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de Inmunología



SEI 43 CONGRESO DE LA SOCIEDAD ESPAÑOLA INMUNOLOGÍA

LEÓN 22-24 SEPTIEMBRE 2022

PALACIO DE CONGRESOS Y EXPOSICIONES DE LEÓN.

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La SEI apoya a los JÓVENES inmunólogos con BECAS para CONGRESOS nacionales e internacionales que nos permiten conocer los últimos avances en el campo.



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Inmunología

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ÍNDICE

COMITÉS 4

BIENVENIDA 6

PROGRAMA

JUEVES 22 7

VIERNES 23 21

SÁBADO 24 40

EXPOSICIÓN COMERCIAL 43

INFORMACIÓN GENERAL 44

PATROCINADORES 46

LIBRO DE ABSTRACTS 47



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Carta de Bienvenida

Estimados compañeros:

Tras casi dos años en los que la pandemia Covid-19 ha reducido al mínimo las interacciones personales, estamos esperanzados en que esta situación pueda ser finalmente revertida por efecto de las vacunas. Esto nos permitirá poder reunirnos de nuevo para debatir y disfrutar presencialmente de la Inmunología en el marco del 43 Congreso de la Sociedad Española de Inmunología, que se celebrará en León en Septiembre de 2022. Durante este tiempo hemos constatado que, si además incorporamos contenidos virtuales, podremos contar con ponentes de alta calidad que de otra manera sería difícil que pudiesen desplazarse hasta aquí.

Con ese objetivo estamos trabajando con ilusión para organizar un congreso de "reencuentro" en el que abordemos las novedades en investigación y asistenciales de nuestra disciplina, haciendo especial hincapié en la investigación traslacional y en la inmunoterapia. Para ello, contaremos con la presencia de científicos y expertos en las diferentes materias que garanticen un contenido de máxima actualidad y calidad científica.

Estamos seguros que nuestra ciudad, con su encanto y tradición cultural, proporcionará un entorno agradable en el que retomar los contactos personales, aprender, compartir y diseñar nuevos proyectos de colaboración.

En breve, a través de la página web de la SEI, dispondréis de información detallada sobre el programa científico, inscripciones, envío de comunicaciones, becas, premios y toda la información referente al congreso.

Un afectuoso saludo,

Jose M. García Ruiz de Morales
Presidente del Comité Organizador

Marcos López-Hoyos
Presidente de la SEI



PROGRAMA

DÍA 1 JUEVES 22 DE SEPTIEMBRE

08:00-08:30 RECOGIDA DE CREDENCIALES Y DOCUMENTACIÓN CONGRESO

08:30-10:00
SALA PALACIO

PLENARIA 1 COVID 19

Moderadores:

María Montoya González. *Centro de Investigación Margarita Salas, CSIC, Madrid*

Marcos López Hoyos. *Inmunología, Hospital Marqués de Valdecilla, Santander*

Ponentes:

- **Tim Thomson**. *Instituto Biología Molecular de Barcelona (IBMB-CSIC)*
  "The inflammosome in COVID-19: a platform for the discovery of inflammosome inhibitors"
- **José Gómez Rial.** *Inmunología, Hospital Universitario Santiago de Compostela*
  "COVID-19:¿Necesitamos un booster (actualizado)?"

10:00-11:00
SALA PALACIO

SESIÓN 1 TRASPLANTE

Moderadores:

Francisco Boix Giner. *Centro de Transfusión, Hospital General Universitario de Valencia*

María Luisa del Río González. *Departamento Sanidad Animal, Universidad de León*

Ponentes:

- **Rafael Correa Rocha.** *Instituto de Investigación Sanitaria Gregorio Marañón, Madrid*
  "A new approach to prevent graft rejection employing a celular therapy with Treg obtained from thymic tissue. Preliminary results of the "first-in- human" clinical trial"
- **Jordi Cano Ochando**. *Mount Sinai School of Medicine, New York, USA*
  "Trained immunity in organ transplantation"



10:00-11:00
SALA PALACÍN 1

SESIÓN 2 EUROPEAN IN VITRO DIAGNOSTIC REGULATION

Moderadores:

Mª Carmen Martin Alonso. *Centro de Hemoterapia y Hemodonación, Valladolid*

Lourdes Mozo Avellaneda. *Inmunología, Hospital Universitario Central de Asturias*

Ponentes:

- **Isabel de la Villa**. *Departamento de Sanidad, Entidad Nacional Acreditación (ENAC), Madrid*
  "In vitro diagnostic medical devices regulation. View from the Spanish accreditation Agency-ENAC"
- **Manuel Hernández González**. *Hospital Universitari Vall d'Hebron, Barcelona*
  "In vitro diagnostic regulation: impact for clinical immunology"
- **Brice Ezzouaouy**. *Beckman-Coulter, Marsella, Francia*
  "In vitro diagnostic regulation: perspective from a manufacturer. Partnering for a smooth transition"

10:00-11:00
SALA PALACÍN 2

SESIÓN 3 CÉLULAS DEL SISTEMA INMUNE

Moderadores:

Juan E. Viñuela Roldán. *Inmunología, Complejo Hospitalario Universitario de Santiago de Compostela*

Jorge Carrillo Molina. *Instituto de Investigación IrsiCaixa, Barcelona*

Ponentes:

- **Miguel López-Botet**. *Instituto Hospital del Mar de Investigaciones Médicas, Barcelona*
  "Adaptive NK cell response to human cytomegalovirus: facts and open issues"
- **Luis Graça.** *Instituto de Medicina Molecular, Lisboa, Portugal*
  "Regulation of antibody production: from cells to populations"

11:00-11:30
SALA PALACIO

INAUGURACIÓN CON AUTORIDADES

11:30-12:00 **PAUSA CAFÉ**



12:00-13:00
SALA PALACIO

PLENARIA 2 TRASPLANTE

Moderadores:

Jose I. Rodríguez Barbosa. *Área de Inmunología, Universidad de León*

Jordi Cano Ochando. *Mount Sinai School of Medicine, New York*

Ponentes:

- **Megan Sykes.** *Columbia Center for Translational Immunology, New York, USA*
  "Combined TCR and RNA sequencing to monitor activities of donor-reactive T cells in human transplant recipients"

13:00-14:00
SALA PALACIO

SIMPOSIO BECKMAN COULTER "APLICACIÓN DE LA CITOMETRÍA DE FLUJO EN ESTUDIOS FUNCIONALES DE LOS ERRORES INNATOS DE LA INMUNIDAD"

Moderador:

Carmen Pérez Robles. *Especialista de Aplicaciones en Citometría de Flujo, Beckman Coulter*

Ponentes:

- **Dra. Lucía del Pino Molina.** *Servicio de Inmunología, CIBERER U767, Hospital La Paz de Madrid*
  "Importancia de la estandarización multiplataforma en citometría de flujo"
- **Jose Antonio Delgado García.** *PhD, Especialista de Aplicaciones en Citometría de Flujo, Beckman Coulter*
  "EUROFLOW STANDARIZATION FOR 8C APPROACHES BECKMAN COULTER DxFLEX INSTRUMENTS"

13:00-14:00
SALA PALACÍN 1

SIMPOSIO DIAGNÓSTICA LONGWOOD "SEGUIMIENTO EN TRASPLANTE ALOGÉNICO"

Moderador:

Jesús Ontañón Rodríguez. *Jefe Inmunología Hospital. Universitario Albacete*

Ponentes:

- **Dr. Marcelo Fernández Viña.** *PhD Professor, Department of Pathology, Stanford University Disease Profiling Laboratory, California.*
  "Incompatibilidad de epítopos en trasplante de órgano sólido"
- **Dr. Miguel Alcoceba.** *Departamento de Hematología, Hospital Universitario de Salamanca*
  Validación del kit de AlloSeq HCT para seguimiento de precursores hematopoyéticos.



13:00-14:00
SALA PALACÍN 2

SIMPOSIO HYRIS "DESARROLLO DE UN TEST DIAGNÓSTICO DE PCR PARA LA MONITORIZACIÓN DE LA INMUNIDAD CELULAR VIRAL"

Moderador:

Jordi Cano Ochando. *Mount Sinai School of Medicine,New York*

Ponente:

- **Matteo Audano,** *PhD. Product Specialist, Hyris SRL*

14:00-15:00
HALL EXPOSICIÓN

VISITA PÓSTER

Pantalla 1. Inmunología Básica. (P-001 – P-016)
Moderadores:
África González Fernández. *CINBIO, Universidad de Vigo*
Alfredo Corell Almuzara. *Facultativo Especialista De Inmunología. Catedrático de Inmunología. Universidad de Valladolid*

Pantalla 2. Inmunidad innata (P-017 – P-033)
Moderadores:
David Bernardo Ordiz. *Instituto de Biomedicina y Genética, CSIC, Valladolid*
Jordi Cano Ochando. *Mount Sinai School of Medicine, New York*

Pantalla 3. Inmunodeficiencias: primarias y secundarias (P-034 – P-065)
Moderadores:
Carmen Cámara Hijón. *Inmunología, Hospital Universitario La Paz, Madrid*
Luis Fernández Pereira. *Inmunología, Hospital San Pedro de Alcántara, Cáceres*

Pantalla 4. Miscelánea (Inflamación - Inmunidad anti-tumoral- Alergias y reacciones de hipersensibilidad) (P-066 – P-076 / P-122 – P-134)
Moderadores:
Jaume Pons de Ves. *Inmunología Hospital Universitario Son Espases, Palma de Mallorca*
Yvelise Barrios del Pino. *Inmunología, Hospital Universitario de Canarias*

Pantalla 5. Inmunoterapia (I) (P-077 – P-099)
Moderadores:
Rafael Sirera. *Departamento de Biología Celular. Universidad Politécnica de Valencia.*
Sara María Calleja Antolín. *Inmunología, Complejo Hospitalario Universitario de León*



Pantalla 6. Inmunoterapia (II) (P-100 – P-120)

Moderadores:

Rafael Sirera. *Departamento de Biología Celular. Universidad Politécnica de Valencia.*

Sara María Calleja Antolín. *Inmunología, Complejo Hospitalario Universitario de León*

15:00-16:00
SALA PALACIO

COMUNICACIONES ORALES 2. INMUNODEFICIENCIAS: PRIMARIAS Y SECUNDARIAS

Moderadores:

Carmen Cámara Hijón. *Inmunología, Hospital Universitario La Paz, Madrid*

Luis Fernández Pereira. *Inmunología, Hospital San Pedro de Alcántara, Cáceres*

CO-07

DISSECTING THE EFFECT OF A NOVEL HYPOMORPHIC *IL2RB* MUTATION ON IMMUNE DYSREGULATION

Olivia Bailey[1], Berenice Cabrera-Martinez[1], Josselyn Garcia-Perez[2], Ryan Baxter[1], Isabel Fernandez[1], Mary Jo Foster[1], Laurant Gapin[1], Ross Kedl[1], **Elena Hsieh[1]**.

[1]University of Colorado Anschutz Medical Campus, Colorado; [2]Cellcarta, Brussels.

CO-08

A TRUNCATED P.Y153X CD247 (TCRZ) VARIANT IS STABLE AND FORMS POTENTIALLY PATHOGENIC CD247 HETERODIMERS IN HETEROZYGOSIS

Rebeca F Megino[1], Ana V Marin[1], Julia Fernandez-Boraita[1], Diego J Jimenez[1], Alejandro C Briones[1], Marta Herrero-Alonso[1], Daniel Chacon-Arguedas[1], Elena Garcia-Martínez[2], Elena Seoane-Reula[2], Carmen Rodriguez-Sainz[2], Jose R Regueiro[1].

[1]Department of Immunology, Ophthalmology and ENT, Complutense University School of Medicine and 12 de Octubre Health Research Institute (i+12), Madrid; [2]Gregorio Marañón Health Research Institute, Madrid.

CO-09

CORRECTING APDS2 SYNDROME-CAUSED MUTATION FOR FUTURE GENE AND CELL THERAPIES

Irene Romayor[1], Marta Inglés-Ferrándiz[1], Myriam Martin-Inaraja[1], Lara Herrera[1], Silvia Santos[2], Miguel Ángel Vesga[2], Jose Ramón Pineda[3], Juan Anguita[4], Luis M Allende[5], Luis I Gonzalez-Granado[5], Cristina Eguizabal[1].

[1]Instituto de Investigación Sanitaria Biocruces Bizkaia, Barakaldo; [2]Centro Vasco de Transfusión y Tejidos Humanos, Galdakao; [3]Universidad del País Vasco (UPV/EHU), Leioa; [4]CIC Biogune, Derio; [5]Hospital Universitario 12 de Octubre, Madrid.



CO-10

HETEROZYGOUS NONSENSE CD247 MUTATIONS SHOW POTENTIALLY PATHOGENIC DOMINANT-NEGATIVE FEATURES AFFECTING TCR EXPRESSION AND FUNCTION

Alejandro C Briones[1], Rebeca F Megino[1], Ana V Marin[1], Daniel Chacón-Arguedas[1], Rebeca Chaparro[1], Carmen Rodríguez-Sainz[2], Elena Seoane-Reula[2], Paloma Sanchez-Mateos[3], Hugh T Reyburn[4], Sarah Henrickson[5], Jose R Regueiro[1], Paula P Cardenas[1].

[1]Department of Immunology, Ophthalmology and ENT, Complutense University School of Medicine and 12 de Octubre Health Research Institute (i+12), Madrid; [2]Inmuno-Alergia Infantil. Unidad de Inmunodeficiencias HGU Gregorio Marañón, Madrid; [3]Instituto de Investigación Sanitaria Gregorio Marañón (IiSGM). Laboratorio de Inmuno-Oncología., Madrid; [4]Department of Immunology and Oncology, National Centre for Biotechnology, CNB-CSIC, Madrid; [5]Division of Allergy and Immunology, Children's Hospital of Philadelphia., Philadelphia.

CO-11

CANDIDIASIS MUCOCUTÁNEA CRÓNICA POR GANANCIA DE FUNCIÓN DE STAT1: EXPERIENCIA DEL HOSPITAL UNIVERSITARIO LA PAZ

Laura Miguel Berenguel, Ricardo Cuesta Martín De La Cámara, Andrea González Torbay, Elisabet Matas Pérez, Ángel Robles Marhuenda, María Bravo García-Morato, Carla Gianelli, Carmen Cámara Hijón, Eduardo López Granados, Rebeca Rodríguez Pena.

Hospital Universitario La Paz, Madrid.

CO-12

SOMATIC GENETIC RESCUE CORRELATING WITH CLINICAL IMPROVEMENT IN A PATIENT WITH *TNFRSF9* (CD137/4-1BB) DEFICIENCY

Roger Colobran[1], Marina Garcia-Prat[2], Alba Parra-Martínez[2], Clara Franco-Jarava[1], Daniel Álvarez De La Sierra[1], Aina Aguiló-Cucurull[1], Jacques G. Riviere[2], Andrea Martin-Nalda[2], Pere Soler-Palacin[2], Ferran Casals[3], Laia Alsina[4], Montserrat Torrent[5], Laura Batlle-Masó[2].

[1]Translational Immunology Research Group, Vall d'Hebron Research Institute (VHIR). Servei d'Immunologia, Servei de Genètica, Hospital Universitari Vall d'Hebron (HUVH), Barcelona; [2]Infection in Immunocompromised Pediatric Patients, Vall d'Hebron Research Institute (VHIR). Unitat de Patologia Infecciosa i Immunodeficiències de Pediatria, Hospital Universitari Vall d'Hebron (HUVH), Barcelona; [3]Department of Genetics, Microbiology and Statistics, Universitat de Barcelona (UB), Barcelona; [4]Clinical Immunology and Primary Immunodeficiencies Unit, Pediatric Allergy and Clinical Immunology Department, Hospital Sant Joan de Déu, Barcelona; [5]Paediatric Hematopoietic Transplant Unit, Hospital Santa Creu i Sant Pau, Barcelona.

15:00-16:00
SALA PALACÍN 1

COMUNICACIONES ORALES 1. INMUNOLOGÍA BÁSICA

Moderadores:

África González Fernández. *CINBIO, Universidad de Vigo*

Alfredo Corell Almuzara. *Facultativo Especialista De Inmunología. Catedrático de Inmunología. Universidad de Valladolid*

CO-01

GENOMEWIDE ANALYSIS OF AID TARGET SPECIFICITY IN HUMAN MEMORY B CELLS

Álvaro Serrano Navarro[1], Ángel Francisco Álvarez Prado[2], Fátima Sánchez Cabo[1], Almudena R. Ramiro[1].

[1]CNIC, Madrid; [2]Ludwig Institute for Cancer Research, Lausanne.

CO-02

RESIDENT MEMORY CD8 T CELLS TRIGGER PDC INFILTRATION AND MATURATION IN THE SKIN

Elena Hernández García[1], Francisco Javier Cueto Rodríguez[2], Ana Redondo Urzainqui[1], Sofía Chayeb Khouili[3], Salvador Iborra Martín[1].

[1]Universidad Complutense de Madrid, Madrid; [2]IdiPAZ - Instituto de Investigación Hospital Universitario La Paz, Madrid; [3]Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid.

CO-03

PHOSPHATASES OF REGENERATING LIVER (PRLS) AS REGULATORS OF ACTIN POLYMERIZATION BALANCE THROUGH THE WDR1/ COFILIN/CORONIN COMPLEX IN T LYMPHOCYTES.

Óscar Aguilar Sopeña[1], Matías Estarás Hermosel[2], Patricia Castro Sánchez[3], Carlos Carrasco Padilla[1], Sergio Alegre Gómez[1], Pedro Roda Navarro[1].

[1]Facultad de Medicina. Universidad Complutense de Madrid. Departamento de inmunología, Madrid; [2]Institute of Molecular Pathology Biomarkers. University of Extremadura., Cáceres; [3]Institute of Immunology and Infection Research. University of Edinburgh, Edinburgh.

CO-04

CHARACTERIZATION OF EXTRACELLULAR VESICLES SECRETED BY THYMIC REGULATORY T CELLS (THYTREG-EVS)

Marta Fernández Castillo[1], Rocío López Esteban[1], Verónica Astrid Pérez Fernández[2], Elena Blázquez López[3], Javier Vaquero Martín[3], Rafael Correa Rocha[1], Marjorie Pion[2].

[1]Laboratorio de Inmuno-Regulación, Instituto de Investigación Sanitaria Gregorio Marañón, Madrid; [2]Grupo de Inmuno-Regulación Avanzada, Instituto de Investigación Sanitaria Gregorio Marañón, Madrid; [3]Laboratorio de Investigación en Hepatología y Gastroenterología, Instituto de Investigación Sanitaria Gregorio Marañón, Madrid.

CO-05

HUMAN INTESTINAL CD103+SIRPα+ CONVENTIONAL DENDRITIC CELLS FROM PATIENTS WITH CROHN´S DISEASE PRIME IL-17+ T-CELLS

Elisa Arribas Rodríguez[1], Carolina González De Castro[1], Aida Fiz López[1], Ángel De Prado Santos[1], Luis Fernández Salazar[2], Jesús Barrio Andrés[3], Sandra Izquierdo[2], Javier García Alonso[3], Beatriz De Andrés[2], José María García Abril[3], Alejandro Romero[2], Javier Sánchez González[3], José Antonio Garrote Adrados[1], Eduardo Arranz Sanz[1], **David Bernardo Ordiz[1].**

[1]Instituto de Biología y Genética Molecular, Valladolid; [2]Hospital Clínico Universitario de Valladolid, Valladolid; [3]Hospital Universitario Rio Hortega, Valladolid.

CO-06

FOLDING OF NEWLY SYNTHESIZED PROTEINS REGULATES JURKAT T CELL METABOLISM AND INTERCELLULAR COMMUNICATION

Amelia Rojas Gómez[1], Sara García Dosil[1], Francisco Javier Chichón[2], Nieves Fernández-Gallego[1], Alessia Ferrarini[1], Enrique Calvo[1], Diego Calzada Fraile[1], Silvia Requena[3], Joaquin Oton[4], Álvaro Serrano[1], Rocío Tarifa[1], Montserrat Arroyo[3], Andrea Sorrentino[4], Eva Pereiro[4], Jesús Vázquez[1], Jose María Valpuesta[2], Francisco Sánchez Madrid[3], Noa Martín Cófreces[3].

[1]CNIC, Madrid; [2]CNB, Madrid; [3]Hospital Universitario de La Princesa, Madrid; [4]ALBA Synchrotron Light Source, Barcelona.

15:00-16:00
SALA PALACÍN 2

COMUNICACIONES ORALES 3. INMUNIDAD INNATA

Moderadores:

Jordi Cano Ochando. *Mount Sinai School of Medicine, New York*

David Bernardo Ordiz. *Instituto de Biomedicina y Genética, CSIC, Valladolid*

CO-13

CYTOTOXIC NKG2DHI NK CELLS ACTIVATED BY CD1C+ DENDRITIC CELLS CONTRIBUTE TO PRIMARY SJÖGREN SYNDROME PATHOLOGY

Ildefonso Sánchez Cerrillo[1], Marta Calvet Mirabent[1], Ana Triguero Martinez[1], Diego Calzada Fraile[2], Cristina Delgado Arévalo[1], Mariel Valdivia[1], Marta Ramírez[2], Enrique Vázquez De Luis[2], Alberto Benguría Filippini[2], Roberto Moreno[2], María Magdalena Adrados De Llano[1], Hortensia De La Fuente Flores[1], Ilya Tsukalov[1], Emilia Roy Vallejo[1], Almudena Ramiro[2], Salvador Iborra[2], Francisco Sánchez Madrid[1], Ana Dopazo[2], Isidoro Gonzalez[1], Santos Castañeda[1], Enrique Martín Gayo[3].

