XVI CONGRESS OF THE CATALAN SOCIETY OF IMMUNOLOGY (SCI)

SOCIETAT CATALANA D'IMMUNOLOGIA
L'Acadèmia

BASIC AND TRANSLATIONAL RESEARCH IN IMMUNOLOGY

Barcelona, November 24 and 25th, 2022

Hybrid meeting

L'Acadèmia
FUNDACIÓ ACADÈMIA DE CIÈNCIES MÈDIQUES I DE LA SALUT DE CATALUNYA I DE BALEARS

Organization Committee (SCI Board)

Welcome to the congress,

On behalf of the organizing committee, we would like to warmly welcome you to the XVIth 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 **Basic and Translational Immunology.**

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Content

Awards to the best communication and to the best poster at the XV Congress SCI 2022, sponsored by SCI

This year SCI sponsors the awards for the best communication (200 €) and for the best poster (100 €) of this congress. The Chairpersons of the different sessions of the congress and the board members of the SCI will select the best oral communications presented, taking into account its scientific value and the aspects related to the presentation. The poster awarded will be chosen by the congress attendees. The results will be announced at the end of the congress.

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Scheme first day

THURSDAY, November 24th	
16:00h – 16:15h	**Welcome to the XVIth CONGRESS of the SCI** ***Pablo Engel*** President of the SCI.
16:15h – 17:00h	Opening Lecture: **Metabolic requirement of regulatory B cells** Speaker: ***Claudia Mauri*** Centre for Rheumatology, Division of Infection and Immunity and Transplantation, University College London, UK Chair: ***Pablo Engel*** University of Barcelona.
17:00h – 17:40h	Plenary Session **B-cell development in a new context: new insights based on inborn errors of immunity** Speaker: ***Mirjam van der Burg*** Dept. of Immunology, Erasmus MC, Rotterdam, the Netherlands. Chair: ***Clara Franco*** Vall d'Hebron Hospital
	Coffee break
18:00h – 20:00h	Oral Communications I: Autoimmunity and Inflammation Chairs: ***Aina Teniente Serra*** Germans Trias i Pujol Hospital and ***Carles Serra-Pagès*** Hospital Clínic de Barcelona. **OC1: MicroRNA signature in children with type 1 diabetes and its role in autoimmunity. *Laia Gomez-Muñoz* *et al.*** **OC2: CDK11 and cyclin D3 exert antagonistic roles in beta cell survival in autoimmune diabetes (T1D). *Conchi Mora* *et al.*** **OC3:Influence of Multiple Sclerosis inflammatory context in the development of a tolerogenic dendritic cell therapy. *Federico Fondelli* *et al.*** **OC4: Longitudinal study of changes induced by cladribine treatment on Innate Lymphoid Cells from patients with multiple sclerosis. *María José Mansilla* *et al.*** **OC5: Oxygen-independent regulation of HIF-1α in ILC3s ameliorates the inflammation of C. rodentium-induced colitis. *Ana Valle-Noguera* *et al.*** **OC6: SEB-induced IL-13 production in CLA+ memory T cells defines Th2 high and Th2 low responders in atopic dermatitis. *Lídia Sans-De San Nicolàs* *et al.*** **OC7: Polyreactive Autoantibodies in the Physiopathology of Sjögren's Syndrome. *Rebeca Gutiérrez-Cózar* *et al.*** **OC8:Autoantibodies to Nuclear Valosin-containing Protein-like (NVL): A Novel Systemic Sclerosis-related Antibody. *Janire Perurena-Prieto* *et al.*** **OC9:Three Novel WAS Mutations with variable clinical presentations. *Teresa Franco-Leyva* *et al.***

Scheme second day

FRIDAY, November 25th	
09:00h – 09:30h	Plenary Session **Connecting myeloid cell metabolism and function** Speaker: ***David Sancho*** Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid Chair: ***Cristina López-Rodríguez*** Immunology Unit, Department of Experimental and Health Sciences, Pompeu Fabra University, Barcelona.
	Coffee break
10:00h – 10:40h	Plenary Session **Functional crosstalk between gut IgA and systemic IgG** Speaker: ***Andrea Cerutti*** Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Barcelona Biomedical Research Park, Barcelona Chair: ***Mónica Martinez Gallo*** Vall d'Hebron Hospital
10:40h – 12:30h	Oral Communications II: Basic Immunology and Immunodeficiencies Chairs: ***Jorge Lloberas*** University of Barcelona and ***Carme Roura*** Autonomous University of Barcelona **OC10: Macrophaging and DNA damage. *Carlos Batlle i Recoder* *et al.*** **OC11: Vitamin C enhances NF-κB-driven DNA demethylation and immunogenic properties of dendritic cells. *Gerard Godoy-Tena* *et al.*** **OC12: Characterization of HLA-DR immunopeptidome presented by dendritic cells pulsed with the breast cancer tumoral cell line MCF-7 lysates. *Gonzalo Lázaro Bermejo* *et al.*** **OC13: The role of Myeloid-derived Supressor cells in STI-mediated enhancement of HIV infection. *Daan K.J. Pieren* *et al.*** **OC14: X-Linked SASH3 Deficiency Presenting as a Common Variable Immunodeficiency *Moisés Labrador-Horrillo* *et al.*** **OC15: Can we predict when to evaluate CVID genetically? *Marina Garcia Prat* *et al.*** **OC16: Detection and evolutionary dynamics of somatic FAS variants in autoimmune lymphoproliferative syndrome: diagnostic implications. *Laura Batlle-Masó* *et al.***

FRIDAY, November 25th

12:30h – 13:10h	Plenary Session **Learning anti-viral immunity in the COVID-19 pandemic scenario** Speaker: ***Estela Paz-Artal*** Instituto de Investigación Sanitaria Hospital 12 de Octubre y Universidad Complutense Madrid. Chair: ***Eva Martinez-Caceres*** Germans Trias i Pujol Hospital, Badalona, Autonomous University of Barcelona.
	Lunch
14:00h – 15:00h	Ordinary General Meeting- Societat Catalana d'Immunologia
15:00h – 15:30h	Company sponsored talk ***Beckman Coulter*** **Experience meets innovation: automate your flow cytometry analysis in the Cytobank platform** Speaker: ***Dr. Zaida Vergara*** Beckman Coulter. Regensburg. Germany.
15:30h – 17:30h	Oral Communications III: Viral Immunity Chairs: ***Ana Angulo*** University of Barcelona and ***María José Buzón*** Vall d'Hebron Research Institute, Hospital Universitari Vall d'Hebron, Barcelona. **OC17: Discovery of a virally-encoded PD-L1 molecule. *Francesc Poblador* *et al.*** **OC18: Functional and Structural Characterization of a New Human Ultrapotent Pan-neutralizing SARS-CoV-2 Monoclonal Antibody. *Giuliana Magri* *et al.*** **OC19: Development of a CAR-T cell therapy for SARS-CoV-2. *Mario Vazquez* *et al.*** **OC20: Limited induction of lung-resident memory T cell responses against SARS-CoV-2 by mRNA vaccination. *Daan K.J. Pieren* *et al.*** **OC21: Humoral and Cellular Immune Responses After a Three-dose Course Of mRNA-1273 COVID-19 Vaccine in Kidney Transplant Recipients: A Prospective Cohort Study. *David Cucchiari, Natalia Egri* *et al.*** **OC22: Characterization of tissue-resident NK cells in a tissue model of HIV infection. *David Perea* *et al.*** **OC23: Distinct NK cell responses define durable control in elite controllers during HIV infection. *Nerea Sánchez-Gaona* *et al.*** **OC24: TCR-independent control of viral-reactivated cells by Cervical CD8+ TRM cells. *Cristina Mancebo* *et al.***

FRIDAY, November 25th	
	Coffee break
17:50h – 19:30h	Oral Communications IV: Tumor Immunology and Immunotherapy Chairs: ***Dani Benítez*** Hospital Clinic de Barcelona and ***Aura Muntasell*** Institut Hospital del Mar d'Investigacions Mèdiques (IMIM) and Autonumous University of Barcelona. **OC25: MYC inhibition by OMO-103 induces immune cell recruitment in preclinical models of NSCLC and modulates the cytokine and chemokine profiles of Phase I patients showing stable disease. *Sílvia Casacuberta-Serra* *et al.*** **OC26: Study of the role of CD38 in the anti-tumoral actions of the LXR pathway. *Joan Font-Díaz* *et al.*** **OC27: Immunomodulatory roles of PARP proteins in cancer. *Miguel A. Galindo-Campos* *et al.*** **OC28: Elucidating the role of the PD-1/PD-L1 axis in CAR-T cell function. *Irene Andreu Saumell* *et al.*** **OC29: Metastatic melanoma (MM) patients with stable disease display reduced Mo-MDSCs levels in peripheral blood. *Maria Iglesias Escudero* *et al.*** **OC30: Immunological biomarkers associated with anal dysplasia in people living with HIV. *Cristina Mancebo* *et al.***
19:30h – 20:10h	Plenary Session **Extending our knowledge on T-cell redirecting strategies for acute leukemia** Speaker: ***Pablo Menéndez*** Josep Carreras Leukemia Research Institute, Barcelona. Chair: ***Gerardo Rodríguez*** Hospital Clinic de Barcelona.
20:10h – 20:20h	**Prize to the best communication and poster** **Closing of the Congress** ***Eva Martínez-Cáceres*** Vice-President of SCI.

1 MicroRNA signature in children with type 1 diabetes and its role in autoimmunity

Laia Gomez-Muñoz1; David Perna-Barrull1; Silvia Rodriguez-Fernandez1; Nati Real2; Aina Valls2; Jacobo Perez3; Raquel Corripio3; Marta Murillo2; Marta Vives-Pi1

1Immunology Department, Germans Trias i Pujol Research Institute; 2Pediatrics Department, Germans Trias i Pujol University Hospital; 3Pediatric Endocrinology Department, Parc Taulí University Hospital, I3PT

Type 1 diabetes (T1D) is an autoimmune disease with complex pathogenesis, being in its nature heterogeneous, thus finding children who maintain good glycemic control after insulin therapy (honeymoon or partial remission (PR) phase) and others who do not. Thus, biomarker discovery and patient stratification are unmet needs essential to understand the courses of this disease and applying for precision medicines. MicroRNAs (miRNAs) are small RNA molecules that negatively regulate gene expression and modulate several biological processes, and are candidate biomarkers for T1D. Therefore, we aimed to identify those differentially expressed miRNAs (DEMs) during the PR phase that could be indicative of immunoregulation and serve as biomarkers for this stage. To that end, miRNA expression profiles in plasma were determined employing small RNA-seq in 17 pediatric patients with T1D at different stages and 17 controls. A total of 14 DEMs with validated target genes were found in PR patients, 11 up- and 3 downregulated in comparison with non-remitters, mainly involved in the immune response, metabolism, stress, and cell death. Of those, miR-30d-5p showed the highest fold change (logFC=3,208) and the lowest P-value (p=0,0016) and was found to present several immune targets. To assess its implication in autoimmunity, we i.p. administered an established miR-30d-inhibitor to Non-Obese Diabetic mice, the preclinical model of T1D. The miR-30d-5p blockade resulted in a significant increase in Treg percentage in the pancreatic lymph nodes, along with an increase both in CD4+ EM and CD8+ CM T lymphocytes and a decrease in the CD4+ CM ones. In the spleen, a decrease in PD-1+ T lymphocytes was observed. Moreover, miRNA inhibition led to a tendency to an increased islet leukocytic infiltrate. In conclusion, the differential peripheral miRNA profile found in patients during the PR phase shows new biomarkers and highlights the immunomodulatory role of miR-30d-5p.

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2 CDK11 and cyclin D3 exert antagonistic roles in beta cell survival in autoimmune diabetes (T1D)

Conchi Mora1,3; Ester Sala1,3; Celia Vived1,3; Júlia Luna1,3; Noemí-Alejandra Saavedra-Ávila1,3; Upasana Sengupta1,3; A. Raúl Castaño2; Sabrina Villar-Pazos4,5; Laura haba6; Joan Verdaguer1,3; Ana B. Ropero7; Thomas Stratmann8; Javier Pizarro9,10,11; Manuel Vazquez-Carrera9,10,11; Angel Nadal4,12; Jill Lahti5

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Pancreatic islets undergo severe inflammatory insult during the progression of the autoimmune diabetes (T1D), leading to profound alterations of islet function and fitness prior to beta cell demise. We found that, both, Cyclin D3, a D-type cyclin involved in cell-cycle progression, and, CDK11, which is a cyclin dependent kinase that is involved in RNA processing, mitosis and apoptosis, are downregulated in the islets during the insult to beta cells in the T1D prone NOD (non-obese diabetic) mouse model. Cyclin D3 deficiency in the NOD mouse exacerbates the diabetes phenotype in a cell-cycle independent fashion. Moreover, cyclin D3 is a natural partner for CDK11p58, a form of CDK11 expressed during mitosis. The complete deficiency in CDK11 is embryonically lethal. We explored the role of CDK11 in T1D and found that NOD mice hemideficient for CDK11, were protected against T1D without exhibiting changes in beta cell proliferation. This protection against diabetes was due to lower susceptibility to cytokine-induced apoptosis. Since cyclin D3 interacts with cyclin D3 as a natural partner, we addressed whether cyclin D3 exerted its protective role in beta cell viability independently of CDK11. This study was conducted by studying the diabetes onset in NOD mice carrying both mutations (the CDK11 hemideficiency and cyclin D3 deficiency). Interestingly, the protection mediated by the CDK11 hemideficiency did not attenuate the exacerbation of T1D caused by cyclin D3 deficiency in NOD mice, which suggests that the mechanism(s)
underlying the cyclin D3 protection may be independent/downstream of the CDK11 pro-apoptotic signaling cascade. CDK11 hemideficiency did not affect neither glucose tolerance or calcium fluxes in pancreatic islets. Therefore, while CDK11 is a natural target in T1D that is presumably repressed in beta cells as a protection mechanism against inflammation-induced apoptosis, the repression of cyclin D3 during the
progression of the autoimmune attack accelerates diabetes onset.

