XVIII Congress of the Catalan Society of Immunology

Cross-talk between anatomical barriers and the immune system

Barcelona, November 21st and 22nd 2024

Hybrid Meeting

Acadèmia de Ciències Mèdiques i de la Salut de Catalunya i de Balears.
Carrer Major de Can Caralleu, 1-7, 08017. Barcelona.

Registration available via the SCI website

PROGRAM

SOCIETAT CATALANA D'IMMUNOLOGIA

FUNDACIÓ ACADÈMIA DE CIÈNCIES MÈDIQUES I DE LA SALUT DE CATALUNYA I DE BALEARS

Welcome to the congress,

On behalf of the organizing committee, we would like to warmly welcome you to the XVIII 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 Cross-talk between anatomical barriers and the immune system.

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SCI President

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Collaborators

Awards to best oral communication and to best poster of the XVIII Congress SCI 2024

The Chairs of the different sessions and the board members of the SCI will select the best oral communication, taking into account its scientific value and the aspects related to the presentation. The congress attendees will elect the awarded poster by voting. The winners will be announced at the end of the congress.

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15:00 - 15:15	**Welcome to the XVIII Congress of the SCI** **Francisco Lozano** (President of the SCI)
15:15 - 16:45	**OPENING SESSION** • **Integration of surfactant in the innate defense of the pulmonary barrier** **Cristina Casals** (Group of Cellular and Molecular Biology in the Alveolus, Department of Biochemistry and Molecular Biology, Universidad Complutense de Madrid, Madrid, Spain) • **Intestinal immune responses shape mucosal barriers: implications for inflammatory bowel disease** **Azucena Salas** (Inflammatory Bowel Disease Unit, Hospital Clínic de Barcelona, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD), Barcelona, Spain). *Chairs: Francisco Lozano, Meritxell Genescà*
16:45 - 17:15	**COFFEE BREAK - POSTERS**
17:15 - 19:00	**ORAL COMMUNICATIONS - SESSION I: Autoimmunity** • **Applying single-cell omics technologies to unravel the impact of E. coli on Crohn's disease immunopathology** **Núria Gumà-Vique** (Hospital del Mar Research Institute, Barcelona, Spain) • **Neutrophil heterogeneity increases in the colon of active Inflammatory Bowel Disease patients in response to local inflammatory cues** **Marisol Veny** (Inflammatory Bowel Disease Unit, Institut d'Investigacions Biomèdiques August Pi i Sunyer (FCRB-IDIBAPS), Barcelona) • **Expanding the landscape of systemic sclerosis-related autoantibodies through RNA immunoprecipitation coupled with massive parallel sequencing** **Janire Perurena-Prieto** (Immunology Division, Vall d'Hebron University Hospital (HUVH), Vall d'Hebron, Barcelona) • **Increasing contribution of mitochondria-derived peptides to the HLA-DR immunopeptidome on different T1D stages** **Manel García-Ayala** (Insitut de Biotecnologia i Biomedicina, Universitat Autònoma de Barcelona, Barcelona) • **Identifying mice Aged-associated B cells (ABCs) in human SLE patients** **Anna Calvet Lacruz** (Hospital de la Santa Creu i Sant Pau, Servei d'Immunologia, Barcelona) • **An innovative nanoparticle-based immunotherapy targeting iTTP: restoring self-tolerance with autoantigen-loaded liposomes** **Daniela Greco** (Ahead Therapeutics SL, Barcelona) • **The importance of nodal and paranodal antibodies in monitoring and treatment in autoimmune nodopathies** **Andrea Bravo Gómez** (Immunology Department. Hospital de la Santa Creu i Sant Pau, Barcelona) *Chairs: Anaís Mariscal, Iñaki Álvarez*

09:30 - 10:10	**PLENARY SESSION I** • **Back to the source in Atopic Dermatitis: CLA+ memory T cells and skin cell interaction allows patient stratification and endotyping** **Luis Santamaría-Babí** (Translational Immunology, Department of Cellular Biology, Physiology and Immunology, Faculty of Biology, University of Barcelona, Parc Científic de Barcelona). *Chairs: Concepción Mora, Iñaki Salvador*
10:10 - 10:40	**COFFEE BREAK - POSTERS**
10:40 - 12:00	**ORAL COMMUNICATIONS - SESSION II: Immunodeficiencies and Inflammation** • **TRAF3 deficiency: From Herpes Simplex Encephalitis to B cell dysregulation syndrome** **Laura Batlle-Masó** (Translational Immunology Research Group, Vall d'Hebron Research Institute (VHIR), Vall d'Hebron, Barcelona) • **A novel pathogenic variant in SASH3 gene in a patient with late-onset CID and immune dysregulation** **Elisabet Pol-Pol** (Servei d'Immunologia. Hospital Universitari Son Espases, Palma de Mallorca) • **Abatacept: our experience in a patient with long-term evolution CTLA4 haploinsufficiency** **Teresa Franco Leyva** (Hospital de la Santa Creu i Sant Pau, Servei d'Immunologia, Barcelona) • **Circulating memory CLA+ T-cell with IL-22 effector function in atopic dermatitis stratifies patients with distinct lesional transcriptomics, plasma proteomics and epidermal hyperplasia** **Irene García Jiménez** (Immunologia Translacional, Departament de Biologia Cel·lular, Fisiologia i Immunologia, Barcelona) • **Exploring the association between eosinophil counts and autoimmunity in Chronic Obstructive Pulmonary Disease** **Alejandro Torvisco Tello** (Biomedicine department, University of Barcelona, Barcelona) • **Non-neutralizing autoantibodies against type I interferons associated with increased risk of thrombotic complications in critical COVID-19 patients** **Mario Framil Seoane** (Servei d'Immunologia, Hospital Clínic, Barcelona) *Chairs: Mónica Martínez, Alexandru Vlagea*
12:00 - 13:00	**ORDINARY GENERAL MEETING - SOCIETAT CATALANA D'IMMUNOLOGIA**
13:00 - 14:00	**LUNCH - POSTERS** **Immunologia al Món Real - Descobreix on podries estar demà** **YOUNG IMMUNOLOGISTS' GROUP MEETING** *Room 8*

14:00 - 14:30	**SPONSORED TALK** • **Update of the new management guidelines for Cytomegalovirus in transplantation. Role of specific cellular immunity against CMV** **Manuel Muro Amador** (Hospital Clinico Universitario Virgen de la Arrixaca, Murcia)
14:30 - 15:15	**PLENARY SESSION II** • **Maping human development one cell at a time** **Roser Vento-Tormo** (Wellcome Sanger Institute; Centre for Trophoblast Research, University of Cambridge, Cambridge, UK) *Chair: Óscar de la Calle Martín*
15:15 - 16:30	**ORAL COMMUNICATIONS - SESSION III: Transplantation and Infection** • **A novel calculated Panel Reactive Antibody (cPRA) score calculator: Enhancing allocation for solid organ transplantation in the catalan population** **Juan Francisco Luchoro** (Department of Immunology, Centre de Diagnòstic Biomèdic, Hospital Clínic de Barcelona, Barcelona) • **Microvascular inflammation (MVI) of the renal allograft in the absence of HLA-DSAs and C4d deposition** **Eduardo Gozálvez González** (Hospital Clinic de Barcelona) • **Detection of potentially allo-reactive NK cells in kidney transplant recipients with or without microvascular inflammation** **Elisenda Alari-Pahissa** (Institut Hospital del Mar d'Investigacions Mèdiques, Barcelona) • **HLA genotype, HLA-G variants, and HLA-B leader dimorphism impact on virus immune response: Refining cell donor selection for virus-specific T cell therapy** **Rut Mora-Buch** (Advanced Cell Therapy Services, Banc de Sang i Teixits, Barcelona) • **Salmonella typhimurium infection model in Wistar rats for immune function analysis** **Ruth Forsten** (Nutrition and Food Safety Research Institute (INSA-UB), Santa Coloma de Gramenet) • **Scorpion venom components target ion channels and Toll-like receptors in macrophages** **Dalila Khemili** (Laboratory of Cellular and Molecular Biology, Faculty of Biological Sciences) *Chairs: Nuria Izquierdo-Useros, Pablo Engel*
16:30 - 17:00	**COOFFE BREAK - POSTERS**
17:00 - 17:30	**SPONSORED TALK** • **Spectral Flow cytometry: A closer step to high dimensional immunology** **Jaime Valentin Quiroga** (IdiPAZ, Hospital Universitario La Paz) **Iñaki Salvador** (Vall d´Hebron instituto de Oncologia (VHIO))

17:30 - 19:00	**ORAL COMMUNICATIONS - SESSION IV: Tumor Immunology** • **Analysis of the HLA-DR Immunopeptidome Derived from Dendritic Cells Pulsed with Tumor Extracts** **Gonzalo Lázaro** (Institut de Biotecnologia i Biomedicina, Universitat Autònoma de Barcelona) • **Therapeutic impact and reactivation of tumor immunity by MYC inhibition in KRAS-driven NSCLC with diverse mutational landscapes** **Íñigo González-Larreategui** (Vall d'Hebron Institute of Oncology (VHIO), Barcelona) • **Use of the TCR (T-cell receptor) as a biomarker for monitoring irAEs (immune-related adverse events) in breast cancer** **Maitane Faus** (Institute of Biotechnology and Biomedicine, Universitat Autònoma de Barcelona) • **Tracking changes in TRBV family repertoire in blood after administration of PD-1+ TILs to identify Tumor-Specific TCRs** **Alejandro Ramírez-Chacón (**Fundació de Recerca Clínic Barcelona, IDIBAPS, Barcelona) • **Characterizing TCR and gene expression diversity in NY-ESO-1-specific HLA-DRB3*02:02-restricted CD4+ T Cells from healthy donors using single-cell RNA and TCR sequencing** **Berta Casanovas-Albertí** (IDIBAPS, Barcelona) • **Differential CD6 mRNA expression associates with adverse prognostic markers in chronic lymphocytic leukemia patients** **Laura Carrillo-Serradell** (Immunoreceptors del Sistema Innat i Adaptatiu, IDIBAPS, Barcelona) *Chairs: José Yélamos, Ester Lozano*
19:00 - 19:40	**PLENARY SESSION III** • **Toward antibody therapeutics with increased tissue selectivity and brain penetration** **Benjamí Oller Salvia** (Institut Químic de Sarrià (IQS), Universitat Ramon Llull, Barcelona) *Chairs: Eva Martínez Cáceres, Laura Martínez*
19:40 - 20:10	**Prize to the best communication and poster - Closing of the Congress** **Eva Martinez Cáceres** (Vice-President of the SCI) EUROIMMUN

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ORAL COMMUNICATIONS - SESSION I: Autoimmunity

Applying single-cell omics technologies to unravel the impact of E. coli in Crohn's disease immunopathology

Núria Gumà-Vique 1,4; Leticia Suárez-García 1; Sonia Tejedor Vaquero 1; Pau Berenguer-Molins 1; Christian Romero 1;Julia Perera-Bel 1; Mar Iglesias 2; Andrea Cerutti 1; Lucía Márquez-Mosquera 3; Giuliana Magri 4

1 Hospital del Mar Research Institute, Barcelona, Spain; 2 Pathology Department, Hospital del Mar, Barcelona, Spain; 3 Department of Gastroenterology, Hospital del Mar, Barcelona, Spain; 4 Immunology Unit, Department of BiomedicalSciences, Faculty of Medicine and Health Sciences

Crohn's disease (CD) is a chronic and relapsing inflammatory condition of the gastrointestinal tract characterized by exacerbated immune responses to the gut microbiota in genetically predisposed individuals. Although a causative microbe has not been identified, Escherichia coli, particularly the adherent-invasive E. coli (AIEC) pathotype, has been proposed to contribute to CD pathogenesis. Yet, it remains unclear whether the presence of E. coli plays a role in disease heterogeneity by triggering distinct adaptive and innate immune responses in the gut mucosa. It is also unknown how the presence of specific microbiome profiles in CD associates with a unique mucosal antibody repertoire. To address these important questions, we initially analyzed the composition of intestinal mucus-embedded microbiota through 16S rRNA sequencing in both non-IBD controls and CD patients. Our preliminary findings confirmed a reduction in mucosal bacterial alpha diversity in CD, with a notable decrease in several beneficial bacterial species, such as F. prausnitzii, alongside a significant increase in mucus-embedded E. coli among a subset of CD patients. Building upon these results, we selected an exploratory cohort comprising non-IBD controls, and CD patients with or without the expanded E. coli population for single-cell RNA and V(D)J sequencing (n=10) to map the gut mucosal immune responses. Our analysis revealed a differential proportion of mucosal immune cell subsets and distinct cellular modules driving inflammation in patients with expanded E. coli. Moreover, these patients display an increased frequency of IgG-expressing plasma cells and extensive B cell clonal expansion. Altogether, our preliminary findings suggest that the presence of E. coli might be promoting a unique inflammatory signature in CD and could potentially trigger mucosal humoral immunity.

ORAL COMMUNICATIONS - SESSION I: Autoimmunity

Neutrophil heterogeneity increases in the colon of active Inflammatory Bowel Disease patients in response to local inflammatory cues

Marisol Veny 1,2; Angela Sanzo-Machuca 1,2; Ana M Corraliza 1,2; Floortje Strobbe 1,2; Alba Garrido-Trigo 1,2; Victoria Gudiño 1,2; Elisa Melón-Ardanaz 1,2; Miriam Esteller 1,2; Iris Teubel 1,2; Maria C. Masamunt 1,2; Ingrid Ordás 1,2; Cristina Prieto 1,2; Àngel Giner 1,2; Maria Esteve 2,3; Albert Martin-Cardona 2,3; Elena Ricart 1,2; Azucena Salas 1,2

1 Inflammatory Bowel Disease Unit, Institut d'Investigacions Biomèdiques August Pi i Sunyer (FCRB-IDIB; 2 Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD), Barcelon; 3 Department of Gastroenterology, Hospital Universitari Mútua Terrassa, Universitat de Barcelona

Neutrophils infiltration is the main histological feature in active inflammatory bowel disease (IBD).Recent data suggests that tissue- and tumour-infiltrating neutrophils can adopt remarkably different phenotypes with different functions. However, the heterogeneity of this population in IBD remains unknown.

Single-cell RNA sequencing data was generated from colonic biopsies of 57 individuals including 20 active Crohn's disease (CD) and 37 active ulcerative colitis (UC) patients. Neutrophils (5380 cells) found in the inflamed colon of CD and UC patients were analysed. We identified 6 different clusters of colonic neutrophils (Figure 1A). In agreement with our previous results we found a population of S100A12/S100A8/S100A9 neutrophils (N0), IFN-response neutrophils (N3) and CCL3/CCL4 neutrophils (N2), in addition to N1.1 and N1.2 neutrophils. N3 neutrophils were enriched in the inflamed colon of CD patients compared to UC (17.2% vs 5.8%, p-value= 0.0021; Figure 1C). Comparative analysis with published datasets revealed that N0, N1.2 and N3 resemble populations of neutrophils found in blood. In contrast, N1.1 and N2-like clusters showed some similarity to umbilical cord blood and tumour-infiltrating neutrophils. Trajectory analysis, originating from N0 (peripheral-like) neutrophils, showed different branches of differentiation, one leading towards N1.2 neutrophils and another one through N1.1 finally branching into N3 (IFN-response) and N2 (CCL3) neutrophils (Figure 1B). Finally, pathway analysis showed that N2 neutrophils presented enriched gene expression of the TNF signalling pathway, and together with the N1.1 state, an enrichment of the IL1β signalling pathway as well. In vitro experiments revealed that TNF and IL1β can induce expression of CCL3 and CCL4, top differentially expressed genes of N2 neutrophils.