[1]Hospital Universitario La Princesa, Madrid; [2]Centro Nacional de Investigaciones Cardiovasculares, Madrid; [3]Universidad Autónoma de Madrid, Madrid.



CO-14

NON-REDUNDANT ROLE OF NF-KB AND INFLAMMASOME SENSORS DRIVING PATHOGENIC DIFFERENTIATION AND ACTIVATION OF MONOCYTES IN RESPONSE TO DISTINCT SARS-COV-2 COMPONENTS

Ilya Tsukalov[1], Ildefonso Sánchez Cerillo[2], Olga Rajas[3], Elena Avalos[3], Gorane Iturricastillo[3], María José Buzón[4], Maritxell Genescà[4], Laura Esparcia[2], Olga Popova[2], Cristina Delgado Arevalo[2], Marta Calvet Mirabent[2], Hortensia De La Fuente[2], Noa Martín Cófreces[2], Ignacio De Los Santos[2], Ceclia Muñoz Calleja[2], Arantzazu Alfranca[2], María José Calzada[2], Isidoro González Alvaro[5], Julio Ancochea[3], Francisco Sánchez Madrid[2], Enrique Martín Gayo[2].

[1]Universidad Autónoma de Madrid, Madrid; [2]Servicio de Inmunología, Hospital Universitario de La Princesa, Madrid; [3]Servicio de Neumología, Hospital Universitario de La Princesa, Madrid; [4]Val d'Hebron Institut de Recerca, Barcelona; [5]Servicio de Reumatología, Hospital Universitario de La Princesa, Madrid.

CO-15

GM-CSF PROGRAMS HEMATOPOIETIC STEM AND PROGENITOR CELLS DURING *CANDIDA ALBICANS* VACCINATION FOR PROTECTION AGAINST REINFECTION

Cristina Bono, Paula Guerrero, Ana Erades, María Luisa Gil, Alberto Yáñez.

Universitat de València, Burjassot.

CO-16

REGULATION OF MACROPHAGE FUNCTION BY AN IFNAR-ISG15-MITOCHONDRIAL AXIS

Gillian Dunphy, Irene Adán Barrientos, Irene Fernández Delgado, Carolina Villarroya Beltri, Andrea Curtabbi, Ignacio Heras Murillo, Aitor Jarit Cabanillas, Miguel Sánchez Álvarez, Miguel Ángel Del Pozo, José Antonio Enríquez, Francisco Sánchez Madrid, David Sancho.

Centro Nacional de Investigaciones Cardiovasculares, Madrid.

CO-17

DIRECT TLR2 SIGNALING THROUGH MTOR AND TBK1 INDUCES C/EBPβ AND IRF7-DEPENDENT MACROPHAGE DIFFERENTIATION IN HEMATOPOIETIC STEM AND PROGENITOR CELLS

Cristina Bono, Pa**U**la Guerrero, Ana Erades, Alberto Yáñez, **María Luisa Gil.**

Universitat de València, Burjasot.



CO-18

OXYGEN-INDEPENDENT REGULATION OF HIF-1α IN ILC3S AMELIORATES THE INFLAMMATION OF C. RODENTIUM-INDUCED COLITIS

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

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

16:00-17:00
SALA PALACIO

COMUNICACIONES ORALES 4. INMUNOTERAPIA

Moderadores:

Rafael Sirera. *Departamento de Biología Celular. Universidad Politécnica de Valencia.*

Sara María Calleja Antolín. *Inmunología, Complejo Hospitalario Universitario de León*

CO-19

ANTIBODY AND T-CELL MEDIATED IMMUNE RESPONSES INDUCED BY CD19 CHIMERIC ANTIGEN RECEPTOR (ARI-0001) IN RELAPSED/REFRACTORY PATIENTS WITH CD19 POSITIVE B-CELL MALIGNANCIES

Ariadna Bartoló - Ibars[1], Europa Azucena González-Navarro[2], Nela Klein - González[3], Berta Casanovas-Albertí[3], Valentín Ortiz-Maldonado[2], Montserrat Torrebadell[4], Marta Español-Rego[2], Maria Castellà[2], Daniel Benítez[2], Raquel Cabezón[2], Jordi Esteve[2], Jordi Yagüe[2], Mariona Pascal[2], Álvaro Urbano-Ispizua[2], Susana Rives[4], Julio Delgado[2], Manel Juan[2].

[1]Fundació Clínic per la Recerca Biomèdica, Barcelona; [2]Hospital Clínic de Barcelona, Barcelona; [3]Instituto de Investigaciones Biomédicas August Pi i Sunyer, Barcelona; [4]Hospital Sant Joan de Déu, Barcelona.

CO-20

PROGRESS TOWARDS A PATIENT-SPECIFIC MONOCLONAL ANTIBODY IN SÉZARY SYNDROME

Marta Herrero Alonso[1], Ana V. Marín Marín[1], Rebeca Fernandez Megino[1], Daniel Chacón Argueras[1], Marcos Viñuela Martín[2], Jose Luis Subiza[3], Jose R Regueiro[1].

[1]Department of Immunology, Ophthalmology and ENT, Complutense University School of Medicine and 12 de Octubre Health Research Institute (i+12), Madrid; [2]Clinical Immunology-Experimental and Surgery Department, Hospital Clínico Universitario San Carlos, Madrid; [3]Inmunotek, Alcalá de Henares.

CO-21

IDENTIFYING SARS-COV-2 'MEMORY' NK CELLS FROM COVID-19 CONVALESCENT DONORS FOR ADOPTIVE CELL THERAPY (RELEASE)

Lara Herrera[1], Myriam Martin-Inaraja[1], Silvia Santos[1], Marta Inglés-Ferrándiz[1], Aida Azkarate[1], Maria Burón[1], Miguel Ángel Pérez-Vaquero[1], Miguel Ángel Vesga[1], Jose Luis Vicario[2], Bernat Soria[3], Carlos Solano[4], Raquel De Paz[5], Antonio Marcos[5], Cristina Ferreras[6], Antonio Pérez-Mártinez[6], Cristina Eguizabal[1].

[1]CVTTH-IIS Biocruces-Bizkaia, Galdakao; [2]Centro de Transfusión de la Comunidad de Madrid, Madrid; [3]ISABIAL, Alicante; [4]Hospital Clínico Universitario de Valencia, Valencia; [5]Hospital la Paz, Madrid; [6]Hospital la Paz-Idipaz, Madrid.

CO-22

DEVELOPING CAR-NK CELLS ON NK-92 CELLS STABLY TRANSFECTED WITH CD16

David Giraldos, Chantal Reina, Nuria Sancho, Isabel Marzo, Javier Naval, Alberto Anel.

Universidad de Zaragoza, Zaragoza.

CO-23

NEXT GENERATION OF TUMOR IMMUNOTHERAPIES BASED IN BACTERIA-TRAINED LYMPHOCYTES

Raquel García Ferreras, Lara Del Campo Milán, Almudena Méndez Pérez, **Esteban Veiga Chacón.**

Centro Nacional de Biotecnología (CNB, CSIC), Madrid.

CO-24

DEVELOPMENT OF NEW TOLEROGENIC PEPTIDE-BASED VACCINE FOR RHEUMATOID ARTHRITIS

Laura Romero Castillo[1], VIlma Urbonaviciute[1], Bingze Xu[2], Rikard Holmdahl[2].

[1]Karolinska Institutet, Estocolomo; [2]Karolinska Institutet, Estocolmo.



16:00-17:00
SALA LOUNGE

REUNIÓN DE GRUPO DE EMBAJADORES GESEI

Moderador:

Isabel Cortegano Jimeno. *Laboratorio de Inmunobiología Centro Nacional de Microbiología - Instituto de Salud Carlos III, Madrid*

16:00-18:00
SALA PALACÍN 1

REUNIÓN TALLER IQ

Moderadores:

Esther Moga Naranjo. *Hospital de la Santa Creu i Sant Pau, Barcelona*

Manuel Hernández González. *Inmunología, Hospital Vall d´Hebron, Barcelona*

16:00-18:00
SALA PALACÍN 2

REUNIÓN TALLER GETHIT

Moderadores:

Jesús Ontañón Rodríguez. *Jefe Inmunología Hospital Universitario Albacete*

Francisco Boix Giner. *Centro de Transfusión, Hospital General Universitario de Valencia*

Ponentes:

- **Dr. Marcelo Fernández-Viña.** Profesor Pathology, Stanford University *"Actualización de la definición de las especificidades HLA a nivel serológico"*
- **Dr. Francisco Boix Giner.** Inmunólogo. Centro Transfusiones Valencia *"Evaluación del beneficio diagnóstico del ADN libre de células derivado de donante (dd-cfDNA) para el trasplante renal".*
- **Dr. Juan Torres Canizales.** Centro de diagnóstico Biomédico (CDB). Servicio Inmunología. H. Clinic Barcelona *"Resultado de la encuesta sobre la prueba cruzada por citometría de flujo en el contexto del trasplante de órganos sólidos "*

17:00-18:00
SALA LOUNGE

COMUNICACIONES ORALES 5. MISCELÁNEA (INFLAMACIÓN/ALERGIAS Y REACCIONES DE HIPERSENSIBILIDAD/ INMUNIDAD ANTI-TUMORAL)

Moderadores:

Jaume Pons de Ves. *Inmunología Hospital Universitario Son Espases, Palma de Mallorca*

Yvelise Barrios del Pino. *Inmunología, Hospital Universitario de Canarias*



CO-25

SPECIFIC CELLULAR REGULATION OF LAMIN A/C IN IMMUNE AND STROMA CELLS AS A THERAPEUTIC TARGET FOR INFLAMMATORY BOWEL DISEASE

Raquel Gómez Bris[1], Beatriz Herrero Fernández[1], Héctor Sánchez Martínez[2], Sara Moran De Bustos[2], Irene Moreno Aperribay[2], Marta Amorós[3], Alberto Del Monte[3], Vicente Andrés[3], Gabriel Criado[2], Alicia Usategui[2], Jose Luis Pablos Alvarez[2], Ángela Saez[4], José María González Granado[2].

[1]Universidad Autónoma de Madrid, Madrid; [2]Instituto de Investigación Hospital 12 de Octubre (imas12), Madrid; [3]Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid; [4]Universidad Francisco de Vitoria (UFV), Madrid.

CO-26

FINDING IMMUNOLOGICAL DIFFERENCES TO HELP DIAGNOSIS AND EARLY TREATMENT OF KAWASAKI DISEASE AND MIS-C (MULTISYSTEM INFLAMMATORY SYNDROME IN CHILDREN)

Rebeca Kennedy-Batalla[1], Elisa Fernández-Cooke[2], Javier Aracil-Santos[3], Jesús Saavedra[4], Alberto García-Salido[5], Manuel José Gómez[6], Teresa Albendea-Gómez[7], Beatriz Escobar[7], Miguel Ángel Cano-Nieto[1], Sofía Mesa[8], Jose Antonio Ruiz-Domínguez[9], Felipe González[4], Silvia Martín-Puig[7], Rafael Correa-Rocha[1].

[1]Instituto de Investigación Sanitaria Gregorio Marañón (IiSGM), Madrid; [2]Unidad de Infectología Pediátrica. Hospital Universitario 12 de Octubre, Madrid; [3]Enfermedades Infecciosas Pediátricas. Hospital Universitario La Paz, Madrid; [4]Pediatría. Hospital Universitario Gregorio Marañón, Madrid; [5]Fundación de Investigación Biomédica Hospital Infantil Universitario Niño Jesús, Madrid; [6]Unidad de Bioinformática. Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid; [7]Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid; [8]Unidad de Urgencias de Pediatría. Hospital Universitario 12 de Octubre, Madrid; [9]Servicio de Urgencias Pediátricas. Hospital Universitario La Paz, Madrid.

CO-27

PD-1 SE EXPRESA EN GRÁNULOS CITOTÓXICOS DE LAS CÉLULAS NK Y SE MOVILIZA RÁPIDAMENTE A LA MEMBRANA CELULAR TRAS EL RECONOCIMIENTO DE UNA CÉLULA TUMORAL

Cecilia Pesini[1], Sandra Hidalgo[1], Maykel Arias[1], Llipsy Santiago[1], Yurena Aguilar[2], Marta Ocariz-Díez[3], Dolores Isla[3], Pilar Lanuza[1], M José Agustín[2], Ariel Ramirez[1], Julián Pardo[1].

[1]Instituto de Investigación Sanitaria de Aragón, Zaragoza; [2]Hospital Universitario Miguel Servet, Zaragoza; [3]Hospital Clínico Univesitario Lozano Blesa, Zaragoza.

CO-28

DEFICIENCY OF SHP1 IN MYELOID CELLS PROMOTES TUMOR GROWTH.

Miguel Galán[1], Elena Hernández-García[2], Francisco J Cueto[1], Ana Redondo-Urzainqui[2], Stefanie K. Wculek[1], Ignacio Heras-Murillo[1], Salvador Iborra[2], David Sancho[1].

[1]Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain., Madrid; [2]Department of Immunology, Ophthalmology and ENT. School of Medicine, Universidad Complutense de Madrid, 28040, Spain., Madrid.



CO-29

BACTERIA EXPRESSING TUMOR ANTIGENS CAN TRAIN CD4+ LYMPHOCYTES FOR AN EFFECTIVE AND SPECIFIC ANTITUMOR IMMUNOTHERAPY

Lara Del Campo Milán[1], Raquel García-Ferreras[1], Almudena Méndez-Pérez[2], Maria Jose Gómez-Sánchez[3], Esteban Veiga[2].

[1]Centro Nacional de Biotecnología, Madrid; [2]Centro Nacional de Biotecnología-CSIC, Madrid; [3]Departamento de Inmunología, Facultad de Medicina, Universidad Complutense de Madrid, Madrid.

17:00-18:00
SALA PALACIO

SESIÓN 4 MUJERES EN LA CIENCIA

Moderadores:

Mª Rosa Juliá Benique. *Inmunología, Hospital Universitario Son Espases, Palma de Mallorca*

María Montoya González. *Centro de Investigación Margarita Salas, CSIC, Madrid*

Ponentes:

- **María Mayan.** *Instituto de Investigación Biomédica, A Coruña*
  "How much are women disadvantaged in science?. The gender data"
- **Ana Fernández Sesma.** *Icahn School of Medicine, New York, USA*

18:00-19:00
SALA PALACIO

PLENARIA 3 METABOLISMO E INFLAMACIÓN

Moderadores:

José María García Ruiz de Morales. *Inmunología, Complejo Hospitalario Universitario de León*

Juan E. Viñuela Roldán. *Inmunología, Complejo Hospitalario Universitario de Santiago de Compostela*

Ponentes:

- **Ruslan Medzhitov.** *Yale School of Medicine, Connecticut ,USA*
  "Metabolism and Inflammation"

19:00-20:00
SALA PALACIO

SIMPOSIO GSK "INMUNOLOGÍA Y VACUNACIÓN DEL ADULTO"

Ponentes:

- **África González Fernández**, *CINBIO (Centro de Investigaciones Biomédicas), Universidad de Vigo*
- **Víctor del Campo Pérez**, *Servicio de Medicina Preventiva del Complexo Hospitalario Universitario de Vigo*



DIA 2 VIERNES 23 DE SEPTIEMBRE

08:30-09:30
SALA PALACIO

PLENARIA 4 RESPUESTA INMUNE INNATA EN LUPUS

Moderadores:

Marcos López Hoyos. *Inmunología, Hospital Marqués de Valdecilla, Santander*

María Mittelbrunn. *Centro de Biología Molecular Severo Ochoa, CSIC, Madrid*

Ponente:

- **Carola García de Vinuesa.** *Francis Crick Institute, Londres, Reino Unido*
  "Innate immune responses en systemic lupus erythematosus"

09:30-10:30
SALA PALACIO

PLENARIA 5 ONE HEALTH

Moderadores:

África González Fernández. *CINBIO, Universidad de Vigo*

Sara María Calleja Antolín. *Inmunología, Complejo Hospitalario Universitario de León*

Ponente:

- **María Neira**. *Departamento de Salud Pública y Ambiente, Organización Mundial de la Salud, Ginebra, Suiza*
  "One health"

10:30-11:30
SALA PALACIO

SESIÓN 5 INMUNOTERAPIA EN CÁNCER

Moderadores:

Rafael Sirera. *Departamento de Biología Celular, Universidad Politécnica de Valencia*

María Mayán. *Instituto de Investigación Biomédica, A Coruña*

Ponentes:

- **Ignacio Melero Bermejo**. *Centro de Investigación Médica Aplicada, Universidad de Navarra*
  "IL 8 and neutrophil extracellular traps (NET) as a part of the wrong inflammation in tumors"
- **Manel Juan Otero**. *Servicio de Inmunología, Hospital Clinic, Barcelona*
  "Treating patients with ATMPs from a public hospital: our experience with the ARI program in Barcelona"



10:30-11:30
SALA PALACÍN 1

SESIÓN 6 INMUNODEFICIENCIAS PRIMARIAS

Moderadores:

Carmen Cámara Hijón. *Inmunología, Hospital Universitario La Paz, Madrid*

Oscar de la Calle Martín. *Inmunología, Hospital de la Santa Creu i San Pau, Barcelona*

Ponentes:

- **Roger Colobran Oriol.** *Servicio de Inmunología, Hospital Vall d´Hebron, Barcelona*
- **María Bravo García-Morato.** *Servicio de Inmunología, Hospital La Paz, Madrid*
  "Current and future challenges in genetic diagnosis of primary immunodeficiencies. Expect the unexpected"

10:30-11:30
SALA PALACÍN 2

SESIÓN 7 MICROBIOMA Y ENFERMEDAD

Moderadores:

Sara María Calleja Antolín. *Inmunología, Complejo Hospitalario Universitario de León*

David Bernardo Ordiz. *Instituto de Biomedicina y Genética, CSIC, Valladolid*

Ponentes:

- **Rachel Caspi.** *National Eye Institute, NIH, Bethesda, Maryland, USA*
  "Microbiota in uveitis"
- **Roger Paredes**. *Instituto Investigación IrsiCAIXA, Hospital Germans Trias I Pujol, Barcelona*

10:30-11:30
SALA LOUNGE

ASAMBLEA DE USUARIOS DEL PROGRAMA GECLID

Moderador:

Mª Carmen Martín Alonso. *Centro de Hemoterapia y Hemodonación, Valladolid*

11:30-12:00

PAUSA CAFÉ



12:00-13:00
SALA PALACIO

PLENARIA 6 ENVEJECIMIENTO E INFLAMACIÓN

Moderadores:

José María García Ruiz de Morales. *Inmunología, Complejo Hospitalario Universitario de León*

África González Fernández. *CINBIO, Universidad de Vigo*

Ponentes:

- **María Mittelbrunn.** *Centro de Biología Molecular Severo Ochoa (CSIC), Madrid*
  "Immunometabolism at the crosroad between inflammation and aging"
- **Santiago Moreno**. *Servicio de Enfermedades Infecciosas, Hospital Ramón y Cajal, Madrid*
  "Inflammation and immunsenescence in HIV infected patients"

13:00-14:00
SALA PALACIO

SIMPOSIO HEALTHINCODE "NUEVOS AVANCES EN LA GENÉTICA APLICADA A INMUNODEFICIENCIAS PRIMARIAS"

Moderadora:

Yvelise Barrios del Pino. Médico. *Especialista en Inmunología. Vocal de la Junta Directiva de la Sociedad Española de Inmunología*

Ponentes:

- **Jean-Laurent Casanova.** *St. Giles Laboratory of Human Genetics of Infectious Diseases, The Rockefeller University, New York*
- **Carlos Rodríguez-Gallego.** *Servicio de Inmunología, Hospital Universitario Dr. Negrín, Las Palmas de Gran Canaria*

13:00-14:00
SALA PALACÍN 1

SIMPOSIO BD "MOLECULAR CYTOMETRY: KEY TOOL FOR EXPANDING OUR KNOWLEDGE OF IMMUNOLOGY. CITOMETRÍA MOLECULAR: HERRAMIENTA CLAVE PARA AMPLIAR NUESTROS CONOCIMIENTOS EN INMUNOLOGÍA"

Moderadora:

Gemma Coma Bravo. *PhD, Marketing Manager BDB Iberia. BD Life Sciences- Biosciences*

Ponente:

- **Werner Rodríguez Acebo.** *Senior Single Cell Solution Architect South Europe. BD Life Sciences- Biosciences*



13:00-14:00
SALA PALACÍN 2

SIMPOSIO GRIFOLS "NUEVAS TENDENCIAS EN LA MONITORIZACIÓN TERAPÉUTICA DE FÁRMACOS BIOLÓGICOS: MEJORAS EN EL FLUJO DE TRABAJO DEL LABORATORIO Y EN LA OPTIMIZACIÓN DE LAS TERAPIAS BIOLÓGICAS"

Moderadora:

Dra. Dora Pascual Salcedo. *Independent Consultant in Clinical Practice*

Ponentes:

- **Dr. Víctor Manuel Navas.** *Sección de Gastroenterología y Nutrición Infantil. Hospital Reg. Univ. de Málaga*
- **Ainhoa Ruiz Del Agua,** *PhD, Especialista de Desarrollo de Producto, Progenika Grifols*

14:00-14:30
HALL EXPOSICIÓN

VISITA PÓSTER

Pantalla 1. Autoinmunidad (I) (P-135 – P-158)
Moderadores:
Lourdes Mozo Avellaneda. *Inmunología, Hospital Universitario Central de Asturias*

