SCI
SOCIETAT CATALANA D'IMMUNOLOGIA
L'Acadèmia

L'Acadèmia

2 & 3 NOVEMBER 2023

XVII ANNUAL SCIENTIFIC CONGRESS
Catalan Society of Immunology

The overreacting immune system against non-self

SCI President,
Dr. Francisco Lozano Soto

Congress Office Contact,
Sr. Xavier Nieves
xaviernieves@academia.cat

A word from the Organizing Committee

Welcome to the congress,

On behalf of the organizing committee, we would like to warmly welcome you to the XVII Societat Catalana d'Immunologia Congress (SCI Congress). We believe that our meeting will present high level scientific knowledge with the contribution of immunologists and different specialists in areas related to The overreacting immune system against non-self.

Dr. Francisco Lozano, SCI President

SOCIETAT CATALANA D'IMMUNOLOGIA
L'Acadèmia

SCI Board

President: Francisco Lozano Soto
Vice President: Eva Martínez Cáceres
Secretary: Andrea García
Treasurer: María José Herrero Mata
Vocal: Iñaki Salvador Corres
Vocal: Carles Serra Pagès
Vocal: Rebeca Gutiérrez Cózar
Vocal: Aina Aguiló Cucurull
Vocal: Mª José Rodríguez Lagunas
Vocal: Anaïs Mariscal Rodríguez

Scientific committee

Pablo Engel Rocamora
Nuria Izquierdo-Useros
Iñaki Álvarez Pérez
Ester Lozano García
José Yélamos López
Concepción Mora Giral

Contents

Awards to best communicator and to best poster of the XVII Congress SCI 2023 - *sponsored by the SCI*:

This year, SCI sponsors the awards for **best communication** and **best poster** of the congress. The Chairpersons of the different sessions and the board members of the *SCI* will select the best oral communication, taking into account its scientific value and the aspects related to the presentation, and the congress attendees will elect the awarded poster. The winners will be announced at the end of the congress.

Collaborators

Consell Català de Formació Continuada Professions Sanitàries

Palex Constant Improvement

UAB Universitat Autònoma de Barcelona

labclinics

Universitat de Barcelona

Mc Graw Hill

cytiva

SCAIC Societat Catalana d'Al·lèrgia i Immunologia Clínica

Sponsors

PLATINUM

QIAGEN

Binding Site
part of Thermo Fisher Scientific

GOLD

BD
BD Biosciences

BECKMAN COULTER

SILVER

Werfen

MA
MULTI ARRAY XPLORER 45k

Miltenyi Biotec

ThermoFisher
SCIENTIFIC

BRONZE

BioSystems

DIAGNÓSTICA
LONGWOOD

EUROIMMUN

CSL Behring
Biotherapies for Life®

A.MENARINI
diagnostics

Condensed Programme: DAY 1

16:00h 16:15h	**Welcome to the XVII CONGRESS of the SCI** *Francisco Lozano* President of the SCI
16:15h 18:00h	**Joint Symposium SCI-SCAIC** Antigen-specific insights into memory B cells in allergic disease ***Menno van Zelm*** Dept. of Immunology, Erasmus University Medical Center, Rotterdam, the Netherlands Nanotechnology in drug and food allergy ***Cristobalina Mayorga*** Nanomedicine Platform, Málaga Biomedical Research Institute and Málaga Regional University Hospital, Málaga, Spain Novel strategies targeting dendritic cells for allergic diseases ***Oscar Palomares*** Dept. of Biochemistry and Molecular Biology, School of Chemistry, Madrid Complutense University, Madrid, Spain **Chairs:** ***Moises Labrador*** Vall d'Hebron University Hospital, Barcelona, Spain; ***Mariona Pascal*** Hospital Clínic of Barcelona , Barcelona, Spain
18:00h 18:30h	**Coffee break @ posters**
18:30h 20:00h	**Oral communications** **Session I: Allergy and Inflammation** Heterogeneous IL-9 production by circulating skin-tropic and non-tropic memory T cells in atopic dermatitis patients ***Irene García-Jiménez et al.*** Tolerance through Macrophages: Alterations in Type 1 Diabetes and Response to Antigen-Specific Immunotherapy ***Ivan Garcia-Loza et al.***

Condensed Programme: DAY 1

18:30h 20:00h	Multiple sclerosis: new approach for the generation of tolerogenic dendritic cells induced with vitamin D3 *Roger Domenech Garcia et al.* Platelets as possible biomarker candidates to differentiate between neurodegenerative disorders. ***Noelia Arias et al.*** Detection of anti-PL7 autoantibodies by a commercial line immunoassay versus gold standard immunoprecipitation ***Janire Perurena-Prieto et al.*** Papel de la incompatibilidad KIR-HLA I en el desarrollo de inflamación microvascular con y sin anticuerpos donante-específicos postrasplante renal ***Judith Federico-Vega et al.*** **Chairs:** ***Iñaki Salvador*** Vall d'Hebron Institute of Oncology, Barcelona, Spain; ***Joan Bartra*** Barcelona Clínic Hospital and University of Barcelona, Barcelona, Spain

Condensed Programme: DAY 2

09:30h 10:10h	**Plenary Session I** **Identifying new regulators of type-2 inflammation** ***Andrew McKenzie*** MRC Laboratory of Molecular Biology, Cambridge, United Kingdom **Chair:** *José Yélamos* Hospital del Mar Research Institute, Barcelona, Spain
10:10h 10:40h	**Sponsored Talk I** Binding Site part of Thermo Fisher Scientific **Hevylite®: a unique biomarker for Monoclonality and Immunoparesis in Multiple Myeloma** ***Rafael Ríos-Tamayo*** Dept. of Hematology, Puerta de Hierro University Hospital, Majadahonda, Spain
10:40h 11:00h	**Coffee break @ posters**
11:00h 12:30h	**Oral communications** **Session II: Basic Immunology and Immunodeficiencies** Deciphering the crosstalk between cDC1 and cytotoxic T-cells and NK-cells ***Georgina Flórez-Grau et al.*** Type I interferonopathies, a macrophage disease ***Carlos Batlle i Recoder et al.*** End-Binding protein 1 regulates the metabolic fate of CD4+ T lymphocytes through the organization of the mitochondrial network ***Álvaro Gómez-Morón et al.*** Selective effects of C/EBPβ in the transcriptional crosstalk between inflammatory signals and pharmacological LXR activation in macrophages ***Estibaliz Glaría et al.***

Condensed Programme: DAY 2

11:00h 12:30h	Role of Skewed X-Chromosome Inactivation in Common Variable Immunodeficiency ***Marina Garcia-Prat et al.*** Expanding the clinical phenotype of TRAF3 haploinsufficiency syndrome ***Blanca A. Urban Vargas et al.*** **Chairs:** *Jorge Lloberas* Biology Faculty, University of Barcelona, Barcelona, Spain; *Concepción Mora* Medicine Faculty, Lleida University, Lleida, Spain
12:30h 13:30h	**Ordinary General Meeting – Societat Catalana d'Immunologia**
13:30h 14:30h	**Lunch @ posters**
14:30h 15:10h	**Plenary Session II** Impact of nutrition on immune responses in allergy and cancer ***Elodie Segura*** Immunotherapy Centre, Curie Institute (INSERM) **Chair:** *Mª José Rodríguez* Faculty of Pharmacy, University of Barcelona, Barcelona, Spain
15:10h 15:40h	**Sponsored Talk II** Monitoring the immunological response in the patient receiving a solid organ transplant. Usefulness of QuantiFERON CMV ***Anna Vila*** Dept. of Nephrology, Germans Trias i Pujol University Hospital, Badalona, Spain; ***María Iglesias Escudero*** Dept. of Immunology, Germans Trias I Pujol University Hospital, Badalona, Spain
15:40h 17:10h	**Oral communications** **Session III: Viral Immunity** Long-term immunodominant T-cell responses to S, Nsp3, NC, Env and M proteins of SARS-CoV-2 during hybrid immunity ***Laia Bernad et al.***

Condensed Programme: DAY 2

15:40h 17:10h	Unvaccinated individuals with severe COVID-19 show a distinctive humoral response characterized by high levels of anti-S2 IgG and IgA antibodies and low avidity anti-RBD IgG responses ***Núria Pedreño-Lopez et al.*** Identification of a Memory-Like NK Cell Population with Enhanced ADCC Activity in HIV Elite Controllers ***Nerea Sánchez-Gaona et al.*** Functional MDSCs are Maintained during Antiretroviral Treatment (ART) and Preclude HIV-1 Reservoir Reactivation ***Ana Gallego Cortés et al.*** HIV Infection Induces Neutralization-Interfering Antibodies that Hamper the Function of Neutralizing Antibodies ***Mª Luisa Rodríguez de la Concepción et al.*** Lack of HIV-associated pathogenicity in Viremic Non-Progressors is related with preserved TLR4 responsiveness ***Ángel Bayón-Gil et al.*** **Chairs:** ***Ana Angulo*** Faculty of Medicine, University of Barcelona, Spain; ***Nuria Izquierdo-Useros*** IrsiCaixa AIDS Research Institute
17:10h 17:30h	**Coffee break @ posters**
17:30h 19:30h	**Oral communications** **Session IV: Tumor Immunology and Immunotherapy** Dual blockade of PD-L1 and TIGIT pathways activated DCs and partially restores proinflammatory function in chronic viral infection ***Maria Lázaro-Díez et al.*** Therapeutic impact and immune modulation by MYC inhibition in KRAS-driven NSCLC with diverse mutational landscapes ***Íñigo González-Larreategui et al.***

Condensed Programme: DAY 2

17:30h 19:30h	Immunotherapy validation of Epstein Barr Virus Specific-T cells expressing the anti-CD19 Chimeric Antigen Receptor (CAR.CD19 ARI-0001) to treat B-cell neoplasms ***Ana Gabriela Lara-de-León et al.*** Targeting of cancer cells by human CD6-based CAR-T/NK cells ***Lucía Aragón Serrano et al.*** Single-cell RNA sequencing temporal analysis reveals a detrimental effect of JAK inhibition on myeloid cells in tofacitinib refractory ulcerative colitis patients ***Elisa Melón-Ardanaz et al.*** Immunomonitoring of intravenous and subcutaneous anti-CD49d treatment in patients with Multiple Sclerosis ***Alex Agundez Moreno et al.*** **Chairs:** ***Ester Lozano*** Biology Faculty, University of Barcelona, Barcelona, Spain; ***Aura Muntasell*** Hospital del Mar Research Institute, Autònoma University of Barcelona, Barcelona, Spain
19:30h 20:10h	**Plenary Session III** In depth analysis of the skin ecosystem: from basic science to drug development ***Nicolas Gaudenzio*** Research Director at Infinity, Toulouse, France and CSO at Genoskin, Toulouse, France, Salem, Massachussets, USA **Chair:** ***Pablo Engel*** Faculty of Medicine and Health Sciences, University of Barcelona, Barcelona, Spain
20:10h 20:20h	**Prize to the best communication and poster - Closing of the Congress** ***Eva Martinez-Cáceres*** Vice-President of the SCI

Session I: Allergy and Inflammation

Heterogeneous IL-9 production by circulating skin-tropic and non-tropic memory T cells in atopic dermatitis patients

Irene García-Jiménez[1] ; Lídia Sans-de San Nicolàs[1] ; Laia Curto-Barredo[2] ; Marta Bertolín-Colilla[2] ; Ignasi Figueras-Nart[3] ; Montserrat Bonfill-Ortí[3] ; Antonio Guilabert[4] ; Anna Ryzhkova[1] ; Marta Ferran[2] ; Giovanni Damiani[5] ; Ramon M. Pujol[2] ; Luis F. Santamaria-Babí[1]

[1]Translational Immunology research group, University of Barcelona, Barcelona, Spain; [2]Dept. of Dermatology, Hospital del Mar Research Institute, Barcelona, Spain; [3]Dept. of Dermatology, Bellvitge University Hospital, L'Hospitalet de Llobregat, Spain; [4]Dept. of Dermatology, General Hospital of Granollers, Granollers, Spain; [5]Italian Center of Precision Medicine and Chronic Inflammation, Milan, Italy

Background: Interleukin (IL)-9 is an important mediator in allergic disease present in atopic dermatitis (AD) lesions and considered to be produced in a temporarily way by skin-homing T cells expressing the cutaneous lymphocyte-associated antigen (CLA) activated by polyclonal activators under artificial conditions. However, its induction by pathogenic AD triggers with patients' samples and relationship with the clinical features of patients have not been addressed.

Methods: Circulating skin-tropic CLA+ and extra-cutaneous CLA- memory T cells in the presence of autologous lesional epidermal cells from moderate-to-severe non-treated adult AD patients were activated with house dust mite (HDM) and staphylococcal enterotoxin B (SEB). Levels of AD-associated mediators (IL-4, IL-5, IL-9, IL-13, IL-17A, IL-21, IL-22, IL-31, IFN-γ, CCL17 and CCL22) in response to both stimuli were measured in culture supernatants and related to the clinical characteristics of the same patients.

Results: CLA+ and CLA- memory T cells cocultures activated with HDM or SEB triggered IL-9 production in an heterogenous manner in a clinically homogenous group of AD patients. Upon allergen exposure, IL-9 was the most produced cytokine in our model, suggesting that HDM is a powerful inductor of IL-9 secretion, whereas SEB mildly induces its production. The IL-9 response to both HDM and SEB enabled patient stratification into producers and non-producers, with the former group exhibiting also higher associated production of other cytokines such as IL-4, IL-5, IL-13, IL-17A and IL-22, and elevated HDM- and S. aureus-specific IgE levels, respectively.

Conclusion: This study demonstrates that HDM and SEB induce heterogeneous non-temporarily IL-9 production by skin-related and extra-cutaneous memory T cells and suggests that the degree of allergen sensitization reflects the heterogenous IL-9 response in vitro, which may allow patient stratification from a clinically homogeneous population.

Session I: Allergy and Inflammation

Tolerance through Macrophages: Alterations in Type 1 Diabetes and Response to Antigen-Specific Immunotherapy

Ivan Garcia-Loza[1,2]; David Perna-Barrull[1] ; Eva Aguilera[3] ; Lidia Almenara-Fuentes[4] ; Maria Vilà[4] ; Daniela Greco[4] ; Miriam Salvado[4] ; Montserrat Mancera-Arteu[4] ; Martí Dalmases[4] ; Sílvia Rodriguez-Vidal[4] ; Michael W. Olszowy[5] ; Jordi Petriz[1] ; Bruna Barneda-Zahonero[4] ; Marta Vives-Pi[1,4]

[1]Dept. of Immunology, Germans Trias i Pujol Research Institute, Badalona, Spain; [2]Biomedical Research Institute Santa Creu i Sant Pau Hospital, Barcelona, Spain; [3]Dept. of Endocrinology, Germans Trias i Pujol University Hospital, Badalona, Spain; [4]Ahead Therapeutics SL, Barcelona, Spain; [5]Sartorius Stedim North America, INC., Arvada, Colorado, United States

Type 1 diabetes (T1D) is caused by a failure of immunological tolerance, an event in which antigen-presenting cells, precisely dendritic cells (DCs) and macrophages (Mφ), are crucial. In this context, antigen-specific immunotherapies have been developed to restore homeostasis in the absence of broad immunosuppression. Particles that mimic apoptotic bodies, i.e., phosphatidylserine (PS) liposomes, have shown great potential to restore tolerance in experimental autoimmunity. Nevertheless, the role of Mφ in these therapies remains poorly understood. Mφ can be divided into different regulatory and inflammatory subsets and are essential in tissue homeostasis. Moreover, Mφ also present antigens to T lymphocytes and secrete various cytokines, making them excellent mediators of immune tolerance. However, limited information on human Mφ in T1D is available. Our aim was to determine the role of Mφ in antigen-specific immune tolerance and their specific particularities in T1D. We conducted a phenotypic and functional characterization of four human monocyte-derived Mφ subpopulations (M0, M1, M2a and M2c) after treatment with PS-liposome nanoparticles in individuals with T1D. Our results revealed different dynamics of liposome capture among Mφ subsets, with preferential uptake by inflammatory (M1) and regulatory (M2c) Mφ, and similar capture rates in patients and controls. Besides, high doses of PS-liposomes did not alter Mφ viability across time. PS-liposome uptake triggered variations in the expression of Mφ membrane molecules related to immunoregulation. Furthermore, the secretion profile of soluble mediators indicated the potential tolerogenic effect of PS-liposomes in inflammatory Mφ and highlighted alterations in the basal levels of expression of soluble mediators in T1D. Finally, functional assays confirmed the tolerogenic imprinting of PS-liposomes in Mφ. These results are consistent with the tolerogenic potential of PS-liposomes and provide new insights into the mechanism of action of this preclinical immunotherapy.

Funding: MINECO (CPP2021-00847) and Ahead Therapeutics S.L.

Multiple sclerosis: new approach for the generation of tolerogenic dendritic cells induced with vitamin D3

Roger Domenech Garcia[1,2,3]; Federico Fondelli[1,2,3]; Eva Martínez Cáceres[1,2,3]

[1]Germans Trias i Pujol Research Institute, Badalona, Spain; [2]Dept. of Cell Biology, Physiopathology and Immunology, Autònoma University of Barcelona, Bellaterra, Spain; [3]Division of Immunology, Germans Trias i Pujol University Hospital, Badalona, Spain

Multiple Sclerosis (MS) is a chronic, inflammatory, autoimmune and demyelinating disease that affects the central nervous system (CNS). The development of tolerogenic dendritic cells (tolDCs) as a therapeutic strategy is on the rise because of their potential to restore tolerance through the specific suppression of autoreactive T cell clones. However, the inflammatory environment of MS patients can alter the potency of tolDCs. In this context, we analysed if the modulation of the aryl hydrocarbon receptor (AHR), one of the key factors in the regulation of tolerogenic functions of tolDCs, could enhance the tolerogenicity of tolDCs induced with vitamin D3 (VitD3-tolDC). At the same time, we studied the effects of dimethyl fumarate (DMF), a leading MS drug, on VitD3-tolDCs in healthy controls and MS patients. Specifically, we characterised the phenotype of VitD3-tolDC modulated by AHR or DMF and analysed the suppressive effect of VitD3-tolDC on allogeneic PBMCs. Our results showed that the modulation of the AHR receptor by its agonist FICZ generated VitD3-tolDC with a mores tolerogenic phenotype than control ones, decreasing the expression of CD83, CD86 and CCR7 markers and increasing the CD14 marker. These phenotypic changes translated into an increased suppression of proliferation of allogeneic PBMCs. On the other hand, DMF did not affect drastically VitD3-tolDCs from HD, while lead to more suppressive tolDC in the case of MS patients. These results suggest that direct AHR agonism can induce more potent VitD3-tolDCs and AHR receptor activation may be a good choice to improve this cell therapy. On the other hand, our data suggest that DMF administration can also improve the functionality of MS-derived VitD3-tolDCs.