We show that active IBD is associated with a highly heterogeneous population of infiltrating neutrophils. Some of them resemble neutrophils in peripheral blood, while others most likely differentiate in response to inflammatory cues present in the colon of active IBD patients.

ORAL COMMUNICATIONS - SESSION I: Autoimmunity

Expanding the landscape of systemic sclerosis-related autoantibodies through RNA immunoprecipitation coupled with massive parallel sequencing

Janire Perurena-Prieto 1,2,3; María Teresa Sanz-Martínez 1,2; Laura Viñas-Giménez 1,2; Claudia Codina-Clavaguera 4,5; Laura Triginer 5; Fernando Gordillo-González 6,7; Eduardo Andrés-León 6; Laura Batlle-Masó 8,9,10; Javier Martin 6; Albert Selva-O'Callaghan 4,5; Ricardo Pujol 3,11; Alfredo Guillen-Del-Castillo 4,5; Carmen Pilar Simeón-Aznar 4,5; Roger Colobran 1,2,3,12

1 Immunology Division, Vall d'Hebron University Hospital (HUVH), Vall d'Hebron Barcelona Hospital Camp; 2 Translational Immunology Group, Vall d'Hebron Research Institute (VHIR), Vall d'Hebron Barcelona Hos; 3 Department of Cell Biology, Physiology and Immunology, Autonomous University of Barcelona (UAB), Bel; 4 Systemic Autoimmune Diseases Unit, Internal Medicine Department, Vall d'Hebron University Hospital (; 5 Systemic Autoimmune Diseases Group, Vall d'Hebron Research Institute (VHIR), Vall d'Hebron Barcelona; 6 Institute of Parasitology and Biomedicine "López-Neyra", CSIC (IPBLN-CSIC), Granada, Spain; 7 Computational Biomedicine Laboratory, Principe Felipe Research Center (CIPF), Valencia, Spain.; 8 Infection and Immunity in Pediatric Patients Research Group, Vall d'Hebron Research Institute (VHIR); 9 Pediatric Infectious Diseases and Immunodeficiencies Unit, Children's Hospital. Hospital Universitar; 10 Pompeu Fabra University (UPF), Barcelona, Spain.; 11 Vall d'Hebron Institute of Oncology (VHIO), Vall d'Hebron Barcelona Hospital Campus, Barcelona, Spai; 12 Department of Clinical and Molecular Genetics, Vall d'Hebron University Hospital (HUVH)

Objectives: Systemic sclerosis (SSc)-related autoantibodies are widely used diagnostic and prognostic biomarkers. This study aimed to develop a new assay for detecting anti-ribonucleoprotein autoantibodies in SSc based on RNA immunoprecipitation (RNA IP) coupled with massive parallel sequencing.

Methods: Serum samples and clinical data were collected from 307 SSc patients. Among these, 57 samples underwent analysis using a new protocol that combines RNA IP with massive parallel sequencing (RIP-Seq). Filtering strategies and statistical outlier detection methods were applied to select RNA molecules that could represent novel ribonucleoprotein autoantigens associated with SSc.

Results: Among the 30,966 different RNA molecules identified by RIP-Seq in 57 SSc patients, 197 were ultimately selected. These included all RNA molecules previously identified by RNA IP, which were found to exhibit high counts almost exclusively in samples positive for the autoantibodies associated to the corresponding RNA molecule, indicating high sensitivity and specificity of the RIP-Seq technique. C/D box snoRNAs were the most abundant RNA type identified. The immunoprecipitation patterns of the detected C/D box snoRNAs varied among patients and could be associated with different clinical phenotypes. In addition, other ribonucleoproteins were identified, which could be potential targets for previously undescribed SSc-related autoantibodies. These include H/ACA box snoRNPs, vault complexes, mitochondrial tRNA synthetases, and 7SK snRNP.

Conclusion: A novel RIP-Seq assay has been developed to detect autoantibodies targeting ribonucleoprotein complexes in SSc patients. This method successfully identified RNA molecules associated with ribonucleoproteins known to be targeted by SSc-related autoantibodies, validating both the assay and the analysis strategy. Additionally, this approach uncovered RNA molecules associated with ribonucleoproteins that were not previously identified as targets of SSc patients' sera, suggesting potential new autoantibody candidates in this disease.

ORAL COMMUNICATIONS - SESSION I: Autoimmunity

Increasing contribution of mitochondria-derived peptides to the HLA-DR immunopeptidome on different T1D stages

Manel García-Ayala 1; Yago A. Arribas 2; Saioa Auzmendi-Aguirresarobe 1; Mercè Rubió-Capdevila 1; Dolores Jaraquemada1; Carme Roura-Mir 1

1 Insitut de Biotecnologia i Biomedicina, Universitat Autònoma de Barcelona, Barcelona, Spain; 2 Inserm U932, Immunity and Cancer Unit, Curie Institute, Paris, France

Mitochondria play a crucial role in β-cell function and homeostasis, with alterations in this organelle reported in type 1 diabetes (T1D) patients. The present study aims to investigate if these alterations lead to modifications in the peptide repertoire derived from b-cells and their impact in T1D development. Human β-cells were exposed to basal, intermediate, and stressing glucose concentrations. β-cell fractions enriched in LAMP1+ vesicles were isolated from each condition for proteome analysis. About 20% of the identified proteins were of mitochondrial origin, with half of them differentially expressed between basal and stressing conditions; these proteins were involved in mitochondrial proteostasis, ATP synthesis, and gene expression. Morphological and mitochondrial respiration assessments confirmed mitochondrial alterations as mitochondrial branch length and number increased from basal to intermediate glucose concentrations but diminished under hyperglycaemic conditions. Basal and maximal respiration rates followed the same trend. Furthermore, pancreata homogenates from a pre-diabetic and an early-T1D donor were used for whole proteome assessment. Out of 1046 mitochondrial proteins identified (15% of the total proteome), 276 were upregulated in early T1D, with 203 detected in the β-cell line LAMP1+ fractions; all these proteins belonged to the previously mentioned functional categories. Finally, HLA-DR4+ moDCs were pulsed with the pancreata homogenates for immunopeptidome analysis, identifying approximately 300 peptides derived from mitochondrial proteins; these were classified as high or medium binders for the DR4 allele. Notably, 88 peptides were unique to the early-T1D immunopeptidome, showing distinct physicochemical properties that may influence their presentation by HLA-DR4. These findings highlight the impact of mitochondrial alterations, which seem to be also present in T1D patients' pancreata, in the peptide MHC-II repertoire. The mechanisms through which these proteins enter the moDC processing and presentation pathways, as well as the role of the identified peptides in T1D progression, require further investigation.

ORAL COMMUNICATIONS - SESSION I: Autoimmunity

Identifying mice Aged-associated B cells (ABCs) in human SLE patients

Anna Calvet Lacruz 1; Teresa Franco Leyva 1; Àngels Santaolalla Tragant 1; Berta Paula Magallares López 2; Oscar de la Calle Martín 1; Laura Martínez Martínez 1

1 Hospital de la Santa Creu i Sant Pau, Servei d'Immunologia; 2 Hospital de la Santa Creu i Sant Pau, Servei de Reumatologia

Systemic lupus erythematous (SLE) is a systemic autoimmune disease characterized by loss of tolerance to nucleic acids and related proteins with the generation of autoantibodies against double-stranded DNA and RNA, leading to immune complexes formation, tissue inflammation and organ damage. Genome-wide association studies and gene expression profiling have highlighted the role of B cells in SLE, consistent with the known expansion of antibody-secreting cells during acute disease flares.

Recently, new B cell subpopulations within the double negative (DN) B cells (CD19+IgD-CD27-) compartment have been described that are prone to differentiate into plasma cells in an extrafollicular manner and are expanded in SLE. These cells require T-bet transcription factor and stimuli via IFNγR and TLR7. Despite many authors have reported similar results, there is no consensus on what markers identify this particular population. These cells were first identified in female mouse models of SLE as aged-B cells (ABCs), since direct correlation with age was found.

Here we describe the analysis of atypical B cell subsets in a cohort of 24 SLE patients and 21 healthy sex and age-matched donors from our centre. We aimed to simplify their identification and to validate the populations in our patient selection. We used the B cell follicle homing receptor CXCR5 and CD11c to define two subpopulations in the DN subset, DN1 and DN2 (mice ABCs) but also to substratify naïve population into resting naïve (rNAV) (CXCR5+CD11c-) and activated naïve (aNAV) (CXCR5-CD11c+) and switched memory (SWM) B cells into CXCR5+CD11c- SWM and CXCR5-CD11c+ SWM cells. Our preliminary results show no correlation with age but significant differences in the proportion of DN2 and aNAV between SLE and healthy donors. Upon stratification according to clinical manifestations, we found stronger association with SLE patients suffering lupus nephritis.

ORAL COMMUNICATIONS - SESSION I: Autoimmunity

An innovative nanoparticle-based immunotherapy targeting iTTP: restoring self-tolerance with autoantigen-loaded liposomes

Daniela Greco 1; Lidia Almenara-Fuentes 1; Maria Vilà 1; David Perna-Barrull 1,2; Inés Hernández Rodriguez 3; Miriam Salvado 1; Montserrat Mancera-Arteu 1; Iván García-Loza 2,4; Silvia Rodriguez-Vidal 1; Martí Dalmases 1; José Tomás Navarro 3; Bruna Barneda-Zahonero 1; Marta Vives-Pi 1

1 Ahead Therapeutics SL, Barcelona, Spain; 2 Germans Trias i Pujol Research Institute, Universitat Autònoma de Barcelona, Immunology Department,; 3 ICO-Hospital Germans Trias I Pujol, Josep Carreras Leukemia Research Institute, Universitat Autònoma; 4 Sant Pau Biomedical Research Institute, Hospital de la Santa Creu i Sant Pau, Neuromuscular Diseases

Immune thrombotic thrombocytopenic purpura (iTTP) is a rare life-threatening autoimmune disease caused by autoantibodies against ADAMTS13, a multidomain metalloprotease that cleaves the polymeric Von Willebrand Factor (vWF). A severe ADAMTS13 deficiency results in the accumulation of ultra-large vWF multimers, which interact with platelets and form aggregates, causing microthrombi. An innovative antigenspecific immunotherapy for autoimmune diseases based on phosphatidylserine (PS)-liposomes has been assessed by Ahead Therapeutics in preclinical models of type 1 diabetes, multiple sclerosis and myasthenia gravis, among others. PS-liposomes loaded with disease-specific autoantigens induce self-tolerance by biomimicking apoptotic cell clearance, halting the autoimmune attack. Driven by the therapeutic potential of the technology, we aimed to explore its application in iTTP by testing ADAMTS13 loaded PS-liposomes in human immune cells and its biodistribution in mice. The highly immunogenic SPACER domain was analyzed using the NetMCHIIpan 4.2 peptide binding affinity prediction tool. A sequence of 33 amino acids was selected as the most adequate for encapsulation. By generating fluorescent PS-liposomesSPACER, we first confirmed their capability to interact with most human immune cells. Furthermore, we showed their crucial role in the tolerogenic modulation of both dendritic cell phenotype and B cell tolerogenic cytokines production. Biodistribution and bioimaging studies with loaded PS-liposomes showed a peak of fluorescence at 1h in mice, indicating rapid uptake, while the bladder peaked at 6h, reflecting an active clearance of the nanoparticles. Finally, no detectable bioaccumulation was observed after 24 hours, highlighting the biodegradable nature of liposomes. Overall, our results confirmed that the platform is safe and effective in re-educating the immune system towards self-tolerance, making it a promising immunotherapy for iTTP. We will next test the therapeutic effect of PS-liposomesSPACERin patient's lymphocytes and in a mouse model of the disease.

Funded by the European Union under Grant Agreement No. 101072729.

ORAL COMMUNICATIONS - SESSION I: Autoimmunity

The Importance of nodal and paranodal antibodies in monitoring and treatment in autoimmune nodopathies

Bravo Gómez, Andrea 1; Lleixa Rodriguez, Cinta 2; Lynton-Pons, Elionor 1; Caballero-Ávila, Marta 2; Pascual Goñi, Elba 2; Martin Aguilar, Lorena 2; Querol Guitierrez, Luis 2; Martinez-Martinez, Laura 1

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Autoimmune nodopathies (AN) are a group of immune-mediated neuropathies associated with antibodies against cell adhesion molecules of the Ranvier node, such as CNTN1, NF155, NF140/186, and CASPR1. The pathogenicity of anti-NF155 and anti-CNTN1 antibodies has been demonstrated in animal models. Moreover, IgG subclasses determination has been particularly important in deciding treatment. We will comment on two illustrative cases of these two ANs.

The first case is a 25-year-old man who at 21 began with peripheral neuropathy symptoms diagnosed with acute inflammatory demyelinating polyneuropathy and treated with intravenous immunoglobulins (IVIg) with clinical improvement. A month later he had a severe relapse, so he was admitted and treated with IVIg and methylprednisolone, maintaining treatment with prednisone at discharge with a mild response. Serum from the first outbreak tested positive for anti-NF155 IgG4 antibodies, so it was decided to start therapy with rituximab, which resulted in a notable and progressive improvement, accompanied by a drop in anti-NF155 titers, allowing a de-escalation in the prednisone dose until its suspension. During the follow-up a minor symptoms relapse was observed with elevated NF155 titers, which prompt us to administered a new dose of rituximab, preventing the appearance of severe symptoms.

The second case is a 73-year-old man who at 62 began with progressive motor-sensory neuropathy with poor response to corticosteroids and IVIg treatment. Anti-CNTN1 screening identified high IgG4 antibodies titters so a cycle of rituximab was performed, achieving negative anti-CNTN1 titers 1.5 years later, remaining negative until today without the need to repeat the treatment.

Our patients represent NF155 AN run with relapses requiring new cycles of rituximab, and CNTN1 AN does not increase titers after rituximab treatment. This highlights the importance of AN antibodies followup, both to treat patients before the onset of symptoms or to avoid overtreatment.

ORAL COMMUNICATIONS - SESSION II: Immunodeficiencies and Inflammation

TRAF3 deficiency: From Herpes Simplex Encephalitis to B cell dysregulation syndrome

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TRAF3, a versatile adaptor protein within the TRAF family, participates in various signaling pathways involving the tumor necrosis factor receptor, toll-like receptor, and retinoic acid-inducible gene I-like receptor families. In 2010, autosomal dominant TRAF3 deficiency was reported in a patient with herpes simplex virus-1 encephalitis, consistent with the role of TRAF3 in type I interferon production. Recently, a novel, completely different clinical phenotype was described in patients with TRAF3 haploinsufficiency (TRAF3HI), characterized by recurrent bacterial infections, autoimmune features, systemic inflammation, and hypergammaglobulinemia. In this study, we conducted a TRAF3-targeted reanalysis of next-generation sequencing data from 800 patients with inborn errors of immunity. Through this reassessment and additional familial investigations, we identified three previously unidentified cases of TRAF3HI within two different families. These individuals harbored stop-gain variants (p.Arg163* and p.Gln407*) and experienced recurrent bacterial infections with hypogammaglobulinemia. Previously, the patients had been diagnosed with common variable immunodeficiency (CVID) and were receiving immunoglobulin replacement therapy. In addition, a TRAF3 start-loss variant (c.3G>A) was identified in a fourth patient, but after familial and molecular studies, it was not considered disease-causing, excluding TRAF3HI in this patient. This study illustrates the usefulness of targeted reanalysis of genes with reported novel phenotypes. We rescued three patients with TRAF3HI, presenting similarities and differences with the previously reported patients. The most significant differences were hypogammaglobulinemia and a CVID-like presentation. These data expand the clinical phenotype of TRAF3HI and pave the way for further investigation into loss-of-function variants in patients with CVID.