3 Influence of Multiple Sclerosis inflammatory context in the development of a tolerogenic dendritic cell therapy

Federico Fondelli[1]; María José Mansilla[1]; Gerard Godot[2]; Silvia Presas-Rodriguez[3]; Cristina Ramo Tello[3]; Esteban Ballestar[2]; Eva Martínez Cáceres[3]

[1] Institut Germans Trias i Pujol; [2] Institut Josep Carreras; [3] Hospital German Trias I Pujol

Introduction: Multiple Sclerosis (MS) is an inflammatory disease affecting the CNS where autoreactive lymphocytes induce demyelination and neurodegeneration. Current treatments reduce inflammation but can't cure the disease. In this context, antigen-specific immunotherapies represent a potentially curative approach, able to re-educate immunity toward homeostasis. Our group developed a tolerogenic dendritic cell product (VitD3-TolDCs) produced from peripheral blood monocytes in presence of Vitamin D3, which showed safety/tolerability in a Phase I clinical trial in patients with MS.

However, given MS-intrinsic inflammation, monocytes could present a proinflammatory phenotype in comparison to Healthy Donors (HD), leading to less-than-ideal VitD3-TolDCs. Indeed, we demonstrated that a combined therapy with IFNB +VitD3-TolDCs improved clinical signs in the EAE model compared to individual treatments, suggesting that inflammation can also affect the potency of VitD3-TolDCs.

Thus, in order to translate this therapy to the clinic, we aim to compare phenotype and functionality of monocytes and VitD3-TolDCs from MS patients and HD, in order to identify pathways that could be modulated to produce stronger 2nd generation VitD3-TolDCs.

Methods: We analyzed via flow cytometry and methylation microarrays monocyte samples from active, naïve MS patients and HD (n=15vs15). Then, we evaluated the capability of VitD3-TolDCs generated from MS-derived monocytes to induce allogeneic PBMCs proliferation in Mixed Lymphocyte Reactions in comparison to HD (n=7vs7).

Results: Flow Cytometry of MS monocytes show an increase in the intermediate subset and increased expression of inflammation markers, while methyl-arrays show changes in MS and enrichment of specific transcription factors binding motifs in unmethylated genes involved in immune activation. Finally, MS-derived VitD3-tolDCs suppress less allogenic proliferation (mean suppression 0.63 (HD) vs 0.35 (MS).

Conclusions: MS monocytes present a proinflammatory phenotype and when used to produce VitD3-tolDCs they generate less powerful tolerogenic cells. Given this, we propose that VitD3-tolDCs potency could be boosted by modulating the identified pathways.

4 Longitudinal study of changes induced by cladribine treatment on Innate Lymphoid Cells from patients with multiple sclerosis

María José Mansilla[1]; Coral Zurera Egea[1]; Federico Fondelli[1]; Aina Teniente Serra[1]; Silvia Presas Rodríguez[2]; Jana Willemyns[1]; Cristina Ramo Tello[2]; Eva M. Martínez Cáceres[1]

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Introduction: Innate lymphoid cells (ILCs) are lymphocytes that lack the expression of an antigen-specific cell receptor. These represent a very small subset in peripheral blood and are divided in three immunoregulatory subsets; ILC1, ILC2 and ILC3, that mimic Th1, Th2 and Th17 $CD4^+$ T-cell subsets, respectively. ILCs are involved in the pathogenesis of some autoimmune diseases, but their role in multiple sclerosis (MS) is still unknown.
Cladribine is an oral MS treatment reported to induce a decrease in both $CD4^+$ and $CD8^+$T-cells, B-cells and Natural Killer cells. However, there is a lack of data regarding the effect of cladribine on ILCs.
Objective: we aimed to analyze the long-term effects of cladribine on ILCs in a 3-year follow-up study.
Methods: 11 MS patients were included. Samples were obtained at Baseline and 3, 6, 12, 18, 24 and 30 months after initiation of treatment. We performed flow cytometry analyses on samples obtained after bulk lysis of 15ml of whole blood. Samples were acquired through FACSFortessa (BDBiosciences) and analysed through FlowJo Software. Statistical analyses and graphs were performed through SPSS and GraphPad softwares.
Results: No significant differences were observed in the main three ILC subsets throughout the follow-up. However, the C-kit^+CD161^-ILC2 subset was significantly increased after 12 months. Also, NCR^+CD161^-ILC3 cells increased throughout time and significantly decreased at the 12-month timepoint**.** Finally, CD161 expression in ILC2s significantly increased throughout time.
Conclusions: Results indicate that cladribine treatment induces changes in several ILC subsets, that could be possibly related with its therapeutical effect. An increased sample size might unravel other subset differences masked due to interindividual variability.

5 Oxygen-independent regulation of HIF-1α in ILC3s ameliorates the inflammation of C. rodentium-induced colitis

Ana Valle-Noguera[1]; María José Gómez-Sánchez[1]; Anne Ochoa-Ramos[1]; Patricia Yagüe Fernández[2]; Blanca Soler Palacios[3]; Virginia Zorita[4]; Berta Braposo Ponce[5]; José María González-Granado[6]; Julián Aragonés[7]; Aranzazu Cruz-Adalia[1]

[1]Universidad Complutense de Madrid; [2]Centro de Investigaciones Biológicas Margarita Salas; [3]Centro Nacional De Biotecnología; [4]Centro Nacional de Investigaciones Cardiovasculares; [5]Centro de Biología Molecular Severo Ochoa; [6] Instituto de Investigación Sanitaria Hospital 12 de Octubre; [7]Hospital Santa Cristina, Fundación de Investigación Hospital de la Princesa

Innate lymphoid cells (ILC3s) are tissue-resident immune cells that play a critical role in preserving the integrity of mucosal tissues and defending against extracellular infections, especially in the gut. ILC3s mainly function by secreting IL-22 and IL-17, however, it is still important to understand the cellular and molecular mechanisms that regulate these cytokines during infectious or inflammatory diseases. This is especially relevant in understanding colitis, which is defined by an imbalance in the relationship between the host, their microbiota, and their immune system. According to new research, hypoxia can alter the activity of intestinal ILC3s in a post-translational oxygen-dependent manner by stabilizing the hypoxia-inducible factor (HIF-1α). To assess the functional significance of this molecular mechanism during infectious colitis, further *in vivo* validation is required by the use of more specialized transgenic knock-out mice. In this study, we examined the response to this infection in new transgenic mice which have HIF-1α gene inactivated in ILC3s (RAG-1KO HIF-1α$^{fl/fl}$ RorγtCre). Due to their failure to upregulate IL-22, the mice had more severe colitis than control mice after contracting *Citrobacter rodentium*. Additionally, *ex vivo* experiments with colon explants from these transgenic animals showed that IL-18, generated by the activation of Toll-like receptor 2 (TLR2), is the cytokine responsible for the IL-22 release through HIF-1α in ILC3s. Importantly, HIF-1α and IL-22 but not IL-17 were transcriptionally activated by sorted ILC3s triggered *ex vivo* with IL-18 and IL-23. Moreover, IL-22 secretion triggered by TLR2 in colon explants was abrogated by the IL-18 binding protein. Our findings offer up new research paths in the field of infectious colitis and the molecular control of ILC3s by demonstrating for the first time that *C. rodentium* may regulate transcriptionally HIF-1α in ILC3s, boosting the release of IL-22 upon IL-18 stimulation.

6 SEB-induced IL-13 production in CLA+ memory T cells defines Th2 high and Th2 low responders in atopic dermatitisMicroRNA signature in children with type 1 diabetes and its role in autoimmunity

Lídia Sans-De San Nicolàs[1]; Ignasi Figueras-Nart[2]; Montserrat Bonfill-Ortí[2]; Carmen De Jesús-Gil[1]; Irene García-Jiménez[1]; Antonio Guilabert[3]; Laia Curto-Barredo[4]; Marta Bertolín-Colilla[4]; Marta Ferran[4]; Esther Serra-Baldrich[5]; Anna Zalewska-Janowska[6]; Yui-Hsi Wang[7]; Michael D. Howell[8]; Ramon M. Pujol[4]; Luis F. Santamaria-Babí[1]

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Atopic dermatitis (AD) is a heterogeneous, chronic, inflammatory, T cell-mediated skin condition characterized by an epidermal barrier deficiency, an altered skin microbiome composition, pruritus and an abnormal immune response. *Staphylococcus aureus*, skin-homing cutaneous lymphocyte-associated antigen (CLA)$_+$ memory T cells and IL-13 are key players in AD. Circulating CLA$_+$ memory T cells reflect the cutaneous abnormalities in AD due to its capacity to recirculate between skin and blood. There is currently no clear *in vitro* model to study the Th2 status in AD. Our purpose was to understand AD functional immune Th2 response heterogeneity through the *S. aureus* enterotoxin B (SEB) activation of CLA$_+$ memory T cells. For this, CLA$_{+/-}$ memory T cells were cocultured with autologous lesional epidermal cells from adult non-treated moderate-to-severe AD patients (n=35) and control subjects (n=8). After 24 hours of stimulation with SEB, IL-13, IL-4, IL-5, IL-17A, IL-22, IFN-g, CCL17 and CCL22 were evaluated. Also, for AD patients plasma and mRNA expression from the lesional skin biopsies were assessed. Circulating
CLA$_+$ memory T cells preferentially produced IL-13, IL-4, IL-17A, IL-22, CCL17 and CCL22 when activated with SEB. The IL-13 response enabled the stratification of a clinically homogeneous population into Th2 high and Th2 low groups. In the Th2 high group, in contrast to the Th2 low, the SEB-induced CLA$_+$ T-cell IL-13 response directly correlated with eczema area and severity index (EASI), plasma CCL17, sIL-2R and *S.aureus*-specificIgE levels, and CCL26 mRNA expression from cutaneous lesions, whereas inverse correlation was found for LCN2 mRNA expression from cutaneous lesions. Our study identifies Th2 high and Th2 low groups, corresponding with disease activity, based on the CLA$_+$ T-cell IL-13 response to SEB, within a clinically homogeneous moderate-to-severe AD population. This translational approach may help to explore the complex heterogeneity of AD pathophysiology form a more functionally point of view.

7 Polyreactive Autoantibodies in the Physiopathology of Sjögren's Syndrome

Rebeca Gutiérrez-Cózar[1]; Manuel Sáez Moya[1]; Joan Puñet-Ortiz[1]; Javier Fernández[1]; Ana Angulo[1]; Pablo Engel[1]

[1]*University of Barcelona*

Sjögren's syndrome (SjS) is a chronic autoimmune disease that primarily affects exocrine glands and is characterized by the presence of anti-nuclear antibodies such as anti-dsDNA and anti-Ro52. Our analysis of the B cell repertoire of NOD.H-2h4 mice, which spontaneously develop a SjS-like disease, showed that these mice presented higher frequencies of autoreactive/polyreactive B cell clones that increase with age, as compared to B6 mice (WT-type) mice. No loss of polyreactivity was observed upon antibody class switching to IgG or somatic mutation. Moreover, strikingly, all anti-Ro52 IgG autoantibodies were polyreactive. We hypothesize that these polyreactive antibodies may play a pathogenic role. We further analyzed if anti-Ro52 antibodies were able to accelerate the disease process using a mouse model of SjS. Anti-Ro52 mouse monoclonal antibodies were injected in autoimmune MRL/lpr mice infected with mouse cytomegalovirus.
Our results show that they induced increased levels of autoantibodies and salivary gland tissue damage.
In conclusion, polyreactive antibodies against Ro52 induce pathogenic phenomena in susceptible mice indicting a role of these antibodies in the pathogenesis of SjS disease.

8 Autoantibodies to Nuclear Valosin-containing Protein-like (NVL): A Novel Systemic Sclerosis-related Antibody

J. Perurena-Prieto[1]; E.L. Callejas-Moraga[2]; A. Guillén-Del-Castillo[3]; L.Viñas-Gimenez[1]; Albert Selva-O'Callaghan[3]; V. Fonollosa-Plá[3]; M. Sanz-Martinez[1]; Roger Colobran[1]; C.P. Simeón-Aznar[3]

[1]Servicio de Inmunología. Hospital Universitario Vall d'Hebron. Barcelona.; [2]Unidad de Enfermedades Autoinmunes Sistémicas. Servicio de Medicina Interna. Hospital Universitari P; [3]Unidad de Enfermedades Autoinmunes Sistémicas. Servicio de Medicina Interna. Hospital Universitario

Introduction: Systemic sclerosis (SSc) specific autoantibodies allow the diagnosis and predict the prognosis of patients with different clinical characteristics. Antinuclear autoantibodies (ANAs) are detectable in 90-95% of SSc patients, however, in a group of patients of our cohort, no SSc-specific autoantibodies were found by available commercial assays. The aim of this study was to describe new SSc-specific autoantibodies by a novel protein immunoprecipitation (IP) assay.

Patients and Methods: In total, 307 SSc patients were evaluated for autoantibodies against Scl70, centromere, RNA-polymerase III, fibrillarin, NOR-90, Th/To, PM-Scl, and Ku by a commercial immunoblot (IB). ANAs were also tested in all patients by immunofluorescence (IIF) on Hep-2 cells. Patients negative for all autoantibodies by IB were analysed by RNA-IP. Patients negative by RNA-IP were then tested by protein IP. For protein-IP, cell extracts metabolically labelled with BONCAT technology were used. Protein bands detected on SDS-PAGE were further analysed by mass spectrometry (MS).

Results: Overall, 68 patients were negative by IB. Anti-U11/U12-RNP, anti-Th/To and anti-Fibrillarin autoantibodies were found by RNA-IP in 9, 6 and one patient, respectively. Of the 51 patients that were tested by protein-IP, 5 showed a band with an approximate molecular weight of 100kDa on SDS-PAGE. These bands were identified as Nuclear valosin-containing protein-like (NVL) by MS. The five patients with
anti-NVL autoantibodies presented a nucleolar pattern by IIF.