Session I: Allergy and Inflammation

Platelets as possible biomarker candidates to differentiate between neurodegenerative disorders

Noelia Arias[1]; Marc Boigues[1]; Pau Pastor[3]; Lourdes Ispierto[3]; Dolores Vilas[3]; Ramiro Álvarez[3]; Marco A. Fernandez- Sanmartín[4]; Katrin Beyer[2]; Eva Martínez Cáceres[1]

[1]Division of Immunology, Germans Trias i Pujol University Hospital and Research Institute, Badalona, Spain; Dept. of Neurosciences, Germans Trias i Pujol Research Institute, Badalona, Spain; Dept. of Neurology, Germans Trias i Pujol University Hospital and Research Institute, Badalona, Spain; [4]Flow cytometry platform, Germans Trias i Pujol Research Institute, Badalona, Spain

Platelets (PLTs) have recently been recognized as immunoregulatory elements with an increasing evidence of their role in the pathogenesis of neurodegenerative disorders. Alzheimer's disease (AD), Parkinson's disease (PD) and Lewy body dementia (DLB) are complex diseases that usually overlap in their neuropathological manifestations preventing a correct clinical diagnosis and management of the disease.

The aim of this project was to explore the interaction of PLTs with other immune cells in these different neurodegenerative disorders and determine the usefulness of PLTs as possible biomarkers. Forty individuals from Germans Trias i Pujol Hospital were analyzed in this pilot study. The percentage of PLTs (CD41+CD61+) attached to Tlymphocytes (CD3+CD4+, CD3+CD8+), B lymphocytes (CD19+), monocytes (CD14+), and their activation grade (CD25+/CD68+) were measured in peripheral blood by flow cytometry.

Results revealed an increase in the percentage of PLTs attached to CD4+ and CD4+CD25+ T lymphocytes in Control (CTRL) and PD compared to DLB. A similar result was obtained with CD19+ and CD19+CD25+ B lymphocytes attached to PLTs in CTRL, PD and AD compared to DLB. Moreover, the percentage of PLTs attached to CD14+ monocytes was higher in AD and CTRL compared to DLB.

The percentage of PLTs attached to B lymphocytes, CD4+ T lymphocytes and monocytes could be a promising biomarker to discriminate between PD and DLB. Moreover, it seems that there is a tendency of a decrease percentage of immune cells attached to PLT in DLB. This difference should be further explored as a tool for the differential diagnosis between DLB and PD at early disease stages, as it is still very difficult to differentiate them. A correct diagnosis and treatment is crucial to improve the quality of life of these patients. Further studies with higher number of participants are needed to validate these results.

Detection of anti-PL7 autoantibodies by a commercial line immunoassay versus gold standard immunoprecipitation

Janire Perurena-Prieto[1,2,3]; Laura Viñas-Giménez[1,2]; María Teresa Sanz-Martínez[1,2]; Alfredo Guillén-Del-Castillo[4]; Carmen P. Simeón-Aznar[4]; Albert Selva-O'Callaghan[4]; Albert Gil-Vila[4]; Ana Villar-Gomez[5]; Ernesto Trallero- Araguas[6]; Roger Colobran[1,2,3,7]

[1]Division of Immunology, Vall d'Hebron University Hospital, Barcelona, Spain; [2]Translational Immunology Group, Vall d'Hebron Research Institute, Barcelona, Spain; Dept. of Cell Biology, Physiology and Immunology, Autònoma University of Barcelona, Bellaterra, Spain; [4]Systemic Autoimmune Diseases Unit, Dept. of Internal Medicine, Vall d'Hebron University Hospital, Barcelona, Spain; [5]Dept. of Pneumonology, Vall d'Hebron University Hospital, Barcelona, Spain; [6]Dept. of Rheumatology, Vall d'Hebron University Hospital, Barcelona, Spain; [7]Dept. of Clinical and Molecular Genetics, Vall d'Hebron University Hospital, Barcelona, Spain

Introduction: anti-synthetase syndrome (ASS) is an autoimmune condition characterized by positivity of anti-aminoacyl-tRNA synthetase (anti-ARS) autoantibodies, interstitial lung disease, myositis, Raynaud's phenomenon, fever, mechanic's hands, and arthritis. Since presence of any anti-ARS, together with only one of the aforementioned clinical features, can lead to diagnosis of AAS, it is essential to have a sensitive and specific assay for detection of anti-ARS. The aim of this study was to evaluate the performance of a commercial assay for detecting anti-PL7, an anti-ARS, using RNA and protein immunoprecipitation (IP).

Materials and Methods: anti-PL7 was assessed by a commercial line immunoassay (LIA). Fourteen patients highly positive for anti-PL7 by LIA were selected and further tested by RNA IP. One of these patients presented positivity for a second anti-ARS, anti-PL12, by LIA, and was also tested by protein IP. ANAs were detected by immunofluorescence (IIF) test performed on HEp-2 cells. IIF patterns were categorized according to the International Consensus on ANA Patterns classification.

Results: only 6 out of 14 (42.9%) patients were confirmed to be anti-PL7 positive by RNA IP. All of the positive patients by IP with available sample were tested by IIF (n=5) and presented a cytoplasmatic dense fine speckled pattern (AC-19). The only anti-PL7 negative patient presenting this pattern was the one presenting positivity for anti-PL7 and anti-PL12 by LIA, and was demonstrated to be positive for anti-PL12 by protein IP.

Conclusions: less than half (43%) of the anti-PL7 positive samples by the commercial assay were confirmed by RNA IP. Anti-PL7 positive samples that do not present the AC-19 IIF pattern, should not be considered as anti-ARS positive for the diagnosis of ASS. Patients positive for more than one anti-ARS and an AC-19 pattern by IIF should be studied by protein IP as double positivity has rarely been described.

Papel de la incompatibilidad KIR-HLA I en el desarrollo de inflamación microvascular con y sin anticuerpos donante-específicos postrasplante renal

Judith Federico-Vega[1]; Elisenda Alari-Pahissa[1]; Victor Bello[4]; Dolores Redondo-Pachón[2]; Maria José Pérez-Sáez[2]; Sara Sanz-Ureña[1]; Ana Faura[2]; Carlos Vilches[4]; Miguel López-Botet[3]; Marta Crespo[1,2]

[1]Hospital del Mar Research Institute, Barcelona, Spain; [2]Hospital del Mar, Barcelona, Spain; [3]Pompeu Fabra University, Barcelona, Spain; [4]University Hospital Puerta de Hierro, Majadahonda, Spain

La inflamación microvascular (MVI) en trasplante renal (TR) acompañada de anticuerpos anti-HLA donante-específicos (DSA) es diagnóstica de rechazo mediado por anticuerpos (ABMR), causa frecuente de pérdida del injerto, aunque MVI sin DSA también tiene impacto en la supervivencia del TR. Las células Natural Killer (NK) podrían tener un papel en el rechazo mediante receptores de HLA-I KIR inhibidores(iKIR) y activadores(aKIR). La presencia en el receptor de genes iKIR con ligando HLA-I propio ausente en el donante se define como incompatibilidad (mismatch,MM) iKIR-HLA-I, y presencia en el receptor de aKIR con ligando HLA-I en el injerto ausente en el receptor se define como MM aKIR-HLA-I. Estos MM implican la existencia de NK potencialmente alorreactivas influyentes en rechazo.

Objetivo: Estudiar la relación entre número de MM KIR-HLA y ABMR/MVI.

Métodos: Seleccionamos 28 receptores con ABMR/MVI en biopsia al año y 40 receptores con biopsia normal/fibrosis intersticial y atrofia tubular (FIAT) leve sin timoglobulina y/o rituximab. Se evaluó genotipo HLA-I de donantes y receptores mediante NGS y genotipo KIR de receptores (KR2DL1,KIR2DL2/L3,KIR3DL1,KIR3DL2,KIR2DS1, KIR2DS2,KIR3DS1,KIR2DS4) mediante PCR-SSO(Luminex). Se consideraron las interacciones IR2DL1/HLA-C2,KIR2DL2&KIR2DL3/HLA-C1, KIR3DL1/HLA-Bw4,KIR2DS1/HLA-C2,KIR2DS2/HLA-C1,KIR3DS1/HLA-Bw4-I80,KIR2DS4/ HLA-A*11/C*02/04/05/16:01. Dada la controversia sobre la capacidad de educación de KIR3DL2, que reconoce HLA-A*03/11, se analizaron los resultados considerando educación y no-educación por KIR3DL2.

Resultados: No encontramos diferencias en número de MM aKIR-HLA-I entre pacientes normal/FIAT y ABMR/MVI. En cuanto MM iKIR-HLA-I, considerando educación KIR3DL2, cuantificamos menor MM iKIR-HLA-I en normal/FIAT(55,00%) respecto ABMR/MVI(60,71%), sin ser estas diferencias estadísticamente significativas. Considerando no-educación KIR3DL2, cuantificamos mayor MM iKIR-HLA-I en pacientes ABMR/MVI(53,57%) respecto normal/FIAT(40,00%).

Conclusión: Considerar o no educación por KIR3DL2 influye en el resultado del análisis genético de MM iKIR-HLA-I en pacientes normal/FIAT y ABMR/MVI. En caso de no considerarla, la incompatibilidad iKIR-HLA-I parece más frecuente en ABMR/MVI, pero se requiere análisis de un mayor número de muestras para corroborarlo.

Deciphering the crosstalk between cDC1 and cytotoxic T-cells and NK-cells

Georgina Flórez-Grau[1]; Mark A.J. Gorris[1]; Jorge Cuenca-Escalona[1]; Johanna Bödder[1]; Daphne Roefols[1]; Tasmin Zahan[1]; Anne van Duffelen[1]; Jasper J.P. van Beek[1]; Kiek Verrijp[1]; Carl G. Figdor[1]; I. Jolanda M. de Vries[1,2]

[1]Dept. of of Tumor Immunology, Radboud Institute for Molecular Life Sciences, Radboud University Nijmegen, Nijmegen, the Netherlands; [2]Dept. of Medical Oncology, Radboud Institute for Molecular Life Sciences, Nijmegen, the Netherlands

Immunotherapy is a novel treatment modality for cancer. Increasing evidence is demonstrating that immunosuppressive factors in the tumor microenvironment strongly reduce the clinical benefit. Several immunosuppressive factors hamper/decrease the presence and activation status of cytotoxic (CD8+) T-cells.

Cytotoxic T-cells have been shown to be directly related to a better prognosis in many cancers such as melanoma. Preclinical data, has shown that a specific dendritic cell (DC) subset known as as conventional DC type 1 (cDC1), is characterized by its superior ability to induce T-cell infiltration and increase cytotoxic T-cell numbers in tumors. In that sense, NK cells are one of the first immune cells to infiltrate the tumor produce chemokines that contribute to cDC1 accumulation.

However, in humans, cDC1 are rare and the role of this subset in recruiting cytotoxic T-cells and NK-cells (cytolytic effector cells) remains unclear. Therefore, we aim to elucidate the mechanisms by which human cDC1 are attracted by T-cells and NK-cells and vice versa. An enhanced influx of CD8+ T-cells and NKcells might improve the efficacy of immunotherapy.

Our results show that cDC1 interact with CD8+ T-cells and NK-cells at two different levels: (i) immaturecDC1 express high levels of XCR1, a key factor for migration towards XCL1 secreted by stimulated cytotoxic T-cells and NK-cells, and (ii) TLR3-matured cDC1, compared to other human DC subsets (cDC2 and pDCs), produce the highest amounts of CXCL9, 10 and 11. These chemokines are associated with the attraction of cytolytic effector cells via CXCR3. Accordingly, in trans-well assays, supernatant of TLR3-activated cDC1 increased the migration of cytotoxic T-cells and NK-cells as compared to the other DC subsets (cDC2 and pDCs).

Altogether, our data suggest that the bidirectional crosstalk of cDC1s with cytotoxic T-cells and NK-cells potentiates cytolytic effector cell attraction and may boost anti-tumor immunity response based on DC vaccination.

Session II: Basic Immunology and Immunodeficiencies

Type I interferonopathies, a macrophage disease

Carlos Batlle i Recoder[1,2]; Dr. Joan Tur[1,2]; Dr. Antonio Zorzano[3,4,5]; Dr. Antonio Celada[1,2]; Dr. Jorge Lloberas[1,2]

[1]Macrophage Biology research group, University of Barcelona, Barcelona, Spain; [2]Dept. of Cell Biology, Physiology and Immunology, University of Barcelona, Barcelona, Spain; [3]Aging and Metabolism research group; [4]Dept. of Biochemistry and Molecular Biomedicine, University of Barcelona, Barcelona, Spain; [5]Institute for Research in Biomedicine , Barcelona, Spain

Type I interferonopathies is a group of Mendelian genotypes originates by a failure of self-versus non-selfdiscrimination of antiviral systems triggered by host DNA and RNA producing large amounts of type I interferons. In inflammatory processes, macrophages produce large amounts of reactive oxygen species (ROS), which serve to eliminate infectious agents, but as a secondary effect they produce DNA damage, which if not repaired can induce cell death. We have seen that macrophage but not any other cells, activated by pro-inflammatory activators such as IFN-γ or LPS, induce the expression of DNA repair systems such as Trex1, Samhd1, Pol-μ, MDA5 or NBS1. Mutations of these proteins, in addition to others, cause type I interferonopathies. Interestingly, the transcription factor IRF1 is necessary for the induction of these genes. Trex1 KO mice have a shortened lifespan relative to controls. However, if they are treated with dexamethasone, an inhibitor of macrophage activation, their half-life is corrected and is similar to controls. Macrophages from Mitofusin 2 (mitochondria fusion protein) KO mice, conditional for macrophages, produce low levels of ROS when activated with pro-inflammatory agents. We have crossed Trex1 KO mice with Mitofusin 2 conditional mice, observing that their life is lengthened. In conclusion, we showed that macrophages play a critical role in type I interferonopathies.

Session II: Basic Immunology and Immunodeficiencies

End-Binding protein 1 regulates the metabolic fate of CD4+ T lymphocytes through the organization of the mitochondrial network

Álvaro Gómez-Morón[1,2]; Silvia Requena-Galindo[2]; Clara Pertusa[2]; Marta Lozano-Prieto[2]; Camila Scagnetti[2]; Manuel Izquierdo[4]; Pedro Roda-Navarro[1]; Noa B Martín-Cófreces[2]

[1]Immunology, Oftalmology and Otorrinolaryngology Dept., School of Medicine, Universidad Complutense ; [2]Immunology Service, La Princesa University Hospital Health Research Institute, Madrid, Spain; [3]Vascular Pathophysiology, Laboratory of Intercellular Communication, Fundación Centro Nacional de In; [4]Instituto de Investigaciones Biomédicas Alberto Sols, CSIC-Autónoma University of Madrid, Madrid

The organization of the mitochondrial network is relevant for the metabolic fate of T cells and their ability to respond to TCR stimulation. This arrangement depends on cytoskeleton dynamics in response to TCR and CD28 activation, which allows the polarization of the mitochondria through their change in shape, and their movement along the microtubules towards the immune synapse.

This work focus on the role of End-binding protein 1 (EB1), a protein that regulates tubulin polymerization and dynamics through the interaction between alpha- and beta-tubulin heterodimers and has been previously identified as a regulator of intracellular transport of CD3-enriched vesicles at the immune synapse. However, the regulatory role of EB1 in T cell metabolism is largely unknown. For this purpose, Jurkat clones stably expressing shCtrl and shEB1, Jurkat cells transiently transfected with Control or EB1 siRNA, and CD4+ T lymphoblasts transfected with control or EB1-specific shRNA were used. Western blot experiments have been performed with these cells to analyze the mTOR signalling pathway, which regulates numerous cellular processes such as survival, protein translation or cell metabolism. Additionally, confocal microscopy assays were assessed to study the distribution of mitochondria and the actin and tubulin cytoskeleton to the immune synapse, and seahorse assays to study mitochondrial respiration and the glycolytic capacity of these cells. In addition, the role of EB1 in activation-induced cell death (AICD) through the Fas/FasL system has been studied.

By unifying the organization of the tubulin cytoskeleton and mitochondria during CD4+ T cell activation, this work highlights the importance of this connection for critical cell asymmetry together with metabolic functions such as glycolysis, mitochondria respiration, and cell viability. EB1-interferred cells show defective metabolic strength in activated T cells, pointing to a relevant connection of cytoskeleton and metabolism in response to TCR stimulation.

Selective effects of C/EBPβ in the transcriptional crosstalk between inflammatory signals and pharmacological LXR activation in macrophages

Estibaliz Glaría[1,2]; Joan Font-Díaz[1,2]; Pol Rodríguez Martínez[1,2]; Juan Vladimir De la Rosa[3]; Antonio Castrillo[3,4]; Jose Manuel Vidal Taboada[5,6]; Josep Saura[5,6]; Carme Caelles[2,7]; Annabel F. Valledor[1,2]

[1]Dept. of Cell Biology, Physiology and Immunology, School of Biology, University of Barcelona, Barcelona, Spain; [2]Institute of Biomedicine of the University of Barcelona, Barcelona, Spain; [3]Biomedicine Unit, University Institute of Biomedical Research, University of Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain ; [4] Alberto Sols Biomedical Research Insitute CSIC- Autónoma University of Madrid, Madrid, Spain; [5]Dept. of Biomedicine, Biochemistry and Molecular Biology Unit, School of Medicine, Vall d'Hebron University Hospital, Barcelona, Spain; [6]Institute of Neurosciences, University of Barcelona, Barcelona, Spain; [7]Dept. of Biochemistry and Physiology, School of Pharmacy and Food Sciences, University of Barcelona, Barcelona, Spain

Macrophages are innate immune cells that play key roles in the host defense against a diversity of insults, but they are also integral tissue components involved in the regulation of metabolism and in the maintenance of homeostasis. Macrophages express abundant levels of liver X receptors (LXRs), which are members of the nuclear receptor family of ligand-dependent transcription factors that act as natural sensors of cholesterol metabolites. Once activated, LXRs control the expression of key regulators of cholesterol, fatty acid and phospholipid homeostasis. Several evidences support both positive and negative crosstalk between LXRs and inflammatory signals in a cell type- and gene-specific manner. A common feature in the macrophage response to inflammatory mediators is the induction of CCAAT/enhancer-binding protein beta (C/EBPβ), a master transcriptional regulator and lineage determining transcription factor in monocytes/macrophages. In this work we have explored the role of C/EBPβ in the cross-talk between inflammatory signals and the macrophage response to pharmacological LXR activation. Whereas inflammatory mediators repress the expression of several LXR-regulated genes involved in lipid metabolism, these effects were conserved after genetic deletion of C/EBPβ. In contrast, synergic effects between inflammatory mediators and LXRs resulted in strong expression of CD38, a multifunctional protein and major regulator of the cellular levels of NAD+, which was abolished in the absence of functional C/EBPβ. The analysis of publicly available ChIP- Seq data identified co-occupancy by C/EBPβ and the LXR subtypes LXRa or LXRb of three genomic regions with enhancer activity in the vicinity of the mouse Cd38 gene, thus supporting the positive cross-talk between these transcription factors on CD38 expression.

