal sites that might contribute to the development of RA.

Keywords: Aggregatibacter actinomycetemcomitans, rheumatoid arthritis, cytolethal distending toxin, mucosal immune response, periodontal disease

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**Loss of bone marrow progenitors affect thymus functionality in lupus-prone mice**

**Nicolás Valdivieso**[1,3], Valeska Simon[1], Justine Castañeda[2], Leonardo Vargas[2], Mario Rosemblatt[1,2,3], Sarah Nuñez[2,3], Daniela Sauma[1,2], María Rosa Bono[1,2]

(1) Laboratorio de Inmunología, Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile
(2) Centro Ciencia & Vida, Santiago, Chile
(3) Facultad de Medicina y Ciencias, Universidad San Sebastián, Santiago, Chile

Systemic lupus erythematosus (SLE), an autoimmune disease affecting mainly females, is associated with multiple immune abnormalities affecting both B and T cells. Functional alterations of the thymus can contribute to immune dysfunction since this organ plays an essential role in the development and maturation of the immune system, particularly in immune tolerance. Several studies have reported thymic atrophy and a decrease in the production of T lymphocytes in patients with SLE and lupus-prone mice; however, the underlying mechanisms that lead to these alterations in SLE are poorly understood. In this work, we explored the effect of disease severity on T lymphocyte development potential in gender-specific lupus-prone (NZBxNZW)F1 mice. Our results show that (NZBxNZW)F1 females with advanced disease symptoms present significant thymic atrophy, marked by decreased organ weight and cellularity and near-complete loss of double-positive thymocytes (CD4+CD8+) compared to age-paired male mice and young female mice of this same strain. Analysis of double-negative T (CD4-CD8-) subpopulations in the thymus indicates a paucity of lymphoid progenitors migrating to the thymus of diseased females. Interestingly, analysis of the bone marrow of diseased female (NZBxNZW)F1 mice showed a complete loss of lymphoid progenitors. Our results suggest that the development of double-positive thymocytes is likely hampered due to an inability of the bone marrow to produce thymus-seeding progenitors in lupus-prone mice.

Keywords: Thymus, Lupus, Bone marrow progenitors, Autoimmunity

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**Myeloid-derived suppressor cells contribute to the development of SLE by secreting Arg1 and iNOS in murine models.**

**Valentina P. I. González**[1,2], Felipe R. Uribe[2], Sebastián A. Echeverria[2], Alexis M. Kalergis[3,4], Jorge A. Soto[2]

(1) Universidad Andrés Bello, Programa Magister en Biotecnología y Ciencias de la Vida, Facultad de Ciencias de la Vida, Av. República 330, Santiago, Chile.
(2) Universidad Andrés Bello, Departamento de Ciencias Biológicas, Facultad de Ciencias de la Vida, Av. República 330, Santiago, Chile.
(3) Pontificia Universidad Católica de Chile, Millennium Institute on Immunology and Immunotherapy, Facultad de Ciencias Biológicas, Alameda 340, Santiago, Chile.
(4) Pontificia Universidad Católica de Chile, Departamento de Endocrinología, Facultad de Medicina, Lira 40, Santiago, Chile.

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease affecting several organs, including the kidney. Overall incidence rates are between 1.5 and 11 cases per 100,000 population, with a higher predisposition in women than men. Myeloid-derived suppressor cells (MDSCs) are a cell type implicated in multiple pathologies, such as cancer or infections, in which they have been characterized as promoting a host-damaging state. In SLE patients, increased serum arginase 1 (Arg1) secretion by MDSCs has been described as contributing to pro-inflammatory responses.
To assess the pro-inflammatory immune response of MDSCs during the development and progression of SLE, two murine models capable of acute and chronic disease development were evaluated. In both, changes in the recruitment of immune populations and the secretion of Arg1 and iNOS by MDSCs were assessed by flow cytometry. In addition, the relative expression of these molecules was assessed by qPCR. Finally, histology was performed to evaluate the renal damage caused by SLE.
The results show changes in MDSC subpopulations' distribution and the secretion of Arg1 and iNOS in different tissues. In addition, an increase in the relative expression of Arg1 and iNOS was observed, as well as increased renal damage.
This work presents new information on how MDSCs influence the progression of the pathology, showing different distributions depending on the subpopulation. Furthermore, through the secretion of molecules such as Arg1 and iNOS, they may be able to present a proinflammatory effect in the development of the pathology.

Keywords: Myeloid-derived suppressor cells, Systemic lupus erythematosus, Autoimmune disease

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**Renal lymphatic vasculature role in immune infiltration and inflammation during lupus development.**

**Valentina Rodríguez-Vergara**[1], Noelia Escobedo[1], Andrés Herrada[1]

(1) Universidad Autónoma de Chile, Instituto de Ciencias Biomédicas, Ciencias de la Salud, 5 Pte. N°1670, Talca, Maule, Talca, Chile

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disease that affects many organs and tissues of the body. In this disease, the kidney is one of the most affected organs, referred to as lupus nephritis, where immune complexes accumulate in the glomeruli, causing inflammation. Kidneys are infiltrated by lymphatic vessels (LV), capable of draining immune molecules and cells from the organ. Previous research describes defects in the LV in autoimmune diseases, however, the morphology or function of the renal LV in SLE has not been explored. The purpose of this investigation is to evaluate the morphology of the renal LV in NZM lupus mice and correlate its status with immune cell infiltration and renal inflammation. The infiltration of immune cells in the kidney was evaluated by flow cytometry. Additionally, immunofluorescence of floating kidney sections was performed to observe and characterize the different immune cells, as well as evaluate and quantify the status of the LV. Renal inflammation was analysed by measuring different inflammatory cytokines by real time PCR. The results show an aberrant morphology and dilation of the renal LV in old NZM lupus mice compared to controls. Furthermore, an increase in the infiltration of T lymphocytes was evident, along with clusters of macrophages in the kidneys of NZM mice. Finally, an increase in IL-12p40 and IL-6 transcripts was evident. Our results suggest that an altered LV morphology correlates with increased infiltration of immune cells and inflammation in kidneys of lupus mice.

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**Immunomodulatory effect of Small Extracellular Vesicles (sEVs) from metabolically reprogrammed Mesenchymal Stem Cells (MSCs) on Memory T-CD4+ cells**

**Yesenia Flores-Elías**[1,2,3], Eliana Lara-Barba[1,3], Yeimi Herrera-Luna[1,3], Noymar Luque-Campos[1,3], Ana María Vega-Letter[4], Patricia Luz-Crawford[1,3]

(1) Laboratorio de Inmunología Celular y Molecular, Centro de Investigación Biomédica, Facultad de Medicina, Universidad de Los Andes, Santiago, Chile.
(2) Facultad de Ciencias, Universidad de Chile, Santiago, Chile.
(3) IMPACT, Center of Interventional Medicine for Precision and Advanced Cellular Therapy, Santiago, Chile.
(4) Escuela de Ingeniería Bioquímica, Pontificia Universidad Católica de Valparaiso, Valparaiso, Chile.

Introduction:An imbalance between proinflammatory and regulatory T-CD4+ cell subpopulations is a common pathogenic feature of different autoimmune/inflammatory diseases and balancing the T-CD4+ cells immune response to self–antigens is an ongoing challenge. The use of MSCs as a therapeutic approach have been broadly documented due to their immunomodulatory capacity. However, clinical outcomes are divergent, and the use of metabolically reprogrammed MSCs towards glycolysis have been proposed to enhance their therapeutic capabilities. One of the mechanisms through which MSCs exert their biological effects relies on secreting sEVs. Therefore, we aimed to investigate whether sEVs from metabolically reprogrammed umbilical cord-derived MSCs (UC-MSC) might exert an enhanced immunosuppressive effects on T-CD4+ cells.
Material and methods: sEVs where isolated from UC-MSCs under basal or reprogrammed by ultracentrifugation and were characterized by NTA and FACS analysis. Memory T-CD4 cells were isolated from PBMC and culture in the presence or absence of sEVs of under basal or glycolytic conditions. The internalization of sEVs on memory T-CD4 cells proliferation and phenotype of proinflammatory (Th1 and Th17 cells) and anti-inflammatory (Treg) cells in memory T-CD4 cells was evaluated by FACS.
Results: Our internalization experiments showed that sEVs are incorporated into memory TCD4+ cells. Furthermore, we found that MSC-derived sEVs and MSC glycolytic-derived sEVs decrease the percentage of IFN$\gamma$ and IL17-producing Th1 or Th17 cells, respectively, while no effects were observed on the percentage of Foxp3+ CD25+ regulatory T cells (Treg).
Discussion:Our ongoing challenge is understanding the mechanisms behind the immunomodulatory abilities of MSCs-derived EVs to potentiate their anti-inflammatory properties.

Keywords: Mesenchymal Stem/Stromal Cells (MSCs), Small Extracellular Vesicles (sEVs), chronic autoimmune/inflammatory diseases, T-CD4+ cells

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# CELLULAR AND MOLECULAR IMMUNOLOGY

**Defective meningeal lymphatic vasculature is associated with CNS B-cell infiltration and depressive behavior in a murine lupus model**

**Alexandra Olate-Briones**[1], Francisca Rodríguez-Arriaza[1], Sofía Albornoz-Muñoz[1], Sabina Rojas[1], Emilia Escalona[1], Andrés Herrada[1], Noelia Escobedo[1]

(1) Universidad Autónoma de Chile, Lymphatic Vasculature and Inflammation Research Laboratory, Instituto de Ciencias Biomédicas, Facultad de Ciencias de la Salud, 5 Poniente N°1670, Talca, Chile

Recently, the presence of meningeal lymphatic vasculature (mLV) has been described in the meninges covering the brain. mLV is a complex network of capillaries and larger vessels responsible for draining immune cells, small molecules, and excess fluid from the central nervous system (CNS) into the deep cervical lymph nodes. mLV dysfunction has been implicated in several neurodegenerative disorders, but the role that mLV in other neurological pathologies such as neuropsychiatric lupus (NPSLE) has not yet been evaluated. Systemic lupus erythematosus (SLE) is a chronic and inflammatory autoimmune disease characterized by the generation of autoantibodies mainly against nuclear components that trigger inflammation. One of the main organs compromised is the brain, which leads to NPSLE, affecting around 12% to 95% of SLE patients. Despite increasing knowledge regarding NPSLE, the different cells and pathways compromised remains mostly unexplored. Here, by using the lupus *FcγRIIb*$^{-/-}$ murine model, we showed that these mice have depressive behavior, as reflected by forced swim test and anhedonia, together with aberrant mLV morphology by immunofluorescence. Additionally, we observed altered meningeal B cell differentiation status and an increase in clusters of B cells in the meninges of *FcγRIIb*$^{-/-}$ mice in comparison with their controls by FACS. These results suggest that defective mLV may contribute to the depressive behavior in a murine model de NPSLE, in part by affecting meningeal B cell status in the CNS.

Keywords: meningeal lymphatic vasculature, neuropsychiatric lupus, B cells

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**Effect of Prevotella copri and its outer membrane vesicles on the polarization of murine and human macrophages**

**Alison Sepúlveda Pontigo**[1], Karissa Chavez Villacreses[1], Cristóbal Madrid Muñoz[1], Isabel Méndez Pérez[1], Francisco Parra[2], Jan Nevermann[2], Juan A Fuentes[2], Katina Schinnerling[1]

(1) Universidad Andres Bello, Laboratory of Translational Immunology, Facultad de Ciencias de la Vida, Av. República 330, Santiago, Chile
(2) Universidad Andrés Bello, Laboratorio de Genética y Patogénesis Bacteriana, Facultad de Ciencias de la Vida, Av. República 330, Santiago, Chile

*Prevotella copri* (*P. copri*) is a strict anaerobic Gram negative bacterium of the intestinal microbiota whose overabundance has been related to the development of rheumatoid arthritis (RA), a disabling autoimmune disease of the synovial joints that affects up to 1% of the world population. Although T helper 1 cell responses to *P. copri* peptides were found in peripheral blood of 40% of RA patients and administration of *P. copri* strains has been shown to promote experimental arthritis in mice, it is not known how this gut bacterium stimulates autoimmune responses in the joint.
In our laboratory, we have discovered that *P. copri* secretes outer membrane vesicles (OMVs), which might be responible for disemination of *P. copri*-derived products and activation of innate immune cells such as macrophages. Thus, we aimed to investigate whether *P. copri* and its OMVs are endocytosed by macrophages and skew their polarization towards a proinflammatory M1 phenotype.
First, we characterized OMVs of *P. copri* in terms of size, morphology and composition using NTA, TEM and proteomics. To analyze endocytosis, we monitored uptake of fluorescently labelled *P. copri* and OMVs into RAW264.7 macrophage cell line and human peripheral blood monocyte-derived macrophages by confocal microscopy and flow cytometry. Moreover, we analyzed the effect of *P. copri* and ist OMVs on polarization of RAW264.7 cells and M0, M1 and M2 monocyte-derived macrophages by flow cytometry.
We found that *P. copri* and its OMVs are readily endocytosed by human and murine macrophages and promote macrophage polarization towards a pro-inflammatory M1 phenotype.

Keywords: Outer membrane vesicles (OMVs), Prevotella copri, Rheumatoid Arthritis, macrophages

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**Control of lysosomal function by the RNase activity of IRE1 in conventional type 1 dendritic cells**

**Amada Arcaya**[1], Alonso Lira[1], José Ignacio Bernales[1], Fabiola Osorio[1]

(1) Universidad de Chile, Laboratorio de Inmunología y Estrés Celular, Facultad de Medicina, Independencia 1027, Santiago, Chile

The unfolded protein response (UPR) is a cellular mechanism safeguarding endoplasmic reticulum (ER) proteostasis. It has been reported that the IRE1/XBP1 axis, in addition to its canonical role, regulates the function of a subtype of dendritic cells (DCs) known as conventional type 1 DCs (cDC1). IRE1, besides activating XBP1s, can degrade certain mRNAs in a process known as RIDD (IRE1-dependent decay). Several RIDD substrates are associated with lysosomal biogenesis. Previous studies in our laboratory have identified Lamp1, a marker of lysosomal maturation, as a novel target of RIDD in cDC1, but it is currently unknown whether Lamp1 degradation impacts lysosomal dynamics and functionality and whether this can modulate antigen presentation to T cells.
**To understand the role of RIDD in the lysosomal function of cDC1, we will study a new point mutation model in the kinase domain of IRE1 in cDCs, which selectively inhibits RIDD activation.** From this, we will generate OP9/DL1-DCs, a new culture system that recapitulates the characteristics of tissue-derived cDCs. The lysosomal dynamics will be assessed by real-time microscopy in cDC1 from mice with the S729A point mutation and lysosomal integrity will be analyzed using Galectin-3 labeling in cDC1.
Functional studies of lysosomal acidity in cDC1 will be conducted using pH-sensitive probes and flow-cytometry. Preliminary assays indicate a decrease in acidity in the genetic models compared to the WT, suggesting a potential dependence on IRE1/XBP1 in the process. Finally, we will perform co-cultures using OT-1 mice to verify the antigen presentation capacity of cDC1.

Keywords: Innate Immunity, IRE1, RIDD, Lamp1, Lysosomal function

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**B-Cell llymphoma-3 (BCL-3) as a cofactor of the Wnt/B-Catenin pathway in the expression of pluripotentiality genes in hepatocarcinoma**

**Araceli Pinto**[1], Rafael Zanabria[1], Javiera Alzaga[1], Jaime Poniachik[1], Elisa Balboa[2], Caroll J. Beltrán[1]

(1) Immunogastroenterology Laboratory, Gastroenterology Unit, Hospital Clínico Universidad de Chile, Santiago, Chile
(2) LIBMED, Medicine School, Faculty of Medicine, University of Chile, Santiago, Chile

**Introduction**: The Wnt/β-catenin pathway is one of the most frequent signaling activated in Hepatocarcinoma (HCC). Its activation triggers pluripotential gene expression, such as EpCAM, which is associated to an aggressive HCC phenotype. Bcl-3 protein, a co-regulator of NF-κB pathway, is upregulated in diverse solid tumor. Bcl-3 plays a role in pluripotency gene expression in colorectal-cancer, as cofactor of Wnt/β-catenin, being this regulatory mechanism unknown in HCC. **Objective:** To evaluate the Bcl-3 expression in HCC, and its regulation of EpCAM expression, as a cofactor of β-catenin. **Methods:** In tumor tissue (TU) and adjacent non-tumor tissue (non-TU) of patients with HCC (N=10), Bcl-3 expression was evaluated by qPCR, Immunoblot and immunofluorescence. EpCAM expression was evaluated *in vitro* in HEPG2, modulated for Bcl-3 expression (pcDNA-Bcl-3 and siRNA) by qPCR and immunoblot. Bcl-3 interaction with β-catenin was determined by Immunoprecipitation. Statistics: Mann Whitney U and Student's T for comparison, significance: $p<0.05$. **Results**: Higher transcript and protein expression of Bcl-3 was observed in TU vs non-TU ($p=0.0067$; $p=0.0463$). A Bcl-3 overexpression cause an increased in Bcl-3 protein ($p=0.0094$), along with elevated EpCAM transcript expression ($p=0.0094$) and protein ($p=0.0105$). Bcl-3 silencing decrease Bcl-3 transcript ($p=0.0435$) and protein expression ($p=0.0055$), as well as EpCAM protein level ($p=0.0201$). The Bcl-3 immuno-staining was observed at perinuclear and cytoplasmic area in TU. Bcl-3/β-catenin didn't co-immunoprecipitated. **Conclusion**:Bcl-3 expression is increased in HCC and play a regulatory role of EpCAM expression *in vitro.* Although direct interaction with β-catenin was not observed, potential associations with other molecules must be explored.

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**Mecanismos detrás de la producción y liberación de trampas extracelulares por neutrófilos equinos en respuesta a diferentes estímulos**

**Bayron Cortés**[1], John Quiroga[1], Claudio Henríquez[1]

(1) Universidad Austral de Chile, Facultad de Ciencias Veterinarias, Campus Isla Teja s/n, Valdivia, Chile

La liberación de trampas extracelulares por parte de neutrófilos (NETosis) es un fenómeno cuyo rol fisiopatológico en varias enfermedades equinas como laminitis y sepsis ha despertado reciente interés. Sin embargo, existe escasa información respecto a los mecanismos involucrados en esta respuesta por parte de neutrófilos equinos. Es por ello, que el propósito de este estudio es caracterizar las vías moleculares involucradas en la NETosis equina, en respuesta a diferentes estímulos *in vitro*.
Para ello, neutrófilos equinos fueron aislados y estimulados con PMA, A32187, factor activante de plaquetas (PAF), GM-CSF, IL-8, LPS y zimosan (OZ). Para evaluar la liberación de trampas, se determino la concentración de ADN liberado por los neutrófilos en el sobrenadante. Además, se realizó inmunofluorescencia de doble tinción para ADN y H4 citrulinada (H4-Cit3). El rol de NADPH oxidasa, especies reactivas de oxígeno mitocondrial, peptidildeiminasas, elastasa neutrofílica y mieloperoxidasa en la NETosis también fue evaluado.
PAF, A23187 y PMA fueron capaces de inducir NETosis, mientras que GM-CSF, IL-8, LPS y OZ fueron ineficaces en inducir la liberación de trampas o H4-Cit3. La inhibición de PAD's mediante Cloroamidina inhibió la liberación de trampas extracelulares independiente del estímulo usado. Las especies reactivas de oxigeno jugaron un rol significativo en NETosis inducida por únicamente por PMA. Además, NE fue requerida para la NETosis inducida por PMA y PAF, pero no A23187. Es notable que MPO no contribuyó a la liberación de ET.
Este trabajo contribuye a caracterizar los mecanismos moleculares involucrados en la NETosis equina en respuesta a diferentes estímulos.

Keywords: neutrophil extracellular traps, equine, molecular mechanism

Funding: Financiamiento, Proyecto FONDECYT 1210839

**IL-33 promotes FoxP3+ T regulatory cells and the production of immune modulatory intestinal metabolites**

**Camila Pinto**[1], Ignacio Jeréz[1], Emilia Morell[1], Javiera de Solminihac[1], María José Moya-Guzmán[1], Karina Pino-Lagos[1]

(1) Universidad de los Andes, Centro de Investigación e Innovación Biomédica, Facultad de Medicina, Santiago, Chile

Intestinal commensal flora and its metabolites are considered factors related to host health and immunity. Interleukin-33 (IL-33) is a tissue-derived cytokine of the IL-1 family, known as alarmin due to its high expression during tissue damage. Several studies place IL-33 as a regulator of immune tolerance by affecting T regulatory cells (Tregs). Our group has demonstrated that IL-33 induces Tregs *in vivo*, preventing the rejection of a skin allograft. Here, we sought to study whether IL-33 modulates immunity under homeostatic conditions. FoxP3-GFP reporter mice received intraperitoneal injections of IL-33. The phenotype of immune cells was evaluated by flow cytometry, and the production of intestinal metabolites of immune interest was subjected to metabolomics analysis. Animals treated with IL-33 showed a reduction on Tregs with pro-inflammatory phenotype (IFNg+ Tregs and IL-17+ Tregs) in the gut. Metabolomic analysis showed a dramatic IL-33 effect on the abundance of intestinal metabolites, highlighting those involved in the Tryptophan (Trp) pathway, such as 5-Hydroxyindoleacetic acid (5-HIAA), Kynurenic acid (KA) among others. Using IL-33R (ST2) KO animals, exogenous KA, and a specific AhR signaling inhibitor, we found that KA induces Tregs *in vitro* in an IL-33 independent manner. We demonstrate that IL-33 induces Tregs in the absence of inflammatory stimulus, and favors the production of intestinal metabolites with known regulatory characteristics. Due to the limited condition of the *in vitro* setting, new *in vivo* experiments will be designed to decipher if IL-33 and Trp pathways connects during the induction of immune regulation.

