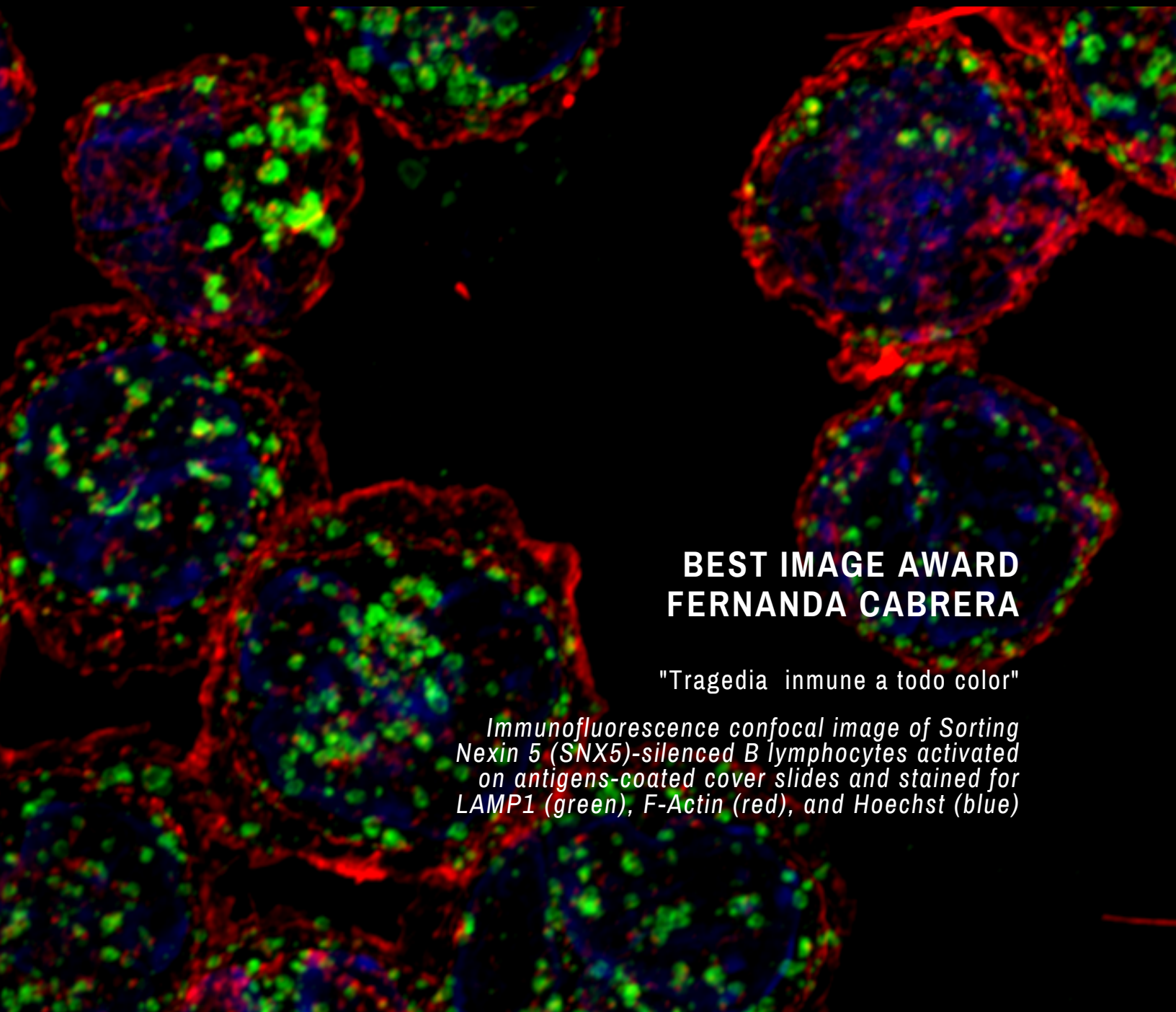
ABSTRACT BOOK

ASOCHIN

5TH ANNUAL MEETING

LAS MAJADAS DE PIRQUE/NOVEMBER 20-21/2022



BEST IMAGE AWARD
FERNANDA CABRERA

"Tragedia inmune a todo color"

Immunofluorescence confocal image of Sorting Nexin 5 (SNX5)-silenced B lymphocytes activated on antigens-coated cover slides and stained for LAMP1 (green), F-Actin (red), and Hoechst (blue)

Agradecemos a todos quienes hicieron de nuestra Reunión Anual una instancia de reencuentro, camaradería y ciencia en tormo a la Inmunología. Una mención especial a nuestros auspiciadores cuyo apoyo fue crucial para llevar a cabo esta misión de juntarnos para compartir y disfrutar de nuestra disciplina querida, la Inmunología.

Nuestra reunión fue todo un éxito y contó con 145 asistentes, 6 simposios y 16 expositores. Además presenciamos una exposición de ciencia y arte en tiempo real a cargo de Infomurales Científicos. Todo esto rodeados del hermoso escenario que nos ofreció el personal, hotel y palacio de Las Majadas de Pirque.

Adjudicamos dos becas de asistencia a alumnos de la Universidad de Chile y de la Universidad Austral de Chile. Asimismo, premiamos a seis alumnos en categoría de presentación de póster y un alumno en la categoría de mejor short talk. Fernanda Cabrera fue la acreedora de la mejor imagen de Inmunología con su fotografía titulada "Tragedia Inmune a Todo Color", la cual decora este libro de Abstracts.

Finalmente, instauramos el Premio a la Trayectoria en Inmunología, el cual fue otorgado a la Dra. María Rosa Bono por su compromiso con la formación de estudiantes y con su constante promoción de la Inmunología en Chile.

Esperamos que para ustedes esta haya sido una memorable instancia que podamos repetir en el futuro.

Directiva Asochin

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FINAL PROGRAM

NOVEMBER 20

8:30 – 10:00 h: REGISTRATION

10:00 – 10:30 h: OPENING CEREMONY Dr. Daniela Sauma

10:30 – 11:30 h: PLENARY LECTURE: Single cell dynamics: from cell biology to immune therapies. Dr. Pablo Vargas. *Sponsored by Pfizer.*
Chair: Dr. Daniela Sauma.

11:30 – 13:00 h: SYMPOSIUM 1: NEUROIMMUNOLOGY
Chairs: Dr. Diego Catalán/Dr. Caroll Beltrán.

11:30 – 12:00 h: Impaired GPR43 signaling on colonic intraepithelial lymphocytes promotes a pro-inflammatory environment in gut mucosa during CNS autoimmunity. Dr. Carolina Prado.

12:00 – 12:30 h: The collaboration of dopaminergic and chemokinergic signaling in T-cells controls the gut homing upon inflammation. Dr. Rodrigo Pacheco.

12:30 – 13:00 h: Beyond Haemostasis - Role of Circulating Platelets During Myelin Repair in Multiple Sclerosis. Dr. Francisco J. Rivera.

13:00 – 14:00 h: LUNCH

14:00 – 15:30 h: SYMPOSIUM 2: TUMOR IMMUNOLOGY
Chairs: Dr. Fermín González/ Dr. Glauben Landkrom.

14:00 – 14:30 h: Crosspriming models for the activation of antitumor immune response. Dr. Claudio Acuña.

14:30 – 15:00 h: Unveiling the cross-talk between transferred and endogenous CD8+ T cells underlying effective antitumor immunity in models of adoptive cell therapy. Dr. Álvaro Lladser.

15:00 – 15:30 h: From T cells with regulatory properties to CD49b as potential target for cancer therapy. Dr. Karina Pino-Lagos.

15:30 – 16:00 h: COFFEE BREAK

16:00 – 17:30 h: SYMPOSIUM 3: MUCOSAL IMMUNOLOGY
Chairs: Dr. Marcela Hernández/Dr. Eduardo Villablanca.

16:00 – 16:30 h: Intestinal immune response, one decision, and two roads: oral tolerance or inflammation. Dr. Carmen Gloria Feijoo.

16:30 – 17:00 h: The immune response as a therapeutic target to control tooth loss. Dr. Rolando Vernal.

17:00 – 17:30 h: The cross-talk between periodontitis and gestational diabetes mellitus. Dr. Alejandra Chaparro.

17:30 – 18:30 h: POSTER PRESENTATIONS

18:30 – 19:30 h: PLENARY LECTURE: Positioning mucosal immune cells and their function in anatomical context. Dr. Eduardo Villablanca.
Chair: Dr. María Rosa Bono.

19:30 – 21:30 h: DINNER

NOVEMBER 21

8:30 – 9:00 h: SYMPOSIUM 4: NEW ASOCHIN MEMBERS PRESENTATIONS
Chairs: Dr. Álvaro Lladser/Dr. Claudio Pérez.

8:30 – 8:45 h: Innate immunity patterns for precision medicine: MicrobeSkin and Skin diseases.
Dr. Carolina Cabalín.

8:45 – 9:00 h: Biomarkers in uveitis.
Dr. Cristhian Urzúa.

9:00 – 9:30 h: SHORT TALKS PRESENTATIONS
Chairs: Dr. Francisco J. Rivera/ Dr. Carmen Gloria Feijoo.

9:00 – 9:15 h: Absence of RAMP3 dysregulates MAIT cell response.
Eleni Phung.

9:15 – 9:30 h: Natural Killer T cells promote antibody class-switch recombination towards IgG subtypes in innate B cells that respond against T-Independent antigens. Pablo A. Palacios.

9:30 – 9:45 h: Metabolism governs the immunomodulatory effect of umbilical-cord derived mesenchymal stem/stromal cells on T-Cells from rheumatoid arthritis patients.
Noymar Luque-Campos.

9:45 – 10:30 h: PLENARY LECTURE: LNP-mRNA platforms: From mRNA-based vaccines to *in vivo* targeting of hematopoietic stem cells. Dr. Rodrigo Mora.
Chair: Dr. Mario Rosemblatt.

10:30 – 11:00 h: COFFEE BREAK

11:00 – 11:15 h: GENEX PRESENTATION

11:15 – 12:00 h: PLENARY LECTURE: Biomedicine, immunology, and vaccines as tools for a new development strategy. Dr. Flavio Salazar.
Chair: Dr. Jennifer Alfaro.

12:00 – 13:00 h: SYMPOSIUM 5: APPLIED IMMUNOLOGY AND VACCINES
Chair: Dr. Flavio Salazar.

12:00 – 12:30 h: Mining humoral immunity as approach to manage Andes hantavirus infection.
Dr. María Inés Barría.

12:30 – 13:00 h: Alpaca-derived nanobody against viral infections. Dr. Naphak Modhiran.

13:00 – 14:00 h: LUNCH

14:00 – 15:30 h: SYMPOSIUM 6: INFLAMMAGING AND AUTOIMMUNITY
Chairs: Dr. Karen Dubois/Dr. Sarah Núñez.

14:00 – 14:30 h: Resolvins in inflammation and cardiac fibrosis.
Dr. Guillermo Díaz.

14:30 – 15:00 h: The link between microbiota and autoimmune inflammation in rheumatoid arthritis. Dr. Katina Schinnerling.

15:00 – 15:30 h: What we have learned from a mouse model of lupus?
Dr. María Rosa Bono.

15:30 – 16:00 h: COFFEE BREAK

16:00 – 17:30 h: SHORT TALKS PRESENTATIONS
Chairs: Dr. Rodrigo Pacheco/Dr. María Isabel Yuseff.

16:00 – 16:15 h: How does the cGAS/STING pathway affect the aging brain?
Daniel Fernández.

16:15 – 16:30 h: Ferritinophagy-mediated ferroptosis drives clinical progression of periodontitis. Alfredo Torres Pérez.

16:30 – 16:45 h: Tubulin acetylation as a mechanosensory target for B cell activation.
Felipe Del Valle.

16:45 – 17:00 h: 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 Valdés.

17:00 – 17:15 h: Renal cell carcinoma tumors are infiltrated by CD4+ T cells with antitumor activity that recognize neoantigens. Farides Saavedra.

17:15 – 17:30 h: STAT3 activation in gingival tissues during health and periodontitis.
Marion Arce.

17:30 – 18:30 h: POSTER PRESENTATIONS

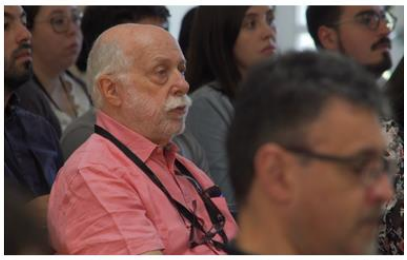
18:30 – 18:45 h: BD PRESENTATION

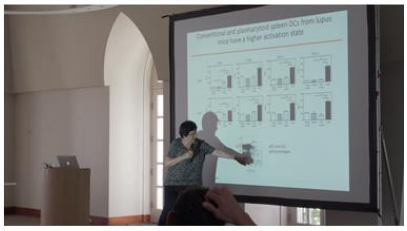
18:45 – 19:30 h: PLENARY LECTURE: Regulation of dendritic cell function by the Unfolded Protein Response. Dr. Fabiola Osorio.
Chair: Dr. Daniela Sauma.

19:30 – 20:00 h: CLOSING CEREMONY

TALKS AND SHORT TALKS






Impaired GPR43 signaling on colonic intraepithelial lymphocytes promotes a pro-inflammatory environment in gut mucosa during CNS autoimmunity.

Carolina Prado[1,2], Rodrigo Pacheco[1,2]

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2.- Universidad San Sebastián, Facultad de Medicina y Ciencia, Providencia, 7510156, Santiago, Chile

Gut microbiota plays a critical role in the regulation of immune homeostasis. Accordingly, several autoimmune disorders have been associated with a dysbiosis in the composition of the gut microbiota. Importantly, the dysbiosis associated to central nervous system (CNS) autoimmunity involves a substantial reduction of bacteria producers of short-chain fatty acids (SCFA). Here we addressed the role of the surface receptor mediated effects of SCFA on mucosal T-cells in the development of CNS autoimmunity. Our results show a sharp and selective reduction of intestinal propionate at the peak of experimental autoimmune encephalomyelitis (EAE) development, which was accompanied by increased IFN-g and decreased IL-22 in the colonic mucosa. Further analyses indicated that GPR43 was the main SCFA receptor expressed on T-cells, which was down-regulated on colonic TCRab$^+$ T-cells upon CNS autoimmunity. The pharmacologic stimulation of GPR43 increased the anti-inflammatory function and reduced the pro-inflammatory features in several TCRab$^+$ T-cell subsets in the colonic mucosa upon EAE development. Furthermore, GPR43 stimulation induced the arrest of CNS-autoreactive T-cells in the colonic lamina propria, thus avoiding their infiltration into the CNS and dampening the disease development. Mechanistic analyses revealed that GPR43-stimulation on mucosal TCRab$^+$ T-cells inhibits their CXCR3-mediated migration towards CXCL11, which is released from the CNS upon neuroinflammation. These findings provide a novel mechanism involved in the gut-brain axis by which bacterial-derived products secreted in the gut mucosa might control the CNS tropism of autoreactive T-cells. Moreover, this study shows GPR43 expressed on T-cells as a promising therapeutic target for CNS autoimmunity.

Funding: FONDECYT 11190251
Keywords: Shor-chain fatty acids, central nervous system, autoimmunity

The collaboration of dopaminergic and chemokinergic signalling in T-cells controls the gut homing upon inflammation

Rodrigo Pacheco[1,2], Francisco Osorio-Barrios[1], Valentina Ugalde[1], Gemma Navarro[3], Javier Campos[1], Carolina Prado[1,2], Iu Raïch[3], Francisco Contreras[1], Ernesto López[1], Alexandra Espinoza[1], Álvaro Lladser[1,2], Rafael Franco[4]

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Introduction. $CD4^+$ T-cells constitute central players in inflammatory bowel diseases (IBD), driving inflammation in the gut-mucosa. Current evidence indicates that CCR9 and the integrin α4β7 are necessary and sufficient to imprint colonic homing on $CD4^+$ T-cells upon inflammation. Interestingly, dopaminergic signalling has been previously involved in leukocyte migration. Despite dopamine levels are strongly reduced in the inflamed gut mucosa, the role of dopamine in the gut-homing of T-cells remains unknown. Here, we studied how the high-affinity dopamine receptors D_3 (DRD3) and DRD5 affect T-cell function and migration upon gut-inflammation.
Methods. Gut-inflammation was induced by transfer of naive T-cells into *Rag1*$^{-/-}$ mice or by administration of dextran sodium sulphate. T-cell migration and function were evaluated by adoptive transfer followed by flow-cytometry analysis. Protein-interaction was studied by Bioluminescence-Resonance-Energy-Transfer analysis, Bimolecular-Fluorescence-Complementation and *in situ* proximity-ligation-assays.
Results. We found the surface receptor providing colonic tropism to effector $CD4^+$ T-cells (Teff) upon inflammation is not CCR9 but the complex formed by CCR9 and DRD5. Assembly of the heteromeric complex was demonstrated *in vitro* and *in vivo* using samples from mouse and human origin. Moreover, we found that DRD3-signalling in Treg attenuates IL-10 production and limits their acquisition of gut-tropism by regulating CCR9 expression. Accordingly, the *ex vivo* transduction of wild-type Treg with a siRNA for *Drd3* induced a potent therapeutic effect abolishing gut inflammation.
Conclusions: Our findings show a critical role of dopaminergic-signalling controlling the gut-tropism of T-cells. We also introduce a new cell-surface module in lymphocytes, which integrates the sensing of multiple molecular cues.

Funding: This work was supported by “Financiamiento Basal para Centros Científicos y Tecnológicos de Excelencia de ANID” Centro Ciencia & Vida, FB210008 (to Fundación Ciencia & Vida). The study was also supported by grants from FONDECYT (FONDECYT-1210013 and FONDECYT-1170093), and from Ferring Pharmaceuticals.
Keywords: CD4+ T cells; inflammatory bowel diseases; Dopamine

Absence of RAMP3 dysregulates MAIT cell response.

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Introduction: RAMP3 is a chaperone protein that modifies G-protein coupled receptors (GPCRs) including chemokine receptors, which are important in immune cell signaling and activation. Although the individual role of RAMP3 in immunity has yet to be explored, *Ramp3* transcripts are highly expressed in mucosal-associated invariant T (MAIT) cells, which are a specialized population of T cells that have characteristics of both innate and adaptive immunity.
Methodology: MAIT cells are activated through bacterial infections. An attenuated live vaccine strain of *Salmonella Typhimurium* (BRD509) is used to infect the lungs and expand MAIT cells, because it contains the riboflavin pathway produces the MAIT cell antigen 5-OP-RU. We analyzed *Ramp3* KO mice lungs through flow cytometry post-BRD509 infection.
Results: *Ramp3* KO mice have higher number of lung MAIT cells with higher levels of IL-17A production, but at the same time, with a higher percentage of apoptosis and lower proliferation. *Ramp3* KO mice also have an increased bacterial burden after vaccination.
Conclusions: RAMP3 is important for the regulation of MAIT cells after a bacterial infection.

Acknowledgements: This research was funded by NIH grant 5R01AI137230-04. We thank the LJI Flow Cytometry Facilities and Sequencing Facilities for technical assistance. NIH-funded equipment was supported by grants S10 RR027366 and S10 OD016262.
Keywords: Ramp3, MAIT

Natural Killer T cells promote antibody class-switch recombination towards IgG subtypes in innate B cells that respond against T-Independent antigens

Pablo A. Palacios[1,2], Francisco F. Otero[1,2], Álvaro Santibáñez[1,2], Cristián Gutiérrez-Vera[1,2], Richard García-Betancourt[1,2], Leandro J. Carreño[1,2]

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Innate B cells stand out for providing protection against T-independent (TI) antigens such as capsular polysaccharides derived from bacteria mainly through IgM antibodies, in a process that doesn't depend on the collaboration with T cells. Interestingly, it has been shown that innate immune cells such as Natural Killer T (NKT) cells are capable of secreting factors and cytokines that promote their differentiation into plasma cells, as well as antibody class switch recombination (CSR). The effector phenotypes of NKT cells are classified according to the cytokines they produced, such as IFN-γ (NKT1), IL-4 (NKT2), IL-17 (NKT17). α-Galactosylceramide (αGC), the reference ligand of NKT cells, and their structural analogues AH10-7 and OCH induce the activation of different subsets of NKT cells, however, the effects of this differential activation of NKT cells on the antibody response against TI-antigens has not yet been evaluated. Here, we evaluated the response against Phosphorylcholine or Ficoll, two different TI antigens, admixed with the ligands of NKT cells and administered intraperitoneally in mice. 7 days after immunization, serum and splenocytes samples were obtained to evaluate the presence of antigen-specific antibodies by ELISA, and to evaluate the expression of different subtypes of IgG in innate B cells and plasma cells trough flow cytometry. αGC and AH10-7 induce the production of IgG1 and IgG3 specific for the antigens, which correlated with the presence of innate B cells and plasma cells expressing high levels of IgG1 and IgG3. This work supports the use of glycolipid as adjuvants against T-independent antigens.

Funding: FONDECYT 1211959, Iniciativa Científica Milenio – ICN09_016: Instituto Milenio en Inmunología e Inmunoterapia (ICN09_016/ ICN 2021_045), FONDEF ID21|10335, ANID-PFCHA/Doctorado Nacional/2021-21211655.
Keywords: NKT cells, Innate B cells, Class-switch recombination

Metabolism governs the immunomodulatory effect of Umbilical-cord derived Mesenchymal stem/stromal cells on T-Cells from Rheumatoid Arthritis patients

Noymar Luque-Campos[1,2], Carolina Pradenas[1,2], Roberto Elizondo-Vega[3], Ana María Vega-Letter[1,2], Yessia Hidalgo-Fadic[2], Farida Djouad[4], Patricia Luz-Crawford[1,2]

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4.- INSERM U1183-Universite de Montpellier, Montpellier, France.

Rheumatoid arthritis (RA) is an autoimmune disease, resulting in inflammation and tissue damage in the joints. The abnormal infiltration and activation of T-CD4 cells are critical for RA development and progression. In this context, Mesenchymal Stem/Stromal Cells (MSC) exhibit broad immunosuppressive abilities on T cells. However, MSC-based clinical trials results are controversial, being critical finding an appropriate stimulus to improve their immunosuppressive properties. Recently, the role of metabolism has emerged as a key regulator of MSC therapeutic capacity. Thus, in this work we evaluated the role of metabolism on MSC immunosuppressive capacities over RA T cells and the mechanism involved in this effect. For that purpose, MSC were metabolically reprogrammed into a glycolytic metabolism (MSC-Glyco) to evaluate their effect, on activated PBMC from RA patients using an *in vitro* model of co-culture or MSCs conditioned medium. The proliferation, activation and pro- or anti-inflammatory phenotypes were evaluated by flow cytometry. Our results showed that MSC-Glyco significantly improves their capacity to inhibit inflammatory T-cells while inducing regulatory T cells with effective suppressive function. Also, they maintain a naïve phenotype and suppress RA memory T-CD4 cells. All these effects were depending on soluble factors. Altogether our data propose the metabolic reprogramming of MSC into glycolysis as an interesting therapeutic strategy to improve their immunosuppressive capacities for RA treatment.