Role of Skewed X-Chromosome Inactivation in Common Variable Immunodeficiencies

Marina Garcia-Prat[1]; Laura Batlle-Masó[1]; Alba Parra-Martínez[1]; Clara Franco-Jarava[2,3,4]; Mónica Martinez-Gallo[2,3,4]; Aina Aguiló-Cucurull[2,3,4]; Janire Perurena-Prieto[2,3,4]; Neus Castells[5]; Blanca Urban[2,3]; Romina Dieli-Crimi[2,3]; Pere Soler-Palacín[1]; Roger Colobran[2,3,4,5]

[1]Pediatric Infectious Diseases and Immunodeficiencies Unit, Vall d'Hebron University Hospital, Barcelona, Spain; [2]Division of Immunology, Vall d'Hebron University Hospital, Barcelona, Spain; [3]Translational Immunology Research Group, Vall d'Hebron Research Institute, Barcelona, Spain; [4]Dept. of Cell Biology, Physiology and Immunology, Autònoma University of Barcelona, Barcelona, Spain; [5]Dept. of Genetics, , Vall d'Hebron University Hospital, Barcelona, Spain

Common variable immunodeficiency (CVID) include a clinically heterogeneous group of disorders mainly characterized by hypogammaglobulinemia, insufficiency of specific antibody production and recurring infections.

Genetics of CVID is complex and include different mechanisms. Monogenic defects only explain a proportion of cases (typically less than 30%) and other models have been proposed including digenic, oligogenic or polygenic inheritance, epigenetic dysregulation or the possible contribution of somatic variants.

In this work, we aimed to assess the role of skewed X-chromosome inactivation (XCI) in CVID. From our cohort of 131 genetically analyzed CVID patients, we selected those female patients who carried rare variants in CVID-associated genes located in chromosome X. We selected four patients carrying heterozygous variants in BTK (n=2), CD40LG (n=1) and IKBKG (n=1) genes.

We analyzed the XCI status using both the classic HUMARA assay and an NGS-based method to quantify the expression of both alleles in the mRNA.

3 out of the 4 patients (75%) had showed a skewed XCI and in all these cases the mutated allele was predominantly expressed. Patient 1 carried a hypomorphic variant in BTK (p.Tyr418His), patient 3 carried a pathogenic variant in CD40LG gene (c.288+1G>A) that caused hyper-IgM syndrome in her son, and patient 4 carried a hypomorphic variant in IKBKG (p.Glu57Lys) and also a heterozygous splice variant in TNFRSF13B (TACI) (c.61+2T>A), making this case an example of a possible digenic contribution to CVID.

These results provide some evidences about the role of skewed XCI as another piece in the complex puzzle of CVID genetics.

Expanding the clinical phenotype of TRAF3 haploinsufficiency syndrome

Blanca A. Urban Vargas[1,2]; Romina Dieli-Crimi Vinuesa[1,2]; Mónica Martínez Gallo[1,2,3]; Marina García Prat[4]; Alba Parra Martínez[4]; Aina Aguiló Cucurull[1,2]; Laura Batlle Masó[4]; Roger Colobran Oriol[1,2,3,5]; Clara Franco Jarava[1,2,3]

[1]Division of Immunology, Vall d'Hebron University Hospital, Barcelona, Spain; [2]Translational Immunology Research Group, Vall d'Hebron Research Institute, Barcelona, Spain; [3]Dept. of Cell Biology, Physiology and Immunology, Autònoma University of Barcelona, Barcelona, Spain; [4]Pediatric Infectious Diseases and Immunodeficiencies Unit, Vall d'Hebron University Hospital, Barcelona, Spain; [5]Dept. of Genetics, Vall d'Hebron University Hospital, Barcelona, Spain

Introduction:
TRAF3 is an adaptor protein that plays a critical role in the regulation of B and T cells. For many years, evidence of its role in humans was limited to a single publication associating TRAF3 deficiency to herpes- simplex encephalitis. A recent study reported TRAF3 haploinsufficiency (TRAF3HI) as causing a novel phenotype presenting with immunodeficiency, autoimmunity, and increased risk of B-cell malignancies. This prompted a re-analysis of genetic data in our patients with undiagnosed inborn errors of immunity(IEI).

Objective
To identify new patients with TRAF3HI, describing the clinical and immunological phenotype.

Materials and Methods
Genetic reanalysis of NGS targeted gene panels from patients with IEI suspicion (n=720), focused in the TRAF3 gene to identify rare and potentially pathogenic genetic variants. We studied candidate variants in affected families using Sanger sequencing. We also performed mRNA and protein analysis.

Results
Initially we identified 2 patients(P1 and P2) carrying likely pathogenic (stop-gain) heterozygous variants in TRAF3. Familial study revealed that the variant was de novo in P1 and inherited from her mother in P2. The mother also presented clinical features compatible with TRAF3HI and she was also included in the study(P3).

Compared with published TRAF3HI patients, our patients shared common features such as respiratory infections, visceromegaly, and intestinal inflammation. Regarding lymphocyte subpopulations, low levels of naive T-cells, low levels of class-switched memory B-cells, and impaired polysaccharide vaccine response were also consistent.

In contrast, our patients had hypogammaglobulinemia and they did not show autoimmunity, malignancy, or B cell hyperactivation.

Conclusion
We present 3 new cases of TRAF3HI, a condition characterized by dysgammaglobulinemia and immune dysregulation. Our study highlights the importance of re-evaluating genetic studies, in this case when novel phenotypes of known genes are described. The patients described here expand the TRAF3Hl phenotype, including common variable immunodeficiency-like presentations.

Long-term immunodominant T-cell responses to S, Nsp3, NC, Env and M proteins of SARS-CoV-2 during hybrid immunity

Laia Bernad[1] ; Raúl Pérez-Caballero[1] ; Athina Kilpeläinen[1] ; Oscar Blanch-Lombarte[1] ; Luis Romero[1] ; Ruth Peña[1] ; Gabriel Felipe Rodriguez-Lozano[1] ; Josep Maria Manresa-Dominguez[2] ; Bonaventura Clotet[1] ; Alex Olvera[1] ; Christian Brander[1] ; Eva Martínez Caceres[2] ; Concepción Violán[3] ; Pere Torán-Monserrat[3] ; Julia G. Prado[1]

[1]IrsiCaixa AIDS Research Institute, Badalona, Spain; [2]Division of Immunology, Germans Trias i Pujol University Hospital, Badalona, Spain; [3]Jordi Gol University Institute for Research in Primary Care, Barcelona, Spain

Characterizing long-term T-cell immunity against SARS-CoV-2 proteome is critical for understanding the implications of virus-specific hybrid immunity in preventing re-infections and advancing vaccine designs. Here, we conducted a high-resolution mapping of T-cell responses towards SARS-CoV-2 proteome over a 2-year follow-up in individuals with hybrid immunity from the ProHEpiC-19 cohort study.

We selected cryopreserved PBMC from 38 healthcare workers, including 19 SARS-CoV-2 infected (CoV2+, tested at 124 days from symptoms onset, DfSO) and 19 uninfected (CoV2-) participants. Longitudinal samples were available from 18 individuals after a 3-dose mRNA vaccination, including 13 CoV2+ (CoV2+Vac+, 825 DfSO) and 5 CoV2- becoming infected during the follow-up (Vac+CoV2+, 302 DfSO). Using a Mega-Matrix approach, we measured the breadth and magnitude of IFNy T-cell responses by ELISpot assay using a 15-mer overlapping peptide (OLP) library of 2,790 SARSCoV-2 peptides in 100 pools. Then, the single peptides were deconvoluted and targeted peptides confirmed by ELISpot.

We identified immunodominant T-cell responses to 13 regions across SARS-CoV-2 proteome within S, Nsp3, NC, Env, and M proteins. A booster vaccination effect was observed in those regions with stronger responses in S and NSp3 proteins. Moreover, CoV2+Vac+ had broader T-cell responses than Vac+CoV2+ showing specific targeting of ORF3a, M, upORFs, Nsp2, 3C_LP, Nsp10 and Hel regions. At the single peptide level, we identified different frequencies in T-cell responses since CoV2+Vac+ showed a preferential targeting of S1 (58%), S2 (17%) and ORF1ab/8 (25%) compared to Vac+CoV2+ with almost sole recognition of S2 (86%).

Our results define immunodominant long-term T-cell responses to S, Nsp3, NC, Env, and M proteins in SARS-CoV2 proteome in a hybrid immunity context and demonstrate broader and specific T-cell responses in CoV2+Vac+ compared to Vac+CoV2+. Overall, we identify differences in long-term T-cell hybrid immunity primed by infection or vaccination with implications for protection from re-infection and future vaccine design.

Unvaccinated individuals with severe COVID-19 show a distinctive humoral response characterized by high levels of anti-S2 IgG and IgA antibodies and low avidity anti-RBD IgG responses

Núria Pedreño-Lopez[1]; Erola Ainsua-Enrich[1]; Susana Benet[2]; Julieta Carabelli[1]; Carlos Ávila-Nieto[1]; Maria Luisa Rodríguez de la Concepción[1]; Dalia Raich-Regué[1]; Ferran Tarrés-Freixas[4,5]; Boris Revollo[2]; Agnes Hernández[6]; Carla Usai[4]; Beatriz Mothe[1,2,5,6,8]; Nuria Izquierdo-Useros[1,7,8]; Julià Blanco[1,5,7,8]; Joaquim Segalés[3,4]; Julia Vergara-Alert[4]; Jorge Carrillo[1,7,8]

[1]IrsiCaixa AIDS Research Institute, Germans Trias i Pujol University Hospital, Badalona, Spain; [2]Lluita contra les Infeccions Foundation, Germans Trias i Pujol University Hospital, Badalona, Spain; [3]Autònoma University of Barcelona, Bellaterra, Spain; [4]Institute of Agricultural Research and Technology - Animal Health Research Center, Bellaterra, Spain; [5]University of Vic – Central University of Catalonia, Vic, Spain; [6]Germans Trias i Pujol University Hospital, Badalona, Spain; [7]Germans Trias i Pujol Research Institute, Badalona, Spain; [8]Center for Biomedical Research in Network of Infectious Diseases - Carlos III Health Institute, Madrid, Spain

The mechanism that causes severe COVID-19 in unvaccinated individuals remains elusive. Here, we characterized the early humoral response elicited in unvaccinated patients with severe (Group S, n=79) and mild (Group M, n=25) COVID-19 to determine whether SARS-CoV-2 induces a distinct immune responses that might be associated with disease enhancement. Samples were taken up to 32 days post-diagnosis. Only individuals from Group S were hospitalized, required oxygenation, and received corticoids, antivirals, and/or monoclonal antibodies.

We determined IgG, IgA, and IgM levels against Spike, Nucleocapsid (N), Envelope, S2, and receptor- binding domain (RBD), and identified that Group S had significantly greater S2-specific IgA and IgG titers compared to Group M (Mann-Whitney T test, p=0.0085 and 0.0265, respectively). IgA levels against the trimeric Spike were also elevated in Group S (p=0.0126). The binding strength of IgG and IgA was also evaluated against S2, RBD, and N. Here, we identified that anti-RBD IgG antibodies had lower avidity in Group S than in M, albeit this reduction was not reflected on the neutralizing capacity of these individuals. To identify potential functional antibody differences, we measured Fc-mediated effector functions, including antibody-dependent cellular phagocytosis (ADCP) and cytotoxicity (ADCC). While Group S RBD- and S2-specific antibodies were capable of mediating significantly greater ADCP activity compared to Group M, these differences faded after normalizing ADCP activity by antibody levels. We did not identify any differences in normalized ADCC.

Our results suggest that severe COVID-19 induces a strong humoral response characterized by high levels of anti-S2 Ig, and anti-RBD IgG antibodies of low avidity. The greater S2-specific ADCP observed in Group S was due to the high levels of anti-S2 IgG and IgA, not because of different functionality. Despite that, the enriched presence of S2-specific antibodies may contribute to the proinflammatory cytokine storm that is typically associated with COVID-19 disease severity.

Identification of a Memory-Like NK Cell Population with Enhanced ADCC Activity in HIV Elite Controllers

Nerea Sánchez-Gaona[1]; Ana Gallego-Cortés[1]; Antonio Astorga-Gamaza[1]; Norma Rallón[2,3]; José Miguel Benito[2,3]; Ezequiel Ruiz-Mateos[4]; Adrià Curran[1]; Joaquín Burgos[1]; Jordi Navarro[1]; Paula Suanzes[1]; Vicenç Falcó[1]; Meritxell Genescà[1]; Maria J. Buzón[1]

[1]Dept. of Infectious Diseases, Vall d'Hebron Research Institute, Barcelona, Spain; [2]Institute for Health Research-Foundation, Autónoma University of Madrid, Madrid, Spain; [3]University Hospital Rey Juan Carlos, Móstoles, Spain; [4]Institute of Biomedicine of Seville, Virgen del Rocío University Hospital, Seville, Spain

The expansion of Memory-like Natural Killer cells (ML-NKs) during HIV infection has been associated with protective effects. Elite controllers (EC), individuals that exhibit spontaneous and drug-free HIV remission, represent a model for a functional cure. Here, we aimed to investigate the phenotype and function of ML-NKs in a cohort of EC with durable HIV control (DC).

PBMCs from n=25 DC, n=25 healthy donors (HD), and n=8 ART-treated subjects (ART) were included in the study. Flow cytometry and dimensionality reduction analyses were employed to characterize the NK phenotype and included the markers: CD16, CD57, NKp30, NKG2C, NKG2A, KIR2DL2/L3, and KLRG1. NK cytotoxicity and activation were evaluated following co-culture with MHC-devoid K562 cells. Functional analyses included the markers IFNg, CD107a and CD69. FACS was employed to isolate NK populations based on the expression of CD16, CD57, NKG2C, NKG2A, and CXCR3. Antibody-dependent cell cytotoxicity (ADCC) was evaluated by co-culturing isolated NK with latently infected ACH-2 cells and HIV+ plasma. Cell killing was calculated as reduction of virally-infected cells.

ML-NKs, characterized by NKG2C and CD57 expression, were significantly expanded in DC and ART, compared to HD. Specifically, DC showed enrichment of an ML-NK subset characterized by the co- expression of NKG2A along with the chemokine receptor CXCR3. ML-NKs from DC exhibited reduced cell activation and degranulation potential upon stimulation, resulting in reduced natural cytotoxic activity when compared to HD and ART. Conversely, DC NKs presented robust ADCC responses against HIV- infected cells, similar to HD. FACS isolation confirmed that ML-NKs expressing NKG2A and CXCR3 receptors elicited strong ADCC responses against HIV-infected cells compared to other NK subsets.

Our results identify a ML-NK population in DC, characterized by the expression of NKG2C, NKG2A, CD57, and CXCR3. Importantly, this population presents enhanced ADCC-mediated killing capacity of HIV-infected cells, offering insights for potential NK-based immunotherapies to control HIV.

Functional MDSCs are Maintained during Antiretroviral Treatment (ART) and Preclude HIV-1 Reservoir Reactivation

Ana Gallego Cortés[1]; Carla Serra Peinado[1]; Laura Luque Ballesteros[1]; Judith Grau Expósito[1]; Joan Rey Cano[1]; Jordi Navarro[2]; Adrià Curran[2]; Joaquín Burgos[2]; Paula Suanzes[2]; Vicenç Falcó[2]; Meritxell Genecà[1]; María José Buzón[1]

[1]HIV Translational Laboratory, Dept. of Infectious Diseases, Vall d´Hebron Research Institute; [2]Dept. of Infectious Diseases, Vall d'Hebron University Hospital

In recent years, new therapeutic strategies aimed at depleting HIV-latent infection by inducing viral reactivation have been unsuccessful in vivo. The inability to reactivate and eliminate all HIV-infected cells could be attributed to the presence of immune-regulatory mechanisms that inhibit viral reactivation and the activity of anti-HIV immune effector cells. We hypothesize that HIV reactivation might be hindered by myeloid-derived suppressor cells (MDSC), a heterogeneous population of immature myeloid cells with high immunosuppressive effects.

Samples from n=14 viremic (VIR) and n=23 ART-suppressed (ART) individuals; and n=10 healthy donors (HD) were included in the phenotypic study. We assessed the frequency of two populations of MDSCs: M- MDSCs (CD3- CD11b+ CD33high HLA-DR- CD14+) and G-MDSCs (CD3- CD11b+ CD33mid HLA-DR- CD14-) using flow cytometry. MDSCs functional status was determined by the expression of Indoleamine 2,3-dioxygenase (IDO) and Arginase-1 (ARG). Samples from n=23 ART patients were used for functional studies in which virally-reactivated CD4+ T cells and MDSCs subpopulations were co-cultured. We assessed viral reactivation and cell activation by measuring the levels of the HIV protein p24 and CD69 expression.

Both VIR and ART cohorts exhibited elevated proportions of MDSCs expressing IDO and ARG compared to HD. M-MDSCs ARG+ and G-MDSCs ARG+ and IDO+ were significantly increased in the VIR group. In ART individuals, G-MDSCs ARG+ failed to normalize regardless of the duration of treatment. Functional assays demonstrated that G-MDSCs significantly reduced HIV reactivation from the latent reservoir ex vivo, which coincided with a modest yet consistent reduction in cell activation. This inhibitory effect was attributed to ARG activity, as the addition of the ARG inhibitor, nor-NOHA, could restore HIV reactivation.

Overall, we found that persistent HIV infection is associated with an expanded population of G-MDSCs ARG+ which impedes viral reactivation. Finding new therapeutic strategies targeting MDSCs could significantly impact the HIV reservoir.

HIV Infection Induces Neutralization-Interfering Antibodies that Hamper the Function of Neutralizing Antibodies

Mª Luisa Rodríguez de la Concepción[1]; Núria Pedreño-López[1]; Julieta Carabelli[1]; Erola Ainsua-Enrich[1]; Carla Rovirosa[1]; Carlos Ávila-Nieto[1]; Carmen Aguilar-Gurrieri[1]; Francesc Cunyat[1]; Edwards Pradenas[1]; Silvia Marfil[1]; Luis Manuel Molinos-Albert[1]; Ferran Tarrés-Freixas[1]; Bonaventura Clotet[1,3,4,5,6]; Julià Blanco[1,4,5,6]; Jorge Carrillo[1,4,6]

[1]IrsiCaixa AIDS Research Institute, Badalona, Spain; [2]Autònoma University of Barcelona, Bellaterra, Spain; [3]Lluita contra les Infeccions Foundation, Germans Trias i Pujol University Hospital, Badalona, Spain; [4]Germans Trias i Pujol Research Institute, Badalona, Spain; [5]Center for Health and Social Care Research, Faculty of Medicine, University of Vic – Central University of Catalonia, Vic, Spain; [6]Center for Biomedical Research in Network of Infectious Diseases - Carlos III Health Institute, Madrid, Spain

Background: The human immunodeficiency virus (HIV) induces a complex humoral response marked by the coexistence of neutralizing (NAbs) and non-neutralizing antibodies (non-NAbs). Although NAbs have been well characterized, little is known about the role of non-NAbs during infection. Non-NAbs can mediate anti-viral Fc-effector functions, such as antibody-dependent cellular cytotoxicity (ADCC) or antibody- dependent cellular phagocytosis (ADCP). However, they can also interfere with the action of NAbs by competing for binding to the Envelope glycoprotein (Env). Therefore, we aimed to determine whether HIV infection promotes the generation of neutralization-interfering antibodies (NiAbs).

Methods: Poor neutralizing plasma samples from chronically viremic HIV-infected (CHI) individuals were selected. The presence of NiAbs in these samples was determined by ELISA, flow cytometry (FACS) and neutralization competition assays. Recombinant Env-specific antibodies were generated by RT-PCR from single memory B cell microcultures or from single Env-specific memory B cells isolated by FACS.