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**Imbalance of Peripheral T cells in cancer survivors with Alzheimer's Disease**

**Camilo Venegas**[1], María A. Gleisner[1], Fabián Tempio[2], Flavio Salazar-Onfray[1,4], Carol San Martin[3], Barbara Bruna[3], Mercedes N. López[2], Maria Isabel Behrens[3]

(1) Laboratorio de Inmunología Antitumoral, Facultad de Medicina, Universidad de Chile, Santiago, Chile
(2) Laboratorio de Regulacion e Inmunologia del Cancer, Facultad de Medicina, Universidad de Chile, Santiago, Chile
(3) Centro de Investigacion Clinica Avanzada, Hospital Clínico Universidad de Chile, Santiago, Chile
(4) Millenium Institute on Immunology and Immunotherapy, Santiago, Chile

Alzheimer's Disease (AD) is the most prevalent neurodegenerative disease in the elderly, while Cancer is the leading cause of death. The immune system is involved in both pathologies. There is evidence of an inverse relationship between cancer and risk of AD, where proinflammatory lymphocytes are thought to participate.
The goal was to perform a phenotypical and functional characterization of lymphocytes from people over 60 years, divided in 6 groups: i) healthy controls ii) mild cognitive impairment (MCI) patients iii) MCI patients with cancer history (Ca+MCI) iv) AD patients v) AD patients with cancer history (Ca+AD) and vi) cancer survivors (Ca).
Blood samples were obtained from patients and processed to collect Peripheral blood mononuclear cells (PBMC). T helper (Th) lymphocytes, cytokine production and memory T cell profiles of patients were characterized by extracellular and intracellular staining of PBMCs, using flow cytometry.
MCI, AD and Ca+AD groups had lower T CD8+ and CD4+ naïve cell counts, compared to healthy controls. Ca+AD group showed lower production of cytokines by Th cells, compared to all groups, while Ca+MCI group exhibited a higher cytokine production by Th and TCD8+ cells, in comparison with MCI group.
These findings suggest that a prior inmune response to cancer may promote an increase in T CD4+ and CD8+ activation and cytokine production by Th1/Th2/Th17 and T CD8+ cells, that can be seen in MCI patients, but is not present in moderate to advanced AD patients.

Keywords: Cancer, Alzheimer, lymphocytes, T helper cells

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**Effect of GelMA hydrogels microstructure on murine Dentritic cells phenotype in vitro.**

**Cristina Padilla**[1,2], Javiera de Solminihac[1], Francisco Kirhman[2], Camila Pinto[1], Javier Enrione[2], Karina Pino-Lagos[1]

(1) Universidad de los Andes, Centro de Investigación e Innovación Biomédica, Facultad de Medicina, Santiago, Chile
(2) Universidad de los Andes, Centro de Investigación e Innovación Biomédica, Facultad de Medicina, Santiago, Chile

The design of scaffolds to promote tissue regeneration is key in tissue engineering (TE). For the design of functional hydrogels, however, it is necessary to comprehend a material at the molecular level to determine how changes in its environment, such as triple-helix formation, can affect its structure and thus its biological response. Gelatin methacryloyl (GelMA) stands as a suitable material for the development of these scaffolds, nevertheless the effect of GelMA hydrogels structure on the immune response has not been extensively studied. Due to the important role of DCs as antigen presenting cells, our objective is to determine the effect of triple-helix formation on GelMA hydrogels structuring, and its effect on DCs phenotype. We produced GelMAs with 2 degrees of substitution (DS) from gelatins of porcine or salmon origin. Prior to the formation of hydrogels, the samples were incubated at 4°C (triple-helix promotion) or 37°C and photocrosslinked with UV light. Hydrogel's structure was characterized by mechanical compression and SEM. DCs (CD11c+) from murine splenocytes were cultured with the hydrogels (18h) and characterized by flow cytometry and ELISA. Results showed no significant changes in DCs phenotype by changing hydrogel's structure; however, a trend towards greater activation and IL-6 release was observed in the presence of hydrogels with lower DS. Future experiments will focus on the effect of the DC-hydrogel interaction in the T CD4+ cell response. This information would help us further understand DC-hydrogel interactions, and possibly modulate a specific immune response while developing functional scaffolds for TE.

Keywords: Hydrogels, Dendritic cells

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**In Silico Affinity Maturation of a Human Anti-MICA Antibody**

**Douglas J. Matthies**[1,2], Karen Toledo-Stuardo[1], María Carmen Molina[1], Gerald Zapata-Torres[2]

(1) Universidad de Chile, Programa Disciplinario de Inmunología, Instituto de Ciencias Biomédicas (ICBM), Facultad de Medicina, Santiago, Chile
(2) Universidad de Chile, Centro de Modelamiento Molecular, Biofísica y Bioinformática (CM2B2), Facultad de Ciencias Químicas y Farmacéuticas, Santiago, Chile

Affinity maturation is a critical step in antibody development that leads to antibodies with increased binding affinity for their target protein. This step has traditionally been performed in vitro, requiring the use of time-consuming and expensive experimental techniques. To address this challenge, new computational methods are employed to accelerate and enhance the antibody development process.
In this study, we used molecular modeling methods to study the interactions between a fully human anti-MICA antibody and its target protein MICA, a tumor-related protein that has been associated with tumor immunesurveillance and evasion. Key residues involved in the anti-MICA antibody/MICA complex formation were observed with residues belonging to CDR H3 contributing most to the complex binding energy. Also, several favorable sites within the CDRs for possible mutations were identified (each site being exhaustively mutated by 18 amino acids). The obtained Fv mutants were further evaluated by means of free energy binding calculations. The Fv mutants that displayed enhanced binding affinities were subsequently chosen for experimental validation.
Our findings provided relevant data to further rationalize and improve the binding capacity of the anti-MICA antibody developed in our laboratory. They also have allowed us to develop an *in silico* affinity maturation protocol that, if combined with experimental techniques, promises to reduce the costs of applying experimental methods associated with the development of not only anti-MICA antibodies, but also of other therapeutic antibodies.

Keywords: antibody, molecular modeling, affinity maturation, MICA, antibody development

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**IL-2 induce autophagy/mitophagy and mitochondrial biogenesis in murine NK cells.**

**Eduardo Velásquez**[1], Yenniffer Ávalos[1], Sophia Mejías[1], Muguete Cisneros[1], Dante Miranda[2], Margarita Montoya[1]

(1) Universidad de Santiago de Chile, Biologia, Química y Biología, Av. Libertador B. 0'Higgins #3363, Santiago, Chile
(2) Universidad de Chile, Bioquimica, Ciencias Químicas y Farmacéuticas, Dr. Carlos Lorca Tobar #964, Santiago, Chile

Natural Killer (NK) cells spontaneously lyse infected or transformed cells and produce significant amount of IFN. Additionally, IL2 activates NK cells inducing a strong cytolytic activity. Upon contact with their target cells, NK cells readjust their mitochondria towards the immune synapsis and rapidly undergo a decrease in mitochondrial membrane potential (m ). We have described that human NK cells stimulated with high doses of IL-2 increased mitochondrial mass and  m at 72h but not at 48h. Considering the importance of mitochondrial metabolism and integrity to accomplish NK cell function and cell viability, we hypothesize that IL-2 increase autophagy/mitophagy in NK cells. To test this hypothesis, we stimulated NK cells purified from c57bl/6 mice with IL-2 (2000U/ml). Our data showed that murine NK cells stimulated with IL-2 increased mitochondrial mass, m and PGC1 expression, like human NK cells. For autophagy analysis, we examined LC3 and p62 expression in NK cells stimulated with IL-2 for 48h using confocal microscopy. Data showed that IL-2 induced an increase in dots/cell of both autophagy markers and a higher Manders coefficient (colocalization), indicating the presence of autophagosomes. To investigate if mitochondria are targeted for degradation through autophagy, we stained NK cells with Mitotracker Deep Red and Lysotracker Red-DND 99. Our results indicate that IL-2 increases the percentage of mitochondria that colocalize with lysosome in NK cells. All these findings suggest that IL-2 induce mitochondrial biogenesis in murine NK cells accompanied by increased autophagy. Additionally, mitochondria/lysosome colocalization suggest an upregulation in mitophagy.

Keywords: IL-2, Autophagy, NK cells

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**The fibrotic microenvironment of Lymph Nodes of Neuraminidase 1 knockout mice affects macrophages polarization.**

**Emilia Escalona**[1], Andrés Herrada Hidalgo[1], Alexandra Olate[1], Sofía Albornoz Muñoz[1], Noelia Escobedo[1]

(1) Universidad Autónoma de Chile, Lymphatic Vasculature and Inflammation Research Laboratory, Instituto de Ciencias Biomédicas, 5 Poniente N°1670, Talca, Chile.

Fibrosis is a common outcome of several pathological diseases characterized by the overproduction of TGF-β and excessive accumulation of fibrous connective tissue, which eventually causes organ failure. The proliferation and maturation of adaptive immune cells occur in secondary lymphoid organs (SLO), and disturbances in this microenvironment can lead to immune system imbalances. Neuraminidase 1 knockout (*Neu1*$^{-/-}$) mouse is a murine model of sialidosis, which has fibrosis in several organs, such as liver, kidney, muscle, and lungs. However, the presence of fibrosis in their SLO or the implications of this process in the differentiation of immune cell subpopulation has not been tested. Here, we evaluated fibrosis markers by histology techniques, qPCR and immunofluorescence in lymph nodes (LN) of *Neu1*$^{-/-}$ mice. We found increased fibrosis markers and higher TGF-β levels in LN of *Neu1*$^{-/-}$ mice. Then, we assessed by flow cytometry, which cells could be involved in TGF-β regulation. Our results suggested that lymphatic endothelial cells and T cells play a role in the regulation of TGF-β signaling in LN of *Neu1*$^{-/-}$ mice. Finally, we studied the effects of TGF-β on LN immune cells. We found an abnormal phenotype (less M1 and M2 markers) in LN-macrophages of *Neu1*$^{-/-}$ mice. Moreover, TGF-β decreased M2 macrophage polarization *in vitro*, suggesting that excessive TGF-β production could be acting like a "brake" at least over M2 polarization of LN-macrophages in *Neu1*$^{-/-}$ mice. This work contributes to understand the effects of a fibrotic environment on LN-macrophages differentiation, which should be considered in fibrotic diseases and TGF-β-dependent treatments.

Keywords: Neu1, Fibrosis, Macrophage polarization

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**Calreticulin-mediated MICA retention as a potential evasion mechanism of immunesurveillance in malignant melanoma cells**

**Fabiola González-Herrera**[1], Marcela Gatica[2], Antonio Serrano[3], Karen Toledo-Stuardo[1], Ivo Campos[1], Douglas J. Matthies[1], Mauricio González-Olivares[1], María José Garrido[1], Carla Díaz[1], Nicolás Fehring[1], Carolina Ribeiro[1], María Carmen Molina[1]

(1) Universidad de Chile, Programa de Inmunología, Laboratorio de Anticuerpos Recombinantes e Imunoterapia, Facultad de Medicina, Independencia 1027, Santiago, Chile
(2) Universidad de La Serena, Facultad de Ciencias
(3) Revvity Inc, 8100 Cambridge Research Park, CB25 9TL, United Kingdom

The NKG2D receptor ligands, MICA and MICB, expressed on the membrane of tumor cells, play a crucial role in Natural Killer (NK) cell-mediated antitumor cytotoxicity. However, tumors employ diverse strategies to evade immune responses. One of these mechanisms include the intracellular retention of NKG2D ligands, which favors tumor progression by dampening NK cell cytolytic functions. Within this context, it has been observed that calreticulin (CRT), an endoplasmic reticulum (ER) chaperone involved in glycoprotein processing, can negatively influence surface protein localization.
In this study we explore whether the intracellular MICA retention involves a direct interaction with CRT in melanoma, depending on MICA glycosylation. Our results, obtained by immunofluorescence, demonstrated the intracellular localization of MICA and MICB in patient-derived melanoma samples. In the BL human melanoma cells, MICA and MICB co-localized with CRT, calcineurin (CNX), and ERp57, endoplasmic ER proteins. Immunoprecipitation assays revealed a double-band migration pattern for MICA, suggesting potential heterogeneity in the glycosylation of this molecule. Notably, the interaction analysis indicated that CRT preferentially interacts with the lower molecular weight band of MICA, suggesting a preference for the less glycosylated form. Furthermore, through ELISA assay, we established a direct interaction between *E. coli*-derived recombinant MICA and CRT. However, when MICA was expressed in eukaryotic HEK293T cells, surface plasmon resonance assays failed to demonstrate any interaction between them, underscoring the impact of post-translational modifications on the intricate interplay between these two proteins. Our findings advance understanding of tumor immune evasion involving NKG2D ligands and CRT interaction, with broader implications for cancer immunology.

Keywords: MICA, Calreticulin, Immunesurveillance, Melanoma

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**Development of reporter cell lines to study FcgRs signaling levels in mild and severe COVID-19 convalescent patients.**

**Felipe Bravo**[1,2], José Luis Garrido[2], Raymond Alvarez[2], María Inés Barría[3]

(1) Universidad de Concepcion, Microbiology, Biological Sciences, Concepcion, Chile
(2) Ichor Biologics, NY, USA
(3) Universidad San Sebastian, Facultad de Medicina y Ciencia, Puerto Montt, Chile

In addition to their neutralizing properties, antibodies can also trigger a range of innate immune functions that are mediated by Fcg receptors (FcgRs), including antibody-dependent cellular phagocytosis (ADCP) and antibody-dependent cell-mediated cytotoxicity (ADCC) (Lu et al 2018). While IgG-mediated FcγR activation is crucial for controlling viral infections in vivo, pathogen-specific antibodies can also enhance pathophysiology by excessive FcγR effector functions and immune complexes formation leading to increased inflammation (ADI) (Lee et al 2020). Importantly, studies have reported ADI effects during severe SARS-CoV-1 and SARS-CoV-2 infections (Liu et al 2019, Shimizu et al 2021).
Since studies have shown that spike-specific IgG could be responsible of the imbalance of the inflammatory response in the more severe cases of COVID-19 (Hoepel et al 2021), here, we generated reporter cell lines that use a NFAT responsive element-driven luciferase expression and independently express FcgRIIA or FcgRIIIA. These cell lines allows us to quantify FcgR signaling levels that originate from the interaction of FcγRs with immune complexes formed with SARS-CoV-2 spike protein and spike-specific IgG from COVID-19 patients serum.
To assess the validity of our strategy, we utilized a cohort of mild and severe COVID-19 convalescent donors (serum) and determined their spike-specific antibody levels and their FcγR signaling levels. We found that FcγR signaling levels correlated with anti-spike IgG levels, moreover, in addition to higher levels of anti-spike IgG, we detected higher levels of anti-spike IgG-mediated FcγRIIa and FcγRIIIa signaling in severe patients when compared to mild COVID-19 patients.

Keywords: SARS-CoV-2, FcGamma, Antibody, Non-neutralizing, Inflammation

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**Phenotype and Mitochondrial Metabolism Assessment of an In Vitro Exhausted CD8+ T Cell Population**

**Felipe Malgue**[1], Juan Saavedra Almarza[1], Solange Gouet Moya[1], José Corrales[1], Eduardo Silva[3], Julio César Cardenas[3], Mario Rosemblatt[1,2], María Rosa Bono[1,2], Daniela Sauma[1,2]

(1) Universidad de Chile, Biología, Ciencias, Las Palmeras 3425, 7800003 Ñuñoa, Región Metropolitana, Santiago, Chile
(2) Fundación Ciencia y Vida, Avenida del Valle Norte 725 Huechuraba, 8580702 Santiago, Región Metropolitana, Santiago, Chile
(3) Center for Integrative Biology, Mayor University, 7510041 Santiago, Chile

In contexts of chronic stimulation, such as cancer, CD8+ T cells differentiate into a state called exhaustion, which reduces their functional activity. Exhausted T cells (Tex) derive from exhausted T precursor cells (Tpex), which have a greater capacity for self-renewal and are more susceptible to immunotherapies. These populations differ by presenting distinct molecular markers and metabolic features, with Tex having a greater capacity to produce cytokines than Tpex and a glycolytic metabolism, while Tpex depends on oxidative phosphorylation (OXPHOS). Metabolic reprogramming has been described to restrict or promote the differentiation of Tpex to Tex.
Our laboratory has developed an in vitro protocol for rapid acquisition of CD8+ T cells with molecular characteristics of exhaustion by chronic stimulation of the T-cell receptor (TCR), identifying cells similar to Tpex and Tex. However, these populations have not been characterized in depth in terms of cytokine production and metabolism. Therefore, our goal is to evaluate these traits in this in vitro model.
Flow cytometry results indicate that the Tex-like population has a greater capacity for cytokine production, along with a greater capacity for glucose consumption and lower mitochondrial membrane potential than the Tpex-like population. Likewise, the results of the measurement of oxygen consumption using Seahorse suggest that Tpex-like cells are dependent on OXPHOS. Based on these results, it can be concluded that the populations generated in vitro have a metabolic and cytokine production profile characteristic of Tpex and Tex cells.

Keywords: T cell, Exhausted, Tpex, Tex, Metabolism

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**Functional studies associated with the mutation of residue S729 of the sensor IRE1 of the unfolded protein response in cDC1 cells cultured in vitro.**

**Javier Lopez**[1], Fabiola Osorio[1]

(1) University of Chile, Department of Immunology, Medicine faculty, Av Independencia 1027, Santiago, Chile

Conventional type 1 dendritic cells (cDC1) are leukocytes that link innate and adaptive immunity. Interestingly, it has been observed that the unfolded protein response (UPR), a pathway controlling the fidelity of cellular proteome, plays a role in cDC1. The IRE1α sensor, a main effector of the UPR, controls different functional aspects in cDC1. IRE1α has an endoribonuclease domain, which acts through two key processes: the generation of the transcription factor XBP1s, and the degradation of diverse mRNAs in a process known as RIDD (Regulated IRE1-dependent decay). Recently a new murine model, known as IRE1 S729A, has been described. This model introduces a point mutation in the kinase domain of IRE1 which inhibits the phosphorylation of a particular residue, preventing RIDD, thus, making it an ideal tool to study selectively RIDD. Here, we studied the effect of this mutation in cultures of cDC1, with the aim of expanding the study of this pathway.
Through flow cytometry and RT-qPCR of cDC1 cultures, we observed that canonical targets of RIDD (such as Cd18 and Lamp1 mRNA) were restored to normal levels in IRE1 S729A cDC1s, suggesting abrogation of this process, without compromising cell survival. Furthermore, a decrease of the splicing capacity of XBP1 was observed by conventional PCR. Finally, through intracellular cytokine labeling (IL-12, IL-6 and TNFα), no changes were observed between the WT and the mutant models under poly(I:C) activation contexts. In conclusion, our results validate this model as a helpful tool for future studies of the RIDD pathway in cDC1.

Keywords: UPR, IRE1, RIDD, cDC1, S729A

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**P2X7R is required for cross-dressing in J774 cells.**

Francisca Espínola[1,2], **Javier Mena**[1,2], Carlos Barrera[1,2], Claudio Acuña[1,2]

(1) Centro de Biotecnología Acuícola - USACH
(2) Universidad de Santiago de Chile, Facultad de Química y Biología

Cross-dressing is an unconventional antigen presentation process that involves the transfer of MHC-peptide complexes from the plasma membrane of an antigen source (infected or cancerous cell for example) to antigen-presenting cells. In this study, we investigated the role of the P2X7 receptor in mediating cross-dressing using apoptotic bodies, with the aim of establishing a model to understand its mechanism.
Based on previous results from our laboratory indicating that P2X7R expression is essential for cross-dressing in dendritic cells (DCs), we used the J774 macrophage cell line to generate an experimental *in vitro* cross-dressing model. We used cellular bodies (CBs) from HEK293T $P2X7^{+}$ or wild-type (wt) cells, made through nutrient deprivation, and labeled their plasma membrane with a fluorescent probe. J774 cells, which naturally express P2X7R, were co-cultured with the labeled CBs, and the transfer of the fluorescent probe from the CBs membrane to the J774 membrane was assessed using confocal microscopy.
Our results reveal that cross-dressing is enhanced when the P2X7 receptor is expressed in both J774 cells and CBs. These findings support the hypothesis that P2X7R expression is a key requirement for the efficient mediation of cross-dressing and the established model will allow us to decipher the mechanism by which P2X7R mediates cellular cross-dressing.

Keywords: Cross-Presentation, Cross-Dressing, P2X7R

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**The role of mast cell in cirrhosis produced by metabolic dysfunction-associated steatotic liver disease (masld)**

**Javiera Favi Coloma**[1], Danette Guiñez Francois[2], Araceli Pinto[1], Alejandra Zazueta[3], Fernanda Jeria[1], Larissa Aleman Franco[1], Benjamín Campusano[2], Laura Carreño[4], Fabien Magne[3], Caroll J. Beltrán[1]

(1) Immunogastroenterology Lab., Gastroenterology Unit, Hospital Clínico Universidad de Chile (HCUCH), Santiago, Chile
(2) Hospital Clínico Universidad de Chile (HCUCH), Gastroenterology Unit, Santiago, Chile
(3) Universidad de Chile, Faculty of Medicine, Santiago, Chile
(4) Hospital Clínico Universidad de Chile, Santiago, Chile

**Introduction:** An increased immune cell activation has been described as central mechanism in liver fibrosis development. A high number of mast cells (MC) has been identified in advanced stages of liver damage from diverse etiologies. However, a comparative study of liver MC infiltration in cirrhosis produced by diverse agents, including MASLD, has not been carried out. **Objective**: To evaluate the MC number in cirrhotic liver tissue from diverse etiologies, including MASLD, and its subsequent association with patient's characteristics. **Methods:** A retrospective, observational study was performed in healthy controls (HC) (N=5), and patients with MASLD (N=10), alcoholic steatohepatitis (AHE) (N=10) and autoimmune hepatitis (AIH) (N=10). The MC number (MC/LPF, low-power field) was determined by immunofluorescence using anti-tryptase antibody in paraffin sections of liver tissue. Image J software was used images processing (blind analysis). Statistics: Kruskal-Wallis for comparisons and Pearson-test for correlations, R Program, considering $p<0.05$ statistically significant. **Results:** A high MC number was observed in MASLD (37.77±13.56, p=0.0019); AHE (50.95±18.11, p=0.00028); AIH (31.36±15.08, p=0.028) vs HC (18.74±2.93). A higher MC number was observed in AHE vs AIH (p=0.017), without differences with MASLD. A positive correlation between MC number/smoking (r=0.437,p<0.01), and MC number/alcohol habit (r=0.449,p<0.01) was observed. **Conclusion:** An increased MC liver infiltration was observed in cirrhotic liver, principally in AHE and MASLD. The lower MC density in HAI could be explained for immunosuppressive therapies. The association of MC density with smoking and alcohol habit suggest a reinforce recruitment of MC precursor by these inflammatory agents.