Funding: This research was supported by ANID-Chile through FONDECYT Regular N°1211353; FONDEF-ID: 21110194 and IMPACT-FB 210024.
Keywords: mesenchymal stem cells, glycolytic metabolism, T cell

Therapeutic LNP-mRNA platforms: From vaccines to potential application in genetic diseases affecting hematopoietic stem cells

J. Rodrigo Mora[1]

(1) Moderna, Immunology, Cambridge, USA

Gene correction of hematopoietic stem cells (HSC) is a promising therapeutic approach for multiple disorders. Current methods, however, require HSC collection from patients, gene correction during ex vivo culture, and re-infusion of corrected HSC into patients conditioned with chemotherapeutic agents. These approaches are complex, and the conditioning creates toxicities. We show that a lipid nanoparticle (LNP) can deliver mRNA encoding a reporter or a gene editing protein to HSC, with one injection transfecting ~25% of mouse HSC, and repeated doses resulting in higher editing efficiencies. We also demonstrate LNP-driven in vivo mRNA delivery to HSC in non-human primates and humanized mice. These results demonstrate a translatable approach to deliver mRNA encoding therapeutic proteins, or gene correcting tools, to HSC that do not require cell culture or toxic conditioning.

Funding: Moderna
Keywords: Hematopoietic, mRNA, LNP

Resolvin-D1 in inflammation and cardiac fibrosis

Guillermo Antonio Díaz Araya[1]

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Aims: Cardiac fibrosis is the excessive deposition of extracellular matrix protein on the cardiac interstitium. Angiotensin-II has been closely linked to hypertension and cardiac remodeling (hypertrophy and fibrosis), by triggering a pro-inflammatory response. Activated fibroblasts are the central cellular effectors in cardiac fibrosis. Resolvin-D1 elicits potent anti-inflammatory and pro-resolving effects. In this study, we examine whether Resolvin-D1 ameliorates cardiac fibrosis Ang II-induced, and the reduction of pro-inflammatory response exerted by fibroblast.
Methods and results: Alzet® osmotic mini-pumps filled with Angiotensin-II were implanted in male C57BL/6J mice for 7 or 14 days. Resolvin-D1 was administered one day after the surgery and during the complete infusion period. Blood pressure, myocardial functional parameters, heart tissue, and plasma were studied. Fibroblast from adult Sprague Dawley rats was used to study mRNA and protein levels of MCP-1, IL-6, TNF-a, IL-10, ICAM-1 and VCAM-1; and adhesion of spleen mononuclear cells to Fibroblast after Angiotensin-II stimulation. In vivo studies showed that after 7 and 14 days, Resolvin-D1 reduced the cytokine plasma levels, ICAM-1 and VCAM-1, the increased neutrophil and macrophage infiltration, cardiac hypertrophy, interstitial and perivascular fibrosis, and hypertension induced by Angiotensin-II. In vitro studies showed that in Fibroblast Angiotensin-II increased IL-6, MCP-1, and TNF-a mRNA levels, but only increased IL-6 and MCP-1 protein levels and monocyte adhesion; and these effects were blocked by Resolvin-D1.
Conclusions: This study unveils novel cardioprotective effects of Resolvin-D1 in Angiotensin-II-induced cardiac remodeling by attenuating cardiac inflammation and CF pro-inflammatory effects.

Funding: FONDECYT 1210627
Keywords: Cardiac fibrosis, Resolvin-D1, Angiotensin II

How does the cGAS/STING pathway affect the aging brain?

Daniel Fernández[1], Jimena Zubieta[1], Camila Sanchez[1], Paola Murgas[1]

1.- Inflammaging Laboratory, Institute of Biochemistry and Microbiology, Faculty of Sciences, Universidad Austral de Chile

The cGAS/STING signaling pathway mediate the response to inflammatory processes by recognizing double-stranded DNA (dsDNA) in the cytosol. This dsDNA can come from pathogens and mutant dsDNA (nucleus and mitochondria). When the cGAS receptor recognizes dsDNA, a downstream signal activates the secretion of type I Interferons and proinflammatory cytokines by STING protein. cGAS/STING pathway is expressed in several immune cells, including microglia in the Central Nervous System and is activation has been involved in neurodegenerative diseases associated with aging.
To evaluate the effect of age in the microglia, we use C57BL6J (WT), cGASKO and STINGKO mice, young and old (3 and 24 month). We found, via western blot, decreased levels proteins for cGAS/STING during aging, in the brain of WT mice. Also, when observed by flow cytometry, there was a reduction in the M1 and M2 phenotype markers in microglia from both KO mice compared with WT. After analyzing with immunofluorescence assay, cryopreserve slides of the brain, we detected a reduction in the microglia in both KO mice at very early ages compared with WT mice. However, in primary culture, the microglia from both KO mice present higher levels of phagocytic ability compared to WT cells. By using Fluoro-Jade-C stain, we observed more neurodegeneration in both young KO mice versus WT, together with alterations in behavior in the memory/cognitive tests (Open Field, Novel object location and recognition).
Finally, or results suggest that the cGAS/STING signaling pathway is essential for the correct microglial functions and the protection against neurodegeneration during aging.

Funding: FONDECYT Project 11190258
Keywords: microglia, aging, cGAS-STING

Ferritinophagy-mediated ferroptosis drives clinical progression of periodontitis

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2.- University of Chile, Laboratory of Experimental Immunology & Cancer, Faculty of dentistry
3.- Karolinska Institute, Department of Medical Biochemistry and Biophysics, Division of Chemistry

Introduction: Periodontitis is one of the most common chronic-inflammatory disease in humans. It's triggered by dysbiotic biofilms eliciting a local response with immuno-inflammatory dysregulation driving a progressive and irreversible periodontal tissue destruction. Previous results have identified a specific profile of DAMPs-proteins during progression of periodontitis suggesting specific cell-death processes related to it.
Objective: To evaluate cell-death processes present in the human GCF during progression of periodontitis.
Methodology: Five patients with periodontitis were monitored weekly in their progression of periodontitis scored as clinical attachment loss (CAL), obtaining GCF samples in each session. Two groups were established: Progression (PG) and non-progression (NP), according to CAL differences during monitoring phase. Proteins were identified with high-throughput proteomic techniques with label free analysis to determine the relative protein abundance, and western-blot analysis were performed to validate results. Enrichment bioinformatic analyses were performed in String-DB, FeRRDB and Shiny GO environment.
Results and Discussion: Proteomic analysis of GCF identified 1504 proteins: 1502 and 1500 proteins in NP and PG, respectively. 48 proteins were exclusive in PG, while 52 were identified in NP. FTH1, a suppressor for ferroptosis were absent in PG while SNCA, a driver for ferroptosis, was exclusively found in PG. Western blot analysis confirmed FTH1 results, suggesting ferritinophagy. ANXA5, an apoptosis marker, and PADI4, a marker for netosis, were more abundant in NP ($P<0.05$). In PG, enrichments analysis showed ferroptosis activity, binding to metallic ions and lipid metabolism.
Conclusions: Ferroptosis is involved in periodontitis progression. This new finding may generate new diagnostic and therapeutic alternatives.

Funding: This study was supported by the Faculty of Dentistry, University of Chile (Grant FIOUCH 17-007 to FEG) and ANID support.
Keywords: Periodontitis, Ferroptosis, Proteomics

Tubulin acetylation as a mechanosensory target for B cell activation

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1.- Immune cell biology laboratory, Pontificia Universidad Católica de Chile, Chile
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To become fully activated, B cells must extract antigens from antigen-presenting cells (APCs) by forming an immune synapse (IS). The B Cell receptor (BCR) controls B cell activation by promoting cell signaling as well as the uptake and processing of antigens (Ags). The mechanism of Ag extraction can be tuned by the physical characteristics of the surface where antigens are found. B cells use mechanical forces to extract Ags from softer surfaces, whereas lysosome-mediated extraction is employed to extract antigens from stiffer substrates. However, the underlying mechanisms that couple mechanosensing in B cells to the mode of Ag extraction remain unknown.
Using Poly-Acrylamide gels with tunable stiffnesses coupled to BCR-ligands, we found that B cells interacting with stiffer substrates exhibited enhanced spreading responses, more actin foci at the synaptic membrane, and higher tubulin acetylation at the center of IS, where lysosomes preferentially localize. Lysosome motility was studied using live-cell microscopy, showing that cells seeded over stiffer substrates exhibited less motile lysosomes, displaying lower mean speed and displacement. When inhibiting HDAC6-mediated tubulin acetylation with SAHA, cells seeded over softer substrates exhibit a phenotype like those interacting with stiffer substrates, suggesting that mechanical signals elicit microtubule post-translational modifications in B cells.
Overall, our results show that B cells respond to mechanical cues by increasing tubulin acetylation, which modulates lysosome trafficking and most likely the capacity of B cells to extract and process immobilized antigens. These results highlight the importance of a molecular pathway that links antigen-extraction mechanisms with mechanical cues from APCs.

Funding: ANID PhD National funding #21191062ANID FONDECYT funding #1221128 Dirección de postgrado ciencias biológicas UC

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

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Lung cancer is the leading cause of death by cancer in Chile with a 5-years survival rate of 19.4% after the diagnosis. Research on the role of the tumor microbiome in cancer has demonstrated that the microbiome can affect cancer cells and modulate cancer immunosurveillance. Although, the underlying functional mechanisms have not been clearly defined. Here we describe how the tumor microbiota could modulate the host immune response to lung cancer. Using proteomics and 16S rRNA sequencing, we functionally characterized host factors and the microbiome of fresh frozen and formalin-fixed paraffin-embedded samples from tumor and non-tumoral adjacent tissue of lung adenocarcinoma. We found tumor proteins to be down-regulated such as T–cell surface glycoprotein CD8 alpha chain, proteins related to T cell activation, and up-expressed such as macrophage migration inhibition factor (MIF) and proteins related to the metabolism of cysteine. Additionally, we observed an intra-tumoral microbiome community enriched in Firmicutes (*Granulicatella* and *Clostridium Sensu stricto 1*) and Actinobacteria (*Micrococcus* and *Corynebacterium*). Our results suggest that MIF up-regulation can inhibit T cell activation by sequestration and depletion of the increased cysteine, necessary for T cell activation. Moreover, increased cysteine from host metabolism can contribute as a substrate for intra-tumoral microbial growth, composed mostly of opportunistic pathogens. We provide an overview of the potential interactions between tumor microbiome and host immunity and how their crosstalk could synergistically favor a local immunosuppressive microenvironment with a poor outcome for the patients.

Funding: Fondecyt 1191526
Keywords: tumor, cancer, microbiome

Human renal cell carcinoma tumors are infiltrated by cytotoxic CD4+ T cells that associate with improved survival

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Introduction: CD4+ T cells play a pivotal role in antitumor immunity through their differentiation into type-1 helper differentiation program. Moreover, increasing evidence indicates a direct cytotoxic function of CD4+ T cells in solid tumors. However, the role of CD4+ T cells in human RCC tumors remain unknown.
Methods: Freshly resected RCC tumors were analyzed by flow cytometry. Effector CD4+ T cells (CD4+CD8-CD45RO+CD25-) were co-cultured with autologous cells from RCC tumors and non-malignant renal tissue (NMRT). Then, OX40 and 4-1BB upregulation and cytokine secretion was measured. As control, HLA-II was blocked in target cells. Association between expression of a cytotoxic CD4+ T cell gene signature and patient survival was analyzed by Kaplan–Meier log-rank test.
Results: The majority of CD4+ T cells infiltrating RCC tumors display an effector phenotype, characterized by PD-1 expression and the ability to produce TNF-α and IFN-γ, consistent with a Th1 differentiation program. Interestingly, effector CD4+ T cells specifically recognize autologous RCC but not NMRT cells in co-culture assays, as measured by OX40 and 4-1BB up-regulation, as well as IFN-g and TNF-a secretion. Moreover, a significant proportion of effector CD4+ T cells displayed a cytotoxic phenotype characterized by expression of granzyme B, CX3CR1 and RUNX3. Strikingly, a cytotoxic CD4+ T cell gene signature associates with better survival in RCC patients.
Conclusions: Our data indicate that tumor-infiltrating effector CD4+ T cells recognize RCC tumor cells and contain a cytotoxic subpopulation that associates with improved survival in RCC patients.

Funding: Centro Ciencia & Vida FB210088, Fondecyt 1119087, Fondecyt 1212070, VRID_PDOC22/07 Universidad San Sebastián. *Co-leading authors.
Keywords: renal cell carcinoma, tumor infiltrating T cells, cytotoxic CD4+ T cells

STAT3 activation in gingival tissues during health and periodontitis

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Introduction STAT3 integrates and transduces the signaling of multiple pro-inflammatory cytokines associated with periodontitis. STAT3 expression on $CD4^+$ T cells is essential for Th17 cell differentiation in gingival tissues, drivers of periodontitis immunopathology. Little is known about the activation of STAT3 (pSTAT3) in other gingival tissue cells or in the early stages of periodontal tissue destruction. We aimed to characterize pSTAT3 in gingival tissues during health and the early stages of periodontitis.
Methods: Standardized gingival tissue biopsies were obtained from healthy and diseased subjects. pSTAT3(Y705) and cell markers (CD45, CD3, CD20, CD31, CD68, MPO, CK5, and Vimentin) were evaluated by immunofluorescence. Analyses were performed with Cellprofiler™ software. An established mice model of periodontitis was used to characterize pSTAT3 in the early stages of periodontitis. Gingival tissues were collected at 2 hours, 1, 3, 5, and 7 days after periodontitis induction. pSTAT3 was detected by Western blot. Bands were quantified, and GAPDH was used as load control.
Results: We detected increased proportions of pSTAT3-positive cells during periodontitis compared to health, explained by a growth in pSTAT3-positive cells from the epithelial and hematopoietic cells, particularly T cells. In mice, pSTAT3 was detected in all evaluated stages of periodontal tissue destruction.
Conclusions: STAT3 activation is higher in periodontitis than in health, explained by a growth in the proportions of pSTAT3-positive cells from the epithelial and T cell compartments. Activation of STAT3 is detected in the early stages of tissue destruction.

Funding: This work was funded by the Chilean Government through the FONDECYT program. Project for Initiation in Research # 11180389. ANID-Subdirección de Capital Humano/Doctorado Nacional/2022-21221003.
Keywords: STAT3, oral mucosa, periodontitis

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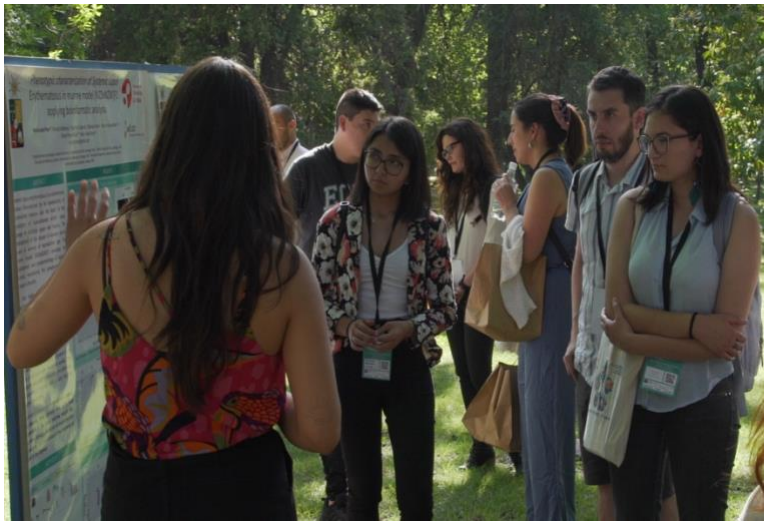

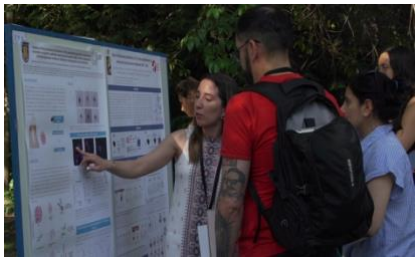


NEUROIMMUNOLOGY

The intranasal infection with human metapneumovirus enhances pro-inflammatory cytokine production in the brains of infected mice.

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Background: The human metapneumovirus (hMPV) is a principal viral agent that causes acute lower respiratory tract infections, mainly affecting pediatric and elderly populations. The symptoms commonly observed in hMPV-positive patients are bronchiolitis and pneumonia, but these patients can also present neurological manifestations, such as encephalitis. Interestingly, respiratory viruses can affect the brain, directly reaching the brain or indirectly through systemic inflammation. This work seeks to evaluate the effects of the hMPV-infection in the brain using a mice model. Methods: BALB/c mice were challenged intranasally with either hMPV (clinical isolate named CZ0107) or non-infectious control (mock). After 3-, 6-, and 14-days post-infection, blood, lung, and brain samples were collected for different evaluations. Results: Despite detecting viral load in the lungs of hMPV-infected mice, no viral load was detected in their brains. Next, a significant increase of pro-inflammatory cytokines was observed in hMPV-infected mice, suggesting a systemic inflammation in these mice. Additionally, it was observed that there was an increase in the relative expression of several cytokines in the brain. However, at protein levels, there is only an increase of pro-inflammatory cytokines in the brain. Conclusion: These results suggest that the infection with hMPV causes alterations in the brain, among which was increased cytokines in brain tissue. Furthermore, it can be suggested that these brain alterations might be caused by a systemic inflammation following an infection by a respiratory virus. Acknowledgment: This work was supported by ANID/CONICYT #21210662 (CAA), ANID/FONDECYT #11221280 (KB) and #1190830 (AMK), Millennium Institute on Immunology and Immunotherapy ICN09_016.

Funding: This work was supported by ANID/CONICYT #21210662 (CAA), ANID/FONDECYT #11221280 (KB) and #1190830 (AMK), Millennium Institute on Immunology and Immunotherapy ICN09_016.
Keywords: human metapneumovirus, neuroinflammation, central nervous system

Deciphering the role of the heteromer formed by dopamine receptors D2 and D3 on regulatory T-cells in gut inflammation

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Introduction. Inflammatory bowel diseases (IBD) involve a chronic inflammation of the gastrointestinal tract, which is driven mainly by effector $CD4^+$ T-cells (Teff). Conversely, regulatory T-cells (Treg) seems to be dysfunctional in IBD. Interestingly, dopamine levels are strongly reduced in the inflamed gut mucosa. the role of dopamine in the gut-homing of T-cells remains unknown. Accordingly, we recently found that the stimulation of the high-affinity dopamine receptor D3 (DRD3) in Treg attenuates their suppressive activity and limits their acquisition of gut-tropism. However, the role of the low-affinity dopamine receptor D2 (DRD2) in Treg remains poorly explored. Here, we studied 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 assessed by Bioluminescence-Resonance-Energy-Transfer analysis, Bimolecular-Fluorescence-Complementation and *in situ*proximity-ligation-assays.
Results. We found that *Drd2*-deficiency in Treg exacerbates colitis manifestation and impairs Treg suppressive activity and reduces their intestinal-tropism. Conversely, *Drd3*-deficiency in Treg improves the suppressive activity, increases the gut-tropism and protected from colitis manifestation. Biochemical analyses provided evidence that DRD2:DRD3 form an heteromeric complex in intestinal Treg and in heterologous systems.
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.

Funding: This work was supported by “Financiamiento Basal para Centros Científicos y Tecnológicos de Excelencia de ANID” Centro Ciencia & Vida, FB210008 (to Fundación Ciencia & Vida). The study was also supported by grants from FONDECYT (FONDECYT-1210013 and FONDECYT-1170093).
Keywords: Dopamine, regulatory T cells, inflammatory bowel diseases

Characterization of T helper lymphocyte profile of patients to study the inverse relationship between Alzheimer's Disease and Cancer

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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, where it may protect against the progression of disease, as well as worsen it. An inverse relationship between AD and cancer has recently been described however little is known about the T helper lymphocyte profile in these settings. We focused on the Flow-Cytometry analysis of the Th1, Th2 and Th17 frequency in samples from 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) patients with cancer history (Ca). Blood samples were obtained from patients and processed to collect Peripheral blood mononuclear cells that were stimulated and then stained with antibodies anti-CD3, CD4, CD8, CD16, IFN-γ, IL-4, IL-17 and analysed by flow cytometry (FACSVerse BD, FlowJo). We found that the Ca+AD group has a lower percentage of total CD4+ T cells and a higher percentage of CD8+ T cells compared to all groups. No significant differences were observed between the groups for the populations of Th1, Th2, Th17, although the percentage of Th1, Th17 and Tc1 (CD8+IFNg+) is lower for the Ca+AD groups probably as a protective effect of previous cancer. Together these results highlight the potential role of the T cell profile in the inverse relationship between AD and cancer.

Funding: IMII P09016F and Fondecyt 1190958
Keywords: Alzheimer's Disease, cancer, T helper

Interferon-Gamma Induces a Tolerogenic Phenotype In Bone Marrow-Derived Dendritic Cells Mediated By Indoleamine 2,3-Dioxygenase 1

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Introduction
Multiple Sclerosis (MS) is an autoimmune disease of the central nervous system. Our previous results have shown that interferon-gamma (IFN-g) suppresses experimental autoimmune encephalomyelitis (EAE), a mouse model of MS, by inducing a tolerogenic phenotype in antigen-presenting cells. Here, we assessed the *in vitro* effect of IFN-g on the differentiation and tolerogenic phenotype of murine bone marrow-derived dendritic cells (BMDCs).
Methodology
BMDCs precursors from mice were differentiated into dendritic cells (DCs) using GM-CSF (20 ng/ml) for 7 days. Lipopolysaccharide (LPS, 1mg/ml) was added during the last 24 h to obtain mature DCs (mDCs). Different concentrations of IFN-g were added starting from day 0, 2 or 4 of differentiation. Cell viability, DC yield, phenotypic profile, and expression of indoleamine 2,3-dioxygenase 1 (IDO-1) were determined by flow cytometry.
Results
The highest cell viability and DC yield were obtained with 50 ng/ml IFN-g added starting from day 2 of differentiation. IFN-g-DCs showed a tolerogenic phenotype characterized by significantly lower levels of CD80, CD86, and MHC-class II molecules than mDCs and higher levels of Programmed Death Ligand 1 (PD-L1) than untreated DCs (UN-DCs) and mDCs. The tolerogenic phenotype of IFN-g-DCs was stable after LPS stimulation. Preliminary results suggest that the tolerogenic effect of IFN-g on DC differentiation would be mediated by induction of indoleamine 2,3-dioxygenase 1 (IDO-1).
Conclusions
Our results suggest that IFN-g induces a tolerogenic phenotype in BMDCS mediated by induction of IDO-1. Further assays will be performed to determine the tolerogenic function of IFN-g-DCs.