Results: Plasma IgGs from CHI individuals were able to block the binding of NAbs to HIV Env, reducing its neutralizing capacity. Fifteen Env-specific recombinant monoclonal antibodies were isolated from a CHI individual. These antibodies were clonally unrelated, and showed limited or non-neutralizing capacity (3/15 and 12/15, respectively). Among non-NAbs, five recognized gp41 and seven targeted gp120. Three non- NAbs were identified as NiAbs as they competed with NAbs for binding to Env, reducing their neutralizing activity.

Conclusions: HIV infection elicits NiAbs, which may be used by the virus as an immune escape mechanism to hamper the development and function of NAbs. The identification of Env regions inducing NiAbs might be crucial for the design of a successful HIV vaccine.

HIV Infection Induces Neutralization-Interfering Antibodies that Hamper the Function of Neutralizing Antibodies

Mª Luisa Rodríguez de la Concepción[1]; Núria Pedreño-López[1]; Julieta Carabelli[1]; Erola Ainsua-Enrich[1]; Carla Rovirosa[1]; Carlos Ávila-Nieto[1]; Carmen Aguilar-Gurrieri[1]; Francesc Cunyat[1]; Edwards Pradenas[1]; Silvia Marfil[1]; Luis Manuel Molinos-Albert[1]; Ferran Tarrés-Freixas[1]; Bonaventura Clotet[1,3,4,5,6]; Julià Blanco[1,4,5,6]; Jorge Carrillo[1,4,6]

[1]IrsiCaixa AIDS Research Institute, Badalona, Spain; [2]Autònoma University of Barcelona, Bellaterra, Spain; [3]Lluita contra les Infeccions Foundation, Germans Trias i Pujol University Hospital, Badalona, Spain; [4]Germans Trias i Pujol Research Institute, Badalona, Spain; [5]Center for Health and Social Care Research, Faculty of Medicine, University of Vic – Central University of Catalonia, Vic, Spain; [6]Center for Biomedical Research in Network of Infectious Diseases - Carlos III Health Institute, Madrid, Spain

Background: The human immunodeficiency virus (HIV) induces a complex humoral response marked by the coexistence of neutralizing (NAbs) and non-neutralizing antibodies (non-NAbs). Although NAbs have been well characterized, little is known about the role of non-NAbs during infection. Non-NAbs can mediate anti-viral Fc-effector functions, such as antibody-dependent cellular cytotoxicity (ADCC) or antibody- dependent cellular phagocytosis (ADCP). However, they can also interfere with the action of NAbs by competing for binding to the Envelope glycoprotein (Env). Therefore, we aimed to determine whether HIV infection promotes the generation of neutralization-interfering antibodies (NiAbs).

Methods: Poor neutralizing plasma samples from chronically viremic HIV-infected (CHI) individuals were selected. The presence of NiAbs in these samples was determined by ELISA, flow cytometry (FACS) and neutralization competition assays. Recombinant Env-specific antibodies were generated by RT-PCR from single memory B cell microcultures or from single Env-specific memory B cells isolated by FACS.

Results: Plasma IgGs from CHI individuals were able to block the binding of NAbs to HIV Env, reducing its neutralizing capacity. Fifteen Env-specific recombinant monoclonal antibodies were isolated from a CHI individual. These antibodies were clonally unrelated, and showed limited or non-neutralizing capacity (3/15 and 12/15, respectively). Among non-NAbs, five recognized gp41 and seven targeted gp120. Three non- NAbs were identified as NiAbs as they competed with NAbs for binding to Env, reducing their neutralizing activity.

Conclusions: HIV infection elicits NiAbs, which may be used by the virus as an immune escape mechanism to hamper the development and function of NAbs. The identification of Env regions inducing NiAbs might be crucial for the design of a successful HIV vaccine.

Lack of HIV-associated pathogenicity in Viremic Non-Progressors is related with preserved TLR4 responsiveness

Ángel Bayón-Gil[1] ; Judith Dalmau[1] ; Itziar Erkizia[1] ; Javier Martínez-Picado[1,2,3]; Maria Carmen Puertas[1]

[1]IrsiCaixa AIDS Research Institute, Barcelona, Spain; [2]Catalan Institutio. for Research and Advanced Studies, Barcelona, Spain; [3]University of Vic – Central University of Catalonia, Vic, Spain

Background: Viremic Non-Progressors (VNPs) are an infrequent group of people with HIV (<0.1%) who naturally preserve CD4+ T cells despite uncontrolled viral replication, by unknown mechanisms. Previous results on single-cell transcriptomics showed a downregulation of interferon (IFN) signature in VNPs during chronic infection compared to standard Progressors (SPs).

Hypothesis: Restrained IFN response in VNPs may be originated by differences in the intrinsic capacity of host cells to produce and/or respond to IFN.

Methods: We selected retrospective samples of 14 VNPs and 22 SPs with similar high viremia (>104 copies/ml) but different CD4+ T-cell decay rate (<10% or >10% CD4+ T-cell loss annually in VNPs and SPs, respectively), and 10 HIV- controls. We phenotyped PBMCs populations by flow cytometry and cultured them in presence of TLR7/9/4 agonists (Imiquimod, ODN2216, or LPS) to evaluate IFNα and IFNγ production. Likewise, we exposed PBMCs to exogenous IFNα or IFNγ and quantified the expression of Interferon Stimulated Genes (ISGs) by qPCR (ISG15, MX1, SIGLEC1, CXCL9, IDO1).

Results: Compared to HIV- controls, PBMCs from both HIV+ groups showed poor IFNα and IFNγ production in vitro upon stimulation via TLR7 or TLR9, which typically sense viral components, and this was partially due to a reduction in plasmacytoid dendritic cells. Conversely, VNPs resembled HIV- controls in LPS-mediated IFNγ production, while SPs showed decreased response to bacterial sensing (pvalue=0.269 vs 0.027). Finally, no differences were detected between groups in ISGs upregulation after exposure to exogenous IFNα or IFNγ.

Conclusions: Beneficial moderation of chronic IFN response in VNPs is not due to intrinsic constraints of host cells to produce IFN after innate sensing, or to respond to IFN. Contrarily, preserved TLR4 responsiveness in VNPs may indicate exposure to lower levels of bacterial components in vivo, which has been associated with better preservation of gut barrier integrity during HIV infection

Session IV: Tumor Immunology and Immunotherapy

Dual blockade of PD-L1 and TIGIT pathways activated DCs and partially restores proinflammatory function in chronic viral infection

Maria Lázaro-Díez[1] ; Miguel Angel Marin[1] ; Valentina Casella[6] ; Laia Bernad[1] ; Eudald Vehí[1] ; Ruth Peña[1] ; Enrique Martin-Gayo[4,5]; Andreas Meyerhans[6] ; Julia G Prado[1,2,3]

[1]IrsiCaixa AIDS Research Institute, Barcelona, Spain; [2]Germans Trias i Pujol Research Institute, Badalona, Spain; [3]Center for Biomedical Research in Network of Infectious Diseases - Carlos III Health Institute, Madrid, Spain; [4]University Hospital la Princesa, Madrid, Spain; [5]Autónoma University of Madrid, Madrid, Spain; [6]Infection Biology Laboratory, Dept. of Medicine and Life Sciences, Pompeu Fabra University, Barcelona, Spain

Background: Chronic viral infections and other pathological conditions, such as cancer, promote overexpression of inhibitory receptors (IRs) on T cells, including PD-1 and TIGIT. The binding of IRs with their cognate ligands on APCs induces cellular-specific inhibitory signalling. Sustained inhibitory signalling and persistent immune activation during chronic infection drive immune exhaustion of antiviral responses. Here, we aim to evaluate the impact of blocking TIGIT and PD-L1 pathways for immune regulation, focusing on myeloid cells in LCMV chronic infection model.

Methods: C57BL/6JOlaHsd mice were infected with LCMVDOC. Once the chronic state is reached, 20 days after infection, 3 intraperitoneal doses of the single (α-PD-L1 or α-TIGIT) or combined treatment (α-PDL1+α-TIGIT) were administered. The mice (n=5/group) were euthanized at 28 days post-infection. Viral infectious units (FFUs) in spleen were assessed, DCs and monocytes in the spleen were immunophenotyped for activation markers and concentrations of 17 soluble cytokines in serum were determined by ELISA multiplex.

Results: No changes in FFUs were observed in α-PD-L1+α-TIGIT and α-TIGIT, although a trend in the reduction of FFUs was found in the α-PD-L1 condition. In DCs, a significant increase in CD40+ frequency and a trend of CD86+ increase in mice under α-PD-L1+α-TIGIT treatment were found. In monocytes, no changes in complementary activation markers CD86, and MCHII were found in any conditions tested. In addition, a significant increase of proinflammatory cytokines (TNF-α, TNFRI, RANTES) and the anti-inflammatory cytokine IL-10 was observed in α-PD-L1+α-TIGIT treatment, compared with single treatments and untreated infected mice.

Conclusions: In summary, our work characterizes the response to the dual blockade of PD-L1 and TIGIT on myeloid cells. The increase in CD40+ expression in DCs and proinflammatory profile indicate the potential of targeting PDL-1/ TIGIT as an immunoregulatory intervention in chronic viral infections.

*MLD and MM contributed equally to this work

Therapeutic impact and immune modulation by MYC inhibition in KRAS-driven NSCLC with diverse mutational landscapes

Íñigo González-Larreategui[1] ; Laura Vera[2] ; Fabio Giuntini[1] ; Lorena Sansegundo[3] ; Sandra Martínez-Martín[3] ; Daniel Capitán[1] ; Manrique Valdés-Bango[1] ; Judit Grueso[1] ; Sergio López[3] ; Erika Serrano del Pozo[1] ; Hugo Thabussot[3] ; Irati Macaya[2] ; Marie-Eve Beaulieu[3] ; Silvestre Vicent[2,4,5]; Sílvia Casacuberta-Serra[3] ; Laura Soucek[1,3,6,7]

[1]Vall d'Hebron Institute of Oncology, Barcelona, Spain; [2]Program in Solid Tumors and Biomarkers, Center for Applied Medical Research, Navarra University, Pamplona, Spain; [3]Peptomyc S.L. Ed. Cellex, Barcelona, Spain; [4]Center for Biomedical Research in Network of Cancer - Carlos III Health Institute, Madrid, Spain; [5] Navarra Institute for Health Research, Pamplona, Spain; [6]University of Barcelona, Barcelona, Spain; [7]Catalan Institution for Research and Advanced Studies, Barcelona, Spain

Introduction: KRAS is the most frequent mutated oncogene in Non-Small-Cell Lung Cancer (NSCLC), linked to poor prognosis and tumor recurrence. Co-mutations in tumor suppressor genes (TSGs), mainly TRP53, KEAP1 and STK11 (LKB1), influence treatment response of KRAS-mutated cancers. MYC, a key transcription factor downstream of KRAS, not only promotes tumor progression and treatment resistance, but also orchestrates tumor immune evasion. Although MYC was long considered undruggable, our lab pioneered the use of Omomyc as the first clinically viable MYC inhibitor. Here, we aim to determine how different TSGs mutations impact the response of KRAS-mutant tumors to MYC inhibition, as well as their effect on the modulation of the tumor immune microenvironment.

Methodology: We used mutant KRAS Lung Adenocarcinoma isogenic cell lines (KLA) CRISPR-edited to knockout TRP53, STK11 or KEAP1 genes. MYC levels were determined by WB, and response to Omomyc was assessed by proliferation, metabolic and transcriptomic assays. For in vivo studies, cells were injected subcutaneously into C57BL/6x129/Sv F1 mice and immune populations were characterized by flow cytometry.

Results: MYC levels were increased in the TSG-edited cells compared to the parental ones, but Omomyc reduced in vitro cell growth, changed the cell cycle profile and modulated tumor immune microenvironmentrelated gene sets across all KLA cell lines. In mice, systemic MYC inhibition displayed different therapeutic efficacy in the different mutational profiles, and the effect of MYC inhibition on remodeling the tumor immune microenvironment varied depending on the specific TSG co-mutation. Importantly, Omomyc enhanced the therapeutic efficacy of different immunotherapies.

Conclusions: Prevalent co-occurring mutations in TSGs in KRAS-driven NSCLC influence the therapeutic effect of MYC inhibition by Omomyc treatment and modulate tumor immune microenvironment. Interestingly, a stronger Omomyc efficacy correlates to an enhanced anti-tumor immune response, positioning it as a potential partner for immuno-oncology treatments.

Session IV: Tumor Immunology and Immunotherapy

Immunotherapy validation of Epstein Barr Virus Specific-T cells expressing the anti-CD19 Chimeric Antigen Receptor (CAR.CD19 ARI-0001) to treat B-cell neoplasms

Ana Gabriela Lara-de-León[1,2]; Rut Mora-Buch[2]; Emma Enrich[2]; Natalia Egri[1,3]; Maria Tomás-Marín[2]; Mireia Antón-Iborra[2,3]; Mireia Uribe-Herranz[1]; Francesc Rudilla[2]; Manel Juan[1,3]

[1]*August Pi i Sunyer Research Institute, Barcelona, Spain;* [2]*Blood and Tissue Bank, Barcelona, Spain;* [3]*Hospital Clinic, Barcelona, Spain*

Chimeric Antigen Receptor T cells against CD19 (CAR-T.CD19) has proven to be one of the most promising treatments for B-cell neoplasms. However, CAR-T therapy has some limitations, such as the lack of cell proliferation and persistence in vivo. CAR-T action could be prolonged and enhanced using virus-specific T cells (VST). Epstein-Barr virus (EBV) is highly prevalent in human population, and it is involved in the pathophysiology of some tumors.

The aim of this project is to optimize and validate a production of EBV-specific T cells off-the-shelf, capable of expressing the CAR.CD19 to ensure proliferation and persistence of ARI-0001 in vivo.

PBMCs from healthy EBV-seropositive donors were activated with overlapping peptides of EBV-specific antigens in G-Rex® 24-well plate. Different culture conditions were tested to optimize T cell specificity and expansion. The optimal condition was replicated 5 times, obtaining a fold expansion in the culture of 16.2±8.9SD, 94.32%±10.84SD of CD3+T lymphocytes, of which 80.2%±30.52SD were CD8+ T cells.
Additionally, more than 80% of CD8+T cells expressed IFN-γ in response to EBV antigens. Production of VSTs has been scaled up under Good Manufacturing Practices (n=1).TCRβ sequencing demonstrated increased clonality in the expanded products and detected specific clonotypes of EBV antigens presented by donor-expressed HLA molecules. EBV-specific T cells transduced with ARI-0001 construct are able to express the CAR.CD19. In vitro cytotoxic assay showed that the CAR-VSTs product is able to kill target cells (NALM6) in co-culture. Since EBV-VSTs, as the intermediate product, are cryopreserved, the expansion of final product (CAR-VSTs) is still under optimization.

In conclusion, we obtained a T cell product highly specific against EBV antigens, with the potential to have anti-tumor activity conferred by CAR.CD19.

Session IV: Tumor Immunology and Immunotherapy

Targeting of cancer cells by human CD6-based CAR-T/NK cells*

Lucía Aragón Serrano[1]; Laura Carrillo i Serradell[1]; Violeta Planells i Romeo[1]; María Velasco de Andrés[1]; Francisco Lozano Soto[1,2,3]

[1]August Pi i Sunyer Research Institute, Barcelona, Spain; [2]Immunology Service, Biomedical Diagnostic Center, Clínic Hospital of Barcelona, Barcelona, Spain; [3]Dept. of Biomedicine, Faculty of Medicine and Health Sciences, University of Barcelona, Barcelona, Spain

Cancer is an unmet clinical need still waiting for more efficient and safer treatments. Current immunotherapies based on adoptive transfer of cells engineered with chimeric antigen receptors (CARs) are gaining attention for its effectiveness against haematological malignancies and to a lesser extend against solid tumours. One of its limitations comes from the difficulty of finding suitable targets, leading to severe on-target and off-tumour toxicities. Indeed, most CAR targets are tumour-associated antigens (TAAs), which are abundant on cancer cells and scarce on healthy tissue. CD6 is a signal transducing transmembrane receptor expressed by all T cells, and some B and NK cell subsets, whose broadly tissue distributed ligands (CD166/ALCAM, CD318/CDCP-1, Galectins 1 and 3) are found overexpressed by malignant cells. This places CD6 as a potential target for novel therapies against haematological and solid tumours. On this basis, we have designed a second-generation CAR whose antigen-binding domain corresponds to the whole extracellular region of human CD6 (CD6-CAR), to lentivirally transduce human primary T cells and lymphoblastoid NK (KHYG-1) cells. Both CD6-CAR T/KHYG-1 cells showed higher cytotoxic activity and cytokine secretion (INF-γ) when co-cultured with high CD166/ALCAM-expressing carcinoma cells of colon (DLD-1) and ovarian (SKOV-3) origin compared to untransduced cells. No differences regarding cytotoxic activity were observed between CD6-CAR transduced and untransduced cells when co-cultured with CD166/ALCAM low (Raji, Daudi) and negative (K562) lymphoblastoid and erythroid cell lines, respectively. This data supports a role for surface CD166/ALCAM dosage-dependence in minimizing off- tumour on-target toxicity, though further in vivo efficacy and toxicity studies are awaited for clarifying this issue.

(*) Work supported by MCIN/AEI /10.13039/501100011033/FEDER, UE (PID2022-140932OB-I00) and AGAUR (2021/SGR/0113). LC-S and VP-R are recipients of fellowships PRE2020-093993 and FPU21/06217, respectively.

Session IV: Tumor Immunology and Immunotherapy

Single-cell RNA sequencing temporal analysis reveals a detrimental effect of JAK inhibition on myeloid cells in tofacitinib refractory ulcerative colitis patients

Elisa Melón-Ardanaz[1]; Marisol Veny[1]; Ana María Corraliza[1]; Alba Garrido-Trigo[1]; Victoria Gudiño[1]; Marc Moro[1]; Ángela Sanzo-Machuca[1]; Miriam Esteller[1]; Maite Rodrigo[2]; Bram Verstockt[3]; Severine Vermeire[3]; Maria Carme Masamunt[1]; Ángel Giner[1]; Ingrid Ordás[1]; Agnès Fernández-Clotet[1]; Elena Ricart[1]; Julià Panés[1]; Azucena Salas[1]

[1]Inflammatory Bowel Disease Unit, August Pi i Sunyer Research Institute, Clínic Hospital of Barcelona, Barcelona, Spain; [2]Dept. of Pathology, August Pi i Sunyer Research Institute, Clínic Hospital of Barcelona, Barcelona, Spain; [3]Dept. of Gastroenterology and Hepatology, Catholic University of Leuven, Leuven, Belgium

Background:
Tofacitinib is an oral JAK1 and JAK3 inhibitor approved for the treatment of ulcerative colitis (UC). Our aim is to identify the cellular subsets and genes involved in the response and/or resistance to tofacitinib using single-cell RNA sequencing (scRNA-seq) and in vitro functional experiments.

Methods:
Colon biopsies from patients with active UC starting tofacitinib were collected at baseline and during follow- up (8-48 weeks) and processed by scRNA-seq. Patients were classified as responders (R) or non-responders (NR) based in clinical, endoscopic and histological criteria. Functional response to tofacitinib in macrophages was investigated using monocyte-derived blood macrophages under different inflammatory stimuli (LPS, TNF and IFNg).