Keywords: mast cells, METABOLIC DYSFUNCTION-ASSOCIATED STEATOTIC LIVER DISEASE, MASLD

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**Metabolic reprogramming of MSC towards glycolysis promotes the transition of macrophages to anti-inflammatory phenotypes**

**María Jesús Araya**[1,2], Ana María Vega-Letter[3], Patricia Luz-Crawford[1,2]

(1) Universidad de Los Andes, Centro de Investigaciones Biomédicas, Facultad de Medicina, Santiago, Chile
(2) IMPACT, Center of Interventional Medicine for Precision and Advanced Cellular Therapy, Santiago, Chile
(3) Pontificia Universidad Católica de Valparaíso, Escuela de Ingeniería Bioquímica, Valparaíso, Chile

Macrophages coordinate inflammatory responses. Proinflammatory-like macrophages increase antigen presentation through the up-regulation of MHC class-II and CD86. By contrast, anti-inflammatory-like macrophages express immunosuppressive marker CD206. During chronic inflammation, the impaired transition between inflammatory and anti-inflammatory macrophages, prevents the resolution of inflammation. Since the immunomodulatory properties of mesenchymal stem/stromal cells (MSC) have gained attention, different methods have been investigated to improve MSC's therapeutic properties. Our group is focused on metabolic reprogramming of MSC towards glycolysis (Glyco-MSC) as a novel way to improve their immunosuppressive potential. Therefore, in this study we evaluate the role of metabolism of MSC on their capacity to modulate macrophage function.
Monocytes and MSC were obtained from the bone marrow of C57BL6 mice. Monocytes were differentiated into macrophages with M-CSF (macrophage colony-stimulating factor) and treated with LPS to induce an inflammatory phenotype. Macrophages were cocultured with Glyco-MSC reprogrammed through pharmacological (oligomycin or 2-deoxy-glucose [2DG]) or genetic (PPARβ-knockout) approaches. We analyzed macrophage function through the expression of MHC class-II, CD86 and CD206 by flow cytometry.
Treatment with LPS increased the expression of CD86 and MHC class-II, corresponding with acquiring an inflammatory activation state. Interestingly, macrophages cocultured with Glyco-MSC (either oligomycin-treated or PPARβ-knockout) increase the expression of CD206, associated with immunosuppressive macrophages. This was reversed when macrophages were cocultured with 2DG-treated MSC, which are metabolically reprogrammed towards oxidative phosphorylation. Our data supports that Glyco-MSC are promising therapeutic tools, showing increased immunosuppressive potential compared to naive MSC. Going deeper into this matter might bring novel insights into counteracting macrophage-mediated inflammatory diseases.

Keywords: macrophages, inflammation, mesenchymal stem/stromal cells, immunomodulation

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**Potential role of Neuropilin-1 on the generation of induced T regulatory cells and their production of small Extracellular Vesicles**

**María José Moya-Guzmán**[1], Javiera de Solminihac[1], Camila Pinto[1], Emilia Morell[1], Cristóbal Villalón[1], Karina Pino-Lagos[1]

(1) Universidad de los Andes, Centro de Investigación e Innvación Biomédica, Facultad de Medicina, Santiago, Chile

T regulatory cells (Tregs) are a subset of CD4+ T cells that modulate the immune response to maintain self-tolerance and immune homeostasis. Naïve T cells can be induced *in vitro* to Tregs (iTregs). Tregs can suppress immune responses through small extracellular vesicles (sEV). Previously, our group described that sEV from Tregs that do not express Neuropilin-1 (*Nrp1KO*) have an altered suppressor function *in vitro* and *in vivo*. Wild Type (*WT*) and *Nrp1KO* naïve T cells were purified using magnetic beads and cultured 5 days with IL-2 and TGFβ (iTregs) +/- Retinoic Acid (RATregs) to induce a Tregs phenotype. The supernatants were ultracentrifugated and sEV were purified using IZON columns. Nanoparticle tracking analysis (NTA) was used to analyze the size and number of particles. *In vitro* modulation assay was performed polyclonally activating CD4+ T cells for 72 h in the presence of sEV from *WT* and *Nrp1KO* iTregs and RATregs. RNA was extracted, and cDNA prepared, to evaluate the expression of IFNγ by qPCR. Corroboration of this modulation was performed by sandwich ELISA assay. iTregs and RATregs from naive CD4+ T cells *Nrp1KO* showed less induction of Tregs phenotype (FoxP3+ cells). Polyclonal activation resulted in cell proliferation, which positively correlates with the amount of isolated RNA . Administration of sEV reduced the proliferation of CD4+ T cells and down-regulated IFNγ expression and production. Nrp1 is required for optimal production of induced-Tregs. No differences were observed on the modulatory effect of sEV from *WT* or *Nrp1KO* iTregs.

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**Pannexin-1 dependent ATP release in response to IL-2 mediate PGC-1α upregulation and mitochondrial biogenesis in NK cells.**

**Muguete Cisneros**[1], Sophia Mejias[1], Dante Miranda[2], Margarita Montoya[1]

(1) Universidad de Santiago de Chile, Biología, Química y Biología, Santiago de Chile, Chile
(2) Universidad de Chile, Bioquímica, Ciencias Químicas y Farmacéuticas, Santiago de Chile, Chile

Natural killer (NK) cells are a subpopulation of lymphocytes involved in clearing viral infections and malignant cells through their cytotoxic and secretory activities. IL-2 stimulates NK cell functions and induces mitochondrial biogenesis depending on the upregulation of the cotranscriptional factor PGC-1α. Besides, it is known that pannexin-1-mediated ATP export and purinergic signaling promote mitochondrial homeostasis and metabolic function in different immune cells. Since high dose of IL-2 promote a strong mitochondrial metabolism, we investigated whether ATP signaling mediates IL-2-induced mitochondrial metabolism. Using purified NK cells from c57bl/6 mice we observed a rapid increase in extracellular ATP concentration (eATP) with the addition of IL-2, which became statistically significant at concentrations over 500 U/mL. This effect was not observed with IL-15, despite sharing $\beta/\gamma_c$ receptor chains. We also observed that ATP release was mediated by pannexin-1 and exported ATP was essential for upregulating PGC-1α expression. Interestingly, hydrolysis of eATP inhibited the increase of mitochondrial mass. Because IL-2 is secreted mainly by T cell and is vital for their proliferation, we explore IL-2 signaling effects on mitochondrial mass in CD4+T cells. Activation of CD4+T cells with anti-CD3/CD28 beads in the presence of additional IL-2 or a neutralizing anti-IL-2 antibody confirmed that autocrine IL-2 signalling participates in CD4+T cell mitochondrial homeostasis. All this data show that high IL-2 doses induce ATP release mediated by pannexin-1, crucial for increasing mitochondrial biogenesis in murine NK cells. Furthermore, our findings suggest that this purinergic signalling might be occurring in CD4+T cells.

Keywords: NK cells, ATP, pannexin-1, IL-2, PGC-1alpha

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**Determination of the binding affinity of antibodies by non-competitive enzyme immunoassay**

**Nicolás Fehring**[1], Karen Toledo-Stuardo[1], Mauricio González-Olivares[1], Douglas J. Matthies[1], Fabiola González-Herrera[1], Yuneisy Guerra[1], Ivo Campos[1], Carla Díaz[1], María José Garrido[1], Francisca Cortés[1], Carolina Ribeiro[1], María Carmen Molina[1]

(1) Universidad de Chile, Programa de Inmunología, Facultad de Medicina, Independencia 1027, Santiago, Chile

One of the most critical stages for developing a therapeutic antibody is the early selection of a candidate and the maturation of its affinity. In this context, non-competitive enzyme-linked immunosorbent assay (ELISA) meets what is required to select candidates quickly at a low cost, using a low amount of antigen and single chain variable fragment (scFv).
The objective of this study was to determine, by ELISA, the affinity constant of four scFv, a wildtype, a mutant and two new candidates directed to different epitopes on MICA protein, an immunotarget in cancer.
Anti-MICA scFvs and MICA were expressed in *E. coli* strain BL21(DE3) cells and purified from inclusion bodies by Ni-NTA affinity chromatography. The eluted proteins were refolded *in vitro* by a diluted dropwise method and further concentrated. A 96-well plate was first coated with MICA at four different concentrations and incubated with different concentrations of scFvs. Antibody binding to MICA was detected with a mouse anti-His antibody-HRP conjugate.
We found that the affinity constants of the analyzed scFvs were between 1 to $3*10^7$ $M^{-1}$; since the mutant antibody showed a slightly higher affinity compared to wildtype scFv, leading us to reject the mutant. On the other hand, we found a significant difference in the binding affinities between the new two candidates.
In conclusion, the determination of the binding affinity by ELISA is a useful, fast, and cost-effective method to select the best therapeutic antibody candidate, even when minor differences are detected.

Keywords: scFv, Antibody, MICA, Binding affinity, ELISA

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**Metabolically reprogrammed Mesenchymal Stem Cells-derived mitochondria as a novel mediator of their immunosuppressives properties on T cells**

**Noymar Luque-Campos**[1,2], Francisca Troncoso-Reyes[1,2,3], Carolina Pradenas[1,2], Yessia Hidalgo[2,4], Jonathan Morales-Reyes[1,2], Felipe Flores[2,4], Felipe Bustamante-Barrientos[1,2], Catalina Asencio[1,2], Constanza Aros[1,2], Daniela Tapia-Molina[1,2], Eliana Lara-Barba[1,2], María Jesús Araya[1,2], Maroun Khoury[2,4], Farida Djouad[5], Patricia Luz-Crawford[1,2]

(1) Laboratorio de Inmunología Celular y Molecular, Centro de Investigación e Innovación Biomédica, Facultad de Medicina, Santiago, Chile
(2) IMPACT, Center of Interventional Medicine for Precision and Advanced Cellular Therapy, Universidad de Los Andes, Santiago, Chile
(3) Faculty of Sciences, Engineering and Technology, Universidad Mayor de Chile, Santiago, Chile
(4) Laboratory of Nano-Regenerative Medicine, Centro de Investigación e Innovación Biomédica, Universidad de Los Andes, Santiago, Chile
(5) INSERM U1183, IRMB, Montpellier, France

INTRODUCTION: Mesenchymal Stromal/Stem Cells (MSCs) are multipotent cells with broad immunosuppressive capacities on memory T-CD4 cells. The glycolytic metabolism on MSCs induce an increase in their therapeutic effects. On the other hand, MSCs-derived mitochondria (mito-MSCs) can be transferred to T-CD4 cells accomplishing therapeutic effects. However, the immunosuppressive effects of metabolically reprogrammed mito-MSCs are undeciphered. Therefore, we evaluated the effect of mitochondria derived from different metabolic status of MSC on the pro- and anti-inflammatory phenotype of memory T-CD4 cells.
MATERIAL AND METHODS: MSC-derived umbilical cord (UC-MSCs) were metabolically reprogrammed into glycolytic metabolism by oligomycin. The mito-MSCs were stained and isolated, and then performed an artificial mitochondrial transfer (known as mitoception) into memory T-CD4 cells from healthy donors. Memory T-CD4 cells were activated using CD3/CD28 after being separated in memory T-CD4 cells that acquired or not mito-MSCs by fluorescence-activated cell sorting (FACS). We analyze between those two groups the proliferation, pro- and anti-inflammatory phenotype, and metabolism by flow cytometry.
RESULTS: Glycolytic MSCs-derived mitochondria exerted an anti-inflammatory effect on memory T-CD4 cells, in terms of an increase of regulatory T cells frequency and anti-inflammatory cytokine IL10. Additionally, we observed a decrease of pro-inflammatory cytokines such as IFNγ and IL17. This effect was associated with a metabolic switch on memory T-CD4 cells that acquired mito-MSC.
DISCUSSION: Altogether our data propose glycolytic MSCs-derived mitochondria as an interesting therapeutic approach for restoring pro-inflammatory memory T-CD4 cells, which are critical mediators in several autoimmune diseases.

Keywords: Mesenchymal Stem Cells, Memory T cells, Metabolism, Mitochondrial transfer

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**Yerba mate (Ilex paraguensis) reduce colitis severity by promoting anti-inflammatory macrophage polarization**

**Sofía Albornoz-Muñoz**[1], Alexandra Olate-Briones[1], Francisca Rodríguez-Arriaza[1], Valentina Rodríguez-Vergara[1], Andrés Herrada[1], Noelia Escobedo[1]

(1) Universidad Autónoma de Chile, Lymphatic vasculature and inflammation research laboratory, Instituto de Ciencias Biomédicas, Facultad de Ciencias de la Salud, 5 Poniente #1670, Talca, Chile

Yerba Mate (YM) *(Ilex paraguariensis)* is a natural herbal with well-described anti-inflammatory capacity and beneficial effects in different inflammatory contexts such as insulin resistance or obesity. However, whether YM could improve other inflammatory conditions such as colitis, or the immune cell population that can be modulated by this plant remains elusive. We studied the effect of YM consumption on the development of dextran sodium sulfate (DSS) - induced colitis in mice. Our results showed that oral administration of YM reduces colitis symptoms and improves animal survival. Decreasing systemic and local inflammation together with increasing infiltration of anti-inflammatory M2 macrophage was observed in the colon of the mice treated with YM. Accordingly, YM promotes M2- macrophage differentiation in vivo. However, direct administration of YM to bone-marrow derived macrophages does not increase M2 macrophage polarization in vitro, suggesting that YM, by an indirect mechanism, is able to skew the M1/M2 ratio. In summary, we show that YM promotes an immunosuppressive environment by enhancing anti-inflammatory M2 macrophage differentiation, reducing colitis symptoms, and suggesting that YM consumption could be a good cost/effective treatment for inflammatory bowel diseases.

Keywords: Yerba mate, DSS-induced colitis, macrophages, inflammation

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**Role of adenosine receptor A2A in the generation of exhausted CD8+ T cells in the tumoral microenvironment**

**Solange Gouet Moya**[1], Juan Saavedra Almarza[1], Felipe Malgue[1], José Corrales[1], Camilo Villaman[2], Alberto Martin[2], Mario Rosemblatt[1,2,3], María Rosa Bono[1,2], Daniela Sauma[1,2]

(1) Universidad de Chile, Departamento de Biologia, Facultad de Ciencias, Las Palmeras 3425, Ñuñoa, Santiago, Santiago, Chile
(2) Centro Ciencia & Vida, Avenida del Valle Norte N° 725, Huechuraba, Santiago
(3) Universidad San Sebastián, Facultad de Medicina y Ciencia, Santiago, Chile

Cytotoxic CD8+ T cells have the potential to eradicate cancer. However, the tumor microenvironment decreases its effector capacity in a process known as exhaustion. Exhausted T cells (Tex) are derived from precursors (Tpex) with anti-renewal capacity. Tpex and Tex exhibit a functional adaptation to the tumor microenvironment, which increases survival but decreases their effector function. These cells are exposed to high concentrations of extracellular adenosine in the tumor niche, a nucleoside that can promote several features associated with Tpex. Our aim was to study the effect of the adenosine receptor A2A (A2AR) on the Tpex and Tex differentiation. Through flow cytometry, it was observed that the use of specific and non-specific A2AR agonists increased stemness markers while decreased exhaustion markers produced by chronic stimulation of OT-I cells. Additionally, the role of A2AR in exhaustion was studied using a conditional KO mouse model specific for CD8+ T cells (A2AR KO). *In vitro* chronic activation of OT-I A2ARKO cells in presence of IL7/IL15 cytokines resulted in higher levels of CD25 and lower of CD73, in addition to an increased Tex frequency related to WT control. In contrast, chronic activation of OT-I A2ARKO in presence of IL2/IL12 cytokines reduced CD73 and CD39 levels, and Tex frequency related to WT control. In line with this, intratumoral PD1+CD8+ T cells and PD1+ memory T cells from tumor-bearing A2ARKO mice were increased compared to WT mice control. These results suggest that adenosine through A2AR may be promoting a more terminally exhausted phenotype in CD8+ T cells.

Keywords: Adenosine receptor, Exhaustion, Tumor, Precursor exhausted, Tumoral Microenvironment

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**Enhanced extracellular ATP levels during IL-2 activation reinforce metabolic profile in NK cell**

**Sophia Mejias**[1], Dante Miranda[2], Margarita Montoya[1]

(1) Universidad de Santiago de Chile, Departamento de Biología, Química y Biología, Chile
(2) Universidad de Chile, Facultad de Ciencias Químicas y Farmacéuticas,, Chile

Different studies have demonstrated the potential of NK cells as vital immunotherapeutic agents in controlling both primary and metastatic cancer dissemination. We described that human NK cells stimulated with high doses of IL-2 exhibited an increased mitochondrial mass and mitochondrial membrane potential in a PGC1α dependent manner. Moreover, we observed that IL-2-induced PGC1α upregulation is dependent on ATP release in agreement with literature that show that ATP signaling promote metabolic function. We decided to study whether sustaining the extracellular ATP reinforces IL-2 signaling on NK cells. This was achieved by inhibiting ectonucleotidases using ARL67156. Purified NK cells from c57bl/6 mice were incubated with ARL67156 for 10min and then with IL-2 for 8 days followed by 4 days of expansion with IL-15. As control we use the same scheme without ARL67156. We also used two additional controls, one incubating for 12 days only with IL-2 and the other only with IL-15. Our results show that the gene expression of PGC1α, CD71, and c- myc, key proteins in NK cell metabolism, are significantly elevated in ARL67156-treated NK cells compared to controls. On the other hand, the gene expression of GLUT1, SRBP1 and IFNγ was similar to that observed in IL-15-treated cells and significantly higher when compared with controls. Interestingly, When we measured cytotoxic activity in a 4-hour assay, we observed similar activity of ARL67156-treated NK cells and those activated with IL-2 or IL-15 alone. These results suggest that increasing extracellular ATP levels can reinforce IL-2-mediated signalling, which could favour NK cell-dependent antitumor action.

Keywords: NK cell, Immunology, metabolism, ATP, cancer

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# COMPARATIVE IMMUNOLOGY

**Identification of T lymphocyte CD8a+ T lymphocytes in Atlantic salmon (Salmo salar)**

**Felipe Barraza**[1], Úrsula Ponce[1], Kevin Maisey[1], Claudia Reinoso[1], Mónica Imarai[1]

(1) Universidad de Santiago de Chile, Centro de Biotecnología Acuícola, Biología, Facultad de Química y Biología, Avenida Libertador Bernardo O'higgins 3363, Santiago, Chile

CD8+ T lymphocytes are critical components of the immune effector mechanisms that have a role in eliminating cells infected by intracellular pathogens. The genes encoding CD8a and CD8b have been identified in several fish species, showing structural similarities. This study was conducted to identify and determine the distribution of the CD8+ T cell population in Atlantic salmon to investigate further its role in immune protection against intracellular bacteria infecting salmon. Then, monoclonal antibodies were produced against CD8a produced as a recombinant protein in *E. coli*. The purified CD8a recombinant protein was used to immunize BALB/C mice and for the generation of mAb-producing cells. Six anti-CD8a-producing hybridomas were obtained and selected for further studies. Two of the mAb obtained were then used to identify lymphocytes in peripheral blood leucocytes (PBL) and immune organs of Rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon using Flow Cytometry. The percentage of CD8+ lymphoid cells in Atlantic salmon PBL, Head kidney, and spleen are approximately 19,4%, 1,5%, 10,3% respectively. The percentage of CD8+ lymphoid cells in Rainbow trout spleen are approximately 20%. CD8a+ lymphoid cells were then sorted to analyze the transcriptional expression profile by RT-qPCR such that we verified the lymphoid nature of CD8+ cells.

Keywords: CD8 T Cells, Atlantic salmon, Hybridoma, Monoclonal antibody, flow cytometry

Funding: Fondecyt 1201664

**β-hydroxybutyrate disturbs carbohydrate metabolism regulations induced by a TLR2/1 agonist in bovine neutrophils**

**John Quiroga**[1], Rafael A. Burgos[1], Pablo Alarcón[1], Daniella Carreta[1], Stefanie Teuber[1], Claudio Henríquez[1], Gabriel Morán[1]

(1) Universidad Austral de Chile, Instituto de Farmacología y Morfofisiología, Facultad de Ciencias Veterinarias, Campus Isla Teja S/N, Valdivia, Chile

In early postpartum, dairy cows suffer a marked negative energy balance with excessive fat mobilization and hyperketonemia, which has been associated with mammary and reproductive infections and linked to neutrophil disfunction. Bovine neutrophils express TLR2 and signaling through TLR2/1 heterodimer has been suggested has pivotal for the recognition and efficient response against Gram positive bacteria. Since neutrophil functional responses are strongly dependent on carbohydrate metabolism, we hypothesize that β-hydroxybutyrate (β-OHB), the major ketone body in the blood of ketotic cows, may alter carbohydrate metabolism adaptations induced following TLR2/1 activation. Neutrophils isolated from 6 healthy non-lactating cows were exposed to normal (0.5 mM), subclinical (2.5 mM) and clinical (5.0 mM) concentrations of β-OHB and then stimulated with 10 µg/mL $Pam_3CSK_4$ (TLR2/TLR1 agonist) *in vitro*. Using qRT-PCR was observed that β-OHB reduced $Pam_3CSK_4$-induced overexpression of glucose transporter 1 (GLUT1), hexokinase 2 (HK2), and hexokinase 3 (HK3) but does not alter the expression of glycogen synthase 1 (GYS1), glycogen branching enzyme 1 (GBE1), glycogen phosphorylase liver isoform (PYGL) and glycogen debranching enzyme (AGL). However, by immunoblot it was observed that, despite not altering phosphorylation of GYS1 (Ser641/645), exposure of neutrophils to β-OHB decreased the phosphorylation of PYGL (Ser15). In addition, a decrease in glucose uptake was detected by flow citometry after exposure to β-OHB in a dose-dependent manner. These results suggest that β-OHB could limit carbohydrate metabolism adaptations induced through TLR2/1 signaling, which could be associated with a lower efficiency in bovine neutrophil response against Gram-positive bacteria.

Keywords: Bovine neutrophils, β-hydroxybutyrate, Carbohydrate metabolism, Ketosis, TLR2/1 activation

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**Efectos del tamoxifeno sobre la polarización de la respuesta inmune Th1 y Th2 en células mononucleares de sangre periférica en equinos.**

**Maksimiano Rodríguez**[1], John Quiroga[1], Bayron Cortés[1], Claudio Henríquez[1]

(1) Universidad Austral de Chile, Instituto de Farmacología y Morfofisiología, Facultad de Ciencias Veterinarias, Campus Isla teja S/N., Valdivia, Chile

El tamoxifeno (TAM) es un fármaco ampliamente utilizado para tratar y prevenir el cáncer de mamas en humanos. Sin embargo, también ha mostrado tener efectos moduladores de la respuesta inmune, siendo sugerido como alternativa terapéutica para el tratamiento de enfermedades inmunomediadas. Se ha explorado al TAM como tratamiento del asma equina, una patología crónica de las vías aéreas de carácter neutrofílico, observándose una disminución en la inflamación y/o funcionalidad pulmonar en los animales tratados. El efecto del TAM sobre los neutrófilos equinos están bien documentados, pero se desconoce sus efectos sobre la polarización del sistema inmune.
Este estudio utilizó células mononucleares de 5 caballos sanos, las que se expusieron a TAM en diferentes concentraciones (0,01 - 10 uM), con o sin estimulación (6 h) con PMA/ionomicina o 0,1% de DMSO. Posteriormente se evaluó la expresión de genes asociados con la polarización de la respuesta inmune (*TBX21, IFN-y, GATA3, IL-4, IL-10, PDCD1, TGF-β, FOXP3, CD274, y CTLA-4*).
El tamoxifeno por sí solo mostró no influenciar la expresión de los genes evaluados, con excepción de *IL-4* con su uso a 10 uM. El estímulo con PMA/ionomicina, mostró favorecer la polarización hacia un fenotipo Th1, lo cual no fue modificado por TAM. Por otra parte, el uso de una concentración de 10 uM mostró tener efecto en la expresión de varios genes, lo cual se correlacionó con una disminución significativa de la viabilidad celular.
Este trabajo contribuye a determinar los efectos inmunológicos del tamoxifeno, como un potencial agente terapéutico para el asma equino.