Ricardo Rojo Amigo. *Inmunología, Complejo Hospitalario Universitario de A Coruña*

Pantalla 2. Autoinmunidad (II) (P-159 – P-181)
Lourdes Mozo Avellaneda. *Inmunología, Hospital Universitario Central de Asturias*

Ricardo Rojo Amigo. *Inmunología, Complejo Hospitalario Universitario de A Coruña*

Pantalla 3. Inmunogenética (P-182 – P-203)
Manuel Muro Amador. *Inmunología, Hospital Clínico Universitario Virgen Arrixaca, El Palmar, Murcia*

Óscar de la Calle Martín. *Inmunología, Hospital de la Santa Creu i San Pau, Barcelona*

Pantalla 4. Trasplante (P-204 – P-229)
Francisco Boix Giner. *Centro de Transfusión, Hospital General Universitario de Valencia*

José ignacio Rodríguez Barbosa. *Área de Inmunología, Universidad de León*



Pantalla 5. Inmunidad frente a las infecciones (P-230 – P-260)

Estanislao Nistal Villán. *Ciencias Farmacéuticas y de la Salud. Universidad CEU San Pablo. Madrid*

Manuel Hernández González. *Inmunología, Hospital Vall d´Hebron, Barcelona*

Pantalla 6. Vacunas (P-261 – P-274)

Jorge Carrillo Molina. *Instituto de Investigación IrsiCaixa, Barcelona*

María Montoya González. *Centro de Investigación Margarita Salas, CSIC, Madrid*

14:30-15:30 SESIÓN 8 INMUNIDAD EN MUCOSAS

SALA PALACIO

Moderadores:

David Bernardo Ordiz. *Instituto de Biomedicina y Genética, Universidad de Valladolid*

Manuel Hernández González. *Inmunología, Hospital Vall d´Hebron, Barcelona*

Ponente:

- **William W. Agace**. *Mucosal Immunology Section, Lund University, Lund, Suecia*
  "Exploring intestinal dendritic cell heterogeneity and function "

14:30-15:30 REUNIÓN GJSEI

SALA PALACÍN 1

Moderador:

Carlos Ávila Nieto. *Grupo de Inmunología (IGG). Institut de Recerca de la Sida (IrsiCaixa), Badalona*

14:30-15:30 COMUNICACIONES ORALES 6. AUTOINMUNIDAD

SALA PALACÍN 2

Moderadores:

Lourdes Mozo Avellaneda. *Inmunología, Inmunología, Hospital Universitario Central de Asturias*

Ricardo Rojo Amigo. *Inmunología, Complejo Hospitalario Universitario de A Coruña*



CO-31

DIFFERENTIAL EFFECTS OF ANTI-TNFα AND ANTI-α4β7 DRUGS ON CIRCULATING DENDRITIC CELLS MIGRATORY CAPACITY IN INFLAMMATORY BOWEL DISEASE

Irene Soleto[1], Samuel Fernández-Tomé[2], Irene Mora-Gutierrez[3], Montserrat Baldan-Martin[1], Cristina Ramirez[1], Cecilio Santander[1], Jose Andrés Moreno-Monteagudo[1], Maria José Casanova[1], Fernando Casals[1], Sergio Casabona[1], Irene Becerro[1], Maria Chaparro[1], David Bernardo[4], Javier P. Gisbert[1].

[1]Gastroenterology Unit, Hospital Universitario de La Princesa and Instituto de Investigación Sanitaria Princesa (IIS-Princesa), Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Madrid; [2]Departamento de Nutrición y Ciencia de los Alimentos, Facultad de Farmacia, Universidad Complutense de Madrid, Madrid; [3]Biobanco del sistema de salud de Aragón, Zaragoza; [4]Mucosal Immunology Lab, Unidad de Exclencia Instituto de Biología y Genética Molecular (IBGM, Universidad de Valladolid-CISC),Centro de Investigación Biomédica en Red de Enfermedadmes Infecciones (CIBERINFEC), Valladolid.

CO-32

CIRCULATING IMMUNE-COMPLEXES OF IGG/IGM BOUND TO B2-GLYCOPROTEIN-I ASSOCIATED WITH COMPLEMENT CONSUMPTION AND THROMBOCYTOPENIA IN ANTIPHOSPHOLIPID SYNDROME

Laura Naranjo[1], Ljudmila Stojanovich[2], Aleksandra Djokovic[3], Laura Andreoli[4], Angela Tincani[4], Maria Maślińska[5], Savino Sciascia[6], Maria Infantino[7], Sara Garcinuño[1], Kinga Kostyra-Grabczak[5], Mariangela Manfredi[7], Francesca Regola[4], Natasa Stanisavljevic[2], Milomir Milanovic[2], Jovica Saponjski[8], Dario Roccatello[6], Irene Cecchi[6], Massimo Radin[6], Maurizio Benucci[9], Daniel Pleguezuelo[1], Manuel Serrano[1], Yehuda Shoenfeld[10], Antonio Serrano[1].

[1]Immunology Department, Hospital Universitario 12 de Octubre, Madrid; [2]Internal Medicine, University Hospital Center Bezanijska Kosa, Belgrado; [3]Cardiology Department, University Hospital Center Bezanijska Kosa, Belgrado; [4]Unit of Rheumatology and Clinical Immunology, ASST Spedali Civili, Brescia; [5]Early Arthritis Clinic, National Institute of Geriatrics, Rheumatology and Rehabilitation, Varsovia; [6]Nephrology and Dialysis Unit (ERK-net Member), Center of Research of Immunopathology and Rare Diseases, San Giovanni Bosco Hospital, Turín; [7]Immunology and Allergy Laboratory, San Giovanni di Dio Hospital, Florencia; [8]Cardiology Department, University Clinical Center of Serbia, Belgrado; [9]Rheumatology Unit, San Giovanni di Dio Hospital, Florencia; [10]Ariel University, Ariel, Israel; Zabludowicz Center for Autoimmune Diseases, Sheba Medical Center, Tel-Hashomer.



CO-33

LOS NIVELES DE IGM FRENTE A FOSFATIDILCOLINA EN SUERO PREDICEN LA RESPUESTA AL TRATAMIENTO CON NATALIZUMAB E INTERFERÓN-β

Maria Cruz Sádaba Argaiz[1], Esther Escudero Lirola[1], Cristina Sebal Neira[1], Elena Urcelay García[2], Rafael Arroyo Gónzalez[3], María Angel García Martínez[2], Francisco J Quintana[4], Roberto Álvarez Lafuente[2], Úrsula Muñoz Morón[1].

[1]Universidad San Pablo CEU, Madrid; [2]Hospital Clínico San Carlos, Madrid; [3]Hospital Universitario Quirónsalud, Madrid; [4]Ann Romney Center for Neurologic Diseases. Brigham and Women's Hospital. Harvard Medical School, Boston.

CO-34

CHARACTERIZATION OF THE INTERACTION OF THE AUTOIMMUNE REGULATOR (AIRE) WITH THE E3-UBIQUITIN LIGASES SIAH1 AND SIAH2

Adrián Tirado Herranz[1], Alba Pastor Moreno[2], María Area Navarro[2], Miguel Marcilla[3], Alberto Paradela[3], Iñaki Álvarez[2].

[1]Hospital Clínic / Institut de Biotecnologia i Biomedicina, Barcelona; [2]Institut de Biotecnologia i Biomedicina, Cerdanyola del Vallès; [3]Centro Nacional de Biotecnología, Madrid.

CO-35

THYROID FOLLICULAR CELLS CAN MODULATE T CELL FUNCTION AND PHENOTYPE IN MIXED AUTOLOGOUS CULTURES; IMPLICATIONS FOR THYROID AUTOIMMUNE DISEASES

Daniel Álvarez De La Sierra[1], Aroa Gómez[2], Irene Bello[2], Carmela Iglesias[2], Anna Petit[3], Óscar González[2], Pablo Moreno[3], Carlos Zafón[2], Ricardo Pujol[2].

[1]Vall d'Hebron Research Institute (VHIR), Barcelona; [2]Hospital Universitario Vall d'Hebron (HUVH), Barcelona; [3]Hospital Universitario de Bellvitge (HUB), Barcelona.

CO-36

ROLE OF POLYREACTIVE AUTOANTIBODIES IN SJÖGREN'S SYNDROME

Rebeca Gutiérrez-Cózar, Manuel Sáez Moya, Joan Puñet-Ortiz, Ana Angulo, Pablo Engel.

Universidad de Barcelona, Barcelona.



14:30-15:30
SALA LOUNGE

COMUNICACIONES ORALES 7. INMUNOGENÉTICA

Moderadores:

Oscar de la Calle Martín. *Inmunología, Hospital de la Santa Creu i San Pau, Barcelona*

Manuel Muro Amador. *Inmunología, Hospital Clínico Universitario Virgen Arrixaca, El Palmar, Murcia*

CO-37

UNDERSTANDING THE ETIOLOGY OF INTESTINAL IMMUNE DYSREGULATION IN A NOVEL LCK VARIANT – OF HUMAN AND MOUSE

Victor Lui[1], Manfred Hoenig[2], Klaus Schwarz[2], **Elena Hsieh[1].**

[1]University of Colorado Anschutz Medical Campus, Colorado; [2]University Medical Center Ulm, Ulm.

CO-38

NUEVAS APROXIMACIONES EN EL DIAGNÓSTICO GENÉTICO DE INMUNODEFICIENCIAS PRIMARIAS: DESARROLLO DE UNA ESTRATEGIA DE ANÁLISIS PARA LA EVALUACIÓN DE VARIANTES DE DOSIS GÉNICA MEDIANTE NGS

Elisabet Matas Pérez, Andrea González Torbay, Maria Ángeles Mori Álvarez, Carmen Rodríguez Jiménez, Ricardo Cuesta Martín De La Cámara, Laura Miguel Berenguel, Carla Gianelli, Carmen Cámara Hijón, Rebeca Rodríguez Pena, Eduardo López Granados, Ángela Del Pozo Mate, María Bravo García-Morato.

Hospital Universitario La Paz, Madrid.

CO-39

MITOCHONDRIAL COMPLEX I REGULATES ANTIGEN CROSS-PRESENTATION IN DENDRITIC CELLS

Elena Priego[1], Michel Enamorado[2], Annalaura Mastrangelo[1], Sarai Martínez-Cano[1], Pablo Rodríguez-Silvestre[3], Johan Garaude[4], Eduardo Balsa[5], Patrycja Kozik[3], Salvador Iborra[6], David Sancho[1].

[1]CNIC, Madrid; [2]National Institute of Allergy and Infectious Diseases, Bethesda; [3]MRC Laboratory of Molecular Biology, Cambridge; [4]Institut national de la santé et de la recherche médicale, Bordeaux; [5]Centro de Biología Molecular Severo Ochoa, Madrid; [6]Universidad Complutense de Madrid, Madrid.

CO-40

ASOCIACIÓN DE LOS NONAPÉPTIDOS PRESENTADOS POR HLA-E CON LA ENFERMEDAD DE BEHÇET

Ángel Luis Castaño Núñez[1], Marco Antonio Montes Cano[2], José Raúl García Lozano[2], Raquel Muñoz García[2], Carmen Morales García[2], Alicia Jurado Orozco[2], María Francisca González Escribano[2].

[1]Hospital Universitario Fundación Jiménez Díaz, Madrid; [2]Hospital Universitario Virgen del Rocío, Sevilla.

CO-41

HEK293F CELL LINE SHOWS DIFFERENT ANTIGEN PRESENTATION FEATURES THAN HEK293

Alba Pastor Moreno[1], María Dolores Area Navarro[1], Erika Scholz[1], Adrián Tirado Herranz[1], Miguel Marcilla[2], Francesc Canals[3], Manel Juan[4], Iñaki Álvarez[1].

[1]Immunology Unit, Department of Cell Biology, Physiology, and Immunology and Institut de Biotecnologia i Biomedicina, Universitat Autònoma de Barcelona, Bellaterra; [2]Proteomics Unit, Spanish National Biotechnology Centre, Madrid; [3]Proteomics Laboratory, Vall d'Hebron Institute of Oncology (VHIO), Universitat Autònoma de Barcelona, Barcelona; [4]Servei d'Immunologia, Hospital Clínic de Barcelona, IDIBAPS, Universitat de Barcelona, Barcelona.

CO-42

HLA-G EN UVEÍTIS: MENOS ES MÁS

Marta Molina Alejandre[1], Christian Vaquero Yuste[1], Ignacio Juarez[1], Marina Begoña Gorroño Echebarria[2], Elisa María Molanes López[3], Fabio Suarez Trujillo[1], Carmen Rodriguez Sainz[4], Eduardo Fernández Cruz[4], Antonio Arnaiz Villena[1], José Manuel Martín Villa[1].

[1]Dpto. de Inmunología, Oftalmología Y ORL. Facultad de Medicina. Universidad Complutense de Madrid, Madrid; [2]Servicio de Oftalmología. Hospital Universitario Príncipe de Asturias, Alcalá de Henares; [3]Dpto. de Estadística e Investigación Operativa. Facultad de Medicina. Universidad Complutense de Madrid, Madrid; [4]Instituto de Investigación Sanitaria Gregorio Marañón, Madrid.

15:30-17:30
SALA PALACIO

REUNIÓN TALLER AI

Moderador:

Mª Luisa Vargas Pérez. *Servicio de Inmunología y Genética. Hospital Universitario de Badajoz*

15:30-16:30
SALA PALACÍN 1

REUNIÓN AEH

Moderador:

Jaume Pons de Ves. *Inmunología Hospital Universitario Son Espases, Palma de Mallorca*

15:30-16:30
SALA PALACÍN 2

COMUNICACIONES ORALES 8. Inmunidad frente a las infecciones

Moderadores:

Estanislao Nistal Villán. *Ciencias Farmacéuticas y de la Salud. Universidad CEU San Pablo. Madrid*

Manuel Hernández González. *Inmunología, Hospital Vall d´Hebron, Barcelona*

CO-43

S PROTEIN VACCINE-INDUCED IMMUNE IMPRINTING REDUCES N PROTEIN ANTIBODY RESPONSE IN SARS-COV-2 INFECTION

Juan Francisco Delgado De La Poza[1], Mónica Vidal[1], Maria Carmen Moya[2], Mateu Espasa[3], Antonio Casabella[3], Manel Seda[2], Rosa María Serrano[2], Pilar Peña[2].

[1]Immunology Unit, Laboratory Service. Parc Taulí Hospital Universitari. Institut d'Investigació i Innovació Parc Taulí (I3PT). Universitat Autònoma de Barcelona, Departament de Medicina., Sabadell; [2]Occupational Health Department. Parc Taulí Hospital Universitari. Institut d'Investigació i Innovació Parc Taulí (I3PT). Universitat Autònoma de Barcelona., Sabadell; [3]Microbiology Unit, Laboratory Service. Parc Taulí Hospital Universitari. Institut d'Investigació i Innovació Parc Taulí (I3PT). Universitat Autònoma de Barcelona. Sabadell. Spain., Sabadell.

CO-44

A DIFFERENTIAL SIGNATURE OF CIRCULATING MIRNAS AND CYTOKINES BETWEEN COVID-19 AND COMMUNITY-ACQUIRED PNEUMONIA UNCOVERS NOVEL PHYSIOPATHOLOGICAL MECHANISMS OF COVID-19

Pedro Martínez Fleta, Paula Vera Tomé, María Jiménez Fernández, Silvia Requena, Emilia Roy Vallejo, Ancor Sanz García, Marta Lozano Prieto, Celia López Sanz, Alicia Vara, Ángel Lancho Sánchez, Enrique Martín Gayo, Cecilia Muñoz Calleja, Arantzazu Alfranca, Isidoro González Álvaro, José María Galván Román, Javier Aspa, Hortensia De La Fuente, Francisco Sánchez Madrid.

HU de la Princesa, Madrid.

CO-45

EXTRACELLULAR VESICLES FROM LISTERIA MONOCYTOGENES-INFECTED DENDRITIC CELLS ALERT THE INNATE IMMUNE RESPONSE

Raúl Izquierdo Serrano[1], **Irene Fernández Delgado[2]**, Olga Moreno Gonzalo[1], Enrique Martín Gayo[2], Diego Calzada Fraile[1], Inmaculada Jorge[1], Emilio Camafeita[1], Francisco Sánchez Madrid[2].

[1]Centro Nacional Investigaciones Cardiovasculares (CNIC) Carlos III, Madrid; [2]Instituto Investigación Sanitaria Hospital Universitario La Princesa (IIS-HUP)-Universidad Autónoma de Madrid (UAM), Madrid.

CO-46

KIR-LIGAND POLYMORPHISM IMPROVED A MORTALITY PREDICTION MODEL INCLUDING CLINICAL DATA OF KNOWN COMORBIDITY IN PATIENTS WITH SARS-COV-2 INFECTION

Jairo Eduardo Niño Ramírez, Maria Nieves Gutierrez Zufiaurre, Miguel Marcos, Mr Bartol Sánchez, Rocío Eiros, Miguel Alcoceba Sanchez, María García Alvarez, Pilar Terradillos Sánchez, Daniel Presa, Juan Luis Muñoz, A López Bernús, E López Sánchez, D González Calle, Pedro L Sánchez, Olga Compán Fernández, Marcos González, Ramón García Sanz, Francisco Boix.

Hospital Universitario de Salamanca, Salamanca.

CO-47

DIFFERENTIAL EXPRESSION OF CHEMOKINE RECEPTORS IN SARS-COV2- SPECIFIC CD4+ T LYMPHOCYTES

Laura Esparcia, Angel Lancho, Noelia Ropero, Enrique Martín, Noa Martin, Francisco Sánchez Madrid, Arantzazu Alfranca.

Servicio de Inmunología, Hospital de la Princesa, Madrid.

CO-48

NATURAL KILLER CELL DEGRANULATION: A DETERMINANT FACTOR IN COVID-19 EVOLUTION

Sara Garcinuño, Francisco Javier Gil Etayo, Esther Mancebo, Marta Lopez Nevado, Antonio Lalueza, Raquel Diaz Simón, Daniel Enrique Pleguezuelo, Manuel Serrano, Óscar Cabrera Marante, Luis M Allende, Estela Paz Artal, Antonio Serrano.

Hospital Universitario 12 de Octubre, Madrid.

15:30-17:30
SALA LOUNGE

REUNIÓN DE GRUPO DE CITOMETRÍA (GECIT)

Moderadores:

Jordi Cano Ochando. *Mount Sinai School of Medicine, New York*

Daniel Lozano Ojalvo. *Assistant Professor. Deparment of Dermatology. Mount Sinai Hospital, New York*

16:30-17:30
SALA PALACÍN 1

REUNIÓN GEIT

Moderador:

Belén Blanco Durango. *Unidad de Inmunoterapia del Cáncer. Hospital 12 de Octubre, Madrid*

Ponentes:

- **Manel Juan.** *Servicio de Immunología. CBD-Hospital Clínic, Barcelona.*
  "Monitorización analítica de CAR-T: para qué y por qué debe hacerse por los inmunólogos de cada centro"
- **Elena Sánchez Zapardiel.** *Unidad de Inmunología. Hospital Universitario La Paz-IdiPAZ, Madrid.*
  "Monitorización CAR-T: recomendaciones de consenso desde la perspectiva del inmunólogo"

16:30-17:30
SALA PLACÍN 2

COMUNICACIONES ORALES 9. TRASPLANTE

Moderadores:

Francisco Boix Giner. *Centro de Transfusión, Hospital General Universitario de Valencia*

José Ignacio Rodríguez Barbosa. *Área de Inmunología, Universidad de León*

CO-49

THE IMPACT OF CD160 DEFICIENCY ON ALLOREACTIVE CD8 T CELL RESPONSES AND ALLOGRAFT REJECTION

Jose-Ignacio Rodriguez Barbosa, Maria Luisa Del Rio Gonzalez.
Section of Immunobiology, University of Leon, Leon

CO-50

NK CELL TRANSCRIPTOME AND PHENOTYPICAL CHANGES AFTER AUTOLOGOUS HSCT: TRANSIENT EXPANSION OF A CD9+ DECIDUAL-LIKE NK CELL SUBSET.

Gabirel Astarloa-Pando[1], Ane Orrantia[1], Iñigo Terrén[1], Ainhoa Amarilla-Irusta[1], Silvia Pérez-Fernandez[1], Itziar Astigarraga[1], Juan J. Mateos-Mazón[1], Juan C. García-Ruiz[1], Marta Riñón[1], Carmen González[2], Alasne Uranga[2], José J. Uriz[2], Mercedes Rey[2], Tomás Carrascosa[3], Enrique Vázquez-De Luis[4], Sergio Callejas[4], Ana Quintas[4], Ana Dopazo[4], Olatz Zenarruzabeitia[1], Francisco Borrego[1].

[1]Biocruces Bizkaia Health Research Institute – Cruces University Hospital, Barakaldo; [2]Biodonostia Health Research Institute – Donostia University Hospital, Donostia; [3]Biocruces Bizkaia Health Research Institute – Galdakao-Usansolo Hospital, Galdakao; [4]Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid.

CO-51

INMUNIDAD ENTRENADA EN PACIENTES CON ENFERMEDAD RENAL CRÓNICA AVANZADA (ERCA) Y EN RECEPTORES DE TRASPLANTE RENAL (RTR): NANOINMUNOTERAPIA PARA SU MODULACIÓN

Marta Chivite Lacaba[1], Cecilia González Cuadrado[1], Marcos J Berges Buxeda[2], Patricia Almendro Vázquez[1], Jara Caro Espada[3], Esther González[3], Alberto Utrero Rico[4], Rocío Laguna Goya[3], Esther Mancebo Sierra[3], Amado Andrés[3], Jordi Ochando[2], Estela Paz Artal[3].