Conclusions: We have identified NVL as a new autoantibody target on SSc patients by a novel protein-IP assay. NVL is an AAA-ATPase involved in multiple cell processes that localizes at nucleoli. Anti-NVL autoantibodies were almost as frequent as anti-Th/To autoantibodies in our cohort. Considering only patients with nucleolar staining by IIF that were negative by IB, 29.4% were positive for anti-NVL autoantibodies. Anti-NVL autoantibodies should be suspected in patients with a nucleolar IIF pattern that are negative by IB.

9 Three Novel WAS Mutations with variable clinical presentations

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Alterations in *WAS* generate 3 well-differentiated clinical entities: Wiskott-Aldrich syndrome (WAS), Xlinked thrombocytopenia (XLT), and X-linked neutropenia (XLN). WAS is caused by complete loss-offunction (LOF) mutations, XLT is due to partial LOF mutations, and XLN arises from gain-of-function (GOF) mutations. We present three patients with different clinical presentations produced by unreported mutations.

The first patient is a 2-year-old Hispanic boy who presented with the classic WAS triad from birth: neutropenia, microthrombocytic thrombocytopenia, and eczema, as well as recurrent infections. *WAS* sequencing revealed a deletion in the exon 2 (c.176del), leading to a frameshift and resulting in a Stop codon 17 amino acids later (p.Pro59Leufs*17). This LOF mutation has not been previously reported.

The second patient is a 40-year-old Caucasian male who was referred from the Haematology department after being treated for a diffuse large B-cell lymphoma. He had suffered from congenital thrombocytopenia and neutropenia, eczema, and autoimmunity before the neoplasia but no genetic studies had been performed. *WAS* sequencing revealed a nonsense mutation in exon 10 (c.1090C>T, p.Arg364*). This mutation was also unreported.

The third patient is an 11-year-old Caucasian boy who presented with congenital neutropenia and macrothrombocytic thrombocytopenia. After ruling out alloimmunity or other bone-marrow defects, the suspicion of XLN was established. *WAS* sequencing showed an unreported missense mutation in exon 9 (c.895G>A, p.G299R), in the XLN-associated mutational region. Functional studies such as viability, migration, phagocytosis, and oxidative capacity performed up to date in the patient's neutrophils seem to indicate that this mutation implies a WASp GOF. The mother is a mutation carrier and shows a partial XLN phenotype.

In conclusion, we have identified three new mutations of the WAS gene: two of them are compatible with WAS of different severity and another in the XLN-associated region that seems to produce a GOF effect.

10 Macrophaging and DNA damage

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Macrophages are phagocytic cells that play an important role in the immune response. They participate in the inflammatory process initially with a pro-inflammatory activity followed by an anti-inflammatory activity critical for the inflammation resolution and the tissue repair.

During the pro-inflammatory step, macrophages will produce toxic molecules such as reactive oxygen species (ROS) to eliminate the pathogens. However, as side effect ROS can produce damage of macrophages such as DNA breaks. Because macrophages need to survive at the inflammatory loci to repair the damaged tissues, they express a large number of molecules to repair DNA damage such as Trex1, Rnase H, Polymerase μ, Samhd1, etc.

Aging is associated with the disfunction and the dysregulation of the homeostatic functions of macrophages,
in a process named macrophaging. This dysregulation reduce the immune responses, and will produce an excess of type I interferons that is associated with autoimmunity.

Here we have found that macrophages from aged mice (18-24 months) in relation to the ones from young mice (8 weeks) produced during pro-inflammatory activation an increased amount of ROS. We also found that the expression of the molecules to repair DNA damage are strongly decreased in macrophages from old mice. Blocking the production of ROS the levels of some of these repairing mechanisms increased. These mechanisms may explain the impairment of macrophages during aging and at least in part the problems of immunodeficiency associated to aging.

11 Vitamin C enhances NF-κB-driven DNA demethylation and immunogenic properties of dendritic cells

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Dendritic cells (DCs) are central in immune responses, and modulation of their function *in vivo* and *ex vivo* are promising strategies to treat different types of cancer, such as acute myeloid leukemia (AML). One example is the *ex vivo* differentiation of autologous monocyte-derived DCs (moDCs) from patients used as DC-based vaccines in several clinical trials that show promising results. DC-based vaccines efficacy can be improved by the addition of vitamin C during the differentiation process. Vitamin C (L-ascorbic acid) is an essential nutrient with pleiotropic functions that can act as a cofactor of Fe-containing hydroxylases such as ten-eleven translocation (TET) enzymes, increasing their DNA demethylation function. In this study, we have analyzed the epigenomic and transcriptomic reprogramming orchestrated by vitamin C in monocytederived DC differentiation and maturation. We have detected extensive demethylation produced by vitamin C treatment, together with concordant gene expression changes during DC maturation. p65, a component of NF-kB complex, interacts with TET2 and is associated with both vitamin C-mediated gene upregulation and DNA demethylation. In addition, we have determined that mature DCs differentiated with vitamin C also have a higher ability to stimulate proliferation of antigen specific T cells when loaded with the antigen, in comparison with those differentiated in the absence of vitamin C. We are currently testing vitamin C potential/capacity to boost DC-based vaccines in an AML mouse model, in which we inject bone marrow derived dendritic cells (BMDCs) that have been differentiated *in vitro* in the presence of vitamin C and loaded with neoantigens. This work provides a potential strategy for the improvement of cell therapies based on DCs vaccines for the treatment of cancer.

12 Characterization of HLA-DR immunopeptidome presented by dendritic cells pulsed with the breast cancer tumoral cell line MCF-7 lysates

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The characterization of tumor antigens that drive the immune response has great potential as a tool to develop new treatments, especially antigen-derived therapies. Despite the importance of cytotoxic T cells, the CD4+ T cell response has gained interest due to its major contribution in the maintenance and orchestration of the anti-tumor immune response. Moreover, due to the epithelial etiology of carcinomas, the presentation of peptides by HLA-II is mediated by antigen presenting cells, mainly dendritic cells (DCs).

Therefore, we have performed the characterization of the peptides presented by different HLA-DR molecules of monocyte-derived DCs (moDCs) from eight healthy donors. Six moDCs samples were pulsed with protein extracts from the breast cancer cell line MCF-7 and two samples of non-pulsed moDCs were used as controls.

The objectives of this study were: (i) to determine whether there are differences between the peptidome presented by pulsed and non-pulsed moDCs, both at peptide and protein levels; (ii) to determine the percentage of peptide and protein overlap between moDCs with different HLA-DR alleles; (iii) to evaluate the influence of the allele combination on antigenic presentation.

The results showed that: (i) the pulse displaces part of the proteins of the moDCs ligandome, since the nonpulsed samples share most of the proteins with the rest of the pulsed moDCs, but not the other way around; (ii) there is a positive correlation between the abundance of an allele in the samples studied and the level of peptide and protein overlap presented by them but not with the sample size; (iii) the immunopeptidome of the pulsed moDCs is modified by different HLA-DR alleles.

In conclusion, the HLA-DR allele combinatorics has some influence on the immunopeptidome presented by moDCs, which should be considered to better design personalized antigen-directed therapies.

13 The role of Myeloid-derived Supressor cells in STI-mediated enhancement of HIV infection

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Sexually transmitted infections (STIs) may affect the development and pathogenesis of other STIs, such as HIV infection. Myeloid-derived suppressor cells (MDSCs) are a diverse population of cells with immunesuppressive effects, which can be generated by certain cytokine environments, including the ones produced during STIs. We hypothesized that MDSCs expand in response to a primary STI, increasing the risk of acquiring a secondary STI such as HIV by suppression of the mucosal immune response.

We obtained cervical samples from healthy women (HC, n=18), or women with acute *Chlamydia trachomatis* infection (CT, n=13), Human Papillomavirus infection (HPV, n=20), Bacterial Vaginosis (BV, n=14), or co-infected (n=12). In these samples, we assessed the cellular immune composition by flow cytometry and the level of eight cytokines. Additionally, we obtained cervical tissue (n=11), which was exposed *ex vivo* to CT or GM-CSF+IL-6 cytokines to assess MDSC expansion and HIV infection by flow
cytometry.

In cervical samples, we found higher frequencies of HLA-DR-CD14- MDSCs in CT compared to HC and a trend towards higher levels of HLA-DR$_{dim}$CD14+ MDSCs in BV patients (p=0.06). Cervical BV samples showed higher levels of several MDSC-associated cytokines including M-CSF, IL-1β, GM-CSF, VEGF-A, and TGF-β, whereas CT samples showed increased levels of M-CSF. *Ex vivo* exposure of cervical tissue to
CT or GM-CSF+IL-6 enhanced subsequent HIV infection (n=7-9, p=0.02 and 0.003, respectively). Moreover, exposure to CT increased the proportion of CD15+ MDSC cells (p=0.07) and increased expression of suppressive mediators.

Pathogen-specific cytokine microenvironments and MDSC phenotypes may be involved in promoting cervical immune suppression in patients with STI. Enhancement of HIV infection after CT infection and increase in potentially suppressive MDSCs suggest a role for MDSCs in increasing the risk of secondary infection. Future efforts include high-dimensional analyses of flow cytometry data and exploring immunesuppression by cervical MDSCs.

14 X-Linked SASH3 Deficiency Presenting as a Common Variable Immunodeficiency

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INTRODUCTION: SASH3 is a lymphoid-specific adaptor protein. In a recent study, SASH3 deficiency was described as a novel X-linked combined immunodeficiency with immune dysregulation, associated with impaired TCR signaling and thymocyte survival in humans. The small number of patients reported to date showed recurrent sinopulmonary, cutaneous and mucosal infections, and autoimmune cytopenia.

CASE DESCRIPTION: We describe an adult patient previously diagnosed with common variable immunodeficiency (CVID) due to low IgG and IgM levels and recurrent upper tract infections. Two separate, severe viral infections drew our attention and pointed to an underlying T cell defect: severe varicella zoster virus (VZV) infection at the age of 4 years and bilateral pneumonia due type A influenzainfection at the age of 38.

RESULTS: Genetic testing using an NGS-based custom-targeted gene panel revealed a novel hemizygous loss-of-function variant in the SASH3 gene (c.505C>T/p.Gln169*). The patient's immunological phenotype included marked B cell lymphopenia with reduced preswitch and switch memory B cells, decreased CD4+ and CD8+ naïve T cells, elevated CD4+ and CD8+ TEMRA cells, and abnormal T cell activation and proliferation. The patient showed a suboptimal response to Streptococcus pneumoniae (polysaccharide) vaccine, and a normal response to Haemophilus influenzae type B (conjugate) vaccine and SARS-CoV-2 (RNA) vaccine.

CONCLUSIONS: In summary, our patient has a combined immunodeficiency, although he presented with a phenotype resembling CVID. Two severe episodes of viral infection alerted us to a possible T-cell defect, and genetic testing led to SASH3 deficiency. Our patient displays a milder phenotype than has been reported previously in these patients, thus expanding the clinical spectrum of this recently identified inborn error of immunity.

15 Can we predict when to evaluate CVID genetically?

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Background and Aims: Common variable immunodeficiency (CVID) is highly variable in terms of clinical presentation, age and severity. Although the polygenic and epigenetic contribution is evident, monogenic causes can explain 15-30% of cases. The aim of this study is to clinically, immunologically, genetically and molecularly characterize CVID patients to offer personalized treatment and preconception counselling.

Methods: Patients who meet the ICON2015 criteria were studied with a custom genetic panel including 323 IEI genes. Previously unreported mutations were functionally validated. All the variables collected were statistically studied to create a predictive model for CVID monogenic forms.

Results: From a cohort of 148 CVID patients (gender ratio (M:F) is 1.16:1, mean age of 43 (18 SD)), 136 were genetically studied. Thirty-six pathogenic mutations were identified (26%): 61% were considered causative (*BTK*, *CTLA4*, *DKC1*, *IKBKG*, *IKZF1*, *LRBA*, *NFKB1*, *PIK3R1*, *RNU4ATAC* and *TNFRSF13B* [homozygous and compound heterozygote] genes) and 39% risk factors (heterozygous *TNFRSF13B* variants). Segregation studies allowed us to identify 11 affected individuals and 14 carriers. Functional studies were performed for some specific variants to confirm its pathogenicity.

Seven (5%) patients in the cohort could benefit from a targeted treatment after genetic diagnosis. Younger age, early age of symptom onset, an "infection plus" phenotype and high transitional B lymphocytes were significantly associated with a positive genetic result (AUC = 0.87).

Conclusions: A causative monogenic defect was identified in 17.6% of the CVID cohort. NGS seems mandatory in young CVID patients, those with early onset of symptoms, a clinical phenotype with infections plus non-infectious features and elevated transitional B cells.

16 Detection and evolutionary dynamics of somatic FAS variants in autoimmune lymphoproliferative syndrome: diagnostic implications.

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Autoimmune lymphoproliferative syndrome (ALPS) is an early-onset disorder presenting with lymphadenopathy, autoimmunity, hepatosplenomegaly, and elevated double-negative ab T cells (DNT). ALPS is mostly associated with genetic variants in the *FAS* gene, which can be somatic in up to 20% of the cases.

Here, we present a patient with early-onset ALPS in whom we identified a somatic pathogenic insertion at the *FAS* gene (c.718_719insGTCG) using Sanger sequencing on a CD3 enriched sample (although the variant was not detected in whole blood using this method). Moreover, we extended the study by exploring the evolutionary dynamics of this somatic variant with NGS. For that, we used blood samples obtained before and during the treatment with immunosuppressive drugs, over five years. By using deep amplicon sequencing (DAS, coverage 20,000-30,000x) we were able to detect the variant in all samples with a variant allele frequency (VAF) ranging from 7% (in pre-treatment samples) to 0.5% (in samples during treatment).

The VAF evolution was significantly correlated to the DNT population frequency (Pearson's R: 0.98), concordant with the preferent location of the *FAS* somatic variants in this population. Additionally, in one of the samples with DNT values within the expected range (0.89%), we performed DAS on DNA from whole blood and purified CD3 cells (using RosetteSep™). When comparing the results, we showed that the VAF of the somatic variant was doubled in CD3 cells (1.6% versus 0.68% in blood).