Keywords: Tamoxifeno, Polarización de la respuesta inmune, Inmunomodulación, Células mononucleares equinas

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**Modulation of genes associated to immune response and tissue repair on Atlantic salmon skin mucosa subjected to mechanical damage**

**Rodrigo Zañartu**[1], María J. Santillán-Araneda[1], Merari Goldstein[1], Catalina Jimenez-Hernandez[1], Silvana Guerra-Arredondo[1], Karina Reichel-Fuentes[1], Andrea Cerda Celis[1], Eva Vallejos Vidal[2], Felipe Reyes Lopéz[1]

(1) Universidad de Santiago de Chile, Fish health and integrative physiogenomics research team, Facultad de Química y Biología, Alameda 3363, Edificio Eduardo Morales Santos, Piso 3, 9170002, Santiago, Chile
(2) Universidad de Las Américas,, Núcleo de Investigación Aplicada en Ciencias Veterinarias y Agronómicas, Facultad de Medicina Veterinaria y Agronomía, Walker Martínez 1360, Piso 3, 8250122 La Florida, Santiago, Chile

The Atlantic salmon (Salmo salar) is the most relevant aquatic productive species in Chile. The intensive culture strategy exposes the fish to skin wounds due to the intrinsic handling practices. Such skin lesions might threaten the fish's health because of its role in protection and defense. Previous research has primarily focused on describing the biological processes on the skin lesion without distinguishing the modulation between the wound area and its perimeter. This study evaluated the kinetics of expression in the wound area and the wound perimeter in Atlantic salmon skin subjected to mechanical damage. We applied the wound using a disposable circular biopsy punch with a diameter of 4 mm below the skin lateral line. We conducted by RT-qPCR the expression of genes involved in tissue repair, and immune response. Fish were sampled at day zero (start of the experiment), 1-hour post-injury (hpi), 6 hpi, 24 hpi, 2 days post-injury (dpi), 4 dpi, and 20 dpi.
In the wound area, our results showed a higher magnitude of expression on a set of the genes evaluated (il-15; tgf-β1; inos; il-6) than in the wound perimeter. The peak of expression varied varied depending on the wound zone and the gene evaluated. Thus, the wound area registered the peak of expression earlier than in the wound perimeter. These results suggest differences in the expression magnitude and modulation of the response in processes associated with pro-inflammation, tissue repair, and angiogenesis.

Keywords: Inmune Response, Skin Wound, Gene expression, Tissue Repair, Atlantic Salmon

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**Identification and characterization of IL-10A e IL-10B in Atlantic salmon**

**Roque Scarpa**[1], Almendra Benavides[1], Felipe Barraza[1], Lizbeth Huaccha[1], Natalia Valdes[1], Kevin Maisey[1], Mónica Imarai[1]

(1) University of Santiago of Chile, Biology Department, Chemistry and Biology Faculty, Alameda 3363, Santiago, Chile., Santiago, Chile

Interleukin (IL)-10 is an immune response suppressor cytokine produced and secreted by T cells, macrophages/monocytes, and dendritic cells (DC), depending on the stimulus type, the tissue affected, and the timing of the immune response. This cytokine controls inflammation by selectively blocking the expression of some cytokine genes, chemokines, and other proteins responsible for producing inflammation. This study was conducted to identify, obtain, and functionally characterize the IL-10 cytokines in Atlantic salmon. Regarding these aims, two sequences hypothetically encoding IL-10 in Atlantic salmon were identified and retrieved from the NCBI database using bioinformatics. The genes encoding IL-10A and IL-10B are paralogs. The *il-10a* and *il-10b* were cloned in the pET-15B vector for expression in *Escherichia coli*. The optimal conditions for the expression induction were standardized, and the recombinant proteins were obtained and purified by fast protein liquid chromatography (FPLC) using HisTrap column. Bioactivity of the protein is now under evaluation, using the antagonistic effect against LPS-induced proinflammatory response in SHK-1 cells.

Keywords: Atlantic salmon, IL-10, cytokines, anti-inflammatory.

Funding: FONDECYT 1201664

# IMMUNITY AND INFECTION

**Atlantic salmon surviving Piscirickettsia salmonis infection elicits an expression profile of cytokines associated with Th1-type response.**

**Almendra Benavides**[1], Ruth Montero Meza[2], Nicole Olate Sanchez[1], Felipe Barraza[1], Mónica Imarai Bahamonde[1]

(1) University of Santiago of Chile, Biology Department, Chemistry and Biology Faculty, Alameda 3363, Santiago, Chile
(2) Norwegian University of Life Sciences, Department of Animal and Aquacultural Sciences, Faculty of Biosciences, Ås, Norway

*Piscirickettsia salmonis* is a bacterium that causes Salmonid Rickettsial Septicemia (SRS) in salmonid fish. Here, we performed *P. salmonis* infection of Atlantic salmon to analyze the type of triggered immune response. Twelve fish of 70 g were tagged and intraperitoneally infected with the bacteria. The clinical signs of infection were registered using a score table. Five of the 12 infected fish survived up end of the experiment (day 30), scoring 0. Blood samples were taken from each fish on days -2, 5, 15, and 30 after infection, and the leukocytes were isolated for analysis. The transcriptional expression for genes of T cell type response was examined in PBL. The signature profile for all 5-survivor fish showed increased expression levels of *ifnγ, tbet, cxcr3,* and *tgfβ* at day 30 compared to initial values. In addition, three of these survivors also showed *il-12* and *tnf-α* increases. At the end of the experiment, transcriptional expression of genes was also evaluated in the head kidney (HK) of the five-surviving fish, which showed higher levels of expression of *ifnγ, tbet, il-12,* and *tgfβ*. The cytokine profile in PBL and HK indicates that a Th1-type response has been induced in surviving fish.Interestingly, all survivor fish also showed *tnf-α* incr ease on day five after the challenge, not observed in fish dying in the experiment. A statistically significant association exists between the TNF's expression and survival outcome. Altogether, results indicate that a Th1-type response in PBL and HK of *P. salmonis* infected salmon might associated with protection.

Keywords: Atlantic salmon, Piscirickettsia salmonis, Th1 response, ifn-γ, cytokines

Funding: FONDECYT 1201664

**Non-neutralizing activity of antibodies against ANDV GP in HCPS survivors**

**Andrea Oyarzún Cisterna**[1], Felipe Bravo[2], Camila Kossack[1], Raymond Alvarez[3], José Luis Garrido[1,3], María Inés Barría[1]

(1) Facultad de Medicina y Ciencia, Universidad San Sebastián, Puerto Montt, Chile
(2) Departamento de Microbiología, Universidad de Concepción, Concepción, Chile
(3) Ichor Biologics LLC, New York, NY, 10065 USA

Andes orthohantavirus (ANDV) is a lethal agent in South America causing human cardiopulmonary syndrome. The knowledge of humoral response in hantavirus infection is limited. To date, no study has examined the antibody (Ab)-mediated innate immune effector response in the context of hantavirus infection in humans. Abs can drive non-neutralizing activities upon Ab–antigen complex formation and multimerization. Here, interactions between the Fc domain of IgG immune complexes are enhanced with Fcγ receptors in innate immune cells. In this study, the Ab response directed against ANDV glycoprotein (GP) was examined in a cohort of survivors of HCPS. We profiled their ANDV GP-specific IgG Ab levels and determined their ability to induce innate immune effector functions such as Ab-dependent NK activation directed towards ANDV GP. We found that survivors have elevated levels of IgG against ANDV GP, which stayed detectable even years after infection. Then, we determine the Ab-mediated NK cell activation. Here, we found that sera from survivors induced significant CD107a upregulation in primary NK cells. We also examined the expression of the pro-inflammatory cytokine IFNγ and the chemokine MIP1β from NK cells, and similar results were obtained. This activity was detected even years after infection in some individuals. Our findings evidence an ample and durable activity of Abs present in sera from the survivor's cohort after ANDV infection, therefore this study contributes to a better understanding of the Ab-mediated innate immune responses, which could be involved in long-term protection against ANDV infection.

Keywords: ANDV, IgG, HCPS, Natural Killer, CD170a

Acknowledgments: We are grateful to the staff of Hospitals from Valdivia, Puerto Montt, and Temuco cities that help us to collect the samples and the participant subjects for their contributions.

**Memory B cells and antibody response in COVID-19: a longitudinal analysis.**

**Camila Kossack**[1], Andrea Oyarzún Cisterna[1], Felipe Bravo[2], Francisco Fuentes[2], Matías Medina[1], Renato Esteban Ocampo Avello[3], Johanna Cabrera[4], Yazmin Pinos[5], Raul O. Riquelme Oyarzun[1,6], Maria Luisa Rioseco[1,6], Sebastian Barria Carcamo[1,6], Mario Calvo Arellano[7], Raymond Alvarez[8], José Luis Garrido[1,8], María Inés Barría[1]

(1) Universidad San Sebastián, Facultad de Medicina y Ciencia, Puerto Montt, Chile
(2) Universidad de Concepción, Departmento de Microbiología, Concepción, Chile
(3) Hospital Regional de Talca, Talca, Chile
(4) Hospital Dr. Hernán Henríquez Aravena, Temuco, Chile
(5) Hospital Base San José, Osorno, Chile
(6) Hospital Puerto Montt Dr. Eduardo Schütz Schroeder, Puerto Montt, Chile
(7) Universidad Austral de Chile, Instituto de Medicina, Valdivia, Chile
(8) Ichor Biologics LLC, New York, NY, 10065, USA

SARS-CoV-2 is the causative agent for the COVID-19 pandemic, which affected more than 648 million and killed over 6.6 million people worldwide. The fact that this virus can cause a wide range of disease severity, along with the emerging variant, vaccine distribution worldwide, and the possibility of reinfection after 3-6 months, keeps this virus in the scope, to understand the mechanism of immune response raised. Our team evidences a positive correlation between disease severity and high levels of IgG cross-reactivity to other betacoronaviruses spike protein, raising the possibility that the high levels and early appearance could have arisen from recall memory against seasonal hCoVs, rather than a de-novo response.
In this work, we analyze the phenotype of memory B cells using flow cytometry, along with the secreted IgG, IgM, and IgA, through ELISA, of a longitudinal cohort of mild, moderate, and severe COVID-19 patients, correlating this data with the patient's profile and vaccine schedule. We obtained different profiles of activated memory B cells and antibody-secreting cells, which correlate with the serology pattern. We also saw a differentiated response according to the vaccine used and the antibody response.
The elevated number of activated memory B cells at an early stage of severe patients along with the high response of antibody-secreting cells might be associated with a memory response. The serology after different immunization platforms has relevant implications in determining vaccine immune response and future immunization schedules to face future threats of COVID-19.

Keywords: SARS-CoV-2, MemoryBcells, Antibody, Vaccines

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**Elucidating the role of ORF8-MHC-I interaction in immune evasion mediated by SARS-CoV-2**

**Carolina Schafer**[1], Javier Mena[1,2], Cristopher Blamey[1], Carlos Mateluna[1,2], Carlos Barrera[2], Douglas J. Matthies[3], Claudio Acuña[2], Ana María Sandino[1]

(1) Laboratorio de Virología, Centro de Biotecnología Acuícola, Facultad de Química y Biología, Universidad de Santiago de Chile, Av. Libertador Bernardo O'Higgins nº 3363, Santiago, Chile
(2) Laboratorio de Bioterapias, Centro de Biotecnología Acuícola, Facultad de Química y Biología, Universidad de Santiago de Chile, Av. Libertador Bernardo O'Higgins nº 3363, Santiago, Chile
(3) Centro de InmunoBiotecnología (Centro IBT), Programa Disciplinario de Inmunología, Instituto de Ciencias Biomédicas (ICBM), Facultad de Medicina, Universidad de Chile, Av. Independencia 1027, Santiago, Chile

The SARS-CoV-2 virus has developed various immune evasion mechanisms, including the ability to reduce antigen presentation by downregulating membrane-bound MHC-I molecules. The ORF8 protein of SARS-CoV-2 has been implicated as playing a critical role in this mechanism. While previous studies have suggested that ORF8 binds to the groove of the MHC-I α chain, the specific regions participating in this interaction have not been fully characterized. The present study aims to elucidate the molecular mechanisms underlying the ORF8-MHC-I interaction and its subsequent impact on MHC-I expression. To identify the putative regions of interaction between ORF8 and MHC-I, we employed bioinformatic techniques, including molecular docking and molecular dynamics simulations. Our results reveal that ORF8 forms a stable bond with the β2m-binding domain of MHC-I, predominantly via hydrophobic interactions situated mainly in the α1 chain of MHC-I. These key interacting residues were subsequently targeted for the generation of ORF8 mutant variants, which are anticipated to restore proper MHC-I-β2m complex formation and functional antigen presentation in future assays. To assess this hypothesis, both wild-type and mutant ORF8 will be expressed in HEK293T cells, followed by evaluation of MHC-I molecule expression on the cell surface via flow cytometry. The combination of bioinformatic tools and experimental approaches used in this study offers the potential for a comprehensive understanding of the functional significance of ORF8-MHC-I interactions. These insights may elucidate one of the immune evasion mechanisms employed by SARS-CoV-2, and potentially lead to the development of targeted therapies to combat COVID19.

Keywords: Immune evasion, SARS-CoV-2, MHC-I, ORF8

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**Pannexin 1 and its implication in antigenic transport and cross-presentation of antigens in dendritic cells**

**Francisco Bravo**[1], Paulina Troncoso[1], Claudio Acuña[1,2], Carlos Barrera[1]

(1) Universidad de Santiago de Chile, Centro de Biotecnología Acuícola, Facultad de Química y Biología, Av. Libertador Bernardo O'higgins 3363, Santiago, Chile
(2) Universidad de Santiago de Chile, Departamento de Biología, Facultad de Química y Biología, Av. Libertador Bernardo O'higgins 3363, Santiago, Chile

The process of antigen cross-presentation is essential for adaptive immunity. A critical step in this process is the transfer of exogenous antigens from phagosomal compartments to the cytoplasm of dendritic cells (DCs) for subsequent presentation to CD8+ T lymphocytes. The trimeric channel Sec61 was believed to facilitate this antigenic transfer for a long time. However, recent evidence questions this idea since inhibitors and anti-Sec61 antibodies did not affect this transfer or antigen cross-presentation. Our study proposes an alternative: Pannexin 1 (Panx1), a Gap Junction family protein highly expressed in DCs. Panx1 forms channels in cell membranes, allowing the transfer of molecules and peptides. Upon phagocytosis, antigens could move to the cytoplasm through the Panx1 pore. Therefore, the basis of this innovative proposal is the detection of Panx1 in phagosomal compartments. Using the Mutu1940 cell line and bone marrow-derived DCs, we detected co-localization of Panx1 with Lamp-1 in phagosomal compartments, after Ph-dependent fluorescent S. aureus phagocytosis using confocal microscopy. This research highlights the potential role of Panx1 in antigen cross-presentation, laying the foundation for future research. Therefore, Panx1 emerges as a promising candidate in cross-presentation mechanics, requiring further exploration to elucidate its exact function.

Keywords: dendritic cells, cross-presentation, pannexin-1, antigen transport

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**Cellular characterization of the lymphocyte immune populations in asymptomatic Atlantic salmon (Salmo salar) naturally infected in a freshwater productive environment with Piscine orthoreovirus (PRV).**

**María J. Santillán-Araneda**[1], Catalina Jimenez-Hernandez[1], Mónica Imarai[2], Kevin Maisey[3], Felipe E. Reyes-López[1], Eva Vallejos Vidal[1,4,5]

(1) Universidad de Santiago de Chile, Centro de Biotecnología Acuícola, Facultad de Química y Biología, 9170002 Estación Central, Santiago, Chile
(2) Universidad de Santiago de Chile, Laboratory of Immunology, Facultad de Química y Biología, Alameda 3363, Estacion Central, Santiago, Chile
(3) Universidad de Santiago de Chile, Laboratory of Comparative Immunology, Facultad de Química y Biología, Alameda 3363, Santiago de Chile, Santiago, Chile
(4) Universidad de Las Américas, Núcleo de Investigación Aplicada en Ciencias Veterinarias y Agronómicas, Facultad de Medicina Veterinaria y Agronomía, Walker Martínez 1360, Santiago, Chile
(5) Universidad de Santiago de Chile, Centro para el Desarrollo de la Nanociencia y Nanotecnología, CEDENNA, Santiago, Chile, Alameda 3363, Santiago, Chile

The salmon industry in Chile faces different challenges, with animal health and disease management being their major concerns. One such pathogen is *Piscine orthoreovirus* (PRV), a double-stranded RNA virus associated with heart and skeletal muscle inflammation in fish. Outbreaks and mortality associated with PRV infection are still marginal in Chile. However, the high number of positive cases suggests an increased occurrence of an asymptomatic persistent infection phenotype. The persistence can involve the release of anti-inflammatory cytokines and the decrease in cell populations associated with the Th1 response. This study aimed to describe the lymphocyte populations in Atlantic salmon naturally infected by PRV in a freshwater farm in southern Chile. To do this, Atlantic salmon grown in a freshwater farm were acclimated to our fish facility conditions. Then, apparently healthy fish were identified with Pit Tags, and blood samples was collected from all tagged fish were examined to diagnose the presence of PRV and other pathogens by one-step RT-qPCR. Peripheral Blood Leukocytes (PBL) from PRV(+) and PRV(-) fish were isolated by double lysis. We characterize the cell populations by flow cytometry, using different specific cell-markers antibodies (CD4; IgM; CD8). Preliminary results of flow cytometry analyses showed for the asymptomatic PRV(+) fish a significant increase in the percentage of $CD4^+$ and $IgM^+/CD4^+$ cells. By contrast, we also registered for the asymptomatic PRV(+) fish, a significant decrease in the $IgM^-/CD4^-$ and $CD8^+$ cells population. These results suggest that asymptomatic PRV(+) fish do not promote the immune response in a $CD8^+$-dependent cytotoxic context

Keywords: Piscine orthoreovirus, lymphocytes, salmonid adaptative immune system, persistent infection, Aquaculture

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**Assessing IRE1 Activation in Dendritic Cells Stimulated by Influenza Virus-Infected Cells**

**María José Vásquez Vidal**[1], Mónica Guzmán[1], Tomás Hernández[2], Ricardo Soto Rifo[2], Fabiola Osorio[1]

(1) Laboratory of Immunology and Celular Stress, Inmunology Program, Institute of Biomedical Science, Faculty of Medicine, University of Chile, Santiago, Chile
(2) Laboratory of Molecular and Cellular Virology, Virology Program, Institute of Biomedical Science, Faculty of Medicine, University of Chile, Santiago, Chile

Conventional type 1 dendritic cells (cDC1s) play an essential role in the antiviral immune response, by activating cytotoxic CD8+ T cells, which eliminate virus-infected cells. cDC1s depend on proper function of the endoplasmic reticulum (ER) to carry out their functions. Protein homeostasis is regulated by the unfolded protein response (UPR).
IRE1 is a conserved and broadly studied UPR sensor, characterized by its constitutive basal activation in the cDC1 lineage. Along with its associated transcription factor, XBP1s, IRE1 regulates key aspects of cDC1s including antigen presentation and cell survival. However, the function of IRE1 in antiviral immunity via cDC1 has been sparsely investigated.
We studied cDCs generated *in vitro* from ERAI reporter mice (ER stress Activated Indicator), which express human XBP-1 sequence fused with VenusFP, measurable by flow cytometry. Upon stimulation with influenza A virus (IAV)-infected MDCK cells, cDC1s did not increase in VenusFP fluorescence compared to cDC1s stimulated by non-infected MDCK cells or unstimulated cDC1s. These results indicate no activation of IRE1 RNase upon recognition of virally-infected cells.
Nevertheless, cDC1 from WT mice increased expression of type I interferon and proinflammatory cytokine after stimulation with virally-infected cells. Future work will aim to elucidate whether the generation of proinflammatory factors by DCs upon activation with viral agonists show dependency on ER stress pathways.

Keywords: Influenza virus, UPR, Dendridic cell, IRE1, XBP1

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**Stimulation of conventional dendritic cells with influenza virus and Zika infected cells induce type-I interferon production and ER stress**

**Mónica Guzmán**[1], María José Vásquez Vidal[1], Tomás Hernández[2], Ricardo Soto Rifo[2], Fabiola Osorio[1]

(1) University of Chile, Institute of Biomedical Sciences, Laboratory of Immunology and Cellular Stress, Avenida Independencia 1027, Santiago, Chile
(2) University of Chile, Institute of Biomedical Sciences, Laboratory of Molecular and Cellular Virology, Avenida Independencia 1027, Santiago, Chile

Conventional dendritic cells (cDCs) are essential for inducing an effective T cell response against viruses. Influenza A (IAV) and Zika virus (ZIKV) are RNA viruses that can lead to severe disease and, in cases of cDCs deficiencies have shown to be fatal. The unfolded protein response (UPR), which is essential to maintain the endoplasmic reticulum homeostasis, is an important regulator of cDCs function, specially the IRE1/XBP1 axis, which has been shown to regulate their activation. It has been demonstrated that many RNA viruses can activate IRE1/XBP1 and manipulate it to their advantage for replication and blocking of immunity, however, the role of IRE1/XBP1 in the activation of cDCs against viruses is still unclear. The aim of this work was to characterize the role of IRE1/XBP1 in the activation of cDCs upon stimulation with IAV and ZIKV infected cells. First, we infected susceptible cells with IAV or ZIKV and then, we stimulated bone marrow-derived OP9/DL1-cDCs, which is an in vitro cell culture that yields dendritic cells with phenotypical properties equivalent to cDCs. We determined that these stimuli increased the levels of Xbp1 splicing, indicating IRE1 activation. Furthermore, cytokine expression was measured in WT and XBP1 cKO cells and, we found that cDCs increased tipe-I interferon and inflammatory cytokines secretion even in the absence of Xbp1. These results suggest that the stimulation with infected cells activates cDCs and IRE1/XBP1. These study model will be useful to determine the role of Xbp1 in the activation of cDCs during the antiviral immune response.