Funding: Acknowledgement ANID/FONDECYT 1191874
Keywords: Interferon-gamma, Tolerogenic-DC, EAE

TUMOR IMMUNOLOGY

Artificial Mitochondria Transfer from Oral Cancer Cell Line HSC-3 Induces an Exhausted Phenotype in CD4+ T Cells

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Oral squamous cell carcinoma (OSCC) is the most frequent type of oral cancer and the OSCC tumour microenvironment (TME) induces impaired T cell responses, promoting an exhausted phenotype and metabolic reprogramming. The mitochondria is the main metabolic organelle and in recent years it has been shown that several cells have the capacity to transfer mitochondria, including cancer cells. However, to date, it has not been evaluated whether mitochondria transfer from cancer cells to T lymphocytes promotes an exhausted phenotype in T helper cells. The aim of this work was to analyse the exhausted phenotype in TCD4+ lymphocytes after artificial transfer of mitochondria (MitoCeption) obtained from the oral cancer cell line HSC-3. Our results showed that TCD4+ lymphocytes that acquired mitochondria had increased expression of 2 inhibitory proteins (TIGIT and CTLA-4) and 3 proteins associated with exhausted phenotype (PD-1, PLD-1 and LAG3), compared to the control group. In addition, the mitocepted lymphocytes exhibited a significant decrease in proliferation compared to control cells. For cytokine analysis, a significant decrease was observed in the mitocepted group for the secretion of IFN-g, TNF-a, IL-10 and IL-4, compared to control cells. In summary, the acquisition of isolated mitochondria from HSC-3 cancer cells by CD4+ T lymphocyte induces effects at the functional level by inducing an upregulation of inhibitory and exhausted phenotype and by inhibiting proliferation and the secretion of Th1 and Th2 cytokines.

Funding: Proyecto Fondecyt Regular 1211480
Keywords: Exhausted Phenotype, Mitochondrial Transfer, Oral Squamous Cell Carcinoma

Natural Killer cell-derived exosome mimetics as alternative nanodrug delivery system for multidrug-resistant lung cancer.

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Introduction: Lung cancer (LC) has the highest mortality rate worldwide. The pathogenesis is multifactorial and targeted therapies are currently recommended treatment; however, patients who don´t qualify for this therapy must resort to classic treatments (e.g. Chemotherapy), although it has limitations (e.g. side effects, chemo-resistance, etc.)
Exosome mimetics (EM), artificially generated vesicles with exosome properties, have been proposed as a potential tool to lower barriers for clinical translation. We propose a formulation of a chemotherapeutic encapsulated in EM generated from natural killer (NK) cells (EM-NK-C) establishing their cytotoxic effects on LC.
Methodology: EM-NK-C were generated by cell extrusion. Morphology was analyzed using atomic force microscopy. Quantification was performed by nanoparticle tracking analysis. Determination of NK and exosome markers were performed by western blot. The cytotoxic effect was determined by MTT in NCI-H1299 and NCI-H1975 cell lines.
Results: The mean size of NK exosomes (EXO-NK), EM and EM-NK-C were within exosome range (<200nm). The presence of all the markers analyzed in the EM and cell lysate was determined, in contrast to the EXO-NK, which didn´t present calnexin and GAPDH. For both cell lines a tendency of dose-response effect is observed when exposed to ratios of 1:1, 1:10 and 1:100 (vesicles:cells).
Conclusion: We established a methodology to generate EM and EM-NK-C. Although, so far, we haven´t been able to establish a cytotoxic effect, further experiments must be carried out to complement these results.

Acknowledgements: We would like to thank Dr. Margarita Montoya (Universidad de Santiago de Chile) for kindly donating NK cell line.
Funding: Beca de Doctorado Nacional, ANID. Convenio Beca UDD - Doctorado en Ciencias e Innovación en Medicina, Facultad de Medicina, Clínica Alemana – Universidad del Desarrollo. Proyecto Fondecy Regular N°1220803.
Keywords: Exo-NK (Exosome from Natural Killer); EM (Exosome Mimetics); Drug encapsulation

CCR5 and Pannexin-1 expression in colorectal cancer and their potential role in disease progression.

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CCR5 is a chemokine receptor showing increased expression in colon cancer, and when blocking promotes antitumor responses. Pannexin-1 is a hemichannel allowing passage of small molecules, such as ATP, essential in proliferation and migration. The activation of CCR5 induces the release of ATP through Pannexin-1 (Panx-1) in CD4+ T lymphocytes, however, a linkage between these two molecules has not been described in cancer. The objective of this study is to determine the CCR5 and Panx-1 expression levels and its relationship with colorectal cancer.
CCR5 and Panx-1 content were analyzed in tumor and healthy mucosa biopsies from CC patients by immunohistochemistry, correlating these molecules with tumor progression. Moreover, to evaluate CCR5 effect on ATP production, , we stimulated CCD481CoN cells with CCL3 in the absence/presence of pharmacological inhibitors of Panx-1 and CCR5 *in vitro*.
Preliminary results show a higher expression of CCR5 and Panexin-1 in tumor cells compared to the epithelium of healthy mucosa (n=27; Wilcoxon signed rank test $p<0.05$), without significant differences in the stroma (tumor vs. healthy tissue). The higher expression of CCR5 and Panx-1 are associated with advanced stages of colon cancer, with a correlation between CCR5 and Panx-1 (Spearman, $p<0.0001$). Furthermore, *in vitro* analysis suggests that activation of CCR5 induces ATP secretion in cell lines mediated by Panx-1 opening (n=3). Our results suggest that CCR5 and Pannexin-1 are related, as both express in tumor and stroma cells and participate in the CC progression.

Funding: Fondecyt iniciación 11190990
Keywords: colorectal cancer, CCR5, Panx-1

Use of HEK293 cells transfected with P2X7R to evaluate the cross-dressing mechanism.

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Antigen cross-dressing has recently gained importance in the induction of antitumor immune responses in vivo (MacNabb et al., 2022). We recently reported that the cross-dressing of apoptotic cells to dendritic cells requires the presence of P2X7R (Barrera et al., 2021). P2X7R has several properties that could be involved in a possible mechanism, such as: inducing cell fusion, triggering cell signaling on cytoskeletal rearrangement, and generation of exosomes. In addition, P2X7R has been described as an apoptotic cell scavenger receptor. In the present work, we seek to generate a model that allows determining the mechanism of cross-dressing mediated by P2X7. We used HEK293 cells that overexpress P2X7R wild type or with specific mutations that suppress certain receptor functions.
HEK293 cells were transfected with different versions of the P2X7 receptor and exposed to apoptotic bodies labeled with CellMask membrane marker fluorophore. Membrane transfer from apoptotic bodies to HEK293 cells was measured using confocal microscopy and flow cytometry. After stimulation with apoptotic bodies, HEK293 cells were evaluated concerning different parameters. These parameters included macropore activation, ethidium bromide incorporation, p38 pathway activation, and phagocytosis by flow cytometry.
To date, we have evaluated the transfection of HEK293 cells with the P2X7 receptor by ethidium bromide incorporation assays by flow cytometry. In addition, the preliminary results of cross-dressing both in confocal microscopy and flow cytometry allow distinguishing the specific membrane transfer to HEK293 cells that express the P2X7 receptor from apoptotic bodies with the presence of P2X7R.

Keywords: P2X7, Cross Dressing

A dendritic cell-mediated crosstalk between transferred and host CD8+ T cells underlies effective antitumor immunity elicited by adoptive cell therapy

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Adoptive cell therapy (ACT) using tumor-specific cytotoxic T lymphocytes (CTLs) has demonstrated great efficacy in hematological cancers. However, ACT does not work in most patients with solid tumors. Hence, understanding the cellular mechanisms underlying effective antitumor immunity in ACT models is key to develop improved cancer immunotherapies. Here, we observed that ACT using *in vitro* activated SIINFEKL(OTI)-specific CD8+ T cells led to total rejection of B16F10-OTI tumors and promoted intratumoral accumulation of both progenitor (PD-1+TCF-1+GzmB-) and differentiated cytotoxic (PD-1+TCF-1+GzmB+) host CD8+ T cells, as compared to untreated controls and mice treated with suboptimal ACT, which results in tumor relapse. Moreover, ACT efficacy was greatly reduced in mice lacking host T cells (RAGKO mice) or mice treated with FTY720, which prevents tumor infiltration of host T cells but not transferred CD8+ T cells. The efficacy of ACT was also decreased by depletion of host CD8+, but not CD4+ T cells. Furthermore, blocking the effector cytokine TNF-α reduced the infiltration of both progenitor and cytotoxic differentiated host, but not transferred CD8+ T cells and impaired ACT efficacy. Mechanistically, ACT promoted activation and migration to draining lymph nodes of tumor-infiltrating type 1 conventional dendritic cells (cDC1). These effects were abrogated by TNF-α blockade. Finally, selective depletion of cDC1 in lymph nodes using Langerin-DTR mice led to decreased efficacy of ACT. Our findings reveal an interplay between transferred and host CD8+ T cells, which underlies effective antitumor immunity in the context of ACT.

Funding: FONDECYT 1212070, Centro Ciencia & Vida FB210008 and PhD. Scholarship ANID 21180968
Keywords: Adoptive cell therapy, host CD8 T cells, cDC1

Nuanced role for dendritic cell intrinsic IRE1 RNase in the regulation of antitumor adaptive immunity

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Introduction: The IRE1/XBP1s axis plays divergent roles in myeloid/dendritic cell (DC) biology. Whereas IRE1/XBP1s activation at the tumor site curtails the function of macrophages and DC subsets, conventional type 1 DC (cDC1) homeostasis requires intact IRE1 RNase activity in steady state. As cDC1s are key inducers of antitumor $CD8^+$ T cell immunity, to elucidate the role of IRE1/XBP1s axis in tumor cDC1s is highly relevant.
Methods: B16 and MC38 tumor cell lines were inoculated subcutaneously into control animals, IRE1 RNase reporter mice (ERAI mice) or conditional knock-out mice lacking XBP1s or double-deficient animals lacking IRE1 RNase and XBP1s in DCs (CD11c-Cre) or cDC1s (XCR1-Cre).
Results: We show that cDC1s constitutively activate IRE1 RNase within B16/MC38 tumor models. Mice dually lacking IRE1 RNase and XBP1s in the DC compartment show normal tumor growth and normal effector T cell responses. In contrast, mice bearing single deletion of XBP1s in DCs display increased melanoma tumor growth and disbalanced effector/terminal exhausted $CD8^+$ T cells, indicating that IRE1 RNase in DCs fine tunes antitumor immunity independently of XBP1s. On transcriptomic level, XBP1 deficient tumor cDC1s decreased expression of mRNAs encoding XBP1s targets and downregulated IRE1 dependent decay (RIDD) substrates. However, selective ablation of IRE1/XBP1s or XBP1s in the cDC1 compartment was not sufficient to alter MC38 growth or T cell immunity.
Conclusion: These data highlight a nuanced role for IRE1 in DCs in tumor immunity, and based on these findings, we speculate that therapeutic targeting of IRE1/XBP1s axis in tumor cDCs will be rather limited.

Funding: FONDECYT #1161212, #1200793, HHMI #55008744, Beca CONICYT-PFCHA/Doctorado Nacional/2017-21170366
Keywords: Dendritic cells, Antitumor immunity, IRE1

Role of CD73 in the phenotype and function of IL-15-expanded murine NK cells

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Natural killer (NK) cells are innate lymphocytes that can directly recognize and eliminate tumor cells and thus are pivotal in the antitumoral immune response. In the tumor microenvironment adenosine is produced by the hydrolysis of extracellular ATP through the action of two ectonucleotidases CD39 and CD73. Adenosine, signals through A2A receptor and is capable of suppressing the cytotoxic activity and cytokine production by NK cells. Recent evidence demonstrates that CD73 ectonucleotidase is expressed in activated NK cells, but the role of this enzyme in NK cells remains unclear. To analyze the function of CD73 ectonucleotidase on NK cell function, we expanded murine NK cells obtained from CD73KO and wild type mice. As previously described, our NK cells cultured with IL-15 proliferated and upregulated CD69 and CD25 activation markers. In addition, after seven days of culture with IL-15, we observed that wild type and CD73KO NK cells upregulate immune checkpoints. Interestingly a lower percentage of NK cells from CD73KO mice upregulate CD39. Moreover, CD73KO NK cells are more proliferative and have an increased glucose uptake than WT NK cells. We conclude that CD73 regulates the expression of CD39 and has a role in the regulation of NK cells cellular metabolism and proliferation.

Funding: ANID/22221673(MGG); ANID/1220196(DS); ANID/1191438(MRB); ANID/BASAL/FB210008 (MR).
Keywords: Natural killer, CD73, Adenosine

The Melanoma Vaccines TRIMELVax and GVAX Limit Myeloid-derived Suppressor Cell Expansion and Tumor-Growth in Mice

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Myeloid-derived suppressor cells (MDSCs) are regulators of the immune system that expand in pathological conditions such as cancer disease. These cells are recruited at the tumor during the establishment of a suppressive tumor microenvironment, which limits the antitumor immune response and it has been identified as a major cause of a poor immunotherapy outcome. In order to understand the immunological mechanisms underlying the success of our melanoma vaccine TRIMELVax, we investigated its effect on MDSCs population in tumor growing mice. To this, C57BL6 mice were challenged with B16F10 melanoma cells and injected with: PBS, TRIMELVax or GVAX. We analyzed blood samples over the course of the experiment and identified polymorphonuclear MDSCs (PMN-MDSCs) and monocytic MDSCs (M-MDSCs) populations in bone marrow, spleen and tumor cells by Flow cytometry. We observed a significant lower circulating levels of cells with a monocytic phenotype in mice treated with TRIMELVax compared to PBS and GVAX, while both vaccines seemed to have a decreasing effect on cells with a neutrophil phenotype. We observed a lower abundance of M-MDSCs in the bone marrow and PMN-MDSCs in the spleen of TRIMELVax and GVAX treated mice compared to the control group. Size-matched tumor analysis revealed a possible advantage of TRIMELVax over GVAX, as TRIMELVax administration seems to result in less PMN-MDSCs recruitment into the tumor microenvironment and a decrease in the tumor volume. Finally, both vaccines showed a limited MDSC expansion and tumor-growth in melanoma challenged mice, which makes them promising candidates for melanoma therapy.

Funding: FUNDING: FONDEF ID16I10148, IMII P09016F, ANID BECA No. 21180633.
Keywords: MDSC, TRIMELVax, immunotherapy

Neutrophils induce NF- kB activation and epithelial-to-mesenchymal transition of breast cancer cells

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Tumor-associated neutrophils (TANs) have been linked to tumor progression as they promote several malignancy characteristics including metastasis, a process that involves the epithelial-to-mesenchymal transition (EMT). However, the underlying mechanism through which TANs promote EMT has not been yet elucidated. Based on previous work showing a relationship between the activation of the NF-kB pathway and EMT, we hypothesized that TANs induce the activation of the NF-kB pathway in tumor cells, causing their EMT.
To this end, we performed co-culture experiments between the neutrophil cell line HL-60N, and breast cancer cell lines MDA-MB-231 and MCF-7 cells, demonstrating that neutrophils favor the expression and production of cytokines induced by NF-kB in tumor cells such as IL-6 and IL-8, and decrease E-cadherin expression. We also observed that neutrophils promote tumor cell migration as measured by wound healing assays. Interestingly, this neutrophil-mediated increase in migration was no longer observed in cell culture experiments where we used a small interfering RNA against P65 to inhibit NF-kB pathway in tumor cells. Finally, using the zebrafish xenograft model, we demonstrate that tumor cells disseminate less to the tail when we use a morpholino that reduces the number of neutrophils in zebrafish.
These results allow us to suggest that neutrophils activate NF-kB pathway in breast cancer cells and that this activation promotes EMT in tumor cells.

Funding: FONDAP 15090007 FONDECYT 1220196
Keywords: EMT, neutrophils, breast cancer cells

Novel role of m6A demethylase FTO in the Tumor microenvironment of colorectal cancer

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Colorectal cancer (CRC) is one of the most frequent cancer worldwide. Patient survival and therapy response are a direct consequence of the tumor microenvironment interactions. The role of the m6A-demethylase FTO has recently been described in CRC cell stemness, and epithelial / mesenchymal transition, however, its function in the tumor microenvironment (TME) and progression remains unclear. This study aimed to evaluate FTO protein expression in tumor and healthy tissue from CRC patients (n=22) with different histological TNM stages (hTNM) by immunohistochemistry. FTO localization was classified in tumor or stroma, associating with clinical and histopathological features to provide a more precise perspective of FTO in the TME. Our study shows that the localization of FTO is highly present in lamina propria cells in healthy mucosa, in lymphoid infiltrates and fibro-immune compartments in the TME, corresponding mostly to CD4+T cells and Iba+macrophages. Additionally, FTO+ tumor cells are increased in early carcinogenic stages (hTNM I vs healthy tissue*) and in moderately differentiated tumors*. Furthermore, stromal FTO+ cells are increased in more invasive stages*(T3) and with low desmoplasia*. Our preliminary results indicate that after exposure to cancer cell-derived conditioned media, nuclear FTO expression is enhanced in normal T cells and fibroblasts, suggesting FTO activity in the TME interaction following a paracrine signal. Together, our findings highlight essential role of FTO in early CRC stages suggesting a TME component interaction. Lastly, research is presently on-going to find new FTO functions in the CRC-TME.(*p<0.05).

Funding: FONDECYT 3190931 (GL), FONDECYT 11190990 (MDLF), FONDECYT 1170834 (MAH). *Project sponsor researchers.
Keywords: Tumor microenvironment, m6A, colorectal cancer

Analysis of the protumoral mechanisms of the periodontal bacterium Fusobacterium nucleatum on growth, epithelial-mesenchymal transition (EMT) and the expression of immunosuppressive markers in cell lines of oral squamous cell carcinoma.

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Background: Oral squamous cell carcinoma (OSCC) is the most common manifestation of oral cancer, and despite the advances in treatments, 5-year survival remains less than 16% in late-stage diagnosis. Periodontitis has been proposed as a risk factor of oral cancer due to the presence of periodontal pathogens, such as *Fusobacterium nucleatum*, that contribute to OSCC progression, however the mechanisms modulated by the tumoral bacteriome remains not fully understood. Methods: Oral cancer cell lines were infected with the periodontal bacteria *Fusobacterium nucleatum* at a MOI 100. To evaluate the effect of the bacteria on tumoral growth of cancer cells, we used the visualization and measure of tumor spheres at a 3, 6, and 10 days post-infection. The expression of EMT markers on oral cancer cells, such as MMP-9 and E-cadherin were analyzed by qPCR, after 6 and 48 h post infection. Finally, the expression of immunosuppressive molecules on OSCC cells induced by the bacteria was evaluated by flow cytometry. Results: A significant increase in the size of tumor spheres infected with the *F. nucleatum* was found at 3, 6, and 10 days post-infection. MMP-9 was significantly elevated in infected cells at 6 hours post infection and E-cadherin was significantly downregulated post infection. Also, infected and non-infected cancer cells highly expressed CD155, PDL-1, however Galectin-9 was significantly elevated only in infected cells. Conclusions: The periodontal bacterium *Fusobacterium nucleatum* could promotes tumor progression of OSCC through increased tumor growth, acquisition of ETM-associated markers, and increased expression of markers associated to tumor immunosuppression.

Funding: Proyecto Fondecyt Regular 1211480, establecimiento de los laboratorios del departamento de Bioquímica Clínica e Inmunología de la Facultad de Farmacia presente en la Universidad de Concepción.
Keywords: Oral Cancer, Periodontitis, Immunosupresssion

NRP1 is required for the immunomodulatory function of CAF

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Introduction: Tumor progression and metastatic spread is modulated by the carcinoma-associated fibroblasts (CAFs) within the primary tumor microenvironment. Little is known about the specific mechanisms by which CAFs would exert its immunomodulatory role on immune cells. Recent studies in our laboratory have shown that Neuropilin 1 (NRP-1) is over-expressed in CAFs; therefore, it is associated with more aggressive cancer. This study seeks to evaluate the potential role of NRP-1 in the immunomodulatory function of CAFs. *Methodology*: CAFs were obtained from patients with metastatic disease and BAF from benign patients. The fibroblasts were functionally characterized by the generation of fibroblast-derived matrices (FDM) and secretory profile by cytokine array. The contribution of NRP-1 to the role of CAFs was determined by siRNA assays. CD8 T lymphocytes were obtained from healthy patients and treated with fibroblast-secretome. Markers were studied by flow cytometry: activation (CD25 and CD69) and repression (LAG3 and PD1). The migration of CD8 T lymphocytes was studied by agarose drop migration assay. *Results*: FDM of CAFs are different in their composition and organization. Furthermore, the secretome of each type of fibroblast presents a differential effect on the activation of CD8 T cells according to the markers highlighted. Regarding migration, it has been observed that the CAFs-secretome can attract lymphocytes. However, it would lead them to apoptosis. *Conclusions*: Our results suggest that NRP1, overexpressed in CAFs, has an immunomodulatory role on CD8 T lymphocytes.
Acknowledgment: Beca Doctorado Nacional ANID 21181427

Funding: BECA DOCTORADO NACIONAL ANID 21181427CORFO NRP1-Cancer 21CVID-171889FONDECYT REGULAR-1221128-TUNING B CELL RESPONSES BY MECHANICAL CUES: ROLE OF CYTOSKELETON REMODELING AND MEMBRANE TRAFFICKING
Keywords: immunomodulatory, tumor microenvironment, carcinoma-associated fibroblasts

CD73 Restrains the Survival and Maturation of Murine Natural Killer Cells

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Natural Killer cells (NK) are innate lymphocytes that recognize and eliminate transformed cells and thus have a relevant role in the antitumor response. Adenosine is mainly produced in the tumor microenvironment by ATP hydrolysis mediated by CD39 and CD73 ectonucleotidases. Recent evidence demonstrates that intratumoral NK cells also express CD73. However, no studies have unraveled the role of this ectonucleotidase on these cells. Here we study the expression of CD73 and its role in the phenotype and function on NK cells. Our results show that spleen NK cells do not express CD73, but this ectonucleotidase is upregulated in the tumor microenvironment upon transfer into tumor-bearing mice. NK cells obtained from CD73KO mice displayed a more immature phenotype than NK cells from WT mice. Upon transfer into the tumor, we observed a lower frequency of CD73KO NK cells than WT NK cells, suggesting that CD73 promotes the survival of NK cells. Accordingly, CD73KO NK cells also presented a decreased expression of CD39 compared to WT NK cells, suggesting that they might be less adapted to survive within inflamed tissues. We observed no changes in the co-expression of immune checkpoints nor activating receptors in tumoral CD73KO NK compared to WT cells. Finally, we demonstrated that CD73KO NK cells presented a similar capacity as WT cells in reducing tumor burden in mice. Our result suggests that CD73 is upregulated in NK cells in the tumor microenvironment and that this ectonucleotidase regulates the survival, maturation and CD39 expression on NK cells.