Taken together, our results evidence that i) somatic genetic variants in *FAS* can be detected and studied with DAS; ii) evolutionary dynamics of somatic variants can be studied in peripheral blood samples regardless of the normalization of the DNT population frequency (<2%); and iii) DAS experiments, combined with an appropriate bioinformatic approach, successfully detect somatic variants in ALPS patients even in samples obtained after several years under treatment.

17 Discovery of a virally-encoded PD-L1 molecule

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Viruses employ an impressive variety of strategies to subvert host immunity. In particular, in herpesviruses, some of these tactics are mediated by viral gene products acquired by horizontal gene transfer from the corresponding hosts and shaped throughout evolution. The programmed death-1 (PD-1) receptor and its ligands, PD-L1 and PD-L2, are cell surface molecules belonging to the immunoglobulin superfamily that play a pivotal role attenuating T-cell responses and regulating immune cell tolerance. Here, we report the first functional PD-L1 homolog gene (*De2*) found in a pathogen. *De2*, captured by a g-herpesvirus from its host during co-evolution around 50 million years ago, encodes a cell-surface glycoprotein that interacts with high affinity and stability with host PD-1. We also find that mutations evolved by the viral protein result in a significant loss of its ability to interact in cis with CD80, an interaction that for PD-L1:CD80 has been reported to block PD-1 inhibitory pathways. Furthermore, employing a fluorescence-based cellular assay, we demonstrate that the viral protein is capable to potently inhibit T-cell signaling. Our observations suggest that PD-L1 homologs may enable viruses to evade T cell responses, favor their replication, and prevent excessive tissue damage. Altogether, our findings reveal a novel viral immunosuppressive strategy and highlight the importance of the modulation of the PD-1/PD-L1 axis during viral infections.

18 Functional and Structural Characterization of a New Human Ultrapotent Pan-neutralizing SARS-CoV-2 Monoclonal Antibody

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In the present study we report the functional and structural characterization of mab17T2, a new highly potent pan-neutralizing SARS-CoV-2 human monoclonal antibody (mAb), isolated from a convalescent COVID-19 individual infected during the first wave of the COVID19 pandemic. mAb17T2 is a class 1 VH1-58/κ3-20 antibody, derived from an RBD-specific IgA canonical memory B cell and developed as a human IgG1 recombinant mAb. Functional characterization reveals that mAb17T2 had an exceptionally high neutralization activity against all SARS-CoV-2 variants tested (WH1, D614G, alpha, beta, gamma, delta, Mu) and Omicron sub-lineages (BA.1, BA.2 and BA.4 and BA.5). Moreover, we demonstrate that mAb17T2 had prophylactic activity against Omicron BA.1 in vivo in K18-hACE2 transgenic mice. 3D reconstruction from cryo-electron microscopy (cryo-EM) indicates that mAb17T2 was able to bind the Omicron BA.1 spike protein with the RBD domains in the up position and recognized an RBD epitope overlapping with the receptor binding motif, as is the case for other previously described neutralizing mAbs, including S2E12. Yet, unlike mAb S2E12, mAb17T2 retains its high neutralizing activity against all omicron sub-lineages tested. These results highlight the importance of microstructure for an antibody's neutralizing activity and identify mab17T2 as a potential candidate for future therapeutic and prophylactic interventions.

19 Development of a CAR-T cell therapy for SARS-CoV-2

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SARS-CoV-2 is a coronavirus known for causing the disease Covid-19. At the beginning of the pandemic SARS-CoV-2 had a high virulence, causing a huge number of severe cases and deaths. Although after the initiation of worldwide vaccination the severity of the cases decreased, there are still several subsets of the population, such as immunocompromised or immunosuppressed individuals, that are at risk of developing severe symptomatology. In order to tackle the lack of medical options for treating these individuals, who cannot eliminate the virus with their own immune system, we decided to develop a Chimeric Antigen Receptor (CAR) T cell therapy directed against the S protein of SARS-CoV-2.

We have generated CAR-T cells with two different single chain variable fragments (scFv) that recognize the receptor binding domain of the Spike protein. We have tested these CAR-T in vitro, with different cell line models to test their specificity. The CAR-T were only capable of recognizing and mediate a cytotoxic response against in-house cell line models that expressed the spike protein on their surface. Moreover, we did also test our CAR-T in cells directly infected with the virus, and they were also capable of mediating a specific response against the infected cells.

Due to the evolution of the virus with time, new variants with mutations in the spike protein have appeared. We also have conducted affinity studies of our scFv for these new viral variants. Our results show that our scFv are capable of recognizing new viral variants such as Delta and Omicron.

20 Limited induction of lung-resident memory T cell responses against SARSCoV-2 by mRNA vaccination

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Resident memory T cells (TRM) present at the respiratory tract may be essential to enhance early SARSCoV-2 viral clearance, thus limiting viral infection and disease. While long-term antigen-specific TRM are detectable beyond 11 months in the lung of convalescent COVID-19 patients after mild and severe infection, it is unknown if mRNA vaccination encoding for the SARS-CoV-2 S-protein can induce this frontline protection.

We obtained cross-sectional paired blood and lung biopsy samples from patients (n=26) undergoing lung resection for various reasons and divided them into four groups: I.) uninfected unvaccinated individuals (n=5), II.) unvaccinated long-term SARS-CoV-2 convalescent individuals (n=9), III.) uninfected and longterm two-dose vaccinated individuals (n=7), and IV.) uninfected and short-term three-dose vaccinated individuals (n=5). We determined the presence of SARS-CoV-2-specific CD4+ and CD8+ T cells in blood and lung samples after exposure of cells to M, N, and S peptide pools, followed by flow cytometry to detect TRM cells expressing interferon (IFN)γ and/or CD107a.

We found that the frequency of CD4+ T cells secreting IFNγ in response to S-peptides was variable but detectable up to 8 months after mRNA vaccination. Moreover, this response was overall similar in the lung of mRNA-vaccinated patients compared to convalescent-infected patients. However, in vaccinated patients, lung responses presented less frequently a TRM phenotype compared to convalescent infected individuals and polyfunctional CD107a+ IFNγ+ TRM were virtually absent.

mRNA vaccines might induce responses within the lung parenchyma, potentially contributing to the overall disease control. However, the robust and broad TRM response established in convalescent-infected individuals may offer advantages in limiting disease if the virus is not blocked by initial mechanisms of protection, such as neutralization.

21 Humoral and Cellular Immune Responses After a Three-dose Course Of mRNA-1273 COVID-19 Vaccine in Kidney Transplant Recipients: A Prospective Cohort Study

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Background.
In kidney transplant recipients, there is discordance between the development of cellular and humoral response after vaccination against SARS-CoV-2. We sought to determine the interplay between the 2 arms of adaptive immunity in a 3-dose course of mRNA-1273 100 µg vaccine.

Methods.
Humoral (IgG/IgM) and cellular (N- and S-ELISpot) responses were studied in 117 kidney and 12 kidneypancreas transplant recipients at the following time points: before the first dose, 14days after the second dose and before and after the third dose, with a median of 203 and 232 d after the start of the vaccination cycle, respectively.

Results.
After the second dose, 26.7% of naive cases experienced seroconversion. Before the third dose and in the absence of COVID-19, this percentage increased to 61.9%. After the third dose, seroconversion occurred in 80.0% of patients. Naive patients who had at any time point a detectable positivity for S-ELISpot were 75.2% of the population, while patients who maintained S-ELISpot positivity throughout the study were 34.3%. S-ELISpot positivity at 42 d was associated with final seroconversion (OR, 3.14; 95% CI, 1.10-8.96; P = 0.032). Final IgG titer was significantly higher in patients with constant S-ELISpot positivity($P < 0.001$).

CONCLUSIONS:
A substantial proportion of kidney transplant recipients developed late seroconversion after 2 doses. Cellular immunity was associated with the development of a stronger humoral response.

22 Characterization of tissue-resident NK cells in a tissue model of HIV infection

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Although NK cells are essential in controlling HIV infection, their role in the main tissues where HIV persists, such as the secondary lymphoid tissue and the gastrointestinal tract, is largely undefined. Here, we have characterized NK cells present in relevant tissues, particularly their memory-like and residency properties, and assessed their functionality in killing HIV-infected cells in a tissue model.
We employed uninfected human tissue resections from gut and tonsils to determine by flow cytometry the expression of the memory marker NKG2C, the residency markers CD49a, CD69, and CD103, and KIRs (KIR2DL1, S1, L2, L3, and S2). Natural cytotoxicity (CD107a and IFN-γ production) was evaluated after co-culturing disaggregated tissue cells with the HLA-negative K562 cell line (tonsil n=11, gut n=7). Last, using the tonsil explant model (n=16), the NK phenotype and natural cytotoxicity were characterized after 5-7 days of HIVBAL ex vivo infection.
Gut tissue CD56+ NK cells (1.8%) were higher in comparison with tonsils (0.38%) and, in both, CD56brightCD16- was the predominant subset. However, the CD56dimCD16+ counterpart was more frequently expressing CD49a, CD103, KIRs, and NKG2C. While gut tissue, with higher expression of CD69 and CD103, showed a reduced stimulatory potential compared with tonsils, tonsil CD16+ NK cells expressing residency markers correlated with decreased HIV levels. Furthermore, two main tonsil populations were associated with significant lower levels of HIV: the CD56brightCD16-CD103+ ($r=-0.54$, $p=0.014$), and the CD56dimCD16+CD69+KIR+ ($r=-0.61$, $p<0.01$), being the latest expanded in the infected culture ($p<0.01$). Importantly, both subpopulations showed high basal cytotoxicity and increased functionality after K562 stimulation.
Our research indicates that higher numbers of cytotoxic NK-resident cells might have an important role in HIV control in lymphoid tissue. Hence, strategies aimed at expanding tissue-resident NKs may lead to the development of targeted therapies directed to the main sites of HIV persistence.

23 Distinct NK cell responses define durable control in Elite Controllers during HIV infection

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Elite Controllers (EC) are people living with HIV (PLWH) that present drug-free control of the infection, representing a model for a functional cure. Hence, understanding their mechanisms of immune-mediated control is of considerable interest. We sought to characterize the NK cell repertoire in EC, including their memory-like properties and functional potential. Samples from n=33 EC (n=20 with durable control (DC), and n=13 with immunological aborted control (AC)), n=25 healthy donors (HD), n=8 PLWH in antiretroviral therapy (ART), and n=7 viremic (VIR) participants were included in the study. Phenotypic studies were performed by flow cytometry and included the markers CD57, CD56, Nkp30, NKG2C, NKG2A, CD16, CXCR3, KIR2DL2/L3, and KLRG1. Natural cytotoxicity and activation were evaluated by IFN-γ, CD107a, CD69 and HLA-DR expression by flow cytometry after co-culturing isolated NK cells with the MHC-devoid K562 cell line with and without IL-15. Antibody-dependent cell-mediated cytotoxicity (ADCC) was assessed after co-culturing NK cells with the latently-infected ACH-2 cell line and HIV+ plasma. NK cells from DC presented lower NKG2A expression and basal cell activation, based on HLA-DR and CD69 expression, compared to AC. In addition, NKG2C+ NK cells, associated to a memory-like phenotype, were expanded in DC compared to HD and AC. Although NK cells from DC showed a marked cytotoxic response compared to unstimulated conditions, natural cytotoxicity was significantly lower compared to HD. However, NK cells from DC showed a strong ADCC, similar to HD. Importantly, DC presented a remarkable ADCC compared to AC and VIR, which was strongly associated with KLRG1 and NKG2D expression and inversely associated with the cytotoxic response presented upon K562 and IL-15 stimulation. Our findings suggest that durable immune-mediated control of HIV infection in EC is defined by NK cell phenotypic and functional attributes, including lower cell activation, an increased memory-NK cell compartment and higher ADCC responses.

24 TCR-independent control of viral-reactivated cells by Cervical CD8+ TRM cells

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Background
The major hurdle to HIV-1 eradication is the establishment of viral reservoirs. In tissues, where most of the HIV burden persists, antiviral resident memory CD8+T cells (TRM) may be critical to eliminate cellular reservoirs. Several reports suggest that certain TRM may have innate-like properties, enabling amplification of inflammation and participation in non-cognate responses. Here we aimed to address if CD8+TRM phenotypes can exert unspecific natural control against the reactivated viral reservoir in tissue.

Methods
Using cervical tissue explants from non-oncogenic hysterectomies (Median= 50years [32-68, IQR], n=27), we established a tissue HIV-reservoir model. We have previously shown that in cervix, >90% of CD69+CD8+T cells are compatible with a CD8+TRM phenotype. HIV-infection, the effectivity of the cART treatment and the establishment and subsequent reactivation of viral reservoirs by a latency reversal agent (Ingenol) was measured by p24 expression using flow cytometry (analyzed by Wilcoxon matched-pairs signed rank test). Tissues were digested and CD4+ and CD8+T cells were isolated and co-culture from to determine the impact of CD8+T cells on viral-reactivated CD4+T cells by quantification of p24 and cell-associated HIV-1 DNA.

Results
Our model showed an increase of HIV infected cells by day 7(n=10, p=0.004) and decrease after 1-2days of ART treatment (n=7, p=0.016); Ingenol treatment increased the expression of p24(n=12, p=0.001) indicating the existence of cell reservoirs. After viral reactivation, addition of CD8+ TRM significantly decreased the frequency of p24-expressing cells (n=11, p=0.042), with a concomitant reduction in viral DNA (n=7, p=0.047). In addition, we detected a positive correlation (r=0.74, p=0.013) between the number of CD8+T cells added to the co-culture and the percentage of killing.

Conclusions
Our results highlight an active role of CD8+TRM phenotypes in limiting tissue viral persistence after viral reactivation, independent of TCR engagement. The mechanism behind remains unknown but future research on this area is warranted.