Keywords: Dendritic cells, cellular stress, RNA viruses, antiviral immunity

Funding: Fondecyt regular 1200793

**Chemokines CXCL9 and CXCL10 promote B cell differentiation into plasma cells and IgG secretion in COVID-19.**

**Romina Quiroga**[1], Sergio Sanhueza Novoa[1], Bárbara Antilef Cáceres[1], Camilo Cabrera[1], Catalina Sepúlveda[1], María Inés Barría[2], Alicia Colombo[3,4,5], Estefanía Nova-Lamperti[1,3]

(1) University of Concepcion, Clinical Biochemistry and Immunology Department, Pharmacy Faculty, Concepcion, Chile
(2) San Sebastián University, Faculty of Medicine and Science, Puerto Montt, Chile
(3) C-19 Genonet ACT210085
(4) University of Chile, Department of Basic and Clinical Oncology, Faculty of Medicine, Santiago, Chile
(5) University of Chile, Clinical Hospital, Pathological Anatomy Service, Santiago, Chile

Several studies have shown that the humoral response against SARS-CoV-2 is exacerbated in COVID-19 patients with acute respiratory distress syndrome (ARDS). These patients also have an exacerbated immune response mediated by a cytokine storm. It is unknown which inflammatory factors support the exacerbated humoral response in severe COVID-19, however B-cells required several cytokines to differentiate into antibody-producing plasma cells. The aim of this study was to identify cytokine storm components positively correlated with the levels of anti-SARS-CoV-2 IgG in patients who had COVID-19, and then, evaluate the effect of these cytokines on plasma-cell differentiation and antibody secretion *in vitro*. Our results showed that at 4-months post-infection, IgG levels were significantly augmented in patients who developed ARDS. In addition, we found that CXCL9&10 levels correlated significantly with IgG levels. Then, a 3-phase protocol was used to differentiate human B cells *in vitro* in the presence or absence of CXCL9&10. B-cells activation was confirmed with CD86 and CD25 upregulation, whereas plasma-cell differentiation was confirmed with the presence of $CD38^{hi}CD27^{hi}$ and $CD138^{+}$ cells. The expression of the CXCL9/CXCL10 receptor (CXCR3) was also upregulated with the activation cocktail used in phase1 and maintained during phase2/3. Regarding CD86 expression, only CXCL9 increased the expression of this activation marker, whereas the presence of CXCL9&10 significantly increased the percentage of $CD38^{hi}CD27^{hi}$ cells, $CD138^{+}$ cells and IgG secretion. Finally, we observed that CXCL9 also increased the expression of CD40L on $CD4^{+}$T-cells. In summary, we demonstrate that CXCL9 and CXCL10 are key players in the humoral response during COVID-19.

Keywords: B cells, plasma cells, CXCL9, CXCL10, IgG antibodies

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# MUCOSAL IMMUNOLOGY

**The transcriptomic profile of atlantic salmon skin epithelium naturally infested with sea louse (c. rogercresseyi) in seawater farming at summer and autumn reveals differential biological functions associated to seasonality**

**Andrea Cerda Celis**[1], Merari Goldstein[1], María J. Santillán-Araneda[1], Mabel Vidal[2], Eva Vallejos Vidal[1,3], Felipe Reyes Lopéz[1]

(1) Universidad de Santiago de Chile, Centro de Biotecnología Acuícola, Facultad de Química y Biología, Avenida Libertador Bernardo O'Higgins 3363, Edificio de Investigación Eduardo Morales, 9170002 Estación Central, Santiago, Chile
(2) Universidad San Sebastián, Departamento de Arquitectura y Diseño, Facultad de Ingeniería, Arquitectura y Diseño, Lientur 1457, 4080871, Concepcion, Chile
(3) Universidad de Las Américas, Núcleo de Investigaciones Aplicadas en Ciencias Veterinarias y Agronómicas, Facultad de Medicina Veterinaria y Agronomía, Avenida Walker Martínez 1360, 8242125 La Florida, Santiago, Chile

Chile is the second largest Atlantic salmon worldwide producer. Like in other countries, Chilean salmon farming is exposed to infection by pathogens present in the aquatic environment. One of them is the infestation by the Chilean sea louse (*Caligus rogercresseyi*), considered the biggest constraint to sustainable growth for this industry. Surprisingly, very few studies have focused on evaluating the health response of Atlantic salmon naturally infested in the environment. The urgency in generating this knowledge takes on particular relevance in the context of climate change and its consequence on ocean warming. In this study, we evaluated the modulation of the epithelial skin transcriptome of Atlantic salmon naturally infested with sea louse. Considering the fish's poikilothermic characteristic, the skin transcriptome was evaluated at summer and autumn. Methodologically, the epithelial skin mucosa was extracted from fish infested and non-infested with caligus in a farming center in southern Chile. Subsequently, the total RNA extracted was evaluated by an RNA sequencing strategy. We found few differential expressed genes (DEGs) in summer than in autumn. In summer, the transcriptome biological interpretation by Reactome showed the presence of processes associated mainly with cell proliferation. On the other hand, in autumn we identified processes associated with immune response, gene expression, membrane trafficking, and cellular response to stimulus. The String-based transcripteractome confirmed the association with a repertoire of cellular responses to stimuli and tissue repair. Collectively, our results shed light on the epithelial skin response in infested Atlantic salmon associated with seasonality

Keywords: Caligus rogercresseyi, Atlantic salmon, aquaculture, environmental impact, epithelial skin transcriptome

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**Dopaminergic signalling through DRD2 and DRD3 regulates the suppressive activity and intestinal tropism of regulatory T lymphocytes**

**Jacob Mora**[1,2], Iu Raïch[2], Valentina Ugalde[1], Carolina Prado[1], Gemma Navarro[3], Rafael Franco[4], Rodrigo Pacheco[1,2]

(1) Fundación Ciencia & Vida, Centro Ciencia & Vida, Av. Del valle 725, Huechuraba, Santiago, Chile
(2) Universidad San Sebastián, Facultad de Medicina y Ciencia, Providencia, Santiago, Chile.
(3) Universidad de Barcelona, Departamento de Bioquímica y Fisiología, Facultad de Farmacia y Ciencia de los Alimentos, Barcelona, España.
(4) Universidad de Barcelona, Departamento de Bioquímica y Biomedicina Molecular, Facultad de Biología, Barcelona, España

**Introduction**. Inflammatory bowel diseases (IBD) are driven by CD4+ effector T-cells (Teff). In contrast, regulatory T-cells (Treg) seems to be dysfunctional in IBD. Interestingly, dopamine levels are strongly reduced in the inflamed gut-mucosa. Dopamine has recently been involved in the gut-homing of T-cells. Accordingly, we found that stimulation of the high-affinity dopamine receptor D3 (DRD3) in Treg attenuates their suppressive activity and limits their acquisition of gut-tropism. The role of the low-affinity dopamine receptor D2 (DRD2) in Treg is still unknown. Here, we study how DRD2 and its interaction with DRD3 affect Treg function upon gut-inflammation.
**Methods**. Gut-inflammation was induced by administration of dextran sodium sulphate. Treg migration was evaluated by transwell-assays and by adoptive transfer followed by flow-cytometry analysis. Treg suppressive-activity was determined by co-culture with Teff and by attenuation of inflammatory colitis. Protein-interaction was evaluated by BRET and PLA. Disruption of the heteromer in Treg was performed by incubation of peptides analogous to the transmembrane segments of DRD2 and DRD3.
**Results**. DRD2-deficiency in Treg exacerbates colitis manifestation, impairs Treg suppressive activity, and limits their gut-homing. Conversely, DRD3-deficiency in Treg improves the suppressive activity, increases the gut-tropism and protected from colitis manifestation. Using biochemical analysis, we detected DRD2 and DRD3 forming heteromeric complexes. The disruption of the DRD2:DRD3 heteromer in Treg decreases their suppressive activity.
**Conclusion**. Our data shows an antagonic effect of DRD2 and DRD3-signalling on Treg and suggests that both protomers form an heteromeric complex that regulates intestinal Treg activity and gut-homing depending on the levels dopamine.

Keywords: Dopamine, Regulatory T cells, Inflammatory bowel diseases

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**The role of tryptase/par-2/pi3k in the regulation of b-cell lymphoma-3 (bcl-3) in the intestinal epithelium.**

**Javiera Alzaga**[1], Araceli Pinto[1], Veronica Torres[1], Caroll J. Beltrán[1,2]

(1) Laboratory of Immunogastroenterology, Gastroenterology Section, Department of Internal Medicine, Hospital Clínico Universidad de Chile
(2) Faculty of Medicine, Universidad de Chile, Santiago, Chile

Introduction: Mast cell (MC) tryptase activates the protease-activated receptor 2 (PAR-2) in the intestinal epithelia, which trigger a tight junction (TJ) restructuring and increase intestinal permeability. The Bcl-3 protein, an atypical member of IkB family, regulates TJ protein expression in intestinal epithelial cell (IEC) lines and PAR-2 activation promotes Bcl-3 upregulation in vitro. PAR-2 activation trigger PI3K signalling, however its consequences in Bcl-3 expression are unknown. Objective: To determinate the signalling induced by Tryptase/PAR-2 activation involved in Bcl-3 expression. Methods: IEC lines (DLD-1,Caco-2) stimulated with tryptase and PAR2-agonist peptides (SLIGKV) in presence/absence of antagonist-receptor peptide (FSLLRY). Bcl-3, β-actin, and PI3K-p/PI3K levels were assessed by immunoblot at short (0 to 60minutes) and long (12 and 24hours) intervals. Tryptase/PAR-2/Bcl-3 expression was evaluated at 24h in the presence of PI3K inhibitor (LY294002). Statistics: T-tests/ANOVA comparisons by GraphPad-Prism software, $p<0.05$ statistically significant. Results: Higher Bcl-3 protein levels were observed at 12 and 24 hours in response to tryptase ($p=0.0075$) and agonist ($p=0.0209$), which was reversed by the antagonist ($p=0.0046$). A time course increase of PI3K-p/PI3K was observed at 15, 30 and 60 min ($p=0.0485$, $p=0.0338$, $p=0.0419$, $p=0.0211$ ), with unchanged Bcl-3 level. A reduced trend of Bcl-3 level with PI3K inhibitor at 24 hours was observed. Conclusion: A PI3K signalling was induced by Tryptase/PAR-2 activation in a short term, being it apparently involved in Bcl-3 expression in a long term. Further research will allow us to elucidate complementary pathways involved in PAR-2/PI3K/Bcl-3 activation, as well as its role in TJ protein expression.

Keywords: PAR-2, IBS, TRYPTASE, MAST CELLS

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**STAT3 activation, histological characterization and bacterial burden during experimental periodontitis progression**

**Joaquin Espinoza**[1], Marion Arce[1,2], Marcelo Rodriguez[1], Valentina Olmedo[1], Catalina Moreno[1], Lukas Restelli[1], Romina Godoy[1], Loreto Abusleme[1,3], Nicolás Dutzan[1,2]

(1) University of Chile, Oral Microbiology and Immunology Laboratory, Faculty of Dentistry, Olivos 943 Independencia, Santiago, Chile
(2) University of Chile, Department of Conservative Dentistry, Faculty of Dentistry, Olivos 943 Independencia, Santiago, Chile
(3) University of Chile, Department of Pathology and Oral Medicine, Faculty of Dentistry, Olivos 943 Independencia, Santiago, Chile

**Introduction**: Periodontitis is an inflammatory disease that destroys tooth-supporting tissues induced by a dysbiotic microbial community. Numerous signal transduction pathways activated during this condition converge on STAT3; a transcription factor whose increased activation (pSTAT3) is associated with other inflammatory diseases but remains poorly studied in periodontitis. We aimed to characterize STAT3 levels, tissue destruction and bacterial detection during periodontitis progression.
**Methods**: The Ligature-induced-periodontitis mice model was used as a progression model. Gingival tissues were collected 1, 3, 5, and 7 days after periodontitis induction. Non-ligated tissues were used as controls. Ligatures were collected for bacterial DNA extraction for total bacterial load quantification. Western blot was used to detect pSTAT3. Additionally, we performed cell counting, collagen quantification, and bacterial detection through H&E, Masson's trichrome, and Brown-Brenn stains, respectively. Gene expression of proteins related to STAT3 signaling (*Rorc* and *Il17*) was determined with RT-qPCR.
**Results**: We observed increased pSTAT3 levels on all experimental days compared to control. A loss of collagen fibers at day 5 and increased cell infiltrate at day 7. The bacterial load was significantly higher starting on day 3. Little bacterial invasion was observed within tissues at any time-point. Gene expression of *Rorc* increased on day 5 and *Il17* on day 7, correlating positively with bone loss.
**Conclusions**: pSTAT3 levels and bacterial load were higher in the early stages of progression. Subsequently, an increase in gene expression of *Rorc* and *Il17* coincided with tissue destruction characterized by loss of collagen fibers, bone loss, and cellular infiltration at later stages.

Keywords: STAT3, tissue destruction, periodontitis

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# NEUROIMMUNOLOGY

**Interferon-gamma Induces Tolerogenic Dendritic Cells with Suppressive Activity in Experimental Autoimmune Encephalomyelitis and Multiple Sclerosis**

**Brian Parra-Tello**[1], Constanza Vilchez[1], Karin Jiménez[1], Camila Covián[1], Luis González[1], Carlos Guevara[2], Rodrigo Naves[1]

(1) Universidad de Chile, Programa de Inmunología, Instituto de Ciencias Biomédicas, Facultad de Medicina, Santiago de Chile, Chile
(2) Hospital Clinico Universidad de Chile, Departamento de neurología y neurocirugía, Facultad de Medicina, Santiago de Chile, Chile

Multiple Sclerosis (MS) is an autoimmune disease of the central nervous system. Our previous results have demonstrated that Interferon-gamma (IFN-g) induces a tolerogenic phenotype and function in murine bone marrow-derived dendritic cells (IFN-g-tol-BMDC). Here, we have evaluated the therapeutic potential of IFN-g-tol-BMDC in Experimental Autoimmune Encephalomyelitis (EAE), a preclinical model of MS. Besides, we determined the impact of IFN-g on the differentiation and function of monocytes-derived dendritic cells (moDC) from MS patients and healthy donors (HD).
EAE mice were i.v. transferred with BMDC differentiated in the presence of IFN-g or IFN-g+LPS at the peak of disease and clinical progression was daily monitored. moDC from MS patients (n=11) and HD (n=9) were differentiated in the absence (UN-moDC) or presence of IFN-g (IFN-g-moDC) or LPS (m-moDC) and the tolerogenic phenotype analyzed by flow cytometry (FC). The tolerogenic function was evaluated in a mixed lymphocyte reaction (MLR) assay by co-culturing IFN-g-moDC from MS patients (n=4) with allogenic peripheral blood mononuclear cells. After five days, proliferation, activation, and apoptosis of T cells were determined by FC.
Our results show that adoptive transfer of IFN-g-tol-BMDC or IFN-g+LPS-tol-BMDC ameliorates clinical symptoms of EAE. Besides, IFN-g-moDC from MS patients and HD show a tolerogenic phenotype characterized by lower levels of CD83, CD80, and CD86 than mDC and higher levels of PD-L1 than UN-moDC. Furthermore, IFN-g-moDC from MS patients inhibit $CD4^+$ and $CD8^+$ T cell activation, proliferation, and apoptosis of $CD4^+$ T cells.
Our results demonstrate that IFN-g induces tolerogenic DC with suppressive activity in EAE and MS.

Keywords: Multiple Sclerosis, Interferon-gamma, Dendritic Cells

Funding: This study was supported by FONDECYT/ANID 1191874 and 1231672.

# TUMOR IMMUNOLOGY

**Host and microbe computational proteomic landscape in oral cancer revealed a key metabolic pathway between Fusobacterium nucleatum and cancer progression**

**Camila Muñoz Grez**[1,2], Mabel Vidal[3], Tamara Rojas[4], Luciano Ferrada[5], Felipe Zuñiga[6], Agustín Vera[1], Sergio Sanhueza Novoa[1], Milovan Acevedo[6], Erik Riquelme[7], Wilfredo González[8,9], Angel Oñate[10], Estefanía Nova-Lamperti[1]

(1) Molecular and Translational Immunology Laboratory, Department of Clinical Biochemistry and Immunology, Pharmacy Faculty, Universidad de Concepcion, Concepcion, Chile.
(2) Faculty of Dentistry and Rehabilitation Sciences, Universidad San Sebastián, Concepción, Chile.
(3) Computer Science Department, University of Concepcion, Concepcion, Chile.
(4) Engineering Faculty, Universidad de Talca, Talca, Chile.
(5) CMA BIO BIO, Faculty of Biological Sciences, Universidad de Concepción, Concepción, Chile.
(6) BIOTER Laboratory, Clinical Biochemistry and Immunology Department, Pharmacy Faculty, Universidad de Concepción, Concepción, Chile.
(7) Respiratory diseases Department, Faculty of Medicine, Pontifical University Catholic of Chile, Santiago, Chile.
(8) Dentistry Faculty, Universidad de los Andes, Santiago, Chile.
(9) Center for Research and Innovation in Biomedicine, Universidad de Los Andes, Santiago, Chile.
(10) Laboratory of Molecular Immunology, Department of Microbiology, Faculty of Biological Sciences, Universidad de Concepción, Concepción

**Background:** Oral squamous cell carcinoma (OSCC) is the most common manifestation of oral cancer. It has been proposed that periodontal pathogens contribute to OSCC progression, Epithelial Mesenchymal Transition (EMT) and modulation of immunosuppressive molecules, mainly by their virulence factors. However, the main periodontal pathogen and its mechanism to modulate OSCC cells remains not fully understood. In this study we identify the main pro-tumoral bacteria in the secretome of biopsies from patients with OSCC and we investigate the most important pro-tumoral bacteria mechanism associated with cancer progression. **Methods:** We analyse the main host-pathogen pathways in the secretome of biopsies from patients with OSCC and healthy controls by performing a host and microbe computational proteomics landscape. Then, oral cancer cells were infected with the main OSCC-associated bacteria and its effects on tumor growth, expression of EMT markers and the induction of immunomodulatory markers was evaluated. **Results:** Proteins from *Fusobacterium nucleatum* were abundant in OSCC samples, whereas proteins associated with EMT and cell proliferation were significantly upregulated in the human cancer proteome. We demonstrated *in vitro* that *F.nucleatum* colonised OSCC cells after infection, inducing tumour spheroids growth, EMT and Galectin-9 upregulation. Then, bacterial proteins and experimental assays revealed that glutamate metabolism was the key host-pathogen metabolic pathway, in which expulsion of L-glutamate by the cystine/glutamate transporter in cancer cells was promoted by *F.nucleatum* to catabolise L-glutamate into butyrate. **Conclusions:** The periodontal bacterium *Fusobacterium nucleatum* promotes OSCC tumor progression by increasing tumor growth, acquisition of ETM-associated markers, Galectin-9 upregulation and L-glutamate metabolism.

Keywords: OSCC, Periodontitis, Fusobacterium nucleatum, L-glutamate

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**Binding capacity of a fully human anti-MICA monoclonal antibody to different gastric cell lines.**

**Carla Díaz**[1], Karen Toledo-Stuardo[1], Fabiola González-Herrera[1], Ivo Campos[1], Jose Rodríguez[2], Samantha Tello[1], Douglas J. Matthies[1], María José Garrido[1], Mauricio González-Olivares[1], Nicolás Fehring[1], Francisca Cortés[1], Vicente Flores[1], Carolina Ribeiro[1], Claudia Altamirano[2], María Carmen Molina[1]

(1) Universidad de Chile, Programa Disciplinario de Inmunología, Facultad de Medicina, Independencia 1027, Santiago, Chile
(2) Pontificia Universidad Católica de Valparaíso, Facultad de Ingeniería, Chile

MICA is a highly polymorphic protein with different allelic variants; it is overexpressed on the membrane of different types of tumor cells, including gastric adenocarcinoma cells. MICA is one of the main ligands to the NKG2D activating receptor on Natural Killer (NK) cells; upon binding to the NKG2D receptor, MICA triggers the cytotoxic and cytolytic NK cell response, resulting in tumor cell lysis. However, as an immune evasion mechanism, MICA can be released into the extracellular medium, where it has the potential to interact with the NKG2D receptor and induce its down-regulation, preventing tumor cell recognition. Thus, neutralization of soluble MICA has been proposed as a therapeutic target in cancer. Our main objective was to evaluate the binding capacity of a fully human anti-MICA monoclonal antibody (mAb) (AcHu-anti-MICA) to allelic variants of MICA expressed on gastric adenocarcinoma cells. Gastric cell lines GES-1, MKN-45 and AGS carrying the allelic variants MICA*008, *009 and *010, respectively, were used to evaluate the binding capacity of the AcHu-anti-MICA compared to a commercial murine anti-MICA mAb by flow cytometry. Although both antibodies detected similar levels of MICA in GES-1 cells, the levels of MICA detected by AcHu-anti-MICA in MKN-45 and AGS cells were higher than those detected by the commercial anti-MICA mAb. In conclusion, AcHu-anti-MICA is able to recognize allelic variants of MICA *in vitro* better than the commercial antibody. The binding capacity of AcHu-anti-MICA to most MICA alleles could be a tool for further development of a therapeutic antibody in oncology.

Keywords: MICA, Anti-MICA Monoclonal Antibody, Gastric Cancer, NK Cells
Funding: Proyecto ANILLO ACT210068, Proyecto FONDECYT N° 1221031.