[1]Fundación para la Investigación Biomédica Hospital 12 de Octubre, Madrid; [2]Centro Nacional de Microbiología, Instituto de Salud Carlos III, Madrid; [3]Hospital 12 de Octubre, Madrid; [4]Icahn School of Medicine at Mount Sinai, Nueva York.

CO-52

COMPARISON OF IMMUNE RECONSTITUTION BETWEEN POST-TRANSPLANT CYCLOPHOSPHAMIDE AND CONVENTIONAL GRAFT-VERSUS-HOST DISEASE PROPHYLAXIS IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

Jennifer Bolaños Gómez[1], Martha Gómez Hernando[2], Jose Luis Caro[1], Carles Serra[1], Eduard Palou[1], María Queralt Salas[2], Alexandra Pedraza[2], Monserrat Rovira[2], Carmen Martínez Muñoz[2], Juan Torres Canizales[1].

[1]Transplant Immunology Section, Immunology Department, Hospital Clínic de Barcelona, Barcelona; [2]Hematopoietic Stem Cell Transplantation Unit, Hematology Department, Hospital Clínic de Barcelona, IDIBAPS, Barcelona.

CO-53

EVALUACIÓN DEL PERFIL DE SENSIBILIDAD A FÁRMACOS INMUNOSUPRESORES EN PACIENTES CON TRASPLANTE RENAL, MEDIANTE EL ENSAYO IMMUNOBIOGRAM® (IMBG) Y MARCADORES DE ACTIVACIÓN LINFOCITARIA

Cecilia González Cuadrado[1], Marta Chivite Lacaba[1], Patricia Almendro Vázquez[1], Alvaro Ortega[2], Teresa Diez[2], Isabel Potero[2], Amado Andrés[3], Julio Pascual[3], Estela Paz Artal[3], Esther Mancebo Sierra[3].

[1]Fundación para la Investigación Biomédica Hospital 12 octubre, Madrid; [2]Biohope Scientific Solutions for Human Health SL, Tres Cantos; [3]Hospital Universitario 12 de Octubre, Madrid.

CO-54

MODULATION OF THE INNATE IMMUNE RESPONSE IN TRASPLANTATION BY TARGETED DELIVERY OF MYCOPHENOLIC ACID BY MUSSEL-INSPIRED NANOPARTICLES

Celia Nieto Jiménez[1], Milena A. Vega Moreno[2], Eva M. Martín Del Valle[2], María Luisa Del Río[3], José Ignacio Rodríguez Barbosa[1].

[1]Section of Transplant Immunobiology and Immunotherapy, Institute of Molecular Biology, Genomics and Proteomics, University of Leon, León; [2]Chemical Engineering Department, Faculty of Chemical Sciences, University of Salamanca, Salamanca; [3]Section of Transplant Immunobiology and Immunotherapy, Institute of Molecular Biology, Genomics and Proteomics, University of Leon, Salamanca.

17:30-18:30
SALA LOUNGE

COMUNICACIONES ORALES 10. VACUNAS

Moderadores:

Jorge Carrillo Molina. *Instituto de Investigación IrsiCaixa, Barcelona*

María Montoya González. *Centro de Investigación Margarita Salas, CSIC, Madrid*



CO-55

MESOPOROUS SILICON MICROPARTICLES (MSMPS) ENHANCE CELLULAR AND HUMORAL IMMUNE RESPONSE TO SARS-COV-2

Ana López Gómez[1], Irene Real Arévalo[1], Elsa Mayol Hornero[1], Ignacio Juárez[1], Beatriz Martín Adrados[1], Raquel González García[1], Raúl Martín Palma[2], Eduardo Martínez Naves[1], Manuel Gómez Del Moral[3].

[1]Universidad Complutense de Madrid, Departamento de inmunología, Facultad de Medicina, Madrid; [2]Universidad Autónoma de Madrid, Departamento de Física Aplicada, Facultad de Ciencias, Madrid; [3]Universidad Complutense de Madrid, Departamento de Biología Celular, Facultad de Medicina, Madrid.

CO-56

PREDICTIVE FACTORS OF THE MAGNITUDE AND LOSS OF ANTI-S ANTIBODY TITERS AFTER TWO DOSES OF BNT162B2 VACCINE IN OLDER PEOPLE: AN EIGHT MONTHS FOLLOW-UP

María Del Mar Pozo Balado[1], Ángel Bulnes Ramos[1], Vanesa Garrido Rodríguez[1], Israel Olivas Fernández[1], Carmen Lozano[2], Ana Isabel Álvarez Ríos[2], Berta González González[2], José Manuel Lara[2], Isabel Maldonado Calzado[2], Encarnación Sánchez Bejarano[2], Juan Antonio Santamaría[3], María Francisca González Escribano[2], Manuel Leal[4], Yolanda M. Pacheco[1].

[1]Instituto de Biomedicina de Sevilla, HUVR, Sevilla; [2]Hospital Universitario Virgen del Rocío, Sevilla; [3]Residencia Hogar de la Santa Caridad, Sevilla; [4]Hospital Viamed Santa Ángela de la Cruz/Hogar Residencia de la Santa Caridad, Sevilla.

CO-57

INMUNOTERAPIA INDUCIDA POR LA VACUNA MV130 EN UN MODELO EXPERIMENTAL DE NEUMONÍA NEUMOCÓCICA

Alejandro Arrabal Sierra[1], Mercedes Rodríguez[1], Carolina Ruíz[1], M. Carmen Prado[2], Isabel Cortegano[1], Laura Conejero[3], María José Ferrandiz[4], Adela G. De La Campa[4], José Luis Subiza[3], María Luisa Gaspar[1], Belén De Andrés[1]

[1]Laboratorio de Inmunobiología - Centro Nacional de Microbiología - Instituto de Salud Carlos III, Majadahonda; [2]Unidad de Citometría de Flujo - Centro Nacional de Microbiología - Instituto de Salud Carlos III, Majadahonda; [3]Inmunotek, Alcalá de Henares; [4]Laboratorio de Genética Bacteriana - Centro Nacional de Microbiología - Instituto de Salud Carlos III, Majadahonda

CO-58

CELLULAR AND HUMORAL RESPONSES FOLLOW-UP FOR 8 MONTHS AFTER VACCINATION WITH MRNA-BASED ANTI-SARS-COV-2 VACCINES

Sergio Gil Manso[1], Diego Carbonell[2], Verónica Astrid Pérez Fernández[1], Rocío López Esteban[3], Roberto Alonso[4], Patricia Muñoz[4], Jordi Cano[5], Ignacio Sánchez-Arcilla Conejo[6], Jose María Bellón[7], Rafael Correa Rocha[3], Marjorie Pion[1].

[1]Grupo de Inmuno-Regulación Avanzanda, IiSGM, HGUGM, Madrid; [2]Servicio de Hematología, HGUGM, Madrid; [3]Laboratorio de Inmuno-Regulación, IiSGM, HGUGM, Madrid; [4]Servicio de Microbiología Clínica y Enfermedades Infecciosas, HGUGM, Madrid; [5]Precision Immunology Institute, Icahn School of Medicine at Mount Sinai, Nueva York; [6]Servicio de Prevención de Riesgos Laborales, HGUGM, Madrid; [7]Servicio de Bioestadística, IiSGM, HGUGM, Madrid.

CO-59

DIFFERENCES BETWEEN B-CELL SUBPOPULATIONS AND NEUTRALIZING ANTIBODIES FOLLOWING BNT162B2 (PFIZER-BIONTECH) VACCINE IN PERSONS WITH AND WITHOUT HISTORY INFECTION OF SARS-COV-2

José Javier Morales Núñez[1], **Saúl Alberto Díaz Pérez[1],** Mariel García Chagollán[1], José Francisco Muñoz Valle[1], Paola Carolina Torres Hernández[1], Saraí Citlalic Rodríguez Reyes[1], Guillermo Santoscoy Ascencio[2], José Julio Sierra García De Quevedo[2], Jorge Hernández Bello[1].

[1]Universidad de Guadalajara - Centro Universitario de Ciencias de la Salud, Guadalajara; [2]Unidad de Patología Clinica - UPC, Guadalajara.

CO-60

MYCOPHENOLATE & MTOR INHIBITORS TREATMENT AFFECTS THE RESPONSE TO MRNA-1273 VACCINATION IN PATIENTS WITH RENAL TRANSPLANTATION

Ignacio Juárez[1], Isabel Pérez-Flores[2], Ana López-Gómez[3], Raquel González-García[3], Belén Peix-Jiménez[2], Manuel Gómez Del Moral[4], Ana Sánchez-Fructuoso[2], Eduardo Martínez-Naves[3].

[1]Dpto. De Inmunología, Oftalmología y ORL, Facultad de Medicina, Universidad Complutense De Madrid, Madrid; [2]Servicio de Nefrología, Hospital Clínico San Carlos, Madrid; [3]Dpto. De Inmunología, Oftalmología y ORL, Facultad de Medicina, Universidad Complutense De Madrid., Madrid; [4]Dpto. De Biología Celular, Facultad de Medicina, Universidad Complutense De Madrid., Madrid.



17:30-19:00
SALA PALACIO

SESIÓN 9 NUEVAS TERAPIAS EN DM I

Moderador:

Sara María Calleja Antolín. *Inmunología, Complejo Hospitalario Universitario de León*

Ricardo Rojo Amigo. *Inmunología, Complejo Hospitalario Universitario de A Coruña*

Ponentes:

- **Francisco León.** *ProventionBio, New Jersey, USA*
  "Targeting the autoimmmune origins of type 1 diabetes"
- **Mattias von Herrath.** *La Jolla Institute for Immunology, California, USA*
  "Induction-maintenance combination therapies for type1 diabetes"
- **Pieter Rottiers**. *Precigen Actobio, Ghent, Bélgica*

17:30-19:00
SALA PALACÍN 1

CASOS CLÍNICOS AI

Moderadores:

Mª Luisa Vargas Pérez. *Servicio de Inmunología y Genética. Hospital Universitario de Badajoz*

Yvelise Barrios del Pino. *Inmunología, Hospital Universitario de Canarias*

CCAI-01

PERSISTENCIA A LARGO PLAZO DE AC ANTIGLIADINA DEAMINADA IGG EN UNA PACIENTE CON APARICIÓN DE NOVO DE DÉFICIT SELECTIVO DE IGA POST DIAGNÓSTICO DE CELÍAQUÍA.

Antonino Cavallo, Paulina Vasquez, Mildred Fabiola Villegas Siles, Andrea Ferranti, Esther Vergara Prieto, Luis Miguel Fernández Pereira.

Hospital San Pedro de Alcántara, Cáceres

CCAI-02

DESARROLLO DE NEUROMIELITIS ÓPTICA EN PACIENTE CON MIASTENIA GRAVIS Y TIMECTOMÍA

Beatriz Rioseras De Bustos, Rocío López Martínez, Raquel Montaña Rodríguez, Manuel Amorin Díaz, Walter Javier Villafani Echazu, Lourdes Mozo Avellaned[1].

Hosptial Universitario Centras de Asturias (HUCA), Oviedo

CCAI-03

ENCEFALITIS ANTI-R-NMDA POST VACUNA SARS-COV-2

Rosana González López, Carmen Botella Martínez, Victor Jiménez Coll, Carlos Sánchez Rodríguez, Pedro Martínez García, Manuel Muro Amador.

Hospital Clínico Universitario Virgen de la Arrixaca, Murcia

CCAI-04

ANTICUERPOS DE ISOTIPO IGA ANTI-MEMBRANA BASAL GLOMERULAR POST-TRASPLANTE RENAL EN PACIENTE CON SÍNDROME DE ALPORT

Marina Segura Guerrero[1], Carles Saus Sarrias[1], Roberto Gozalbo Rovira[2], María Luísa Vargas Pérez[3], Juan Bautista Saus Mas[2], María Rosa Julià Benique.

Hospital Universitario Son Espases. Instituto de Investigación Sanitaria Illes Balears (IdISBa)[1]; Facultad de medicina y Odontología. Universidad de Valencia, Valencia[2]; Complejo Hospitalario Universitario de Badajoz, Badajoz[3]

CCAI-05

HETEROGENEIDAD CLÍNICA EN EL SÍNDROME POLIGLANDULAR AUTOINMUNE DE TIPO 1, MÁS ALLÁ DEL DEFECTO MONOGÉNICO

Elisabet Matas Pérez, Laura Miguel Berenguel, Andrea González Torbay, María Bravo García Morato, Pilar Nozal Aranda, Rebeca Rodríguez Pena.

Hospital Universitario La Paz, Madrid

CCAI-06

ANTICUERPOS ANTI-SRP EN UNA PACIENTE CON ESCLEROSIS SITÉMICA, CIRROSIS BILIAR PRIMARIA Y SIN SIGNOS DE MIOPATÍA INFLAMATORIA.

Roberto Pariente Rodríguez, Elena Manterola Navarro, Carlota García-Hoz Jiménez, Jesús Loarce Martos, Anaís Mariscal Rodríguez, **Miren Garbiñe Roy Ariño.**

Hospital Universitario Ramón y Cajal, Madrid

CCAI-07

SÍNDROME DE COTARD (DELIRIO NIHILISTA) COMO DEBUT DE ENCEFALITIS AUTOINMUNE

Antonio Trujillo Aguilera, Raquel Bernardo Serrano, Ana María Navas Romo, Rocío Aguado, Sara Cantisán, Aurora Jurado Roger.

Hospital Universitario Reina Sofía, Córdoba

17:30-19:00
SALA PALACÍN 2

CASOS CLÍNICOS ID

Moderadores:

Carmen Cámara Hijón. *Inmunología, Hospital Universitario La Paz, Madrid*

Juana Gil Herrera. *Inmunología, Hospital Universitario Gregorio Marañón, Madrid*



CCID-01

DIAGNÓSTICO DE SÍNDROME DE DELECIÓN 22Q11 EN EDAD ADULTA

Laura Miguel Berenguel, Ricardo Cuesta Martín De La Cámara, Andrea González Torbay, Elisabet Matas Pérez, María Bravo García-Morato, Carmen Cámara Hijón.

Hospital Universitario, La Paz, Madrid

CCID-02

DEFICIENCIA DE TAP1 EN PACIENTE CON INMUNODEFICIENCIA Y LESIONES GRANULOMATOSAS CUTÁNEAS CRÓNICAS

Aurora Fernández Galván[1], Mar Llamas Velasco[1], Javier Fraga[1], Daniel Arroyo Sánchez[2], Oscar Cabrera Marante[2], Luis M Allende[2].

Hospital la Princesa, Madrid[1]; Hospital 12 de Octubre, Madrid[2]

CCID-03

SINDROME DE VEXAS. UN NUEVO INVITADO EN LAS ENFEMREDADES INFLAMATORIAS.

Ana Lucia Jimenez[1], Ernesto Tovar[1], Mariano Andres[1], Anna Mensa[2], Juan Arostegui[2], Francisco M. Marco[1].

Hospital General Universitario de Alicante Dr. Balmis, Alicante[1]; Hospital Clinic de Barcelona, Barcelona[2].

CCID-04

ALOTRASPLANTE DE DONANTE NO-EMPARENTADO COMO TRATAMIENTO EXITOSO EN UN CASO DE INMUNODEFICIENCIA PRIMARIA POR SÍNDROME DE WISKOTT-ALDRICH CON DISREGULACIÓN INMUNE

Antonio Costa Anzola, Antonio Trujillo, Juan Molina, Ana Navas, Aurora Jurado.

Hospital Universitario Reina Sofía, Córdoba.

CCID-05

NEUTROPENIA CONGÉNITA POR DEFICIENCIA DEL RECEPTOR DE G-CSF

Carmen Morales García[1], Alicia Jurado Orozco[1], Pilar Ortiz Aljaro[1], Antonia Rodriguez Villa[2], José Manuel Lucena Soto[1].

Hospital Universitario Virgen del Rocío, Sevilla[1]; Hospital Universitario Reina Sofía, Córdoba[2].

CCID-06

A PROPÓSITO DE UN CASO FAMILIAR DE SÍNDROME DE HIPER-IGE CON NIVELES NORMALES DE IGE

Elisabet Matas Pérez, Andrea González Torbay, Ricardo Cuesta Martín De La Cámara, Teresa Del Rosal Rabes, María Bravo García-Morato, Carmen Cámara Hijón.

Hospital Universitario La Paz, Madrid



CCID-07

DÉFICIT AUTOSÓMICO RECESIVO PARCIAL DEL RECEPTOR DEL IFN-γ: EFECTO FUNDADOR EN ISLAS CANARIAS

Raquel Muñoz García[1], Carmen Morales García[1], Ana Reyes Domínguez Acosta[2], Maria Del Pino Santana Falcón[2], Inmaculada Zorio Reyes[2], Carlos Rodriguez Gallego[2].

Hospital Universitario Virgen del Rocío, Sevilla[1]; Hospital Universitario Doctor Negrin, Las Palmas de Gran Canaria[2].

19:00-20:00
SALA PALACIO

ASAMBLEA GENERAL SEI

Moderador:

Carmen Cámara Hijón. *Inmunología, Hospital Universitario La Paz, Madrid*



DIA 3 SABADO 24 DE SEPTIEMBRE

08:15-09:15
SALA PALACIO

PLENARIA 7 CAR-T EN AUTOINMUNIDAD

Moderadores:

Luis Fernández Pereira. *Inmunología, Hospital San Pedro de Alcántara, Cáceres*

Eva Martínez Cáceres. *Inmunología, Hospital Germans Trias, Barcelona*

Ponentes:

- **George Schett.** *Friedrich Alexander University Erlangen-Nuremberg & Universitätsklinikum, Alemania*
  "CAR-T in systemic lupus erythematosus"

09:15-10:15
SALA PALACIO

PLENARIA 8 CELIACA

Moderadores:

Eduardo Arranz Sanz. *Instituto de Biomedicina y Genética, Universidad de Valladolid*

Luis Fernández Pereira. *Inmunología, Hospital San Pedro de Alcántara, Cáceres*

Ponente:

- **Detlef Schuppan.** *Institute of Translational Immunology, Johannes Gutenberg University Medical Center, Mainz, Alemania*
  "Sick with wheat: celiac disease, type 2 allergy and ATT sensitivity"

10:15-10:30

PAUSA CAFÉ

10:30-11:30
SALA PALACIO

SIMPOSIO PATROCINADO "ACTUALIZACIÓN EN LA FISIOPATOLOGÍA Y EL TRATAMIENTO DEL ANGIOEDEMA HEREDITARIO"



10:30-11:30
SALA PALACÍN 2

SIMPOSIO DIAGNÓSTICA LONGWOOD " EL PODER TERAPÉUTICO DE CAR-T, CAR-NK "GENICAR". EL FUTURO DE LA TERAPIA CELULAR PARA EL TRATAMIENTO DEL CÁNCER"

Moderador:

Francisco Boix Giner. *Facultativo Especialista en Inmunología, Hospital General Universitario de Valencia*

Ponente:

- **Dr. Alejandro Madrigal,** *MD PhD FRCP FRCPath DSc HonDSci FMedSci. Professor of Haematology, UCL Cancer Institute*

11:30-13:00
SALA PALACIO

SESIÓN 10 NEUROINFLAMACIÓN

Moderadores:

Eva Martínez Cáceres. *Inmunología, Hospital Germans Trias, Barcelona*

Álvaro Prada Iñurrategui. *Inmunología, Hospital Universitario de Donostia*

Ponentes:

- **Juan Carlos Gómez Esteban**. *Servicio de Neurología, Hospital Cruces, Bilbao.*
  "Autoimmune dysautonomias"
- **M. Villar.** *Servicio de Inmunología, Hospital Ramón y Cajal, Madrid.*
  "Effects of B -cell depletion in multiple sclerosis. Immunological and clinical aspects"
- **Álvaro Prada Iñurrategui.** *Servicio de Inmunología, Hospital Universitario Donostia, San Sebastian*
  "Anti NMO and anti MOG antibodies in NMOSD and MOGAD"

11:30-13:00
SALA PALACÍN 1

SESIÓN 11 INFLAMACIÓN TH2

Moderadores:

Jaume Pons de Ves. *Inmunología, Hospital Universitario Son Espases, Palma de Mallorca*

Yvelise Barrios del Pino. *Inmunología, Hospital Universitario de Canarias*

Ponentes:

- **Ana Giménez-Arnau.** *Servicio de Dermatología, Hospital del Mar, Barcelona*
  " Urticaria crónica: bases patogénicas y estrategias terapéuticas:"
- **María Luisa Baeza**. *Servicio de Alergología, Hospital Gregorio Marañón, Madrid*
  "Anaphylaxis"



11:30-13:00
SALA PALACÍN 2

SESIÓN 12 DIVULGACIÓN/INMUNOMEDIATICOS

Moderadores:

Rafael Sirera. *Departamento de Biología Celular, Universidad Politécnica de Valencia*

Lourdes Mozo Avellaneda. *Inmunología, Hospital Universitario Central de Asturias*

Ponente:

- **Alfredo Corell Almuzara.** *Departamento de Pediatría e Inmunología, Universidad de Valladolid*
- **África González Fernández**. *CINBIO, Universidad de Vigo*
- **Carmen Cámara Hijón**. *Servicio de Inmunología, Hospital Universitario La Paz, Madrid*

13:00-14:30
SALA PALACIO

PLENARIA 9 SINDROMES HEMOFAGOCÍTICOS

Moderadores:

Juana Gil Herrera. *Inmunología, Hospital Universitario Gregorio Marañón, Madrid*

Oscar de la Calle Martín. *Inmunología, Hospital de la Santa Creu i San Pau, Barcelona*

Ponentes:

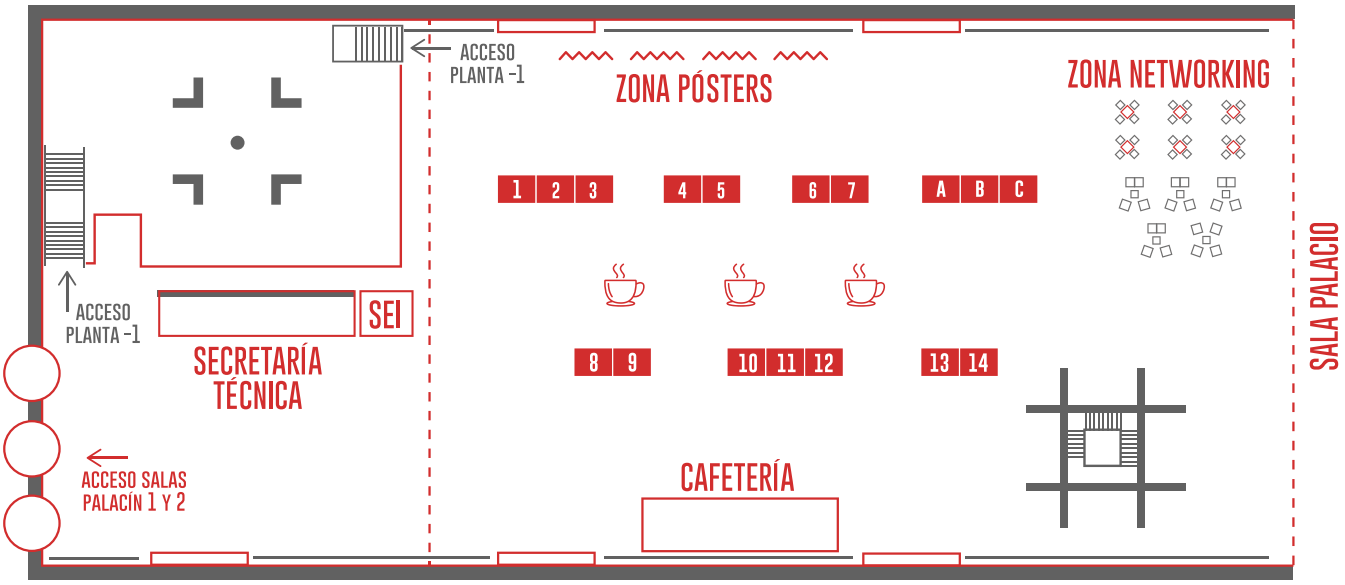
- **Michael Jordan.** *Cincinnati Children´s Hospital Medical Center, Ohio, USA*
  "Primary Hemophagocytic Lymphohistiocytosis: Updates on Pathogenesis and Treatment"
- **Jan Inge Henter**. *Karolinska University Hospital, Stockholm, Suecia*
  "Update on secondary hemophagocytic syndromes"

14:30-15:00
SALA PALACIO

ACTO FIN CONGRESO Y ENTREGA DE PREMIOS

15:00 COMIDA CLAUSURA CONGRESO

EXPOSICIÓN COMERCIAL



STAND	EMPRESA
1	STEMCELL™ TECHNOLOGIES
2	BONSAILAB
3	sysmex
4	Binding Site

STAND	EMPRESA
5	EUROIMMUN a PerkinElmer company
6	DIAGNÓSTICA LONGWOOD
7	Miltenyi Biotec
8	BECKMAN COULTER Life Sciences

STAND	EMPRESA
9	werfen
10-11-12	Palex Constant Improvement
13	BD
14	MABTECH Capture | Detect | Discover

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B	EMBAJADORES SEI
C	biotechne



INFORMACIÓN GENERAL

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ORGANIZA

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Calle Castelló 128, 7º, 28006 Madrid
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Fax. +34 913 023 926
Email: sei2022@pacifico-meetings.com

HORARIO DE SECRETARÍA

- **Jueves 22 de septiembre:** de 08:00h a 20:00h
- **Viernes 23 de septiembre:** de 08:00h a 20:00h
- **Sábado 24 de septiembre:** de 08:00h a 15:00h

ENTREGA DE DOCUMENTACIÓN

La retirada de documentación se efectuará mediante la presentación del bono de confirmación de su inscripción en horario de secretaría.
La documentación se entregará exclusivamente al titular de la misma.

NUEVAS INSCRIPCIONES

Se aceptarán nuevas inscripciones en horario de secretaría, de acuerdo a las cuotas establecidas.



CERTIFICADOS DE ASISTENCIA

Los certificados se entregarán **ONLINE,** a partir del martes **27 de septiembre** (una vez finalizado.
el congreso), a través del enlace que se publicará en la web www.sei2022.com

DISTINTIVO

Con la documentación se entregará un distintivo personalizado, que deberá llevar visible durante todo el congreso.

Su presentación es imprescindible para la entrada a las salas y exposición comercial para su control.

ENTREGA DE PRESENTACIONES Y PRUEBAS AUDIOVISUALES

Habrá un espacio habilitado donde podrán entregar el soporte de su presentación, para ser luego proyectada directamente en la sala correspondiente, debiendo entregarla con tiempo suficiente, y como mínimo, **dos horas antes del inicio de su sesión, o en el café o almuerzo anterior**. Una vez empezada la sesión podrán entregar su presentación al técnico de sala, pero no se podrá probar en pantalla.

Las presentaciones de PowerPoint que incluyen video deben incluir también el software y Codex correspondientes para su visualización dinámica. Recuerde que los videos necesitan un tiempo mínimo para su descarga por lo que se ruega especialmente que todas las presentaciones con video se entreguen con la debida antelación. Por favor, informe al técnico en el momento de su carga y compruebe que se visualiza correctamente.

EXPOSICIÓN COMERCIAL

Palacio de Congresos de Exposiciones de León

IDIOMA

Las sesiones serán en español o inglés, **no habrá traducción simultánea.**

SEGURO

El Comité Organizador del presente Congreso declina toda responsabilidad en caso de accidentes, enfermedad, pérdida o daño de los objetos personales que puedan ocurrir durante la celebración del Congreso o pre y pos viaje. Se recomienda a los asistentes que tengan contratados los seguros que consideren oportunos.



PATROCINADORES

PATROCINADOR PLATINUM

DIAGNÓSTICA LONGWOOD

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43 CONGRESO DE LA SOCIEDAD ESPAÑOLA DE INMUNOLOGÍA

COMUNICACIONES ORALES

Sesión Comunicaciones Orales 1. Inmunología Básica

CO-01

GENOMEWIDE ANALYSIS OF AID TARGET SPECIFICITY IN HUMAN MEMORY B CELLS

Serrano Navarro, Álvaro[1]; Álvarez Prado, Ánfel Francisco[2]; Sánchez Cabo, Fátima[1]; R. Ramiro, Almudena[1].

[1]CNIC, Madrid; [2]Ludwig Institute for Cancer Research.Lausanne, Suiza

After antigen recognition mature B lymphocytes can undergo immunoglobulin (Igs) secondary diversification in germinal centers (GCs) through somatic hypermutation (SHM) and class switch recombination (CSR). SHM introduces point mutations in the variable regions of Igs and can generate higher affinity antibodies, while CSR exchanges the constant region of Igs to allow the expression of alternate isotypes. Both SHM and CSR are initiated by Activation Induced Deaminase (AID) through the deamination of C on Ig DNA, thus generating a U:G mismatch that is repaired by different mechanisms. However, AID activity is not exclusively restricted to Ig loci; instead, it can target other regions, known as off-targets, leading to mutations and chromosome translocations with oncogenic potential. The mechanisms underlying the specificity of AID activity are not well understood.

To address this question, we have evaluated AID activity in human memory B cells, which generally are GC experienced, using a DNA capture protocol coupled with UMI-based high throughput sequencing. We sequenced more than 20.000 regions in the genome, accounting for 12.6Mb, which cover the first exon of all described coding genes. Error corrected sequencing (ECS) method reduced the technical error below 1/10.000 mut/bp threshold. We were able to identify 49 off-target candidate regions in two donors and found that switched memory B cells have a higher mutational load at off-targets than unswichted memory B cells. Off-targets harboured on average 1.28e-4 mutations/bp, which had a distinct mutational footprint found by de-novo extraction of mutational signatures. In addition, we found that the identified off-targets are among the top expressed genes in the GC B cells. Thus, our data reveal for the first time an unbiased set of AID off-targets in normal human B cells. The potential impact of these mutations on lymphoma development is currently under investigation.



CO-03

PHOSPHATASES OF REGENERATING LIVER (PRLS) AS REGULATORS OF ACTIN POLYMERIZATION BALANCE THROUGH THE WDR1/COFILIN/CORONIN COMPLEX IN T LYMPHOCYTES.

Aguilar Sopeña, Óscar[1]; Estarás Hermosel, Matías[2]; Castro Sánchez, Patricia[3]; Carrasco Padilla, Carlos[1]; Alegre Gómez, Sergio[1]; Roda Navarro, Pedro[1].

[1]Facultad de Medicina. Universidad Complutense de Madrid. Departamento de Inmunología; [2]Institute of Molecular Pathology Biomarkers. University of Extremadura.; [3]Institute of Immunology and Infection Research. University of Edinburgh.

PRLs group (PRL-1, 2 and 3) is formed by three highly-homolog proteins that belong to dual specificity phosphatases family. Their role in lymphocytes is mainly unknown, despite having similar expression to other phosphatases which are important in their function. We have previously reported that PRLs are relevant in the effector function and activation of T lymphocytes, regarding interleukin secretion and immune synapse architecture.

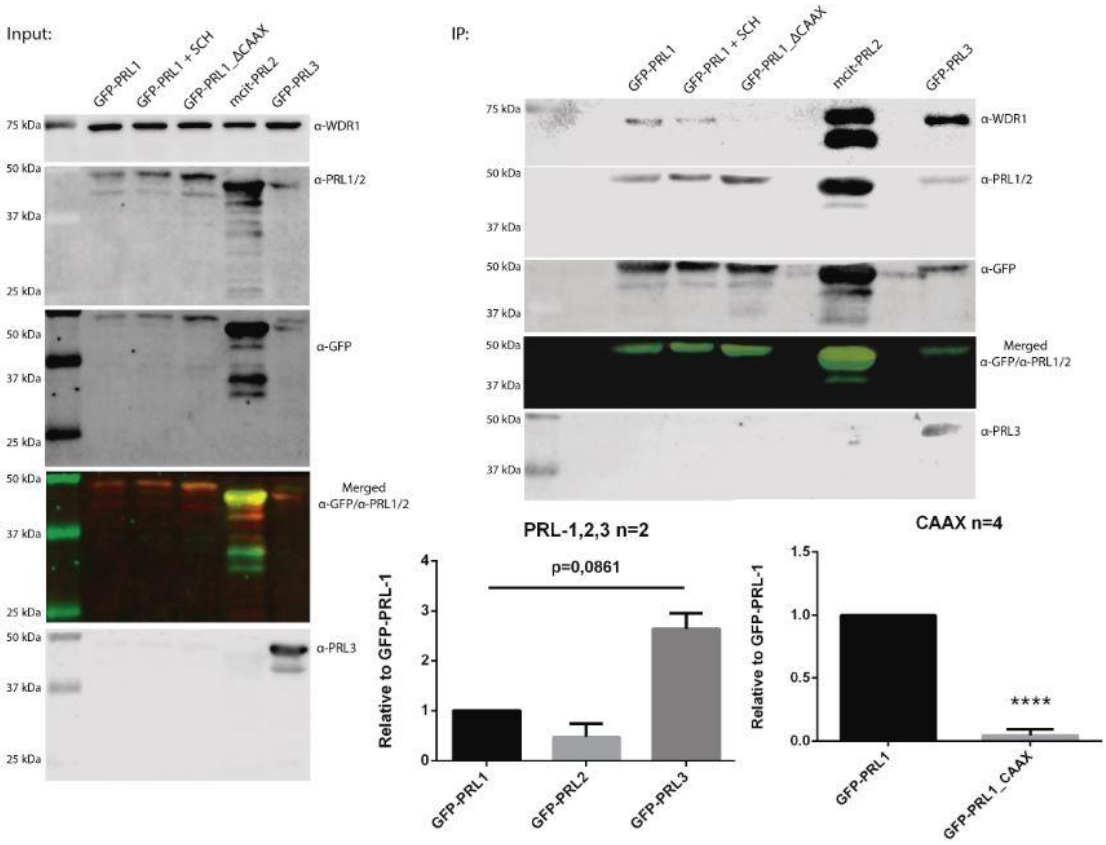
However, there are still many questions to answer about PRLs and the mechanism by which they are regulating lymphocytes activity. We thought that identifying substrates of PRLs could help in our task, since there is a lack of knowledge about that in the bibliography. To do so, we develop a proteomic assay of an immunoprecipitation of GFP-PRL-1 in activated Jurkat cells. Interestingly, the three proteins of the complex WDR1/cofilin/coronin had high scores. This complex is a regulator of the actin polymerization process, which is crucial to T lymphocyte biology.

As a consequence, we tried to evaluate the possible interaction between PRLs and WDR1, because it has been described an immunodeficiency due to WDR1 mutations. Our data in both HEK and Jurkat cells confirmed that PRLs, either overexpressed or at endogenous levels, interacted with WDR1. This interaction is dependent on the farnesylation state of PRLs in their C-terminal motif, since a mutant GFP-PRL-1 protein in this motif and an inhibitor of farnesyl transferase disrupted the interaction.

Additionally, confocal microscopy assays in activated Jurkat cells through TCR stimulation demonstrated how WDR1 is polarized to the immune synapse and let us to define its distribution compared to F-actin and PRL-1/2.

Lastly, we performed immunoprecipitation assays with β-actin in Jurkat cells in which PRL-1/2 appeared to be with the actin filament alongside WDR1.

All together, we propose that PRLs affects several aspects of T lymphocytes biology by regulating actin filament dynamics.



WDR1 immunoprecipitated with GFP-PRLs in HEK cells. (**A**) Immunoprecipitation assay in HEK cells transfected with GFP-PRL-1, GFP-PRL-1_CAAX, mcit-PRL2 or GFP-PRL3 plasmids and western blot analysis. SCH accounts for a treatment with a general inhibitor of farnesyl transferases. at 1µM. Each antibody used is indicated. The input (100 µg) correspond to cells lysates and inmunoprecipitates (IP) were obtained with a GFP trap. (**B**) The mean +/- the standard deviation of WDR1/GFP ratio is shown. Samples were compared by a two-tailed one-sample t-test. **** p<0,0001.



CO-04

CHARACTERIZATION OF EXTRACELLULAR VESICLES SECRETED BY THYMIC REGULATORY T CELLS (THYTREG-EVS)

Fernández Castillo, Marta[1]; López Esteban, Rocío[1]; Pérez Fernández, Verónica Astrid[2]; Blázquez López, Elena[3]; Vaquero Martín, Javier[3]; Correa Rocha, Rafael[1]; Pion, Marjorie[2].

[1]Laboratorio de Inmuno-Regulación, Instituto de Investigación Sanitaria Gregorio Marañón, Madrid; [2]Grupo de Inmuno-Regulación Avanzada, Instituto de Investigación Sanitaria Gregorio Marañón, Madrid; [3]Laboratorio de Investigación en Hepatología y Gastroenterología, Instituto de Investigación Sanitaria Gregorio Marañón, Madrid.

Introduction

Regulatory T cells (Tregs) are currently used in therapy and, in most cases, are obtained from peripheral blood. However, a different cellular profile is observed by using the human thymus as a novel Treg source. These cells (thyTreg cells) seem to manifest more suitable features for therapy than peripheral Treg cells, such as purity, suppressive capacity, stability, and cell numbers without extensive proliferation.

Extracellular vesicles (EVs) constitute an important cell-communicating mechanism that has been broadly studied during the last years. In immunology, regulatory EVs from mesenchymal or Treg cells are thought to be pivotal in maintaining the immune homeostasis. Therefore, EVs are thoroughly studied as a potential treatment for a great variety of diseases. For that, EVs show characteristics that make them an interesting alternative to cell therapies:

- A small size allowing a wider distribution, reaching more tissues.
- There is no risk of differentiation, modification and proliferation because of their lack of nucleus.
- More feasible storage and stability.

The current project lies in understanding EVs-derived thyTreg (thyTreg-EVs), their properties and characteristics.

Materials and Methods

Firstly, thyTreg cells were purified from human thymuses and later cultured and activated. EVs were obtained from the conditioned media by different isolation methods such as Immunocapture, Ultrafiltration and Size Exclusion Chromatography and further characterized by Western Blot and Nanoparticle Tracking Analysis.

Results

EVs from thyTreg cells were characterized by the presence of EV-enriched proteins (CD9 and

TSG101) by Western Blot. Size distribution was also studied, observing two main EVs subpopulations, one of which proved to be small EV-like (30-150 nm). These findings show that thyTreg-EVs with a good purity grade can be isolated from this source, opening new possibilities regarding EV therapy.

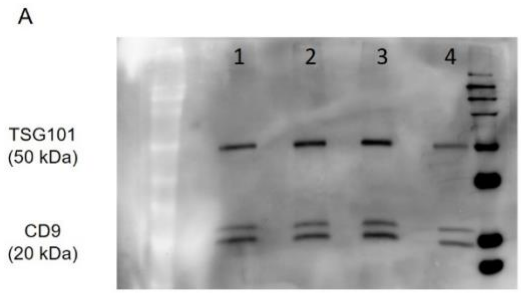


Figure A. CD9 (20 kDa) and TSG101 (50 kDa) molecules identified by Western Blot Analysis in four independent experiments.

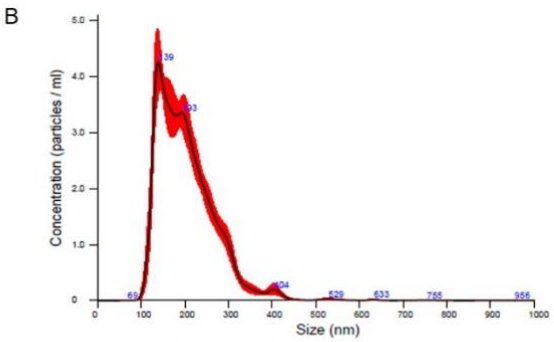


Figure B. Size distribution obtained after Nanoparticle Tracking Analysis. Two main populations are observed (139 nm and 193 nm). The concentration was $5{,}29 \cdot 10^9$ particles/mL.



CO-05

HUMAN INTESTINAL CD103+SIRPA+ CONVENTIONAL DENDRITIC CELLS FROM PATIENTS WITH CROHN´S DISEASE PRIME IL-17+ T-CELLS.

Arribas Rodríguez, Elisa[1]; González De Castro, Carolina[1]; Fiz López, Aida[1]; De Prado Santos, Ángel[1]; Fernández Salazar, Luis[2]; Barrio Andrés, Jesús[3]; Izquierdo, Sandra[2]; García Alonso, Javier[3]; De Andrés, Beatriz[2]; García Abril, José María[3]; Romero, Alejandro[2]; Sánchez González, Javier[3]; Garrote Adrados, José Antonio[1]; Arranz Sanz, Eduardo[1]; Bernardo Ordiz, David[1].

[1]Instituto de Biología y Genética Molecular, Valladolid; [2]Hospital Clínico Universitario de Valladolid; [3]Hospital Universitario Rio Hortega, Valladolid.

Human intestinal conventional dendritic cells (cDC) maintain the balance between immunity and tolerance. However, their specific function in patients with Crohn´s disease (CD) is scarce. Hence, we hereby decided to assess their phenotype in inflamed and non-inflamed tissue from patients with CD referred to non-inflamed controls.

Tissue resections from patients with ileocolonic CD and controls (healthy tissue from proximal colonic cancer patients) were obtained, being subsequently enzymatically digested. Total intestinal cDC were identified within singlet viable leukocytes as CD14-CD64-HLA-DR+CD11c+. Three cDC subsets were sorted based on the expression of CD103 and SIRPα+ (CD103-SIRPα+; CD103+SIRPα+; CD103+SIRPα-) while total macrophages were sorted as CD14+CD64+HLA-DR+. cDC subsets and macrophages were further cultured with allogeneic CD4+ naïve T-cells to assess their Th1, Th17 and Treg phenotype.

All 3 intestinal cDC subsets from controls stimulated naïve T-cells as opposed to intestinal macrophages. Indeed, cDC stimulated T-cells produced IL-10 and co-expressed FoxP3, being this capacity increased in both CD103+ subsets. Moreover, all three ileal cDC subsets stimulated T-cells more efficiently than their paired colonic counterparts, although the acquired T-cell profile did not differ among tissues. None of the cDC subsets, however, induced the expression of IFNγ or IL-17. Referred to CD patients, all three colonic cDC subsets were more stimulatory than their counterparts from control tissue, while CD103+SIRPα+ cDC from inflamed ileum primed the generation of IL-17+ responding T-cells.

We have demonstrated that all human intestinal cDC prime the generation of Tregs, being this capacity increased in CD103+ cDC. Indeed, ileal cDC were more stimulatory than their paired colonic counterparts, suggesting an increased capacity of the ileum to generate Tregs.



Nevertheless, this processed was dysregulated in the inflamed tissue from patients with CD were CD103+SIRPα+ cDC seem to have been reprogramed by the tissue microenvironment to prime the generation of Th17 T-cells hence driving disease progression.