Funding: ANID/1220196; ANID/BASAL/FB210008, ANID 22190463
Keywords: Ectonucleotidase (CD73), NK Cells, Tumor Microenvironment

Therapeutic TRIMELVax vaccine induces highly proinflammatory immune cell recruitment and early inflammatory gene expression pattern in treated mice.

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TRIMELVax is a new immunotherapeutic technology based on heat shock-conditioned melanoma cell lysates (TRIMEL) combined with the adjuvant CCH. This vaccine showed efficacy in reducing tumor growth and increasing survival in mice models of melanoma. Although the clinical impact of this vaccine raises great expectations, many of the immunological factors involved in its effectiveness are unknown. In this work, we focused on studying how TRIMELVax regulates early inflammatory events in the tissue microenvironment at the administration site. To achieve this, C57Bl/6 mice were injected in the hind footpad with TRIMELVax, PBS or Gvax. Animals were euthanized, biopsies were obtained to analyze innate immune cells by FACS, and the expression of cytokine and chemokine genes by qPCR. We found that TRIMELVax induces a specific profile in innate immune cells, highlighting a rapid recruitment of neutrophils, an increase in M1, monocytes, cDC1, LCs and mo-DCs, as well as a decrease in M2. Besides, the RT-qPCR array showed that TRIMELVax compared to GVax induces the up-regulation of genes such as pro-inflammatory cytokines, the chemokines CxCl1, 9, 10, 11 and the cytokines IL15 and 17 that lead a recruitment of neutrophils, macrophages and dendritic cells to the site of injection; as well as the overexpression of Ccl19 that allows the migration of DCs to draining lymph nodes. Taken together, these results suggest that TRIMELVax induces a rapid and specific activation of the innate immune system, which would lead to the coordination of a sustained and effective adaptive immune response against malignant melanoma tumors.

Funding: FUNDING: FONDEF ID16I10148, IMII P09016F, ANID BECA No. 21180633.
Keywords: Melanoma, Vaccine, Immunity

Role of adenosine produced by CD73 in the establishment of exhausted and precursor exhausted CD8+ T cells.

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The functional activity of cytotoxic CD8+ T lymphocytes is reduced in the tumor niche, through a process known as 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 PD1 checkpoint blockade therapies. Several features of Tpex including their stemness have been described to be induced by the adenosine-producing ectoenzyme CD73 in CD8 T cells. However, the relationship between the CD73/adenosine axis and Tpex/Tex differentiation has not been studied. Thus, our aim is to evaluate 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. Multiparametric flow cytometry analysis of tumor-infiltrating T cells in B16F10 melanoma tumors revealed a higher expression of CD73 in Tpex compared to Tex. Also, *in vitro* chronic activation of CD73-deficient OT-I cells resulted in a lower expression of the immune checkpoints CD39 and TIM-3, and higher levels of TCF-1 compared to OT-I cells, suggesting that CD73 may be promoting exhaustion. In contrast, the adenosine receptor A2AR antagonist (SCH58261) promoted a higher frequency of Tex cells, whereas the adenosine analogue NECA reduced Tex compared to cultures with vehicle control. In conclusion, while CD73 is expressed by intratumoral Tpex and promotes the development of Tex *in vitro*, adenosine has the opposite effect reducing Tex differentiation, suggesting different roles for CD73 and adenosine in T cell exhaustion.

Funding: This work was supported by: PhD ANID scholarship: 21201553 (JSA); María Ghilardi PhD Scholarship (SG), ANID/ FONDECYT 1220196; ANID/BASAL/FB210008.
Keywords: Exhaustion, Adenosine, T cells

MUCOSAL IMMUNOLOGY

The Role of Intestinal Goblet Cells in Irritable Bowel Syndrome (IBS)

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IBS is a gut-brain axis disorder, characterized by loss of intestinal barrier function. Goblet cells (CC) are epithelial cells specialized in the secretion of mucus, the protective layer of the mucosa. The role of CC in IBS has not been fully elucidated. We aim to evaluate the differences in number and morphometric characteristics of intestinal CCs between patients with IBS and healthy control (HC). METHODS: In ileal and colonic mucosa of IBS patients (n=22, 8 IBS-D; 5 IBS-C; 5 IBS-M; 3 IBS-I) and HC (n=12), the density (number of cells /area) and CC ultrastructure (activity, vesicle fusion, vacuole diameter and cell apical opening length), was characterized by PAS/Alcian-blue staining and transmission electron microscopy, respectively. T-test and ANOVA test for comparisons, significance $p<0.05$. RESULTS: Increased CC density ($p=0.0461$) and decreased vacuole diameter ($P=0.005$) in colon of IBS patients, mainly in IBS-M phenotype ($p=0.0068$), was observed with no changes in the ileum and in other variables analyzed for both intestinal segments. CONCLUSION: The alterations in the density and form of CC observed in the colon of patients with IBS suggest the presence of an alteration in this innate immune mechanism of the intestinal mucosa. Future research directed to evaluate the relationship between these alterations, the microbiota composition and immune cell activation of intestinal mucosa must be explored.

Funding: FONDECYT 1181699.
Keywords: Goblet cells, IBS

Evaluation of anti-inflammatory and mitochondrial effect of CoQ10 and alpha-ketoglutarate in colitis models.

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Introduction: Ulcerative colitis (UC) is an inflammatory bowel disease characterized by exacerbated intestinal immune response, with macrophages being importantly involved. Additionally, lower energy production capacity has been associated with downregulated tricarboxylic acid cycle and decreased respiratory complex function in UC patients' colonic tissue. Supplementation with alpha-ketoglutarate (α-KG) and CoenzymeQ10 (CoQ10) improve energy production with antioxidant effects, although their role in mitochondrial reprogramming and inflammation resolution in UC monocyte-macrophages is unknown. Aim: To evaluate the effect of CoQ10 and cell-permeable α-KG derivative on inflammatory surface markers and mitochondrial function in monocytes-macrophages UC models.
Methods: peripheral blood monocytes (PBM) were enriched from active UC patients (n=3) and healthy subjects (HS) (n=3), by negative selection (Rosettesep). Inflammatory environment was induced with LPS (10 ng/mL, 12 hrs). THP1-differentiated macrophages with PMA (M0), and inflammatory phenotype (M1) was induced with LPS (0.1ng/mL) + IFNg (20ng/mL) (24 h). Cells were co-treated with CoQ10 (10mM) and/or α-KG (1mM) and inflammatory stimuli. Phenotype and inflammatory markers (PBM= CD14-CD16-CD86-CD163-HLADR; THP1-Mf= CD40-CD80-CD163) and mitochondrial function (membrane potential(Δ?m)-mtROS) were measured by flow cytometry. PBM supernatant cytokines (IL10-IL6-IL8-TNF-IL1B-L12) were measured by cytometric bead array (BD).
Results: CoQ10 and α-KG tend to reduce HLADR whilst increasing CD163 in HC-PBM. Furthermore, α-KG tends to increase Δ?m in HC. COQ10 and α-KG reduced mtROS in UC-PBM and HC-PBM; α-KG and COQ10 reduced CD40, CD80 and mtROS in THP1-Mf. Moreover, α-KG tends to reduce TNFα, increasing IL10 in UC and HS-PBM.
Conclusion: Our preliminary data suggest that CoQ10 and α-KG reduce inflammatory and mitochondrial dysfunction markers.

Funding: Fondecyt 3210367, 11201322, 1220702, Anillo ACT210097
Keywords: Ulcerative colitis, Macrophages, antioxidants

The IRE1/XBP1s axis activation in DCs regulates intestinal Th17 differentiation

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Introduction: The intestinal immune system is constituted by different cell types. Perturbations in this equilibrium is associated with the development of intestinal diseases. Type 1 dendritic cells (cDC1) are fundamental for maintaining tolerance in the gastrointestinal tract. The sensor IRE1 of the unfolded protein response (UPR) and its associated transcription factor XBP1s are reported to regulate the survival of cDC1 and in the gastrointestinal tract. However, little is known about the role of IRE1 signaling in maintaining the tolerogenic role of cDC1 in the intestine.
Methods: Using conditional knock-out mice for the RNase domain of IRE1 or XBP1 in DCs (CD11c-Cre) we analyzed the role of this UPR branch in the control of cDC function in the gastrointestinal tract.
Results: Mice bearing IRE1 deletion in cDC ($IRE1^{trunc\text{-}DC}$ mice) exhibit a marked accumulation of Th17 cells in the small intestine lamina propria (siLP), which is accompanied by Th17 hallmarks consisting in increased neutrophil accumulation and epithelial crosstalk. Interestingly, $IRE1^{trunc\text{-}DC}$ animals present a marked delay in the progression of experimental autoimmune encephalomyelitis (EAE), indicating that Th17 present in these mice are not proinflammatory. Mechanistically, we found that siLP cDCs from $IRE1^{trunc\text{-}DC}$ mice produce high levels of IL-6, a cytokine involved in Th17 priming. Finally, we found that these effects are not dependent on XBP1s, as $XBP1^{\Delta DC}$ mice present a decrease frequency of Th17 in the siLP.
Conclusions: We unconver a novel regulatory mechanism controlling Th17 homeostasis in the intestine, which is dependent on the IRE1 sensor of the UPR in cDCs.

Funding: FONDECYT #1161212, #1200793, HHMI #55008744
Keywords: Dendritic cells, Th17 cells, UPR

IL-33 favors Foxp3+ T regulatory cells and the production of intestinal metabolites linked to immune regulation

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Introduction. Intestinal commensal flora and its metabolites have been considered factors related to host health and immunity. Interleukin-33 (IL-33) is a tissue-derived nuclear cytokine of the IL-1 family, known as alarmin due to its high expression in endothelial and epithelial cells exposed to tissue damage or encounter with pathogens. Recent studies place IL-33 as a new regulator of immune tolerance by affecting T regulatory cells (Tregs). As seen by our group and others, administration of IL-33 into transplanted animals facilitates graft acceptance. Methodology. FoxP3-GFP reporter mice were treated intraperitoneal injections of IL-33. The immunological capacity of IL-33 was evaluated by the ability to induce Tregs and the production of intestinal metabolites of immune interest (metabolomics). Results. The administration of IL-33 upregulates the frequencies of Tregs in mesenteric lymph nodes, reducing the capacity to produce IFNg and IL-17 compared to the control group. Metabolomic analyzes identified a total of 579 differential metabolites, of which 12.5% showed significant variations between the treatment and control groups. Heatmaps and KEGG pathway enrichment analysis show the robust effect of IL-33 on metabolites production and their involvement on amino acid synthesis, respectively. Search on public literature indicates that several of these metabolites are involved in immune processes. Discussion. IL-33 favors Tregs presence and stimulates the production of regulatory intestinal metabolites, complementing several reports in which this cytokine is involved in gut immune tolerance.

Funding: Fondecyt Regular Grant #1210654
Keywords: IL-33, T regulatory cells, intestinal metabolites

The Mast Cell Role In B-Cell Lymphoma-3 (Bcl-3) And Zonula Occludens-1 (Zo-1) Expression In The Intestinal Epithelia Of Irritable Bowel Syndrome

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Irritable bowel syndrome (IBS) is a gut-brain axis disorder characterized by an increased intestinal permeability that is associated to epithelial tight junction (TJ) disorganization. An elevated mast cell activity in IBS induces the release of proteases that activate receptors (PAR2) located in the basolateral side of the epithelia that produce TJ proteins restructuring by unknown signaling mechanism. Bcl-3 is a regulatory protein of NF-kB genes transcription, which is elevated in the intestinal epithelia of IBS patients. Mast cell tryptase via PAR2 induces Bcl-3 expression *in vitro*, as well as the Bcl-3 overexpression displace the immunolocalization of TJ protein ZO-1 from the membrane to cytoplasm. The mast cell role on Bcl-3 expression and its consequences on ZO-1 expression *in vivo* is unknown. Methods: The expression of Bcl-3 and ZO-1 was evaluated in ileum and colon of $Kit^{W\text{-}sh/W\text{-}sh}$(wsh) mast cell deficient and wild type mice (wt)(n=6/group), by WB and immunofluorescence. T-test and Pearson for comparisons and correlations, respectively, significance $p<0.05$. Results: Despite no differences Bcl-3 expression($p=0.5750$) and ZO-1($p=0.1813$) between wsh and wt by both WB and IFI, a positive correlation between the Bcl-3 and ZO-1 immune staining ($r=0.9195$, $p=0.0403$) was observed in wt. Conclusion: The presence of mast cells does not affect the expression of Bcl-3 or ZO-1 in the epithelium at basal condition. The positive immune staining correlation between both proteins suggests that Bcl-3 controls the expression of ZO-1 by independent mechanism. Further research in IBS mice model will elucidate the role of mast cells in this regulatory signaling of TJ restructuring.

Funding: FONDECYT 1181699
Keywords: Bcl-3, Tight Junction, Mast Cell

RATreg-derived extracellular vesicles promote immune suppression and prevent alveolar bone loss during periodontitis: potential role of CD73-mediated adenosine production

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Introduction: Extracellular AMP hydrolysis prompted by CD73 ecto-5'-nucleotidase generates adenosine, a potent immune suppressor which limits mucosal inflammation. Murine regulatory T cells induced in the presence of retinoic acid (RATregs) and their secreted extracellular vesicles (RATEVs) are enriched in CD73, which endows them acellular immunomodulatory functions. Periodontitis is triggered by a deregulated inflammatory host immune response which promotes bone resorption, process that largely relies on the IL-17/RANKL axis.
Aim: To evaluate RATEVs immunosuppressive capacity and CD73's role over T cells function *in vitro* and their effect on periodontitis-induced immune response/alveolar bone resorption.
Methods: RATregs and RATEVs were isolated and characterized. CD73 enrichment on RATEVs was evaluated by Western Blot, imaging and conventional flow cytometry (FC), whereas its enzymatic activity was tested by adenosine and phosphate production assays. RATEVs immunosuppressive capacity was assessed evaluating their effect over T cells proliferation and activation, whereas the relevance of RATEVs-derived CD73 by adding the specific CD73 inhibitor. We also evaluated RATEVs effect over periodontitis-associated immune response and alveolar bone loss on a ligature-induced murine model using FC and morphometric/histological analysis, respectively.
Results: RATregs and RATEVs showed high CD73 expression and AMPase activity. Particularly, RATEVs dampened $CD4^+$ T cell proliferation and activation in the presence of AMP, which was partially reverted by the addition of CD73 inhibitor. During periodontitis, RATEVs reduced CD25, IL-17 and RANKL expression and alveolar bone resorption.
Conclusion: Enzymatically active CD73 is released from RATregs within EVs, which suppress T cell responses and could be involved in preventing periodontitis-induced alveolar bone loss.

Funding: Regular Fondecyt 1220999 (R.V) and 1210654 (K.P-L). ANID National PhD Scholarship 21180841 (C.R)
Keywords: Tregs, Periodontitis, Extracellular vesicles

Human metapneumovirus infection affects intestinal immunity and microbiota composition in a murine model

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Respiratory infections are one of the main causes of morbidity and mortality worldwide, mainly in children, immunocompromised people, and the elderly. Several respiratory viruses can induce intestinal inflammation and alterations in the composition of the intestinal microbiota. Human metapneumovirus (hMPV) is one of the major respiratory viruses contributing to infant mortality in children under 5 years of age worldwide and the effect of this infection at the gut level has not been studied. Here, we analyzed the distal effects of hMPV infection on intestinal microbiota and inflammation in a murine model, analyzing several post-infection times (days 1, 3 and 5). A group of C57BL/6 mice was infected intranasally with a dose of $1x10^6$ PFU of hMPV and mice inoculated with a non-infectious supernatant (Mock) were used as a control group. Although hMPV does not have the ability to infect the intestine, we observed significant changes in the expression of proinflammatory cytokines in intestinal tissue analyzed by qPCR at days 1 and 3 post-infection compared to the control group. Concordantly, changes in the frequency of different myeloid innate immune cell populations were observed in the colon of hMPV-infected mice, which were analyzed by flow cytometry. Additionally, significant changes were observed in the abundance of the genus Bacteroides in the intestinal microbiota of hMPV-infected mice, using 16S qPCR and 16S sequencing. Therefore, these results indicate that hMPV can affect intestinal immunity and the microbiota and further research is required to understand the mechanisms inducing these distal effects in the intestine.

Funding: FONDECYT de iniciación 11200764
Keywords: Inflammation, Gut-lung axis, Intestinal microbiota

APPLIED IMMUNOLOGY AND VACCINES

Evaluation of immune response induced by a recombinant BCG-SARS-CoV-2 vaccine.

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Background: SARS-CoV-2 is the virus that caused COVID-19, which to date has generated millions of deaths worldwide. The most important antigens for this virus are its structural proteins: Spike, Envelope, Membrane, and Nucleoprotein. In this line, our laboratory generated vaccines against SARS-CoV-2 using Bacillus Calmette-Guérin (BCG) as a vector expressing these structural proteins. BCG is a vaccine administered to prevent tuberculosis. Interestingly, countries, where the BCG vaccine is administered at birth, have reported lower infection rates and a decrease in COVID-19-related deaths. Here, we evaluated the safety and immune response induced by a recombinant BCG vaccine expressing the SARS-CoV-2 nucleoprotein (rBCG-N-SARS-CoV-2) in a murine model. Methods: BALB/c mice were immunized with $1x10^5$ CFU of rBCG-N-SARS-CoV-2 vaccine to evaluate safety and immunogenicity parameters. Lymphocytes were purified and co-cultured with dendritic cells, and activation markers were evaluated. The co-culture supernatants and serum samples were analyzed using ELISA to evaluate cytokines and antibodies. Results: Immunization with the rBCG-N-SARS-CoV-2 vaccine was safe and promoted activation of antigen-specific $CD4^+$ and $CD8^+$ T cells and induction of specific antibodies against nucleoprotein. Conclusion: BCG is a promising platform that can potentially be used to control emerging respiratory pathogens, including SARS-CoV-2. Also, this vaccine induces a marked antigen-specific immune response mainly characterized by a T_h1-type cellular immune response and secretion of IFN-y and IL-2 cytokines. We believe that rBCG-N-SARS-CoV-2 is an excellent candidate to combat the COVID-19 pandemic. Acknowledgment: COPEC UC 2020.R.001, ANID-Millennium Institute on Immunology and Immunotherapy. CONICYT/ANID scholarship #21190183 for N.M.G.; and #21210336 for M.A.R.

Funding: This Work was supported by COPEC UC 2020.R.001, ANID-Millennium Institute on Immunology and Immunotherapy. CONICYT/ANID scholarship #21190183 for N.M.G.; and #21210336 for M.A.R.
Keywords: Recombinant BCG, SARS-CoV-2, COVID-19

Contribution of two-dose vaccination to the reduction of COVID-19 cases, ICU hospitalizations and deaths in the total population of Chile.

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Background: A large number of clinical studies have shown that is why this work evaluates the impact of the initial mass vaccination campaign with two doses in the population of Chile, and how these reduce adverse epidemiological outcomes due to SARS-CoV-2 infection.
Methods: Publicly available epidemiological data ranging from February 3rd 2021, to September 30, 2021 were used to build GAMLSS models that explain the beneficial effect of up to two doses of vaccination on the following COVID-19-related outcomes: new cases per day, daily active cases, daily occupied ICU beds and daily deaths.
Results: The results suggest that the first and second doses of the vaccine, and the statistical interaction between the two, are strong and statistically significant predictors of new daily COVID-19-related cases, daily active cases, ICU hospitalizations and deaths. They also explain ICU bed occupancy by age range, and how these have been favored to a greater or lesser extent by vaccination.
Conclusions: Our models highlight the importance of completing vaccination schedules to reduce adverse outcomes during the pandemic. In recent analyses, the results seen with this model are very similar when booster and fourth doses are added to the model. This work highlights the importance of achieving full vaccination status (two doses) and reinforces the notion that a second dose provides greater non-additive protection, but questions whether two doses alone are sufficient to stop the pandemic.

Funding: This work was supported by the following funding agencies: ANID-Subdirección de Capital Humano/Doctorado Nacional, Agencia Nacional de Investigación y Desarrollo (ANID), Millennium Institute on Immunology and Immunotherapy.
Keywords: COVID-19 vaccination, ICU hospitalizations, Explanatory models

Role of Microbiota in Modulating the Immune Response to SARS-CoV-2 vaccination in elderly people

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The vaccination is one of the most effective strategies to prevent infectious diseases in aged people. However, adult population strongly decreases their capacity to induce protective immunity against infections. Given the close functional link between the microbiota and the immune system, evidence suggests that the gut microbiota influences the immune response, modulating the ability to generate an efficient response to vaccination. Here we demonstrate that there a high correlation between the composition of the gut microbiota and the ability to generate an efficient immune response to SARS-CoV-2 vaccination in elderly people. Our data suggests that a high microbial diversity directly impact the efficiency of the immune response. In addition, our results identify specific microbial communities differentially represented in people with low or high immune response, which could play a key role in modulating this response. Our data demonstrate that strategies to modify the microbiota in aged people might be novel therapeutic strategies to increase the response capacity of the immune system and the effectiveness of vaccination, reducing the susceptibility to infections and their complications. This information can be used as predictive biomarker of the immune response and to design strategies to restore or modify the composition of the gut microbiota to stimulate the immune system, reducing the risk of infections and increasing the general health of a healthy aged individuals, improving their quality of life.