**The interplay between transferred and endogenous CD8+ T cells determines the efficacy of adoptive cell therapy against solid tumors**

**Diego Figueroa**[1], Felipe Galvez-Cancino[2], Andrés Hernández-Oliveras[1], Juan Pablo Vega[1], Felipe Flores[3], Sofia Hidalgo[1], Felipe Ardiles[1], Fabiola Osorio[3], Vincenzo Borgna[1,4,5], Álvaro Lladser[1,5]

(1) Fundacion Ciencia & Vida, Laboratory of Immunoncology, Santiago, Chile
(2) UCL Cancer Institute, Immune Regulation and Tumor Immunotherapy Group, Research Department of Haematology, Santiago, Chile
(3) Universidad de Chile, Laboratory of Immunology and Cellular Stress, Facultad de Medicina, Santiago, Chile
(4) Hospital Barros Luco Trudeau, Servicio de Urología, Santiago, Chile
(5) Universidad San Sebastián, Facultad de Medicina y Ciencias, Santiago, Chile

Adoptive T cell therapy (ACT) has proven to be an effective treatment for hematological cancers, however it remains ineffective against solid tumors. The mechanisms underlying protective immunity in solid tumors are not fully understood. Here, we show that adoptive transfer of TCR-transgenic $CD8^+$ T cells led to eradication of established mouse melanoma tumors, concomitantly with intratumoral accumulation of endogenous $CD8^+$ T cells exhibiting both progenitor-exhausted (Tpex) and terminally-differentiated (Tdif) phenotypes. Interestingly, ACT-mediated tumor elimination was dependent on endogenous $CD8^+$ T cells but not $CD4^+$ T cells. Furthermore, ACT promoted protective host antitumor immunity against rechallenge with melanoma cells lacking ACT-targeted antigen. Administration of cyclophosphamide prior to ACT as a lymphodepleting conditioning regimen severely impaired host antitumor immunity and abrogated protection against rechallenge. Mechanistically, ACT induced TNF-α-dependent maturation and lymph node migration of type 1 conventional dendritic cells (cDC1). Moreover, tumor eradication and accumulation of endogenous Tpex and Tdif cells relied on TNF-α and cDC1. Interestingly, enrichment of Tpex, Tdif, TNF-α-signaling and cDC1 gene signatures in human melanoma tumors was associated with favorable responses to ACT and improved survival. Our findings reveal a TNF-α- and cDC1-dependent interplay between transferred and endogenous $CD8^+$ T cells that determines the efficacy of ACT to eradicate solid tumors. This work also highlights the need to develop targeted lymphodepleting regimens that spare host T cells and cDC1s to unleash the full potential of ACT.

Keywords: Adoptive cell therapy, Solid tumors, Endogenous CD8 T cells

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**“Role of the intra-tumor microbiome in the non-small cell lung cancer immune microenvironment through a multi meta-omics analysis in Chilean patients”**

**Ivania Valdes**[1,4], Alberto Martin[2,3], Eduardo Martinez[5], Erick Riquelme[4]

(1) Universidad Mayor, Center for Integrative Biology, Facultad de Ciencias, Camino La piramide 5750, huechuraba, Santiago, Chile
(2) Universidad San Sebastian, Escuela de Ingeniería, Facultad de Ingeniería, Arquitectura y Diseño, Avenida Del Condor 720, Santiago, Chile
(3) Fundación Ciencia & Vida, Centro Científico y Tecnológico de Excelencia Ciencia & Vida, Laboratorio de Redes Biológicas, Avenida Del Condor 720, Santiago, Chile
(4) Pontificia Universidad Católica de Chile, Departamento de Enfermedades Respiratorias, Facultad de Medicina, Marcoleta 391, Santiago, Chile
(5) Corporación CGNA, Las Heras 350, Temuco, Chile

Lung cancer is the main cancer death in Chile and worldwide. The tumor microbiome´s role in cancer has underscored its capacity to influence and modulate immune surveillance, although the functional mechanisms remain elusive. Here, we outline how the tumor microbiota can impact the host´s immune response to lung cancer. We explored the microbiome in lung adenocarcinoma tumors and adjacent non-tumoral tissues. Within the tumor microenvironment, we identified an enrichment of microbiome members from class *Gammaproteobacteria* and *Actinobacteria*, specifically genera *Pseudomonas* and *Corynebacterium* in undifferentiated samples. Furthermore, a frequent depletion of class *Verrucomicrobiota* with genus *Akkermansia* was observed in the same samples using 16S-rRNA sequencing. In a subset of these samples using proteomics, the gene-ontology analysis revealed an enrichment of proteins related to the Interferon pathways, whereas we observed a downregulation of proteins such as T-cell surface-glycoprotein CD8 and HLA-class I histocompatibility antigen, related to MHC protein complex binding function, associated to immune evasion through a decrease in antigen presentation. Interestingly, we found 6 bacteria-associated peptides from *Pseudomonas* and *Streptomyces* enriched in the tumor known to be negatively associated with cancer. Metabolic analysis showed 10 bacteria-associated metabolites enriched in the tumor, highlighting within these, adenosine and inosine two known metabolites involved in inducing immunosuppression on T-cells in the tumor microenvironment. In summary, our multi-omics and bioinformatic analyses reveal the existence of a distinctive intra-tumoral microbiome capable of promoting immune suppression by altering the tumor microenvironment’s metabolic profile, and together with the immune evasion profile collectively could contribute to the tumor progression.

Keywords: Tumor microbiome, Cancer, Omics

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**Differential cellular release mechanisms of MHC class I polypeptide-related sequence A (MICA) alleles and their effects on the regulation of the NKG2D receptor on NK cells**

**Karen Toledo-Stuardo**[1], Silvia López-Borrego[2], Carmen Campos[2], María Carmen Molina[1], Mar Valés-Gómez[2]

(1) Universidad de Chile, Programa de Inmunología, Facultad de Medicina, Avda. Independencia 1027, Santiago, Chile
(2) Centro Nacional de biotecnología (CNB)-CSIC, Department of Immunology and Oncology, C. Darwin, 3, 28049, Madrid, Spain

MICA is a stress-induced membrane ligand engaged with NKG2D receptors on NK cells activating the cytotoxic response.However, this molecule can be released in a soluble form by metalloprotease action or bound to extracellular vesicles(EVs),which would have a down-regulator effect on NKG2D,altering the cytotoxicity of NK cells. There are multiple MICA protein variants,whose biochemical characteristics and immunological effects have been studied only in a limited number of proteins.
Our main objective was to study the cellular mechanisms of release and effects of MICA variants on the NKG2D receptor on NK cells.
Methodologically, biochemical and cellular approaches were used in both transfectant systems with MICA variants (*002,*008,*009,*011and*019)and tumor cell lines.The release mechanisms were evaluated using metalloprotease inhibitors.The effect of MICA variants on the NKG2D expression was analyzed by flow cytometry.
The MICA variants showed a differential release profile after the inhibition of metalloproteases.While MICA*008 was not affected,other variants were mainly recruited into EVs with a reduced release of soluble form.The membrane expression of MICA*008 compromised a glycosylphosphatidylinositol(GPI) anchor, whereas other MICA variants were GPI-independent, showing a differential cellular release mechanism. Both soluble and EV forms of MICA can induce the downregulation of NKG2D receptors. However,this effect would be more potent by the*002 and*008 variants bound to EVs.
These results indicate, for the first time,the differential release mechanisms,and down-regulatory effects on NKG2D receptors by MICA variants. Also,these findings would be important when selecting patients to be treated with anti-MICA antibodies that avoid the release of MICA from the membrane,whose variants could be released through EVs, augmenting the immune evasion mechanisms.

Keywords: MICA, NKG2D RECEPTOR, NK CELLS, CANCER

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**Co-Expression of Immunohistochemical Markers MRP2, CXCR4, and PD-L1 in Gallbladder Tumors Is Associated with Prolonged Patient Survival**

**María A. Gleisner**[1,2], Andrés Tittarelli[3], Omar Barría[1,2], Evy Sanders[1,2], Anna Bergqvist[1,2], Daniel Uribe[4], Mabel Vidal[5,9], Jorge Vergara[6], Ignacio Niechi[4], Cristian Pereda[1,2], Iván Flores[1,2], Cristian Carrasco[7], Claudia Quezada[1], Flavio Salazar-Onfray[1,2,8]

(1) Millennium Institute on Immunology and Immunotherapy, Santiago, Chile
(2) Universidad de Chile, Disciplinary Program of Immunology, Institute of Biomedical Science, Faculty of Medicine, Santiago, Chile
(3) Universidad Tecnológica Metropolitana, Programa Institucional de Fomento a la Investigación, Desarrollo e Innovación, Santiago, Chile
(4) Universidad Austral de Chile, Laboratorio de Biología Tumoral, Instituto de Bioquímica y Microbiología, Valdivia, Chile
(5) Universidad de Concepción, Molecular and Traslational Immunology Laboratory, Concepcion, Chile
(6) Universidad Tecnológica Metropolitana, Departamento de Informática y Computación, Santiago, Chile
(7) Hospital Base de Valdivia, Subdepartamento de Anatomía Patológica, Valdivia, Chile
(8) Karolinska Institute, Department of Medicine Solna, Stockolm, Sweden
(9) Universidad de Concepción, Departamento de Informática y Computación, Concepcion, Chile

Gallbladder cancer is the most malignant neoplasm in the biliary tract. Asia and Latin America have high mortality and highest prevalence in middle-aged Chilean women. The limited therapeutic options for GBC require the identification of targetable proteins with prognostic value for improving clinical management support. We evaluated the expression of targetable proteins, including three epithelial tumor markers, four proteins associated with multidrug and apoptosis resistance, and eleven immunological markers in 241 primary gallbladder adenocarcinomas. We investigated correlations between tumor marker expression, the primary tumor staging, and GBC patients' survival using automated immunohistochemistry, a semi-automatic method for image analysis, univariate and multivariate statistical analyses, and machine learning algorithms. Our data show a significant association between the expression of MRP2, CXCR4, and PD-L1, and a better prognosis for patients with late-stage primary tumors. The expression of the MRP2/CXCR4/PD-L1 cluster of markers discriminates among short-, medium-, and long-term patient survival, with an ROC of significant prognostic value. Moreover, a high MRP2/CXCR4/PD-L1 co-expression is associated with increased survival time (30 vs. 6 months) in GBC patients, regardless of tumor stage. Hence, our results suggest that the MRP2/CXCR4/PD-L1 cluster could potentially be a prognostic marker for GBC.

Keywords: Gallbladder cancer, multivariate analyses, immunological markers, survival

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**Development of a completely human monoclonal antibody targeting MICA for cancer immunotherapy**

**María José Garrido**[1], Karen Toledo-Stuardo[1], Fabiola González-Herrera[1], Jose Rodríguez[2], Samantha Tello[1], Douglas J. Matthies[1], Mauricio González-Olivares[1], Ivo Campos[1], Nicolás Fehring[1], Carla Díaz[1], Fabián Tempio[1], Carolina Ribeiro[1], Claudia Altamirano[2], María Carmen Molina[1]

(1) Universidad de Chile, Programa de Inmunología, Instituto de Ciencias Biomédicas, Facultad de Medicina, Santiago, Chile
(2) Pontificia Universidad Católica de Valparaíso, Escuela de Ingeniería Bioquímica, Valparaíso, Chile

Cancer is one of the leading causes of death worldwide. During the last years, immunotherapy has had great advances that allowed the development of novel specific therapies using monoclonal antibodies (mAbs). MICA is a cell-surface protein that is overexpressed under cellular stress in different types of cancer. It is recognized by the NKG2D receptor, which triggers cytolytic activation of Natural Killer (NK) cells. However, tumors have developed several strategies to evade the immune response, such as proteolytic removal of MICA, which induces down-regulation of the NKG2D receptor on NK cells, thus compromising their function.
The aim of this study was to generate a fully human anti-MICA mAb and to analyze its capacity to bind to soluble MICA, as well as its ability to block MICA and NKG2D receptor interaction. The antibody was constructed from an antibody fragment (scFv) targeted to the α1 non-polymorphic region of MICA, produced in CHO-S cells, and purified with protein G column-coupled HPLC. The antibody binding and blocking capacity were assessed by ELISA. Additionally, *in vivo* binding of anti-MICA was studied using NOD SCID gamma mice employing IVIS-ILUMINA imaging, after establishing MICA-transfected B16F10 tumor models.
The results demonstrated that our anti-MICA mAb binds to the soluble form of MICA and competes for MICA binding with the NKG2D receptor. Additionally, our findings elucidate its biodistribution pattern at the tumor site in the murine model. These findings hold promise for the designed mAb as a potential biopharmaceutical against MICA-expressing tumors, contributing to the evolving landscape of cancer immunotherapy.

Keywords: Recombinant Antibody, Cancer Immunotherapy, MICA, NKG2D

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**Development of a mini-antibody directed against the alpha 2 domain of MICA**

**Mauricio González-Olivares**[1], Karen Toledo-Stuardo[1], Fabiola González-Herrera[1], Ivo Campos[1], Jose Rodríguez[2], Samantha Tello[1], María José Garrido[1], Douglas J. Matthies[1], Nicolás Fehring[1], Carla Díaz[1], Carolina Ribeiro[1], Claudia Altamirano[2], María Carmen Molina[1]

(1) Universidad de Chile, Programa de Inmunologia, Facultad de Medicina, Independencia 1027, Santiago, Chile
(2) Pontificia Universidad Catolica de Valparaíso, Escuela de Ingeneria Bioquimica, Facultad de ingeniería, Valparaiso, Chile

Soluble MICA protein is considered an oncotarget due to its potential capacity to promote tumor immune evasion by internalizing the cytotoxicity-associated NKG2D receptor on Natural Killer cells. In this sense, neutralization of extracellular MICA with antibodies may become an interesting strategy to counteract this mechanism, which has been shown to favor tumor progression. The aim in this work was to generate a single-chain fragment variable (scFv) antibody that binds to the α2 domain of MICA.
We used phage display technology to generate an anti-MICA scFv antibody; evaluation of transfected *E. coli DH5α* clones was performed by PCR and phage ELISA. The homology of the sequences of each selected scFv was compared with antibody germline genes by IgBlast. The scFvs were expressed in the pET_52b vector using *E. coli BL21* for their production, and were further refolded and analyzed by size exclusion column.
We obtained two scFvs against the α2 domain of MICA, which shared 98.5% gene sequence identity with the IGKV2-30*01 and IGHV1-46*01 antibody families; differences were detected only in the CDRs of the scFv. The recombinant scFvs yield reached 1 mg/mL with a purity of 90%; the folding of the scFvs was optimized in a buffer with L-Arginine. Finally, two high-affinity scFvs were produced, as evaluated by ELISA.
In conclusion, the phage display method successfully allowed the generation of scFv antibodies directed towards MICA. This innovative technology is useful in the development of high-affinity antibodies targeting various molecules, thereby advancing the field of therapeutic antibodies.

Keywords: scFv, MICA, NKG2D, Antibody, Phage display

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**Functionally heterogeneous tumor-reactive tissue-resident memory CD8+ T cells infiltrate human renal cell carcinoma tumors.**

**Sofia Hidalgo**[1,6], Andrés Hernández-Oliveras[1], Jimena Tosello-Boari[2,3], Javiera Reyes-Álvarez[1], Ernesto López[1], Yoann Missolo-Koussou[2,3], Wilfrid Richer[2,3], Sergio Hernández[1], Farides Saavedra[1], Eduardo Roa[1], Diego Figueroa[1], Christine Sedlik[2,3], Luis Alarcón[4], Camélia Radulescu[5], Yves Allory[5], Daniela Sauma[6], Vincenzo Borgna[1,4,7], Eliane Piaggio[2,3], Álvaro Lladser[1,7]

(1) Centro Cientifico y Tecnologico de Excelencia Ciencia & Vida, Fundacion Ciencia & Vida, Santiago, Chile.
(2) INSERM U932, PSL Research University, Institute Curie Research Center, Paris, France.
(3) Department of Translational Research, PSL Research University, Institut Curie Research Center, Paris, France.
(4) Hospital Barros Luco Trudeau, Santiago, Chile.
(5) Department of Pathology, Hôpital Foch, Suresnes, France.
(6) Laboratorio de Inmunología, Facultad de Ciencias, Universidad de Chile.
(7) Facultad de Medicina y Ciencia, Universidad San Sebastián.

Tissue-resident memory $CD8^+$ T (Trm) cells mediate potent innate and adaptive immune responses and has been associated with a better prognosis in several solid cancers. However, the tumor reactivity and heterogeneity of Trm cells in renal cell carcinoma (RCC) patients has not been studied. In this study, memory $CD8^+$ T cells infiltrating RCC tumors were analyzed by flow cytometry as well as bulk and single-cell RNA sequencing (RNAseq) coupled to TCR sequencing. Also, sorted $CD103^+CD69^-$ and $CD103^+CD69^+$Trm cells and circulating CD103-CD69- CD8+ T cells were co-cultured with autologous cells from RCC tumors and non-malignant renal tissue to assess tumor recognition. We observed high frequencies of $CD69^+CD103^-$ and $CD69^+CD103^+$ Trm cells, which express higher levels of tumor reactivity- and tissue residency-associated markers than circulating $CD8^+$ T cells. Interestingly, both Trm subsets specifically recognized autologous RCC cells. Flow cytometry and bulk RNA sequencing analyses demonstrated that the $CD69^+CD103^+$ Trm subset expresses an effector and progenitor program, whereas the $CD69^+CD103^-$ Trm subset displays a terminally differentiated signature. Moreover, we found by scRNAseq a higher heterogeneity in Trm cell populations, including proliferating, progenitor and terminally differentiated Trm cells, which were also highly clonal expanded in RCC patients. These data evidence the presence of tumor-reactive Trm cells with distinct functional states in RCC patients.

Keywords: Renal cell carcinoma, Tumor Immunology, Memory CD8+ T cells

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# EXTENDED ABSTRACTS

**Characterization and humanization analysis of Immunoglobulin New Antigen Receptor Variable Domains (VNARs) Derived from Cartilaginous Fishes Inhabiting the Chilean Coast to be Used in Biomedical Applications.**

**Richard Olivares Olivares**[1,2,3], Angello Retamal Díaz[1,2,3], Angélica Bravo[3], Carlos Bustamante[4], Margarita Lay[1,2,3]

(1) Universidad de Antofagasta, Departamento de Biotecnología, Facultad de Ciencias del Mar y Recursos Biológicos, Av. Universidad de Antofagasta#02800, Antofagasta, Chile
(2) Universidad de Antofagasta, Instituto Milenio en Inmunología e Inmunoterapia, Av. Universidad de Antofagasta#02800, Antofagasta, Chile
(3) Universidad de Antofagasta, Centro de Investigación en Inmunología y Biotecnología Biomédica de Antofagasta (CIIBBA), Av. Universidad de Antofagasta#02800, Antofagasta, Chile
(4) Universidad de Antofagasta, Instituto de Ciencias Naturales Alexander von Humboldt, Laboratorio de Biología Pesquera, Facultad de Ciencias del Mar y Recursos Biológicos, Av. Universidad de Antofagasta#02800, Antofagasta, Chile

**Introduction:** Antibodies are powerful tools in research and diagnostics. Particularly, monoclonal antibodies have widely been used to detect various pathogenic diseases due to their higher purity and specificity which gives them multiple functions such as targeting protein-protein interactions and identifying single members of protein families.
The antibody is a structural complex composed of two heavy chains and two light chains. The heavy chain of a conventional antibody consists of three constant domains (CH1, 2 and 3) and one variable domain (VH), while the light chain consists of one constant domain (CL) and one variable domain (VL). VH is the responsible for antigen recognition. However, the efficacy of conventional antibodies could be limited by their large molecular size (≈150kDa) and complex structure, where their paratopes may not be able to access certain antigens, resulting to poor binding affinity. Degeneration by high temperatures and humidity are the main factors affecting sensitivity that could simply lead to false positive results. To overcome these problems, exploration of new antigen binders with natural heat-stability is extensively needed for improving the current diagnostic platform. In recent years, single domain antibodies have been in the spotlight of researchers due to their great therapeutic and diagnostic potential. These antibodies are found in camelids (HcAb or VHH) and cartilaginous fishes (IgNAR or VNAR). *Mustelus mento* as well as *Mustelus whitneyi* are cartilaginous fishes found on the Chilean coasts and share taxonomic similarities with *Mustelus canis* and *Triakis scyllium* where new antigen receptor immunoglobulin (IgNAR) has already been reported. Each chain in IgNAR consists of five constant domains followed by one variable domain. The variable domain of IgNAR, or referred to as VNAR, contains only two complementarity-determining regions (CDRs), as known as CDR1 and 3. Therefore, it allows VNAR diversity to be achieved by long variable CDR3 that protrude with additional diversity in CDR1 and connected through two hypervariable regions (HV), HV2 and 4, which confers VNRAs the formation of special paratopes that can access confined epitopes.
VNARs are so far the smallest natural immunoglobulin-based protein scaffolds, with a molecular mass of about 12kDa, in the animal kingdom. Due to their small size, high specificity for a cognate antigen, and high physiochemical stability, these antibody domains have been considered as promising candidates for biomedical development.
In this study, we propose the development of an innovative platform for the generation of variable single chain (VNAR) antibodies from a Chilean cartilaginous fish to be used in biomedical therapies.

**Aims: (1)** To identify and describe VNAR domain sequences in *M. mento* and *M. whitneyi* using conserved regions from previously described cartilaginous fishes. **(2)** To develop VNARs libraries and characterize the natural coding sequence for single-domain antibodies of *M. mento* and *M. whitneyi*. **(3)** To design and propose *In Silico* potential methods for humanization to be used in biomedical applications.

**Methods:** The design of degenerate primers and characterization of IgNAR expression in *Mustelus Mento* and *Mustelus whitneyi* was performed by bioinformatics assays. The obtainment of VNARs was done by using RT-PCR, bacterial transformation, and characterized by Sanger sequencing. Finally, the design of the humanization strategy for IgNAR variable domain sequences was performed through bioinformatic software's.

**Results:** Degenerate primers were design to amplify and characterize *M. Mento* and *M. whitneyi's* IgNAR variable domain sequences. VNARs library was done by clone VNARs PCR products, using three pairs of specific primers, and were successfully transformed into chemically competent cells (Figure I & II). The coding sequences for VNAR domains of *M. mento* and *M. whitneyi* displayed similarities with sequences from the variable light chain of the human immunoglobulin.
**Discussion:** The identification and characterization of VNARs in *M. mento* and *M. whitneyi* may open new opportunities for the generation of therapeutic and diagnostic agents. The ability of these VNARs to access confined epitopes, due to the uniqueness of their paratopes, suggests that they could be especially valuable in detecting molecular targets that are difficult for conventional antibodies to reach due to their size and complexity. In addition to VNARs characterization, we have highlighted the importance of humanizing these sequences for biomedical applications. The *In Silico* humanization strategy is a fundamental step to ensure compatibility with human biological systems and to minimize potential immunogenic responses. However, it is crucial to emphasize that the successful implementation of this strategy will require meticulous analysis of both the VNAR sequences and the factors that provide stability and efficacy.