ORAL COMMUNICATIONS - SESSION II: Immunodeficiencies and Inflammation

A novel pathogenic variant in SASH3 gene in a patient with late-onset CID and immune dysregulation

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SASH3 (Sterile Alpha Motif [SAM] and Src Homology 3 [SH3] Domain-Containing Member 3) is a lymphocyte-specific adaptor protein encoded by an X-linked gene. To date, SASH3 deficiency has been described in six patients with Combined Immunodeficiency (CID), recurrent sinopulmonary and mucocutaneous infections, as well as autoimmune cytopenia, associated with impaired TCR signaling and thymocyte survival.

We present a 40-year-old male who has presented with multiple episodes of autoimmune hemolytic anemia and thrombocytopenia, mainly in childhood, together with recurrent respiratory infections, hypogammaglobulinemia and T-lymphopenia. Initially diagnosed with common variable immunodeficiency (CVID), genetic analysis revealed a novel pathogenic variant in the SASH3 gene. Immunophenotyping of peripheral blood sample was performed by flow cytometry. The genetic analysis was carried out using whole exome sequencing (WES). SASH3 protein expression was assessed by western blotting using a lysate from peripheral blood mononuclear cells.

Immunophenotypic analysis revealed reduced CD4+ and CD8+ T-lymphocyte counts, as well as CD19+ Blymphocytes. Additionally, a decreased B-cell memory compartment and severe hypogammaglobulinemia, including IgG, IgA, IgM and IgE were observed. The distribution (percentage values) of NK, Treg, and follicular T cells was within the normal range.
The genetic test revealed a novel hemizygous pathogenic variant in the SASH3 gene (c.954_960del p.G319RfsTer94) leading to premature stop codon. Western blot analysis confirmed the absence of SASH3 expression in the patient's blood cells.

We describe a novel loss-of-function variant in the SASH3 gene in a patient with late-onset CID and immune dysregulation.

ORAL COMMUNICATIONS - SESSION II: Immunodeficiencies and Inflammation

Abatacept: our experience in a patient with long-term evolution CTLA4 haploinsufficiency

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CTLA4 inhibits lymphocyte T activation by competing against CD28 for CD80/86 binding. CTLA4-haploinsufficiency presents with enteropathy, autoimmunity and infections.

We describe a Venezuelan 46-year-old male referred to our Immunology department for severe corticodependent enteropathy since he was 15, previously misdiagnosed as refractory coeliac disease. He had suffered from intestinal opportunistic infections, susceptibility to Papillomavirus with digital warts and CBP-like hepatopathy with anti-M2 autoantibodies. Duodenal biopsy described atrophied mucosa and inflammatory infiltrates in the lamina propria. Immunological studies showed hypogammaglobulinemia, <1% B cells, low percentage of naïve T cells and elevated B-CD21low cells. Genetic analysis by NGS virtual panel for primary immunodeficiencies revealed an heterozygous missense mutation in CTLA4 c.308G>T, p.C103F, diagnosing CTLA4-haploinsufficiency. Sanger sequencing in the patient and family confirmed the mutation and an autosomal dominant inheritance.

Intravenous immunoglobulins normalized the levels without improving his symptoms. Our patient had been unsuccessfully treated with oral budesonide, azatropin, infliximab, sirolimus and monthly abatacept. Only short-term partial response to vedolizumab allowed temporary withdrawal of corticosteroids.

Abatacept is a CTLA4-immunoglobulin fusion protein that antagonizes T lymphocyte activation. Its usefulness had been described in patients with CTLA4 and LRBA mutations. Long-term studies showed that weekly doses of abatacept exhibited more disease control than monthly intervals after 6 months of treatment. Consequently, targeted therapy was reconsidered: administering a loading dose followed by weekly 125mg subcutaneous abatacept combined with daily 20mg of prednisone.

We monitored response by number of CD25+ T cells, naïve/effector T cells ratio and inflammatory biomarkers. After 7 months with weekly subcutaneous abatacept, we achieved resolution of the warts and great reduction in daily depositions. His inflammatory biomarkers have normalized and the ratio naïve/effector T cells is stable. The number of CD25+ T cells increased up to 98% after the loading dose and has progressively normalized. These results are compatible with a good response.

ORAL COMMUNICATIONS - SESSION II:
Immunodeficiencies and Inflammation

Circulating memory CLA+ T-cell with IL-22 effector function in atopic dermatitis stratifies patients with distinct lesional transcriptomics, plasma proteomics and epidermal hyperplasia

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Background: IL-22 is increased in the sera and lesional skin of atopic dermatitis (AD) patients, and a relevant cytokine in the course of the disease, as indicated by the anti-IL-22 therapy in humans. Current understanding on T-cell-derived IL-22 in AD mostly relies on animal models, and the polyclonal activation and intracellular staining of peripheral lymphocytes.

Methods: We have developed a translational approach that allowed the stratification of AD patients (n=60) into IL-22 producers (IL22P) and non-producers (IL22NP). This method is based on the coculture of autologous lesional epidermal cells with circulating memory T cells expressing the cutaneous lymphocyte associated antigen (CLA+) or its negative counterpart (CLA-), activated with house dust mite (HDM) allergen.

Results: Bulk RNA-seq of lesional skin revealed that the IL22P group was enriched for immune-associated signaling pathways (e.g., leukocyte mediated immunity, receptor signaling pathway via JAK-STAT). Contrarily, the IL22NP group was enriched for biological processes related to skin development. Histological analysis identified that skin lesions from IL22P patient exhibit considerably higher epidermal thickness compared with IL22NP patients and control subjects. The levels IL-8, a cytokine recognized to be triggered by IL-22, produced by HDM-activated CLA+ T-cell cocultures were increased and directly associated with IL-22 content in the IL22P group. Assessment of clinical characteristics indicated elevated HDM-specific IgE plasma levels in IL22P patients. Furthermore, IL22P patients present heightened CCL20 plasma levels, a chemokine enhanced by IL-22, than the IL22NP group.

Conclusions: Our innovative approach functionally distinguishes phenotypically, and molecularly moderateto-severe adult AD patients based on the CLA+ T-cell IL-22 response induced by HDM allergen. This translational approach might be of help for the identification of biomarkers, such as CCL20, thus facilitating the selection of patients that may benefit from IL-22-directed therapies.

ORAL COMMUNICATIONS - SESSION II: Immunodeficiencies and Inflammation

Exploring the association between eosinophil counts and autoimmunity in Chronic Obstructive Pulmonary Disease

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Introduction. Chronic obstructive pulmonary disease (COPD) is a major public health problem because of its high prevalence and associated morbi-mortality. COPD is defined by airflow obstruction and associated to an abnormal immune response to smoke. In patients with severe airflow limitation and/or emphysema an increase in autoantibodies and a pulmonary Th17 response has been reported. Moreover, treatment with anti-IL-4, but not anti-IL-5, reduces exacerbations in COPD patients with high eosinophil counts, suggesting an underlying altered immune process.

Aim. To explore the association between the titer of several autoantibodies, the smoking status, eosinophil counts and disease severity in patients with COPD.

Methodology. The level of autoantibodies (ANCAs, ANAs, ATs) and blood eosinophil counts was determined in 283 COPD patients, additionally, in 183 patients we determined the sputum eosinophil counts. We performed multivariable linear regressions evaluating the association between eosinophil percentage and autoantibodies, adjusting for age, sex, smoking status and packs/year. Auto-reactivity and smoking status stratifications were also performed.

Results. In our study, the level of autoantibodies was not associated to the level of FEV1. But we observed significant associations between the % of blood eosinophils and levels of AT+ (p=0,005). In sputum eosinophil % was associated to serum levels of ANCA (+) (p=0,003) and elevated (>1/320) ANA titers (p=0,01). Stratifying by smoking status, we observed stronger associations in current smokers (CS) between blood eosinophil % and AT+ (p=0,018), and between sputum eosinophil % and high (>1/320) ANA titer (p=5,57e-11).

Conclusion. Our findings show that levels of certain autoantibodies are associated to current smoking and eosinophil percentage, suggesting that the level of eosinophils is just a marker of a more profound immune dysfunction in patients with COPD. Future studies will have to address the association with emphysema, and the inflammatory status of the patient.

ORAL COMMUNICATIONS - SESSION II:
Immunodeficiencies and Inflammation

Non-neutralizing autoantibodies against type I interferons associated with increased risk of Thrombitic complications in critical COVID-19 patients

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Background and Aims: During the COVID-19 pandemic, high levels of neutralizing antibodies against type I interferons (nAAB) were found in nearly 15% of patients with severe COVID-19, mainly men. These antibodies impair the antiviral response and lead to a poorer prognosis. However, the role of nonneutralizing antibodies (nnAAB) remains unclear. This study describes the evolutive data of critical COVID-19 cases based on the presence or absence of nonneutralizing IgG autoantibodies against type I interferons (IFN α2 and ω).

Patients and Methods: A retrospective study of ICU-admitted COVID-19 patients (n=275) from March 2020 to March 2021 analyzed autoantibodies against IFN-α2 and IFN-ω using ELISA. Neutralizing ability was assessed through Dual-Luciferase reporter assays. Demographic, clinical, and evolutive data during hospitalization were recorded and laboratory data variables were collected at ICU admission.

Results: Among 275 patients, 49 (17.8%) tested positive for anti-type I IFN antibodies, with almost half being neutralizing (26 [53.1%]), predominantly among men (91.3%). Non-neutralizing autoantibodies were significantly associated with thrombotic complications compared to the absence of autoantibodies (11 [47.8%] vs. 42 [18.3%]; p=0.002), with four times higher odds (OR 4.054 [95% CI 1.638-9.972]). There was also a higher tendency of cardiovascular complications (8 [34.8%] vs. 43 [18.8%]; p=0.098), especially in fatal cases (6 [46.2%] vs. 26 [21.7%]; p=0.081). No other clinical complications were statistically significant.

Conclusions: Non-neutralizing autoantibodies against type I interferons could confer an increased risk of thrombotic complications in critical COVID-19 patients, likely through the prolongation of cytokine halflife and subsequent disruption of vascular homeostasis. Stratifying antibodies based on their neutralization capacity may prove useful in predicting thrombotic and cardiovascular events. Further research is warranted to validate these findings in other infectious and autoimmune diseases.

ORAL COMMUNICATIONS - SESSION III: Transplantation and Infections

A Novel Calculated Panel Reactive Antibody (cPRA) Score Calculator: Enhancing Allocation for Solid Organ Transplantation in the Catalan Population

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Purpose: Calculated panel reactive antibody (cPRA) score represents the possibility of encountering an incompatible donor for solid organ transplant candidates. This study aims to evaluate a novel calculator designed to improve compatibility assessment in the Catalan population and by considering HLADQB1/DQA1 or DPB1/DPA1 associations not accounted for in existing calculators.

Methods: A cohort of 1000 deceased donors from Catalonia was analysed, focusing on HLA-A, B, C, DRB1, DQB1, DQA1, DPB1, DPA1, and DRB345 loci typing. The HLA genotype was compared with Single Antigen Bead (SAB) assay results from 398 patients within the kidney transplant waiting list. A custom computer script written in R language was created for identifying any unacceptable antigen matches between patients and donors. The cPRA represented the percentage of donors with incompatible HLA typing relative to patient serum profiles. Notably, the calculator incorporated distinctions between DQB1/DQA1 and DPB1/DPA1 associations to enhance estimation precision compared to existing calculators.

Results: Our calculator exhibited a high Lin's concordance correlation coefficient (rho = 0.92, 95% CI 0.91-0.93) when compared to the Eurotransplant Reference Laboratory (ERL) calculator. A limit of agreement was established to identify samples requiring further investigation, with 24 samples falling below this threshold. These samples exhibited positive reactions to certain DQ or DP beads but lacked specificity for individual DQB1, DQA1, DPB1, or DPA1 alleles. Mean difference between these samples was −55.39 ($p < 0.001$), so our calculator increased the apparent compatibility in these patients with positivity to single DQB1/DQA1 and DPB1/DPA1 associations.

Conclusions: The novel calculator demonstrates optimised performance in predicting donor-recipient compatibility within the Catalan population, offering additionally improved precision through the consideration of single HLA-DQ or DP associations. Further validation and refinement may enhance its utility in clinical practice, ultimately benefiting organ allocation outcomes.

ORAL COMMUNICATIONS - SESSION III: Transplantation and Infections

Microvascular inflammation (MVI) of the renal allograft in the absence of HLA-DSAs and C4d deposition

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One of the main reasons for graft loss following a kidney transplant is currently thought to be antibodymediated rejection (ABMR) of the renal graft. Three histologic criteria have been used to diagnose it since its recognition in 2001: 1) histology consistent with humoral rejection, which is characterized by microvascular inflammation (MVI); 2) serological evidence of circulating HLA-DSAs; and 3) evidence of antibody interaction with the endothelium based on C4d deposition.

However, an increasing number of cases that only fulfil the first criteria are being recognized in the literature. That is the subject of this case clinic report, in which two patients diagnosed with this phenotype of rejection (iMVI) will be presented. In this context, due to the absence of HLA-DSAs and C4d deposition in the presence of MVI, to ascertain the aetiology of the rejection process and choose the most effective treatment plan, research on MICA antibodies and KIR/HLA-I compatibility is requested. The KIR study's operation in both patients will also be explained, as will the reasons why the NK cell might be one of the cellular effectors involved and how its activation brought on by a "missing self" scenario might explain the chronic vascular damage observed in these two patients.

Ultimately, the findings from both investigations will be showcased. While only one of the two patients exhibits a "missing self" condition, both of them complain to the absence of anti-MICA. The ambiguity of the results will be discussed, with particular attention to the fact that a wide range of factors interact to determine the allograft's outcome rather than just one. As a result, a deeper and more comprehensive understanding of the pathophysiology of the process is necessary to guide the best therapeutic approach that best suits the patient's circumstances.

ORAL COMMUNICATIONS - SESSION III: Transplantation and Infections

Detecció de cèl.lules NK potencialment aloreactives en receptors de transplantament renal amb o sense inflamació microvascular

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Introducció: La detecció en un receptor de trasplantament de ronyó (RTR) d'un gen de Receptor tipus Immunoglobulina de cèl.lules Assassines (Killer-cell Immunoglobuline-like; iKIR) inhibidor específic per un HLA-I propi absent en el donant es defineix com "desajustament" o mismatch iKIR-HLA-I (iKIR-MM), i és més freqüent en RTR amb rebuig mediat per anticossos donant-especifics (DSA), el segell de la qual és la inflamació microvascular (microvascular inflammation; MVI) ⩾2, que també pot ocórrer en absència de DSA. Hem comparat pacients amb MVI⩾2 o MVI ⩽1 pel nombre de cèl·lules NK circulants amb combinacions d' iKIR potencialment al·lorreactives.