Funding: NAM21I0059
Keywords: microbiome, sarcov2, aging

Evaluation of the immunogenic profile of a Tobacco Mosaic Virus-associated vaccine expressing immunogenic epitopes of Canine Parvovirus in Nicotiana benthamiana.

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Background. Canine parvovirus (CPV) is a major pathogenic burden in canines with a high mortality rate in unvaccinated puppies. CPV is traditionally classified into three antigenic variants (CPV-2a, CPV-2b, and CPV-2c) based on the amino acid composition of the VP2 protein. Currently, various mutations are described in the receptor binding area or in the regions of greatest antigenicity of the VP2 protein giving rise to new viral variants that favor immune escape, affecting the protective immunity of traditional vaccines composed of the original CPV-2 or CPV-2b variant.
Aim. To develop a tobacco mosaic virus (TMV)-associated vaccine expressing immunogenic peptides of CPV viral variants expressed in *N. benthamiana* with the ability to stimulate an adaptive immune response *in vitro* and *in vivo* in a murine model.
Results. Mice vaccinated with the experimental formulation presented a Th1 response profile, characterized by increased levels of IgG2a and overexpression of INF-γ compared to the control group.
Conclusions. Our results demonstrate that vaccines using plant viral vectors (TMV) for antigen expression possess the ability to adequately stimulate an immune response in mice, being a potential platform for veterinary vaccines development.
Acknowledgments. to the CONICYT National Doctoral Scholarship, Chile (21180754). Project partially funded by the Corporación de Fomento de la Producción, Santiago, Chile (Grant number 18-COTE-97956).

Funding: CONICYT National Doctoral Scholarship, Chile (21180754). Project partially funded by the Corporación de Fomento de la Producción, Santiago, Chile (Grant number 18-COTE-97956).
Keywords: vaccines, tobacco mosaic virus, nicotiana benthamiana

Evaluation of fibrin matrix as biological support in cell therapy

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Introduction: In Cell Therapy, the injection of cells inside a biological matrix would offer an advantage, compared to cells in suspension by increasing cell retention in affected tissues speeding up the desired biological effect. The matrix must be biodegradable, biocompatible and inert to the cells used in biological therapies.
Objective: To build a matrix based on fibrin polymer that serves as a biological support for cells used in reparative or antitumor therapies.
Materials and methods: Macrophages M0 and M1 were cultured for 72 hours in fibrin matrices constructed from plasmatic fibrinogen and polymerized with thrombin. Subsequently, cell viability was measured using the AO/IP method and confocal microscopy, and gene expression in cultured macrophages was performed RT-qPCR of IL-1β, Arginase-1 and type I collagen. Additionally, the concentration of D-dimer in the fibrin matrix supernatant was measured to determine its biodegradation.
Results: M0 and M1 macrophages maintain a viability greater than 90% in the fibrin matrix (5 mg/ml) and compared to conventional culture, Macrophages express a greater amount of IL-1β, Arginase-1 and type I collagen after 72 hours. Compared to matrix without cells, supernatants from macrophage-carrying matrix contained a higher D-dimer concentration.
Conclusion: The fibrin matrix maintains the viability and functionality of the inserted cells and degrades over time, which could be considered a good delivery vehicle for cell therapies.
Projection: Generate *in vivo* model to assess the fibrin matrix immunogenicity and the functionality *in situ* of the inserted cells inside matrix.

Funding: Proyecto OAIC 17/18Oficina de apoyo a la Investigación Científica, Hospital Clínico Universidad de Chile
Keywords: Fibrin Matrix, cell therapy, delivery cell

AUTOIMMUNITY AND INFLAMMAGING

LPS-induced thymic involution in the [NZBxNZW]F1 murine model of Systemic Lupus Erythematosus.

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Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by the hyperactivity of autoreactive T and B lymphocytes. Ours results in the murine model of lupus [NZBxNZW]F1 show that female mice, when they develop lupus, produce anti-dsDNA antibodies, loss of B cell progenitors in the bone marrow, significant weight loss, fatal kidney damage, and thymic involution. It has been observed that the production of anti-dsDNA antibodies and reduction of thymus cellularity are induced in the face of inflammatory processes such as infections.
Here we study the effect of successive treatments with LPS on the development of lupus symptoms in young females of the [NZBxNZW]F1 model.
Thymus involution and the presence of B cell progenitors in bone marrow were studied by flow cytometry, while ELISA evaluated anti-dsDNA antibodies and corresponding isotypes.
Our results show that mice produce anti-dsDNA antibodies four days after LPS injection, loss of double-positive thymocytes, increased frequency of B-lymphocytes in the thymus, and loss of B-lymphocyte progenitors in the bone marrow. However, 30 days after injection with LPS, we observed a decrease in autoantibodies and recovery of thymus and bone marrow cellularity.
In conclusion, a dose of LPS reproduces the autoantibody production, thymic involution, and bone marrow aplasia observed in female mice when they develop the fatal disease. These results suggest that infectious/inflammatory processes would accelerate the appearance of characteristics associated with SLE.

Funding: Funded by FONDECYT1191438 (MRB), FONDECYT 1220196 (DS), ANID/BASAL/FB210008 (MRB/DS/MR).
Keywords: Systemic lupus erythematosus, Thymic involution, Bone marrow aplasia

Induction of regulatory iNKT cells with glycolipid encapsulated into liposomes: a novel strategy to prevent inflammation and mucus production during allergic asthma.

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Invariant NKT (iNKT) cells have attracted attention because of their ability to be activated specifically by glycolipid antigens. The activation of iNKT cells (mainly NKT10 cells, a novel iNKT cell subset with IL-10-dependent regulatory function) with α-galactosylceramide (α-GalCer) can protect against inflammatory diseases. Nevertheless, the strong activation of iNKT cells elicited by α-GalCer exhibit limited therapeutic efficacy, mainly due to the induction of a mixed pro- and anti-inflammatory cytokine response. Since iNKT cells can be differentially activated by α-GalCer analogs, it is highly important to determine which α-GalCer analogs will expand NKT10 cells. Firstly, we identified NKT10 cells in hCD1d-KI mice (a partially humanized murine model for NKT cell responses). Hence, we evaluated different experimental conditions, such as immunization schemes, glycolipid activators of iNKT cells, and the uses of glycolipid delivery systems. We observed a significant expansion of NKT10 cells only in hCD1d-KI mice treated with α-GalCer at seven days, like the proliferation of NKT10 cells reported during the immunization scheme of 30 days. In addition, it was observed that incorporating the α-GalCer analog: AH10-7 into liposomes remarkably increased the expansion of NKT10 cells. Finally, we evaluated the anti-allergic effect of liposomes containing OVA and AH10-7(Lp/OVA/AH10-7). We observed a significant decrease in the inflammatory score and the number of mucus-producing cells in the lungs of mice with allergic induction treated with Lp/OVA/AH10-7. Our results demonstrated that AH10-7 contained in liposomes it's an excellent candidate to induce expansion of NKT10 cells and reduce lung inflammation and goblet cell hyperplasia.

Funding: Funded by FONDECYT Nº 1211959, Millennium Institute of Immunology and Immunotherapy P09/016-F, Copec-UC 2017.J.924 and CONICYT-PFCHA/Doctorado Nacional/2017-21170084 to R.G.

Small Extracellular Vesicles from metabolically reprogrammed Mesenchymal Stem Cell as a potential immunosuppressive mechanism

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MSCs are multipotent fibroblast-like cells that exert different biologic functions, including tissue repair and immunosuppressive activity, making them attractive for autoimmune disease treatment. The immunomodulatory activity of MSC, is mediated mainly by paracrine factors. However, the release of small extracellular vesicles (sEV) by these cells has been demonstrated as a principal mechanism by which MSCs perform their biological effects.
Our studies in human umbilical cord MSCs showed that metabolic reprogramming to glycolysis significantly improves their immunoregulatory capacity on proinflammatory T cells (Th) by inducing T regulatory cells (Treg). Here we evaluated the effect of different fractions obtained after the differential centrifugation to obtain sEVs from glycolytic or non-glycolytic MSCs over T proinflammatory and T regulatory cells.
We found that the MSC glycolytic conditioned medium, significantly decreased the proliferation of $CD4^+$ T and reduced $CD4^+$ IFN-γ^+ type 1 helper T (Th1) cells. This fraction also induced $CD4^+CD25^+Foxp3^+$ regulatory T cells (Treg). Moreover, the sEV fraction showed a trend to decrease T cell proliferation and induce regulatory T cells. On the other hand, fractions with less soluble or depleted factors did not affect the proliferation of Th1 and Treg cells. Thus, our findings suggest that paracrine factors of glycolytic MSC, specifically their sEVs, can activate T cells by inhibiting inflammatory immune responses and inducing anti-inflammatory responses.

Funding: This research was supported by the ANID from Chile through the grants FONDECYT Regular N°1211353; FONDEF ID: 21110194; and IMPACT FB 210024.

Effects of Platelet Rich Plasma (PRP) on the repolarization of inflammatory-type macrophages (M1) towards a reparative profile (M2)

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Introduction: Macrophages participate in transition from inflammation to tissue repair, however, their persistence as inflammatory cells correlates with tissue damage observed in autoimmune or inflammatory pathologies. On the other hand, PRP is a blood preparation used in treatment of inefficient tissue repair caused by chronic inflammation and decreased cell renewal. PRP preparations contains anti-inflammatory molecules that could repolarize M1 macrophages towards M2 profiles favoring tissue repair.
Objective: To evaluate the effect of PRP on pro-inflammatory macrophages phenotype as background to validate the clinical utility of preparation in inflammatory or autoimmune pathologies.
Methodology: We generate Monocyte-derived inflammatory macrophages with LPS and IFN-g, and then, they were stimulated with PRP for 48 hours. By flow cytometry, we measured CD86 and CD206 markers of M1 and M2 macrophages, respectively. In addition, we quantify by qRT-PCR the expression of IL-1β, TNF-α, IL-10, TGF-β, ARG1 and COL1A1.
Results: M1 macrophages treated with PRP show an increase in cell membrane expression of CD206 and a decrease in CD86 compared to untreated M1 macrophages. Furthermore, we observed a significant increase in gene expression of IL-10, TGFβ, TNF-α, and a reduction of IL-1β in PRP-treated macrophages. There were no changes in the genic expression of ARG1 or COL1A1 in macrophages with and without PRP.
Conclusion: Proinflammatory macrophages treated-PRP change their phenotype towards repair profiles. Functional studies are necessary to verify the biological effect of PRP on macrophage or other cells involved in reparative process.

Funding: Proyecto OAIC 17/18 Oficina de apoyo a la Investigación Científica Hospital Clínico Universidad de Chile
Keywords: Platelets Rich Plasma, Macrophages, regenerative medicine

Characteristic lymphocyte responses to Prevotella copri protein fractions in patients with rheumatoid arthritis

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Rheumatoid Arthritis (RA) is an autoimmune disease that affects 0.5-1% of the world population. Several genetic and environmental risk factors for the development of RA have been identified, including microbial dysbiosis. Recent findings highlight the relevance of *Prevotella (P.) copri*, a member of gut microbiota, in the immunopathogenesis of RA. *P. copri* is overrepresented in patients with new-onset RA and has been shown to promote the development of arthritis in susceptible mice, as well as to induce specific lymphocyte responses in RA patients.
To gain further insights into the nature and origin of immunodominant *P. copri* antigens, we analyzed lymphocyte responses towards distinct protein fractions and outer membrane vesicles (OMVs) from *P. copri*.
Peripheral blood mononuclear cells of RA patients and healthy or osteoarthritis subjects were stimulated for 18 hours with *P. copri* protein fractions of membrane, periplasm, cytoplasm and OMVs and the percentage of activated memory CD4+ T cells producing IFN-g or TNF-α was determined by flow cytometry. Specific antibodies to *P. copri* protein fractions were detected in serum samples by ELISA.
While healthy subjects showed T helper cell responses to *P. copri* antigens of the cytosolic fraction, *P. copri* membrane fraction stimulated Th1 cell responses particularly in RA patients. Furthermore, RA patients presented an increase of IgA, and IgG antibodies to *P. copri* protein fractions where absent in healthy controls.
The results suggest that a differential response to *P. copri* antigens might contribute to autoimmune inflammation in RA patients.

The authors thank ANID-Chile for financial support (FONDECYT11220882, PAI77180094).
Funding: FONDECYT11220882 and PAI77180094 from ANID-Chile
Keywords: Rheumatoid arthritis, Prevotella copri, Lymphocyte response

Phenotypic characterization of Systemic Lupus Erythematosus in murine model [NZBxNZW]F1 applying bioinformatic analysis

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Systemic lupus erythematosus is an autoimmune disease characterized by the hyperactivity of autoreactive immune cells that leads to the production of autoantibodies which cause damage to multiple organs and tissues. The development of the disease in humans occurs mainly in women of reproductive age. The murine model [NZBxNZW]F1 emulates the development and symptomatology of lupus in humans, maintaining the prevalence of the disease in females.
In this study, we sought to demonstrate statistically the phenotypic difference between healthy and diseased mice that may be relevant to understand the development and, potentially, the initial phenotypic abnormalities of lupus disease. To determine this, we perform a multiparametric analysis of recollected data applying bioinformatic tools.
The weight and the presence of protein in the urine of animals were monitored weekly. The appearance of autoantibodies was determined by ELISA. Flow cytometry was used to determine the phenotype and number of immune cells present in each organ at selected age estates.
Our results show that when female mice develop the disease, they produce anti-dsDNA antibodies, loss of B cells progenitors in the bone marrow, weight loss, fatal renal damage, depletion of double positive thymocytes and increase in the frequency of B cells in the thymus.
In conclusion, there is a significant reduction in the progenitors of B cells and double positive T cells in diseased mice compared to healthy mice. The development of the disease begins at 20 weeks or 5 months old. Weight loss correlates with severe disease progression, culminating in mouse death.

Funding: FONDECYT 1191438 (MB); ANID/BASAL/FB210008 (TPA/MRB/MR)
Keywords: Autoimmune disease, Systemic Lupus Erythematosus, Bioinformatics

CELLULAR AND MOLECULAR IMMUNOLOGY

CPAF from Chlamydia trachomatis alters the host proteome and the peptide repertoire presented by MHC-I molecules

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MHC-I molecules present peptides to CD8+ T cells and also are involved in the predisposition to several autoimmune diseases. Reactive arthritis (ReA) is strongly linked with *Chlamydia trachomatis* infection and MHC-I. However, how the MHC-I/peptide complex can contribute to the pathology is not well understood. *Chlamydia* is an intracellular pathogen and one of its main pathogenic factors is CPAF. This enzyme is secreted to the cytosol of the host and there are no data regarding about its effect on the generation/destruction of ligands presented by MHC-I molecules.
293-T-REx cells stably transfected with CPAF were induced with doxycycline and its effects on several known substrates were assayed by western blot and its unknown effects were evaluated by label-free quantitation (LFQ) using a Q-Exactive plus mass spectrometer. Moreover, MHC-I complexes were purified by immunoprecipitation and the eluted peptides were analyzed by MS. CPAF is highly active degrading known substrates such as Vimentin and RFX5, but also showed profound effects on the cellular proteome altering the expression of many proteins with a wide range of functions. More than 4000 MHC-I-restricted peptides were identified showing that the peptidome is altered when CPAF is induced, increasing the diversity, the amount and the characteristics of the presented peptides.
As conclusion, CPAF modifies the proteome, and more importantly, the MHC-I-associated peptidome, altering the degradation of host proteins, and the characteristics and composition of the MHC-I-associated immunopeptidome. These data suggest that the generation of “new ligands” could be a new pathogenic mechanism of C. *trachomatis* in ReA.

Funding: Fondecyt Initiative 11170969, Fondequip Initiative EQM 190142
Keywords: MHC-I, Mass spectrometry, Immunopeptidomics

Role of the Unfolded Protein Response in the immune regulation of liver tissue

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The UPR is an adaptive mechanism, coordinated by three sensors: IRE1, PERK and ATF6, responsible of preserving protein homeostasis in the ER under stress conditions. The liver is a tissue with a high secretory demand, due to its prominent role in systemic metabolism. It is known that the liver presents high basal expression levels of transcriptional factors involved in the regulation of UPR, such as Xbp1, also the hepatic immune system is constantly exposed to massive loads of antigens from the systemic circulation and the intestine, to which it must remain tolerant. Under this context, this study tries to elucidate whether the UPR is activated in immune cells infiltrating the liver.
Using Tabula Muris, we analyzed single cell expression patterns of different UPR genes in immune cells present in liver tissue. In addition, using conditional knockout mice for ATF6 and Xbp1 (CD11c-Cre), we analyzed the role of both branches of the UPR in DCs infiltrating the liver. We observed high basal expression levels of molecular chaperones and canonical targets of the UPR in macrophage, B lymphocyte and NK cell populations. On the other hand, from FACS analysis, we identified a broad diversity of myeloid and lymphoid cells present in the liver. Mice lacking ATF6 or XBP1 had normal liver DC frequencies compared to WT mice. However, there was a slight upward trend in the cDC1 population together with a slight decrease in the cDC2 population in ATF6 KO mice. These results will be further explored in settings of metabolic challenge.

Funding: FONDECYT #1161212, #1200793, HHMI #55008744
Keywords: UPR, Liver, ATF6

Differential Expression of TLR-2 and -9 an Inflammatory Profile of Peripheral Monocytes in Apical Periodontitis

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Introduction: Apical periodontitis (AP) is the chronic destruction of the peri-radicular tissues due to endodontic infection. Emerging evidence sustains a key role of monocytes in human chronic diseases, but their contribution to the AP-systemic burden is unknown. We aimed to determine the expression of Toll-like receptor (TLR)-2 and -9 and to explore the inflammatory profile of peripheral monocytes in individuals with AP and controls.
Methodology: Cross-sectional study. Otherwise healthy individuals with AP and controls consulting at the Dental Clinic, Universidad de Chile were included. Peripheral monocytes were isolated through Ficoll gradient and negative selection immunoassay and cultured for 24 hours. The mRNA expression of TLR-2 and -9 was determined by qPCR and the secretory profile of monocytes was explored by Proteome Profiler. Results were analyzed with Prism Graphpad ($p<0.05$).
Results: Monocytes from AP patients demonstrated a higher TLR-2 expression while TLR-9 was down-regulated compared to controls ($p<0.05$). Out of 35 cytokines, only 23 were detected in the supernatants of monocytes. In AP 10 cytokines were down-regulated, while 7 cytokines were up-regulated including CXCL1, CXCL10, IL-1beta, IL-6, and TNF-alpha.
Conclusions: Expression of TLR-2 and TLR-9 was up-regulated and down-regulated respectively along with differential cytokine profiles associated with chemotaxis and inflammation in peripheral monocytes from AP individuals

Funding: This study was funded by FONDECYT 1200098 from ANID, the Chilean government. MJB is a recipient of the scholarship ANID 21210551, from the Chilean Government. AF is a recipient of the scholarship CONICYT 21181377, from the Chilean Government.
Keywords: periapical periodontitis, toll-like receptors, monocytes

Role of mitochondria in the formation and function of the B cell Immunological Synapse

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Interaction B cells with immobilized antigens results in the formation of an immunological synapse (IS), where local lysosome secretion can facilitate antigen extraction. During IS formation, mitochondrial division, and metabolic activity increase, however the effect of this reorganization on the IS remains unknown. Importantly, mitochondria also establish interactions with lysosomes to regulate cellular homeostasis and therefore we asked whether mitochondria played a role at the IS of B cells, in terms of extraction and presentation of antigens. To this end, we activated B cells with antigen-coated beads, labeled and quantified the recruitment of mitochondria and lysosomes to the IS and evaluated the effect of inhibition of Drp1, GTPase involved in mitochondrial division, by using mdivi-1. Additionally, we measured the capacity of B cells to extract antigen under these conditions by quantifying the amount of antigen remaining on beads. Antigen presentation was evaluated be measuring levels of IL-2 produced by co-cultured B cells and T cells. Our results show that mitochondria are recruited to the IS upon interaction with beads containing BCR ligands and their recruitment is compromised when Drp1 is inhibited by mdivi-1. Additionally, polarization of lysosomes to the IS is not affected in the presence of mdivi-1, however, the antigen extraction capacity of these cells decreased compared to control conditions. Accordingly, antigen presentation to T cells was also impaired in B cells treated with mdivi-1. In conclusion, we unveiled a role for mitochondria in B cells, where their recruitment to the IS is necessary for efficient lysosome-mediated antigen extraction.

Funding: ANID: Beca doctorado nacionalANID: Fondecyt regular 1221128Dirección de Postgrado UC
Keywords: mitochondria, lysosomes, drp1

Characterization of CD36 expression and trafficking in B lymphocytes during activation and hepatocytes with Niemann Pick Type C (NPC) disease

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Niemann-Pick type C disease (NPC) is lysosomal storage, progressive and fatal disease that mainly affects the liver and the central nervous system. At the cellular level, lysosomal dysfunction is caused by the deficiency of cholesterol transporters NPC1 or NPC2 in lysosomes. An imbalance in B cell activation triggers inflammation and the production of autoantibodies, where antibodies against gangliosides associated with locomotor problems have been reported in NPC patients. Importantly, activation and production of high affinity antibodies by B cells relies on lysosomes, used for extraction and processing of antigens. Lysosome dysfunction in B cells in NPC and the function of CD36, which mediates the uptake of lipids have not been evaluated. To this end, we used a B cell line, which was treated with an NPC1 inhibitor (U18) and activated with antigens immobilized on beads or glass coverslips to generate an immunological synapse (IS), where we evaluated the localization of CD36 and lysosomes by immunofluorescence. The results show that CD36 and lysosomes accumulate at the IS in activated B cells, but this was impaired when NPC1 was inhibited. We anticipate that lysosome dysfunction affects the extraction and processing of antigens, and CD36 could promote this imbalance. We also evaluated the expression of CD36 in hepatocytes cell lines treated with U18. The results show an increase in the expression of CD36, which in obesity is associated with lysosomal dysfunction. Thus, in NPC, CD36 could mediate lysosomal defects in hepatocytes and B cells leading to inflammation and damage of the liver.