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Keywords: Nanobodies, IgNAR, VNAR, Single-domain antibodies, Antibodies

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**Regulation of microRNA degradation by the unfolded protein response sensor IRE1 in dendritic cells.**

**Alonso Lira**[1], Fabiola Osorio[1], María Julieta Gonzalez[2]

(1) Universidad de Chile, Programa de Inmunología, Facultad de Medicina, Av. Independencia 1027, Santiago, Chile
(2) Universidad de Chile, Programa de Biología Molecular y Celular, Facultad de Medicina, Av. Independecia 1027, Santiago, Chile

The Unfolded Protein Response (UPR) is a vital cellular stress pathway that maintains proteostasis of the endoplasmic reticulum (ER). IRE1 and XBP1s, a main arm of the UPR, are well-studied components which in addition to their canonical roles in protein folding, they also regulate the development and function of conventional type 1 dendritic cells (cDC1). cDC1s play pivotal roles in antitumor and antiviral responses and interestingly, in steady state, these cells display constitutive IRE1 activation.
IRE1 contains an endoribonuclease (RNase) domain which perform two important functions: it mediates the unconventional splicing of *Xbp1* mRNA, prompting the translation of the transcription factor XBP1s (XBP1 spliced), master regulator of ER homeostasis. And second, the IRE1 RNase domain also degrades various mRNAs and microRNAs containing the consensus sequence *CUGCAG*, in a process known as RIDD (*Regulated IRE1 Dependent Decay*). Although it is described that RIDD is important for cDC1 function, recent evidence indicates that this process could also influence the levels of microRNAs (miRNAs) that are critical for development, survival and inflammation. However, to date, no evidence connecting IRE1 activity and miRNA levels has been made in the cDC1 lineage. Thus, **a key question to ask is whether IRE1 through RIDD, can degrade miRNAs associated with cDC1 function. In this study, we explore the interplay between RIDD activation and miRNAs degradation in cDC1, with a specific focus on miRNAs linked to their pro-inflammatory function (miR-155 5p) and survival (miR-125a 5p).**

We carried out bioinformatic analyses using the RNAfold platform which revealed that both miR-125a and miR-155 possess recognition sequences for IRE1 RNase activity within the required secondary structure context (stem-loop). Then, as cDC1 source, we studied a bone marrow DC culture system termed 'OP9-DL1 DCs' which recapitulates the characteristics of cDC1 present in lymphoid tissues and organs. To model the function of the RNase domain of IRE1, we used different colonies of conditional knock-out mice including CD11c-Cre XBP1 fl/fl (XBP1ΔDC) mice, which lacks XBP1s in DCs and exhibits constitutive activation of RIDD in cDC1 and the CD11c-Cre x IRE1fl/fl mouse (IRE1TRUNCΔDC) which lack the RNase domain of IRE1 in DCs, thereby inhibiting RIDD.

Studies assessing expression of miR-155, a pro-inflammatory miRNA that indirectly induces the production of the pro-inflammatory cytokines IL-12, IL-6 and TNF-a, showed that miR-155 levels are reduced in cDC1s lacking XBP1 (which spontaneously activate RIDD) in resting and poly I:C-activated state. Concomitantly, these cells exhibit a reduction in the expression of IL-12, IL-6 and TNF-a in a RIDD-dependent manner (previous reported findings from our lab, Medel, B *et al* (2023). The Unfolded Protein Response Sensor IRE1 Regulates Activation of In Vitro Differentiated Type 1 Conventional DCs with Viral Stimuli. *International Journal of Molecular Sciences*). Future experiments will include the validation of direct miR-155 targets by western blot and inhibition of the RNase activity of IRE1 through pharmacological / genetic tools. Additionally, we will use molecular mimetics of miR-155 in cDC1 from XBP1ΔDC mice to determine whether there is a recovery in the pro-inflammatory cytokine production upon miR-155 overexpression. Following the same approach, we will employ miR-155 inhibitors in cDC1s from WT mice to assess whether a similar decrease in the production of pro-inflammatory cytokines occurs, as observed in cDC1 with active RIDD.

Studies aiming to assess cDC1 survival showed that XBP1 deficient cDC1s display increased viability compared to WT counterparts in resting and poly I:C activated conditions. In this context, expression of the pro-apoptotic microRNA miR-125a in in XBP1-deficient cDC1 showed a tendency towards decrease and a tendency towards increase in IRE1-deficient cDC1s. To obtain a clearer picture, we will determine expression of the 3p variant of miR-125a , which is also described to be targeted by RIDD. Therefore, future experiments will be to meausure the levels of miR-125a 3p expression in cDC1 in contexts of RIDD activation/inhibition.

Future studies include RNA sequencing (RNAseq) of miRNAs in RIDD-activated and non-activated sorted cDC1 cells and *in vitro* cleavage assays will explore IRE1 ability to cleave miRNAs with consensus cleavage sequences. This project will highlight novel insights on the regulation of miRNA homeostasis in cDC1s through RIDD, establishing a new regulatory axis that can be further explored for therapeutic purposes.

Keywords: IRE1, UPR, microRNAs, cDC1, Innate Immunity

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**Serum hepatic transaminases as biomarkers of Nonalcoholic Fatty Liver Disease (NAFLD) in Apical Periodontitis**

**David González-Quintanilla**[1], María José Bordagaray[1], Elizabeth Pellegrini[1], Mauricio Garrido[1], Patricia Hernández[1], Alejandra Fernández[2], Thomas Villalobos[1], Marcela Hernández[1]

(1) University of Chile, Dentistry, Olivos 943, Independencia, Santiago, Chile
(2) Andrés Bello University, Dentistry, Santiago, Chile

**Introduction**
Prevalent oral diseases impacting periodontal tissues encompass periodontitis and Apical Periodontitis (AP), a leading cause of adult tooth loss. Both are chronic inflammatory conditions resulting in the degradation of dental support tissues and alveolar bone. AP entails the chronic inflammation and deterioration of periapical tooth tissues due to interaction between endodontic infection and the host's immune-inflammatory response. Typically asymptomatic, AP is diagnosed through the presence of an osteolytic apical lesion of endodontic origin (ALEO) visible on periapical radiography. AP may induce low-grade systemic inflammation and bacterial translocation, linking it to non-communicable systemic diseases [1].
Nonalcoholic fatty liver disease (NAFLD) is a common condition consisting of fat deposits in the liver of individuals who consume limited or no alcohol (≤30 ethanol grams per day in men, and ≤20 in women). It presents an estimated prevalence of 24% and leads to liver cirrhosis and malignancy [2]. NAFLD is multifactorial and closely related to metabolic syndrome, including diabetes, hypertension, and obesity. NAFLD can also be influenced by alterations in the microbiota and inflammation. Liver diseases can be detected by hematological tests, especially leaking of serum aminotransferases levels, including alanine aminotransferase (AST) and aspartate aminotransferase (ALT), as surrogate biomarkers [2].
Some studies have explored the association between NAFLD and periodontitis, although it remains a subject of debate. Studies in animal models suggest that periodontitis and/or periodontal microbiota may exacerbate NAFLD through insulin resistance and hepatic inflammation in mice [3]. Epidemiological studies have suggested that periodontitis, especially periodontal pocket depth and *P. gingivalis*, is related to NAFLD and higher levels of aminotransferases [3].

**Methods**
In this cross-sectional study, conducted with the approval of the ethical-scientific committee of the Faculty of Dentistry at the University of Chile and the Central Metropolitan Health Service, 59 participants aged 18 to 40 were recruited between 2016 and 2022. Inclusion criteria required that individuals were healthy, with at least one tooth exhibiting apical periodontitis. The control group met the same criteria, excluding individuals with obesity, diabetes, hypertension, pregnancy, advanced periodontitis, recent drug use, or daily alcohol consumption above certain limits.
The study assessed alcohol consumption using the AUDIT-C questionnaire validated for the Chilean population and conducted clinical examinations that included demographic parameters. Periodontal and endodontic evaluations were performed, including periodontal probing depth, clinical attachment level, and bleeding on probing. The Decayed/Missing/Filled Teeth (DMFT) index was assessed, and a comprehensive dental radiograph was taken. All participants received dental prophylaxis before sample collection.
Fasting blood samples were collected and sent to the laboratory to evaluate lipid profiles and high-sensitivity C-reactive protein (hsCRP) levels. Levels of transaminases (ALT and AST) were measured, and levels of inflammatory biomarkers (IL-10, IL-17A, IL-22, IL-9, IL-4, and TNF-α) were determined using a multiplex panel.
Data analysis included statistical tests and correlations, and a *post hoc* power analysis was conducted.

**Results**
Regarding systemic levels of ALT, it was observed that individuals diagnosed with AP had significantly higher levels compared to controls, $p < 0,05$. AST levels were also elevated in individuals with AP compared to controls, $p < 0,05$.
Furthermore, moderate correlations were found between AST levels and the number of apical lesions in individuals with AP, as well as an inverse moderate correlation between AST levels and IL-22 levels in the participants' serum, $p < 0,05$.
Finally, potential modifying factors of ALT and AST levels were explored. It was found that the diagnosis of AP and IL-22 levels were the only factors influencing ALT levels, while AST were influenced by the diagnosis of AP and the severity of periodontitis. However, when applying a multivariate model that included the diagnosis of AP, the severity of periodontitis, and IL-22 levels as adjusting covariates, it was observed that ALT levels were significantly influenced by all three factors, $p < 0,05$.

**Discussion**
AP has been associated with various non-communicable diseases, potentially through inflammation or bacterial translocation [1], although its relationship with NAFLD has not been studied. The enzymes AST and ALT, released due to liver parenchymal destruction, are often used as surrogate markers for NAFLD [2]. In this study, we demonstrated for the first time that in individuals with AP, serum levels of AST and ALT are significantly higher than in controls. Even though there is no evidence in AP, some studies in periodontitis support this association. In periodontitis, higher levels of serum ALT and AST were associated with periodontal diseases diagnosis [2,4,5]. Periodontitis and AP share common characteristics as microbiological etiology, alveolar bone integrity compromise, systemic inflammatory burden establishment [4], and possibly in their association with NAFLD.
In conclusion our results suggests a potential association between AP and liver damage were IL-22 might play a compensatory role, emphasizing the importance of oral pathologies in overall health.

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**Characterization of the anti-inflammatory activity of Quillaic Acid from Quillaja Saponaria Molina.**

**Said Alamos Musre**[1], Carolina Otero Acuña[1], Adrian Moreno Vilches[2]

(1) Universidad Andrés Bello, Escuela de Química y Farmacia, Facultad de Medicina, Abate Molina 140 primer piso, Santiago, Chile.
(2) Universidad Andrés Bello, Centro de Biotecnología Vegetal, Facultad de Ciencias de la Vida, Republica 330 tercer piso, Santiago, Chile.

**Introduction:** The incidence of inflammatory diseases in the actual population has been on rise in the recent years[1,2]. In the search for treatment options, plant-based natural drugs are gaining popularity due to their effectiveness and low incidence of side effects[3]. Among these natural drugs, quillaic acid (QA) extracted from *Quillaja saponaria* Molina bark has demonstrated topical anti-inflammatory effects in *in vivo* models[4-6].

**Objectives:** To evaluate the effect of quillaic acid on classical components of the inflammatory response.

**Methodology:** A murine macrophage cell line (RAW264.7) was used to study cell viability assays, COX-2 enzyme expression analysis by Western blot, and evaluation of the effect of QA on NF-κB nuclear translocation using immunofluorescence assays. Three independent experimental assays were performed, and in all cases, a two-way ANOVA statistical analysis was applied.

**Results:** QA exhibits an inhibitory effect on COX-2 enzyme expression as result of the ability of QA to inhibit NF-κB nuclear translocation.

**Discussion and Conclusions:** As a first approach, QA cytotoxicity was evaluated, and it was observed that at 25, 75 and 125 µM does not trigger cell death. To assess the anti-inflammatory effect of QA, a western blot was performed to detect COX-2 enzyme expression, a key factor in the development of inflammation and associated pain. Due to the observed decrease in COX-2 expression, the master transcription factor NFκB, which is upstream of COX-2 expression, was evaluated to elucidate whether the anti-inflammatory effect associated with QA is mediated through NFκB or not. Finally, it can be concluded that QA exerts its anti-inflammatory effect is by inhibiting NF-κB nuclear translocation, preventing the expression of classical enzymes involved in the inflammatory response in a dose-dependent manner.

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Keywords: anti-inflammatory properties., quillaic acid, Quillaja Saponaria

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**Porphyromonas endodontalis DNA induces proinflammatory response via TLR-9 and implicates TLR-2 and/or 4 in human macrophages.**

**J Lucero**[1,2], A Fernández[3], M Reyes[1], A Hoare[1], M Bordagaray[1], L Yañez[3], D Bravo[3], Elizabeth Pellegrini[1], A Escobar[1], Marcela Hernández[1]

(1) Facultad de Odontología, Universidad de Chile.
(2) Facultad de Odontología, Universidad Finis Terrae.
(3) Facultad de Odontología, Universidad Andrés Bello.

**Introduction:** Species of the genus *Porphyromonas* are recognized as part of the central agents for the development and progression of apical periodontitis (AP)(Belstrøm et al., 2014; Pérez-Chaparro et al., 2014). Bacterial DNA effects differ due to unmethylated oligodeoxynucleotide CpG motifs(Dalpke et al., 2006; Li et al., 2023). The effect of *P. endodontalis* (*Pe.*) DNA on the inflammatory response and the receptors involved are unknown.

**AIM:** To evaluate *in vitro* if *Pe.* induces a proinflammatory response mediated by TLR-9 and involvement of TLR-2 and 4 in human macrophages.

**Method:** THP-1-monocytes were differentiated into macrophages and stimulated with 100 ng/µl *Pe*.-DNA (ATCC 35406[*Pe*.ATCC]) and clinical isolate-DNA(*PeC*). Gene expression (TNF-α, IL-1β, IL-6, IL-12, IL-10, TLR-9, -2, -4) and their participation were determined by RT q-PCR. Macrophages were pretreated with TLR-9 inhibitor (INH-18) or TLR-2/4 inhibitor (OxPAPC) and stimulated with TLR-9 agonist(ODN2006). NF-kB nuclear translocation was determined using immunofluorescence and western blot assays (WB). Statistical analyses (SA) of the results were performed (ANOVA/Kruskal-Wallis), $P<0.05$ was significant.

**Results:** Macrophages stimulated with DNA from *Pe*.ATCC induced increases in TNF-α and IL-6 mRNA, whereas the *PeC* induced increases in TNF-α, IL-1β, IL-6, and IL-10 mRNA above the unstimulated control. NF-kB nuclear translocation increased in response to stimuli with *Pe*. DNA. The percentage of macrophage's nuclear NF-kB increases in response to *Pe*. DNA, being higher with the *PeC* ($P<0.05$) than *Pe*.ATCC. WB shows a trend of higher NF-kB activation than *Pe*.ATCC. In addition, there was TLR-9 involvement in IL-6 gene expression by *Pe.* DNA. Our results also suggest TLR-2 and 4 involvements in the DNA-induced response of *Pe.*, which was evidenced by decreased expression of TNF-α, IL-1β, and IL-6 upon inhibition of these receptors.

**Discussion: *Pe.*-DNA induces gene expression of TNF-α, IL-1β, IL-6 and IL-10.** Stimulation with *P. gingivalis* (*Pg.*)-DNA of THP-1-monocytes and macrophages induces significantly elevated concentrations of the proinflammatory cytokines IL-1β-TNF-α-IL-6 and IL-8, and anti-inflammatory IL-10-IL-4 and TGF-β, compared to unstimulated cells(Blancas-Luciano et al., 2023; Lin et al., 2022; Sahingur et al., 2012). *Pe.* DNA associates with cytokines of the M1 profile and thus could potentiate the local and systemic proinflammatory response in PA.
***Pe.*-DNA induces NF-kB activation via TLR-9.** TLR-9 is the most overexpressed endosomal DNA sensor in inflamed gingival tissues in periodontitis and an increase in its expression is generated upon stimulation with *Pg.*-DNA THP-1-monocytes(Sahingur et al., 2010; Zhan et al., 2014). Similarly, increased levels of TLR-9 have been reported in ALEOS compared to healthy periodontal ligaments(Fernandez et al., 2022). Studies support the activation of the NF-kB pathway in cell models with monocytes and macrophages(Mussbacher et al., 2023; Noursadeghi et al., 2008; Stacey et al., 2016), through the recognition of *Pg.* and *Tannerella forsythia* bDNA by TLR-9(Sahingur et al., 2010).
**TLR-9 participates in *Pe.*-DNA induced IL-6 gene expression.** TLR-9 activation in macrophages has a more pronounced effect on IL-6 levels than other cytokines(Kim et al., 2015). Scavenger receptors can censor CpG DNA in macrophages(Zhu et al., 2001), as is the NLRP3 sensor of the inflammasome and its activation induces the production of the cytokines IL-1β and IL-18 (Man et al., 2016).
**TLR-2 and TLR-4 participate in *Pe.*-DNA induced gene expression of TNF-α, IL-1β, and IL-6.** TLR-4 and TLR-9 would act synergistically in LPS- and CpG DNA-induced TNF-α production in murine macrophages(De Nardo et al., 2009). Similarly, the interaction between TLR-2 and TLR-9 determines the production of IL-12 family cytokines in microglia stimulated with Gram-positive bacteria(Holley et al., 2012). Furthermore, mice deficient for TLR-2, TLR-4, or TLR-9 do not show susceptibility to *Mycobacterium tuberculosis* infection. The above suggests a joint involvement of the receptors(Kim et al., 2015; Saiga et al., 2011). TLR-4 can be internalized and stimulate intracellular pathways and TLR-2 can also activate NF-kB from endosomes in human monocytes(Mussbacher et al., 2023; Noursadeghi et al., 2008; Stacey et al., 2016). The endocytic machinery would be critical in the interaction of these pathways; however, they are not fully characterized(Marongiu et al., 2019). *Pe.* DNA induces a proinflammatory effect on human macrophages via TLR9, TLR2, and TLR4.

Keywords: Porphyromonas endodontalis, macrophages, apical periodontitis

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**Immunotherapy based on heat-conditioned melanoma cell lysate promotes the maintenance of stem-like CD8+ T cells by diminishing the acquisition of their exhausted state**

**Consuelo Merino González**[1], Camila Fuentes[1], Fabián Tempio[1], Amarilis Perez[1], Flavio Salazar-Onfray[1], Mercedes N. López[1]

(1) Universidad de Chile, Programa de Inmunología, Instituto de Ciencias Biomédicas (ICBM), Facultad de Medicina, Santiago, Chile.

**Introduction:** The effective activation of the antitumor immune response is essential for the neoplastic cells elimination, with the cytotoxic $CD8^+$ T cells infiltration being key in the process. However, their mere presence does not ensure an effective antitumor response. The immunosuppressive tumor microenvironment and constant and persistent T cell receptor (TCR) stimulation by tumor associated antigens, drives a specific epigenetic program on T cells, which diminishes their function hence impeding a proper antitumor response called "T cell exhaustion" [1]. Exhausted T cells are characterized by an altered transcriptional program which translates into diminished effector function and the co-expression of multiple inhibitory receptors, such as programed cell death protein 1 (PD-1), and the transcription factor thymocyte selection-associated high mobility group box (TOX) [2,3]. Interestingly, it has recently been described that the expression of the transcription factor TCF-1 defines a subset of exhausted T cells with stemness properties, such as the ability to both self-renew and generate the entire spectrum of more differentiated cells, which have been associated with to a better therapy response [4,5].
In this regard, our group, developed an Immunotherapy based on heat-conditioned human melanoma cell lysate (TRIMEL) combined with *Concholepas concholepas* hemocyanin (CCH) called Tvax, where Heat-conditioned tumor cell lysates provides a complex source of tumor associated antigens and contains several DAMPs, such as calreticulin and HMGB1 hence inducing DCs maturation, and promoting cross-presentation of tumor derived antigens required for establishing an antitumor response [6]. Our previous results using B16-F10 murine melanoma model showed that Tvax (plus B16-F10 lysate for preclinical use) immunotherapy reduces the tumor growth rate and $CD8^+$ T cells infiltration [7]. Interestingly, the use of only murine B16-F10 lysate plus CCH (B16vax), in the absence of human lysate, lacks antitumoral effect, although a high presence of $CD8^+$ T cells within the tumor site was described (Unpublished results). This indicates that even though $CD8^+$ T cells tumor infiltration is necessary for an effective antitumor response but not sufficient. Hence, other variables such as their phenotype and/or functionality may be critical. Therefore, we propose that Tvax would preserve TCF-1 expression on tumor $CD8^+$ T cells, allowing the maintenance of stem-like $CD8^+$ T cell subsets by reducing the acquisition of their exhausted state.

**Methods:** For therapeutic assays, C57BL6 mice were subcutaneously inoculated with $2{,}5x10^4$ B16.F10 cells in lower right flanks. Mice were then immunized subcutaneously on lower left flanks on days 1, 6 and 12 post-tumor challenge with corresponding treatments: (1) 1:1 mixture of HS-conditioned B16.F10 cell lysate with TRIMEL + CCH (2) lysates of HS-conditioned B16F10 cells + CCH or (3) PBS. On day 18 after challenge, mice were sacrificed, and tumors and lymph nodes were analyzed for cell phenotyping by flow cytometry. Acquisition and analysis were performed on LSR Fortessa X-20 (BD Biosciences), and subsequent analysis was made with FlowJo10 software (FlowJo).

**Results:** Our results showed that immunization with Tvax induces a significant decrease in the exhausted population ($PD\text{-}1^+TOX^+$) like B16vax treatment, both compared with the control group, but only Tvax treatment showed a significant increase in TCF-1 expression, both in the total $CD8^+$ T cell population and in the exhausted population, compared with the B16vax and the control group (Figure 1). Furthermore, although treatment with Tvax and B16vax shows a significant increase in the effector population compared to the PBS group, only Tvax induces a significant increase in granzyme-b (GZM-B) expression in the stem-like population ($TCF1^+$) (Figure 2).

**Discussion:** These results suggest that immunization with Tvax induces a particular phenotypic and functional characteristics in intratumoral $CD8^+$ T cells, given by a less exhausted phenotype, greater TCF-1 expression and better effector capacity than B16vax treatment and the control group, which could explain, at least in part, the effective antitumor response we observed with Tvax treatment.

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Keywords: Cancer Immunotherapy, CD8+ T cells, Exhaustion, TCF-1, Progenitor exhausted T cell

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**PD-L1 expression is associated with FTO expression and activity in human in-vitro models and tissues from Chilean patients of colorectal cancer.**

**Diego Irribarra**[1], Antonia Domínguez[1], Rocio Cofré[1], Veronica Fuentes[1], Consuelo Santibañez[1], Marjorie De la Fuente[1], Karen Dubois-Camacho[2,3], Victor Cortes[2,4], Antonella Sanguineti[2,4], Mario Abedrapo[4], Daniela Sauma[5], Marcela Hermoso[2,3], Glauben Landskron*[1]

(1) Laboratorio de Investigación en Biomedicina, Escuela de Medicina Universidad Finis Terrae., Santiago, Chile
(2) Laboratorio de Inmunidad innata, Programa de Inmunología, ICBM, Facultad de Medicina, Universidad de Chile., Santiago, Chile
(3) Department of Gastroenterology and Hepatology, University Medical Center Groningen, University of Groningen, Netherlands.
(4) Departamento de Cirugía, Hospital Clínico Universidad de Chile.
(5) Laboratorio de Inmunología, Departamento de Biología, Facultad de Ciencias, Universidad de Chile.