Results:
Contrary to R, scRNA-seq analysis demonstrated an increase in the proportions of neutrophils, M1-macrophages and inflammatory-monocytes in NR after treatment. The number of anti-inflammatory M2-macrophages remained similar, but their transcriptional signature was different depending on the response. NR M2 macrophages upregulated the expression of M1-related genes (CLEC5A, INHBA) during follow-up, while in R tofacitinib induced upregulation of tolerogenic genes (IGF1, IL10RA). In in vitro monocyte-derived macrophages tofacitinib was able to block the inflammation induced by IFNg and TNF. However, the response to LPS was intensified by tofacitinib as seen by the increased expression of genes including M1-like markers (INHBA, CLEC5A) and cytokines (CXCL1, IL6). LPS-stimulated macrophages produced higher levels of the anti-inflammatory IL10 gene compared to the other stimuli. The transcriptional profile of LPS-stimulated macrophages treated with a blocking anti-IL-10 antibody (Cuevas, Víctor D et al, 2022) highly correlated with the genes upregulated in M2-macrophages from tofacitinib NR patients.

Conclusions:
Our study identifies LPS-activated macrophages as a main cellular subset driving resistance to tofacitinib. We demonstrate that, through blockade of IL-10 signaling, tofacitinib promotes the hyperactivation of macrophages in response to LPS which, in contrast to IFNg or TNF, heavily rely on IL-10 regulation.

Session IV: Tumor Immunology and Immunotherapy

Immunomonitoring of intravenous and subcutaneous anti-CD49d treatment in patients with Multiple Sclerosis

Alex Agundez Moreno[1,2]; Silvia Presas Rodríguez[1] ; Aina Teniente Serra[1,2,3]; Cristina Ramo Tello[1] ; Eva Martínez Cáceres[1,2,3]

[1]Germans Trias i Pujol University Hospital, Badalona, Spain; [2]Germans Trias i Pujol Research Institute, Badalona, Spain; Dept. of Cell Biology, Physiology and Immunology, Autònoma University of Barcelona, Barcelona, Spain; [4]Mataró Hospital, Mataró, Spain; [5]Sant Joan Despí Moisès Broggi Hospital, Sant Joan Despí, Spain; [6]Santa Creu i Sant Pau Hospital, Barcelona, Spain; [7] General Hospital of Granollers, Granollers, Spain

Background
Natalizumab (NTZ) (Tysabri®) is a humanized monoclonal antibody that selectively binds the α4-integrin (CD49d), preventing the migration into the central nervous system. NTZ is administrated intravenously, but recently, in 2021, the EMA accepted subcutaneous administration. It is indicated for the treatment of Multiple Sclerosis (MS), being one of the most effective and tolerated treatment to reduce the activity of the disease. Adversely, its use is associated with a higher risk of developing Progressive Multifocal Leukoencephalopathy, produced by JC virus reactivation, as a consequence of immunosurveillance blocking. For this reason, immunomonitoring NTZ-treated patients is a must.

Objective
To identify differences in the percentage of receptor occupancy (CD49d R.O.) between patients that changed their route of administration from intravenous (IV) to subcutaneous (SC).

Methods
Longitudinal ongoing study in 27 RRMS patients treated with NTZ, two groups were established: standard dose (SID) and extended interval dose (EID). Peripheral blood samples were analysed through quantitative flow cytometry to determine CD49d R.O. in CD4+, CD8+ T cells and CD19+ B cells. Patients were analysed at 3 different time points in SID and 2 different time points in EID.

Results
By date 20 from the 27 patients included in the study were analysed: 4 SID and 16 EID. When comparing the change from IV to SC administration no significant differences were seen neither for CD49d R.O. and nor for clinical scores. EID patients showed receptor saturation percentages around 60% and higher number of CD49d molecules compared to those treated in SID with saturation percentages around 80%.

Conclusions
Our study shows that the change of administration route from IV to SC is safe, maintains CD49d molecules, bound NTZ molecules, and % R.O. stable over time. Additionally, the change to SC administration does not induce clinical alterations, suggesting SC administration as the best option for NTZ-treated patients.

Anti-phospholipid antibodies analysis: assessment laboratory algorithm since two years of implementation

Clara Esteve Cols[1]; Marina Carbonell Prat[1]; Laura Puigví Fernández[1]; Míriam Ruiz Fernández[1]; Diana Regidor López[1]; Maria Teresa Massaro[1]; Sílvia Miró Cañís[1]; Alba Cebollero Agustí[1]; Miguel Ángel Benítez Merelo[1]

[1]*CLILAB Diagnòstics, Vilafrnaca del Penedès, Spain*

Introduction
Laboratory markers of Anti-Phospholipid Syndrome(APS) are: Lupic Anticoagulant(LA) and four antiphospholipid antibodies (aPL): anti-cardiolipin IgG (aCLG) and IgM (aCLM), anti- β2glycoprotein I IgG (aβ2-GPIG) and IgM (aβ2-GPIM). However, aCLs have been described in 10% of general population, in infections and malignancies. To minimize cross-reactions and thus facilitate interpretation, we have created an algorithm including LA, aCLG and aβ2-GPIG. In case of negative result, IgM isotype is extended.

Targets
To analyse agreement between aPL and LA, and to assess our algorithm.

Materials and methods
Requests with APS study were analysed between 2021 and 2022. For aPLs analysis, isotype and levels (low 20-40CU, and high >40CU) have taken into account. aPLs were measured by chemiluminiscence with BIOFLASH platform, and LA with dilute Russell virus venom coagulometric reagents(dRVVT) and Clotting Time (SCT) Silica with ACLTOP750 platform.

Results
972 requests were analysed. LA agreement with each aPL is:70.6% at low levels of aCLG, and 88.6% at high levels (Table 1); 90.5% at low levels of aβ2-GPIG, and 93% at high levels (Table 2); 75.6% at low levels of aCLM, and 81.8% at high levels (Table 3); 75% at low levels of aβ2-GPIM, and 88.2% at high levels (Table 4).

Patients with positive LA have higher levels of aβ2-GPIG(1687CU) than aCLG, aCLM and aβ2-GPIM (355.52CU; 167.3CU and 169.5CU). 22% have low levels of aCLG, compared to 58.5% with low levels of IgM aPL. 9.5% with negative IgG aPL (85.18%) have some positive IgM aPL.

Conclusions
aβ2-GPIG have better agreement with LA, especially at high levels, as well as higher positivity rates. These results agree with the better specificity of aβ2-GPIG than aCLG described.

Less than 10% of IgG aPL negative has one of the IgM aPL positive and with low levels, so its interpretation might be complicated mainly in contexts of infections or malignancies. These results support the usefulness of our algorithm.

Association of GDF-15 levels with clinical and laboratory features in SSc. Our center's experience.

Elionor Lynton-Pons[1]; Andrea Bravo Gómez[1]; Esther Ortiz[1]; José Luís Tandaipan[1]; Laura Martínez-Martínez[1]; Iván Castellví[1]; Anaís Mariscal[1]

[1]*Santa Creu i Sant Pau Hospital, Barcelona, Spain*

Introduction:
Growth differentiation factor (GDF)-15, also known as macrophage inhibitory cytokine (MIC)-1, is a secreted member of the transforming growth factor (TGF)-β cytokine superfamily. Elevated levels of GDF-15 have been found in pathological conditions involving inflammation and/or oxidative stress including cancer, diabetes, cardiovascular, pulmonary, and renal disease. Increased levels of this cytokine have also been found in autoimmune diseases such as autoimmune hepatitis, systemic lupus erythematosus, rheumatoid arthritis or systemic sclerosis (SSc). In SSc, previous studies have found higher levels of serum GDF-15 (sGDF-15) related to diffuse SSc, pulmonary involvement, and the extent of skin sclerosis. Here we aimed to associate sGDF-15 levels with clinical and laboratory features in those patients with GDF-15 request in our laboratory and SSc diagnosis.

Methods:
We received sGDF-15 requests from 42 patients with SSc between January 2020 and September 2023. We measured sGDF-15 levels by enzyme-linked immunosorbent assay and associated them with clinical and laboratory features: SSc type (limited, diffuse or pre-SSc), extent of skin sclerosis, lung involvement or myopathy, and autoantibody status.

Results:
We found higher sGDF-15 levels in SSc patients than in healthy controls (HC) (2107 ± 1417pg/mL SSc vs 644.8 ± 354.2 pg/mL HC, $p < 0.001$). Levels of sGDF-15 were higher in SSc patients with pulmonary involvement according to the percentage of diffusing capacity of the lungs for carbon monoxide (% DLCO) lower than 60% (2829 ± 1574 pg/mL DLCO ≤60 vs 1329±727 pg/mL DLCO >60, $p < 0.01$). We also found a correlation of sGDF-15 levels with %DLCO ($r = -0.599$, $p<0.001$) and forced vital capacity (FVC)/DLCO ratio ($r = 0.414$, $p < 0.05$).

Conclusion:
Increased levels of sGDF-15 could be reflecting lung damage and it may be used as a biomarker of pulmonary involvement severity in SSc patients.

Síndrome de Rowell. A propòsit d'un cas

Roger Randolfe Ricart[1]; Eduardo Villegas Martín[2]; Jorge Eguía Núñez[2]

[1]Hospital del Mar, Barcelona, Espanya; [2]Laboratori de Referència de Catalunya, El Prat del Llobregat, Espanya

Introducció
La síndrome de Rowell (SR) és una malaltia infreqüent caracteritzada per lesions de lupus eritematós (LE) i eritema multiforme (EM) en pacients amb anticossos antinuclears (ANA) positius patró clapat, sobretot dones caucàsiques vora els 30 anys.

Els criteris diagnòstics van ser classificats per Zeitouni (2000) en majors (LE, lesions similars a EM, ANA positius) i menors (eritema perni, anticossos anti-Ro/SSA o anti-La/SSB, FR positiu). Posteriorment van ser revisats per Torchia (2012) – majors: LE cutani crònic, almenys un anticòs positiu (ANA, anti-Ro/SSA, anti-La/SSB), lesions tipus EM amb immunofluorescència directa negativa; menors: absència de desencadenants infecciosos o farmacològics i de localització típica de EM (acral, mucoses), i presència d'almenys un criteri de LE.

Cas clínic
Dona de 31 anys de Filipines que ingressa per dolor muscular predominantment a escàpules i extremitats inferiors orientant-se com possible miopatia inflamatòria, però amb mínima elevació dels enzims musculars.

Presenta febre, i a l'analítica inicial s'observa anèmia, limfopènia i augment dels enzims hepàtics.

Passades 48h apareixen lesions papulars eritematoses a cara i extremitats amb afectació palmoplantar. La pacient refereix també odinofàgia, de manera que el diagnòstic s'orienta com eritema multiforme en context de quadre viral vs SR.

La PCR panviral resulta negativa, i la pacient compleix criteris diagnòstics de LE, tant clínics (febre inexplicada, probable LE cutani subagut, artromiàlgies, alopècia no cicatricial, proteïnúria) com immunològics (ANA positius, C3 i C4 disminuïts, anticossos anti-DNA positius, anticoagulant lúpic positiu).

La biòpsia de les lesions cutànies revela canvis compatibles amb dermatitis crònica liquenoide superficial, amb component purpúric, i no mostra troballes diagnòstiques d'eritema multiforme.

Discussió
La pacient compleix els criteris majors de Zeitouni, i el criteri menor de positivitat per als anticossos anti-Ro i anti-La. Compleix també tots els criteris, majors i menors, de Torchia. Per tant, atenent les dues classsificacions, el diagnòstic final és de SR.

A maternal diet rich in polyphenols and fiber ameliorates the offspring acute inflammation challenge later in life.

Sergi Casanova-Crespo[1,2]; Daniela Ceballos-Sánchez[1,2]; Maria José Rodríguez-Lagunas[1,2]; Malén Massot-Cladera[1,2]; Margarida Castell-Escuer[1,2,3]; Francisco José Pérez-Cano[1,2]

[1]Dept. of Biochemistry and Physiology, Faculty of Pharmacy and Food Science, University of Barcelona, Barcelona, Spain; [2]Research Institute of Nutrition and Food Safety, University of Barcelona, Barcelona, Spain; [3]Center for Biomedical Research in Network of Pathophysiology of Obesity and Nutrition - Carlos III Health Institute, Madrid, Spain

Fiber and polyphenols are bioactive components of a healthy diet that can modulate the immune system and exhibit anti-inflammatory properties. However, little is known about the role of these components during gestation and lactation on the infant's health. The main objective of this work was to assess the potential anti-inflammatory role of a maternal diet rich in polyphenols and fiber during the pre-gestation, gestation, and lactation periods on an acute inflammation process in their offspring later in life.

7-week-old rats were fed an experimental diet rich in polyphenols and fiber Healthy diet (HD group) or a reference diet (REF group) during pre-gestation (3 weeks), gestation (3 weeks) and lactation (3 weeks).The pups were weaned from their mothers on day 21 of life and feed a reference diet until they become young adults (7 weeks of age). During that period, their food and water intake were monitored three times a week, and fecal samples were collected weekly to measure pH and moisture content. At the end of the study, a local inflammation was induced by injecting carrageenin into the rats' paws. The inflammatory process was monitored for 6 h using a plethysmometer.

The body weight of the animals was similar between groups, but some changes in fecal pH,and moisture content appeared due to the maternal diet. In addition, at the end of the study, a lower paw inflammation was observed in the offspring of rats from the HD group compared to the REF group ($p<0.05$).

In conclusion, polyphenols and fiber intake in mothers show an immunoprogramming potential in their offspring by reducing an inflammation process later in life. These effects may be associated with alterations
at the epigenetic level and changes in the microbiota composition. Thus, these and other immune mechanisms should be further explored.

Antibodies against the flotillin-1/2 complex in patients with multiple sclerosis

Cinta Lleixà[1,2,3]; Teresa Franco-Leyva[1]; Marta Caballero-Ávila[2]; Elba Pascual-Goñi[i2]; Lorena Martín-Aguilar[2]; Esther Moga[1]; Luis Brieva[4]; Sergio Martínez-Yélamos[5]; Luis Querol[2,3]; Laura Martínez-Martínez[1]

[1]Dept. of Immunology, Santa Creu i Sant Pau Hospital-Autònoma University of Barcelona, Barcelona, Spain; [2]Neuromuscular Diseases Unit, Biomedical Research Institute Santa Creu i Sant Pau, Barcelona, Spain; [3]Center for Biomedical Research in Network of Rare Diseases - Carlos III Health Institute, Madrid, Spain; [4]Dept. of Neurology, Arnau de Vilanova University Hospital, Lleida, Spain; [5]Multiple Sclerosis Unit, Dept. of Neurology, Bellvitge Biomedical Research Institute, Bellvitge University Hospital, Barcelona, Spain

Background: Multiple sclerosis (MS) is a tissue-specific autoimmune disease of the central nervous system in which the antigen(s) remains elusive. Antibodies targeting the flotillin-1/2 (FLOT–1/2) complex have been described in 1-2% of the patients in a recent study. Other candidate antigens as anoctamin-2 (ANO2) or neurofascin-155 (NF155) have been previously described in MS patients, although their clinical relevance remains uncertain.

Objective: Our study aims to analyse the frequency and clinical relevance of antibodies against NF155, ANO2 and the FLOT-1/2 complex in MS.

Methods: Serum (n=252) and CSF (n=50) samples from 282 MS patients were included in the study. The control group was composed of 260 serum samples (71 healthy donors and 189 with other neuroinflammatory disorders). Anti-FLOT1/2, anti-ANO2 and anti-NF155 antibodies were tested by in-house cell-based assays (CBA) using transfected-HEK293 cells. Anti-FLOT1/2antibodies were also assessed by commercial CBA (Euroimmun).

Results: We identified 6 MS patients with antibodies against the FLOT-1/2 complex (2.1%) by both commercial and in-house CBA, and 1 MS patient with antibodies against ANO2 (0.35%). All MS patients were negative for anti-NF155 antibodies. Three of the anti-FLOT1/2 positive patients showed anti-FLOT-1/2 positivity in other serum samples extracted at different moments of their disease. IgG subclasses of anti-FLOT-1/2 antibodies were predominantly IgG1 and IgG3.

Conclusion: We confirm that antibodies targeting the Flotillin-1/2 complex are present in a subgroup of patients with MS. Further studies are needed to understand the clinical and pathological relevance of anti-FLOT-1/2 autoantibodies in MS.

Fiber and polyphenol-enriched diet during pregestation, gestation and lactation periods modulates maternal spleen and mesenteric lymph node cell phenotypes

Daniela Ceballos-Sánchez[1,2]; Sergi Casanova-Crespo[1,2]; Maria Jose Rodríguez-Lagunas[1,2]; Margarida Castell[1,2,3] ; Magdalena Massot-Cladera[1,2]; Francisco Jose Pérez-Cano[1,2]

[1]Dept. of Biochemistry and Physiology, Faculty of Pharmacy and Food Science, University of Barcelona, Barcelona, Spain; [2] Institute of Research in Nutrition and Food Safety, University of Barcelona, Barcelona, Spain; [3]Center for Biomedical Research in Network of Pathophysiology of Obesity and Nutrition - Carlos III Health Institute, Madrid, Spain

Understanding the effects of dietary compounds on the immune system during critical life stages such as gestation and lactation is crucial. Among these components, fiber and polyphenols have emerged as significant influencers. Fiber can modulate the immune response through microbiota, while polyphenols possess anti-inflammatory and antioxidant properties. This study aims to explore the impact of a fiber and polyphenols-enriched diet (HFP) on lymphocyte phenotype in spleen (SPL) and mesenteric lymph nodes (MLN) cells, shedding light on potential immunomodulatory effects for maternal health. Five groups of female rats were categorized based on their intake period of HFP diet: the pregestation period (PG group), the gestation period (G group), the lactation period (L group), or during all three periods (PGS group). The reference group was fed the standard diet during all three periods (REF group). At the end of lactation (21 days after birth), SPL and MLN were obtained and lymphocytes isolated. The cells were phenotyped using antibodies conjugated to diverse fluorochromes to analyze the following subsets: T cells (Tc -both TCRαβ or TCRγδ-, Th and NKT), NK, and B cells. At MLN, the groups with HFP during lactation (PGS or S) showed lower T:B lymphocyte ratio and higher NK proportion than those of PG and G groups. However, no changes in NKT proportion were observed due to the dietary interventions. A different effect was observed in the SPL: the HFP diet did not modified either the T:B ratio or the NK proportion but the NKT proportion in the PGS group was lower than that in the P, G and S groups. Overall, it is evidenced the impact of a particular maternal diet on the phenotype of their lymphocytes both at mucosal and systemic levels and these changes are dependent on the particular period of dietary intervention (pregestation, gestation or lactation).