Funding: Fondecyt regular #1221128 (MIY) and Fondecyt regular #1190334 (SZ)
Keywords: B cell activation, lysosome dysfunction, CD36

The thymus supports the differentiation of memory B cells via an unconventional pathway

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The thymus harbors a small population of B cells that mediate negative selection. In mice without immunization, numerous thymic B cells have undergone Ig-class-switch. Moreover, we have shown that a significant proportion of thymic B cells express memory markers CD73 and PDL2, supporting that this population is programmed towards a memory phenotype. It is not clear if this process is independent of B cell stimulation by external antigens and which signals drive their differentiation into memory phenotype.
We generated mice with reduced microbiota through perinatal antibiotic treatment to evaluate the influence of antigenic exposure on the generation of memory B cells ($CD73^+$, $CD73^+PD\text{-}L2^+$) and class-switched B cells ($IgG2b^+$, IgA^+) in the thymus and peripheral organs. To establish if the appearance of thymic memory B cells is correlated with memory formation in the periphery, we evaluated Ig-class-switch cells in spleen and thymus in neonatal mice. Finally, we analyzed thymic B cells from OT-II mice to evaluate if the acquisition of memory phenotype requires T-B-interaction during negative selection.
Mice with reduced microbiota exhibit decreased memory B cells in the spleen and gut-associated lymph nodes. Interestingly, this subset remains unchanged in the thymus. Furthermore, Ig-class-switched cells appear first in the thymus within the first days of life, prior to their appearance in the spleen. Finally, thymic B cells from OT-II mice, show decreased memory differentiation.
Our results show that the thymus supports the differentiation of memory B cells via an unconventional pathway, independent of external antigen exposure through interaction with developing T cells.

Funding: FONDECYT 11221128 (SN), FONDECYT 1220196 (DS), FONDECYT 1191438 (MB), ANID/BASAL/FB210008 (MRB/DS/MR), and Beca ANID magister nacional 2020/ 22201364 (JC)
Keywords: Thymic B cells, Memory B cells, Differentiation

Effect of Mitochondrial Transfer derived from mesenchymal stem cells on postnatal and adult immune cells

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Mitochondria (MT) imbalance is associated with several pathologies. Mitochondrial transfer (MitoT) from Mesenchymal Stem Cells (MSC) has the potential to rescue MT deficient cells in diseases such as cancer, lung and heart injuries, as well as immune and inflammatory diseases. In the present work, we explore the effect of the MitoT from umbilical cord MSCs (UC-MSC) through an artificial transfer or co-culture procedure with postnatal and adult mononuclear cells derived from umbilical cord (UCB-MC) and peripheral blood (PBMC), respectively.
In PBMC, by using FACS, we observed that ~40% of CD45+ cells are permissive to MT. Subsequently, by FACS, we evidenced MitoT to CD3+ T cells, CD 19+ B cells, and CD56+ natural killer cells. Also, we observed a higher transfer to CD4+ T helper (60%) than in CD8+ T cytotoxic cells (20%). Additionally, we observed that MT plays a protective role in T lymphocytes following apoptosis induction by an immunotoxin (Staurosporine, STP). Additionally, we observed that MitoT to T CD4+ naïve cells induce Treg differentiation (~25% of MitoT+ cells vs. 0.5%).
As for postnatal immune cells, MitoT from MSC to CD34- cells from UCB, a dose-dependent MitoT to CD3+ T cells, CD19+ B cells, CD56+ natural killer cells, and CD11c+ dendritic cells. Consequently, with our results of PBMC, in CBMC, we observed that MitoT protects T lymphocytes from STP-induced apoptosis.These results open new avenues for the development of therapies based on cells and organelles for immune-mediated diseases.

Funding: FONDECYT Regular 1211749-ANID—Basal funding for Scientific and Technological Center of Excellence, IMPACT, #FB210024.
Keywords: cell-based therapy, Mitochondrial Transfer, Mitochondria

Circulating Mitochondria isolated from healthy donors inflict immunosuppressive effect on CD4-T cells

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Introduction: Circulating mitochondria are a component of the blood in human donors. However, the characterization and function of these extracellular mitochondria have not been elucidated. CD4-T cells are key players of the immune response due to their potential to regulate other immune cells. Mitochondria also have a crucial role on CD4-T cells modulating their metabolism and function. Here we evaluated the potential of an isolated fraction from blood containing circulating mitochondria, named CirMito, to regulate the activation and proliferation of CD4-T Cells from human donors.
Methodology: In this work, CirMito and CD4-T cells were obtained from blood of healthy donors. First, CirMito was characterized by flow cytometry and TEM analysis. Then, we performed *in vitro* experiments of CD4-T cells with CirMito under activation and differentiation conditions; four days later, activation level and differentiated phenotype were evaluated, also proliferation and cell cycle were analyzed.
Results: Flow cytometry revealed that CirMito maintained mitochondria's classic qualities, such as TOM20 expression, positive Mitotracker staining, and modulable membrane potential. TEM analysis showed the presence of mitochondria-like double membrane structures. *In vitro* experiments showed that CD4-T cells cultured with CirMito significantly decreased their activation and differentiation. This was correlated with a proliferation reduction and G0/G1 phase arrest of the cell cycle.
Conclusions: CirMito has immunosuppressive potential on CD4-T cells. These CirMito could contribute to the cellular homeostasis of the individuals and might be a powerful tool for long-distance intercellular communication.
Acknowledgments: We are grateful for the grants that supported this research.

Funding: Fondecyt de Iniciación #11221017Fondecyt Regular #1201420 Fondecyt Regular #1211749IMPACT, Center of Interventional Medicine for Precision and Advanced Cellular Therapy, Santiago, Chile. ANID—Basal #FB210024
Keywords: Mitochondria, CD4+ T cells, Blood

SWAP70 regulates actin cytoskeleton dynamics at the immune synapsis and participates in the mechanosensitive function of B lymphocytes

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Recognition of immobilized antigens by B cells leads to the formation of an immune synapse (IS), where local actin cytoskeleton rearrangements occur to antigen extraction. B cells respond to the mechanical properties of the surface where antigens are localized, by modifying actin cytoskeleton dynamics. We sought to investigate the proteins that could be involved in coupling mechanosensing properties of B-cells to the regulation of actin dynamics. SWAP70 associates with the actin and has been described as an immunomodulator because SWAP70-deficient mice develop autoimmunity, however, its role in the immune synapse of B cells has not been evaluated.
We activated B cells with latex beads coated with B cell receptor (BCR) ligands, which simulate the formation of an IS and analyzed the localization of SWAP70. Our results show that SWAP70 is recruited to the IS to where actin is depleted. This suggests that SWAP70 participates in regulating actin dynamics at IS in response to BCR activation. Additionally, we observed that recruitment of SWAP70 to the non-specific ligands bead contact site also occurs, although to a lesser extent, suggesting that the contact with surfaces promotes SWAP70 re-localization in response to mechanical stimuli.
Altogether, these findings suggest that SWAP70 could regulate actin cytoskeleton dynamics at the IS and participate in the mechanosensitive function of the B cells. Elucidating how SWAP70 regulates the formation of an IS in response to mechanical cues will help to understand the pathophysiological events of autoimmune diseases that involve the loss of the mechanosensing properties in B cells.

Funding: Proyecto FONDECYT No 1221128.
Keywords: SWAP70, Actin, Mechanosensing

HMGB1 modulates the immune synapse of B lymphocytes to promote cell migration

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Production of high-affinity antibodies relies on the capacity of B cells to uptake antigens from the surface of presenting cells and expose them as peptides to specific T cells. Extraction of immobilized antigens by B cells requires the formation of an immune synapse (IS), which can be tuned by local cues originating from the cell microenvironment. For instance, B cells infiltrate and perform their effector functions in tumours, where they are exposed to soluble components secreted by tumour cells, including the protein HMGB1. HMGB1 activates signaling pathways that could affect IS organization and function; therefore, we decided to evaluate the effect of HMGB1 on these parameters. To this end, we assessed cell spreading and lysosome trafficking by immunofluorescence during B cell activation with immobilized antigens in the presence of HMGB1. Additionally, we studied the signalling pathways triggered by HMGB1 in B lymphocytes and the induction of migration by an under-agarose migration assay. Our results show that cell spreading and recruitment of lysosomes to the centre of the IS during B cell activation decrease in the presence of HMGB1. Additionally, we observed that HMGB1 signals through ERK1/2 and induces migration in B cells. Interestingly, B lymphocytes with migratory-like morphology, induced by HMGB1, showed delocalisation of Exo70 and GEF-H1 from the centrosome, which are essential to establish an IS. These results suggest that HMGB1 could act as a signal to restrict IS formation to promote B cell migration, which might be used by tumour cells to inhibit the immune response against cancer.

Funding: Beca de Doctorado Nacional, ANID 2020, 21200382.Dirección de Posgrado, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile. FONDECYT, 1221128.
Keywords: B cell, Immune synapse, HMGB1 cell migration

Evaluation of the Immunosuppressive properties of vesiculated mitochondria secreted from umbilical cord mesenchymal stomal cells (MSC)

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The therapeutic function of MSCs is achieved through cell-to-cell contact-dependent and independent mechanisms, including the release of paracrine factors, such as soluble molecules and extracellular vesicles (EVs). Also, MSCs possess the ability to donate mitochondria through cell-to-cell contact known as mitochondrial transfer. Artificial or co-cultured mitochondrial transfer from MSC increases oxidative phosphorylation in T cells, regulating their function and activating anti-inflammatory genetic pathways. Similarly, when isolated mitochondria from MSC are transferred to PBMCs, the proliferation capacity is inhibited through Treg induction. On the other hand, recent studies evidenced the presence of extracellular functional mitochondrion in peripherical blood, suggesting a physiological cell-to-cell free communication pathway. However, it is still unknow if MSC secrete vesiculated mitochondria (VesMito) and whether they are energetically functional or if they can modify the metabolic and transcriptional expression of a potential acceptor cell. First, we have demonstrated that isolated microvesicles (MVs) from the MSC- conditioned contain mitochondrial proteins and structures. Furthermore, we hypothesize that VesMito can emulate the immunosuppressive properties of an artificial or contact-dependent mitochondrial transfer to PBMCs. To assess this, isolated MSC-derived MVs were characterized in terms of their morphology, molecular markers, physical dimensions, and metabolic activity. The isolated MVs were incubated with PBMCs to see cellular subpopulations with increased affinity for these vesicles. Finally, immunosuppressive properties were evaluated in terms of proliferative capacity, pro-inflammatory cytokine secretion and surface membrane markers expression.

Funding: FONDECYT Regular 1211749
Keywords: Microvesicles, Mitochondria transfer, MSC

Effects of seasonal photoperiods on antigen-dependent immune responses in rainbow trout (Oncorhynchus mykiss)

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En este estudio, buscamos revelar los efectos potenciales de los regímenes de fotoperiodo en la inmunidad en salmónidos. Investigamos los efectos de los fotoperiodos artificiales estacionales, que imitan los solsticios verano e invierno y equinoccios, y un régimen de luz continúa usado en acuicultura (i) sobre las poblaciones de leucocitos de riñón anterior (HK) de trucha arcoíris, mediante citometría de flujo y (ii) la respuesta mediada por linfocitos T, evaluando los perfiles de expresión de genes marcadores por RT-PCR tiempo real. Se observó que los tratamientos de fotoperiodo inducen cambios en las poblaciones de leucocitos de HK, siendo el solsticio de verano (16L:8D) el que presentó un mayor porcentaje de células T CD4-1^{+} (Th) y de otras células linfoides no identificadas. Además, el fotoperiodo estacional (aunque de forma limitada) afecta la expresión de los genes marcadores evaluados, observándose diferencias en los niveles de *il-14/13a* y *il-10a* en el régimen 16L:8D, comparados con las otras condiciones estudiadas. En truchas inmunizadas con VP1r (proteína del Virus de la Necrosis Pancreática Infecciosa), se observaron respuestas únicas dependiendo del fotoperíodo. Las truchas mantenidas en 16L:8D presentaron una respuesta inmune de tipo 1, mientas que las mantenidas en 8L:16D mostraron respuestas de tipo 2. Los peces mantenidos en fotoperiodos 12L:12D y 24L:0D fueron hipo-respondedores. En conclusión, el fotoperiodo influye profundamente en el tipo de respuesta inmunitaria antígeno dependiente en peces, lo que puede impactar positiva o negativamente en los mecanismos de protección y desarrollo de memoria en salmónidos después del encuentro con patógenos o vacunación.

Funding: Agradecimientos a Fondecyt 1201664, Agencia Nacional de Investigación y Desarrollo (ANID)Proyecto Beca CONICYT, Doctorado Nacional Folio n° 21160061
Keywords: Fotoperiodo, Salmónidos, Citoquinas

Anti-inflammatory effect of boldine on macrophages stimulated with periapical exudate and heat-inactivated Porphyromonas endodontalis

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Introduction
Asymptomatic apical periodontitis (AAP) is a chronic inflammatory condition characterized by the destruction of the apical periodontium due to a polymicrobial infection of the endodontic dental canals. Boldine, is an alkaloid identified in boldo *(Peumus boldus)*, could have potential as a new therapy to treat AAP because of its anti-inflammatory properties. Our objective was to evaluate its anti-inflammatory effect and the optimum occasion of boldine administration in human macrophages emulating endodontic conditions.
Methodology
In this in vitro study, THP-1-differentiated macrophages were exposed to different concentrations of boldine and their viability was analyzed. Posteriorly, cells were stimulated either with periapical exudates from AAP patients or heat-inactivated *P. endodontalis* and exposed simultaneously or not to boldine. To assess the optimal occasion of boldine administration, another group of cells was pretreated with this alkaloid and subsequently stimulated with heat-inactivated *P. endodontalis*. The mRNA levels of TNF-α, IFN-γ, and IL-6 were determined by qPCR, and the activity of MMP-2 and MMP-9 in the supernatants was determined by zymography. Statistical analyzes were performed with STATA V12.
Results and conclusions
Boldine up to 100 ug/ml was safe based on macrophage viability. Apical exudates and heat-inactivated *P. endodontalis* resulted in increased gene expression and/or activity of previously evaluated mediators compared to unstimulated condition ($p<0.05$). In contrast, boldine pretreatment and simultaneous exposure to bacterial stimuli reduced the gene expression of cytokines evaluated, and the activity of MMP-2 and -9 in their active-form and pro-form ($p<0.05$). Therefore, boldine has the potential as anti-inflammatory intracanal medication in periapical diseases of endodontic origin.

Funding: FONDECYT 1200098
Keywords: Anti-inflammatory, Boldine, Macrophages

Anti-inflammatory iNKT cells activation by a novel liposomal formulation induces expansion of regulatory B cells

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Invariant Natural Killer T (iNKT) cells have become an attractive target for the generation of new immunological therapies, given their ability to secrete pro- and anti-inflammatory cytokines rapidly after their activation. Such cytokines can activate and modulate different immune cells, including the induction of the differentiation of B cells into regulatory B cells (Bregs).
Bregs cells possess the ability to modulate the immune response, promoting the reduction of inflammatory states and the restoration of immunological tolerance. Although it has been established that pro-inflammatory cytokine leads to the expansion of Bregs cells, it has not been evaluated whether anti-inflammatory cytokines can promote an increase in the frequency and regulatory activity of these cells.
In order to evaluate if anti-inflammatory cytokines secreted by activated iNKT cells lead to the expansion and activation of Bregs cells, we administered different liposomal formulations containing anti-inflammatory iNKT cells ligands and ovalbumin in a murine model.
Our results indicate that the administration of such liposomal formulations induce the activation of iNKT cells, leading to differential secretion of a wide range of cytokines, including IL-10. Such activation has led to the expansion and activation of antigen-specific Bregs cells. Furthermore, we have demonstrated that anti-inflammatory iNKT cells ligands that induce higher secretion of IL-10 by these cells cause a higher expansion of Bregs.
These initial results are fundamental for the generation of novel strategies aiming to decrease the inflammatory response and restore an adequate immune response in pathologies where it is altered, such as allergic asthma.

Funding: FONDECYT 1211959, Iniciativa Científica Milenio - ICN09_016: Instituto Milenio en Inmunología e Inmunoterapia (ICN09_016/ ICN 2021_045), FONDEF ID21I10335, ANID-PFCHA/Doctorado Nacional/2020-21202280.
Keywords: iNKT cells, Regulatory B cells, Liposomes

Role of the IRE1-XBP1 axis on lysosomal function in murine dendritic cells

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The unfolded protein response (UPR) is a cellular mechanism safeguarding endoplasmic reticulum (ER) proteostasis. IRE1 and its transcription factor XBP1s are the most studied branch of the UPR, which besides its canonical role, it also regulates the function of one subtype of dendritic cells (DCs) termed conventional type 1 DC (cDC1). Notably, in addition to activate XBP1s, the RNase domain of IRE1 can degrade diverse mRNAs in a process called RIDD (regulated IRE1 dependent decay). Among the described RIDD substrates there are mRNAs coding for proteins involved in lysosomal biogenesis, which is a key process in the cross-presentation of antigens to cytotoxic lymphocytes. However, despite this evidence, the role of IRE1 in phago-lysosomal dynamics and function has not been examined.
Here, we explore the interplay between IRE1 activation and phago-lysosomal maturation in cDC1s using OP9/DL1-DCs, a novel culture that recapitulates features of tissue derived cDCs.Using flow cytometry, qPCR and epifluorescence microscopy, we show that expression of the lysosomal marker Lamp1 is directly controlled by IRE1 through RIDD. PhagoFACS organelle-cytometry analysis in cDC1s indicate that in absence of IRE1, individual phagosomes alter their maturation process, accumulate Lamp1 at faster rates and degrade more OVA antigen. Furthermore, *in vivo* studies show that IRE1 is necessary for cross presentation of dead cells. Interestingly, these changes are not observed in XBP1-deficient cells, suggesting that IRE1 through its RNase domain coordinates lysosomal dynamics independently of the transcription factor.

Funding: Fondecyt 11611212HHMI 55008744
Keywords: lysosomes, dendritic cells, unfolded protein response

IXA4 as a novel drug for the activation of the IRE1/XBP1 axis in type 1 conventional DCs.

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The UPR is a conserved cell stress control mechanism with crucial roles in reticulum endoplasmic proteostasis. IRE1/XBP1 axis of the UPR, beside their canonical stress regulation function, controls the development and immune functions of cDC1. Interestingly, this axis is constitutively active in cDC1. Despite these antecedents, the action mechanisms of the pathway are uncertain and its impact on the development of these cells is unknown. New technologies and drugs have emerged that seek to facilitate the elucidation of these interrogants. Among them, the drug IXA4, which is a specific activator of IRE1 that does not induce reticular stress. In this project, we explored the effect of IXA4 on IRE1 activation and its role in cDCs development.
We used OP9/DL1-DCs, a novel culture that recapitulates features of tissue derived cDCs. Using ERAI mice, which reports IRE1 RNase activation by flow cytometry, we validated the splicing of XBP1 on IXA4 treated cells. Additionally using PCR and qPCR, we saw an upregulation on XBP1s confirming its effect at a transcriptional level. We explored the effect of IXA4 treatment on OP9/DL1-DCs differentiation during the days 3 and 6 of culture. Although our cytometry results did not show a preponderance of the IRE1 axis during the differentiation of cDCs subpopulations. This data highlights IXA4 as a novel drug that activates the IRE1 axis on cDCs. Further studies are required to assess a definitive interplay between this axis activation and the cDCs differentiation process.

Funding: Fondecyt 11611212HHMI 55008744Beca Anid 21211552
Keywords: IXA4, cDC1, Unfolded Protein Response

CHARACTERIZATION AND DIFFERENTIATION OF NKT10 LYMPHOCYTES: AN IN VITRO MODEL.

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Natural killer T (NKT) cells constitute a new subgroup of T lymphocytes that are specifically activated by antigens of glycolipid nature, such as α-Galcer, mediated by CD1d by antigen-presenting cells. Among them, 4 effector subtypes named NKT1, NKT2, NKT17 and, recently, NKT10 are described. In inflammatory and osteolytic diseases, NKT10 lymphocytes can promote immune-regulatory responses through the local production of IL-10. Thus, this work aims to characterize and differentiate NKT10 cells from spleen NK1.1 cells using an *in vitro* differentiation protocol. NK1.1+ cells were isolated from spleens of C57BL/6 mice by immunomagnetic separation/depletion. NK1.1+ cells were supplemented with IL-2 or α-Galcer for 14 days to promote differentiation to NKT10 lymphocytes. At day 14, in NKT10 lymphocytes, IL-10 levels were quantified by ELISA and *e4bp4*, *il-10*, *plzf* levels by qPCR. The NKT10 population was characterized by visualization of TCR, IL-10, E4BP4 and NK1.1 by immunofluorescence and determined the percentage of NKT10 cells. Supplementation of NK1.1+ cells with IL-2 and α-Galcer induced NK1.1+TCR+E4BP4+IL-10+ cells. In addition, higher levels of *e4bp4* and *il-10* expression and IL-10 secretion compared to IL-2 supplemented NK1.1+ cells. *In vitro* supplementation with IL-2 and α-Galcer in NK1.1+ cells promotes differentiation to IL-10- producing NKT10 lymphocytes.

Funding: Fondecyt Regular 1211959, Fondecyt Regular 1220999.
Keywords: NK1.1 cells, α-Galcer, IL-10

The increase of periodontal-derived extracellular vesicles is related to gestational diabetes during pregnancy: A cross-sectional study.

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Introduction: Periodontitis is an inflammatory disease affecting around 45-50% of global population and over 60% of pregnancies, being associated with an increased risk of development of preeclampsia, preterm birth and gestational diabetes mellitus (GDM). The links mechanisms between periodontitis and GDM remain unclear. We have postulated that periodontitis-derived extracellular vesicles (EVs) present in the gingival crevicular fluid (GCF), could modulate GDM risk. EVs are secreted by cells, carrying molecules such as proteins and non-coding RNA, being important mediators of cell-to-cell communication. Aim: to characterize GCF-derived EVs (GCF-EVs) from GDM vs healthy pregnancies. Methods: A cross-sectional study was conducted. Pregnant women were recruited at 24-32 gestational week. Demographic, obstetric and periodontal data were recorded, and oral glucose tolerance test was conducted for GDM diagnose. GCF samples were collected and EVs isolated by Exoquick. EVs size and concentration were calculated using a Nanoparticle Tracking Analysis. Expression of GCF-EVs markers (CD63, CD9, CD81, cytochrome c, syntenin and VLA-4) were assessed using multiplex technology. Results: Eighty-nine women were recruited and 33.7% of them were diagnosed with GDM. The GCF-EVs total concentration was higher in GDM vs healthy pregnancies ($p=0.04$). Furthermore, when comparing EVs distribution, higher exosome population ($p=0.04$) while similar micro-vesicles concentration ($p=0.06$), was observed in GDM pregnancies. In addition, surface EVs markers, such as tetraspanins CD9 and CD63, were lower in GDM-GCF-EVs ($p=0.04$ and $p=0.05$, respectively). Conclusion: A higher concentration of GCF-EVs (total EVs and exosomes), and different surface markers were observed in GDM, suggesting a role of periodontal EVs in GDM development.