Sesión Comunicaciones Orales 2. Inmunodeficiencias: primarias y secundarias

CO-07

DISSECTING THE EFFECT OF A NOVEL HYPOMORPHIC *IL2RB* MUTATION ON IMMUNE DYSREGULATION

Bailey, Olivia[1]; Cabrera-Martinez, Berenice[1]; Garcia-Perez, Josselyn[2]; Baxter, Ryan[1]; Fernandez, Isabel[1]; Foster, Mary Jo[1]; Gapin, Laurant[1]; Kedl, Ross[1]; Hsieh, Elena[1].

[1]University of Colorado Anschutz Medical Campus, Colorado, USA; [2]Cellcarta.Brussels, Belgium

Human inborn errors of immunity (IEIs) are a class of genetic disorders caused by monogenic germline mutations that impair the function of the encoded protein. We recently described a novel IEI due to an *IL2RB* homozygous mutation (p.Pro222_Gln225Del) in two siblings who suffered from multi-organ autoimmunity and CMV susceptibility. This mutation resulted in decreased cell surface protein expression and increased serum IL-2/15 levels, with concomitant increased baseline STAT5 phosphorylation (pSTAT5) but poor pSTAT5 response to IL-2 and IL-15 stimulation. Counterintuitively, this IL-2Rβ hypomorphic mutation led to an expansion of memory $CD8^+$ T and immature $CD56^{bright}$ NK cells, which express the highest levels of IL-2Rβ and require IL-2/15 signaling for survival and differentiation. Yet, their maturation and function appeared impaired, suggesting that both intrinsic (hypomorphic receptor) and extrinsic (serum cytokine milieu) mechanisms were at play. To further interrogate these receptor instrinsic/extrinsic mechanisms we developed a mouse model that harbors the homolog mutation ($Il2rb^{Mut/Mut}$). Notably, when mutant bone marrow cells reconstituted a WT host, serum IL-2/15 levels and IL-2Rb protein surface expression partially normalized, though IL-2/15-induced signaling remained hypofunctional. Additionally, the dysregulated mutant IL-2Rb-driven CD8+ T cell immunophenotype normalized but the NK phenotype did not. These data highlight the asymmetrical effect that a partial IL-2Rb defect has on CD8 T and NK cell IL-2/15-dependent signaling and downstream cellular processes. These results imply that strategies to modify serum IL-2/15 levels may serve as a therapeutic approach to treating IL-2/15 signaling defects.



CO-08

A TRUNCATED P.Y153X CD247 (TCRZ) VARIANT IS STABLE AND FORMS POTENTIALLY PATHOGENIC CD247 HETERODIMERS IN HETEROZYGOSIS

Fernández Megino, Rebeca[1]; Marin, Ana V[1]; Fernandez-Boraita, Julia[1]; Jimenez, Diego J[1]; Briones, Alejandro C[1]; Herrero-Alonso, Marta[1]; Chacon-Arguedas, Daniel[1]; Garcia-Martínez, Elena[2]; Seoane-Reula, Elena[2]; Rodriguez-Sainz, Carmen[2]; Regueiro, Jose R[1].

[1]Department of Immunology, Ophthalmology and ENT, Complutense University School of Medicine and 12 de Octubre Health Research Institute (i+12), Madrid; [2]Gregorio Marañón Health Research Institute, Madrid.

The T-cell receptor (TCR) consists of a variable heterodimer, two invariant CD3 heterodimers and a single invariant homodimer (TCRζ/CD247). CD247 is essential for TCR expression and signaling through its three ITAMs (immunoreceptor tyrosine-based activation motifs). Thus, homozygous null mutations in *CD247* (–/–) are associated with primary immunodeficiency, but carriers (+/–) show no clinical features despite decreased surface TCR levels. Such cases help to understand human TCR biology. We studied an 8-year-old female with chronic refractory immune thrombocytopenia associated with CMV primo-infection. The patient inherited a hitherto undescribed heterozygous mutation in *CD247* (c.459C>A, p.Y153X) predicted to truncate the third ITAM. The carrier but asymptomatic mother had high IgE and RF, past episodes of lymphopenia and recurrent canker sores.
Our aim was to stablish a cell model to recapitulate p.Y153X carrier T cells and analyze if it may affect TCR expression, structure and/or function with pathogenic relevance.

PBMC were immortalized using HTLV-1. Extra and intracellular TCR/CD3/CD247 phenotype was performed and compared between immortalized and primary CD4+ T cells by flow cytometry. Western blot was used to analyze CD247 proteins.

HTLV-1 T-cell lines fully recapitulated the CD247 phenotype of p.Y153X carrier primary T cells, showing normal surface TCR levels, in contrast to +/– T cells. Western blot analyses showed a normal-sized CD247 protein (14.5 kDa), but also a smaller 14.3 kDa band, likely p.Y153X, giving rise to three CD247 dimer forms: two dominant dimers (a normal homodimer and a normal/p.Y153X heterodimer) and a minor p.Y153X homodimer.

We have thus developed a reliable cell model to further study TCR assembly and function in this case and shown that p.Y153X is stable and may form frequent variant surface TCR ensembles that could be the involved in the patient`s pathology, in contrast to null mutation carriers who build only normal CD247 dimers.



CO-09

CORRECTING APDS2 SYNDROME-CAUSED MUTATION FOR FUTURE GENE AND CELL THERAPIES

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APDS2 syndrome is a genetic disorder classified as a primary immunodeficiency and caused by mutations in *PIK3R1* gene. Such alteration results in a dysfunctional immune system, affecting NK cells among others. Giving the lack of specialized non-toxic long-term treatments against APDS2 syndrome, we aim to develop novel strategies to uncover effective specific-therapies directed to APDS2 patients by correcting the mutation in their own cells. For this purpose, we use the CRISPR/Cas9 molecular tool to edit the genome of an APDS2-derived iPS cell line recently generated in our laboratory. According to the characteristics of the iPS cells, we utilize nucleofection as the transfection method for CRISPR/Cas9 complex internalization, which supplies a less aggressive and more reproducible gene editing technology. Finally, we use corrected APDS2-derived iPS to differentiate them into hematopoietic stem cells expressing the CD34+ marker. These hematopoietic progenitors could be also differentiated into the different types of blood cells, including NK cells. To this extent, we will be able to obtain not only normal NK cells, but also a personal source of healthy transfusable components from the APDS2 patient. As a whole, our work represents an innovative therapy that will improve current treatments, providing an exceptional and powerful tool to study APDS2 syndrome as well as personalized gene and cell therapy. Moreover, our findings could be extrapolated to the research of similar pathologies, facilitating the development of advanced targeted gene and cell therapies.



CO-10

HETEROZYGOUS NONSENSE CD247 MUTATIONS SHOW POTENTIALLY PATHOGENIC DOMINANT-NEGATIVE FEATURES AFFECTING TCR EXPRESSION AND FUNCTION

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The TCR has a single invariant homodimer (TCRζ/CD247) that is essential for expression and signaling through its three ITAMs (immunoreceptor tyrosine-based activation motifs). Thus, homozygous null mutations in CD247 are associated with immunodeficiency, but +/– carriers show no clinical features despite decreased surface CD3 (50% vs normal). It is unclear if carriers of CD247 variants show dominant-negative effects. We analyzed the impact of heterozygous CD247 mutations in patients with signs of immunodeficiency or autoimmunity.

A 6-month-old boy with recurrent infections carried a *CD247* mutation (p.Q101X) predicted to ablate the last two ITAMs. Primary T cells showed a moderate reduction of surface CD3 expression (85%, with UCHT1 MoAb) but a severe reduction of intracellular CD247 (20% with H146, an ITAM3-specific MoAb). A second patient with the same mutation, a 50-year-old woman with autoimmune alopecia, showed a similar reduction in CD3 (70%) but normal iCD247 expression (90%) and increased (400%) or normal induction of activation markers CD69 and CD25 in response to anti-CD3/CD28, respectively.

To evaluate Q101X impact in TCR expression and function, Jurkat cells (JK) and CRISPR/Cas9-edited CD247-deficient JK (ZKO), were lentivirally transduced with plasmids carrying normal CD247 or mutations ablating three (Q70X, associated to immunodeficiency), two (Q101X), or one (Q142X, ClinVar-reported) ITAM, to compare Q101X with more (Q70X) or potentially less (Q142X) severe variants. Transduction of the mutations into ZKO could not restore normal surface CD3 expression (11%, 22% and 61%, respectively), whereas in JK they reduced it (9%, 18% and 38%), and both effects occurred in an ITAM number-dependent fashion. All six transfectants showed similarly reduced CD69 induction (25-50%).

Our results suggest that variant homodimers lacking ITAMs are defective for normal TCR assembly and may exert a dominant-negative effect in normal TCR expression and signaling which, in turn, may cause disease, as shown for Q70X and Q101X.



CO-11

CANDIDIASIS MUCOCUTÁNEA CRÓNICA POR GANANCIA DE FUNCIÓN DE STAT1: EXPERIENCIA DEL HOSPITAL UNIVERSITARIO LA PAZ

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El síndrome de ganancia de función de STAT1 (STAT1 GOF) es una enfermedad genética con penetrancia completa y expresividad clínica muy variable, siendo la candidiasis mucocutánea crónica (CMC) el fenotipo clásico y la presentación clínica más común, frecuentemente asociada a infecciones no fúngicas, distintas formas de autoinmunidad y procesos inflamatorios.

Se realiza un estudio retrospectivo de 6 pacientes de edades comprendidas entre 20 meses y 77 años, estudiados en el Hospital Universitario La Paz entre los años 2015 y 2022. Se revisan las características epidemiológicas, presentación clínica y tratamientos, así como los hallazgos inmunológicos y genéticos.

A nivel clínico, todos los individuos presentaron candidiasis y la mitad sufrió infecciones bacterianas de repetición del tracto respiratorio, dos de ellos con bronquiectasias. Las manifestaciones autoinmunes fueron variadas, desarrollando hidrosadenitis supurativa (2/6), enfermedad de Addison (1/6), hipotiroidismo autoinmune (1/6) y trombopenia autoinmune (1/6). Respecto a los tratamientos, todos han recibido antifúngicos y antimicrobianos, una paciente requiere gammaglobulina inespecífica periódica, corticoides y metotrexato, y un paciente recibe baricitinib. Lo más característico del estudio inmunológico fue la disminución de linfocitos Th17 y aumento de Th1, que se observó en el 100% de los pacientes estudiados, así como linfopenia en 5/6. En cuanto al estudio genético, se detectaron variantes en el gen *STAT1* en todos los pacientes. Mediante el ensayo de fosforilación de pSTAT1, pudimos catalogarlas como variantes generadoras de ganancia de función.

El síndrome STAT1 GOF es una entidad clínica a considerar ante un paciente con candidiasis de inicio temprano, infecciones bacterianas del tracto respiratorio, manifestaciones de autoinmunidad y citopenia Th17. En nuestra experiencia, el ensayo de fosforilación tiene una gran utilidad para validar el estudio genético. Los tratamientos con inhibidores de la vía JAK/STAT suponen una nueva opción terapéutica, principalmente en pacientes con inflamación y/o autoinmunidad.



CO-12

SOMATIC GENETIC RESCUE CORRELATING WITH CLINICAL IMPROVEMENT IN A PATIENT WITH *TNFRSF9* (CD137/4-1BB) DEFICIENCY.

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Reversion mosaicism is a naturally occurring event involving spontaneous correction of a pathogenic mutation in somatic cells. TNFRSF9 (CD137/4-1BB) deficiency is a recently described inborn error of immunity (IEI) characterized by lymphocytic defects with early-onset Epstein-Barr virus (EBV)-associated lymphoma.

We report a patient who at 12 years of age developed a severe EBV-associated hemophagocytic lymphohistiocytosis episode. No genetic defects were found at that time, and she underwent HSCT from an HLA identical brother with good engraftment. In the following 8 years the patient could not control the EBV, with recurrent reactivations, lymphoproliferation and EBV-associated smooth muscle tumor. Then, the patient experienced a spontaneous decrease in EBV viral load. Using wole exome sequencing (WES) we identified a novel homozygous stop-gain variant in *TNFRSF9*. Strikingly, the HSCT donor was also homozygous but without overt clinical symptoms (incomplete penetrance). The presence of small, unexpected peaks in Sanger sequencing of blood samples lead us to suspect a possible somatic reversion event. Using deep amplicon sequencing (a next generation sequencing approach) we confirmed the presence of two independent somatic reversion events: a second-site mutation in the same codon (leading from a STOP codon to a missense variant) and a "back mutation" (leading from a STOP codon to the wild-type sequencing). The revertants were specifically located at CD8-T cells (absent in neutrophils, B cells and CD4-T cells), the main cell type involved in EBV control. Single-cell RNA sequencing experiments in purified CD8 T cells are ongoing.

We report the first case of reversion mosaicism in CD137 deficiency associated with clinical improvement, which may point the way to gene therapy strategies in this IEI.



Sesión Comunicaciones Orales 3. Inmunidad innata

CO-13

CYTOTOXIC NKG2DHI NK CELLS ACTIVATED BY CD1C+ DENDRITIC CELLS CONTRIBUTE TO PRIMARY SJÖGREN SYNDROME PATHOLOGY

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Sjögren syndrome (pSS) is characterized by pathogenic Th17 adaptative immune responses in salivary glands (SG) but the contribution of innate natural killer (NK) and dendritic cells (DC) to the pathology remains unclear.

PBMCs from 38 pSS patients and 42 healthy donors were used for phenotypical analysis by FACS. Function of NK cells from these individuals was tested by co-culture with target K562 cells. CD1c+ cDC from pSS were characterized by RNA-seq analysis and by functional co-culture assays with autologous NK cells. Expression of ligands for NK cell receptors on cDC was analyzed basally and after stimulation with poly I:C in the presence of siRNAs specific for RIG-I/DDX60. Finally, influence of NK cell enrichment in pathogenic adaptative Th17 cells in the SG and impact of anti-NK1.1-depleting antibody was determined in a mouse SS model.

Enriched proportions of transitional CD16+ CD56hi NK cells characterized by increased expression of NKG2D ($p<0.0001$), IFNg and TNFa ($p=0.01$) and high cytotoxic activity were found in pSS. In addition, we detected significantly basal higher expression ($p<0.0001$) of the activating NKG2D ligand MICab on CD1c+ cDC from pSS, which exhibited transcriptional signatures enriched on RIG-I/DDX60 and IFN target genes. Such CD1c+ cDC phenotype was associated with higher ability to activate cytotoxic NK cells *ex vivo*. Importantly, expression of MICab was efficiently induced on cDCs from pSS after stimulation with poly I:C and was dependent on RIG-I, DDX60. Importantly, transitional NK cells NKG2Dhi and CD1c+ cDC expressing higher levels of NKG2D ligand were also detected in vivo in the SG of mice injected with poly I:C and NK cell depletion was associated with reduced levels of IL-17 cells in this tissue.



Our study suggests and active role of NK cells during pSS pathology and provides novel mechanisms of activation mediated by DC that could represent future therapeutic targets.



CO-14

NON-REDUNDANT ROLE OF NF-KB AND INFLAMMASOME SENSORS DRIVING PATHOGENIC DIFFERENTIATION AND ACTIVATION OF MONOCYTES IN RESPONSE TO DISTINCT SARS-COV-2 COMPONENTS

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Background:

Inflammatory CD16+CD14+ transitional and CD16HiCD14lo non-classical monocytes (Mo) associate with severe COVID-19. Previous studies suggest that Mo exposed to SARS-CoV-2 Spike protein might induce the activation of the inflammasome. However, specific innate sensors mediating the activation or differentiation of Mo in response to different SARS-CoV-2 components remain poorly characterized.

Methods:

Mo from healthy donors (n=15) were stimulated with SARS-CoV-2 peptide pools of Spike protein-1 (S1), Receptor Binding Protein (RBD), Nucleocapsid protein (NP). Differentiation of Mo subsets and activation were assessed by FACS analysis of CD14, CD16, CD40 and CD86 and IL-1 b was evaluated in culture supernatants. Experiments were sometimes performed in the presence of specific inhibitors for NF-kB/Caspase1 (Parthenolide), NLRP3 (MCC950), caspase 1,5,9 (Z-WEHD-FMK) or siRNAs targeting NLRP3, NLRP1, NLRC4. Bronchoalveolar lavage (BAL;n=13) paired blood samples (n=8) from severe COVID-19 patients were included in some experiments. Finally, functionality of Mo stimulated with SARS-CoV-2 components was analyzed by *in vitro* co-cultures with isolated allogenic T cells from healthy donors. Pathogenic CD4+ T cells were identified by expression of IL-4, IL-17, IFN-g.

Results: SARS-CoV-2 S1 but not NP and RBD induced Mo differentiation into transitional and non-classical subsets, however all components induced IL1-b secretion. Interestingly, differentiation of Mo from peripheral blood and COVID-19 BAL into these subsets was observed exclusively in response to S1 and was dependent on NF-kB activation, while the NLRP3 inflammasome was required for the induction of CD86+ classical Mo in response to S1. In contrast, NLRC4 and NLRP3 regulated CD86 upregulation in Mo after stimulation with RBD and NP. Such activation/differentiation patterns were associated with differential abilities



of Mo primed with S1, RBD and NP to induce pathogenic IFNg+IL-4+ CD4+ T-cells *in vitro*.

Conclusion: Distinct SARS-CoV-2 components differentially mediate activation, subset generation and functional activity of Mo and could represent new targets against COVID-19 pathology.



CO-15

GM-CSF PROGRAMS HEMATOPOIETIC STEM AND PROGENITOR CELLS DURING *CANDIDA ALBICANS* VACCINATION FOR PROTECTION AGAINST REINFECTION

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Universitat de València.

Trained immunity is defined as the adaptation of the innate immune system to mount a faster and greater response against a secondary challenge. We previously demonstrated that this concept can be applied not only to mature myeloid cells, but also to hematopoietic stem and progenitor cells (HSPCs), making them important players in fighting against infection.

In this work, we have studied the mechanisms that drive trained immunity in HSPCs during a primary infection with a low-virulence, non-germinative strain of *Candida albicans* (PCA2) that causes a mild infection and confers protection from a lethal reinfection with a virulent *C. albicans* strain.

In order to characterize transcriptomic changes in specific HSPC subsets during the PCA2 infection, we performed scRNA-seq of Lin^- c-Kit^+ cells isolated from the bone marrow of non-infected mice, the bone marrow of 24-h PCA2-infected mice, the bone marrow and spleen of 7-day PCA2-infected mice, and the bone marrow and spleen of mice reinfected with a virulent strain of *C. albicans* for 24 h. These analyses showed changes in biological pathways involved in the generation of trained immunity, in the activation of an emergency myelopoiesis response, and in immune cell responses against infections, such as regulation of cytokine production. In fact, we found that, upon reinfection, HSPCs are trained to produce greater amounts of proinflammatory cytokines. In addition, by performing adoptive transfer experiments, we showed that PCA2-trained HSPCs contribute to protection against reinfection. Finally, blocking GM-CSF demonstrated its involvement in HSPC training and in the protection induced by PCA2 infection.
Overall, these findings reveal a fundamental role for HSPCs in the protective response provided by trained immunity. Mechanistically, autocrine GM-CSF activation of HSPCs is responsible for the trained phenotype and essential for the PCA2-induced protection.

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CO-16

REGULATION OF MACROPHAGE FUNCTION BY AN IFNAR-ISG15-MITOCHONDRIAL AXIS

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Centro Nacional de Investigaciones Cardiovasculares, Madrid.

Type I Interferons (IFN-I) are multi-functional cytokines required for the initiation, but also the resolution, of many immune responses. IFN-I bind to their receptor (IFNAR) leading to activation of IFN Stimulated Genes (ISGs) and immune modulation. Upon stimulation of bone marrow-derived macrophages with STING/MAVS agonists, which strongly induce IFN-I, we observed a decrease in mitochondrial membrane potential (MMP). The decrease in MMP was also observed with recombinant IFN-I but not with other pattern recognition receptor agonists. This effect of IFN-I relied on IFNAR signalling and the ubiquitin-like protein ISG15, which has been previously been found to modify several mitochondrial proteins. One effect of the IFN-I induced decrease in MMP was an increase in efferocytosis by BMDMs and peritoneal macrophages, dependent on ISG15. In vivo, ISG15-deficient mice showed decreased clearance of apoptotic thymocytes upon dexamethasone administration, indicating decreased efferocytosis. Decreased MMP also lead to activation of the mitochondrial protease OMA1 and the induction of mitochondrial fission, decreasing the contact sites between the mitochondria and the Endoplasmic Reticulum, signalling hubs known as Mitochondrial Associated Membranes (MAMs). Preventing mitochondrial fission or the decrease in MAMs, using ISG15-deficient or OMA1-deficient cells, enhanced ISG induction upon IFN-I stimulation without affecting the primary response to STING stimulation. These data indicate that IFNAR-ISG15 signalling can modulate key macrophage functions, promoting the resolution of inflammation.