CD3G mutation finding challenges a patient's common variable immunodeficiency diagnosis

Andrea Carolina Bravo Gómez[1] ; Teresa Franco Leyva[1] ; Anna Marin Marin[2] ; Rebeca Fernández Megino[2] ; Anna Calvet Lacruz[1] ; José R. Regueiro González-Barros[2] ; Laura Martínez-Martínez[1] ; Óscar de la Calle Martín[1]

[1]Immunology Service, Santa Creu i Sant Pau Hospital, Barcelona, Spain; [2]Dept. of Immunology, Ophthalmology and ORL, Complutense University of Madrid, Madrid, Spain

T-cell receptor-CD3 complex consists in an αβ or γδ TCR heterodimer associated with CD3δε, CD3γε and ζ–ζ dimers. Mutations in any CD3 chain can have a significant effect on the TCR-CD3 complex. CD3γ (CD3G) mutations produce a Combined Immunodeficiency (CID).

We present a 39-year-old female with a history of recurrent flu and fever since childhood. At 15 years old, after 3 seizure episodes, she was started on Carbamazepine (CBZ). After 2 years of treatment, she developed recurrent pneumonias with 3 hospital admissions and hypogammaglobulinemia was detected. Despite discontinuation of CBZ treatment, neither hypogammaglobulinemia nor infections improved.

At 20 years of age, the increase in IgM together with the absence of IgG and IgA led to the suspicion of a hyper-IgM syndrome. That was ruled out after genetic study (CD40, CD40L, AID). Given the suspicion of primary immunodeficiency (PID), she was started on immunoglobulin replacement therapy, attaining IgG levels >700mg/dL and controlling the infections. The diagnosis changed to Common Variable Immunodeficiency (CVID) with an altered T cell compartment (inverted CD4/CD8).

At 39 years old, NGS virtual panel for PID was performed, with no clear pathogenic finding, but with several mutations of uncertain significance (VUS). Of these, an heterozygous CD3G missense mutation (c.473A>G, p.Gln158Arg) in the ITAM motif was the most potentially pathogenic. Flow-cytometry and functional studies showed altered T lymphocyte function and lower intracellular expression of the CD3gamma. Additionally, familial mutant segregation was studied in her descents: her eldest son (10 y.o.) who had frequent non-severe infections in early childhood inherited the mutation while her youngest son (6 y.o.) was wild-type.

Clinical features in patients with CD3G mutations range from healthy individual to life-threatening CID, as reported by Regueiro et al. Our mutation could explain the patient's clinical picture. Ongoing studies are being carried out to validate our hypothesis.

Generation of CRISPR/CAS9-mediated PD-1 knock-out ARI-0001 CAR-T cells

Blanca Tocornal Garnica[2] ; Carles Serra Pagès[1,2]; Europa Azucena González Navarro[1,2]; Manel Juan Otero[1,2]

[1]*Immunology Service, Biomedical Diagnostic Center, Clínic Hospital of Barcelona, Barcelona, Spain;* [2]*August Pi i Sunyer Research Institute, Clínic Hospital of Barcelona, Barcelona, Spain*

Chimeric antigen receptor (CAR)-T cell therapy has shown great promise in treating hematological malignancies. However, current viral gene delivery methods have several limitations such as the inaccurate insertion of the CAR transgene. CRISPR/Cas9 gene editing technology allows insertion of CAR transgenes to specific genomic sites of primary human T cells. By extension, this targeted insertion permits the replacement or disruption of relevant endogenous genes such as immune checkpoint molecules. PD-1 is an inhibitory surface receptor that inhibits T cell function when binding to its ligand PD-L1. This binding produces negative signals, induces apoptosis and reduces immunocompetence of T cells, which permits immune evasion of cancer cells. Disruption of PDCD1 (the PD-1 coding gene) restores T cell function and its capacity to eliminate cancer cells. The objective of this project is to direct the ARI-0001 CAR construct insertion through CRISPR/Cas9 targeting system into the PD-1 locus of the T cell genome, in order to generate PD-1 knock-out anti-CD19 CAR-T cells.

SYK mutations in a patient with Immune Dysregulation

Anna Calvet Lacruz[1] ; Teresa Franco Leyva[1] ; Andrea Carolina Bravo Gomez[1] ; Àlex Bofí Cuadros[1] ; Laura Martínez Martínez[1] ; Óscar de la Calle Martín[1]

[1]*Immunology Service, Santa Creu i Sant Pau Hospital, Barcelona, Spain*

We present a 48-year-old man referred to our Immunology service for hypogammaglobulinemia and lymphopenia. His clinical history included respiratory infections with bronchiectasis, ulcerative colitis, Raynaud's phenomenon, acne and seborrheic dermatitis, oral and genital Condyloma acuminatum, HPV+ oral and pubic squamous cell carcinoma, non-ischemic dilated cardiomyopathy and osteoporosis. All these events were suggestive a profound immune dysregulation.

The genetic analysis by NGS virtual panel for Inborn Errors of Immunity revealed an heterozygous missense variant in exon 10 of SYK c.1183G>A (p.Val395Ille). This variant had only been reported in databases in three subjects with unknown clinical association. Sanger sequencing showed 5 additional SYK polymorphisms, 3 intronic and 2 exonic synonymous [c.1302G>C (p.Arg434=), c.1338G>A (p.Leu446=)]. Familiar segregation stablished two haplotypes in the patient: the maternal that included 2 intronic [c.1391+58 C>T, c.1391+69 C>T] and the 2 exonic synonymous variants, and the paternal, with the missense variant and the other intronic [c.1182-7 C>G]. The brother carried only the paternal haplotype despite showing no clinical symptoms. The father could not be studied since he had died from pancreatic cancer.

Mutations in SYK were described in 2021 in 6 patients with clinical features matching our patient's. The immune phenotype alterations were also compatible: inverted CD4/CD8 ratio, peripheral CD4+ and CD8+ T cells skewed towards effector-memory cells (CXCR7-) with absence of naive T cells. All of these SYK mutations, including ours, are located in the kinase domain of the protein. Published functional studies proved spontaneous phosphorylation, enhanced downstream signalling and higher production of proinflammatory cytokines. Functional studies to demonstrate the gain-of-function effect of our patient's mutation are still in progress. The complexity of familial segregation would need further studies. However, all these above-mentioned commonalities support the hypothesis that our patient's missense variant is responsible for his clinical manifestations.

Macrophage characterization in muscle biopsies

Andrea Carolina Bravo Gómez[1] ; Elionor Lynton Pons[1] ; Esther Ortiz Losada[1] ; Rosa Maria Blanco Soto[2] ; Paula Llarch Cegarra[1] ; Esther Peña Sendra[3] ; Eduard Gallardo Vigo[2] ; Anaís Mariscal Rodriguez[1]

[1]Immunology Service, Santa Creu i Sant Pau Hospital, Barcelona, Spain; [2]Dept.of Neuromuscular Diseases, Biomedical Research Institute Santa Creu i Sant Pau, Barcelona, Spain; [3]Confocal Microscopy Platform, Biomedical Research Institute Santa Creu i Sant Pau, Barcelona, Spain

Idiopathic inflammatory myopathies (IIM) are a group of immune-mediated diseases characterized by muscle weakness and the presence of inflammatory infiltrates in the muscle biopsy. Muscle biopsies have a significant role in the diagnosis and classification of IIM, allowing the identification of specific histopathological features. The characterization of inflammatory infiltrates is included in these features, for which leucocyte phenotyping is made. Between the inflammatory cells broadly detected in the muscle biopsy characterization are macrophages. Therefore, macrophages are not only key players in skeletal muscle's inflammatory response but also in tissue repair processes.

In this research project, we investigated the localization and surface markers expression of macrophages in muscle biopsies from patients with IIM and compared them to, neurogenic diseases and other myopathies.

To achieve this goal, we developed a double immunofluorescence staining to differentiate M1 (proinflammatory) and M2 (anti-inflammatory/repair) macrophages using two primary antibodies from the same host. We observed two distinct patterns of macrophage localization: localized infiltrates invading the muscle fibers and dispersed endomysial infiltration. The localized infiltrates displayed a lower proportion of M2 macrophages compared to the endomysial pattern. When we further analyzed according to the pathology, we found that IIM biopsies had the lowest proportion of M2. Our findings suggest a prevailing proinflammatory environment in the affected muscles of IIM patients. Additionally, the distribution of M2 macrophages differed among the diagnostic groups, indicating distinct immune responses and underlying mechanisms in skeletal muscle pathology.

This study enhances our understanding of macrophage involvement in muscle pathologies and highlights the potential of macrophage analysis for clinical applications. Further investigations with larger cohorts are needed to validate these findings and explore macrophage subtypes in specific subgroups of muscle pathologies.

Effect of immunogenic gliadin peptide 33-mer and its fragments on the cytokine production by human intestinal biopsies of coeliac patients and peritoneal macrophages

Marina Girbal Gonzalez[1,2]; Laura del Amo-Maestro[3] ; Soraia R. Mendes[3] ; Arturo Rodríguez-Banqueri[3] ; Laura GarzonFlores[3] ; Tibisay Guevara[3] ; Ulrich Eckhard[3] ; María José Rodríguez-Lagunas[1,2]; Àngels Franch[1,2]; F. Xavier GomisRüth[3] ; Francisco J. Pérez-Cano[1,2]

[1]*Section of Physiology,Dept. of Biochemistry and Physiology, Faculty of Pharmacy and Food Sciences, University of Barcelona, Barcelona, Spain;* [2]*Research Institute of Nutrition and Food Safety, University of Barcelona, Barcelona, Spain;* [3]*Proteolysis Laboratory, Dept. of Structural Biology, Molecular Biology Institute of Barcelona, Barcelona, Spain*

Background and objectives: Recently, we have demonstrated the ability of a prolyl endopeptidase to degrade the gliadin-immunotoxic peptide 33-mer into smaller and theoretically less immunoreactive fragments. The goal of the present study was to evaluate ex vivo and in vitro the immune response of 33-mer and its cleavage peptides.

Materials and methods: For the ex vivo approach, human intestinal biopsies (n= 14) were obtained from coeliac disease patients on a gluten free diet and varying Marsh indexes from the Hospital Mútua de Terrassa. For the in vitro studies, rat peritoneal macrophages (PMs) were obtained and cultured at 106 cells/mL per well and incubated for 24 h to allow their attachment. Both intestinal biopsies and PMs were stimulated with 33-mer or the fragments generated after the prolyl endopeptidase digestion (3-, 7- and 9-mer mixed or separately) at 0.25 mM and 4.35 mM. Equal volumes of medium, lipopolysaccharide and two random peptides (6- and 8-mer) were also added as controls. After 24 h, supernatants were collected and stored at -80 °C. A Luminex Multiplex Assay was used to quantify 18 different cytokines secreted by the intestinal biopsies as a response to each stimuli. In the case of the PMs, an ELISA assay was performed for IL-6 and TNF-α determination.

Results: The intestinal biopsies from coeliac patients did not show, in our experimental conditions, any statistically significant modulation of cytokines production due to the 33-mer addition nor the digested peptides. However, in PMs, there was a significant rise in the production of both IL-6 and TNF-α that was not observed when the PMs were stimulated with the fragments.

Conclusion: Besides the lack of evidence in human intestinal biopsies from coeliac patients, the in vitro results indicate that the use of a prolyl endopeptidase capable of degrading the gliadin-immunotoxic peptide 33-mer could be effective in reducing its pro-inflammatory effect.

Shaping the effect of maternal diet and breast milk composition on the infant microbiota and defensive capacity against infections in early life

Karla Rio-Aige[1,2]; Marta Selma-Royo[3] ; Raul Cabrera-Rubio[3] ; Sonia González[4,5]; María J Rodríguez-Lagunas[1,2]; María Carmen Collado[3] ; Francisco J Pérez-Cano[1,2]

[1]Physiology Section, Faculty of Pharmacy and Food Science, University of Barcelona, Barcelona, Spain; [2]Nutrition and Food Safety Research Institute, Barcelona, Spain; [3]Institute of Agrochemistry and Food Technology, National Research Council, Valencia, Spain; [4]Dept. of Functional Biology, Faculty of Medicine, University of Oviedo, Oviedo, Spain; [5]Diet, Microbiota and Health Group, Institute of Health Research of the Principado de Asturias, Oviedo, Spain; [6]Dept. of Pediatrics, University of Valencia, INCLIVA Biomedical Research Institute, Valencia, Spain

The nutritional status and dietary composition of mothers during pregnancy and breastfeeding, significantly influence the short- and long-term health outcomes of their offspring. Nevertheless, the precise underlying mechanisms remain incompletely understood. This study aimed to evaluate the influence of maternal dietary choices on the well-being of their children by investigating the relationship between maternal diet, immune components present in breast milk, the composition of neonatal gut microbiota, and the occurrence of gastrointestinal and respiratory infections.

To achieve this objective, two approaches were employed: a observational and longitudinal birth cohort MAMI (NCT03552939) where data of infant infections during the first year of life are available and a preclinical nutritional intervention using a neonatal rat model of rotavirus (RV)-induced acute gastroenteritis performed on 5th day of life. In both cases, two dietary patterns were studied, one characterized by a high content of dietary fiber and plant-based protein (D1) and the other one with lower fiber content and a higher proportion of animal protein (D2). In both clinical and preclincial studies, immune factors in maternal milk and plasma were analyzed using ELISA and multiplex immunoassays, and microbiota profiling was obtained by V3-V4 16S rRNA gene amplicon sequencing on the Illumina platform.

In the clinical study, the infants from D1 mothers had a lower number of infections during the first year of life. The preclinical approach aligned with this results and pups from D1 mothers showed a reduction in the severity of the induced diarrhea. Some associations between neonatal gut microbiota, specifically alpha microbial diversity, as well as with immune factors in maternal milk and plasma, such as particular IgG subclasses, IgA or Th1/Th2 balance, were found.

Our results highlight the critical role of maternal diet during pregnancy and lactation in preventing infections in their descendance.

Efecto de la Ciclosporina A sobre la función reguladora de las células Treg *in vitro*

Daniel Acevedo Gomez[1]

[1]*Sant Joan de Déu Research and Teaching Foundation, Esplugues del Llobregat, Spain*

Actualmente, se han descrito más de 450 formas de errores congénitos de la inmunidad (ECI), con amplios fenotipos superpuestos que van desde una mayor susceptibilidad a las infecciones hasta desregulación inmune significativa. La desregulación inmune es un problema importante en pacientes con ECI, que actualmente carece de un enfoque terapéutico efectivo. La evidencia actual sugiere que las células T reguladoras (Treg) pueden tener una importante implicación en dicha desregulación inmune.
Los inmunomoduladores como la ciclosporina A (CsA), se utilizan como tratamiento de las manifestaciones de desregulación en los ECI. Nuestro objetivo es evaluar los mecanismos por los cuales la CsA impacta en la función Treg in vitro y ampliarlo a más fármacos inmunosupresores de uso común.

Paraneoplastic neurological syndromes: a case report

María Cárcel Pérez[1] ; Ana Gómez Núñez[2] ; Jorge Eguía Núñez[2] ; Eduardo Villegas Martín[2]

[1]Sant Joan de Reus University Hospital, Reus, Spain; [2]Catalan Reference Laboratory,El Prat del Llobregat, Spain

Background – Aim
Paraneoplastic neurological syndromes (PNS) include a heterogeneous group of neurological disorders that occur in direct relation to tumor development. However, they are not directly caused by the tumor, they are related to immunological mechanisms characterized by the presence of antineuronal antibodies. Serological detection of antineuronal antibodies may allow early detection of a hidden and potentially treatable cancer.
Here we report the case of a 44-year-old patient with headache, disorientation and behavioral disorder. Microbiological analysis of cerebrospinal fluid excluded the presence of infectious pathology. Cervical computed tomography showed a small mass suspicious for a potential thymoma.

Methods
Laboratory tests for neuronal autoimmunity were performed on serum by indirect immunofluorescence (IIFT) on cerebellum, hippocampus, nerves, intestinal tissue, pancreas and transfected cells with NMDAR, CASPR2, AMPAR, LGI1, DPPX, GABABR and IgLON5. Immunoblot were also used to confirm the results.

Results:
A pattern compatible with anti-Hu and anti-CV2 was detected and verified by immunoblot. Immunoblot also showed positivity for anti-titin antibodies, which were confirmed by enzyme immunoassay.
The analysis of neuronal surface IIFT on hippocampus and cerebellum showed a pattern compatible with anti-AMPAR, being confirmed with IIFT on transfected cells.

Conclusions:
This is a case of paraneoplastic encephalitis with anti-AMPAR, anti-Hu, anti-CV2 and anti-titin antibodies, associated with the presence of a thymoma.
Anti-AMPAR encephalitis is clinically characterized by a subacute disturbance of short-term memory loss, confusion, abnormal behavior and seizure. Most patients with anti-AMPAR encephalitis are associated with tumors. Presence of other antineuronal antibodies is common in patients with anti-AMPAR antibodies.
The patient was treated with high-dose corticosteroids and underwent a thymectomy. After a few months, the patient showed no neurological symptoms.
In conclusion, antineuronal antibodies can be a useful tool in the diagnosis of paraneoplastic syndromes, as well as in the detection of early-stage tumors.

Impact of COVID-19 vaccination on the immune profile of breast milk in lactating women

Blanca Grases-Pintó[1,2]; Karla Rio-Aige[1,2]; Maria José Rodríguez-Lagunas[1,2]; Margarida Castell[1,2]; Carles Lerin[1,2] ; Maria Carmen Collado[4] ; Francisco José Pérez-Cano[1,2]; on behalf of MilkCORONA study team[4]

[1]Dept. of Biochemistry and Physiology, Faculty of Pharmacy and Food Science, University of Barcelona, Barcelona, Spain; [2]Institute of Research in Nutrition and Food Safety, Barcelona, Spain; [3]Dept. of Endocrinology, Sant Joan de Déu Research Institute, Sant Joan de Déu Hospital, Esplugues de Llobregat, Spain; [4]Institute of Agrochemistry and Food Technology-National Research Council, Paterna, Spain

Our aim was to investigate the impact of coronavirus disease (COVID-19) vaccination on the immunological profile of breast milk (BM) and assess whether the impact differs between different types of vaccines. The study enrolled 83 healthy lactating women and was conducted in Spain, the vaccine distribution was Pfizer (n=31), Moderna (n=28), and Oxford (n=24). A baseline measurement of immunoglobulins (Ig) and cytokine (CK) concentrations were taken at baseline (before 1st dose of the vaccine) and after the two diferent doses (28 days after each dose). The concentration of Ig and CK were measured using immunoassays and Luminex technology. A strong reactivity was observed for IgA for all vaccines ($p<0.05$); there was an average 19.3% increase after vaccination. A vaccine-dependent response was observed with IgA, IgG and the IgG subclasses. Oxford was the only vaccine that induced a significant increase in IgA concentration (25.6%) after both doses, and Pfizer was the only vaccine that induced an increase in IgG1 (21.8%) and IgG3 (73.7%) concentration after one dose. A decrease in the detection level for nearly all cytokines was observed for all three vaccines. Furthermore, the extent of the decrease in the percentage detection of IL-27, IL-1alfa, Il-4, Il-13, IFN-gamma and IL-18 differed between the vaccine types. COVID-19 vaccination impacts the IgA and IgG concentrations in BM, and that their response is vaccine dependent. These results are in agreement with previous studies and supports the current recommendation for the continuation of breastfeeding after maternal vaccination. The impact of vaccination on CK is not clearly established and requires further studies. Also, future reserch is needed to assess how the changes in BM immune composition affects the breastfed infant's immunity to COVID-19 infection.