Mètodes: Se seleccionaren RTR sense rebuig cel·lular i sense tractar amb timoglobulina ni rituximab, amb (n=21) o sense (n=41) MVI⩾2 als 12-36 mesos després del trasplantament. Les cèl·lules NK es van analitzar abans i després del trasplantament (3 i 12-36 mesos). Es consideraren les interaccions KIR2DL1-HLA-C2, KIR2DL2/3-HLA-C1, KIR3DL1-HLA-Bw4 i KIR3DL2-HLA-A*11/03. Les cèl·lules NK NKG2A (-) amb almenys un iKIR específic d'HLA-I propi i sense cap iKIR específic d'HLA-I del donant es consideraren MM+.

Resultats: El nombre de cèl·lules MM+ NK abans del trasplantament fou major en pacients amb MVI⩾2, correlacionà amb el grau de MVI i s'associà amb una disminució de la supervivència de l'empelt lliure de MVI⩾2, independentment dels DSA post-TR. Les diferències foren especialment marcades cèl·lules MM+ NK que expressaven un fenotip de diferenciació adaptativa associada a la infecció per HCMV.

ORAL COMMUNICATIONS - SESSION III: Transplantation and Infections

HLA genotype, HLA-G variants, and HLA-B leader dimorphism impact on virus immune response: Refining cell donor selection for virus-specific T cell therapy

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The current use of virus-specific T cell therapy for treating viral infections in immunocompromised patients highlights the need for rapid identification of compatible cell donors with optimal virus-specific T cell responses. This study aims to characterize cytomegalovirus (CMV), Epstein-Barr virus (EBV), Adenovirus (AdV), and BK virus (BKV)-specific T cell responses in relation to class I and class II HLA genotypes of donors, using samples from a cell donor registry (ReDoCel). Peripheral blood mononuclear cells from cell donors were stimulated with peptide pools from viral proteins, and interferon-gamma production was analyzed using ELISpot and validated by flow cytometry. Our findings provide an overview of the T cell responses to main viral proteins, correlated with HLA alleles in healthy donors. Notably, certain alleles were associated with either enhanced or diminished T cell responses. Furthermore, our results suggest that HLAG 3'UTR variants influence the CMV-specific T cell response. Additionally, HLA-B leader dimorphism, specifically the presence of threonine (T) at position 2, influences the BKV-specific immune response, resulting in higher T cell activity. This study contributes valuable insights into virus-specific T cell responses linked to donor HLA genotypes, improving the selection of optimal cell donors for patient-specific therapy.

ORAL COMMUNICATIONS - SESSION III: Transplantation and Infections

Salmonella typhimurium infection model in Wistar rats for immune function analysis

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Introduction: Infection models are crucial tools for investigating immune responses and understanding how various factors such obesity andother metabolic disorders affect disease susceptibility and severity.

Objective: The aim of the study was to develop a reliable and reproducible Salmonella typhimurium infection model in rats to study immune dynamics.

Methods: 8-week-old Wistar rats were either left uninfected or infected with one of three doses (D) of S. Typhimurium, with D1 receiving 105CFU/mL, D2 receiving 107CFU/mL, and D3 receiving 108 CFU/mL.Body weight, chow, and water intake were recorded daily, and on day three postinfection, organ morphometry and hematological parameters were evaluated. Mesenteric lymph node (MLN) cells were isolated and characterized by flow cytometry, and plasma immunoglobulin (Ig) levels were assessed by multiplex immunoassays.

Results: While no significant changes in body weight or physiological parameters were observed across doses, the highest infection dose (D3) resulted in a reduction in the relative proportion of circulating lymphocytes and an increase in granulocytes. D3 also induced notable shifts in lymphocyte subpopulations, including an elevated proportion of cytotoxic T cells and altered CD8αβ and CD8αα expression pattern. Plasma Ig analysis revealed a significant increase in IgG, particularly IgG2c, along with elevated IgM levels only in the D3 group correlating with a pronounced Th1-associated response.

Conclusion: The infection model using the highest dose provides a robust framework for investigating immune function in conditions associated with increased infection risk, such as obesity.

ORAL COMMUNICATIONS - SESSION III: Transplantation and Infections

Scorpion venom components target ion channels and Toll-like receptors in macrophages

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Scorpion venom components are ion channels modulators, known for their ability to stimulate the immune system through the neuroendocrine-immune axis and by interacting with ion channels and transporters expressed in both innate and adaptive immune cells. Adding to that, scorpion venom toxins are able to target innate pattern recognition receptors (PRRs). Macrophages are key components of the inflammatory response induced by scorpion venom and potassium channels are pivotal for the regulation of their immunomodulatory responses. The present study was undertaken to investigate the effect of scorpion venom on KV channels in murine resident peritoneal macrophages and analyse the signalling cascade, upstream of inflammatory cytokines expression after venom-stimulation of mouse alveolar macrophages (MHS). Data of electrophysiological assays revealed a differential block of KV current between resting and LPS-activated macrophages. Scorpion venom significantly reduced KV current amplitude and the use dependent decline, decreased the degree of inactivation and decelerated the inactivation process of KV current in LPS-activated macrophages. The venom exerted a similar blocking effect on KV1.3 current compared to KV current in LPSactivated macrophages, indicating a direct current inhibition mechanism by targeting KV1.3 subunits. Interestingly, scorpion venom induced significant increase of TNF-α, IL-1β, and MIP-2 expression in alveolar macrophages cell line MH-S. The pre-treatment with inhibitors showed that cytokine increase involves TAK1, IKκ-β, and ERK1/2 pathways, similarly to Toll-like receptors activation. These findings provide evidence that scorpion venom could be endowed with immunomodulatory potential by targeting macrophage voltage-gated potassium channels and TLR signalling.

ORAL COMMUNICATIONS - SESSION IV: Tumor Immunology

Analysis of the HLA-DR Immunopeptidome Derived from Dendritic Cells Pulsed with Tumor Extracts

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The anti-tumor immune response is critical for eradicating cancer cells and is frequently associated with positive patient outcomes. The presence of Tumor-Infiltrating Lymphocytes (TILs) serves as a biomarker, underscoring the necessity of interaction between the immune system and the cancerous tissue. CD4+ T lymphocytes play a crucial role during the initial phases of tumor development as key orchestrators of the immune response. Effector T cell activation depends on the specific interaction between T-Cell Receptors (TCRs) and Major Histocompatibility Complex (MHC) class II peptide complexes on Antigen-Presenting Cells (APCs). Our previous research demonstrated a greater homogeneity of CDR3 motifs among CD4+ T cells, compared to CD8+ T cells, suggesting a less diverse immunopeptidome presented by MHC-II molecules. This high overlap of class II peptides among different pulsed Dendritic Cell (DC) samples has been previously observed in a preliminary study.

We collected immunopeptidomics data from DCs obtained from HLA-DR1 and HLA-DR11 donors. These DCs were pulsed with protein extracts derived from three different molecular breast cancer subtypes: luminal A (LA), luminal B (LB) and triple-negative (TN). The main objective was to confirm the previously observed peptide and protein overlap and to identify class II tumor-associated peptides.

The results demonstrated that: (i) DR1 binds most peptides with high affinity, whereas DR11 exhibits a lower peptide-binding strength; (ii) a significant proportion of ubiquitous proteins was detected; (iii) proteome data from the cancer subtypes facilitated the identification of certain class II peptides derived from cancer-relevant proteins: acid ceramidase and lumican in LA-pulsed samples; ER resident protein 29 in LB-pulsed samples; and Talin-1 in TNBC-pulsed samples.

Overall, the presentation of tumor antigens depends not only on the protein source but also on the combination of HLA-DR alleles. Such analyses are essential for proposing cross-patient interventions of CD4+ TILs through the design of therapies utilizing peptide-pulsed DCs.

ORAL COMMUNICATIONS - SESSION IV: Tumor Immunology

Therapeutic impact and reactivation of tumor immunity by MYC inhibition in KRAS-driven NSCLC with diverse mutational landscapes

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KRAS is the leading mutated oncogene in NSCLC and concomitant mutations in tumor suppressor genes (TSGs) influence treatment response. MYC is downstream of KRAS, driving tumor progression and immune evasion. Although considered undruggable, our lab has pioneered Omomyc as the first-in-human MYC inhibitor. Here, we show how TSG mutations influence the tumor immune microenvironment (TIME) and affect the response of KRAS-mutant tumors to MYC inhibition (MYCi).

KRAS-Lung-Adenocarcinoma isogenic cell lines were CRISPR-edited to knockout TRP53, STK11 or KEAP1 genes. MYC levels were determined by WB, differential expressed genes by RNAseq and response to Omomyc by proliferation and transcriptomic assays. In vivo, lines were inoculated in C57BL/6x129/Sv F1 mice and tumors were treated intravenously with Omomyc. The TIME was characterized by FACS and RNAseq.

Upregulation of MYC and MAPK cascade was observed in vitro in the TSG-edited cells, along with downregulation of inflammatory and immune-related pathways. In vivo, tumors of all mutational profiles evolved towards an immunosuppressive phenotype over time. Interestingly, each TSG mutation uniquely shaped the TIME. In vitro, Omomyc treatment reduced cell growth and modulated genes associated with lymphocyte-mediated immunity across all lines. In mice, systemic MYCi resulted in different therapeutic responses and TIME modulation for each profile. Notably, in the parental and the p53-mutant tumors, MYCi reactivated the anti-tumor immune response, with p53-mutant tumors showing earlier modulation. In contrast, Stk11- and Keap1-mutant tumors exhibited more subtle TIME changes. Importantly, in profiles with stronger immune-modulatory effect, Omomyc treatment induced the expression of immune-activating TNFSF members and significantly enhanced the efficacy of TNFSF-targeting immunotherapies.

Prevalent co-occurring TSGs mutations in KRAS-driven NSCLC create an immunosuppressive TIME and influence the therapeutic effect of MYCi. Interestingly, stronger Omomyc efficacy correlates with enhanced tumor immune reactivation, and its combination with immunotherapies leads to remarkable therapeutic effects, positioning it as a promising partner for immuno-oncology.

ORAL COMMUNICATIONS - SESSION IV: Tumor Immunology

Use of the TCR (T-cell receptor) as a biomarker for monitoring irAEs (immune-related adverse events) in breast cancer

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The activation of tumoral infiltrating lymphocytes (TILs) following immune checkpoint inhibitor (ICI) treatment is unpredictable due to unclear molecular targets of their receptors. While tumor-specific lymphocytes attack cancer cells, bystander lymphocytes can also react post-ICI. Tumor-associated antigens may trigger autoreactive lymphocytes to attack other organs, leading to immune-related adverse events (irAEs). However, these irAEs are often not detected promptly due to insufficient molecular monitoring after initial treatment, complicating early identification and personalized treatment for affected patients.

The study aims to analyze the TCR repertoire in primary breast cancer tumor tissue samples and pericardium affected by irAEs, using paraffin-preserved samples. The central hypothesis suggests that tracking the TCR could serve as a predictive biomarker to monitor dominant TILs in the primary biopsy and their evolution during ICI-treatment.

To achieve this, sequential samples from a breast cancer patient who developed pericarditis following pembrolizumab (anti-PD-1) treatment were analyzed. An experimental protocol was developed and optimized for the TCR repertoire analysis in paraffin-embedded samples, using mRNA purification techniques with magnetic beads, encompassing an initial tumor biopsy, two follow-up biopsies, a surgical biopsy, a non-sentinel lymph node biopsy, and a pericardium biopsy affected by irAEs. The analysis identified several shared clonotypes among different samples, highlighting two that remained present in all tumor biopsies and in the affected pericardium. These results suggest that TCR repertoire characterization may be useful as a biomarker both for treatment response and predicting irAEs.

This study emphasizes the importance of optimizing experimental techniques as refining these methods will provide a clearer understanding of TCR repertoire dynamics and its relationship with immune response to treatment and to the onset of irAEs. Ultimately, these findings reinforce the potential of the TCR as a biomarker in oncology, not only for initial diagnosis but also for long-term monitoring of therapeutic response and immune-mediated adverse effects.

ORAL COMMUNICATIONS - SESSION IV: Tumor Immunology

Tracking changes in TRBV family repertoire in blood after administration of PD-1+ TILs to identify Tumor-Specific TCRs

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Breast cancer remains the leading cause of cancer-related deaths among women. ACT-TILs face two challenges: increasing specificity and preventing relapses. Tracking changes in T-cell Receptor (TCR) repertoire post-TIL therapy can be a valuable strategy for identifying tumor-reactive TCRs. These TCRs can then be utilized to design transgenic TCRs (t-TCRs).

The aim of this project is to track changes in the repertoire of TRBV families within TIL product and in the blood of patients post-therapy to identify **tumor-specific TCRs** and generate a second product by transducing autologous T-cells with transgenic TCRs (t-TCRs).

For this purpose, gDNA was extracted from three samples from NZ-1014-005, patient enrolled in TILS-001 trial (NCT05451784): PD-1+ TIL final product (FP); pre-treatment blood (SCR, screening); and blood from 28 days after treatment (d28). Multiplex PCR was performed to amplify the CDR3β . Second PCR introduced sample-specific barcodes and adapters for NGS. MIXCR© and Immunarch software were used to analyze the clonotypes.

- We identified 3266, 25417 and 4039 unique clonotypes, repeated more than once, from FP, SCR and d28, respectively. A total of 208 clonotypes were found across all three time points.
- TRBV27, TRBV5-6, TRBV6-1 and TRBV7-9 families increased in frequency on day 28 compared to the final product and screening, whereas TRBV12-3, TRBV15, TRBV19, TRBV20-1, TRBV29-1 and TRBV6-5 families decreased in frequency.
- Ten unique clonotypes increased their frequency above 1% at day 28. Only 4 out of the 10 clonotypes contained genes from the upregulated TRBV families, whereas 5 contained genes from the downregulated TRBV families.
- The two most abundant clonotypes in the final product drastically decreased in frequence at day 28 post-treatment.

In conclusion, monitoring changes on frequency of unique clonotypes, instead of focusing on TRBV families, allows for the identification of upregulated CDR3β sequences, which could serve as potential candidates for the design of t-TCR T cells.