CO-17

DIRECT TLR2 SIGNALING THROUGH MTOR AND TBK1 INDUCES C/EBPB AND IRF7-DEPENDENT MACROPHAGE DIFFERENTIATION IN HEMATOPOIETIC STEM AND PROGENITOR CELLS

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During an infection, hematopoiesis is altered to increase the output of mature myeloid cells to fight off the pathogen. Despite convincing evidence that hematopoietic stem and progenitor cells (HSPCs) can sense pathogens directly, more mechanistic studies are needed to reveal whether pattern recognition receptor (PRR) signaling initiates myeloid development directly, or indirectly through the production of cytokines by HSPCs that can act in an autocrine/paracrine manner, or by a combination of both direct and indirect mechanisms. In this study, we have used an *in vitro* model of murine HSPCs to study myeloid differentiation in response to the TLR2 ligand Pam_3CSK_4 and showed that, besides indirect mechanisms, TLR2 stimulation of HSPCs promotes myelopoiesis directly by initiating a MyD88-dependent signaling. This direct differentiation program involves a combined activation of the transcription factors PU.1, C/EBPβ and IRF7 driven by TBK1 and PI3K/mTOR. Notably, downstream of MyD88, the activated TBK1 kinase can activate mTOR directly and IRF7 induction is mediated by both TBK1 and mTOR. TLR2 signaling also induces NF-κB dependent IL-6 production that may further induce indirect myeloid differentiation. Our results have identified the direct signaling pathways and the transcription factors involved in macrophage development from HSPCs in response to TLR2 engagement, a critical process to trigger a rapid immune response during infection. This study defines the mechanism by which direct macrophage differentiation is induce upon microbial sensing by HSPCs. Understanding the biology of emergency myelopoiesis induced by TLRs may aid in devising new therapeutic strategies to treat infections and hematologic diseases. TLR2-activated pathways could be targeted to boost innate immune replenishment during infection and to induce the terminal differentiation of myeloid leukemic cells.

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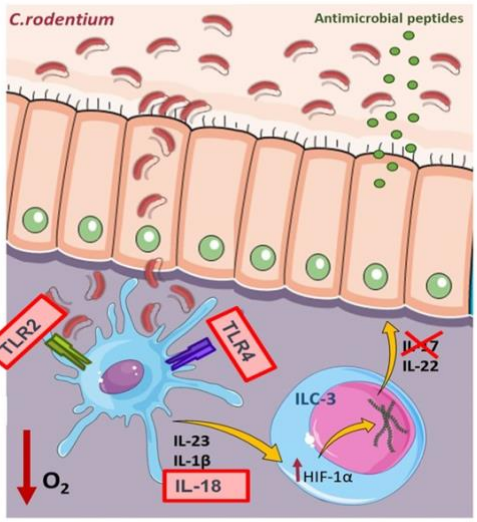
CO-18

OXYGEN-INDEPENDENT REGULATION OF HIF-1A IN ILC3S AMELIORATES THE INFLAMMATION OF C. RODENTIUM-INDUCED COLITIS

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

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Innate lymphoid cells type 3 (ILC3) are tissue-resident immune cells that are fundamental in maintaining mucosal integrity and protecting against extracellular infections, particularly in the gut. ILC3s primarily act by secreting IL-22 and IL-17, but the cellular and molecular processes that control them need to be better characterized. This is especially significant when it comes to understanding colitis, which is defined by an imbalance in the connection between the host, their microbiota, and immune system. According to a new research, hypoxia can modulate the activity of intestinal ILC3s via stabilizing hypoxia-inducible factor (HIF-1 a a) in a post-translational manner. This molecular mechanism, however, needs further validation *in vivo* using more specific transgenic knock out mice to evaluate its functional significance during the development of infectious colitis. In this research, we identified a new molecular pathway that modulates HIF-1a transcriptionally in ILC3s after *Citrobacter rodentium* infection. We assessed the response to this infection in novel transgenic mice designed for specific HIF-1α gene inactivation in ILC3s (RAG-1KO HIF-1a$^{fl/fl}$ RortCre). Mice showed a more severe colitis after *C. rodentium* infection than control mice due to the inability to upregulate IL-22. Moreover, colon explants from these transgenic mice revealed that IL-18, produced most likely by the activation of Toll Like Receptors, is the cytokine responsible for the IL-22 secretion through HIF-1a in ILC3s. Importantly, sorted ILC3s activated *ex vivo* with IL-18 and IL-23 induced the transcription of HIF-1a and IL-22, but not IL-17. Our findings show for the first time that *C. rodentium* can modulate transcriptionally HIF-1a in ILC3s, promoting the secretion of IL-22 upon IL-18 stimulation, opening up new research pathways in the field of infectious colitis and the molecular regulation of these cells.


C.rodentium
Antimicrobial peptides
TLR2
TLR4
IL-17
IL-22
ILC-3
IL-23
IL-1β
IL-18
HIF-1α
O2



Sesión Comunicaciones Orales 4. Inmunoterapia

CO-19

ANTIBODY AND T-CELL MEDIATED IMMUNE RESPONSES INDUCED BY CD19 CHIMERIC ANTIGEN RECEPTOR (ARI-0001) IN RELAPSED/REFRACTORY PATIENTS WITH CD19 POSITIVE B-CELL MALIGNANCIES

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Background: ARI-0001 cells express a second-generation CAR that combines the anti-CD19 single-chain variable fragment (scFv) originated from A3B1, a murine monoclonal antibody, conjugated with the transmembrane and co-stimulatory domains. The Spanish Agency of Medicine has approved under hospital exemption this treatment for patients with actue lymphoblastic leukemia (ALL) older than 25 of age. Although the efficacy and safety has been demonstrated, it has been poorly investigated the relevance of the immunogenicity induced by CAR-T19 cells.

Methods: Regarding humoral response, the detection of human anti-murine antibodies (HAMA) in patients' sera was assessed by a cell-based fluorescence assay. Afterwards, a cytotoxicity assay was performed to study the effect of HAMA on the CART-cell function. To study the cellular response, possible HLA-specific CAR19 immunogenic peptides were predicted *in silico*. These peptides were used to assess the presence of reactive T cells (peptide specific) in patients.

Results: Twenty out of 47 patients (42.5%) tested positive for the presence of anti-CAR antibodies after a year of the first or second infusion. Despite the fact that 42.5% had anti-CAR antibodies, only some of these patients' HAMA positive had neutralizing effect on ARI-0001 cells *in vitro*. Concerning cellular immune response, a set of 103 prioritized immunogenic peptides specific for HLA patients derived from scFv and CAR19 domains (68 HLA-I peptides and 35 HLA-II peptides) were chosen for assessing the T-cell activation. It has been observed a T-cell activation (IFNg secretion).



Conclusions: These data suggest that our CAR19 induces humoral and cellular immune responses. On the one hand, anti-CAR antibodies had been detected in 42,5% of patients. Moreover, these antibodies had a neutralizing effect on ARI-0001 cells. On the other hand, it has been detected reactive T-cell against HLA-specific CAR19 peptides. Thus, immunogenicity induced by ARI-0001 cells could be clinically important, specially before considering a second infusion.



CO-20

PROGRESS TOWARDS A PATIENT-SPECIFIC MONOCLONAL ANTIBODY IN SÉZARY SYNDROME

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Sézary Syndrome (SS) is an aggressive ultra-rare leukemic form of cutaneous T-cell lymphoma characterized by circulating malignant CD4 T lymphocytes (SS cells). Patients with SS have poor prognosis and current treatments show high rates of relapse. Thus, there is an unmet need for an efficient treatment. SS cells have unique clonal potentially targetable surface epitopes, including its TCR, which we aim to generate specific mouse monoclonal antibodies (mAbs) against SS cells.

SS patient primary sorted SS (tumoral) and non-SS (healthy) T cells using CD4 and PD-1 were expanded in vitro using different immortalization procedures, such as exposure to HTLV-I, HVS or irradiated allogeneic feeder cells (PBMC and EBV-B cells from different donors). Non-SS cells were stained with deep red cell tracker and mixed 1:1 with SS cells for mAb reactivity screening by flow cytometry. Mice were immunized with SS cells and Ab producing hybridomas were generated by fusion of splenic mice with immortal myeloma cells (SP2)

We showed that in vitro expanded SS cell lines recapitulate the clonal malignant TCR by PCR and Western blot. Regarding mAbs, a total of 203 hybridomas were obtained after fusion and two hybridomas (M5F8 and M9A9) consistently recognized immortalized SS slightly better than non-SS T cells, in comparison to an anti-CD3 hybridoma (Leu-4) as positive control. We are currently cloning the hybridomas and characterizing their specificity.

In conclusion, we have generated two mAbs specific for SS cells that could become patient-specific reagents to treat SS.



CO-21

IDENTIFYING SARS-COV-2 'MEMORY' NK CELLS FROM COVID-19 CONVALESCENT DONORS FOR ADOPTIVE CELL THERAPY (RELEASE)

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COVID-19 disease is the manifestation of syndrome coronavirus 2 (SARS-CoV-2) infection, which is causing a worldwide pandemic. This disease can lead to multiple and different symptoms, being lymphopenia associated with severity one of the most persistent. Natural killer cells (NK cells) are part of the innate immune system, and play a key role fighting against virus-infected cells. Unlike T lymphocytes, NK cells are not known for their specific memory population. Nevertheless, studies regarding NK cell population in other viral infections, such as cytomegalovirus, have shown specific memory population of these cells. These NK cells are particularly re-activated when facing viral-specific peptides. In this study, we determined the phenotype of NK cells after COVID-19 and the main characteristic of SARS-CoV-2-specific-like NK population in the blood of convalescent donors. CD57+ NKG2C+ phenotype in SARS-CoV-2 convalescent donors indicates the presence of 'memory'/activated NK cells as it has been shown for cytomegalovirus infections. Moreover, this “memory” population responds to the presence of SARS-CoV-2 peptides. Although the existence of this population is donor dependent, its expression may be crucial for the specific response against SARS-CoV-2. As a result, it gives us a tool for selecting the best donors to produce off-the-shelf living drug for cell therapy to treat COVID-19 patients under the RELEASE clinical trial (NCT04578210). NK cells from selected donors’ have been isolated, frozen and storage. NK cell arm of RELEASE phase 1 clinical trial has been initiated in several patients.



CO-22

DEVELOPING CAR-NK CELLS ON NK-92 CELLS STABLY TRANSFECTED WITH CD16

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Natural Killer (NK) cells are a promising adoptive cellular therapy against cancer due to their ability to lyse infected or transformed cells in an antigen-independent manner. Human allogenic NK cells produced from healthy donor peripheral blood cells should be expanded and activated with specific feeder cell and cytokines to become a viable treatment, generating expanded NK cells (eNK). Previous results from our research group showed a moderate cytotoxicity effect of eNK cells over several Multiple Myeloma (MM) cell lines, which could be increased combining the eNK cells with the anti-CD38 monoclonal antibody Daratumumab, approved for use in MM patients. However, there's often a noteworthy variability between each series of experiments because of the donor's genetic background. To avoid that variability, we chose the highly cytotoxic cell line NK-92 as a more stable model. However, NK-92 cells are unable to induce Antibody Dependent Cell Cytotoxicity (ADCC) since these cells do not express CD16. We transduced NK-92 cells with the high affinity CD16 variant and a fluorescent reporter using lentiviral supernatant. The new cell line obtained, NK-92-CD16, showed more cytotoxicity alone and especially in combination with Daratumumab than both parental cells and eNK cells. In order to maximize their cytotoxic potential and clinical use, we decided to transfect these NK-92-CD16 cells with an anti-SLAMF7 chimeric antigen receptor (CAR). Although CAR expression and stability has been achieved, CAR-NK-92-CD16 cells did not display greater cytotoxicity than NK-92-CD16 at least in vitro, although they could have a better targeting potential in vivo. In addition, since NK-92 cells should be irradiated for its clinical use, we also checked if irradiation impaired their functionality.



CO-24

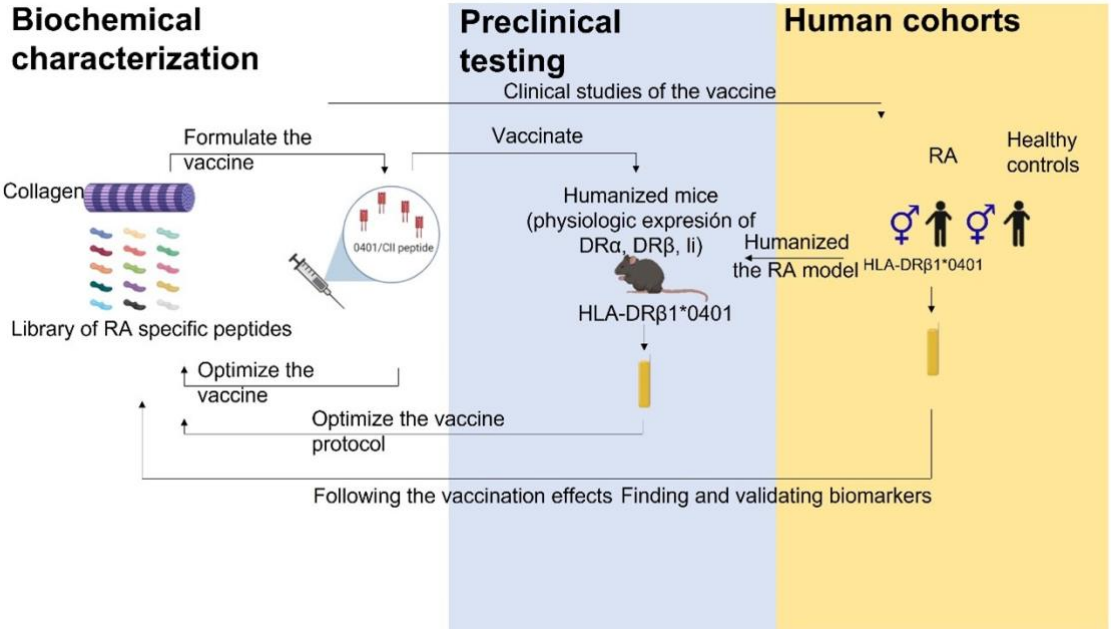
DEVELOPMENT OF NEW TOLEROGENIC PEPTIDE-BASED VACCINE FOR RHEUMATOID ARTHRITIS

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Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease, characterized by inflammation of peripheral joints and bone destruction due to synovial tissue infiltration with immune cells, secretion of proinflammatory cytokines, and matrix metalloproteinases. Interestingly, around the onset of the disease, autoantibodies against citrullinated proteins cross-react with joint proteins, such as type II collagen (Col2). Currently, the medical treatment of established RA is the neutralization of inflammatory mediators such as TNFα. However, these treatments remain problematic, largely because of their lack of immune specificity and side-effects. Hence, there is a need for the development of more specific, safer, and more effective therapeutic interventions targeting the initiating immune mechanisms. The genetic association of RA with certain MHC class II (MHCII) alleles indicate T cells as central players and, consequently, an important target for therapeutic intervention in the disease. We suggest a tolerogenic peptide-based vaccination as a new therapeutic option for the prevention of RA and, possibly, treatment of early/established disease. In this sense, we have previously shown that modulation of Col2-reactive T cells by vaccination with soluble mouse MHCII molecules in complex with the glycosylated Col2 peptide 259-273 efficiently prevents collagen-induced arthritis (CIA), as well as also therapeutically cures the disease. Our current focus is to develop vaccines based on human MHCII molecules, improve them and investigating the mechanism of the vaccine induce tolerance, through our unique humanized mouse strains that will provide new opportunities to develop an effective vaccine against RA in humans.




Biochemical characterization
Preclinical testing
Human cohorts
Clinical studies of the vaccine
Formulate the vaccine
Vaccinate
RA
Healthy controls
Collagen
0401/CII peptide
Humanized mice (physiologic expresión of DRα, DRβ, Ii)
Humanized the RA model
HLA-DRβ1*0401
HLA-DRβ1*0401
Library of RA specific peptides
Optimize the vaccine
Optimize the vaccine protocol
Following the vaccination effects
Finding and validating biomarkers



Sesión Comunicaciones Orales 5. Miscelánea (Inflamación / Alergias y reacciones de hipersensibilidad / Inmunidad anti-tumoral)

CO-25

SPECIFIC CELLULAR REGULATION OF LAMIN A/C IN IMMUNE AND STROMA CELLS AS A THERAPEUTIC TARGET FOR INFLAMMATORY BOWEL DISEASE

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The maintenance of intestinal homeostasis is crucial to prevent the development of inflammatory bowel disease (IBD). It has been shown that gut homeostasis strictly depends on the interplay between several cell types including immune and non-immune cells. However, the mechanism of action of intestinal homeostasis-regulating cells remains largely unknown. Here, we explore the specific role of several of these immune cells and stroma cells by regulating the levels of lamin A/C, a structural, epigenetic, and transcriptional regulator, in a mouse model of IBD with dextran sulfate sodium salt (DSS).

DSS-colitis development was measured in mice that lack of lamin A/C (Lmna-/-) in all immune cells (Vav1-Cre), T cells (CD4-Cre), some myeloid cells (LysM-Cre), and stroma cells (Col1a2-Cre) or in mice overexpressing lamin A/C in T cells (Lmna++/++ CD4-Cre). Mice were exposed to one or two cycles of DSS to preferably stimulate innate or adaptive immunity, respectively.

Our experiments show that, in comparison to wild-type mice (Lmna+/+), the absence of lamin A/C in T cells, in myeloid cells, or stroma cells upon one dose of DSS protects from colitis. The protection appears to be more accentuated when mice that lack lamin A/C in T cells are exposed to two doses of DSS, hence, highlighting the key role of lamin A/C in the adaptive immunity in colitis. In accordance, lamin A/C overexpression in T cells provokes much more colitis upon two doses of DSS. The lack of lamin A/C in all immune cells protects from colitis upon one or two doses of DSS.

In conclusion, lamin A/C levels in immune cells and stroma cells seems to be important in colitis evolution: its absence protects against colitis while its overexpression worsen the disease, converting lamin A/C regulation in certain immune cells in a potential therapeutic target.



CO-26

FINDING IMMUNOLOGICAL DIFFERENCES TO HELP DIAGNOSIS AND EARLY TREATMENT OF KAWASAKI DISEASE AND MIS-C (MULTISYSTEM INFLAMMATORY SYNDROME IN CHILDREN)

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The recent COVID-19 pandemic was first thought to spare children from health deprivation caused by infection with SARS-CoV-2. However, soon a new syndrome resembling Kawasaki Disease (KD) was reported: Multisystem Inflammatory Syndrome in Children (MIS-C). The aim of this study is to provide new biomarkers for both diseases in order to facilitate diagnosis and reduce the time-lapse until treatment is provided – which will reduce the risk of developing severe cardiovascular complications.

An extensive immune system characterization by flow cytometry and serum protein characterization by a multiplex technology (Olink) was performed from fresh blood samples of patients with acute MIS-C (n=19) and KD (n=10). For protein characterization we also analysed recovery samples for these groups (n=19 and n=8, respectively).

Besides the already described lymphopenia in MIS-C, we found additional significant immune differences in both groups. Although lymphocyte numbers (cells/ml) were lower in MIS-C, percentages of activated T-CD4+ and T-CD8+ cells were higher compared to KD. Moreover, when comparing activated T cells in MIS-C and KD individually, regulatory T cells (Treg) showed the highest levels. These data suggest a stronger response of T cells in MIS-C, and higher Treg activity in both groups, which could reflect the response to the excessive inflammation. Ratios previously described in other inflammatory conditions were evaluated: MIS-C showed higher neutrophil/lymphocyte and Th17/Treg ratios than KD, suggesting higher inflammatory conditions in this group. In addition, monocyte and dendritic cells (DCs) numbers were decreased in MIS-C relative to KD. Parallel to these inflammatory cellular profiles, we identified increased levels of inflammatory cytokines in plasma of patients during the acute phase of the disease compared to recovery samples. Moreover, IL-6, which is one of the main

cytokines involved in cytokine storm in adult COVID-19, was higher in MIS-C suggesting, again, stronger inflammatory conditions in this pathology compared to KD.



CO-27

PD-1 SE EXPRESA EN GRÁNULOS CITOTÓXICOS DE LAS CÉLULAS NK Y SE MOVILIZA RÁPIDAMENTE A LA MEMBRANA CELULAR TRAS EL RECONOCIMIENTO DE UNA CÉLULA TUMORAL

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La contribución de PD-1 a la regulación de la actividad de las células NK es un tema controvertido, con resultados ambiguos en cuanto a su expresión y papel en la modulación de su actividad anti-tumoral. Nuestro estudio presenta nuevas evidencias sobre el mecanismo implicado en la regulación de la expresión de PD-1 en la membrana de las células NK y sus consecuencias funcionales durante la eliminación de células tumorales. A diferencia de las células NK de pacientes con cáncer, las de donantes sanos no expresan PD-1 en la membrana celular. Sin embargo, al incubar estas últimas con células tumorales, aumenta la expresión de PD-1 en membrana, al mismo tiempo que se produce la movilización de CD107a a la superficie. Este hallazgo sugiere la movilización de PD-1 a la membrana celular durante la degranulación de las células NK tras el contacto con células diana. De hecho, observamos presencia de PD-1 citosólico en células NK recién aisladas de donantes sanos, y que este colocaliza con CD107a y granzima B, indicando que PD-1 se expresa en los gránulos citotóxicos y se moviliza a la membrana tras el reconocimiento de la célula diana. La movilización de PD-1 a la membrana reduce significativamente la actividad antitumoral de las células NK frente a células tumorales que expresan PD-L1 *in vitro* e *in vivo,* revirtiéndose parcialmente con anticuerpos bloqueantes de PD-1. Estos resultados indican que células NK de individuos sanos expresan PD-1 asociado a gránulos citotóxicos, movilizándose rápidamente a la membrana celular después de la interacción con las células diana tumorales. Este nuevo hallazgo ayuda a comprender cómo se regula la expresión de PD-1 en la membrana de las células NK y las consecuencias funcionales durante la eliminación de las células tumorales, lo que ayudará a diseñar inmunoterapias contra el cáncer basadas en células NK más



CO-28

DEFICIENCY OF SHP1 IN MYELOID CELLS PROMOTES TUMOR GROWTH.