25 MYC inhibition by OMO-103 induces immune cell recruitment in preclinical models of NSCLC and modulates the cytokine and chemokine profiles of Phase I patients showing stable disease

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Despite the promise of targeted therapies and immunotherapy, many cancer patients do not respond to treatment and are still in need of effective therapeutic options. We propose a revolutionary strategy based on the inhibition of MYC, a central molecule that drives tumor progression and immune evasion. Although MYC has long been considered undruggable, we have demonstrated the safety and dramatic therapeutic potential of its inhibition using a MYC dominant negative, termed Omomyc. We showed that Omomyc abrogates tumor progression in a Non-Small Cell Lung Cancer (NSCLC) mouse model, modulates chemokine/cytokine profiles and recruits T cells to the tumor site. These results have granted further development of Omomyc towards ongoing Phase I/IIa clinical trials (MYCure).

In our preclinical models, the infiltrating T cells consequence of Omomyc intranasal treatment are mainly CD4+ T cells expressing different immune-modulating molecules, suggesting that Omomyc induces the expansion of this tumor-reactive cell population. In addition, Omomyc-treated animals display higher proportions of Th1-Th17 hybrid population, effector/memory T cells, cytolytic NK cells and activated dendritic cells. Importantly, this immune stimulatory effect is also observed upon systemic intravenous administration in KRAS-mutated NSCLC models bearing concomitant mutations in common tumor suppressor genes. Finally, we confirmed that Omomyc treatment also induces CD4+ and/or CD8+ T cell recruitment in PBMC-humanized NSCLC models. Most notably, immune engagement was also seen in Phase I patients receiving OMO-103 (the first Omomyc-derived drug product) and showing stable disease. In particular, they display a cytokine signature that was not observed in patients with progressive disease. Interestingly, a predictive cytokine signature at baseline was identified as associated to sensitivity to OMO-103, adding further insight in the cross-talk between MYC activity and immune modulation.

Our preclinical and clinical findings support the therapeutic opportunity to induce a potent antitumor immune response in NSCLC by pharmacological MYC inhibition with Omomyc.

26 Study of the role of CD38 in the anti-tumoral actions of the LXR pathway

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Liver X Receptors (LXRs) are members of the nuclear receptor family of transcription factors.LXRs are activated by specific agonists, including several derivatives of cholesterol metabolism and high affinity synthetic agonists, such as T0901317. Once activated, LXRs regulate gene transcription both positively and negatively. We have reported that activation of the LXR pathway trough the intraperitoneal administration of T0901317 suppresses tumor growth in a murine model of syngeneic Lewis lung carcinoma. In this context, LXR activity reduced the intratumoral abundance of regulatory T cells (Treg), most probably due to the LXR-specific suppression of the CCL17-CCL22/IRF4 axis in tumor-associated macrophages (TAMs), involved in Treg recruitment. Our group has also identified CD38 as a transcriptional target gene for LXRs. CD38 is a multifunctional transmembrane protein that is widely expressed in immune cells. Its expression is upregulated by pro-inflammatory cytokines, endotoxins, and interferons. CD38 can function either as a receptor or as an enzyme. The primary enzymatic function of CD38 is the synthesis of adenosine diphosphate (ADP) ribose from nicotinamide adenine dinucleotide (NAD+). Interestingly, increased mRNA expression of CD38 was detected in TAMs stimulated ex vivo with the LXR agonist. In addition, in vitro studies indicate that factors secreted by cancer cells cooperate with LXR signalling in the induction of CD38 expression. Interestingly, T0901317 failed to reduce the intratumoral Treg levels and to suppress tumor progression in CD38-deficient mice, suggesting that CD38 expression may be important for the antitumoral effects of pharmacological LXR activation. This data provides new insights on the role of the LXR-CD38 axis in the tumor microenvironment.

27 Immunomodulatory roles of PARP proteins in cancer

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Poly(ADP-ribose) polymerase-1 (PARP-1) and PARP-2 are enzymes which, in response to DNA damage, transfer ADP-ribose chains onto amino acid residues of target proteins (PARylation), resulting in modulation of protein function. Many targets of PARP-1/2-dependent PARylation are involved in the DNA damage response and hence, the loss of these proteins disrupts a wide range of biological processes, from DNA repair and epigenetics to telomere and centromere regulation. The central role of these PARPs in DNA metabolism in cancer cells has led to the development of PARP inhibitors as new cancer therapeutics. However, a cancer is not just made up of cancer cells and the tumour microenvironment also includes multiple other cell types, particularly stromal and immune cells. Interactions between these cells-cancerous and non-cancerous-are known to either favour or limit tumourigenesis. We have examined how specific PARP-1-deficiency or PARP-2-deficiency impacts in c-Myc-driven tumour development in a mouse models of B cell lymphoma .In this model, the c-Myc oncogene is driven by the immunoglobulin heavy enhancer giving rise to B-cell lymphomas. Interestingly, PARP-2 deficiency prevented c-Myc–driven B-cell lymphoma in mice, whereas PARP-1 deficiency accelerated tumour progression. At cellular level, PARP-2 limited replication stress of c-Myc–overexpressing B cells, whereas PARP-1 affects the cross-talk between tumour cells and the immune system. Indeed, PARP-1-deficiency induces a proinflammatory response, and an increase in regulatory T cells likely contributing to immune escape of B-cell lymphomas, resulting in an acceleration of lymphomagenesis. These findings pinpoint specific functions for PARP-1 and PARP-2 in c-Myc-driven lymphomagenesis with antagonistic consequences that may help inform the design of new PARP-centred therapeutic strategies with selective PARP-2 inhibition potentially representing a new therapeutic approach for the treatment of c-Myc-driven tumours.

28 Elucidating the role of the PD-1/PD-L1 axis in CAR-T cell function

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CAR-T cell therapy of solid tumours faces several hurdles, including dysfunction of the therapeutic T-cells. Even though the inhibition of the PD-1/PD-L1 axis by T-cell genetic engineering is being actively investigated, there is still controversy about long-lasting PD-1 inhibition on CAR-T cell exhaustion. Here, we hypothesized that consequences may be influenced by the CAR design and PD-L1 levels expressed in the tumour microenvironment. To elucidate the role of the PD-1/PD-L1 axis in CAR-T cell function, we generated a preclinical model of cancer cell lines expressing varying PD-L1 levels. Cancer cells were CRISPR-engineered to eliminate PD-L1 expression, sorted and transduced with lentiviral vectors encoding PD-L1 under the control of constitutive promoters of low, medium, or high intensity and generated PD-1 KO CAR-T cells using CRISPR-Cas9 targeting HER2 (4D5.5-CD28z) or mesothelin (M11-CD28z). Here, we show that even low expression of PD-L1 in tumour cells could significantly impair CAR-T cell effector functions in vitro, and that cytokine secretion was improved by genetic or pharmacological inhibition of the PD1-PD-L1 axis. In vivo, we observed that while CAR-T cells could induce complete responses (CR) in 87% of mice bearing PD-L1-negative ovarian tumours (PD-L1 KO), the percentages of CR in animals with PD-L1 expressing tumours (PD-L1 low, high or wild-type) were below 25%. PDCD1 ablation in HER2-CAR-T cells showed a significantly enhanced antitumour effect compared to CAR-T cells alone or even in combination with checkpoint inhibitors, with > 87% of animals with PD-L1-expressing tumours exhibiting CR. We confirmed our observations using engineered CAR-T cells against other cancer types expressing HER2 (HCC1954-breast) or mesothelin (Capan2-pancreatic). Our results show that, in a context where PD-L1 is expressed by tumour cells, the deletion of PD-1 in CAR-T cells can restore CAR-T cell functions, suggesting an overall beneficial effect on CAR-T cell therapy.

29 Metastatic melanoma (MM) patients with stable disease display reduced Mo-MDSCs levels in peripheral blood

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Introduction: Myeloid-derived suppressor cells (MDSCs) are increased in late-stage tumors promoting metastasis by exerting their immunosuppressive functions. Recently, treatment with anti-PD1 and/or anti-CTLA-4 has improved the prognosis of metastatic melanoma (MM). In this regard, the immune effect of cancer immunotherapy on MDSCs is poorly understood. The aim of this study was to examine Mo-MDSCs frequencies in peripheral blood in relation with clinical outcomes in MM patients under immunotherapy.

Material and methods: Peripheral blood from 38 MM patients included in a clinical trial authorised by the Ethics Committee of the University of Regensburg was collected before administration of the first dose of Nivolumab and/or Ipilimumab. Patients were classified according to the iRECIST criteria into complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD). Samples were acquired with a NaviosTM cytometer. Mo-MDSCs were defined as CD14+ CD33+ CD11b+ CD15- HLADR-/low lin- cells.

Results: We found no association between Mo-MDSCs levels and checkpoint blockade adverse events (hepatitis and colitis), sex, presence of IgG anti-CMV and stage of the disease. On the contrary, we observed that Mo-MDSCs frequencies in patients with stable disease (n=7, median 50.92 IQR 49.63-71.49) were lower compared to those who progress (n=15, median 77.95 IQR 67.24-83.39) (p=0.026) and those who have partial response (n=11, median 78.98 IQR 66.84-80.35) (p= 0.0185).

Conclusion: Further studies are required to understand the immune response to immunotherapy and if MDSCs could serve as a biomarker to predict prognosis in MM patients under immunotherapy treatment.

30 Immunological biomarkers associated with anal Dysplasia in people living with HIV

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Background
Early detection of squamous intraepithelial lesion (SIL) is essential to limit anal cancer development and progression. Men who have sex with men (MSM) living with HIV are at high risk for SIL and therefore, anal cancer. Here, we aimed to identify the local immunological mechanisms involved in the development of anal dysplasia that could be critical for prevention, diagnosis and development of novel treatments.

Methods
A cross-sectional study of 54 anal biopsies obtained from 47 MSM living with HIV who participated in an anal screening program was performed. In these samples, we assessed multiple lymphocyte and myeloid immunological subsets by flow cytometry, in addition to histological examination. Selected potential biomarkers were further assessed by immunohistochemistry.

Results
Resident Memory T cells expressing CD103 were less frequent in pathological biopsies (Low/High-grade-SIL (LSIL/HSIL)), with a more pronounced effect on the CD4+T cell subset (p=0.024). Increases in the frequency of Natural Killer cells (NK) expressing CD16 (p=0.030) and overall NK activation measured by HLA-DR (p=0.018), were also associated with pathological samples. Furthermore, potentially immune suppressive subsets, including CD15+CD16+ neutrophils, gradually increased as the anal lesion progresses (p=0.012). Staining of CD15 by immunohistochemistry confirmed the association between the presence of this biomarker in the epithelium and SIL, with a sensitivity of 80% and specificity of 71% (AUC 0.762) for the correlation with HSIL.

Conclusions
Immunological tissue analyses revealed a complex immunological environment where the balance between resident effectors and immune suppressive subsets was tilted towards the second in pathological samples. Neutrophil infiltration determined by CD15 staining, may represent a valuable biomarker associated to the grade of dysplasia.

Abstracts
Posters

1 Neprosin decreases gluten-derived inflammatory response in macrophages through proteolysis of the highly immunogenic gliadin peptide 33-mer

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Background and objectives: Neprosin is a prolyl endopeptidase capable of degrading the gliadin-immunotoxic peptide 33-mer into smaller and theoretically less immunoreactive fragments [1]. Previous studies have shown that 33-mer peptides can arrange into supramolecular structures that can interact with macrophages through the TLR-2/-4 dependent pathways. This causes an immune response that includes the secretion of TNF-α. The goal of the present study was to evaluate in vitro the immune response generated by the 33-mer cleavage peptides produced by neprosin (fragments).

Materials and methods: Peritoneal macrophages (PMs) were obtained from Lewis rats, cultured at 106 cells/mL per well and incubated for 24 h to allow their attachment. After that, the gluten-derived peptide 33-mer or the fragments generated by neprosin (3-mer, 7-mer and 9-mer) were added at two different concentrations (0.25 mM and 4.35 mM). Equal volumes of medium and lipopolysaccharide (LPS) were also added as negative and positive controls, respectively. After 24 h, supernatants were collected and stored at -80 °C. An ELISA assay was performed to quantify the TNF-α secreted by PMs as a response to each stimulus.

Results: Neither the33-mer peptide nor the fragments added at low concentration induced a significant release of TNF-α. However, high concentration of 33-mer provoked a significant increase in TNF-α secretion as compared to medium, suggesting a pro-inflammatory action of the peptide. In contrast, the fragments derived from the 33-mer degradation caused a release of significantly lower TNF-α levels than those induced by the peptide.

Conclusion: These results indicate that the use of the enzyme neprosin could be effective in reducing the pro-inflammatory effect of the gluten-derived peptide 33-mer, thus suggesting its potential as an active agent in the context of the coeliac disease.

2 Development of T-cell receptor (TCR)-modified T-cells for the recognition of NY-ESO-1 peptide presented on HLA-DRB3*02:02

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Natural transgenic T-cell receptors (tTCR) represent an emergent type of cell therapy against cancer, recognizing peptides expressed both on the intracellular and extracellular level. The capacity to recognize intracellular antigens constitutes a significant advantage in comparison to chimeric antigen receptor (CAR) therapy that can only recognize extracellular surface proteins, which are difficult to define as Tumor Associated Antigens (TAA), especially on solid tumors. However, it must be taken into consideration that TCR therapies against TAAs could potentially generate off-tumor on-target toxicity. In order to prevent this risk, this work is focused on New York Esophageal Squamous cell carcinoma Origin 1 (NY-ESO-1), from the cancer/testis antigen family, expressed on several types of tumors but not relevantly on healthy tissues besides testicles. Several tTCR clinical trials targeting NY-ESO-1 have been performed, but all of them are focused on peptide presentation to class I HLA (Human Leukocyte Antigen) molecules. Therefore, we also focus our work on TCR recognizing peptides presented on class II HLA, collaborating with other T-cells focused against class I HLA. Developing a TCR that is able to recognize NY-ESO-1 presented on HLA-II, we are looking for TCR against the peptide presented by the allele HLA-DRB3*02:02 (DR52b), expressed approximately by half of the Caucasian population in a similar way to HLA-A*02:01 (the most frequent allele of the class I HLA), for which many more studies have been performed. Other studies determined that stimulation of T-cells from patients with NY-ESO-1 peptides triggers a CD4+ T-cell-specific immune response, restricted to HLA-DR52b and with a preserved TCR repertoire. It remains important to develop research enabling the generation of new TCR therapies that can recognize intracellular antigens presented by HLA-II, that can be useful to a high percentage of population and that can be applicable to several types of tumors.