Evaluation of a novel particle-based assay for detection of SLE-related autoantibodies

Daniel Lorca-Arce[1,2]; Albert Pérez-Isidro[1,2]; Judit Becerra[1]; Maria José Martínez[1]; Roberto Ríos-Garcés[2,3]; Gerard Espinosa[2,3]; Ricard Cervera2,3; Carmen Andalucía4; Odette Viñas-Gomis1,2; Estibaliz Ruiz-Ortiz[1,2]

[1]Immunology Department, Biomedical Diagnostic Center, Clínic Hospital of Barcelona, Barcelona, Spain; [2]August Pi i Sunyer Research Institute, Barcelona, Spain; [3]Department of Autoimmune Diseases, Clínic Hospital of Barcelona, Barcelona, Spain.; [4]Research and Development, Headquarters Technology Center Autoimmunity, Werfen, San Diego, CA, USA

Background: Autoantibodies are a hallmark of systemic lupus erythematosus (SLE). Among them, anti-dsDNA, anti-Sm and anti-Ribosomal-P have been demonstrated to be the most specific, being anti-dsDNA and anti-Sm antibodies included in the 2019-ACR/EULAR SLE Classification Criteria. Whereas, enzyme-linked immunoassay (ELISA) and chemiluminescence assays (CIA) are widely established in immunology laboratories, new technologies such as particle-based multi-analyte technology (PMAT) allows the detection of multiple autoantibodies with different specificities simultaneously.

Aim: To compare the results of anti-dsDNA and anti-Sm autoantibodies by the two different diagnostic technologies available in our laboratory: CIA and PMAT.

To establish the relationship between the presence of anti-dsDNA, anti-Sm and anti-Ribosomal-P autoantibodies with SLE clinical manifestations measured by SLEDAI-2K.

Method: Overall, 378 patients were included in the study, divided into three groups: 192 SLE patients; 164 patients with other autoimmune diseases and 22 healthy-controls. Anti-dsDNA and anti-Sm autoantibodies levels were studied by CIA (QUANTA Flash®, Werfen, USA) as well as PMAT (Aptiva CTD Essential, Werfen, USA). PMAT also includes anti-Ribosomal-P antibodies.

Results: Anti-dsDNA and anti-Sm results (CIA) showed a substantial agreement with PMAT (Cohen´s kappa=0.662 and 0.671, respectively).

Anti-dsDNA autoantibodies by PMAT showed a positive strong correlation with cSLEDAI-2K ($p<0.001$) and a moderate negative correlation with C3 and C4 levels ($p<0.001$). Anti-Sm and anti-Ribosomal-P autoantibodies showed a positive weak correlation with disease activity measured by SLEDAI-2K ($p<0.001$ and $p=0.001$, respectively) and a negative weak correlation with C3 and C4 levels ($p<0.001$ and $p=0.001$, respectively).

Finally, anti-Sm autoantibodies positive results (PMAT) were associated with renal involvement although no differences in mean titers were observed. In addition, hypocomplementemia was associated with osteomuscular manifestation only due to C3 consumption.

Conclusion: Our study shows a substantial agreement between PMAT and CIA and an optimal overall clinical performance.

Leukocyte determination in semen using flow cytometry.

Germán Julià Agulló[1] ; Santiago Garcia Navarro[2] ; Maria Mosquera Olle[1] ; Mila Martinez Rubio[1] ; Yolanda Sanchez Salinas[1]

[1]*Parc Taulí Health Corporation Consortium, Sabadell, Spain*; [2]*Autònoma University of Barcelona, Barcelona, Spain*

The presence of leukocytes in semen is associated with infection of the genital tract and low sperm quality that can become causes of male infertility. Currently, there are no evidence-based reference values for CD45+ cells in the semen of fertile men. The leukocyte count is determined microscopically, by round cell counting and by peroxidase staining. According to the World Health Organization (WHO) Laboratory Manual for the Examination and Processing of Human Semen (Sixth Edition, 2021) the consensus threshold value for the peroxidase-positive leukocyte count is 1x106 cells/ml. Which implies a higher concentration of total leukocytes, since not all leukocytes are peroxidase positive. Polymorphonuclear cells can also be determined visually on Papanicolau smear or by immunohistochemical staining of semen with anti-CD45.

The aim of this study is to determine the presence of leukocytes using flow cytometry in semen samples.

The determination of leukocytes was carried out by staining with anti-CD45 monoclonal antibody. The granulocytes, monocytes and lymphocytes populations, were determined according to their complexity in the SSC axis.

Using our cytometry panel, we were able to determine granulocyte, monocyte and lymphocyte populations in all semen samples. In addition, we observed that post-vasectomy samples have significantly higher levels of monocytes.

Double Positivity MPO PR3: let's put light in the darkness

Lydia García Serrano[1] ; Sergi Cantenys Molina[1] ; Adrián García García[1] ; Jordi Bas Minguet[1]; Francisco Morandeira Rego[1]

[1]*Bellvitge University Hospital, Hospitalet del Llobregat, Spain*

Background and Aims:
Anti-neutrophil cytoplasmic antibodies (ANCA) are mainly direct against myeloperoxidase (MPO) or proteinase-3 (PR3). The aim of this study was to compare the characteristics of patients with MPO+PR3+ double positivity versus single-positive.

Methods:
Retrospective study of 105 serum samples MPO+PR3+ (DP), MPO+PR3- (MPO+) or MPO-PR3+ (PR3+) analyzed by chemiluminescence immunoassay. Clinical data of ANCA-associated vasculitis (AAV), nonAAV autoimmune disease, inflammatory bowel disease (IBD) and organ involvement were collected and compared between DP and MPO+ or PR3+ patients.

Results:
DP patients show lower anti-MPO values than MPO+ (43 CU (IQR 30-96) vs 153 CU (IQR 62-434); p<0.001) and lower anti-PR3 values than PR3+ (52 CU (IQR 34-176) vs 166 CU (IQR 52-605); p=0.004) (Fig.1).

AAV diagnosis was more frequent in both MPO+ (80% vs 29%; p<0.001) and PR3+ groups (86% vs 29%; p<0.001) than DP. Non-AAV autoimmune disease diagnosis was more frequent in DP group than MPO+ or PR3+ (43% vs 20%; p<0.001 in both cases). DP group present more IBD cases than MPO+ (17% vs 0%; p=0.025).

Regarding organ involvement, a higher percentage of renal involvement was observed in both MPO+ and PR3+ patients than in DP (71% vs 40%; p=0.004 in both cases). DP patients present a higher percentage of gastrointestinal involvement than MPO+ (23% vs 6%; p=0.040). DP present a lower incidence of lung (40% vs 69%; p=0.016), cutaneous (0% vs 26%; p=0.002) and ophthalmological (0% vs 34%; p<0.001) involvement than PR3+.

Conclusions:
In those patients with an MPO+PR3+ double-positive result, the probability of a vasculitis diagnosis is lower than in patients with simple positivity, although a diagnosis of non-AVV autoimmune disease is more likely. Thus, double positivity may suggest an unspecific immune activation state.

Generation and functional analysis of engineered NK cells designed to combat bacterial infections

Violeta Planells-Romeo[1] ; Laura Carrillo-Serradell[1] ; Lucía Aragón-Serrano[1] ; María Velasco-de-Andrés[1] ; Francisco Lozano[1,2,3]

[1]August Pi i Sunyer Research Institute, Barcelona, Spain; [2]Immunology Service, Santa Creu i Sant Pau Hospital, Barcelona, Spain; Biomedical Diagnostic Center, Clínic Hospital of Barcelona, Barcelona, Spain; [3]Dept. of Biomedicine, Faculty of Medicine and Health Sciences, University of Barcelona, Barcelona, Spain

Bacterial infections can lead to severe life-threatening situations (septic shock) mainly as a result of immunedebilitating disorders (e.g. diabetes mellitus, cancer, AIDS), aggressive medical/surgical interventions (e.g., organ transplantation, prosthesis replacements, immunosuppressive drugs), emergence of antimicrobial resistant (AMR) strains, and shortage of new antibiotics in the pipeline of the pharma industry. In this context, there is an urgent need of new strategies for treating bacterial infections regardless of their AMR status. Adoptive transfer of either normal or engineered immune cells offers the possibility of treating immune-mediated disorders such as infectious diseases. To this end, we have taken advantage of the broadspectrum microbicidal activity of NK cells and the bacterial recognition properties of CD6 -a lymphoid scavenger-like receptor - to generate NK cells expressing a CD6-based chimeric antigen receptor (CD6CAR). Such CD6CAR encompasses the whole extracellular region of CD6 fused to the transmembrane region of CD8 and a cytoplasmic tail including the activation motifs of the 4-1BB/CD137 and CD3ζ co-stimulatory receptors. The achievement of stable CD6CAR expression in KHYG-1 cells, a leukemic cell line derived from natural killer cells, demonstrated an increase in their cytotoxic activity against the Gram-positive bacteria Staphylococcus aureus when compared to untransduced KHYG-1 cells. Similar results were obtained when using CD6CAR transduced cord blood derived NK cells (CBNKs). These results constitute first evidence on the prospect of developing a CD6-based adoptive CAR NK cell immunotherapy against bacterial infection and warrants further in vitro and in vivo assays to better characterize its efficacy and safety.

(*) Work supported by MCIN/AEI /10.13039/501100011033/FEDER, UE (PID2022-140932OBI00) AGAUR (2021/SGR/0113). LC-S and VP-R are recipients of fellowships PRE2020-093993 and FPU21/06217, respectively.

Evaluating the Role of KIR3DL2 in Natural Killer Cell Education and Inhibition

Elisenda Alari Paissa[1,2]; Roc Pomarol Moyà[1] ; Judith Federico Vega[2] ; Marta Crespo Barrio[2] ; Carlos Vilches[3]

[1]Pompeu Fabra University, Barcelona, Spain; [2]Hospital del Mar Research Institute, Barcelona, Spain; [3]University Hospital Puerta de Hierro, Majadahonda, Spain

Natural Killer (NK) cells are innate lymphocytes with an important role in eliminating virus-infected and tumor cells, regulated by a balance between activating and inhibitory receptors. Among them, inhibitory Killer-cell Immunoglobulin-like Receptors (iKIRs) and CD94/NKG2A bind human leukocyte antigen (HLA) class I molecules. Along their differentiation NK cell subsets acquire different combinations of these receptors, whose interaction with self-HLA-I molecules enhances their responsiveness to target cells lacking the corresponding HLA-I ligand (missing self), through a process termed "NK cell education". Beyond their importance in the response to pathological cells, iKIR-HLA-I-mismatching may promote NK cell alloreactivity in transplantation. The function of several iKIR (KIR2DL1, KIR2DL2/3 and KIR3DL1) in NK cell education and inhibition is well established. By contrast the role played by KIR3DL2, reported to bind HLA-A*03 and -A*11 alleles and HLA-B*27 homodimers, remains unclear.

To investigate this issue, purified NK cells from HLA-genotyped donors were incubated with HLA-I deficient targets or with T cell blasts with various HLA-I genotypes. NK cell degranulation and TNFa production by subsets defined according to their expression of iKIRs and NKG2A were assessed. Our preliminary results show that KIR3DL2 single positive NK cells were inhibited by HLA-A*03 or -A*11+, but not HLA-B*27+ T blasts, supporting the role of the receptor in NK cell inhibition. On the other hand, KIR3DL2+ NK cell subsets not predicted to be educated by any other iKIR nor NKG2A, displayed a modest but significant increase in functional activity in donors expressing HLA-A*03 or -A*11, but not HLA-B*27, compared to those from donors lacking those ligands, suggesting that KIR3DL2 may contribute to NK cell education, yet to a lesser extent than other iKIRs.

Impaired Regulation by IL-35 in Systemic Sclerosis

Rubén Osuna-Gómez[1]; Ivan Castellví[2]; Maria Mulet[1]; Mª Àngels Ortiz[1]; Douglas E. Brough[3]; Helen Sabzevari[3]; Roshanak T. Semnani[3]; Silvia Vidal[1]

[1]Dept. of Dept. of Inflammatory Diseases, Biomedical Research Institute Santa Creu i Sant Pau, Barcelona, Spain; [2]Dept. of Rheumatology and Systemic Autoimmune Diseases, Santa Creu i Sant Pau Hospital, Barcelona, Spain

This study investigated the role of IL-35 in systemic sclerosis (SSc) patients, focusing on CD4+ T cell response and immunomodulatory cytokine production. By comparing the cytokine levels in healthy donors (HD) and SSc patients using ELISAs, we found a significantly lower plasma IL-35 concentration in the SSc patients (52.1 ± 5.6 vs. 143 ± 11.1, $p < 0.001$). Notably, the IL-35 levels showed a negative correlation with TGF-β ($p < 0.001$) and IL-17 ($p = 0.04$). Assessing the IL-35R expression across cell types in the SSc patients and HDs via flow cytometry, we found higher levels on monocytes (40.7 + 5.7 vs. 20.3 ± 1.9, $p < 0.001$) and lower levels on CD8+ T cells (61.8± 9.2 vs. 83.4 ± 0.8, $p < 0.05$) in the SSc patients. The addition of recombinant IL-35 to stimulated peripheral blood mononuclear cells reduced the IL-17+CD4+ T cell percentage (9.0 ± 1.5 vs. 4.8 ± 0.7, $p < 0.05$) and increased the IL-35+CD4+ T percentage (4.1 ± 2.3 vs. 10.2 ± 0.8, $p < 0.001$). In a Treg: Tresponder cell Sco- culture assay with HD and SSc samples, rIL35 decreased the cell proliferation and levels of IL-17A (178.2 ± 30.5 pg/mL vs. 37.4 ± 6.4 pg/mL, $p < 0.001$) and TGF-β (4194 ± 777 pg/mL vs. 2413 ± 608 pg/mL, $p < 0.01$). Furthermore, we observed a positive correlation between the modified Rodnan skin score (mRSS) and TGF-β ($p < 0.001$), while there was a negative correlation between mRSS and IL-35 ($p = 0.004$). Interestingly, higher levels of plasmatic IL-35 were detected in individuals with limited disease compared to those with diffuse disease (60.1 ± 8.0 vs. 832.3 ± 4.1, $p < 0.05$). These findings suggest that IL-35 exhibits anti-inflammatory properties in SSc and it may serve as a marker for disease severity and a therapeutic target.

Molecular challenges in the diagnosis of X-linked chronic granulomatous disease: CNVs, intronic variants, skewed X-chromosome inactivation, and gonosomal mosaicism

Laura Batlle-Masó[1,2]; Jacques G Rivière[1,2]; Clara Franco-Jarava[1,2]; Andrea Martín-Nalda[1,2]; Marina Garcia-Prat[1,2]; Alba Parra-Martínez[1,2]; Aina Aguiló-Cucurull[1,2]; Neus Castells[1,2]; Monica Martínez-Gallo[1,2]; Pere Soler-Palacín[1,2]; Roger Colobran[1,2]

[1]*Vall d'Hebron Research Institute, Barcelona, Spain;* [2]*Vall d'Hebron University Hospital*

Chronic granulomatous disease (CGD) is a prototypical inborn error of immunity affecting phagocytes, in which these cells are unable to produce reactive oxygen species. CGD is caused by defects in genes encoding subunits of the NADPH oxidase enzyme complex (CYBA, CYBB, CYBC1, NCF1, NCF2, NCF4); inflammatory responses are dysregulated, and patients are highly susceptible to recurrent severe bacterial and fungal infections. X-linked CGD (XL-CGD), caused by mutations in the CYBB gene, is the most common and severe form of CGD. In this study, we describe the analytical processes undertaken in 3 families affected with XL-CGD to illustrate several molecular challenges in the genetic diagnosis of this condition: in family 1, a girl with a heterozygous deletion of CYBB exon 13 and skewed X-chromosome inactivation (XCI); in family 2, a boy with a hemizygous deletion of CYBB exon 7, defining its consequences at the mRNA level; and in family 3, 2 boys with the same novel intronic variant in CYBB (c.1151 + 6 T > A). The variant affected the splicing process, although a small fraction of wild-type mRNA was produced. Their mother was a heterozygous carrier, while their maternal grandmother was a carrier in form of gonosomal mosaicism. In summary, using a variety of techniques, including an NGS-based targeted gene panel and deep amplicon sequencing, copy number variation calling strategies, microarray-based comparative genomic hybridization, and cDNA analysis to define splicing defects and skewed XCI, we show how to face and solve some uncommon genetic mechanisms in the diagnosis of XL-CGD.

Changes in Treg and Breg cells in a healthy pediatric population

Yiyi Luo[1,2,3]; Daniel Acevedo[1,2,3]; Alexandru Vlagea[2,4]; Anna Coodina[5]; Ana García-García[1,2,3]; Angela Deyà-Martínez[1,2,3]; Celia Martí-Castellote[1,2,3]; Ana Esteve-Solé[1,2,3]; Laia Alsina[1,2,3,6]

[1]Clinical Immunology and Primary Immunodeficiencies Unit, Dept. of Allergy and Clinical Immunology, Sant Joan de Déu Hospital, Esplugues de Llobregat, Spain ; [2]Clinical Immunology Unit, Sant Joan de Déu Hospital-Clínic Hospital of Barcelona, Barcelona, Spain; [3]Study Group for Immune Dysfunction Diseases in Children, Sant Joan de Déu Research Institute, Barcelona, Spain; [4]Dept. of Immunology, Biomedic Diagnostic Center, Clínic Hospital of Barcelona, Barcelona, Spain; [5]Biobank at Sant Joan de Déu Hospital, Esplugues de Llobregat, Spain; 6Dept. of Surgery and Medical Specializations, Faculty of Medicine and Health Sciences, University of Barcelona, Barcelona, Spain

The interpretation of clinical diagnostic results in suspected inborn errors of immunity, including tregopathies, is hampered by the lack of age-stratified reference values for regulatory T cells (Treg) in the pediatric population, and a consensus concerning which Treg immunophenotype to use. Regulatory B cells (Breg) are an important component of the regulatory system that have been poorly studied in the pediatric population. We analyzed 1) the correlation between the three immunophenotypic definitions of Treg (CD4+CD25hiCD127low, CD4+CD25hiCD127lowFoxP3+, CD4+CD25hiFoxP3+), and with CD4+CD25hi, and 2) the changes in Treg and Breg frequency and their maturation status with age. We performed peripheral blood immunophenotyping of Treg and Breg (CD19+CD24hiCD38hi) by flow cytometry in 54 healthy pediatric controls. We observed that Treg numbers varied depending on the definition used, the frequency ranging between: 3.3–9.7% for CD4+CD25hiCD127low, 0.07-1.6% for CD4+CD25hiCD127lowFoxP3+ and 0.24-2.83% for CD4+CD25hiFoxP3+. The correlation between the three definitions of Treg was positive for most age ranges, especially between the two intracellular panels and with CD4+CD25hi vs CD4+CD25hiCD127low. Treg and Breg frequency tended to decline after 7 and 3 years onwards, respectively. Treg maturation status increased with age, with a decline of naïve Treg and an increase in memory/effector Treg from age 7 onwards. Memory Breg increased progressively from age 3 onwards. In conclusion, the numbers of Treg frequency span a wide range depending on the immunophenotypic definition used despite a good level of correlation existing between them. The decline in numbers and maturation process with age occurs earlier in Breg than Treg.