ORAL COMMUNICATIONS - SESSION IV: Tumor Immunology

Characterizing TCR and gene expression diversity in NY-ESO-1-specific HLA-DRB3*02:02-restricted CD4+ T Cells from healthy donors using single-cell RNA and TCR sequencing

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Antigen-specific natural transgenic T-cell receptors (tTCRs) have been successfully isolated against NYESO-1 119-143, a cancer/testis antigen that is minimally expressed in healthy tissues but broadly present in various tumors, making it a promising target for immunotherapy. Moreover, NY-ESO-1 avoids off-tumor ontarget toxicity which is a common risk of therapies targeting tumor-associated antigens (TAAs). Unlike CAR T-cell therapies, which are restricted to targeting extracellular proteins, tTCRs enable the recognition of peptides derived from both intracellular and extracellular proteins, broadening their therapeutic potential. TCR-based immunotherapies are usually directed towards HLA class I alleles, which imply an elevated risk of HLA class I downregulation, a common mechanism by which tumour cells evade immune detection. To address this, our study focused on the HLA-DRB3*02:02 allele, which is expressed in approximately 50% of the Caucasian population. CD4+ T cells were pre-sensitized and expanded in vitro using NY-ESO-1119-143-pulsed monocytes. Specific T cells expressing CD4 and CD154 markers were sorted and underwent multiple rounds of expansion to increase their numbers and enhance specificity. After 30 days of culture, a highly specific population of CD4+ CD25- T cells targeting NY-ESO-1119-143 presented on HLADRB3* 02:02 was obtained. Purity assessments revealed a sustained purity of 97%, with specificity significantly improving after the second sorting round. Single-cell RNA sequencing (scRNAseq) and T-cell receptor sequencing (scTCRseq) were successfully performed, and the data were analyzed to identify potential TCR candidates for further therapeutic development. These findings demonstrate the feasibility of isolating specific TCRs from CD4+ T cells that target NY-ESO-1119-143 in the context of HLADRB3*02:02, supporting the potential for this strategy to be developed as an effective immunotherapy targeting NY-ESO-1119-143-expressing tumors.

ORAL COMMUNICATIONS - SESSION IV: Tumor Immunology

Differential CD6 mRNA expression associates with adverse prognostic markers in chronic lymphocytic leukemia patients

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CD6 is a signal transducing receptor expressed by all T cells and a subset of B (B1a) and NK(CD56dim CD16+) cells. It physically associates with the antigen-specific clonotypic receptor of T (TCR) and B1a (BCR) cells, where it modulates the activation and differentiation signals delivered along lymphocyte development and upon peripheral antigen recognition, likely through interaction with its reported ligands (i.e., CD166/ALCAM, CD318/CDCP-1, galectin 1 and 3). CD6 is also well expressed in some B cell malignancies (e.g., CLL), though little is known on whether it plays any role in their biological and clinical behavior. Limited information available on this regard shows that CD6 crosslinking provides CLL cells with anti-apoptotic signals. Within this framework, the purpose of this study was to evaluate the potential prognostic effect of CD6 differential expression in a CLL patient cohort. The study, that includes 270 CLL patients from the CLL-ES ICGC study with available RNA-Seq data, shows that low CD6 expression associates with unmutated IGHVstatus and predictive of shorter time to first treatment in a uni- and multivariable model along with the lymphocyte count and the CLL International Prognostic Index. Further gene set enrichment analyses (GSEA) showed association of low CD6 expression with upregulation of MYC-regulated, mitotic spindle-related and RNA splicing-associated genes, all of them being positively related to cancer progression. Interestingly, CD38 and ITGA4 (CD49d), two widely studied adverse prognostic markers in CLL, were significantly upregulated in the CD6 low group in agreement with immunophenotyping data. These results reinforce the notion that CD6 may play a pivotal role in neoplastic B cell biology and lays the groundwork to further explore CD6 expression in the context of CLL prognostication.

POSTERS

Perfil de sensibilització molecular de la població d'estudi de CLILAB Diagnòstics: àrea territorial Penedès-Garraf, Anoia, part del Barcelonès i del Baix Llobregat

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Introducció: Pel diagnòstic de la patologia al·lèrgica és important detectar polisensibilitzacions. Per això, el laboratori ha disposar de microarrays que permetin analitzar simultàniament diverses fonts al·lergèniques.
Objectiu: Analitzar les freqüències de sensibilització molecular a la nostra població d'estudi. Materials i mètodes: S'han analitzat 867 microarrays(ALEX de MacroArray Diagnostics GmbH). S'han classificat els al·lèrgens entre: inhalants, alimentaris, marcadors de reactivitat creuada. S'ha calculat el percentatge de sensibilització i la mitjana de IgE específica dels positius(⩾ 0.35kU/L).
Resultats:
Inhalants:
Pòl·lens: 36.6% a Ole e1(□=11.76 kU/L), 34.9% a Fra e1(□=11.35 kU/L), 31.60% a Phl p1(□=10.20 kU/L), 29.3%(□=5.99kU/L) a Cyn d1, 24.40%(□=7.65kU/L) a Lol p1.
Epitelis: 32.40%(□=11kU/L) a Fel d1, 15.20%(□=9.50kU/L) a Can f1 i 17.50%(□=7.18kU/L) a Can f5.
Àcars: 30.45%(□=11.34kU/L) a Der f1, 39.50%(□=23.61kU/L) a Der f2, 33.6%(□=13.37kU/L) a Der p1, 39.40%(□=23.47kU/L) a Der p2, 39.7%(□=12.74kU/L) a Der p23, 17.40%(□=6.3kU/L) a Gly d2, 11.50%(□=7.33kU/L) a Lep d2.
Espores: 19.20%(□=16.76kU/L) a Alt a1
Alimentaris
Origen vegetal: sensibilització primària baixa i en context de sensibilitzacions a LTP i proteïnes d'emmagatzematge.
Origen animal: sensibilització baixa a proteïnes de la llet de vaca i a l'ou: Bos d4(2.2%; □=1.59kU/L), Bos d5(1.9%; □=5.94kU/L), Bos d8(1.7%; □=13.72kU/L); Gal d1(1%; □=8.22kU/L), Gal d2(2.10%; □=4.82kU/L), Gal d3(2.2%; □=5.31kU/L), Gal d4(1.40%; □=4.34kU/L). Sensibilització primària a peixos i mariscos baixa i en context de sensibilitzacions a tropomiosines i β-parvalbúmines.
Marcadors de reactivitat creuada
Sensibilització a LTP: 22,3%(□=8.95kU/L) a Pru p3.
Conclusions: La nostre població presenta sensibilització a al·lèrgens inhalats, principalment al pol·len del freixe, de la olivera, d'algunes gramínies i als àcars. També es detecta elevada sensibilització a LTPs. La sensibilització a llet i ou és baixa, probablement degut al baix percentatge de població pediàtrica que inclou la nostre població.

POSTERS

Complement activation - related pseudoallergy (CARPA): A case report

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Background: Practice reveals the relative high frequency of non-IgE mediated severe hypersensitivity reactions (SHR). Since the 80s, Paclitaxel, has been associated with SHR, some fatal, which is why patients receiving this treatment must be pre-treated with corticosteroids and antihistamines, even though this measure has not been proven 100% effective. We present a case of anaphylaxis to Paclitaxel associated to direct complement activation (DCA).

Case summary: A 62 years old female diagnosed with endometrial carcinoma, underwent cytoreduction surgery and subsequent adjuvant treatment with Paclitaxel-Carboplatin. After first infusion of Paclitaxel she presented anaphylactic shock that reverted after treatment with corticosteroids, antihistamines, adrenaline and cardiorespiratory support. Analytical results showed: tryptase 8.0 ug/L, tIgE 642.0 kU/L, C3 1.13 g/L (nv 0.87-1.70 g/L), C4 0.28 g/L (0.11-0.54 g/L) and CH50 17 U/mL (nv 28-60 U/mL). Prick test and basophil activation test to paclitaxel were negative after 1 week of the reaction. Further Complement studies also detected increased levels of anaphilotoxins C3a (24.4 µg/mL; nv 6.7-11.7 ng/ml) and C5a (103 ng/mL; nv 11.5-77.8 ng/mL), as well as of membrane attack complex (MAC) sC5b-9 (1298 ng/mL (nv 187-525 ng/mL). For drug readministration, desensitization could not be achieved since a new anaphylactic shock appeared.

Conclusion: Based on SHR occurring upon the first contact with paclitaxel, no evidence of specific IgE could be obtained and significantly increased anaphylotoxins and soluble MAC serum levels, we consider this to be a SHR mediated by DCA. Non-IgE mediated SHR involving DCA are relatively frequent, but little considered when making in vitro diagnosis. So far there is no difference in the medical management of IgEmediated and non IgE-mediated anaphylaxis. Therefore, it would be important to differentiate them and to consider the possibility of implementing preventive treatments directed against complement that could prove to be more effective than current preventive treatment.

POSTERS

Chronic lymphocytic leukemia patients with non-hematologic autoimmune disease present particular clinical and immunological alterations in Th17 and Treg cells.

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Chronic lymphocytic leukemia (CLL) presents with severe immune dysfunction, increasing the risk of autoimmune disorders (AID). While autoimmune cytopenia (AIC) has been extensively described in patients with CLL, the clinical characteristics and pathogenesis behind non-hematologic AID are poorly understood.

We seek AID in a retrospective single-center series of 907 patients with a confirmed diagnosis of CLL. After a median follow-up of 6.6 years (0.1-36.4), 10.9% presented non-hematologic AID, 5.1% AIC, and 1.2% both AIC and non-hematologic AID. One of the most frequent non-hematologic AID was psoriasis (2.5%). Patients with CLL and non-hematologic AID had a significantly longer time to first treatment (TTFT) (13.8 vs 3.6 years; $p<0.001$) and overall survival (OS) (15.1 vs 9.9 years; $p=0.005$) than patients with CLL and AIC.

Blood sambles were collected to study T/NK cell populations in patients with CLL with and without psoriasis; non-CLL individuals with psoriasis were included as a control. Patients with CLL and concomitant psoriasis showed significantly higher counts of Th17 and Treg cells [Th17 263 cells/mL vs 142 cells/mL ($p=0.009$); Treg 66 cells/mL vs 30 cells/mL ($p=0.01$)] and significantly better prognosis (TTFT at 15 years 65.7% vs 48.1%, $p=0.04$), compared to those with CLL without AID. T cell populations in individuals with psoriasis was similar to those patients with CLL and psoriasis, except in Tregs that were higher in the CLL population. Using an unsupervised hierarchical clustering including CLL with and without AID, we found a cluster exclusive of patients with CLL and psoriasis characterized by higher counts of Th17 and Treg cells and lower counts of T-CD8+ and T-γδ cells.

The expansion of Th17 and Treg cells in patients with CLL and psoriasis could explain the lower risk of leukemic progression. Functional studies are ongoing.

POSTERS

Modified radioimmunoassay versus ELISA to quantify anti-acetylcholine receptor antibodies in a mouse model of myasthenia gravis

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In mouse models of myasthenia gravis (MG), anti-acetylcholine receptor (AChR) antibodies can be quantified to monitor disease progression and treatment response. In mice, enzyme-linked immunosorbent assay (ELISA) is the gold standard to quantify these antibodies. However, this method requires antigen purification, which is both time-consuming and expensive. In humans, radioimmunoassay (RIA)-which is more sensitive than ELISA-is commonly used to quantify AChR antibodies. At present, however, no commercial RIA kits are available to quantify these antibodies in mice. The aim of this study was to compare a modified commercial human RIA kit to two ELISA methods to detect AChR antibodies in an experimental autoimmune mouse model of MG (EAMG). C57BL/6 J mice were immunized with purified AChR from Tetronarce californica (T-AChR). Serum samples were analyzed by RIA and two ELISAs (TAChR and purified mouse AChR peptide [m-AChR]). The modified RIA showed excellent sensitivity (84.1 %) and specificity (100 %) for the detection of AChR antibodies. RIA showed a good agreement with TAChR ELISA (κ = 0.69) but only moderate agreement with m-AChR ELISA (κ = 0.49). These results demonstrate the feasibility of modifying a commercially-available RIA kit to quantify AChR antibodies in EAMG. The advantage of this technique is that it eliminates the need to develop the entire methodology inhouse and reduces inter and intra-laboratory variability.

POSTERS

Nanoparticle Immunotherapy for Myasthenia Gravis: Effects of Autoantigen-Loaded Liposomes in a Mouse Model

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Myasthenia Gravis (MG) is a rare chronic neuromuscular autoimmune disease characterized by antibodies targeting the acetylcholine receptor (AChR), leading to fatigue and muscle weakness. An innovative immunotherapy developed by Ahead Therapeutics, based on phosphatidylserine (PS)-liposomes loaded with disease-specific autoantigens, has proven effective in various preclinical models of autoimmune diseases. Currently, it is being tested in the Experimental Autoimmune Myasthenia Gravis (EAMG) mouse model, induced by the administration of purified T-AChR. Previously we demonstrated that PSAChR-liposomes significantly reduced the production of autoantibodies to AChR and improved MG symptoms. The aim of this study was to assess the safety, absence of side effects, and therapeutic effects of PSAChR-liposomes in EAMG mice. On one hand, PSAChR-liposomes were i.v. injected in EAMG mice, and biochemical, hematological, and coagulation parameters were determined at the end of the study. The weight and histology of organs of interest were assessed. On the other hand, EAMG mice treated with liposomes and control groups performed a Treadmill Fatigue Test once a week, where mice were forced to run to exhaustion on a gradually accelerating treadmill, receiving shocks when they stopped. The results show that our liposomes do not affect biochemical, hematological, and coagulation (Prothrombin Time) parameters. As expected, PSAChR-liposomes only mesenteric lymph nodes weight was altered when compared to control group, correlating to liposome concentration. No effect was observed on thymus, spleen, cervical lymph nodes, liver, or kidney weight. Interestingly, mice treated with PSAChR-liposomes (iv) significantly ran longer distances, received fewer shocks, and showed higher exercise tolerance in comparison to the control group. In summary, the study confirms the safety of PSAChR-liposomes, revealing no major impact on organ weight or adverse side effects, while halting disease progression.

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POSTERS

Comparative Evaluation of Methods for Measuring Anti-Transglutaminase IgA Antibodies: Impact on Celiac Disease Management and Monitoring

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Introduction: Anti-transglutaminase IgA antibodies are crucial for diagnosing celiac disease and monitoring adherence to a gluten-free diet. However, different methods for detecting these antibodies lack standardization, potentially complicating patient follow-up and management.

Objective: To evaluate various methods for measuring anti-transglutaminase IgA antibodies to ensure confidence in case of a change in methodology due to public tender processes.

Methods: We compared 45 samples categorized as negative (6), non-pathological (15), and pathological at low and high levels (24). The anti-transglutaminase IgA antibodies were measured using three different instruments (two technologies): Phadia250 (FEIA), Bioflash (CLIA), and Zenit Prime (CLIA). We performed statistical analyses (agreement and regression) for pairwise comparisons, investigated discrepant samples, and conducted follow-up to determine which method was closest to the clinical decision point.

Results: The Cohen's kappa agreement coefficient was 0.79 (Phadia and Bioflash), 0.86 (Phadia250 and Zenit Prime), and 0.93 between both CLIA systems. The Spearman correlation coefficients were 0.85 (Phadia and Bioflash), 0.86 (Phadia250 and Zenit Prime), and 0.97 between the two CLIAs. Bioflash results were consistently higher (seven times more elevated), suggesting that conversion formulas should be applied for patient follow-up. We used the normality thresholds established by the manufacturers (10 for ELIA and Zenit Prime CLIA; 20 for Bioflash). The highest number of discrepancies occurred between Phadia250 and Bioflash (n=6). For three samples, Phadia yielded negative results while Bioflash detected positive results, which were later confirmed as celiac disease patients. No diagnosis was confirmed for samples positive by Phadia but negative by both CLIA systems.

Conclusions: All three methods show good qualitative agreement. CLIA is more sensitive, particularly for long-term monitoring, as it offers a broader measurement range. Findings support lowering ELIA positivity to five and suggest establishing a grey zone for CLIA tests as well.