3 Changes in immune composition of human milk from SARS-CoV-2 infected women

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The COVID-19 pandemic caused by SARS-CoV-2 increased concerns about potential mother-to-infant transmission of the pathogen, including via human milk (HM). In this regard, previous studies from our research consortium (MILKCORONA) have demonstrated the absence of SARS-CoV-2 RNA in milk from women with COVID-19 and the presence of anti-SARS-CoV-2 IgA and IgM. In addition, vaccination against the virus also increased these specific antibodie. Finally, HM composition was also affected, as shown by changes in the metabolome and the metallome. We aim now to study the impact of SARS-CoV-2 infection on the immune composition of HM in terms of cytokine profile and immunoglobulinome. For this purpose, a prospective multicenter longitudinal study (April–December 2020, trial registration
numberNCT04768244) in 63 mothers with SARS-CoV-2 infection and/or who have recovered from COVID-19 was launched. A prepandemic control group (n=63) was also included. Immune composition of HM was analyzed by a bead immunoassay (Luminex®) targeting 18 cytokines involved in the Th1/Th2/Th9/Th17 responses and an antibody isotyping panel directed to IgA, IgM, IgG1, IgG2, IgG3, IgG4 and IgE. Maternal SARS-CoV-2 infection modulated the immunoglobulome and cytokine profile of HM samples compared to non-infected milk samples.Regarding the immunoglobulinome, SARS-CoV-2 infection was associated with a significant increase in total IgA, main isotype found in HM, at the expense of IgG and IgM, whose concentrations were lower than in the pre-pandemic group. In this regard, lower concentration of all IgG subclasses was observed in the infected participants that overall showed higher Th2-type Ig and, consequently, a reduction in the Th1/Th2 balance. With respect to cytokine composition, although a high variability was found among samples, the abundance and proportion of some of them were affected. Our study confirms that HM Ig and cytokine composition from women with COVID-19 is affected. Further studies are needed to investigate the impact of these changes on infant growth and immune development.

4 Intracellular HIV-TAT initiates a cellular senescence programme in a Jurkat T-cell model

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Background: Current treatments for HIV achieve undetectable viral loads and increase life expectancy. However, this is accompanied with signs of accelerated aging and higher prevalence of age-related comorbidities.
TAT (Trans-Activator of Transcription) is a key protein for viral replication and pathogenesis. Two forms exist through multiple splicing: 1 exon form with 72aa (TAT72) and 2 exons form with 101aa (TAT101). Only TAT101 mediates additional cellular effects compatible with a cellular senescence program: cell cycle arrest, altered cell metabolism and increased pro-inflammatory mediator release. The role of HIV-TAT in aging is currently unknown.

Aim: Research in aging has identified key biomarkers that can track cellular senescence. We aim to determine whether intracellular HIV-TAT alters such markers, producing a senescent phenotype in TAT-expressing cells.

Methods: Jurkat TET-off cell lines stably transfected with HIV-TAT or an empty vector (control) were used to model HIV-TAT intracellular expression, which can be silenced with Doxycycline (DOX). A combination of Flow Cytometry, qPCR and Western Blotting was used to address the following senescence biomarkers: BCL-2, CD87, p21, p16INK4A, γ-H2AX, SAβ-GAL, IL-6 and PAI-1. Cell cycle was assessed by KI-67 and DAPI staining by flow cytometry.

Results: TAT101 expression increased BCL-2, CD87 and p21 protein and mRNA levels, compared to TAT72 and TEToff control cells. Increased mRNA levels of PAI-1 and IL-6 were also detected. Western blotting and Flow Cytometry revealed increased phosphorylation of the Histone H2AX (γ-H2AX). Finally, TAT101 cells showed reduced levels of KI-67 and an increase of cells in G1 phase. DOX was unable to reduce any of the increased markers.

Conclusions: Intracellular, full-length TAT expression increase senescence biomarkers and reduce cell proliferation suggesting that TAT may initiate a cellular senescence program which may contribute to HIV pathogenesis.

5 Changes in the immunoglobulin profiles after synbiotic administration in pregnant and lactating rats

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Pregnancy and lactation are crucial periods for the offspring`s development. During this time, specific nutritional requirements are needed to promote an adequate fetal and neonatal development. Scientific evidence demonstrates the impact of the maternal diet during gestation and lactation on the neonatal immune system. Furthermore, prebiotics and probiotics have long been used to prevent and ameliorate intestinal disorders. The aim of this study was to evaluate the effect of the supplementation with a synbiotic during gestation and lactation on the immune system of the dams and the influence on milk composition.

For this, Lewis rats were administered once a day with a synbiotic (SYN group) or the vehicle (REF group) during gestation (21 days) and lactation (21 days) and morphometric and immune variables were analyzed. The synbiotic supplementation did not affect the weight of the mothers but increased the relative weight of the small intestine and the caecum. In terms of immune variables, different immunoglobulin (Ig) (IgA, IgM and total IgG and IgG subtypes) were quantified in several compartments. In plasma, only an increase in IgG2c levels was observed. Furthermore, the same Ig isotype was also increased in milk, thus inducing an increase in total IgG, the most abundant Ig in rat's milk. Interestingly, this change was not observed in salivary gland or in mesenteric lymph nodes (MLN) where no other Ig was affected. However, in MLN an increase in IgM levels could be observed due to the SYN supplementation and a tendency to increase IgA and IgG2b was also found.

In conclusion, this study shows that the maternal supplementation with a synbiotic during gestation and lactation is safe for the dams. Synbiotic influenced the maternal immune system and increases the richness in the milk immune components, thus potentially influence the protective role of milk on the newborn health outcomes.

6 Galectins -1, -3 and -9 are present in breast milk

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Galectins (Gal) are a family of conserved soluble proteins with high affinity for β-galactoside structures. They have been recognized as important proteins in immune responses and they are also pivotal to enable successful pregnancy. Although there is data reporting that human breast milk (BM) glycans are selectively bound by Gals, little is known about the Gal presence in the breast milk. In this study Gal-1, -3 and -9 concentrations were evaluated by Multiplex immunoassays in BM samples at days 7 and 15 postpartum from the MAMI cohort (n = 23). Data regarding mother and infant characteristics were collected. A major finding in the current study is that Gal-1, -3 and -9 were detected for the first time in the transitional breast milk from all the mothers at a concentration of 14.55 ng/mL, 60.15 ng/mL and 166.07 pg/mL, respectively. In addition, the levels of Gal-1 and -9 were about 1.6 times lower than those found in umbilical cord plasma (UCP) at birth. However, the concentration of Gal-3 was 3.5 times higher than that observed in UCP. No differences were found comparing the two breastfeeding points, indicating that Gals levels in BM could be more stable in time than other immune molecules. Finally, Gals were associated with maternal and infant characteristics, such as the pregnancy weight gain, the maternal diet, the infant growth and the infant infections over 1 year. In conclusion, Gals are present in BM and would be involved in certain developmental aspects of early life. Finally, the understanding of the presence of Gals in BM and the underlying molecular mechanisms could contribute to the design of interventions in order to sustain infant health and to prevent infant infections.

7 Influence of supplementation with extra virgin olive oil during gestation and lactation on breast milk's immune factors

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Maternal breast milk plays a key role providing the newborn with passive immunity and stimulating the maturation of infant's immune system, protecting them from many diseases. It is known that diet can influence the immune system of the lactating mothers and the composition of their breast milk . It has been recently described that extra virgin olive oil (EVOO) metabolites are present in EVOO supplemented rats breast milk (1). However further studies are needed to ascertain their impact on infant's health.

The aim of this study was to establish if a supplementation with EVOO during gestation and lactation has an impact on breast milk immune composition and to stablish the relationship among EVOO metabolites present in breast milk and the immunoglobulinome.

For this 10 mL/Kg of EVOO or refined oil (control oil) or water (reference group) were administered orally once a day to rats during gestation and lactation periods. Immunoglobulin (Ig) concentrations in breast milk were quantified by a ProcartaPlex® multiplex immunoassay (IgA, IgG1, IgG2a, IgG2b, IgG2c2, IgM). Moreover, in mammary gland IgA levels and IgA gene expression were also quantified by ELISA and RTPCR, respectively. Finally, the immune components were correlated with EVOO metabolites in breast milk.

EVOO group showed higher IgA levels in both breast milk and mammary gland than in the REF group. In addition, the gene expression of IgA in mammary gland was also boosted by EVOO consumption. Additionally, a positive correlation was observed between IgA and phenolic compounds found in EVOO.

Overall, supplementation with EVOO to rats during gestation and lactation showed a positive impact on breast milk immune composition, which possibly may influence infant's health.

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8 Impact of intensive exercise on immune function: Effect of diets enriched in flavonoids.

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Exhausting exercise can impair immune function thus increasing the risk of infection. Flavonoids may prevent these alterations, given that they have shown, among others, antioxidant and immunomodulatory properties. The aim of this study was to assess the influence of dietary interventions with cocoa (a rich source of flavan-3-ols) and hesperidin (a flavanone found in oranges) on the antibody levels, the composition and function of spleen lymphocytes as well as the adipose tissue structure after an intensive training and an exhausting exercise bout.
For this purpose, Lewis rats were fed either a standard diet (REF), a diet containing 10% cocoa (C10) or a diet containing 10% cocoa plus 0.5% hesperidin (CH) for 6 weeks. In this period, animals undertook an intensive running training on a treadmill, involving three trainings and two exhaustion tests per week, or remained as a sedentary control group. At the end, blood samples, white adipose tissue (WAT), brown adipose tissue (BAT) and spleen were obtained 24h after performing a regular training (trained groups) and immediately after carrying out an exhaustion test (exhausted groups).
The final exhaustion increased the concentration of IgG (IgG2b and IgG2c) in serum and decreased the release of interleukin (IL)-10 and IL-2 by spleen lymphocytes in all dietary conditions. The C10 and the CH diet prevented the decrease in spleen T helper proportion induced by the final exhaustion test. Exhausted animals that received the REF diet, but not C10 and CH groups, had lower adipocyte relative area in WAT than their sedentary counterparts. In addition, both diets lowered the number of lipid droplets found in BAT.
Overall, exhausting exercise modified the serum antibody levels, the composition and function of spleen lymphocytes, and the adiposity. Some of these alterations were modulated by a diet enriched in cocoa and hesperidin.

9 Optimization of the Multiplex Immunoassay for the determination of antidsDNA antibodies in the Systemic Lupus Erythematosus diagnosis.

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INTRODUCTION
Systemic Lupus Erythematosus is a chronic, multisystemic and autoimmune disease. Presents a wide spectrum of clinical manifestations and has a complex diagnosis. It is characterized by the presence of antidsDNA antibodies, usually determined in the clinical laboratory by Multiplex Immunoassays. The main problem with these tests lies in a very high number of false positive results.

AIM
In this project, a comparative study has been carried out between the Multiplex Immunoassay and other two techniques, to analyze the sensitivity and specificity of Multiplex and determine a new positivity cut-off point.

MATERIAL AND METHODS
A total of 311 patient serum samples were analyzed by Multiplex Immunoassay (BioPlex 2200 System, Bio- Rad), Indirect Immunofluorescence on Crithidia luciliae and Immunoblot (IF Sprinter and EUROBlot Master, Euroimmun). Samples with a value ≥ 11 IU/mL for anti-dsDNA antibodies by multiplex were considered positive. A new cut-off value of anti-dsDNA antibody concentration was selected using the 95th percentile to get less than 5% of false negatives.

RESULTS AND CONCLUSIONS
The comparative analysis revealed that the multiplex turned out to be very sensitive but not very specific. The cut-off point established by the commercial company proved to be very low for our population. In contrast, IIF was highly specific. The trend obtained was that as the concentration of anti-dsDNA antibodies increased by multiplexing, the percentage of positive samples that were confirmed by the other techniques also increased.
It is necessary to carry out at least two techniques, one to confirm positive results for anti-dsDNA antibodies (IIF) and another to monitor the disease and its treatment (Multiplex).
With the determination of the new cut-off point at 25 IU/mL for the Multiplex Immunoassay the specificity of the technique would greatly increase, reducing the number of false positives (72.6% less). The laboratory's Autoimmunity service will implement the new cut-off point.

10 Study of the presence of anti-quaporin 4 (AQP4) antibodies in elderly patients with frailty syndrome.

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INTRODUCTION AND AIMS

Frailty is a progressive geriatric syndrome in which the ability to cope with stress factors is significantly reduced. Elderly people are more vulnerable to the development of disease, functional decline and dependency. Under conditions of hyperosmotic stress, the body is able to compensate for intracellular dehydration by several mechanisms. One of these is the concentration of urine by increasing the expression of aquaporins, transmembrane protein channels that allow water reabsorption at the level of the renal tubule. Specifically, AQP2 and APQ4 are involved in this process. Elderly people with frailty syndrome have impaired ability to concentrate urine, but the cause is still unknown. This results in a progressive increase in hyperosmotic stress, which could lead to progressive muscle dehydration and functional deterioration. AQP4 and AQP7 are present in myocytes, and the alteration in their expression could also promote dehydration. Given that anti-AQP4 antibodies are known to be present in some autoimmune diseases such as neuromyelitis optic, this project aimed to study whether the presence of anti-AQP4 antibodies may influence the development of frailty syndrome in the elderly.

MATERIAL AND METHODOLOGY

A total of 97 serum samples from patients over 70 years old were analysed. They were processed by indirect immunofluorescence on cells transfected with AQP-4 (EU90) (IF Sprinter and IFT Anti Aquaporin-4 kit, Euroimmun). Samples showing clear fluorescence specific for EU90 cells were considered positive.