**Introduction:** Colorectal cancer (CRC) is one of the most frequent and deadly cancers worldwide, and a determining factor in cancer prognosis and immune evasion is the expression of the immune checkpoint markers PD-1 and PD-L1, often upregulated in many cancers. The mechanisms underlying PD-1/PD-L1 overexpression are not completely understood, but recent studies have shown that the N6-methyladenosine (m6A) modification could regulate its expression impacting the efficacy of ICI blockade treatment. The m6A-demethylase protein called FTO regulates PD-L1 expression in an IFN-independent manner in CRC cell lines. Preliminary work from our lab shows increased FTO levels in tumor cells and stroma compared to healthy tissue, particularly in T cells, macrophages and fibroblasts from the tumor microenvironment (TME). However, the association between FTO expression and PD1/PDL-1 has not been described in tissue from CRC patients and whether modulating FTO-demethylase activity could impact PDL-1 expression.
The aim of this study was to associate the expression of PD1 and PD-L1 with FTO in tissue samples from colorectal cancer patients and further assess PD-L1 expression mediated by FTO activity in-vitro.

**Methods:** Eight patients were included with informed consent. FTO, PD-1, PDL-1 immunohistochemistry was performed in 4µm-thick histological sections fixed in formalin derived from tumor tissue and adjacent benign colon tissues from the surgical specimen of the same patient who underwent resective surgery.
Additionally, the colon adenocarcinoma cell line HCT116 was treated to 10µM of FB23-2 (FTO inhibitor), 10µM of dimethyl-2-oxoglutarate (DMKG, FTO substrate) and 2mM of butyrate for 6 hours, for further determination of PD-L1 by western blot. For western blot analysis, cultured cells were lysed in RIPA buffer supplemented with phosphatase and protease inhibitors. Lysates were subjected to SDS-PAGE, transferring the separated proteins to a PVDF membrane, then exposed to primary anti-PDL1 antibody (Cell Signaling, cat.13684) and GAPDH as loading control.

**Results:** The results of immunohistochemistry show a positive nuclear immunoreactivity for FTO in the tumor and stroma from all colorectal cancer tissues. In all benign adjacent tissues it was localized in the gut-associated lymphoid tissue (GALT) and epithelial cells.
Positive membranous immunoreactivity for PD-1 was observed in 4 CRC tissues located in the GALT and, notably, in the tumor stroma. In 6 benign adjacent tissues it was restricted to the GALT.
Positive membranous and cytoplasmic immunoreactivity for PDL-1 was observed in 7 CRC tissues localized in tumor cells and stroma. In 6 adjacent benign tissues it was only localized in epithelial cellsIn the cell line HCT116, western blot analysis showed an increase in PD-L1 protein levels in cells treated with DMKG compared to control cells (DMSO). On the other hand, a slight decrease of PD-L1 was observed in cells treated with butyrate and FB23 compared to the control.

**Discussion:** DMKG is a substrate of the FTO enzyme, whose demethylase activity on mRNA has been associated with positive regulation of PD-L1 in cancer models. Moreover, FB23-2 is a reported inhibitor of FTO catalytic activity. These findings could indicate that modulating substrate availability of FTO enzyme regulates its demethylase activity on PD-L1 mRNA, increasing the levels of this protein. This effect could not be limited to tumor cells but also occur in the TME, as PD-L1 was also observed in the tumor and stroma from our samples, suggesting the association between FTO demethylase activity and increased PD-L1 expression in the TME of colorectal cancer.
Taken together, our results support a regulatory role of FTO activity on PD-L1 expression in colorectal cancer cells, with implications for immune evasion. These preliminary findings suggest that FTO may have potential as a therapeutic target, however, further research is needed to confirm this hypothesis.

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Keywords: FTO, immune evasion, Colorectal cancer, PD1, PDL1

**Different roles of CD73 and adenosine in the establishment of exhausted CD8+ T cell populations in the tumor niche.**

**Juan Saavedra Almarza**[1], Solange Gouet Moya[1], Felipe Malgue[1], José Corrales[1], Camilo Villaman[2], Alberto Martin[2], Mario Rosemblatt[1,2,3], María Rosa Bono[1,2], Daniela Sauma[1,2]

(1) Universidad de Chile, Departamento de Biología, Facultad de Ciencias, Santiago, Chile
(2) Centro Ciencia & Vida, Avenida del Valle Norte 725, Huechuraba, Santiago, Chile
(3) Universidad San Sebastián, Facultad de Medicina y Ciencia, Santiago, Chile

**Background** The functional activity of cytotoxic CD8+ T cells is reduced in the tumor niche through exhaustion. Exhausted CD8+ T cells (Tex) derive from precursor exhausted T cells (Tpex), which present an enhanced self-renewal capacity and are responsible for the proliferative burst in anti-PD1 therapies [1]. Several features of Tpex, including stemness, have been described as induced by the adenosine-producing ectoenzyme CD73 in CD8+ T cells [2]. However, the relationship between the CD73/adenosine axis and Tpex/Tex differentiation has not been studied. Here we evaluated the role of CD73 and adenosine in the development of Tpex and Tex within the tumor niche and under *in vitro* conditions of chronic activation.

**Methods** We generated *in vitro* exhausted T cells using a previously reported protocol [3] based on chronic activation of OT-I cells with their cognate peptide. To study the role of CD73 in CD8+ T cell exhaustion, we differentiated OT-I cells in the presence of the CD73 catalytic inhibitor APCP or vehicle control. To this end, we also co-transferred OTI (WT) and OTI/CD73KO (CD73KO) naïve CD8+ T cells (CD44lowCD62L+) to B16F10-OVA tumor-bearing mice and analyzed intratumoral exhausted populations by FACS. We evaluated the contribution of adenosine in CD8+ T cell exhaustion *in vitro* through the addition of the specific A2AR inhibitor SCH58261, the A2AR agonist NECA, or the specific A2AR activator CGS-2168 to chronically activated OTI cells. Finally, we analyzed the role of A2AR in exhaustion *in vivo* through the intraperitoneal administration of the specific A2AR inhibitor, SYN115 or vehicle control, in B16F10-OVA tumor-bearing mice.

**Results** *In vitro* chronic activation of OT-I cells generated Tpex and Tex-like cells. Tpex expressed higher levels of CD73 than Tex in these cultures. In agreement, intratumoral Tpex had higher expression of CD73 compared to Tex. *In vitro* chronic activation of OT-I cells in the presence of APCP did not affect T cell exhaustion, but the frequency and number of *in vivo*-generated Tpex from CD73KO cells was higher than their WT counterpart in B16F10-OVA tumor-bearing mice. In line with this, we observed a reduced frequency of Tex differentiated from CD73KO cells compared to WT cells. In contrast, while the frequency of Tex cells did not change in the presence of SCH58261, NECA and CGS-21680 reduced Tex and exhausted markers and increased stemness markers like CD62L and CD73, compared to vehicle control. Moreover, TCF1 expression decreased while TOX and CD39 increased in tumor-bearing mice treated with SYN115 related to the control.
**Conclusion** While CD73 restricts the establishment of Tpex *in vivo*, adenosine has the opposite effect, reducing Tex differentiation and promoting Tpex features, suggesting different roles for CD73 and adenosine in CD8+ T cell exhaustion.

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Keywords: CD8+ T cells, Exhaustion, CD73, Adenosine

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INDICE

A Escobar ..... 60
A Fernández ..... 60
A Hoare ..... 60
Abel Vásquez ..... 14, 19, 20
Adnane Achour ..... 9
Adrian Moreno Vilches ..... 59
Agustín Vera ..... 49
Alberto Martin ..... 36, 50, 63
Alejandra Fernández ..... 14, 57
Alejandra Herrera ..... 22
Alejandra Rivera ..... 14
Alejandra Zazueta ..... 33
**Alexandra Olate-Briones** ..... 25, 36
Alexis M. Kalergis ..... 23
Alicia Colombo ..... 45
**Alison Sepúlveda Pontigo** ..... 25
Almendra Benavides ..... 40, 41
**Alonso Lira** ..... 12, 26, 56
Álvaro Lladser ..... 9, 50, 53
**Amada Arcaya** ..... 26
**Amarilis Perez** ..... 19, 61
Ana María Sandino ..... 42
Ana María Vega-Letter ..... 24, 33
Andrea Cerda Celis ..... 39, 46
**Andrea Oyarzún Cisterna** ..... 41, 42
Andrés Caicedo ..... 13
Andrés Hernández-Oliveras ..... 50, 53
Andrés Herrada ..... 23, 25, 36
Andrés Tittarelli ..... 9, 19, 51
Angel Oñate ..... 49
Angélica Bravo ..... 16, 54
Angello Retamal Díaz ..... 16, 54
Anna Bergqvist ..... 51
Antonella Sanguineti ..... 17, 62
Antonia Domínguez ..... 17, 62
Antonio Serrano ..... 30
**Araceli Pinto** ..... 26, 33, 47
Augusto Manubens ..... 20
**Bárbara Antilef Cáceres** ..... 13, 45
Barbara Bruna ..... 28
**Bayron Cortés** ..... 27, 39
Belén Flores ..... 22
Benjamín Campusano ..... 33
Boris Riveros Rodriguez ..... 14
**Brian Parra-Tello** ..... 48
Camélia Radulescu ..... 53
Camila Covián ..... 48
Camila Fuentes ..... 61
Camila Kossack ..... 41, 42
Camila Muñoz Grez ..... 13, 49
Camila Pinto ..... 15, 27, 28, 34
Camilo Cabrera ..... 13, 45
**Camilo Venegas** ..... 28
Camilo Villaman ..... 36, 63
Carla Díaz ..... 30, 35, 49, 52
Carlos Barrera ..... 32, 42, 43
Carlos Bustamante ..... 16, 54
Carlos Guevara ..... 48
Carlos Mateluna ..... 42
Carmen Campos ..... 51
Carol San Martin ..... 28
Carolina Manosalva ..... 10
Carolina Otero Acuña ..... 59
Carolina Pradenas ..... 35
Carolina Prado ..... 46
Carolina Ribeiro ..... 30, 35, 49, 52
Carolina Rivera ..... 15
**Carolina Schafer** ..... 42
Caroll J. Beltrán ..... 26, 33, 47
Catalina Asencio ..... 35
Catalina Jimenez-Hernandez ..... 39, 43
Catalina Moreno ..... 47
Catalina Sepúlveda ..... 45
Cecilia Canales ..... 14
Chris Gabriela Quinteros ..... 22
Christine Sedlik ..... 53
Claudia Altamirano ..... 49, 52
Claudia Cortes ..... 14
Claudia Durán-Aniotz ..... 9
Claudia Quezada ..... 51
Claudia Reinoso ..... 38
Claudio Acuña ..... 32, 42, 43
Claudio Henríquez ..... 27, 38, 39
Constanza Aros ..... 35
Constanza Vilchez ..... 48
Constanza Zurita ..... 19
**Consuelo Merino González** ..... 61
Consuelo Santibañez ..... 17, 62
Cristian Carrasco ..... 51
Cristian Pereda ..... 9, 19, 51
**Cristina Padilla** ..... 28
Cristóbal Madrid Muñoz ..... 25
Cristóbal Villalón ..... 34
Cristopher Blamey ..... 42
D Bravo ..... 60
Danette Guiñez Francois ..... 33
Daniel Escobar ..... 14, 19, 20
Daniel Soto ..... 14, 20
Daniel Uribe ..... 51
Daniela Cárdenas ..... 19
Daniela Castillo ..... 14, 22
Daniela Sauma ..... 11, 17, 22, 31, 36, 53, 62, 63
Daniela Tapia-Molina ..... 35
Daniella Carreta ..... 38

Dante Miranda ..... 34, 37
**David González-Quintanilla** ..... 57
Diego Díaz Dinamarca ..... 14, 19, 20
**Diego Figueroa** ..... 50, 53
**Diego Irribarra** ..... 17, 62
Douglas J. Matthies ..... 30, 35, 42, 49, 52
Eduardo Martinez ..... 50
Eduardo Roa ..... 53
Eduardo Silva ..... 31
Eliana Lara-Barba ..... 24, 35
Eliane Piaggio ..... 53
Elisa Balboa ..... 26
Elizabeth Pellegrini ..... 14, 57, 60
Emilia Escalona ..... 25
Emilia Morell ..... 27, 34
Emilio Seguel ..... 22
Erick Riquelme ..... 50
Erik Riquelme ..... 49
Ernesto López ..... 53
Estefanía Nova-Lamperti ..... 13, 14, 45, 49
Eva Vallejos Vidal ..... 15, 39, 43, 46
Evy Sanders ..... 51
Fabian Salazar ..... 20
Fabián Tempio ..... 9, 28, 52, 61
Fabien Magne ..... 33
**Fabiola González-Herrera** ..... 30, 35, 49, 52
Fabiola Osorio ..... 12, 26, 32, 44, 50, 56
Farida Djouad ..... 35
Farides Saavedra ..... 53
Felipe Ardiles ..... 50
**Felipe Barraza** ..... 38, 40, 41
**Felipe Bravo** ..... 31, 41, 42
Felipe Bustamante-Barrientos ..... 35
Felipe E. Reyes-López ..... 15, 43
Felipe Flores ..... 35, 50
Felipe Galvez-Cancino ..... 50
**Felipe Malgue** ..... 31, 36, 63
Felipe R. Uribe ..... 23
Felipe Reyes Lopéz ..... 39, 46
Felipe Zuñiga ..... 13, 14, 49
**Fermín E. González** ..... 9, 19
Fernanda Jeria ..... 33
Flavio Salazar-Onfray ..... 9, 28, 51, 61
Florencia Vicencio ..... 22
Francisca Cortés ..... 35, 49
Francisca Espínola ..... 32
Francisca Espinoza ..... 13
Francisca Rodríguez-Arriaza ..... 25, 36
Francisca Troncoso-Reyes ..... 35
**Francisco Bravo** ..... 43
Francisco Fuentes ..... 42
Francisco Kirhman ..... 28
Francisco Parra ..... 25
Gabriel Morán ..... 38
Gemma Navarro ..... 46
Gisselle Barra ..... 14, 19
Glauben Landskron ..... 17, 62
Heriberto Garcia-Escorza ..... 14
Ignacio Jeréz ..... 27
Ignacio Niechi ..... 51
Isabel Méndez Pérez ..... 22, 25
Iu Raïch ..... 46
Iván Flores ..... 9, 19, 51
**Ivania Valdes** ..... 50
Ivo Campos ..... 30, 35, 49, 52
**J Lucero** ..... 60
**Jacob Mora** ..... 46
Jaime Poniachik ..... 26
Jan Nevermann ..... 25
Janepsy Díaz ..... 14, 19, 20
Javier Enrione ..... 28
**Javier Lopez** ..... 32
**Javier Mena** ..... 32, 42
Javiera Alzaga ..... 26, 47
**Javiera de Solminihac** ..... 15, 27, 28, 34
**Javiera Favi Coloma** ..... 33
Javiera Reyes-Álvarez ..... 53
Jimena Tosello-Boari ..... 53
**Joaquin Espinoza** ..... 47
Johanna Cabrera ..... 42
John Quiroga ..... 10, 27, 38, 39
Jonathan Morales-Reyes ..... 35
Jonnathan Grossolli Galvez ..... 14
Jorge A. Soto ..... 23
Jorge Vergara ..... 51
José Corrales ..... 31, 36, 63
José Ignacio Bernales ..... 26
José Luis Garrido ..... 31, 41, 42
Jose Miguel Pacheco ..... 13
Jose Rodríguez ..... 49, 52
Juan A Fuentes ..... 25
Juan P. Miranda ..... 9
Juan Pablo Vega ..... 50
Juan Saavedra Almarza ..... 31, 36, 63
Julio César Cardenas ..... 31
Julio Santelises ..... 14
**Justine Castañeda** ..... 11, 22
Karen Dubois-Camacho ..... 17, 62
Karen Toledo-Stuardo ..... 30, 35, 49, 51, 52
Karin Jiménez ..... 48
Karina Pino-Lagos ..... 15, 27, 28, 34
Karina Reichel-Fuentes ..... 15, 39
**Karissa Chavez Villacreses** ..... 22, 25
Katina Schinnerling ..... 22, 25
Kevin Maisey ..... 38, 40, 43
L Yañez ..... 60
Larissa Aleman Franco ..... 33
Laura Carreño ..... 33
Leonardo Vargas ..... 22
Liliana Lamperti ..... 13, 14

Lizbeth Huaccha ..... 40
Loredana Arata Reyes ..... 14
Loreto Abusleme ..... 47
Luciano Ferrada ..... 13, 49
Luis Alarcón ..... 53
Luis Ardiles ..... 14
Luis González ..... 48
Lukas Restelli ..... 47
M Bordagaray ..... 60
M Reyes ..... 60
Mabel Vidal ..... 15, 46, 49, 51
**Maksimiano Rodríguez** ..... 39
Mar Valés-Gómez ..... 51
Marcela Gatica ..... 30
Marcela Hermoso ..... 17, 62
Marcela Hernández ..... 14, 60
Marcelo Rodriguez ..... 47
Margarita Lay ..... 16, 54
Margarita Montoya ..... 34, 37
María A. Gleisner ..... 9, 19, 28, 51
María Carmen Molina ..... 30, 35, 49, 51, 52
María Inés Barría ..... 31, 41, 42, 45
Maria Ines Becker ..... 20
Maria Isabel Behrens ..... 28
María J. Santillán-Araneda ..... 15, 39, 43, 46
**María Jesús Araya** ..... 33, 35
**María José Bordagaray** ..... 14, 57
María José Garrido ..... 30, 35, 49, 52
María José Moya-Guzmán ..... 15, 27, 34
**María José Vásquez Vidal** ..... 44
María Julieta Gonzalez ..... 12, 56
Maria Luisa Rioseco ..... 42
María Rosa Bono ..... 11, 22, 31, 36, 63
Maria Teresa Valenzuela ..... 14
Mario Abedrapo ..... 17, 62
Mario Calvo Arellano ..... 42
Mario Rosemblatt ..... 11, 22, 31, 36, 63
Marion Arce ..... 47
Marjorie De la Fuente ..... 17, 62
Maroun Khoury ..... 21, 35
Matías Medina ..... 42
Mauricio Bittner ..... 22
Mauricio Garrido ..... 14, 57
Mauricio González-Olivares ..... 30, 35, 49, 52
Mauricio Hernández ..... 13
Mayarling F Troncoso ..... 20
**Merari Goldstein** ..... 15, 39, 46
Mercedes N. López ..... 9, 28, 61
**Michal Díaz Samirin** ..... 20
Michelle Salazar ..... 20
Milovan Acevedo ..... 49
Milton Larrondo ..... 9
Mónica Guzmán ..... 44
Mónica Imarai ..... 38, 40, 41, 43
**Muguete Cisneros** ..... 34
Mychel Urra ..... 14
Nancy Vasquez ..... 22
Natalia Santis ..... 19
Natalia Valdes ..... 40
Nicolás Dutzan ..... 47
Nicolás Fehring ..... 30, 35, 49, 52
Nicolas Muena ..... 19
Nicolás Valdivieso ..... 11, 22
Nicole D Tischler ..... 19
Nicole Olate Sanchez ..... 41
**Nicole Ossandon** ..... 13
Noelia Escobedo ..... 23, 25, 36
Noymar Luque-Campos ..... 24, 35
Omar Barría ..... 51
Pablo Alarcón ..... 10, 38
Pablo Castro-Córdova ..... 21
**Pablo Díaz** ..... 14, 19, 20
Patricia Hernández ..... 57
Patricia Luz-Crawford ..... 13, 24, 33, 35
Paulina Troncoso ..... 43
Pia M. Vidal ..... 13
**Rafael A. Burgos** ..... 10, 38
Rafael Franco ..... 46
Rafael Zanabria ..... 26
Raul O. Riquelme Oyarzun ..... 42
Raymond Alvarez ..... 31, 41, 42
Renato Esteban Ocampo Avello ..... 42
Ricardo Soto Rifo ..... 44
**Richard Olivares Olivares** ..... 16, 54
Rocio Cofré ..... 17, 62
Rodrigo Naves ..... 48
Rodrigo Pacheco ..... 46
Rodrigo Puentes ..... 14, 19
**Rodrigo Zañartu** ..... 39
Romina Falcón ..... 19
Romina Godoy ..... 47
Romina Quiroga ..... 13, 45
**Roque Scarpa** ..... 40
Rosana Benitez ..... 14
Ruth Montero Meza ..... 41
Sabina Rojas ..... 25
**Said Alamos Musre** ..... 59
Samantha Tello ..... 49, 52
Sarah Nuñez ..... 11, 22
Sebastián A. Echeverria ..... 23
Sebastian Barria Carcamo ..... 42
Sebastián Irarrázaval ..... 22
**Sebastián Valdés-Cabrera** ..... 21
Sergio Hernández ..... 53
Sergio Lavandero ..... 20
Sergio Sanhueza Novoa ..... 13, 45, 49
Silvana Guerra-Arredondo ..... 39
Silvia López-Borrego ..... 51
Sofía Albornoz-Muñoz ..... 25, 36
Sofia Hidalgo ..... 50, 53

Solange Cisterna ..... 13
Solange Gouet Moya ..... 31, 36, 63
Sophia Mejias ..... 34, 37
Stefanie Teuber ..... 38
Tamara Rojas ..... 49
Thilo Kaehne ..... 15
Thomas Villalobos ..... 57
Tomás Hernández ..... 44
Úrsula Ponce ..... 38
Valentina Olmedo ..... 47
**Valentina P. I. González** ..... 23
**Valentina Rodríguez-Vergara** ..... 23, 36
Valentina Ugalde ..... 46
Valeria Vasques-Saez ..... 14
Valeska Ormazabal ..... 14
Valeska Simon ..... 11, 22
Veronica Fuentes ..... 17, 62
Veronica Torres ..... 47
Vicente Flores ..... 49
Victor Cortes ..... 17, 62
Vincenzo Borgna ..... 50, 53
Wilfredo González ..... 13, 49
Wilfrid Richer ..... 53
Yazmin Pinos ..... 42
Yeimi Herrera-Luna ..... 24
**Yesenia Flores-Elías** ..... 24
Yessia Hidalgo ..... 35
Yoann Missolo-Koussou ..... 53
Yuneisy Guerra ..... 35
Yves Allory ..... 53

## SPONSORS