POSTERS

Evaluating the Consistency of IgE Ratio 1 Across Different Measurement Platforms in the management of Allergy

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Background: IgE ratios, such as ratio 1 (we-sIgE/tIgE) and ratio 2 (c-sIgE/we-sIgE), are widely recognized in the literature as valuable diagnostic tools for identifying and managing allergic conditions. Several studies emphasize the need to use the same technique to measure IgE values for both ratios. However, since platforms for measuring total IgE are calibrated against the WHO international standard, significant differences in total IgE levels between platforms should not be expected. Thus, ratio 1 should theoretically remain unaffected by the platform or technology used.

Objective: With advancing technology and the increasing use of commercial platforms for allergy diagnosis, this study aims to assess whether significant differences exist in the IgE ratio 1 when calculated using different platforms for measuring total and specific IgE.

Methods: 83 clinical samples were analyzed. Specific IgE was measured using the ImmunoCAP System (Phadia, Uppsala, Sweden), while total IgE was measured using two platforms: the ImmunoCAP System and Atellica (Siemens Healthcare GmbH, Erlangen, Germany). IgE ratios were then calculated for each sample. Statistical analyses, including linear regression and concordance evaluations, were performed to assess potential differences between the two platforms.

Results: Regression analysis showed no statistically significant differences in IgE ratio1 between the two platforms used to measure total IgE ($p > 0.05$). There was strong agreement between the methods, with regression lines showing close alignment and minimal variation.

Conclusions: The use of different platforms for total and specific IgE measurement does not significantly affect IgE ratio 1. These findings allow laboratories to select platforms based on logistical needs without compromising diagnostic accuracy. Further studies are required to evaluate other platforms and IgE ratio 2.

POSTERS

Decoding the tolerogenic function of secreted IgD in monocytes

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Secreted IgD (SIgD) emerges from IgD class-switched plasma cells predominantly located in the nasopharyngeal mucosa. SIgD is the second least abundant antibody in peripheral blood, and its function is still poorly understood.

SIgD can bind to basophils trough a multi-protein receptor. Antigen-specific IgD interaction attenuate basophil IgE-degranulation, and activate the release of tolerogenic cytokines that amplify a protective response. Here, we aim to evaluate the impact of SIgD binding in circulating monocytes. We hypothesized that this interaction enhances the tolerogenic characteristics of monocytes, priming them for their differentiation into dendritic cells (DCs) or macrophages. Circulating monocytes from healthy subjects were analyzed by flow cytometry, IF and WB to assess the monocyte interaction with SIgD. MS was performed to characterize the putative receptor. The signaling program was dissected by culturing monocytes with IgD mAbs and antigens, and then analyzed by RNA-seq and ELISA. Approximately 85% of circulating CD14+ classical monocytes specifically bind and internalize high levels of SIgD through an endocytic and FcR-independent pathway. MS results revealed that SIgD interacts with PRPRT, LRRK2, and HBP, potentially facilitating its binding to monocytes. Upon internalization of the SIgD-Ag complex, there is differential expression of gene products that shape the monocyte phenotype into a tolerogenic profile that correlates with the secretion of IL-10 and BAFF after 24/48h culture.

Our results suggest that the interaction of SIgD with monocytes trigger a tolerogenic signaling program that may help attenuating the responses towards airborne antigens in the nasopharyngeal mucosa.

POSTERS

Multivalent anti-PD-L1 protein nanoparticles as immunotherapy in colorectal cancer

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Immune checkpoint inhibitors targeting the PD-1/PD-L1 pathway prevent T cell inactivation by blocking the interaction between PD-1 on effector T cells and PD-L1 on tumor cells, reactivating the immune response against tumors. Monoclonal antibodies have shown high efficacy, especially in dMMR/MSI colorectal cancer, but face limitations such as low selectivity and side effects. Peptides present a promising alternative due to better tumor penetration, fewer immune-related side effects, and lower production costs, though challenges remain, such as degradation susceptibility and rapid renal clearance.

This study investigated the potential of three multivalent anti-PD-L1 protein nanoparticles (npCLP002, npPEP1, and npPEP2), assembled from peptides that have previously demonstrated tumor growth inhibition by blocking PD-1/PD-L1 interaction in murine colorectal cancer models. Each peptide was incorporated into a modular protein structure containing the peptide sequence, GFP, and a histidine tail, which enabled nanoparticle assembly via zinc divalent cation coordination.

The binding capacity of these anti-PD-L1 nanoparticles to murine colorectal cancer cells overexpressing PDL1 (CT26) was demonstrated in vitro through flow cytometry and confocal microscopy. Their antitumor effect was then evaluated in vivo in a syngeneic murine colorectal cancer model (BALB/c). Intravenous administration of the nanoparticles effectively inhibited tumor growth. Flow cytometry analysis of extracted tumors revealed that all nanoparticles reduced the number of tumor-infiltrating CD4+ T cells. Additionally, npPEP1 and npPEP2 increased the number of tumor-infiltrating CD8+ T cells. Immunohistochemistry further confirmed that npPEP1 increased CD8+ T cells and elevated perforin secretion within the tumor microenvironment. These antitumor effects were comparable to those of the anti-PD-L1 monoclonal antibody used as a control, positioning these nanoparticles as promising alternatives to antibodies, and overcoming the limitations associated with peptides.

POSTERS

Dual PSMA-ICAM1 CAR-T for Advanced prostate cancer treatment

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Introduction: Prostate cancer is the most prevalent malignancy among men. Prostate-specific membrane antigen (PSMA) has emerged as a promising target for CAR-T cell therapies due to its elevated expression on prostate cancer cells. However, PSMA CAR-T therapies present challenges such as dose-limiting toxicities, "on-target, offtumor" effects, and difficulties in sustaining an effective immune response within the highly immunosuppressive tumor microenvironment. To address these issues, dual CAR-T therapy has been developed to enhance T-cell specificity and efficacy. This project aims to develop a Dual PSMA- ICAM-1 CAR-T therapy for advanced prostate cancer treatment, with enhanced antitumor activity, safety and efficacy compared to existing PSMA-specific CAR-T cells. Indeed, elevated levels of Intercellular adhesion molecule-1 (ICAM-1) are found in various carcinomas and their associated stroma, such as in prostate cancer, making it a viable target for dual CAR-T therapies.

Methodology: Initial studies validated the binding capacity of PSMA and ICAM-1 single-chain variable fragments (scFvs) to their respective antigens and their ability to activate CAR responses in second-generation CAR constructs. The PSMA scFv sequence (provided by Dr. Rosato) and the ICAM-1 scFv sequence (from the hybridoma cell line RM3A5 developed in our institution) were used to develop the CAR constructs. Evaluation included ex vivo expansion potential and in vitro antitumor activity using patient-derived tumor cell lines.

Results: Both the second-generation PSMA CAR and ICAM-1 CAR have demonstrated the ability to bind their respective targets, triggering intracellular signaling pathways that activate CAR-T cells, ultimately leading to the destruction of target cells. Specifically, PSMA CAR-T cells exhibited cytotoxic effects against prostate cancer cell lines in vitro, and ICAM-1 CAR-T cells showed the same when derived from ICAM-1 knockout T-cells.

Conclusions: CARs targeting PSMA and ICAM-1 demonstrate the potential for developing Dual PSMA-ICAM-1 CAR-T therapies.

POSTERS

Novel biomarkers in IgG4-related disease

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IgG4-related disease (IgG4-RD) is a chronic, multiorgan fibroinflammatory condition characterized by an infiltration of IgG4 plasma cells in the affected tissue and elevated blood IgG4 levels. The highly heterogeneous manifestations and the incompletely understood pathogenesis make IgG4-RD diagnosis and management challenging, requiring the identification of reliable biomarkers for early diagnosis, monitoring, and an appropriate therapeutic approach.

We hypothesized that a more detailed analysis of novel biomarkers (plasmablasts, other B cell subsets, and cytokines) in IgG4-RD patients could improve clinical and histological classification, accurate diagnosis and management, and predict treatment response. To this end, we conducted two studies from a 31-patient cohort: a transversal study assessing biomarker expression at a specific disease time point, and in the other we compared the biomarkers from naïve patients at disease onset to age- and sex-matched healthy controls.

Our findings showed no differences among the four phenotypic groups; however clustering patients by clinical features revealed significant variations in biomarker expression. Patients with glandular involvement had decreased IgG4 and IgG levels, alongside low IL-13, IL-5, and IL-4, but elevated IL-21, IFN-γ, and IL-10. Those with lumbago exhibited increased IL-5 levels. In patients with kidney involvement, IL-10 and IL-13 levels were elevated, while IL-1β and IL-5 were low. Thorax involvement correlated with high CRP and IgE, and retroperitoneal fibrosis also showed elevated CRP. Histologically, patients with infiltration had low IgG4 and high C4 levels. A negative correlation was found between the responder index and certain B cell subsets, alongside low eosinophil levels in active disease. Interestingly, methotrexate effectively reduced plasmablasts levels in IgG4-RD patients. Naïve patients had high IL-21, IL-4, IL-5, and IL-1β levels, with low IL-10 and IFN-γ compared to healthy controls.

In conclusion, these biomarkers could enhance histological classification, diagnostic accuracy, and treatment response in IgG4-RD patients, though further validation is needed.

POSTERS

Novel PLCG2 Variant Linked to Autoinflammation and Immune Dysregulation in a Family: Clinical Overlap of APLAID/FCAS3

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Mutations in PLCG2, which encodes a phospholipase, are associated with autosomal dominant disorders with some overlapping features: PLCy2-associated antibody deficiency and immune dysregulation syndrome (PLAID/APLAID, when associated with autoinflammation) and familial cold autoinflammatory syndrome (FCAS3).

We present a family with autoinflammation and immune dysregulation. The index case is a 48-year-old woman, initially misdiagnosed with lupus, presenting with episodes of recurrent fever, oral ulcers, abdominal pain, several aseptic cystitis and two episodes of pericarditis. She has two children, aged 17 and 13 years. The youngest developed hemifacial and glottic oedema, knee arthritis, chronic urticaria, prolonged fever, oral ulcers and hyper-IgD. His symptoms worsened with nocturnal myalgias, facial rash and Raynaud's phenomenon. The eldest exhibited chronic diarrhoea, recurrent fever and otitis, skin rash and a longstanding buccal infection. Additionally, her mother and maternal aunt describe similar symptomatology.

Genetic analysis using an NGS Virtual Panel for Primary Immunodeficiencies revealed a novel heterozygous missense mutation in PLCG2 c.239A>G, p.Asn798Ser. Sanger sequencing confirmed the mutation in the mother, both children and affected relatives, compatible with autosomal dominant inheritance.

Immunological studies showed low IgG2, reduced CD4/CD8 ratio and decreased class-switched CD27+ memory B lymphocytes in both children, and low B and NK cell counts in the mother. Acute phase reactants were within normal limits outside of flares. The other relatives were only genetically tested. The mother’s treatment with hydroxychloroquine and Dapsone had been ineffective. Neither child responded to colchicine or Anakinra.

This novel variant suggests a phenotypic overlap between APLAID/PLAID and FCAS3, without antibody deficiency in the patients. Recently, G-CSF has been shown to drive autoinflammation in APLAID, rather than the commonly believed implication of the NLRP3 inflammasome. Therefore, the demonstration of this potential mechanism in our patients would explain the poor response to treatment. In addition, this case broadens the spectrum of PLCy2-related disorders.

POSTERS

Detection of anti-cN-1A autoantibodies in the clinical practice: one centre experience

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Autoantibodies directed against cytosolic 5'nucleotidase 1A (cN-1A) have been used to help distinguish patients with sporadic inclusion body myositis (sIBM) from other idiopathic inflammatory myositis (IIM). However, they can also be found in other systemic autoimmune diseases (SAD), particularly Sjögren's syndrome and Systemic lupus erythematosus.

This study aims to analyse our cohort of anti-cN-1A positive patients using an in-house immunoblot (IB) and to evaluate if quantification of these autoantibodies by ELISA is useful to differentiate anti-cN-1A positive in sIBM from anti-cN-1A positive in other diseases.

Clinical data from 96 anti-cN-1A positive patients by in-house IB was analysed. The most frequent disease group was IIM (28%), being sIBM the most prevalent (30%) among them. However, there were other SAD (24%), organ-specific autoimmune diseases and other pathologies. Sixty-nine anti-cN-1A positive by inhouse IB with available samples were also tested by ELISA, and 54% were confirmed. No significant differences in anti-cN-1A OD were found between IIM, other SAD or other pathologies. Interestingly, we found that the presence of concomitant anti-Ro52 with anti-cN-1A do associate with higher anti-cN-1A levels.

As a conclusion, we found that anti-cN-1A autoantibodies are found in a very heterogeneous group of patients, showing no relevant value in the diagnostic of sIBM when the pre-test probability is low.

POSTERS

A maternal diet enriched with fiber and polyphenols modulates the rat immunoglobulin profile in their offspring at young age

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The Mediterranean Diet (MD) is rich in bioactive compounds, such as fiber and polyphenols, which have been shown to modulate the immune system and may have an immune programming effect. This study aims to evaluate the potential impact of a maternal diet high in polyphenols and fiber during pre-gestation, gestation, and lactation on the immunoglobulin (Ig) profile in the offspring later in life.

The intervention was conducted on 7-week-old rats, with one group receiving an experimental diet enriched with polyphenols and fiber, resembling the Mediterranean Diet (MD group), while the other group was provided a reference diet (REF group). The dietary intervention spanned the pre-gestation (3 weeks), gestation (3 weeks), and lactation (3 weeks) periods. After weaning, the offspring were allowed to mature until reaching young adulthood (7 weeks of age). At that time, Ig concentrations were analyzed using multiplex immunoassay techniques coupled with flow cytometry from plasma and mesenteric lymph node (MLN) samples.

The maternal diet rich in polyphenols and fiber is capable of modifying the Ig pattern in the offspring. Although no significant differences were found in the overall plasma Ig concentrations, there was a significant increase in both IgG and IgM levels in MLN. Additionally, the concentrations in MLN of both the IgG2a and IgG2c isotypes levels were higher in the offspring of mothers that consumed the experimental diet, without affecting their Th1/Th2 isotype associated balance.

As a conclusion, a maternal diet rich in polyphenols and fiber may exert a positive effect on immune programming in the offspring, as it promotes an increase of Ig concentration in MLN during adulthood. These and other immune effects should be further explored.

POSTERS

Cocoa supplementation prevents changes in lymphocyte phenotype in a mice model of coeliac disease

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Background and objectives: Cocoa consumption has been associated with some health benefits including prevention of cardiovascular disease, insulin resistance or modulation of immune function. Nevertheless, a potential role of cocoa in coeliac disease (CD) has never been researched before. To this end, the aim of this work was to evaluate the impact of cocoa intake on the phenotype of mesenteric lymph node lymphocytes (MLNLs) from mice with a predisposition to CD in a gluten-containing diet.

Materials and methods: DQ8-Dd-villin-IL-15tg mice were divided in three groups (n = 14) receiving a gluten free diet (REF), a diet containing gluten and oral administration (p.o.) of a 20 mg gliadin ball/thrice a week (GLI) or the latter with an addition of defatted cocoa (p.o.) at 5g/kg bodyweight prior to gliadin intake (GLI+COCOA). The nutritional intervention lasted 25 days during which multiple pathophysiological and immunological markers were tracked. At day 25, MLNLs were extra- and intracellularly stained using antimouse MoAbs conjugated to various fluorochromes and analyzed by flow cytometry.