Funding: FONDECYT 1211471
Keywords: Extracellular vesicles, gestational diabetes mellitus, periodontitis

Unfolded protein response sensor ATF6 regulate the cytokine expression but not costimulatory molecules in dendritic cells

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Background. Dendritic cells (DCs) are key in the coordination of the antiviral immune response mediated by CD8+ T. The priming of LT-CD8+ requires activated-DCs, and this activation is partially regulated by the unfolded protein response (UPR), which a cellular mechanism that regulates the fidelity of the cellular-proteome. The UPR axis regulated by ATF6 can induce the expression of inflammatory factors in infectious settings, but their overall contribution over DCs functions is largely unknown. In this project, we evaluated the role of ATF6 over the cytokine-expression and costimulatory molecules in a specific setting with TLR7-ligands plus palmitic-acid for DCs activation in a novel murine model ATF6 knock-out in DCs.
Method. The primary culture of GMCSF-derived DCs (GM-DCs) was established from transgenic mice deficient in ATF6, which has been activated with viral-agonist and lipid acids. The activity of the three branches of the UPR, cytokine and costimulatory molecules expression were analyzed by qPCR and flow cytometry. Additionally, the ATF6 expression and immune-cell population in ATF6-cKO were measured by qPCR and flow cytometry respectively.
Result. GM-DCs stimulated with TLR7- ligand plus palmitic-acids induce a strong activation of the three branches of UPR, together with a massive IL-23 expression. In contrast, TLR7-ligand alone induce a poorly UPR activation. Additionally, the deficiency of transcription factor ATF6 in GM-DCs decreased the cytokines expression of IL-6 and IL-12, but not TNFα. Interestingly, the ATF6 deficiency increase the transcription of IFNb1 but not IFNa4. Furthermore, costimulatory molecules CD86 and CD40 didn´t change their surface expression in GM-DCs activated.

Funding: FONDECYT 1200793, HHMI 55008744, CONICYT/Doctorado Nacional/2018- 21181584
Keywords: Dendritic cell, ATF6, TLR7/Palmitic acid

Class-switching recombination induced by Natural Killer T (NKT) cells in the context of a T-independent humoral response.

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The activation of B cells in a T-independent (TI) context does not require the cooperation of $CD4^+$ helper T lymphocytes, because TI antigens such as capsular polysaccharides or highly repeated macromolecules cause a cross-linking of B cell receptors (BCR) inducing their activation directly. Typically, this response only induces the production of IgM, however it has been reported that innate immune cells are able to induce antibody class-switch recombination towards different isotypes such as IgG subtypes. In this context, Natural Killer T (NKT) cells are innate-like immune cells that interact with B cells and produce different cytokines that could also induce class-switch recombination. NKT cells can be activated by the prototypical lipid α-galactosylceramide (αGalCer) presented in the context of CD1d molecule which is expressed by antigen presenting cells such as B cells. This induces the rapid production of mixed cytokines with proinflammatory, and anti-inflammatory properties defined as a Th0-like response. Interestingly, several analogues of αGalCer have been synthesized to induce a polarized cytokine response, as AH10-7 which induces a proinflammatory cytokine response, and OCH that induces an anti-inflammatory response. In this work, we observed that the administration of different analogs of α-GalCer contained in liposomal nanoparticles together with TI antigens (administered in a soluble format or in these liposomes) can modulate the response of B cells by inducing isotype changes, increasing serum IgG1 and IgG3 antibody titer in treatments that had the AH10-7 analog in their composition. These results provide attractive aspects for the use of αGC analogs as vaccine adjuvants.

Funding: FONDECYT 1211959, Iniciativa Científica Milenio – ICN09_016: Instituto Milenio en Inmunología e Inmunoterapia (ICN09_016/ ICN 2021_045), FONDEF ID21|10335
Keywords: NKT cells, T-independent humoral response, Class switch recombination.

Kinetics of IgG subtypes modulated by iNKT cell activation with analogous ligands in C57BL/6

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B cells activation requires the uptake of antigens through B cell receptor (BCR) and their presentation on the cell surface through the MHC for recognition by primed T lymphocytes. Invariant Natural Killer T (iNKT) is a non-conventional T cell that can help B cells promoting Class Switch Recombination (CSR) when these are activated by α-Galactosylceramide (α-GalCer); there are α-GalCer analogous ligands that can activate iNKT cells. In this work it was proposed the administration of liposomal nanoparticles containing different analogues of α-GalCer and the ovalbumin (OVA) protein anchored on their surface, can modulate the CSR generated from the B cells and iNKT cells interactions. α-GalCer-analogues can modulate the response of iNKT cells to cytokine production, generating Th1-type profiles, like AH10-7, or Th2-type profiles, like OCH; while α-GalCer can generate a mixed or Th0 profile. Antibody production in response to a 60-day post immunization protocol with two doses was evaluated by ELISA tests. The results show IgG3 levels peak at 14 days post second immunization, with AH10-7 generating the highest production of these antibodies; while the kinetics of IgG1 evidences AH10-7 and α-GalCer produces comparable antibodies levels, registering their highest point on day 7 post second immunization, and then undergo a decrease in their production. These data suggest AH10-7 enhances IgG3 production, while OCH causes a slight increase in all titers by performing a finer modulation. The study on iNKT cells allows to project them as a mechanism of immunotherapy to improve the response of B cells to pathogens.

Funding: Funded by FONDECYT 1160336, ICGEB CRP-CHL17-06-EC, Millennium Institute on Immunology and Immunotherapy P09/016-F
Keywords: iNKT Cells, α-GalCer analogues, liposomal nanoparticles

Effect of Th1-type and Th2-type activation of iNKT cells in Class-Switch Recombination of antibodies

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T helper (T_h) cells provide co-stimulatory molecules and cytokines that are directly involved in Class-Switch Recombination (CSR) of B cells. A non-conventional T cell, named invariant Natural Killer T cells (iNKT), can help B cells promoting CSR when these are activated by *α-Galactosylceramide* (α-GalCer). Unfortunately, a-GalCer is a glycolipid that induce a plethora of mixed cytokines by iNKTs meaning an ambiguous contribution to CSR. The design of a-GalCer-analogues, AH10-7 and OCH, has driven the cytokine production of iNKT cells toward a TH1-bias or Th-2 bias, respectively. Nevertheless, the effect that this polarization of cytokines has on CSR is unknown. Here, we evaluated the effect produced by the administration of AH10-7 and OCH delivered in liposomes (LPs) with Ovalbumin (OVA)-anchored, as protein model, on CSR of mice B cells. We measure circulating anti-OVA antibodies on sera, splenic class switched-B cells and iNKT cells by flow cytometry. The results show that LPs/OVA/AH10-7 produced an increase of circulating IgG2c$^+$, correlating them with more expansion of IgG2c$^+$ B cells than controls. On the other hand, LPs/OVA/OCH produced comparable levels of IgG2b with AH10-7 and α-GalCer, even though this ligand promoted the lesser expansion of iNKT cells. These results suggest that it is possible to polarize the humoral response toward IgG2c or IgG2b isotypes when AH10-7 or OCH are administrated, respectively. Our findings position iNKT cells as a potential immunotherapy tool to improve a B cells response against pathogens or restricting the harmful production of autoantibodies.

Funding: FONDECYT Nº 1211959, Iniciativa Científica Milenio - ICN09_016: Instituto Milenio e Inmunología e Inmunoterapia (ICN09_016/ICN 2021_045). FONDEF ID21I10335, Beca ANID Doctorado Nacional 2018 Nº 21180465.
Keywords: switched-B cells, humoral response, iNKT ligands

TNF-Alpha Induces M1 Macrophage and Antigen Presenting Cell Phenotype in the Rainbow Trout Cell Line Rts11

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In higher vertebrates, polarization to M1 macrophages by IFNγ and TNFα is characterized by the induction of proinflammatory and destructive activity. The role of TNFα in polarization M1 and antigen presentation in fish macrophages is poorly characterized, however TNFα is a powerful proinflammatory cytokine released by these cells during infection. In teleost, there are no studies that have characterized the role of TNFα on macrophage functionality. The aim of this work was to study at the phenotypic level whether rainbow trout macrophages stimulated with TNFa acquire the M1 phenotype (iNOS$^+$) and express molecules associated with antigen presentation to T helper lymphocytes. For this purpose, cells from *Oncorhynchus mykiss* cell line RTS11, were induced with 10 ng/ml of rTNFα (6, 24, 48, 72h) and the differential expression of M1 (iNOS$^+$ IL-1β$^+$) and antigen presentation markers (MHCII, CD83, CD80/86) was analyzed by RT-qPCR, flow cytometry and Immunofluorescence. At the transcriptional level, up-regulation of both M1 and antigen presentation markers was demonstrated. Flow cytometry and epifluorescence analyses confirmed the upregulation of iNOS and IL-1β at the phenotype level of M1 cells. On the other hand, at the protein level, a tendency to increase MHCII, CD83 and CD80/86 surface molecules was evidenced, and induction studies in the presence of antigens are required to verify the effect of TNFα. These results obtained *in vitro* are a contribution to the knowledge of fish immunity and have interesting applications for the improvement of antimicrobial activity in salmonids. Funding: Fondecyt 1191763, National Research and Development Master Program ANID/2022-22221529.

Funding: Fondecyt 1191763, National Research and Development Master Program ANID/2022-22221529.
Keywords: Fish macrophage, Macrophage polarization, Fish TNFα

Structural analysis of iNKT cell stimulation by α-GalCer-derived C6”-modified ligands in partially humanized mice

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Invariant natural killer T cells are unconventional T cells that upon stimulation secrete a wide array of cytokines. iNKT cells express a semi-invariant α/β TCR with the ability to recognize glycolipid ligands presented by surface molecule CD1d. α-Galactosylceramide can induce a potent yet diverse cytokine response, thus, to obtain a more polarized cytokine response α-GalCer-derived ligands with specific chemical modifications have been synthesized for immunotherapy applications.
Our aim was to identify ligands that induce a potent Th1-like biased response by activation of iNKT cells. C6”-modified α-GalCer derivatives were evaluated both in vitro via stimulation of iNKT cell hybridomas and in vivo by injection in CD1d knock-in mice. Ligands such as AH10-7, AH17-5 and AH17-6 were found to induce a potent Th1-like response in vivo due to high IFNγ secretion levels detected in serum of stimulated animals and by high cytokine production by iNKT cells observed by flow cytometry. By analyzing expanded iNKT cells following injection of different ligands, we demonstrate that the TCR repertoire is diverse and influenced by ligand structure. Structural analysis evidence important differences in ligand recognition in the context of human CD1d compared to mouse CD1d. Our results are in line with previously reported differences between mouse and human lipid antigen presentation in the context of CD1d and serve as a base for the immunotherapeutic potential of ligands such as AH10-7, AH17-5 and AH17-6 which could be evaluated in further assays with the partially humanized, human CD1d knock-in model.

Funding: Proyecto IMII P09/016-F.FONDECYT Nº 1211959.Beca Doctorado Nacional ANID 21171172.
Keywords: NKT cells, Immunotherapy, Innate immunity

Characterization of small extracellular vesicles obtained from different subsets of T regulatory cells

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Introduction. T regulatory cells (Tregs) act as modulators of the immune response and use the release of small extracellular vesicles (sEV) as one of the mechanisms of suppression. Tregs can be classified based on their origin: thymic or natural Tregs (nTregs) and induced Tregs (iTregs). Our group have shown that nTregs release sEV harboring Neuropilin-1 (Nrp1), a protein required for skin transplantation tolerance. The characteristics of sEV produced by other types of Tregs is unknown. Materials and methods. nTregs and naïve T cells were purified using magnetic beads. nTregs were cultured for 48h and iTregs were generated with IL-2 and TGF-b alone (iTregs) and complementing the media with retinoic acid (RATregs). sEV were purified using IZON columns. Size and number of particles were calculating using the Nano-tracking analysis (NTA) equipment. Suppression assay was performed polyclonally activating splenocytes for 72h in the presence of sEV obtained from the three types of Tregs. This assay and cell phenotype were analyzed by flow cytometry. Results. sEV obtained from the three-types of Tregs did not show differences in particle´s number or size. Also, sEV´s T cell proliferation blockade was dose-dependent and nTregs-derived sEV show the less effective inhibition. Discussion. Treg cells secrete sEV as part of their immune suppression mechanisms. Our results suggest that Tregs-EVs induced are most suppressors than the nTregs-EVs.

IMMUNITY AND INFECTION

Ex-vivo human term placental NF-κB and NLRP-3 inflammasome activation by Porphyromonas gingivalis-lipopolysaccharide and hyperglycemia

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Introduction: An epidemiological association between Periodontitis and Gestational Diabetes Mellitus has been reported. The translocation of periodontal bacteria into the placenta has been described, and placental proinflammatory activity generates positive feedback for hyperglycemia. The objective of this study was to explore the synergistic proinflammatory effect of *Porphyromonas gingivalis*-lipopolysaccharide and hyperglycemia in human-term placental explants. Methods: Healthy term pregnant women were recruited (n=7), placental chorionic villi explants were obtained and stimulated with the following conditions: 1) Normoglycemia, 2) Hyperglycemia, 3) Normoglycemia and commercially ultra-purified *P. gingivalis*-lipopolysaccharide (LPS), 4) Hyperglycemia and *P. gingivalis*-LPS (Dual stimuli). Toll-like receptor-4 (TLR-4) and cytokines IL-6, IL-1β, and TNF-α mRNA expression was explored by RT-qPCR; NF-κB (Phospho-p65/total-p65) and inflammasome NLRP-3 protein expression by Western Blot, and nuclear localization of NF-κB (p65 mean fluorescence intensity) through immunofluorescence. Statistical analysis of Friedman's or ANOVA multivariate comparison was performed according to the normality of the data distribution (alpha 0.05). Results: *P. gingivalis*-LPS increased IL-1β (p=0.001), and dual stimuli increased TNF-α (p=0.015) mRNA expression. TLR-4 and IL-6 mRNA expression showed a trend to increase with the dual stimuli (p=0.35 and 0.375, respectively). *P. gingivalis*-LPS increased NF-κB phosphorylation (p=0.017) and NLRP-3 protein expression (p<0.0001). Moreover, the dual stimuli increased the nuclear localization of NF-κB in c. villi explants (p<0.001). Conclusions: In these experimental conditions *P. gingivalis*-LPS and hyperglycemia synergistically increased placental proinflammatory activity, evidenced by NF-κB and NLRP-3 inflammasome pathway activation.
Acknowledgments: Grant FONDECYT 1211471 and Doctorado Nacional grant 2019-21190319, ANID, Chile.

Funding: Grant FONDECYT REGULAR 1211471, ANID Chile; and Doctorado Nacional grant 2019-21190319, ANID, Chile.
Keywords: Human term placental explants, Porphyromonas gingivalis, hyperglycemia

Patients who develop insulin resistance 4-months post-COVID-19 exhibited higher basal NETosis and higher miRNA-21-5p expression in comparison with COVID-19 patients without metabolic alterations.

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Long COVID-19 has been associated with alterations in the glucidic metabolism and the presence of metabolic-related miRNA. Since neutrophils responses depends on glycolysis, it is unclear whether the formation of neutrophil extracellular traps (NETosis) is also affected in patients with Long COVID-19 that developed insulin resistance post infection. In addition, miRNA-21-5p have been described as a direct gene regulator of the innate immune response against COVID-19 and the presence of insulin resistance. In this study, we evaluated NETosis at 4-months post-COVID-19 and its association with the development of insulin resistance and the expression of circulating miRNA-21-5p. Sixty post-COVID-19 patients were recruited from Víctor-Rios-Ruiz Hospital, and Long COVID-19 was evaluated 4-months after acute phase. Serum samples were used to determine glycemia, insulin and HOMA. Vital NETosis was measured in neutrophils from patients at basal condition and in response to TLR agonists using flow cytometry with SytoxBlue and Live/Dead dye. miRNA-21-5p expression was measured with qPCR. From our cohort, 19 patients were excluded because they exhibited previous alteration, 25 patients develop new IR and 16 remains without glucidic alteration. Our analysis showed that COVID-19 patients with IR-Post-COVID-19 have a significant increment of vital NETosis in basal condition, reducing the capacity to respond efficiently to TRL3/7/8 agonists. We also found a significant increment of miR-21-5p expression in IR-Post-COVID-19 patients and a significant and positive correlation between miR-21-5p and vital NETosis. These results demonstrated that patients who develop IR post-COVID-19 exhibited higher basal NETosis and higher miRNA-21-5p expression in comparison with patients without alterations.

Funding: Funding: PROYECTO COVID1005, ACT210085 y Fondecyt Regular 1211480.
Keywords: NETosis, Long COVID-19, miRNA-21-5p

Analysis of the Unfolded Protein Response Ire-1alpha Patwhay in Dendritic Cells Infected with Herpes Simplex Virus Type 1

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Herpes simplex virus type 1 (HSV-1) infections are lifelong and highly prevalent in the human population. These viruses persist in the host, eliciting either symptomatic or asymptomatic infections that may occur sporadically or in a recurrent manner through viral reactivations. HSV-1 can negatively modulate the function and viability of dendritic cells (DCs), eliciting their apoptosis after infection. Recently, we found that HSV-1 activates the unfolded protein response (UPR) IRE-1alpha pathway in DCs, producing the splicing of *XBP-1* mRNA. However, IRE-1alpha may also elicit regulated IRE1-dependent mRNA decay (RIDD), a process that helps overcome ER stress, but also can trigger a proapoptotic responses. Currently, it is unknown whether RIDD is activated in HSV-1 infected DCs and if it modulates infection. Using RT-qPCR, we observed reduced levels of the *bloc1s1* mRNA, and increased caspase-2 activity in HSV-1-infected DCs, suggesting RIDD activity. Interestingly, HSV-1-infected DCs lacking XBP-1, but preserving IRE-1alpha endonuclease activity display significant cell viability as assessed by flow cytometry. To date, our results suggest that RIDD is likely activated in HSV-1-infected DCs and may play additional roles to XBP-1 in the phenotype observed in HSV-1-infected DCs

Funding: Authors are supported by FONDECYT grant #1190864 and the Millennium Institute on Immunology and Immunotherapy #ICN09_016.
Keywords: UPR, HSV, Dendritic cells

The offspring gestated in hypothyroxinemia suffers a more severe herpes simplex virus type 1 infection and a higher inflammation in central nervous system tissue

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Hypothyroxinemia (HTX) is an asymptomatic thyroid hormone deficiency that is highly common during pregnancy, and which can affect the offspring's immune response. Previous studies show how the progeny gestated in HTX suffers enhanced experimental autoimmune encephalitis (EAE). In this study we analyzed the response of the progeny gestated in HTX to herpes simplex virus type 1 (HSV-1) infection, a virus that can enter the central nervous system (CNS), cause chronic neuroinflammation, and establish latency in neurons. The aim of this work was to determine if the progeny gestated in HTX suffer more severe infection with a higher inflammation in the CNS and in the peripheral nervous system (PNS) after herpes simplex encephalitis (HSE) signs. Importantly, we observed that only the offspring gestated in HTX exhibited manifestations of HSE. Furthermore, we found that the number of viral copies in offspring gestated in HTX was higher in the CNS than in the offspring gestated in euthyroid conditions. Lastly, the relative expression of cytokines was analyzed by RT-qPCR, finding a reduced expression of some proinflammatory cytokines in the brain of the offspring gestated in HTX in comparison to mice gestated in euthyroid conditions, while in the trigeminal ganglia, some proinflammatory cytokines had an increased expression in offspring gestated in HTX. The results of this study show that the offspring gestated in HTX have a different immune response towards HSV-1 infection. Acknowledgements to FONDECYT #1191300, #1190864 and the Millennium Institute on Immunology and Immunotherapy #ICN09_016.

Funding: FONDECYT #1191300, FONDECYT #1190864, Millennium Institute on Immunology and Immunotherapy #ICN09_016.
Keywords: HSV-1, hypothyroxinemia, herpes simplex encephalitis

Herpes Simplex Virus Type 1 Elicits Lipid Droplet Accumulation In Dendritic Cells Modulating Negatively Their Function

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Herpes simplex virus type 1 (HSV-1) is a prevalent human pathogen that produces lifelong infection through latency in neurons. HSV-1 infects dendritic cells (DCs), deteriorating their viability, and affecting their maturation and capacity to activate T cells. Lipid droplets (LDs) are neutral lipid-rich organelles mainly related to energy reservoirs, although also with immune system regulation, wherein LD accumulation in DCs impairs T cell activation. Here, we report that HSV-1 induces LD accumulation in DCs determined by confocal and transmission electron microscopy. RT-qPCR analyses reveal that HSV-1 infection significantly modulates the expression of neutral lipid metabolism associated-genes in DCs. Moreover, the inhibition of triacylglycerol (TAG) and cholesterol ester (CE) biosynthesis reduces HSV-1 release from DCs, while fatty acid transport protein (FATP) inhibition reduces viral protein expression and HSV-1 yield. Importantly, the inhibition of CE synthesis and FATP recovers the viability of DCs and promotes IL-2 and IFN-*gamma* secretion by $CD8^+$ virus-specific T cells in DC-T cell co-cultures. Finally, the inhibition of CE synthesis promoted dermal DC migration to the draining lymph node and virus-specific T activation. Taken together, our results suggest that HSV-1 induces LD accumulation and neutral lipid metabolism alterations in DCs that negatively impact their viability and capacity to activate virus-specific T cells.