RESULTS AND CONCLUSION

All samples diluted 1:10 (according to the manufacturer's instructions) were negative for anti-AQP4 antibodies. To confirm the results, they were retested undiluted and 100% of the samples were negative.

Based on the data obtained in this study, we conclude that failure of urine concentration in elderly people with frailty syndrome probably is not associated with the presence of anti-AQP4 IgG.

11 Usefulness of serum calprotectin as biomarker in pediatric rheumatic diseases.

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Introduction
Pediatric rheumatic diseases are a diverse group of illnesses characterized by inflammation and autoimmunity. Due to the complexity and progressive course of these disorders, specific biomarkers are desirablefor patient follow up and therapeutic decisions. In the past few years, serum calprotectin has emerged as a potential biomarker.
Objectives
The main objectives of the present study were:
1. To assess the correlation between serum calprotectin levels and clinical inflammation in pediatric rheumatic patients.
2. To compare serum calprotectin quantification by two different methods: Enzyme Immunoassay (EIA) and Chemiluminescence Enzyme Assay (CLIA).
3. To evaluate the concordance of each method with several clinical variables.
Material and Methods
First, we studied 72 samples from 37 patients (3 to 23 years old) with rheumatic diseases, mainly Juvenile Idiopathic Arthritis (JIA). Serum calprotectin concentration measured by EIA (Bülmann®) was compared with clinical inflammation in terms of JADAS27 index, and with other general inflammation markers (Creactive protein (CRP) and Erythrocyte Sedimentation Rate (ESR)). Second, we studied 144 samples from 41 patients with similar characteristics. Serum calprotectin measured by EIA and by CLIA (QUANTA Flash®) were compared and correlated with sex, age, clinical remission, number of inflamed joints, and JADAS27 index.
Results
Our study showed that serum calprotectin is a biomarker with high specificity (78.2%) and sensitivity (80%) (Cut-off: 3,36 µg/ml) in our cohort. We found a good correlation between EIA and CLIA results ($r=0.87$). However, we observed significant differences in values >4 µg/ml ($p<0.01$). Both methodologies were associated with clinical remission and JADAS27 index.
Conclusions
Serum calprotectin is a usefulness biomarker to evaluate clinical inflammation in pediatric patients with rheumatic diseases. Ongoing studies will tell us if serum calprotectin is also helpful in therapeutic decisions.

12 CDC-FCXM: Detection of donor-specific HLA-antibodies and their complement-dependent cytotoxicity by flow cytometry.

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Introduction: Detection of preformed complement-binding donor-specific HLA-antibodies (DSAs) before transplantation is an important factor to prevent organ rejection. The complement-dependent-cytotoxicity (CDC) assay is the conventional technique for detecting these DSAs. However, it has several limitations such as low sensitivity, long-time technique, and observer-dependent interpretation.

Aim: Develop a technique able to detect the presence of DSAs and their CDC ability by flow cytometrybased procedure.

Method: Donor cells were cultured with HLA-sensitized recipient sera using 96-well plates. Then, cells were washed 3 times and were incubated with rabbit complement (RC). Afterward, lymphocyte populations were incubated with anti-CD3 and anti-CD19 fluorochrome-labeled antibodies, FITC-conjugated goat F(ab)2 anti-human IgG, and 7-actinomycin D (7-AAD). IgG binding was reflected through the mean fluorescence intensity (MFI) and cytotoxicity was calculated as the percentage of 7AAD-positive cells within T cells or B cells.

Results: Successive experiments have established that the optimum conditions and incubation times required for the crossmatch were as follows: 100.000 donor cells in 25mL incubated with 50mL of recipient serum for 30 minutes. Incubation with 100mL of RC was required for an additional 30 minutes. Finally, an incubation time of 30 minutes was set as optimal for labeling the cells with a titrated mix of monoclonal and 7AAD antibodies for subsequent analysis by flow cytometry. Tests were performed with sera from HLA sensitized patients, demonstrating that the technique can detect the cytotoxic capacity of IgG anti-HLA class I and/or II antibodies and discriminate them from other IgM antibodies. (Figure 1)

Conclusion: We describe the optimization of a flow cytometry-based procedure for the determination of both cytotoxicity and IgG HLA antibodies binding to donor-derived lymphocytes, without magnetic separation of T and B lymphocytes, and lower times than the conventional CDC technique, that can improve pre-transplant risk assessment and progress organ allocation efficiency.

13 The immunoregulatory role of IL-35 in patients with interstitial lung Disease.

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Pulmonary fibrosis involves various types of immune cells and soluble mediators, including TGF-β and IL-35, a recently identified heterodimeric cytokine that belongs to the IL-12 cytokine family. However, the effect of regulatory IL-35 may play an important role in fibrotic diseases. The aim of this paper is to explore the immunoregulatory role of IL-35 in the development of fibrosis in interstitial lung disease (ILD). To gain better understanding of this issue, the concentrations of IL-35 and different profibrotic cytokines in fibrotic (F-ILD) and non-fibrotic (NF-ILD) patients by ELISA were compared to that of intracellular IL-35 and IL-17 on CD4+ T cells stimulated in the presence of BAL or with different ratios of recombinant IL-35 (rIL-35) and TGF-β (rTGF-β), which were evaluated by flow cytometry. We observed that BAL concentration of IL-35 was lower in F patients and was negatively correlated with concentrations of TGF-β and IL-17. In supplemented cell cultures, BAL from NF but not F patients enhanced the percentage of IL-35+CD4+ T cells and decreased the percentage of IL-17+CD4+ T cells. The percentage of IL-35+CD4+ T cells correlated positively with BAL concentration of IL-35, but correlated negatively with BAL concentrations of IL-17 and TGF-β. After adjusting the concentrations of recombinant cytokines to establish TGF-β:IL-35 ratio of 1:4, an enhanced percentage of IL-35+CD4+ T cells but a decreased percentage of IL-17+CD4+ T cells was observed. After adding recombinant IL-35 to the BAL from F patients until a 1:4 ratio of TGF-β:IL-35 was reached, a significantly increased percentage of IL-35+CD4+ T cells and a decreased percentage of IL-17+CD4+ T cells were found. These results suggest that IL-35 may induce an anti-fibrotic response, regulating the effect of TGF-β and the inflammatory response on CD4+ T cells.

14 Targeting HIV reservoirs in a novel human intestinal model of persistent infection.

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HIV establishes a persistent infection in lymphoid and mucosal tissues due to the presence of latently HIVinfected cells not susceptible to antiretroviral therapy (ART). "Shock and kill" therapies have emerged as promising approaches for eliminating the HIV-latent infection, but their effectiveness in distinct anatomical reservoirs remains unclear. Here, we have developed an intestinal tissue model to characterize immune cell reservoirs and assess strategies directed to impact viral persistence in this anatomical compartment.

Human intestinal tissue resections from uninfected donors were obtained from routine surgeries. To develop the best model, we assayed different time points for HIV infection, ART initiation and viral reactivation. HIV-reservoir cells were first identified by quantification of the HIV intracellular protein p24 in CD4+ T subsets after viral reactivation with the latency reversal agents (LRAs) PMA+Ionomycin. Furthermore, the effect of different LRAs, including Ingenol (ING), Romidepsin (RMD), Panobinostat, AZD5582, IL-15, and the combination of ING+RMD, was evaluated in Naïve (TNA), Central Memory (TCM), Effector Memory (TEM), Resident Memory (TRM, CD69+ and/or CD103+) and Memory CD127+(TM-CD127+) CD4+ T cells. The optimal model consisted in 9 days of HIVBAL infection of tissue blocks on top of gelatin sponges and the introduction of ART from day 6. HIV caused a significant decline in CD4+ T cells that was partially reverted with ART. Addition of LRAs on day 8 revealed the presence of viral reservoir cells in all tested CD4+ T subsets, which differed in their reactivation potential. Both ING alone and in combination with RMD reactivated HIV in TCM, TEM, and TRM-CD69+ cells; whereas IL-15 and AZD5582 were strongly active in TRM-CD69+ and TM-CD127+.

Overall, this tissue model recapitulates major features of HIV infection, ART treatment, and viral reactivation. Besides identifying LRAs acting in different tissue CD4+ T cells, this model may be useful for testing tissue-specific immune-related approaches.

15 Immune reconstitution in patients who received cyclophosphamide vs conventional treatment as graft-versus-host disease prophylaxis after allogeneic hematopoietic stem cell transplantation.

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Introduction: Graft versus host disease (GVHD) is every day less frequent in patients with allogeneic hematopoietic cell transplantation (allo-HCT) due to prophylaxis regiment applied. Post-transplantation highdose cyclophosphamide (PTCy) is being increasingly used for GVHD prophylaxis across various donor types. However, immune reconstitution (IR) comparison with conventional GVHD prophylaxis has not been well studied. The aim of this study is to determine how PTCy influence IR after allo-HCT and its potential impact on clinical outcomes.
Materials and methods: 100 adults diagnosed with malignant haematological diseases, treated with allo-HCT from 2018-2020 were included. Dynamics of absolute lymphocytes (ALC), monocytes cell counts (MNC) and CD4+, CD8+ subpopulations were collected. Acute and chronic GVHD, relapse, overall survival (OS) and non-relapse mortality (NRM) were analysed during the first year after transplantation.
Results: PTCy was used in 67 patients. Main differences in the patterns of immune cell dynamics between groups were detected in the first 100 days post-transplantation (Figure 1, A and B). Patients who received PTCy had low persistent ALC at day +45 (131vs.371cells/µL, $p<0.001$), d+70 (447vs.641cells/µL, $p<0.001$), and d+90 (570vs.817 cells/µL, $p>0.05$), respectively. PTCy treated patients had less chronic GVHD
(24%vs.46%, $p=0.039$) and NRM (18%vs.39%, $p=0.027$), without difference in acute GVHD, relapse and OS after 1 year (Table 1) (Figure 2, A and B). There were no differences in MNC between groups at any time during the follow up. CD8+ subpopulation was higher than CD4+ without reaching their threshold during the first year.
Conclusions: PTCy as GVHD prophylaxis influences peripheral lymphocyte expansion, favouring lower allorecognition, which manifests as less GVHD disease and better clinical outcomes. Analysis of early IR could provide useful information for post-transplant immunosuppressive treatment or adaptive cell therapy to optimize clinical outcomes.

16 Cellular response to SARS-CoV-2 mRNA-1273 Moderna vaccine in patients with treatment-induced immunosuppression.

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Immunosuppressed patients have a higher risk of undergo infections by different pathogens. A large number of studies show the effect of immunosuppression on humoral immune response to SARS-CoV-2 vaccination. Nevertheless, the study of cellular immune response has been largely forgotten. Here we present the results of 2 different studies evaluating the cellular immune response of patients with 2 different pathological conditions triggering the use of immunosuppressive therapies: myasthenia gravis patients and allotransplanted patients. All patients were vaccinated with mRNA-1273 Moderna COVID-19 vaccine with 2 doses following the requirements of our health centre and the vaccine availability during the study course.To assess the cellular immune response, blood samples were collected from the patients after the second dose of the vaccine.
Myasthenia gravis patients at 2 months and allogeneic transplant patients at one and three months. Cellular response was positive in 73.47% of myasthenia gravis patients 2 months after second dose vaccination and 66% of allotransplanted patients at both 1 and 3 months after second dose. Nonimmunosuppressed patients showed higher cellular response rates in both myasthenia gravis (93%) and allotransplanted patients (77%) compared to immunosuppressed (72.29% and 55%, respectively).
Furthermore, myasthenia gravis patients treated with combined therapy (corticosteroids in combination with another immunosuppressant) reported lower levels of cellular response. Similarly, allotransplant recipients who had a lower cellular response were those treated with corticosteroids. Our results demonstrate, as expected, a reduced cellular immune response to SARS-CoV-2 mRNA-1273 vaccine in immunosuppressed patients. Moreover, the lowest levels of cellular response were found in corticosteroid-treated patients, demonstrating the impact of using this type of immunosuppressant on the achievement of an adequate cellular immune response to SARS-CoV-2 mRNA vaccination.

17 Autoantibodies detection by multiparametric assay in patients with Systemic Autoimmune Diseases.

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Introduction
Systemic autoimmune diseases (SAID) are a heterogeneous group of diseases characterized by an immune response against self-antigens. Autoantibodies are a hallmark of most SAID being some included in classificatory criteria. Recently, multiparametric technologies for autoantibody detection have been proposed to improve the accuracy of the immunological diagnosis of SAID. Particle-based-multi-analyte technology (PMAT-Aptiva) is a multiplexed system based on the coupling of different antigens to paramagnetic particles with unique signatures. It allows us to obtain results from different autoantibodies simultaneously. The aim of this study was to evaluate the PMAT-Aptiva CTD-Essential diagnostic performance in a cohort of patients with SAID from the Hospital Clínic de Barcelona. Patients and Methods: Study cohort includes consecutive samples of patients with and without SAID (n=222, n=70, respectively). Diagnoses of patients were Systemic Lupus Erythematosus (n=45), Sjögren Syndrome (n=20), Systemic Sclerosis (n=87), Mixed Connective Tissue Disease (n=6), anti-Synthetase Syndrome (n=4), Overlap Syndromes (n=28) and other SAID (n=32). Anti-dsDNA, SmD1, Ribo-P, Ro/SSA, La/SSB, Ro52, U1-RNP, Jo1, Cenp-B, Scl70, DFS70 autoantibodies were measured by PMAT-Aptiva CTD-Essential and compared to the classical methods employed by our laboratory (EIA, CIA, FEIA or Immunoblot). Results Using PMAT-technology, positive-likelihood ratio for each biomarker ranges from 1.9 to >50.0 being the best (>10) for anti-SmD1, Jo1, Cenp-B and Ribo-P. Specificities range between 73.0%-100% for all biomarkers whereas sensitivities range between 43.2%-80.0% excluding anti-SmD1 (15.9%), Ribo-P A good agreement (Cohen kappa >0.7) was observed for all autoantibodies, except for anti-RiboP (EIA), Sm (CIA) and Jo1 (Immunoblot). CIA results for anti-Jo1 autoantibodies show good correlation.
Conclusions PMAT-Aptiva CTD-Essential is a suitable option to detect autoantibodies and to improve SAID diagnosis, showing a good sensitivity and specificity.
The discrepancies observed for anti-Sm and Jo1 autoantibodies can be due to the different nature of the antigen used in each method.