Results: Neither gliadin challenge (GLI) nor the cocoa supplementation (GLI+COCOA) had an effect onthe proportion of the main MLNLs populations -B, T, NK or NKT cells- with respect to the REF group.However, MLNLs from animals in the GLI group had a lower global percentage of IL-2 and IFN-ɣproducing cells and within T cells or CD8α-CD4+ T cells than those from REF animals. In addition, a smaller proportion of CD44+ lymphocytes in both NK and NKT cell subpopulations was found in GLI animals with respect to REF ones. In all cases, cocoa supplementation (GLI+COCOA) was able to prevent such change.

Conclusion: Cocoa administration was capable of preventing some of the phenotypical changes in the lymphocytes from MLN associated to gliadin intake in animals with predisposition to CD. This indicates a potential immunomodulatory effect of cocoa in CD.

POSTERS

Clinical and functional characterization of memory CLA+ T-cell response in moderate-to-severe atopic dermatitis patients with high serum levels of Lactate Dehydrogenase

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Background: Lactate Dehydrogenase (LDH) is a well-known serum biomarker associated with atopic dermatitis (AD) disease activity. However, no studies have characterized the clinical features nor the memory CLA+ T-cell effector function in moderate-to-severe AD patients in relation to high or low serum LDH levels.

Methods: A cohort of 56 non-treated adult moderate-to-severe AD patients were stratified into LDHhigh (LDH>206 kU/L) or LDHlow (LDH>206 kU/L) according to the median levels of LDH detected in blood. Next, clinical characteristics and memory CLA+/- T-cell responses were compared between groups and associated with serum LDH levels. CLA+/- T-cell responses were assessed by the coculture of purified circulating memory T cells with autologous lesional epidermal cells activated with house dust mite (HDM) extract.

Results: Patients with LDHhigh serum levels are younger, exhibit increased disease severity, blood eosinophil numbers, and total, HDM- and Staphylococcus aureus-specific IgE plasma levels, compared to LDHlow. More importantly, in contrast to LDHlow, in LDHhigh patients LDH is directly associated with EASI and eosinophilia. Additionally, the number of AD patients suffering from allergic rhinitis and conjunctivitis is significantly higher in the LDHhigh group. The effector function of memory CLA+ T cells activated with HDM showed that LDHhigh patients produced significantly higher levels of IL-13 and IL-9, whereas extracutaneous memory CLA- T cells produced higher amounts of IL-5.

Conclusion: LDH stratification allows identifying LHDhigh AD patients as young individuals with a more severe disease, higher eosinophilia, total IgE, HDM- and S. aureus-specific IgE, prevalence of well-known atopic comorbidities such as allergic rhinitis and conjunctivitis. Furthermore, LDHhigh individuals have a preferential production of IL-13 and IL-9 in CLA+ T cells, and IL-5 by CLA- T cells, which can be related to their higher prevalence of allergic comorbidities.

POSTERS

VitD3tolDC reduce the transmigration of allogeneic CD4+ T cells in an in vitro transwell assay

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Background: Tolerogenic dendritic cells (tolDCs) have emerged as a promising therapeutic strategy for autoimmune diseases like multiple sclerosis (MS). The tolerogenic capacity and clinical potential of tolDCs is achieved through different mechanisms such as induction of T-cell deletion, anergy, or induction of regulatory T cells, which might be able to trigger long-lasting tolerogenic circuits. However, it is not known how tolDCs can mitigate migration of T cells. This study aims to evaluate the ability of Vitamin D3(VitD3)-tolDCs to inhibit the migration of tolerized allogeneic CD4+ T lymphocytes in an in vitro transwell assay.

Methods: VitD3-tolDCs were generated by isolating CD14+ monocytes from buffy coats and inducing differentiation with GMCSF+IL4 in the presence of VitD3 (tolerogenic agent). Phenotypic and proliferation assays were conducted to confirm the functionality of the VitD3-tolDCs. To assess their impact on T cell migration, tolDCs were co-cultured with allogeneic CD4+ T cells for five days. After this time, CD4+ cells were collected and placed in a Boydenn chamber transwell system for six hours. A cocktail of chemokines (CCL20, CXCL16, CXCL9, CCL5, CCL4, CCL17, CCL21) was used as a chemoattractant to simulate CNS inflammatory environment.

Results: VitD3-tolDCs exhibited a functional tolerogenic phenotype, reduced allogenic proliferation and presented a significant number of Tregs following coculture with CD4+ T cells compared to mature dendritic cells (mDCs) differentiated in absence of VitD3. We observed a 29.8 % reduction in the migration of CD4+ T lymphocytes that had been co-cultured with VitD3-tolDCs, compared to those cultured with mDCs (n=5).

Conclusion: VitD3-tolDCs, in comparison to mDCs, effectively reduce the migration of allogenic CD4+ T lymphocytes in transwell assays, using a chemokine cocktail that simulates CNS inflammation. This finding suggests that tolDCs may help to mitigate T cell-mediated inflammation in the CNS, offering a potential therapeutic advantage for treating MS.

POSTERS

CRISPR/Cas9-Engineered PD-1 disrupted anti-CD19 CAR-T cells

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Chimeric Antigen Receptor (CAR) T cell therapy targeting CD19 has demonstrated significant potential in the treatment of B-cell malignancies, such as leukemia and lymphoma. However, current viral-based gene delivery systems exhibit several drawbacks, including the random integration of the CAR transgene into the genome. CRISPR/Cas9 genome editing technology offers a precise alternative, enabling site-specific transgene insertion and facilitating the modification or disruption of key endogenous genes, such as those involved in immune checkpoint regulation. The interaction between Programmed Cell Death protein 1 (PD-1) and its ligand PD-L1 has been shown to impair T cell function, leading to T cell exhaustion and subsequent immune evasion by cancer cells. Disruption of the PDCD1 gene, which encodes PD-1, has been associated with the restoration of T cell activity and enhanced tumor cell clearance. The primary aim of this study is to generate anti-CD19 CAR-T cells with PD-1 disruption through the application of CRISPR/Cas9 gene editing technology. Primary T cells were isolated and stimulated from buffy coats derived from blood samples. Two days later, cells were electroporated with the CRISPR/Cas9 system targeting the first exon of PDCD1. The homology-directed repair template (HDRT) encoded the anti-CD19 CAR construct with two homology arms on either side. CAR expression was assessed out by flow cytometry at day 6 after electroporation. CRISPR/Cas9-generated CAR-T cells killing activity was compared to those generated via lentiviral transduction against the CD19-positive NALM6 cell line. CAR-T cells produced by both methods exhibited a strong killing response against the target cells. This study highlights the feasibility and efficacy of the CRISPR/Cas9 gene editing system for precise CAR transgene insertion, enabling the efficient production of cytotoxic PD-1 disrupted anti-CD19 CAR-T cells.

POSTERS

Synbiotics during pregnancy and lactation: Enhancing maternal and offspring gut and immune function with Bifidobacterium breve M-16V and scGOS/lcFOS supplement

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Previous studies showed that maternal synbiotic supplementation to rats during pregnancy and lactation enhances some aspects of systemic immune function in both mothers and their offspring. This study aimed to evaluate the effects of this same synbiotic on the intestinal barrier function.

For that, pregnant Lewis rats were orally administered with Bifidobacterium breve M-16V and a mixture of short-chain galacto-oligosaccharides (scGOS) and long-chain fructo-oligosaccharides (lcFOS) (SYN group) or vehicle (REF group) daily throughout pregnancy and lactation. The effects of the supplementation were assessed in both mothers and offspring at the end of the lactation period. Intestinal morphology was evaluated using hematoxylin-eosin staining, gene expression was analyzed by quantitative PCR (qPCR), and intestinal immunoglobulin A (IgA) levels were measured by ELISA.

Maternal synbiotic administration induced higher intestinal weight, increased villi height and crypt depth and reduced villi width in mothers. Notably, higher weight of the intestine and a reduction in villi width was also observed in the offspring of the SYN group. The gene expression of Muc3 or Muc2 was upregulated in mothers and pups, respectively. Whereas maternal intestinal IgA levels were not affected by the supplementation, an increase in intestinal IgA was detected in the offspring of the SYN group at the end of lactation.

In conclusion, maternal synbiotic supplementation during pregnancy and lactation positively influences the immune function and structure of the intestine in both mothers and offspring. This demonstrates that some effects produced directly by the synbiotic in the mother can also be induced in her offspring. This opens new strategies for modulating immunity in early life without directly targeting the infant.

POSTERS

Evaluating the effects of a brown seaweed supplement on immune and haematological changes in obese rats infected with Salmonella

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Obesity increases susceptibility to infections and disease severity by disrupting metabolism and impairing immune function (1). High-fat diets (HFD) are linked to obesity development and contribute to these negative effects (2). This study aimed to evaluate whether a brown seaweed supplement could mitigate these effects by assessing immunoglobulin (Ig) plasma levels and haematological variables in a Salmonella typhimurium infection model using adult male and female Wistar rats. The study was conducted over a period of 12 weeks. Animals were divided into three groups based on their diet: a healthy control group fed a reference diet (REF), an obese control group fed a HFD (HFD), and a treatment group fed the HFD with a daily brown seaweed supplement starting from week 8 (SW). At week 12, rats were orally infected with 108 CFU of S. typhimurium, while continuing the dietary interventions during the infection period. Blood and plasma samples were collected three days post-infection for haematological variables by Spincell3 automated analyser; and for Ig analysis using multiplex immunoassays, respectively. Haematological variables did not differ between the REF and HFD groups. However, SW animals showed lower proportions of lymphocytes and monocytes and higher proportions of granulocytes compared to the HFD group, along with lower lymphocyte levels and higher mean corpuscular volume. Regarding Ig levels, HFD females tended to have higher proportions of IgG1 and IgG2a compared to REF animals. SW females also presented increased proportions of IgG2a compared to REF females. In males, SW animals showed a lower Th2-related Ig response than the REF group, with no significant differences in the Th1/Th2 ratio. In conclusion, the brown seaweed supplement modulates immune responses and blood parameters in obese rats, with sex-specific effects. Further research is needed to confirm its efficacy in mitigating HFD-induced alterations.

POSTERS

Microvascular inflammation (MVI) of the renal allograft in the absence of HLA-DSAs and C4d deposition

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One of the main reasons for graft loss following a kidney transplant is currently thought to be antibodymediated rejection (ABMR) of the renal graft. Three histologic criteria have been used to diagnose it since its recognition in 2001: 1) histology consistent with humoral rejection, which is characterized by microvascular inflammation (MVI); 2) serological evidence of circulating HLA-DSAs; and 3) evidence of antibody interaction with the endothelium based on C4d deposition.

However, an increasing number of cases that only fulfil the first criteria are being recognized in the literature. That is the subject of this case clinic report, in which two patients diagnosed with this phenotype of rejection (iMVI) will be presented. In this context, due to the absence of HLA-DSAs and C4d deposition in the presence of MVI, to ascertain the aetiology of the rejection process and choose the most effective treatment plan, research on MICA antibodies and KIR/HLA-I compatibility is requested. The KIR study's operation in both patients will also be explained, as will the reasons why the NK cell might be one of the cellular effectors involved and how its activation brought on by a "missing self" scenario might explain the chronic vascular damage observed in these two patients.

Ultimately, the findings from both investigations will be showcased. While only one of the two patients exhibits a "missing self" condition, both of them complain to the absence of anti-MICA. The ambiguity of the results will be discussed, with particular attention to the fact that a wide range of factors interact to determine the allograft's outcome rather than just one. As a result, a deeper and more comprehensive understanding of the pathophysiology of the process is necessary to guide the best therapeutic approach that best suits the patient's circumstances.

POSTERS

Designing in vitro platforms to study transendothelial T cell migration in colorectal cancer

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Over the years, a better understanding of the tumor microenvironment (TME) in colorectal cancer (CRC) has highlighted its critical role in tumor development and progression. The infiltration of cytotoxic T lymphocytes into the tumor is one of the most predictive factors of prognosis in CRC. Therefore, great interest has been focused on understanding the ability of T lymphocytes to cross the endothelial barrier, navigate the stroma and access the tumor, and how the TME affects this migration. However, studying this process in vivo is extremely difficult and costly, and standard in vitro models are often too simplistic to fully recapitulate the journey of T cells from the blood stream to the tumor. To this end, we use a tiered approach developing increasingly complex in vitro models to dissect the mechanisms underlying transendothelial migration and T cell motility within the stromal microenvironment. Specifically, we investigate distinct migration steps using: (i) a Transwell model cultured with endothelial cells mimicking the leukocyte endothelial transmigration, (ii) and primary T cells embedded in hydrogels mimicking the migration through the stromal compartment. To accurately reflect the signaling cascades present during the early stages of CRC, we use conditioned media from Apc-/- intestinal organoids and compare T cell responses in these models with those in conditioned media from wild-type intestinal organoids. Finally, (iii) a Transwell model that combines the stromal compartment and intestinal organoid to effectively recapitulate the stromal T cell migration dynamics in the presence of an intestinal epithelium. Each of these models will help understand the effect of the different barriers and TME in the T cell migration, providing a toolbox to study the mechanical and biochemical signals during immune cell recruitment within a complex tissue-like CRC model.

POSTERS

Contribution of the different proteasomes to the conformation of the repertoire presented by HLA-I

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The proteasome is the major component of the ubiquitin-proteasome system. It is a large, multi-subunit complex that degrades the vast majority of the proteins located in the cytosol and nucleus. It is also involved in class I antigen presentation pathway, as it degrades cytosolic proteins into peptides that are, thereafter, transported to the ER where they bind MHC-I molecules. Finally, complexes are transported to the plasma membrane, where they show the peptides to CD8+ T lymphocytes. The catalytic 20S proteasome is formed by 4 heptameric rings, the two outer rings are constituted by α subunits, while the inner rings are composed by β subunits. The catalytic activity of the proteasome resides in 3 of the 7 β subunits: β1, β2 and β5, leading to caspase-like, trypsin-like and chymotrypsin-like activities, respectively. In addition to the standard or constitutive proteasome (containing β1, β2 and β5), other proteasomes have been described such us the immunoproteasome (β1i, β2i and β5i), thymoproteasome (β1i, β2i and β5t) as well as intermediate proteasomes: type I (β1, β2 and β5i) and type II (β1i, β2 and β5i).

The objective of this work is to study the specificity of the intermediate proteasomes and their contribution to the conformation of the HLA-I immunopeptidome. To accomplish that purpose, different transfectants expressing exclusively one type of proteasome were generated in HEK-293 cell line. The 20S proteasome was purified from the transfectants, and their specificity was determined by digestion of synthetic or fluorogenic peptides. The type I intermediate proteasome showed a higher tryptic activity. The HLA-I immunopeptidome from intermediate proteasome type I-expressing cells was characterized, showing a reduction of peptides containing small aliphatic residues and an increase of peptides with basic residues at PΩ. Cryo-EM of the intermediate proteasomes has been performed and the data is being analyzed.