Funding: The authors are funding by FONDECYT #1190864 and IMII #ICN09_016. MAF is an ANID fellow #21191390.
Keywords: Herpes simplex type 1, Lipid droplets, Dendritic cells

MicroRNAs in the regulation of inflammatory response in apical periodontitis

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Introduction: MicroRNAs (miRNAs) play a crucial role in regulating inflammation. This study aimed to determine the expression of miR-181-5p, miR-16-5p, miR-150-5p, and miR-146a-5p and their association with the transcriptional regulation of inflammatory genes in asymptomatic and symptomatic forms of apical periodontitis.
Methodology: Cross-sectional study. Periodontal apical tissues (PATs) were obtained from volunteers scheduled for tooth extraction with a diagnosis of asymptomatic apical periodontitis (AAP, n=16), symptomatic apical periodontitis (SAP, n=18), or healthy periodontal ligament (HPL, n=15). Total RNA was extracted, and the miRNAs for miR-181-5p, miR-16-5p, and miR-146a-5p and miR-150-5p and mRNAs for VEGF-A, TRAP, NFkB, and HIF-1a expression were analyzed using qRT-PCR. The miR-155-5p and 18S-ribonucleic RNA were used to normalize the expression of miRNA and mRNA, respectively. Multiple modeling were performed to understand the influence of miRNA and apical inflammation on gene transcription.
Results: miR-181-5p, miR-16-5p, and miR-146a-5p were downregulated, whereas miR-150-5p was upregulated in AAP and SAP compared to HPL ($p<0.05$). The multivariate analysis in apical inflammation revealed that miR-16-5p downregulated the IL-6 and HIF-1a mRNA expression, whereas miR-150-5p enhanced the VEGF-A mRNA expression ($p<0.05$). Finally, AAP influenced TRAP mRNA expression as both AAP and SAP influenced NFkB mRNA expression ($p<0.05$).
Conclusions: miR-181-5p, miR-16-5p, miR-146a-5p downregulation, and miR-150-5p upregulation might contribute to SAP clinical presentation. Moreover, miR-16-5p and miR-150-5p regulate the transcriptional activity of pro-inflammatory genes in apical inflammation.
Acknowledgment: Thanks to the Doctorado Nacional grant 21181377 and 2019-21190319, ANID, Chile.

Funding: Grant FONDECYT 1200098 and Grant FONDECYT 1211471, ANID, Chile.
Keywords: periapical periodontitis, MicroRNAs, inflammation

Cytotoxicity NK-hub genes involved in COVID-19 disease progression distinguish mild from severe outcomes

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Introduction: Diverse clinical manifestations are associated with severe acute respiratory coronavirus 2 (SARS-CoV-2) infection, involving several dysfunctions triggered by a broad spectrum of immune responses in the human. The study of transcriptional programs displayed by immune cells can aid in the discovery of immune functions associated with severity progression.
Methodology: We performed a longitudinal RNA-seq analysis of PBMCs at three different sampling times to identify transcriptional programs underlying the effective immune response mounted during SARS-CoV-2 pathogenesis (0, 7, and 28 days after recruitment). Using Differentially Expressed Genes, Gene Set Enrichment Analysis, and Weighted Gene Co-expression Network Analysis, we compared the transcriptional programs of PBMCs isolated from mild outpatients to those obtained from severely hospitalized COVID-19 donors from southern Chile.
Results: We discovered transcripts that were consistently associated with a specific pathway of Natural Killer [NK] cell-mediated cytotoxicity in mild patients during acute phase. In this regard, we discovered critical NK hub-genes that distinguished mild from severe progression, including activating and inhibitory receptors such as *KLRC3*, *KLRC1*, *KIR3DL2*, as well as other cytotoxicity-related genes such as *KLRD1*, *CD247*, and *IFNG*. We also described an interconnected immune response associated with cytokine-cytokine receptor interaction and Th1/Th2-cell differentiation as part of a transcriptional program being preferentially executed across mild patients.
Conclusions: Our results not only unveil a transcriptional program that engages multiple regulatory checkpoints of innate NK cell cytotoxic activity, but also suggest that its early fate-commitment triggers a proper adaptive immune response linked to better resolution of COVID-19.
Acknowledgment: COVID-19 South Chile Group.

Funding: COVID0422FONDEF ID20I10192
Keywords: NK-cytotoxicity, Longitudinal-RNASeq, COVID19-Severity

Role of Cyclooxygenases Cox-1 and Cox-2 over the Function of Herpes Simplex Virus Type 1-Infected Dendritic Cells

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Herpes simplex virus type 1 (HSV-1) is a prevalent microorganism that produces lifelong infections in humans by establishing latency in sensory and autonomic neurons. HSV-1 also infects dendritic cells (DCs), which are professional antigen-presenting cells that initiate and regulate antiviral immune responses. Importantly, HSV-1 modulates DC function and kill these cells. Cyclooxygenases (COXs) are host enzymes that metabolize arachidonic acid into prostaglandin G2 (PGG2), which is subsequently converted into prostaglandin H2 (PGH2). PGH2 which acts as a precursor for PGE2, PGD2, and PGI2 synthesis, as well as thromboxane (TXA2), which are involved in inflammatory and non-inflammatory processes. COXs and their products have been reported to participate in the modulation of pro-inflammatory responses in Kaposi's sarcoma herpes virus (KSHV) infections. Furthermore, COX-2 products can suppress the functions of DCs, natural killer cells and T cells. We explored the role of COXs over the modulation of DC function after infection with HSV-1. Importantly, we found that HSV-1 infection significantly modulates the expression of COX-2 in infected-DCs, as determined by RT-qPCR and Western Blot, and that the pharmacological inhibition of COX-2 recovers the viability of DCs infected with this virus and promotes T cell activation. Interestingly, the pharmacological inhibition of COX-2 did not impact the yield of HSV-1 plaque-forming units. These results indicates that HSV-1 induces COX-2 expression in DCs, which relates to cell death. Authors are supported by FONDECYT grant #1190864 and the Millennium Institute on Immunology and Immunotherapy #ICN09_016.

Funding: FONDECYT grant #1190864 and the Millennium Institute on Immunology and Immunotherapy #ICN09_016.
Keywords: Cyclooxygenase, Dendritic cells, herpes simplex virus

Sustained presence of proinflammatory chemokines CXCL9 and CXCL10 are associated with higher anti-SARS-CoV-2 IgG antibodies levels, but not with RBD-specific B cell percentages in patients with Long COVID-19

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Background: It has been reported that the humoral response and cytokine secretion is exacerbated in patients with severe COVID-19. However, it is unknown which cytokines are associated with the exacerbated humoral immune response during and after COVID-19. The aim of this study was to determine the association between cytokines and the humoral response of patients who had COVID-19 with different severity degree.
Methods: 60 COVID-19 patients were recruited from Víctor-Ríos-Ruiz Hospital during acute phase at 4-months post-infection. Serum samples were collected to measure IgG levels (Nucleocapsid + Spike) using commercial CLIA assays and cytokines and chemokines by Cytometric Bead Array (CBA). Percentage of B cell subsets and RBD-specific B cells were measured by flow cytometry.
Results: Anti-SARS-CoV-2 IgG levels were significantly associated with severity and lung structural damage. B cell subsets and RBD-specific B cells were not associated with severity or pulmonary sequelae. Moreover, the data revealed that proinflammatory chemokines CXCL9 and CXCL10 were significantly correlated with Anti-SARS-CoV-2 IgG levels, but not with the percentage of RBD-specific B cells.
Conclusions: Severe COVID-19 patients produced higher IgG levels than patients with mild disease, possibly due to an exacerbated immune response, associated with sustained levels of proinflammatory chemokines CXCL9 and CXCL10. This exacerbated response was associated with structural lung damage post-COVID-19. RBD-specific B cells were not associated with Anti-SARS-CoV-2 IgG levels, disease severity or chemokine levels.

Funding: COVID1005 project, Fondecyt Regular 1211480, ACT210085
Keywords: Long COVID-19, IgG anti-SARS-CoV-2, CXCL9 and CXCL10

Circulating Levels of Cxcl-9 and Platelets are Augmented in Patients with Long-Term Pulmonary Dysfunction 4- and 12- Months after Covid-19

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SARS-CoV-2 is the etiological agent of COVID-19. Severe COVID-19 is characterized by marked cytokine release and pulmonary dysfunction; however, it is still unknown which mediators support post-COVID-19 pulmonary dysfunction. Our aim was to determine the signaling pathways associated with long-term pulmonary dysfunction in patients with COVID-19.
Our cohort of 60 patients with COVID-19 were followed up at 4- and 12-months post infection. Pulmonary sequelae were analyzed at both time points using computed tomography (CT), diffusing capacity of carbon monoxide (DLCO), spirometry, 6-minute walk test (6MWT) and handgrip strength test (HGS). Cytokine analysis was performed using flow cytometric bead array in serum and cell counts were obtained from blood tests. Furthermore, signaling pathways associated with pulmonary dysfunction were identified by serum proteomics analysis using TimsTOF-Pro.
Fourteen patients with CT and abnormal DLCO were classified as patients with long-term pulmonary dysfunction (LTPD), who also presented more abnormalities in spirometry tests, 6MWT and HGS than patients without sequelae or patients with CT only. Analyzing inflammatory parameters, we observed that CXCL-9 and platelet aggregation were significantly increased in LTPD patients in comparison with other groups. In addition, the main signaling pathways identified by proteomics in LTPD patients were associated with an immunothrombotic state related to heart damage and a decreased Th_1 response mediated by IFN-γ regulation.
Conclusion: Patients with LTPD presented a restrictive lung condition, with greater fatigue, lower aerobic capacity and muscle strength 4 months post-COVID-19. This was associated with immunothrombosis and alterations in Th_1-type proinflammatory pathways.

Funding: PROYECTO COVID1005, ACT210085 y Fondecyt Regular 1211480.
Keywords: cytokine storm, post COVID-19 syndrome, pulmonary sequelae

Activation of the IRE1 axis in conventional dendritic cells type 1 upon stimulation with poly I/C

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Conventional type 1 (cDC1) dendritic cells are crucial for eliciting a cytotoxic T cell response against infection with influenza virus. Remarkably, although cDC1s are not directly infected by the virus, these cells can capture and process virally infected cells for the initiation of immunity. In this context, the unfolded protein response (UPR) sensor IRE1 is a critical regulator of cDC1 function, which also can be activated by viral infection various cell types. However, it is unclear which is the contribution of IRE1 to antiviral immunity via cDC1.
Using culture of bone marrow-derived OP9/DL1-cDC1s, we determined that these cells activate the IRE1 branch of the UPR upon viral recognition using the viral RNA agonist poly I/C, using the ERAI reporter mice for IRE1 RNase activity and qPCR. These data suggest that cDCs activate the UPR upon viral recognition. Current work is aiming to elucidate the role of this UPR branch in the recognition of influenza-infected cells and its consequence in innate and adaptive immunity. We will further dissect the role of the axis through the study of transgenic mice deficient for the RIDD branch only in settings of viral infection.

Funding: Proyecto Fondecyt 1200793
Keywords: Dendritic cell, Influenza Virus, Unfolded protein response (UPR)

INDEX

A

Aaron Fierro · 30
Akos Vegvari · 16
Alba Mendis · 10
Alberto Martin · 18
Aldo Figari · 82
Alejandra Chaparro · 82, 91, 96
Alejandra Fernández · 67, 96
Alejandra Gleisner · 25, 35, 41
Alejandra Herrera · 62
Alexandra Espinoza · 9, 24
Alexis M. Kalergis · 23, 50, 52, 53, 93, 95
Alexis Veliz-Ahumada · 55
Alfredo Sierra Cristancho · 49
Alfredo Torres Pérez · 16
Almendra A. Castillo · 93, 94
Alonso Lira · 79, 80
Álvaro Lladser · 9, 19, 32
Álvaro Santibáñez · 11, 59, 78, 84, 85, 86, 88
Amada Arcaya · 66
Amarilis Pérez Baños · 35, 41
Amber Philp · 48
Amy R. Howell · 88
Ana Maria Madrid · 44
Ana María Vega-Letter · 12, 60
Andrés Caicedo · 28
Andrés Hernández · 19
Andrés Tittarelli · 41
Angel Oñate Contreras · 38
Angela C Court · 71
Angélica Michea · 16
Angeline Chen · 10
Anilei Hoare · 82, 91
Antonella Sanguineti · 37
Antonia Dominguez · 37
Antonia Geisse · 46
Araceli Pinto-Leon · 44, 48
Areli J. Navarro · 93, 94, 95, 98

B

Bárbara Antilef Cáceres · 28, 92, 99, 100
Barbara Bruna · 25
Bastián Lillo · 53
Benjamín Basterrechea · 30
Benjamín Diethelm Varela · 53
Bernardita Medel · 79, 101
Brandon Martínez · 42
Brian Parra-Tello · 26, 34, 40
Bruce Cassels · 77

C

Camila Estay · 45
Camila Muñoz Grez · 28, 38
Camila Pinto-Leiva · 47, 89
Camila Sanchez · 15
Camilo Cabrera · 28, 92, 99, 100
Camilo Venegas · 25, 56
Carlos Alvarez · 65
Carlos Barrera-Avalos · 31
Carlos Flores · 48
Carol San Martin · 25
Carolina Pradenas · 12
Carolina Prado · 9, 26, 46
Carolina Reyes · 48
Carolina Rivera · 89
Carolina Rojas Pérez · 49, 89
Carolina Schäfer · 59, 86, 88
Caroll J. Beltrán · 44, 48
Catalina A. Andrade · 23, 50
Catalina Araneda · 30
Catalina Moreno · 20

Ch

Christina Schuh · 29

C

Claudia A. Riedel · 94
Claudio Acuña-Castillo · 31
Claudio Pérez · 56, 61
Claudio Quevedo · 97
Constanza Méndez · 53
Constanza Vilchez · 26
Cristián Gutiérrez-Vera · 11, 59, 78, 84, 86, 88
Cristian Pereda · 35, 41
Cristian Valenzuela · 87
Cristóbal Madrid · 62
Cristobal Riveros · 44

D

Daniel Betancur Castro · 38

Daniel Ernst · 39
Daniel Fernández · 15
Daniel Pacheco · 62
Daniel Rivas · 85
Daniela Parada · 30, 45
Daniela Ponce · 25
Daniela Sauma · 34, 36, 40, 42, 58, 70
Daniela Schneider · 59
Daniela Siel · 55
Daniela Simian · 45
Darío Donoso · 75
David De Gonzalo · 92
David González · 77
Dayesi López · 94
Débora Zamorano · 77
Diego Figueroa · 32
Diego Irribarra · 71
Diego Rebolledo · 53
Dolores Busso · 82, 91
Dominique Fernández · 33, 46, 66, 83
Douglas J. Matthies · 88

E

Eduardo A Catalán · 50
Eduardo I. Tognarelli · 95
Eduardo Martinez · 18
Eduardo Roa · 32
Eduardo Silva · 72
Eduardo Tognarelli · 94, 98
Eleni Phung · 10
Eliana Lara-Barba · 60
Eliseo Eugenin · 37
Eliseo Parra · 71
Emilio Seguel · 62
Erick Riquelme · 18, 38, 54
Ernesto López · 9
Estefania Nova Lamperti · 28, 38, 54, 92, 99, 100
Eva Rekus · 34
Eva Vallejos-Vidal · 76

F

Fabian Tempio · 25
Fabiola Osorio · 32, 33, 46, 66, 79, 80, 83, 93, 101
Farid Llerena · 100
Farida Djouad · 12
Farides Saavedra · 19
Faryd Llerena · 92, 99
Felipe A. Cancino · 93, 94, 95, 98
Felipe Aguilera · 97
Felipe Del Valle · 17, 79
Felipe Flores-Santibáñez · 32, 33
Felipe Gálvez-Jirón · 47
Felipe Malgue Morales · 42
Felipe Melo-Gonzalez · 50
Felipe Ramírez · 87
Felipe Reyes-López · 76
Felipe Zamorano · 62
Felipe Zuñiga Albarti · 28, 38
Félix Urra · 45
Fermín González · 16
Fernanda Antilén · 59
Fernanda Cabrera Reyes · 69
Fernando Figueroa · 72
Flavio Salazar-Onfray · 25, 35, 41
Florencia Valdés · 77
Francesca Velarde · 71
Francisca Espínola · 31
Francisca Gutierrez · 46, 83
Francisca Hofmann · 32
Francisco Contreras · 9
Francisco F. Otero · 11, 84
Francisco Fuentes-Villalobos · 97
Francisco J. Rivera · 48
Francisco Osorio-Barrios · 9

G

Gabriel Ascui · 10
Gabriela Oses · 48
Gemma Navarro · 9, 24
Gerald Zapata-Torres · 88
Glauben Landskron · 30, 37, 45
Gonzalo Labarca · 92, 99, 100
Gonzalo Vásquez · 30, 37, 45
Guillermo Antonio Díaz Araya · 14

H

Hector Molina · 45
Hilde Cheroutre · 10
Humberto Reyes · 53

I

Ignacio Jérez · 47
Ilan Camhi · 30
Isabel Méndez · 62
Isidora D Suazo · 50
Iu Raïch · 9, 24
Ivania Valdes · 18, 54

J

J. Rodrigo Mora · 13
Jacob Mora · 24
Jaime Díaz-Zúñiga · 81
Jaime Lastra · 100

Javier Campos · 9
Javier Cerda · 39
Javier López · 80
Javiera Carrasco-Rojas · 29
Javiera De Solminihac · 47, 49, 89
Javiera Jiménez · 94
Javiera Reyes · 19
Javiera Sepúlveda-Álfaro · 50
Jessica Astorga · 77
Jihye Han · 10
Jimena Zubieta · 15
Joaquín Espinoza · 20
Jonathan Lillo · 61
Jonathan Morales · 83
Jorge A. Soto · 23, 50, 52
José Antonio Jara Sandoval · 29
José Corrales Bermúdez · 42
Jose Ignacio Bernales · 79
Jose Luis Garrido · 97
Juan Fuentes · 62
Juan Pablo Araya · 35, 41
Juan Pablo Bozo Olea · 68
Juan Pablo Saavedra Almarza · 42
Juan Pablo Vega · 32
Justine Castañeda · 70

K

Karen Bohwmald · 23
Karen Dubois-Camacho · 30, 45
Karina Pino-Lagos · 47, 49, 89
Kathleen Caron · 10
Katina Schinnerling · 62
Klaas Nico Faber · 45

L

Lara J. Monteiro · 91
Leandro J. Carreño · 11, 59, 78, 81, 84, 85, 86, 88
Leonardo Saénz · 55
Leonardo Vargas · 58
Lesley Smyth · 49
Lilian Poblete · 70

Ll

Lluis Tort · 76

L

Loreto Abusleme · 20
Lucas Cereceda · 72
Lucía Valenzuela-Pérez · 48
Luciano Ferrada Cofré · 28, 38
Luis Alarcón · 19
Luis González-Osuna · 49
Luis Mercado · 87
Luisa F. Duarte · 94, 95

M

Mabel Vidal · 92
Macarena Badilla-Alfaro · 44
Marcela Hermoso · 30, 37, 45
Marcela Hernández · 67, 77, 82, 91, 96
Marcelo Rodriguez · 20
Marco Azua · 87
Marco Fraga · 19, 92, 99, 100
María Francisca Gutierrez · 66
Maria García · 92
Maria Ines Barria · 97
Maria Isabel Beherens · 25
María Isabel Yuseff · 17, 39, 68, 69, 73, 74, 79
Maria Jesus Santillan Araneda · 87
María José Bendek · 82, 91, 96
Maria Jose Bordagaray · 67
María José Pino · 58, 63
María José Vásquez Vidal · 101
María Luisa Mizgier · 82, 91
Maria Luisa Rioseco · 97
María Rosa Bono · 33, 34, 40, 42, 46, 58, 63, 70
Mario A. Ramírez · 52
Mario Abedrapo · 37
Mario Calvo · 97
Mario Henríquez · 92, 99, 100
Mario Rosemblatt · 34, 40, 42, 58, 63, 70
Marion Arce · 16, 20
Marjorie De la Fuente · 30, 37, 45
Maroun Khoury · 71, 75
Matias Medina · 97
Mauricio Hernandez · 100
Mauricio Zambra · 37
Merari Simei Goldstein Vasquez · 76
Mercedes Lopez · 25
Michelle García Reyes · 49
Miguel L. Allende · 36
Mitchell Kronenberg · 10
Moira García Gómez · 34, 40
Mónica A. Farías · 93, 95, 98
Monica Guzman · 101
Monica Imarai Bahamonde · 76
Montserrat Reyes · 20
Muriel Nuñez · 39

N

Nancy Vásquez · 62
Nicolás Dutzan · 20
Nicolás M.S. Gálvez · 52

Nicolás Valdivieso · 58, 63
Noymar Luque-Campos · 12, 60

O

Omar Barría · 35, 41
Omar P Vallejos · 50
Oreste Corrales Vázquez · 39, 74
Orlando Ramírez · 29
Ornella Realini · 82, 91

P

Pablo A. González · 52, 53, 93, 94, 95, 98
Pablo A. Palacios · 11, 59, 78, 84, 86, 88
Pablo Besa · 62
Pablo Castro-Córdova · 71
Paola Murgas · 15
Patricia Luz-Crawford · 12, 28, 60, 71
Paulina Espinosa · 58, 63
Pedro H Silva · 50
Pedro Leal · 24
Pía Vidal · 24
Pieta Mattila · 17

R

Rafael Franco · 9, 24
Raul Riquelme · 97
Ricardo Ibáñez · 62
Richard García-Betancourt · 11, 59, 78, 81, 84, 86, 88
Roberto Elizondo-Vega · 12
Rodrigo Pacheco · 24, 26, 46
Rolando Vernal Astudillo · 49, 81
Roman Zubarev · 16
Romina Falcón · 35, 41
Romina Quiroga · 28, 92, 99, 100

S

Samanta Melgar-Rodríguez · 81
Sara Hernández-Pérez · 17
Sarah Nuñez · 70
Sebastián Aguayo · 29
Sebastian Barria · 97
Sebastian Castro · 77
Sebastián E. Illanes · 82, 91
Sebastián Fuentes-Retamal · 45
Sebastián González · 59
Sebastián Irarrázaval · 62
Sergio Muñoz · 53
Sergio Sanhueza · 28, 92, 99, 100
Shilpi Chandra · 10
Silvana Valdebenito-Silva · 37
Silvana Zanlungo · 69
Sofia Hidalgo · 19
Sofia Monje · 82
Sofie Rennen · 33
Solange Cisterna · 28
Solange Gouët · 42
Sonia Vidal · 55
Sophie Janssens · 33
Steven A. Porcelli · 88
Susan M. Bueno · 50, 52, 53

T

Teemly Veronica Contreras Palacios · 73
Ting-Fang Chou · 10
Tomás Perez-Acle · 63

V

Valentia Wong-Benito · 76
Valentina Mora · 23, 65
Valentina Ugalde · 9, 24
Valeria Ramirez · 82, 91
Valeska Simon · 58, 63
Vincenzo Borgna · 19, 32
Violeta Kallens · 36
Viviana Montecinos · 39

W

Wilfredo González · 28, 38

Y

Yazmin Pinos · 97
Yeimi Herrera-Luna · 60
Yessia Hidalgo-Fadic · 12

Z

Zoé K. Engels · 35