18 Retrospective revision of non-classical ANCA specificities

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Introduction: As it is well known, the two major antigens against anti-neutrophil cytoplasmic antibodies (ANCA) are proteinase 3 (PR3) and myeloperoxidase (MPO), however other ANCA specificities such as cathepsin G, elastase, lactoferrin and bactericidal permeability-increasing protein (BPI) may be detected.
Objective: The objective of this study was to establish the prevalence of minority ANCA specificities in our cohort analyzed between January 2020 and April 2022.
Methods: We processed 8730 ANCA determinations by IFA, 3303 MPO and 3169 PR3 determinations by CLIA. In addition, we analyzed 111 human sera with positive IFA without MPO and/or PR3 specificity and samples with discordant results for minor ANCA (cathepsin G, elastase, lactoferrin and BPI) by ELISA.
Results: Concordance between methods CLIA and ELISA for PR3 (n=69) was moderate degree of agreement (K=0.54) and for MPO (n=66) was medium degree of agreement (K=0.38). From the CLIA MPO+PR3+ samples (n=10), 50% were double positive by ELISA and 50% were double negative. From the double positives by ELISA 80% were also elastase+ and from the double negatives 20% of sample was cathepsin G+ and BPI+.
From the CLIA MPO-PR3- (n=56), 64.28% were double negative by ELISA and, from these, 83.33% showed ANCA positivity by IFA with different patterns (cANCA: 23.33%, pANCA: 36.67%, xANCA: 33.33%, Undetermined: 6.67%). Thus, of the 30 MPO-PR3- by both methods with ANCA IFA +, 7 were elastase+ being 85.71% pANCA and 14.29% xANCA. In addition, one sample was lactoferrin+ with xANCA pattern, 2 BPI+ with cANCA pattern and 1 elastase+/lactoferrin+/BPI+ with pANCA pattern.
Conclusion: The correlation between the two methods of detection of MPO and PR3 specificity used in our laboratory has a medium agreement. Furthermore, in our cohort samples, elastase was the majority specificity in samples MPO-PR3- by both methods with ANCA IFA positive.

19 NKG2A blockade as a potential target to improve NK cell-mediated ADCC in HER2 positive breast cancer.

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In primary HER2+ breast cancer patients, we have described an association between tumor-infiltrating Natural Killer (NK) cell numbers and the achievement of pathological complete response to neoadjuvant treatment with anti-HER2 antibodies (trastuzumab, pertuzumab), supporting the contribution of antibodydependent NK cell-mediated cytotoxicity (ADCC) to their clinical efficacy. We hypothesize that enhancing NK cell function could improve clinical outcomes in HER2+ breast cancer. Phenotypic and transcriptomic characterization of the lymphocytic infiltrate in patient-derived breast carcinoma samples showed that CD16+ NK cells resembled peripheral blood cytotoxic NK cells and displayed high surface expression of NKG2A, TIGIT and TIM3 while lacking PD1 and CTLA-4. In addition, NKG2A expression was also detected in a significant fraction of CD8+T lymphocytes concomitantly present, supporting the suitability of targeting NKG2A as a strategy to potentiate both, NK and CD8 T cell anti-tumor activity triggered by anti-HER2 antibodies in HER2+ breast tumors. In preclinical models, trastuzumab/pertuzumab-triggered NK cell-mediated ADCC promoted an adaptive response in bystander breast cancer cells, characterized by the up-regulation of HLA-I, HLA-E and PD-L1. In cocultures and 3D spheroid models, blockade of NKG2A-HLA-E axis enhanced trastuzumab/pertuzumabtriggered NK cell cytotoxicity against HER2+ breast cancer cells. We established a humanized in vivo model for analysing direct and antibody-dependent NK cell-mediated anti-tumor function against GFP+/Luc+ HCC1954 breast cancer xenografts in NOD/Scid/ɣc-/- (NSG) mice. As compared to control groups, mice receiving the combined treatment including intratumoral injections of NK cells and intraperitoneallydelivered anti-HER2 antibodies showed the largest tumor growth inhibition. Tumor-infiltrating NK cells obtained from resected tumors maintained the expression of NKG2A and promoted the up-regulation of HLA-E in bystander tumor cells. Incoming experiments will address whether NKG2A blockade in vivo enhances the anti-tumor activity of anti-HER2 mAbs.

20 Detection of M2-type anti-mitochondrial antibodies against specific subtypes in the diagnosis of Primary Biliary Cholangitis in patients with discordant/low positive results.

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Introduction: Primary biliary cholangitis (PBC) is a chronic autoimmune liver disorder characterized by inflammation of intrahepatic bile ducts and ultimately cirrhosis.
M2-type anti-mitochondrial antibodies (M2-AMA) are specific and, together with biochemical cholestasis, are sufficient for diagnosis, without the need for liver biopsy. M2-AMA recognise mainly the E2 subunits of the 2-oxo-acid dehydrogenase complex: PDC-E2, OGDC-E2 and BOADC-E2. The aim of this study is to determine if the use of E2 subunits separately (sep-E2), by Dot-Blot, can improve M2-AMA detection in cases with low positive or discordant results showed by methods without sep-E2.
Material and methods: Patients with suspicion of PBC were routinely evaluated with indirect immunofluorescence on Rat-Triple-Tissue, ELISA and Dot-Blot assay to detect M2-AMA. None of those methods included sep-E2 as autoantigens. Patients with low positive or discordant results between techniques without sep-E2 (n=24) were analyzed on sep-E2 by Dot-blot. All of them had showed reactivity in the routine Dot-blot assay against a fusion protein formed by the immunogenic domains of the three E2 subunits mentioned, but had a negative result for native M2 antigen with PDC-E2 as main component. In addition, clearly positive patients (n=10) in these three assays as controls were also analyzed on sep-E2.
Results: Dot-Blot (sep-E2) positive M2-AMA results in discordant/low positive results group were as follows: native-PDC (6/24; 25%), PDC-E2 (6/24; 25%), OGDC-E2 (2/24; 8.33%) y BCOADC-E2 (17/24; 70.83%). All cases except one showed a positive result. Isolated reactivity was only observed against PDCE2 (4/6; 66.67%) and BCOADC-E2 (13/17; 76.47%). In clearly positive cases, OGDC-E2 and BCOADCE2 were observed in combination with PDC (native o recombinant).
Conclusions: The good correlation of the results obtained with the sep-3E2 and non sep-3E2 methods shows that either of those methods could be used for PBC diagnosis.

21 Potential false positive anti-HLA antibodies against self antigens detected by Single Antigen Luminex technology. How frequent are they?

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Introduction: False positive anti-HLA antibodies can decrease the chance of finding a suitable donor and undergo patients to unnecessary immunosupression. Therefore, the accurate identification of them is crucial in the context of transplantation. The aim of this study was to determine the prevalence of these false HLA autoantibodies in a cohort from recipients on kidney transplant waiting list.
Materials and methods: We analyzed the records from a total of 5570 Single Antigen (SAB) results performed from 2019 to 2020. First, results with calculated panel-reactive antibody ≠ 0 (n= 2560) were selected. Then, patients with at least two determinations in this period (n=1147) were filtered and finally those patients having a high-resolution HLA typing for class I and/or class II were analyzed in detail. The presence of a null allele in the recipient was discarded. The positivity criteria in the SAB were the recommended by the manufacturer (Immucor).
Results: None out of 253 patients showed anti-HLA class I antibodies against self HLA alleles. For class II, we detected potential auto-HLA antibodies in 14 out of 159 patients (10.7%): 6 DQ, 3 DRB3, 1 DRB4, 3 DPB1 and 1 DRB1. Median Fluorescence Intensity (MFI) was similar among each couple of sera except in one case. The MFI range was between 813 and 22423. Highest positive serum of each patient was checked with the SAB kit from the other manufacturer (One Lambda) and in all cases except one the results were negative against these specific alleles.
Conclusions: False anti-HLA class II autoantibodies were relatively frequent, whereas we did not detect any case in class I. In order to confirm that these antibodies are recognizing cryptic or denatured antigens, these positive results should be verified by the use of a cellular crossmatch and/or a different SAB kit.

22 Three novel HLA alleles found with Next Generation Sequencing.

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Next generation sequencing (NGS) for HLA typing has allowed the study of more patients faster, cheaper, with higher resolution and less ambiguities than previous techniques. It has brought to light new HLA alleles that were previously unlikely to be found. In this study, we present three new HLA alleles detected in our center.
First, a novel HLA-DRB1 allele was found in an international donor for hematopoietic stem cell transplantation (HSCT) that was matched to a patient in our hospital. He had been previously typed in an international center as HLA-DRB1*04:02, 11:01. The new allele is a missense variant respect to HLADRB1* 11:01:01:01 (c.244C>T, p.Ser82Leu). This change is found in exon 2, thus, the potential mismatch with the original patient should be assessed before transplantation. Second, another novel HLA-DRB1 was found in a patient with an inborn error of immunity who was typed in case HSCT were needed. She showed a variant to the common HLA-DRB1*14:54: c.654A>T, p.Arg218Ser. This base change is present in other HLA-DRB1 alleles. Third, a novel HLA-A allele, similar to HLA-A*24:02:01:01 was found in a patient with myelofibrosis and his son, as part of the familial study for related-HSCT. The change is in c.1094-1G>A. This is a splice acceptor site before the small exon 8. Further studies should be performed to assess the potential effect of this variant in the resulting protein. As shown, NGS has allowed us the identification of two new HLA-DRB1 alleles and one HLA-A allele. All three novel alleles have been reported and are currently waiting for its own nomenclature. In the future, more novel HLA alleles are expected to arise from NGS studies.

23 CASP10 mutation with Autoinflammatory presentation instead of ALPS in an Ecuadorian girl.

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Autoimmune lymphoproliferative syndrome (ALPS) is an inborn error of immunity (IEI) of immune dysregulation characterized by lymphadenopathy, splenomegaly and autoimmunity. It is caused by defects in genes involved in the FAS-mediated apoptosis pathway. FAS mutations are responsible for the majority of the reported cases but other genes such as FASL or CASP10 have been associated. Here we present a 12 years old girl from Ecuador who referred fever, cephalea and arthralgias characteristic of an autoinflammatory disease. No adenopathies, hepatosplenomegaly nor autoimmunity were found. She was initially treated with colchicine and iron supplementation with insufficient clinical response. A genetic test for IEI was performed revealing the variant CASP10 c.1202_1208del (p.Cys401Leufs*15) in heterozygosis. This deletion located in the exon 9 results in a shortened form of the protein. This CASP10 variant has been previously described in the literature in two patients. One patient presented a classical ALPS with impaired FAS-induced apoptosis together with syndromical features suggesting other genetic defects. The other patient presented with respiratory infections, dermatitis, inflammatory bowel disease and hypogammaglobulinemia. That symptomatology was compatible with an IEI of immune dysregulation, but not with the classical ALPS. In conclusion, the variant CASP10 c.1202_1208del (p.Cys401Leufs*15) presents different clinical manifestations in the three patients reported. This points out the complexity of the genotype-phenotype relation in IEIs, especially in heterozygous mutations. Therefore, wide genetic studies in atypical cases become highly useful.

24 Serum calprotectin in COVID-19 patients.

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Aim
The aim of the study is to evaluate the levels of serum calprotectin between healthy controls and COVID-19 patients.

Material and methods
Serum samples were collected from 16 healthy controls and 50 COVID-19 hospitalized patients, 17 with severe evolution and 33 mild evolution, at two times: hospitalization (T0) and 11 days post hospitalization (T1). Serum samples were stored at -80 Cº and serum calprotectin was subsequently determined with the Chemoluminiscent immunoassay QUANTA Flash® Circulating Calprotectin, according to the manufacturer's specifications. Statistical analyses were performed with Graphpad prism v5.0 software.

Results
COVID-19 patients showed statistically higher levels of serum calprotectin at T0 and T1 compared with healthy controls. Statistically significant differences have been detected in serum calprotectin of patients with COVID-19 between the time of hospitalization and 11 days later.

Discussion
Elevated levels of serum calprotectin observed in COVID-19 patients could reflect that neutrophils participate in the COVID-19 pathophysiology.s

25 Impact of functional CD5 gene variants on B cell transcriptome.

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CD5 is a signal-transducing transmembrane receptor expressed by all T cells and B1a cells —a subset involved in production of natural polyreactive antibodies— and some T- and B-cell malignancies, the later mainly including B cell chronic lymphocytic leukemia (CLL) and mantle cell lymphoma.
CD5 is physically associated to the T and B cell receptor complex (TCR, BCR), and is involved in down-regulating intracellular signaling by such receptors upon specific antigen recognition. Two allelic CD5 variants differing in one amino acid (Ala471>Val) at the cytoplasmic region have been described, which respectively confer lower or higher TCR/BCR down-modulatory activity. Such CD5 polymorphism has prognostic value in autoimmune (i.e., psoriasis and SLE) and neoplastic (i.e., melanoma, prostate cancer or CLL) disorders.
To characterize the functional consequences of CD5 variation in B cell physiology we analyzed the transcriptomic profile of a B cell line (Daudi) stably expressing the CD5 Ala471 or Val471 variants, and of CLL patients homozygous for either variant available from the International Cancer Genome Consortium (ICGC) database.
RNA-Seq analysis showed that B cells expressing the CD5 Ala471 variant upregulate gene sets related to glucose metabolism, amino acid transport and several signaling pathways, while those expressing Val471 upregulate genes related to histones and cholesterol synthesis.
Taken together, the data would agree with CD5 A471 variant associating with a metabolic state resembling that of activated lymphocytes, while V471 would associate with that of resting lymphocytes. Moreover, they strongly support CD5 receptor as a molecule deeply involved in lymphocyte function.

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