POSTERS

Epigenetic Regulation of Microglia and Infiltrating Myeloid cells in the Central Nervous System of Mice with Experimental Autoimmune Encephalomyelitis

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Multiple sclerosis (MS) is an inflammatory and neurodegenerative disease of the central nervous system (CNS), causing damage to myelin, oligodendrocytes, and axons. The early stage is marked by lymphocyte and macrophage infiltration into the CNS, which decreases but persists in later stages. Microglia, the CNS's tissue-resident macrophages, are also activated early and become more involved as the disease progresses. The role of microglia in MS remains complex, as they adopt a degeneration-associated/pro-inflammatory state while also providing benefits, such as phagocytosing neurotoxins and myelin debris, and supporting oligodendrocyte progenitor cell recruitment and maturation.

We aim to elucidate longitudinal changes in microglia and infiltrating myeloid cells in the CNS of mice with experimental autoimmune encephalomyelitis (EAE), an archetypical MS animal model. We studied the epigenetic regulation of these cells by isolating DNA at four time points (baseline, acute, remission, and chronic) along the EAE pathology in SJL/JRj mice and performing methylation analysis.

We used SeSAMe to analyse the raw data of the DNA samples of microglia and myeloid cells and obtained 9504 (delta-beta-value $\geqslant$ 0,1; FDR $\leqslant$ 0,05) and 11524 (delta-beta-value $\geqslant$ 0,2; FDR $\leqslant$ 0,05) differentially methylated positions, respectively, comparing all the different time points. We composed a heatmap to visualize our data and clustered them. With these clusters, we performed gene ontology analysis using the software GREAT (Stanford University) and we analysed enrichment of consensus binding motifs of transcription factors using HOMER (UCSD).

We found remarkable changes in the acute and in the progression to the chronic phase impacting promising genes that possibly play a role in the activation state of microglia and myeloid cells during the clinical relapse in the EAE model. The next step is to confirm this with our transcriptomics data and in vitro experiments.

POSTERS

Impact on different Immune Cell Compartments of monogenic mutations in the CVID spectrum

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Common variable immunodeficiency (CVID) is a primary immunodeficiency characterized by hypogammaglobulinemia and impaired immune responses, leading to recurrent infections, autoimmune disorders, and increased risk of malignancies. Despite advances in understanding CVID, its pathogenesis remains unclear, with monogenic mutations explaining only 20% of cases.

Mutations in NFKB1 and TACI are the most common genes mutated in monogenic forms within the spectrum of CVID, affecting key aspects of immune function. NFKB1 encodes a transcription factor essential for immune regulation, while TACI is critical for B cell function and antibody production. To explore the underlying molecular mechanisms, we employ full-spectrum cytometry to analyze peripheral blood mononuclear cells (PBMCs) from CVID patients with NFKB1 and TACI mutations, non-monogenic patients, and healthy controls. Our analysis focuses primarily on the B-cell compartment, while also considering alterations in T cells and NK cells.

Previous studies suggest that activation reveals significant transcriptomic differences emerge when cells are activated. Thus, a key aspect of our study is the examination of transcriptomic changes and immune cell phenotypes following specific stimulations. Therefore, we set up different combinations of stimuli, time points and concentrations trying to mimic the activation of different immune cell compartments. We ultimately selected a combination of B-cell (BAFF-60mer), T-cell (α-CD3/CD28), and myeloid (LPS) stimuli at 48h to assess gene expression and signaling pathways that may contribute to immune dysfunction in CVID.

Our findings are expected to shed light on the complex pathogenesis of CVID by identifying both shared and unique molecular pathways in patients with and without specific mutations. These insights could advance our understanding of immune dysregulation in CVID, potentially revealing new therapeutic targets for improving outcomes in affected patients.

POSTERS

Análisis descriptivo y retrospectivo del inmunofenotipo de las subclases del linfocito T en pacientes con diagnostico de EPID

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Las enfermedades pulmonares intersticiales difusas (EPID) comprenden un grupo heterogéneo de trastornos pulmonares, caracterizados por la inflamación y fibrosis del tejido pulmonar. Dentro de su patogénesis, el sistema inmunológico, en particular los linfocitos T, desempeña un papel crucial. No obstante, el perfil inmunofenotípico de los linfocitos T en pacientes con diagnóstico probable/definitivo de EPID no está completamente caracterizado.

Este estudio retrospectivo tiene cómo objetivo analizar el fenotipo del linfocito T en pacientes con sospecha diagnóstica/diagnostico establecido de EPID, (neumonía en organización criptogénica(NOC), sarcoidosis y neumonitis por hipersensibilidad(NH)) para una mejor adecuación de tratamientos y una comprensión más profunda de su papel en la progresión de la enfermedad.

Métodos: Se realizó un análisis retrospectivo de 40 pacientes en los que se había realizado el estudio de poblaciones linfocitarias en lavado broqueoalveolar y tenían diagnóstico/sospecha de NOC, sarcoidosis y NH 2023-2024 en el HUVH. El estudio se realizó mediante citometría de flujo, analizando las subpoblaciones de linfocitos junto a un panel de fenotipo avanzado T. Los datos clínicos, demográficos y el inmunofenotipaje fueron recolectados de los historiales médicos.

Resultados: Los pacientes se clasificaron desde el punto de vista clínico en grupos: NOC(n=17), sarcoidosis (n=19) y NH (n=12). El 95% de los linfocitos T tenían fenotipo efector (CD45+CCR7+). La comparación multivariable de los grupos mostró diferencias significativas en la polarización Thelper, encontrando en el grupo de sarcoidosis un aumento significativo del porcentaje de la población Th1-Th17(CCR3+CXCR6+)(NOC, p= 0.0073, NH p=0.0012) comparado con el resto de diagnósticos.

Conclusiones: Este estudio retrospectivo muestra alteraciones significativas en el perfil inmunofenotípico de los linfocitos T en pacientes con EPID, lo que sugiere una posible implicación en la patogénesis y el diagnóstico de estas enfermedades. Detectar estas alteraciones podría ayudar a crear nuevas estrategias de tratamiento enfocadas en el sistema inmunológico para mejorar el manejo de los pacientes con EPID.

POSTERS

Immune monitoring of multiple myeloma patients: beyond hypogammaglobulinemia

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Immune monitoring of multiple myeloma patients: beyond hypogammaglobulinemia Background: Multiple myeloma (MM) treatment has improved significantly, with 5-year relative survival increasing from 37% to 62%. However, novel therapies are associated with increased infection risk, often due to induced hypogammaglobulinemia. Recent studies have revealed reactivation of pathogens typically associated with T-cell depletion and linked low CD4+ T cell counts to poor prognosis in patients with newly diagnosed multiple myeloma.

Objective: To investigate the impact of MM therapies on immune cell subsets in peripheral blood, beyond classical monitoring methods, to assess potential infection risks.

Methods: A retrospective analysis of 49 MM patients diagnosed between 2022 and 2024 was conducted. Patient records were reviewed for demographic data, disease characteristics, treatment history, and clinical outcomes. Immunophenotyping data from peripheral blood samples were analyzed. Patients were classified based on treatment regimens, lines of therapy, and years of disease. Infection occurrences were recorded.

Results: A progressive decline in CD4+ T cell counts was observed among MM patients over the course of their disease. This reduction significantly correlated with the number of treatment lines received and years of disease. Patients treated with CAR-T cell or bispecific antibodies had lower T CD4+ cell counts but also had longer disease duration and more lines of treatment. Patients receiving T cell engagers also had a higher incidence of infections.

Conclusion: These findings highlight the need for comprehensive immune monitoring in MM patients, particularly those receiving advanced therapies such as T cell engagers or CAR-T cell or with prolonged disease. Regular assessment of immune cell subsets especially CD4+ T cells, besides of the classical monitoring multiple myeloma may help identify patients at higher risk for infections. By adopting this holistic perspective, clinicians can better assess immune competence, guide prophylaxis decisions, and potentially improve outcomes in this vulnerable patient population.

POSTERS

Genetic variants in TCF3 and GATA2 genes in a patient with common variable immunodeficiency, uveitis, neurosensorial hearing loss and monocytosis

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Common Variable Immunodeficiency (CVID) is the most prevalent symptomatic primary immunodeficiency, characterized by hypogammaglobulinemia and inflammatory, autoimmune, and neoplastic disorders. TCF3 (Transcription factor E2- alpha) deficiency has been related to a profoundly hypogammaglobulinemia and B cell depletion. However, milder forms have been described in TCF3 haploinsufficiency mutations.

We present a 72-year-old female diagnosed with CVID, bilateral uveitis, neurosensory hearing loss, and chronic myelomonocytic leukaemia, which eventually progressed to acute myeloid leukaemia. The initial diagnosis was CVID, but genetic testing using next-generation sequencing (NGS) revealed a monoallelic predicted loss-of-function (pLOF) TCF3 mutation.

At the age of 65 years the patient presented with unexplained ocular pain, redness, photophobia, asthenia, vertigo and sudden hearing loss, suggesting the diagnosis of Cogan´s syndrome. MRI revealed labyrinthitis. Treatment with corticosteroids improved her symptoms but deafness persisted, requiring a cochlear implant. Leucocytosis and absolute monocytosis were noted in routine blood counts. Bone marrow analysis revealed 2% myeloid blasts and 20% and 10% of mature and immature monocytes respectively, leading to a chronic myelomonocytic leukaemia diagnosis. The disease progressed to acute myeloid leukaemia and the patient died aged 72 years.

Genetic analysis identified a novel heterozygous c.1874delA p.K625Rfs*Ter20 variant in the TCF3 gene resulting in a premature stop codon. In silico prediction showed LOF with high confidence. Additionally, a monoallelic c.457G>A p.G153R variant in the GATA2 gene, classified as a variant of uncertain significance, was identified.

We describe a patient carrying a monoallelic pLOF TCF3 mutation, presenting with B cell defects, reduced serum immunoglobulin levels, and recurrent non-severe infections. The patient’s chronic myeloid leukemia may be linked to TCF3 haploinsufficiency, as hematologic malignancies have been suggested as part of this mutation’s clinical spectrum.

POSTERS

Activation features of circulating innate lymphoid cells in human Type 1 Diabetes

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Innate lymphoid cells (ILC) are lymphocytes of the innate arm of immunity that lack rearranged specific lymphocyte receptors. Functionally, three groups of helper ILC have been described i.e. ILC1, ILC2 and ILC3, that mirror the adaptive T helper type 1, 2 and 17 transcriptional and effector programs. As first line defense, ILCs are enriched in barriers and peripheral tissues but they are also present in circulation at low frequency.

Type 1 Diabetes (T1D) is an autoimmune disease caused by the destruction of insulin-producing pancreatic beta cells. Subjects with T1D develop a metabolic dysfunction and require exogenous daily insulin treatment. Self-reactive T lymphocytes targeting beta cell antigens are well recognized pathogenic effectors in T1D, but innate immunity cells also participate in the disease pathogenesis at early steps.

Here we investigated the immune alterations of circulating ILC1, ILC2 and ILC3 subsets from a small cohort of adult patients with T1D with less than two years of disease progression (n=7), in comparison with age-matched controls (n=11). We applied spectral cytometry in patients with T1D and healthy donor-derived ILCs ex vivo and upon mitogen stimulation, analyzing the frequencies of the ILC subsets and their expression of activation (CD69, HLA-DR) and memory (CD45RO) cell markers. We developed an in vitro model of ILC culture mimicking hyperglycemic conditions.

Results indicate a significantly higher expression of HLA-DR in ILC1 and ILC3 from patients with T1D compared with controls. Upon phytohemmaglutinin stimulation, patient-derived ILC3 showed a statistically higher expression of CD45RO than control ILC3. We did not observe phenotypical differences in ILC cultured in high vs low glucose medium.

Our data indicate that circulating ILC1 and ILC3 subsets have an activated phenotype in human T1D probably reflecting the autoimmune process at a peripheral level and prompt us to continue dissecting the immunological features of ILCs in human T1D.

POSTERS

Challenges in results interpretation: Why clinical phenotype matters in autoantibody detection

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Background: Systemic sclerosis (SSc) is an autoimmune disease characterized by fibrosis of the skin and internal organs. The disease's pathophysiology involves autoantibodies that target intracellular proteins, with the most common being anti-centromere (CENP-A/B), anti-Scl70, and anti-RNA polymerase III (RP3), which are included in the 2013 ACR/EULAR classification criteria. These autoantibodies are detected in the laboratory using various methods: enzyme immunoassay (EIA), particle-based multi-analyte technology (PMAT), and Dot-Blot.

Aims: This study aims to establish a correlation between different methods used for the detection of these autoantibodies and their predictive value. Patients and Methods: We analyzed serum samples from 41 patients collected between January and July 2024 from key departments at Hospital Clínic, including Pulmonology, Rheumatology, Dermatology, and Autoimmune Diseases services, among others, as well as from Granollers Hospital. The detection methods used included EIA for RP3, PMAT for Scl70 and CENP-B, and Dot-Blot for all three autoantibodies (RP3, Scl70, and CENP-B). Additionally, indirect immunofluorescence using HEp2 cells and clinical history data were available for analysis.

Results: For anti-CENP-B autoantibodies, all 10 cases tested (100%) returned positive results using both PMAT and Dot-Blot, regardless of the PMAT value (>5 FLU). Moreover, all cases exhibited a compatible AC-3 pattern on HEp-2 cells. In the case of anti-Scl70 autoantibodies, positive concordance was observed in 5 out of 14 cases (35.7%), with values ranging from 20 to 371 FLU. For anti-RP3 antibodies, 7 out of 22 cases (31.8%) showed positive results on both EIA and Dot-Blot.

Conclusions: The concordance of results for anti-CENP-B autoantibodies was excellent in our cohort, while the concordance for anti-Scl70 and anti-RP3 autoantibodies was not as robust. In cases where results differed between methods (particularly with low to moderate values), the presence of compatible clinical expressions in patients is crucial for enhancing the predictive value of the obtained results.

POSTERS

Deciphering virus-host interactions at the entry site in pigs infected with lethal or attenuated African swine fever virus strains

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African swine fever (ASF) is a pandemic disease causing important worldwide economic and social consequences. The control of the disease, caused by the African swine fever virus (ASFV), is hampered due to the lack of effective vaccines and the poor knowledge on protective immunity. Up to date, live attenuated vaccines (LAV) are the only ones conferring solid protection, despite the associated biosafety concerns. However, the mechanisms associated with the LAV-induced protective immunity are not well characterized. To better understand the early innate immune events induced upon LAV vaccination, in this study we intranasally vaccinated pigs with the LAV Ba71ΔCD2, and temporal immune responses induced locally were compared to pigs lethally infected with the parental ASFV BA71 strain. Virus dynamics across several lymphoid and non-lymphoid tissues collected at 0, 2, 3, 4, and 7 days post-infection/vaccination was analysed by qPCR. Absolute numbers of the main immune cell subsets quantified by flow cytometry showed a clear distinction on their kinetics in lethally infected and vaccinated pigs. Indeed, pigs infected with the virulent virus showed a broad depletion of several immune cells, while in vaccinated pigs their numbers were maintained. Importantly, cytotoxic cells appeared as a distinctive marker of a favourable outcome in vaccinated animals. Altogether, these results contribute to a better understanding of the early protective immunity induced by an intranasal LAV, and thus reveal the immune sequential events to be targeted when developing ASF vaccines.

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