Evolution of portosystemic collaterals and hyperdinamic circulation after removal of the etiological agent in two animal models

Albert Guinart-Cuadra[1]; Anna Brujats[2]; Héctor García-Calderó[3,4]; Claudia Pujol[2]; Laura Casani[1]; Elisabet Cantó[1]; Rúben Osuna-Gómez[1]; Elisabet Sánchez A.[1,4]; Silvia Vidal[1]; Eilmar Alvarado-Tapias[1,2,4]; Candido Villanueva[1,2,4]

[1]Dept. of Inflammatory Diseases, Biomedical Research Institute Santa Creu i Sant Pau, Barcelona, Spain; [2]Santa Creu i Sant Pau Hospital-Autònoma University of Barcelona, Barcelona, Spain; [3]August Pi i Sunyer Research Institute, Barcelona, Spain; [4]Center for Biomedical Research in Network of Liver and Digestive Diseases - Carlos III Health Institute, Madrid, Spain

Portosystemic collaterals (PC) are one of the main drivers of portal hypertension. However, it is not clear whether the elimination of the etiological agent of liver disease could modify the presence of these collaterals (reversed or persistent). The aim of this study was to evaluate the evolution of PC, portal pressure (PP), and inflammatory markers in two Sprague Dawley models (Liver disease model CCL4 Group, portal hypertension model PPVL Group).

The results showed that PP was significantly higher in the PPVL group compared to control groups. However, ligature removal in the PPVL group (PPVL-LO) significantly reduced PP.

In the CCL4 group, the number of PC (assessed using CD105 and VEGF) decreased in subgroups that received treatment for a longer time. This suggests that the withdrawal of the etiological agent (CCL4) may reverse the development of PC.

Regarding inflammatory markers, in the CCL4 group, an early increase in IL-6, IL-10, TNF-alpha, and VEGF was observed, followed by a decrease after CCL4 withdrawal. In the PPVL group, no significant changes in inflammatory markers were observed.

In both models, the withdrawal of the etiological agent was associated with the reversion of the number of mesenteric collateral veins. In the CCL4 model, an early increase in systemic inflammatory markers was particularly observed, as well as their reduction after the withdrawal of the etiological agent.

Mast cell activation profile and TFG13 detection discriminate food anaphylaxis versus sensitization

Laia Ollé[1,2]; Lucía García-García[2]; Joan Bartra[2,3,4,6]; Europa Azucena González-Navarro[5]; Maria Pérez[2]; Jordi Roca- Ferrer[2]; Mariona Pascal[2,4,5,6]; Margarita Martín[1,2,4]; Rosa Muñoz-Cano[2,3,4,6]

[1]Biochemistry and Molecular Biology Unit, Dept. of Biomedicine, Faculty of Medicine and Health Sciences, University of Barcelona, Barcelona, Spain; [2]Clinical and Experimental Respiratory Immunoallergy Research Group, August Pi i Sunyer Research Institute, Barcelona, Spain; [3]Dept. of Allergy , Clínic Hospital of Barcelona, Barcelona, Spain; [4]Asthma, Adverse Drug Reactions and Allergy Network - Health Outcomes-Oriented Cooperative Research Networks, Carlos III Health Institute, Madrid, Spain; [5]Dept. of Immunology, Biomedical Diagnostic Center, Clínic Hospital of Barcelona, Barcelona, Spain; [6]Dept. of Medicine, Faculty of Medicine and Health Sciences, University of Barcelona, Barcelona, Spain

Background
The prevalence of food allergy (FA) has increased significantly, and the risk of developing anaphylaxis is unpredictable. Thus, discriminating between sensitized patients and those at risk of having a severe reaction is of utmost interest.

Objective
To explore mast cell activation pattern and T follicular helper (TFH) 13 presence in sensitized and food anaphylaxis patients.

Methods
Patients sensitized to Lipid transfer protein (LTP) were classified as anaphylaxis or sensitized depending on the symptoms elicited by LTP-containing food. CD34+-derived MCs from patients and controls were obtained, sensitized with pooled sera, and challenged with Pru p 3 (peach LTP). Degranulation, PGD2, and cytokine/chemokine release were measured. The TFH13 population was examined by flow cytometry in the peripheral blood of all groups. In parallel, LAD2 cells were activated similarly to patients' MCs.

Results
A distinguishable pattern of mast cell activation was found in anaphylaxis compared to sensitized patients. Robust degranulation, PGD2, and IL-8 and GM-CSF secretion were higher in anaphylaxis, whereas TFG- b and CCL2 secretion increased in sensitized patients. Concomitantly, anaphylaxis patients had a larger TFH13 population. MC activation profile was dependent on the sera rather than the MC source. In agreement with that, LAD2 cells reproduce the same pattern as MCs from anaphylactic and sensitized patients.

Conclusion
The distinct profile of mast cell activation allows to discriminate between anaphylaxis and sensitized patients. Pooled sera may determine mast cell activation independently of mast cell origin. Besides, the presence of TFH13 cells in anaphylaxis patients points to an essential role of IgE affinity.

Dysregulated neutrophil extracellular traps formation in sepsis

Maria Mulet[1]; Ruben Osuna-Gómez[1]; Carlos Zamora Atenza[1]; Iris Artesero[2]; Marc Arús[3]; Paula Vera-Artazcoz[4]; Alejandra Cordón[4]; Noelia Vilalta[3]; Andrés Abril[5]; Antoni Moliné[6]; Indalecio Morán[4]; Joaquín López-Contreras[2]; Silvia Vidal[1]

[1]Dept. of Inflammatory Diseases, Biomedical Research Institute Santa Creu i Sant Pau, Barcelona, Spain; [2]Infectious Disease Division, Dept. of Internal Medicine, Santa Creu i Sant Pau Hospital, Barcelona, Spain; [3]Unit of Haemostasis and Thrombosis, Santa Creu i Sant Pau Hospital, Barcelona, Spain; [4]Dept. of Intensive Care, Santa Creu i Sant Pau Hospital, Barcelona, Spain; [5]Dept. of Emergency Care, Sant Joan de Déu Hospital, Esplugues de Llobregat, Spain; [6]Dept. of Emergency Medicine, Santa Creu i Sant Pau Hospital, Barcelona, Spain

The migration and antimicrobial functions of neutrophils seem to be impaired during sepsis contributing to the dysregulation of immune responses and the pathogenesis of the disease. However, the role of neutrophil extracellular traps (NETs) remains to be fully elucidated. This study aimed to analyze the sequential phenotypic and functional changes in neutrophils following the diagnosis of sepsis. We prospectively enrolled 49 septic and 18 non-septic patients from the intensive care unit (ICU) and emergency room (ER), as well as 20 healthy volunteers (HV). Baseline blood samples from septic and non-septic patients were collected within 12 h of admission to the hospital. Additional septic samples were drawn at 24, 48 and 72 h after baseline. Neutrophil phenotype was analyzed by flow cytometry and NET formation was measured by fluorescence. We found that neutrophils from septic patients exhibited increased CD66b, CD11b and CD177 expression at baseline compared with non-septic patients and healthy volunteers (HV) controls. Notably, the expression of CD66b in septic patients progressively decreased until 72 h of follow-up, reaching levels similar to those of HV. Functionally, neutrophils from septic patients displayed reduced NET formation at baseline compared to non-septic patients and HV. A similar pattern of NETosis dynamics was observed among septic patients, consisting of a first reduction of NET formation after 24 h followed by an increase at 48 h and another reduction at 72 h. Assessing a decision tree model, our study showed that CD11b expression and NETosis values are useful variables to discriminate septic from non-septic patients. We conclude that sepsis induces changes in neutrophil phenotype and function that may compromise the effective capacity of the host to eliminate pathogens.

αCD4 CAR-T cells produce an in vitro effective cytotoxic effect against CD4+ T cells

Campos-Gonzalez G[1]; Tirado N[2,3]; Martinez-Picado J[1,4,5,6,7]; Velasco-Hernandez T[2,3]; Salgado M[1,4,6]

[1]IrsiCaixa AIDS Research Institute, Badalona, Spain; [2]Josep Carreras Leukaemia Research Institute, Badalona, Spain; [3]Advanced Therapies - Health Outcomes-Oriented Cooperative Research Networks, Carlos III Health Institute, Madrid, Spain; [4]Germans Trias i Pujol Research Institute, Badalona, Spain; [5]University of Vic - Central University of Catalonia, Vic, Spain; [6]Center for Biomedical Research in Network of Infectious Diseases - Carlos III Health Institute, Madrid, Spain ; [7]Catalan Institution for Research and Advanced Studies

Background: Chimeric Antigen Receptor (CAR) technology has become a well-established therapy for different leukaemias. Recently, efforts have been made to demonstrate that CAR-T cells have therapeutic potential to achieve a sterilizing cure for human immunodeficiency virus (HIV). Here, we have designed, developed, and validated a CAR targeting CD4, the main receptor for the HIV envelope with the hypothesis that eliminating all CD4+ cells would avoid any possibility of viral rebound.

Methods: We designed a second-generation CAR construct containing a single-chain variable fragment (scFv) against CD4 followed by a CD8 transmembrane domain, co-stimulation domain 4-1BB and intracellular domain CD3ζ. We infected isolated healthy CD8+ T cells with lentiviral particles containing the CAR and a mock to produce αCD4 CAR-T cells. We co-cultured them at different effector:target (E:T) ratios with autologous CD4+ T cells. We evaluated the cytotoxicity produced in vitro by αCD4 CAR-T cells at 24 and 48 hours.

Results: We successfully generated CAR-T cells expressing αCD4 specific extracellular domain. From all lentiviral infected cells, 94.7% expressed the αCD4 extracellular domain. αCD4 CAR-T cells produced a significant cytotoxic effect towards CD4+ T cells in comparison to mock transduced CAR-T cells. At 24h, αCD4 CAR-T cells effectively killed CD4+ T cells eliminating 71.2% (SD = 23.6) of CD4+ T cells at 1:1 ratio and killing 8.8% (SD = 7.7) of CD4+ T cells at 1:8 ratio. After 48h, αCD4 CAR-T cells had produced a higher cytotoxic effect eliminating 95.5% (SD = 5.0) of CD4 T+ cells at 1:1 ratio and killing 38.9% (SD = 25.5) of CD4+ T cells at 1:8 ratio compared to mock transduced CAR-T cells.

Conclusions: αCD4 CAR-T cells specifically eliminate healthy donor CD4+ T cells in vitro. This tool could facilitate HIV remission strategies based on full depletion of CD4+ cells containing latent proviruses prior to potential autologous hematopoietic stem cell transplantation.

Molecular sensitization pattern to dust mites with multiplex allergy test ALEX2

Mariona Pascal[2,3,4]; Joan Bartra[1,3,4]; Rosa Muñoz-Cano[1,3,4]; Sònia Gelis[1]; Anais Bello[2]; Rocío Lara[2]; Uma Bolos[2]; Daniel Martí[2]; Kimberly Solis[1]

[1]Dept. of Allergology, Respiratory Clinical Institute, Clínic Hospital of Barcelona, Barcelona, Spain; [2]Dept. of Immunology, Biomedical Diagnostic Center, Clínic Hospital of Barcelona, Barcelona, Spain; [3]August Pi i Sunyer Research Institute, Barcelona, Spain; [44]Asthma, Adverse Drug Reactions and Allergy Network, Inflammatory diseases Network - Health Outcomes-Oriented Cooperative Research Networks, Carlos III Health Institute, Madrid, Spain

Introduction: Dust mites (DM) are the most frequent indoor allergens causing respiratory allergy. Group 1,2 and Der p 23 are major allergens, but less is known about prevalence and relevance of other components. Certain DM allergens have been associated with shrimp allergy (SA). We aimed to determine the DM sensitization profile and its clinical relevance using wide panel of allergens.

Methods: Patients sensitized only to DM by ALEX2 (MacroArrayDiagnostics) from September 2021-October 2023 in Hospital Clínic were included. D.pterynosinus (DP,Der p 1,2,5,7,10,11,20,21,23), D.farinae(DF,Der f 1,2)(i.e.,house dust mites), storage-mites (SM): B.tropicalis(BT,Blo t 5,10,21), G.domesticus(GD) (Gly d 2), A.siro (AS), L.destructor (LD, Lep d 2) and T.putrescentiae (TP) (extract,Tyr p 2). Clinical history was reviewed for respiratory and SA.

Results: Among 250 individuals tested,34(14%) were sensitized only to DM. 5 had allergic rhinoconjunctivitis(RC)(3 moderate/persistent),3 asthma(2 severe) and 4 RC+asthma. 22 asymptomatic. 24(70%) were sensitized to DP&DF, 10(29%) only DP. 17(50%) were also sensitized to SM,with GD(32%) the most frequent (BT(20%),LD(20%),TP(20%),AS(11%)). At molecular level, DP/DF sensitization was: group 1 and 2 (70%, respectively),Der p 23(70%),Der p 5(29%) and Der p 21(20%). Sensitization to others was sporadic or undetected. For BT,Blo t 5 was the most frequent,always with Der p 5+. Those sensitized to DP/DF&SM did not show more severe allergy compared to those only DP/DF+. No correlation between number of positive components and clinical relevance or severity was found. Four(12%) reported SA (2 anaphylaxis) and only one was Der p 10+(tropomyosin). One anaphylactic was Der p 5&21+ and the other only Der p 1+. One sensitized to Der p 20(arginine-kinase) had SA,but also Der p 5&21+.

Conclusions: DM sensitization goes beyond group 1,2 and Der p23 allergens.SM occurs with DP/DF+, GD is the most frequent. Blo t 5 occurs with Der p 5+.SA is minor among them, not always associated to tropomyosin. Larger cohorts should confirm these data.

Long-term shared and differential immune features of patients with pulmonary sequelae or long COVID

Alejandro Torvisco Tello[1]; Tamara Cruz[1,2]; Estibaliz Ruiz-Ortiz[4]; Odette Viñas[4]; Núria Mendoza[1]; Gema M Lledo[5]; Lídia Perea[1,2]; Núria Albacar[1,2,3]; Alvar Agustí[1,2,3]; Jacobo Sellares[1,2,3]; Oriol Sibila[1,2,3]; Rosa Faner[1,2,6]

[1]August Pi i Sunyer Research Institute, Barcelona, Spain; [2]Center for Biomedical Research network of infectious Diseases, Spain; [3]Respiratory Clinical Institute, Clínic Hospital of Barcelona, Barcelona, Spain; [4]Dept. of Immunology, Clínic Hospital of Barcelona, Barcelona, Spain; Dept. of Autoimmune Diseases, Clínic Hospital of Barcelona, Barcelona, Spain; [6]Dept. of Biomedicine, Immunology Unit, University of Barcelona, Spain

Introduction. While most patients recover from an acute infection of SARS-CoV-2, 10-20% of them experience long-term effects. These include chronic pulmonary sequelae (PS), defined by impairment of lung function and/or structure, and long COVID (LC), defined the persistence or development of new symptoms 3 months after the initial infection that persists for more than 2 months and cannot be explained by an alternative diagnosis. Here, we explored the long-term (1 year) immune-related similarities and differences between PS and LC.

Methods. We studied 113 patients 1 year after hospital discharge because of COVID-19, 31 recovered without post-COVID symptoms, 51 with PS and 31 with LC. Using the Olink multiplex proximity extension arrays, we determined the plasma levels of 96 inflammatory markers and 96 organ damage proteins in these patients. Additionally, a panel of autoantibodies (anti-nuclear, anti-cytoplasmatic and anti-IFN) and autoimmune-related proteins was measured.

Results. We observed higher levels of autoantibodies and organ-damage proteins in LC than in PS patients. However, both LC and PS in comparison to fully recovered individuals, presented elevated levels of proteins implicated in anti-microbial immune response functions. Finally, in those with PS, the levels of CCL3 and CCL19 were significantly increased vs. fully recovered individuals, (6.02 ±0.3 vs 6.70±0.4 and 8.52±0.35 vs 8.86±0.6) and their increase between 6 to 12 months, correlated negatively (-0.33 and -0.31) with the degree of pulmonary impairment (i.e. % DLCO).

Conclusions. Our results suggest that, compared to fully recovered participants, those with LC show higher systemic inflammation, features of autoreactivity and an extended repertoire of organ-damage related proteins, while those with PS had a more specific inflammation with CCL3 and CCL19 as promising biomarkers. However, both LC and PS patients presented an anti-microbial immune response suggesting persistent viral reservoir as a common mechanism that can perpetuate the inflammatory response.

Comparación prueba cruzada virtual y por citometría de flujo en la evaluación del paciente candidato a trasplante renal

Juan Francisco Luchoro Cerdá[1]; Silvia Rica[1]; Nuria Exposito[1]; Alexandra Manchón[1]; Jose Luis Caro[1]; Eduard Palou[1]; Juan Torres Canizales[1]

[1]*Transplant Immunology Section, Dept. of Immunology, Clínic Hospital of Barcelona, Barcelona, Spain*

La prueba cruzada por citometría de flujo (FC-XM) y la prueba cruzada virtual (VXM) son herramientas fundamentales para detectar anticuerpos específicos del donante (DSA). Sin embargo, existen datos limitados entre la correlación y la capacidad del vXM para predecir los resultados del FC-XM.
Objetivo: Evaluar la precisión de la predicción de la VXM con los resultados de FC-XM para la detección de DSA en el pre-trasplante renal.

Materiales y métodos: 1201 pruebas cruzadas en 1149 pacientes candidatos a trasplante renal (donante cadáver= 859, vivo= 290) durante el año 2022 fueron incluidas. Resultados de FC-XM fueron categorizados por positividad en linfocitos T y/o B, y DSA fueron clasificados en clase I y clase II, por locus HLA, y en función de la MFI obtenida en el SAB.

Resultados: Se detectaron DSA en 145 (DSA clase I=28, clase II= 89, Clase I+II=28). Para DSAs clase I con MFI superior a 5000, el FC-XM tiene una sensibilidad (S) de 83,7% mientras para DSA clase II tiene una
S=40,5% (VPP=43,7%, VPN=99,7%). El AUC de predicción del VXM para FC-XM fue de 0.885 para DSA clase I, 0,744 (S=84,7%, E=79,5%, MFI=3779) para DSA clase II (S=80,5%, E=62,8%, MFI=5978) y 0.895 para clase I+II (S=90,5%, E=78,6%, MFI=4000). Al comparar MFI por locus, las MFI cutoff para
FC-XM positivo fueron: HLA-A=2439, HLA-B=2013, HLA-DR=3299 y HLA-DQ=7985 y HLA-DP=17790.

Conclusiones: FC-XM muestra una buena correlación con SAB para anti-HLA de locus HLA-A, B y DR, y pero inferior para antígenos de baja expresión como DQ/DP, siendo necesario incrementar el cutoff de estos locus para mejorar la precisión de VXM para predecir el FC-XM. Los resultados presentados confirman con previos estudios donde VXM y el MFI del SAB pueden predecir un FC-XM positivo y que podría aportar un mejor riesgo inmunológico ante la detección de DSAs previos al trasplante.

To all Sponsors, Speakers, Chairs and Curious Listeners,
THANK YOU for making this Congress possible!

Catalan Society of Immunology

2023

Best regards from the Organizing Committee of the Congress

Programme Brochure put together by Inés Vanhille Campos