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Introduction:

Tyrosine kinases and phosphatases mediate the phosphorylation state of proteins and control cell homeostasis. Although the phosphoprotein tyrosine phosphatase SHP1 acts as an immune checkpoint in CD8+T cells, its pharmacological inhibition has no effect against tumor development in clinical trials, suggesting that SHP1 may play opposing roles in different cells in the tumor microenvironment. Here we assessed the effect of depleting SHP1 in $CD11c^+$ cells in the anti-tumoral response.

Methods:

We subcutaneously inoculated different tumors in CD11cCre SHP1flox mice (CD11cΔSHP1). Tumor growth was assessed and immune infiltrates were analysed by flow cytometry. To dissect the specific role of SHP1 in conventional type 1 (cDC1s) and type 2 (cDC2) dendritic cells, we adoptively transferred cDC1s or cDC2s from WT or CD11cΔSHP1 mice loaded with tumour lysates containing OVA to WT mice. Then, lymph node cells were restimulated with OVA257-264 or OVA323-339 peptides to reveal the CD8 and CD4 OVA-specific T cell response, respectively. Also, we depleted macrophages by clodronate liposome injection into WT or SHP1KO mice, and monitored the effect in tumor growth.

Results:

B16 tumors grew similarly in WT and CD11cΔSHP1 mice, but the immune cell infiltrates were reduced in CD11cΔSHP1 mice. Cancer-bearing CD11cΔSHP1 mice showed an impaired rejection of highly immunogenic tumours and a deficient response to PD1 blockade immunotherapy of MC38 tumors, compared with WT mice. However, vaccination with SHP-1-deficient cDCs induced normal or even increased cross-priming of OVA-specific IFN-γ+ CD8+ T cells. Notably, depletion of macrophages with clodronate-liposomes reverted the deficient rejection of MC38 tumors treated with anti-PD1 in CD11cΔSHP1 mice.

Conclusion:

Our results show that SHP1 depletion in CD11c+ cells impairs anti-tumor immunity and suggest that CD11c+ macrophages, rather than cDCs, are responsible for this effect



CO-29

BACTERIA EXPRESSING TUMOR ANTIGENS CAN TRAIN CD4^{+} LYMPHOCYTES FOR AN EFFECTIVE AND SPECIFIC ANTITUMOR IMMUNOTHERAPY

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Transphagocytosis has been described by our group as a mechanism by which conventional CD4+ T lymphocytes can capture and kill bacteria from previously infected dendritic cells. Bacteria capture “trains” CD4+ T cells (from now on bacteria trained T cells; BacT) that acquired novel abilities as antigen presenting cells. BacT cross-present bacterial antigens to naïve CD8+ T cells, triggering a potent and specific cytotoxic response, with great perspectives for being furtherly used as a cancer immunotherapeutic resource 1,2. In the present work we aimed to evaluate the efficacy of BacT cell immunotherapy in a model of mouse melanoma. We genetically engineered different strains of recombinant Listeria Monocytogenes (L.) by using the integrative plasmid vector pPL2, to express antigens from B16-F10 mouse melanoma, or OVA. Hence, Listeria-TRP2 express TRP2, a well-known antigen expressed in murine and human melanomas 3. Listeria-NeoAg express a tandem of 5 previously described immunoactive neoantigens 4. Listeria-OVA was also used as a tumor non-specific control. Melanoma tumors were implanted by subcutaneous injection of 400K B16-F10 cells per mouse. Tumor growth was monitored daily for 13 days post inoculation. Mice were weighed every two days and inspected for health, ulcers, and survival every day. Animals that met humane end point criteria were euthanized and the deaths recorded for the survival curve analysis. Mouse spleen naïve CD4+ cells were isolated and trained with either Listeria-OVA, Listeria-TRP2 or Listeria-NeoAg. BacT CD4+ cells, were then isolated by density gradient centrifugation and freezed until intravenous injection once the tumor has been formed (around day 6 after tumor implantation). Treatment with either bactT-TRP2, bacT-NeoAg or a mixture of both, significantly retarded tumor growth compared with bactT-OVA group, and extended lifespan by 26%.



Sesión Comunicaciones Orales 6. Autoinmunidad

CO-31

DIFFERENTIAL EFFECTS OF ANTI-TNFA AND ANTI-A4B7 DRUGS ON CIRCULATING DENDRITIC CELLS MIGRATORY CAPACITY IN INFLAMMATORY BOWEL DISEASE

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Inflammatory bowel disease (IBD) is a and chronic disorder that includes ulcerative colitis (UC) and Crohn's disease (CD). Both diseases show an uncontrolled intestinal immune response that generate tissue inflammation. Dendritic cells (DC) are antigen presenting cells that play a key role in the tolerogenic maintenance of the gastrointestinal mucosa. It has been reported that DC are recruited by intestinal mucosa in a CCR2 dependent manner and in IBD patients, this recruitment is more prominent than in healthy controls (HC), but the specific migration mechanism is still unknown. Here we study if anti-TNFα and anti-α4β7 drugs modify the GI mucosa recruitment capacity of DC in the same manner. For that Peripheral blood mononuclear cells were obtained from 15 patients per group (HC, UC and CD active and quiescent). Then, the expression of homing markers and the circulating DC migratory capacity toward CCL2, CCL25 and MadCam1 from the different study groups were determined. Then, cells were cultured in presence of anti-TNFα or anti-α4β7, hence assessing the differential effect of biological drugs (if any) over the DC subset in the different groups. Our data reveal that cDC2 ($CD1c^+$) express more surface markers than other DC, the expression of CCR9 within this subset was expanded in both quiescent groups and integrin β7 was modulated by anti-α4β7. CCL2 showed a clear chemoattract effect but it was not modulated by biological drugs. Anti-TNF increased the migratory capacities in almost all groups. So, we described for the first time a cDC2 subset as a potential diagnostic marker in IBD. DC from IBD patients express a different migration pattern than HC, being the expression of these molecules modulated by anti-α4β7. CCR9 seem to elicit a protective function over IBD. The CCR2/CCL2 recruitment mechanism is not modulated by biological drugs.

Keywords: biological drugs; inflammatory bowel disease; dendritic cells.



CO-32

CIRCULATING IMMUNE-COMPLEXES OF IGG/IGM BOUND TO B2-GLYCOPROTEIN-I ASSOCIATED WITH COMPLEMENT CONSUMPTION AND THROMBOCYTOPENIA IN ANTIPHOSPHOLIPID SYNDROME.

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Background.

Antiphospholipid syndrome (APS) is a multisystemic autoimmune disorder characterized by thrombotic events and/or gestational morbidity in patients with antiphospholipid antibodies (aPL). In a previous single-center study, APS-related clinical manifestations that were not included in the classification criteria (livedo reticularis, thrombocytopenia, leukopenia) were associated with the presence of circulating immune-complexes (CIC) formed by beta-2-glycoprotein-I (B2GP1) and anti-B2GP1 antibodies (B2-CIC). We have performed a multicenter study on APS features associated with the presence of B2-CIC.

Methods.

A multicenter, cross-sectional and observational study was conducted on 303 patients recruited from six European hospitals who fulfilled APS classification criteria. Prevalence of B2-CIC (IgG/IgM isotypes) and its association with clinical manifestations and biomarkers related to the disease activity were evaluated.

Results.

B2-CIC prevalence in APS patients was 39.3%. B2-CIC-positive patients with thrombotic APS presented a higher incidence of thrombocytopenia (OR: 2.32, p=0.007), heart valve thickening and dysfunction (OR: 9.06, p=0.015) and triple aPL positivity (OR: 1.83, p=0.027), as well as lower levels of C3, C4 and platelets (p-values: <0.001, <0.001 and 0.001) compared to B2-CIC-



negative patients. B2-CIC of IgM isotype were significantly more prevalent in gestational than thrombotic APS.

Conclusions.

Patients with thrombotic events and positive for B2-CIC had lower platelet count and complement levels than those who were negative, suggesting a greater degree of platelet activation. B2-CIC could be a possible biomarker of disease activity in APS patients.



CO-33

LOS NIVELES DE IGM FRENTE A FOSFATIDILCOLINA EN SUERO PREDICEN LA RESPUESTA AL TRATAMIENTO CON NATALIZUMAB E INTERFERÓN-B

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Antecedentes: Hemos desarrollado un ELISA ultrasensible que ha demostrado que el 90% de los pacientes con esclerosis múltiple (EM) presentan IgM frente a fosfatidilcolina en suero (IgMPCs), siendo la técnica más sensible para el diagnóstico de la enfermedad. Los pacientes con EM son tratados con inmunosupresores, pero en un porcentaje considerable el tratamiento no es efectivo.

Objetivo: Analizar el papel de los anticuerpos IgMPCs como biomarcador de respuesta al tratamiento.

Materiales: Muestras pareadas de suero de 95 pacientes con EM obtenidas antes y después de 6 meses de tratamiento. Los pacientes fueron clasificados en respondedores, si no había nuevos brotes ni aumento de la discapacidad, y en no respondedores cuando no se cumplían esos criterios.

Resultados: Los niveles de IgMPC en suero antes de comenzar el tratamiento con natalizumab fueron significativamente mayores en los pacientes que posteriormente respondían a este fármaco (0.618±0.065) que en los que no respondían al mismo (0.327±0.054; p=0.020). El 93% de los pacientes en cuartil 4 respondían al tratamiento con natalizumab. Los niveles de IgMPC en suero aumentaban después de seis meses de tratamiento con interferon-β en los pacientes que no respondían a este fármaco (23.72% ± 15.95%), por el contrario, los pacientes que respondían a interferón-β mostraron un descenso significativo de la concentración de IgMPC en suero (-26.63 ± 8.95; p=0.007). Un 84,6% de los pacientes que respondían al tratamiento con interferon-beta mostraron disminución de estos anticuerpos. En sólo un 27,3% de los pacientes que no respondían al tratamiento se observó disminución de IgMPC en suero.

Conclusiones: Los niveles de IgMPC antes de comenzar el tratamiento son un biomarcador de respuesta al natalizumab y el descenso de los niveles de IgMPC tras seis meses de tratamiento son un biomarcador de respuesta al interferón-β



CO-34

CHARACTERIZATION OF THE INTERACTION OF THE AUTOIMMUNE REGULATOR (AIRE) WITH THE E3-UBIQUITIN LIGASES SIAH1 AND SIAH2

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The Central Tolerance is a process based on the elimination of autoreactive thymocytes in the thymus. To generate a non-reactive T cell repertoire is necessary the expression of, ideally, the whole proteome of the organism by the medullary thymic epithelial cells (mTECs). The Autoimmune Regulator (AIRE) is expressed in mTECs. It leads the ectopic expression of a wide set of tissue restricted antigens (TRAs) to avoid the eggression of potentially autoreactive T cells to the peripheral tissues and thus produce autoimmune responses. AIRE is a multi-domain protein has been involved in different activities: regulation of the expression machinery, RNA processing, maturation and differentiation of mTECs, apoptosis and cell cycle regulation. AIRE also acts reading epigenetics marks, leading to the expression of specific genes. Previous findings of our group showed differences in the proteome between WT and AIRE-transfected epithelial cells. AIRE-transfected cells showed increased levels of pro-apoptotic proteins and chaperones and lower amounts of some cytoskeleton-interacting proteins. One increased protein was Calcyclin-binding protein/SIAH1 interacting protein (CACYBP/SIP), an adaptor protein involved in ubiquitination. The Seven in Absentia Homologue (SIAH) E3-ubiquitin ligase family is involved in proteasome-mediated degradation. It contains 2 active members, SIAH1 and SIAH2, that have shared and specific targets. SIAH1 is expressed in response to genotoxic damage or apoptotic signals, while SIAH2 is increased under hypoxia conditions. SIAH-interacting proteins contain a consensus motif (PxAxVxP) present in CACYBP/SIP and AIRE. These data suggest that AIRE interacts with SIAH through this motif. Here, we studied the impact of AIRE transfection on the proteome of HEK-293 cells. The results confirmed the increase of CACYBP/SIP in HEK-293. AIRE interacts with both active members of SIAH through the so-called SIAH1-interacting motif. This interaction may define a new role for AIRE as a member of a degradation complex including SIAH, or may describe an AIRE-degradation pathway.



CO-35

THYROID FOLLICULAR CELLS CAN MODULATE T CELL FUNCTION AND PHENOTYPE IN MIXED AUTOLOGOUS CULTURES; IMPLICATIONS FOR THYROID AUTOIMMUNE DISEASES

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PD-L1 expression by parenchymal cells have been proposed as an important mechanism of peripheral tolerance that is utilized by neoplastic cells to escape anti-tumoral immune response, this being under intense investigation; however, but much less attention has been paid to its role in common autoimmune diseases.

PD-L1 expression by thyroid follicular cells (TFCs) in glands from patients with Hashimoto Thyroiditis (HT) and Graves' disease (GD) was previously reported by our group. Similar findings were simultaneously reported in islet beta cells from human and mouse diabetic pancreas.

Interestingly, PD-L1 expression by TFC from autoimmune glands is, however, much less intense that HLA class II "ectopic" expression in autoimmune glands *in vivo*, but arises spontaneously in TFC as plated in culture and is increased by IFN-gamma, this pointing to PD-L1 *in vivo* inhibition. In order to better understand PD-L1 expression regulation, autologous mixed TFC-PBMC cultures have been set using samples from Graves` disease and multinodular goiter (MNG) patients, and cadaveric organ donors. In this setting, TFCs have been shown to suppress CD3/CD28 induced T cell proliferation at different culture ratios. Additional experiments using transwell demonstrated that the observed effect was contact dependant. Unexpectedly, T cell proliferation inhibition produced by TFCs was not mediated by PD-L1/PD-1 axis signaling, as treatment of mixed cultures with Nivolumab, a blocking antibody against PD-1, had no effect on T cell proliferation.

In order to identify possible immunecheckpoint ligands and other mediators expressed by TFCs or other parenchymatous thyroid cells, single cell RNA-seq (scRNA-seq) was performed on 5 GD and 4 MNG thyroid patients previously depleted of CD45+ cells. Additional negative immunecheckpoints has been identified in TFCs from GD and MNG thyroids, including IDO1, CD276 (B7-H3), PVR or NECTIN2. Further experiments must be done to assess their role in the regulation of immune responses when expressed by TFCs.



CO-36

ROLE OF POLYREACTIVE AUTOANTIBODIES IN SJÖGREN'S SYNDROME

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



Sesión Comunicaciones Orales 7. Inmunogenética

CO-37

UNDERSTANDING THE ETIOLOGY OF INTESTINAL IMMUNE DYSREGULATION IN A NOVEL LCK VARIANT – OF HUMAN AND MOUSE

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Partial T cell defect primary immunodeficiency disorders (PIDD) are a heterogeneous group of genetic disorders characterized by incomplete reduction of T cell number and/or function. Unlike severe T cell immunodeficiencies, partial T immunodeficiencies are commonly associated with hyper-immune dysregulation, including autoimmunity and inflammatory complications. Lymphocyte cell-specific protein-tyrosine kinase (LCK) is a Src family tyrosine kinase that initiates T cell receptor (TCR) signaling. Given its role in TCR signal transduction, LCK is critical to T cell development and function. Here we report the clinical and immunological phenotypes of a novel LCK variant which results in partial T cell defects and immune dysregulation.

A novel homozygous mutation in LCK (p.Pro440Ser, LCK P440S) was identified in two siblings who were born to consanguineous parents, presenting with recurrent viral and fungal infections, and early-onset pulmonary and gastrointestinal inflammatory complications. The immunological phenotype included T lymphopenia, impaired vaccine responses, and increased CD21lo B cells. The LCK P440S mutation results in decreased protein expression and defective TCR signaling. To address questions pertaining to T cell development and immune dysregulation, a mouse model harboring the murine homolog of this mutation was created (Lck mut mouse). The Lck mut mouse demonstrated impaired thymocyte development with concomitant T cell lymphopenia, decreased regulatory T cells, and spontaneously developed colitis, recapitulating the human phenotype. While Lck knock-out (KO) mice replicate the T cell aberrations in Lck mut mice, Lck KO mice do not develop colitis, suggesting that partial loss of Lck function drives colitis. Our findings explain the abnormal immune system development in the human P440S patients, help advance our understanding of gastrointestinal inflammation in the context of perturbed T cell development/function arising from defective TCR signaling and establish a model system with which to develop therapies against monogenic gastrointestinal inflammation.

CO-38

NUEVAS APROXIMACIONES EN EL DIAGNÓSTICO GENÉTICO DE INMUNODEFICIENCIAS PRIMARIAS: DESARROLLO DE UNA ESTRATEGIA DE ANÁLISIS PARA LA EVALUACIÓN DE VARIANTES DE DOSIS GÉNICA MEDIANTE NGS

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Introducción:

Los avances metodológicos en el campo de la genética han permitido la descripción de nuevas entidades clínicas y la ampliación de fenotipos en otras ya conocidas. El siguiente reto diagnóstico consiste en la detección de variantes de dosis génica que, hasta el momento, solo pueden ser evaluadas mediante técnicas cuantitativas.

Los objetivos de este trabajo son mostrar el desarrollo de una estrategia de filtrado para el análisis de variantes de dosis génica mediante NGS utilizando el programa VarSeq y comparar su sensibilidad con la estrategia de rutina y con las técnicas *gold standard.*

Métodos:

Para establecer la estrategia de filtrado mediante VarSeq se recopilaron 25 muestras con deleciones conocidas, que fueron usadas para definir los parámetros del filtrado. Las 25 deleciones habían sido detectadas por MLPA, array-CGH y/o PCR de puntos de corte. Todas ellas fueron evaluadas por la nueva estrategia diseñada para VarSeq como por la estrategia de rutina, basada en los *softwares* XHMM y LACONv.

Resultados:

Para el análisis por VarSeq se establecieron 4 grupos de filtrado atendiendo al número de exones implicados (uno o más) y a la cigosidad de la deleción (homo/hetero-cigosis). Los parámetros de filtrado seleccionados fueron CNV-State, #Samples, #Target, Avg-Ratio y Avg-Z-Score. En la evaluación de 25 muestras con deleciones conocidas, el análisis con VarSeq presentó mayor sensibilidad que la estrategia de rutina (95,5% vs 63,6%). Tres deleciones no fueron detectadas mediante la herramienta VarSeq, dos por encontrarse en una región no capturada. Se observó una buena correlación con las técnicas *gold standard* (Fig.1).

Conclusiones:

El análisis de variantes de dosis génica usando VarSeq mejoró la sensibilidad de la estrategia de rutina. Su principal limitación es que únicamente permite la evaluación de variantes en regiones capturadas. La secuenciación masiva podría ser incorporada como una herramienta de *screening* de variantes de dosis génica próximamente.

Sensibilidad de diferentes técnicas en la detección de variantes de dosis génica. Grado de concordancia entre el resultado validado y el resultado de la técnica. Cig, cigosidad; ID, Identificación; Homo, Homocigosis; Hemi, Hemicigosis; Hetero, Heterocigosis.

Cig	Gen	ID	Deleción	MLPA	Array-CGH	PCR de puntos de corte	Análisis de deleciones por NGS Estrategia de rutina (XHMM y LACONv)	Análisis de deleciones por NGS Nueva estrategia (VarSeq)
Homo /Hemi	*DCLRE1C*	1	NC_000010.11: g.(14942271_14945217)_(15020312_?)del					
		2	NC_000010.11:g.(14939614_14942271)_(15020312_?)del					
	BTK	3	NM_000061.3:c.(335_456)_(1437_1606)del					
		4	NM_000061.3c.(179_284)_(335_456)del					
		5	NC_000023.11: g.(101395849_101386099)_(101386099_101375253)del					
		6	NM_000061.3:c.(335_456)_(857_915)del					
	CYBB	7	arr Xp21.1p11.4(37358919-37787309)x0					
		8	NM_000397: c.1291_1462-726del					
	DOCK8	9	NM_203447.4:c.(53+932_156+31)_(1280_1316)del					
	ACP5	10	NC_000019.10:g.(?_11575165)_ (11577130_?)del					
	CD19	11	NC_000016.10: g.28937789_28939853delinsCAGT					
Hetero	DCLRE1C	12	NC_000010.11: g.(14942271_14945217)_(15020312_?)del					
	BTK	13	NM_000061.3c.(179_284)_(335_456)del					
		14	NC_000023.11: g.(101395849_101386099)_(101386099_101375253)del					
	CYBB	15	NC_000023.11:g(?_37780066)_(37811524_?)del					
		16	NM_000397: c.1291_1462-726del					
	DOCK8	17	NM_203447.4:c.(53+932_156+31)_(1280_1316)del					
	CD19	18	NC_000016.10: g.28937789_28939853delinsCAGT					
	CTLA4, CD28, ICOS	19	arr 2q33.1q33.3(203287959-207476290)x1					
	NCF1	20	NC_000007.14:g.(?_74777146)_(74788745_?)del					
	C1INH	21	NM_000062.3:c.(79_648)_(648_754)del					
	NFKB1	22	NC_000004.12:g.(?_102567068)_(102613511_?)del					
	SLC29A3	23	arr[GRCh37] 10q22.1(72974144_73996728)x1					
	PEPD	24	NM_000285.4:c.(325_329+2286)del					
	TBX1	25	arr[GRCh37] 22q11.21(18,889,039-21,505,417)x1					

Concordancia completa con la deleción validada
Concordancia parcial con la deleción validada
No detección de la deleción validada



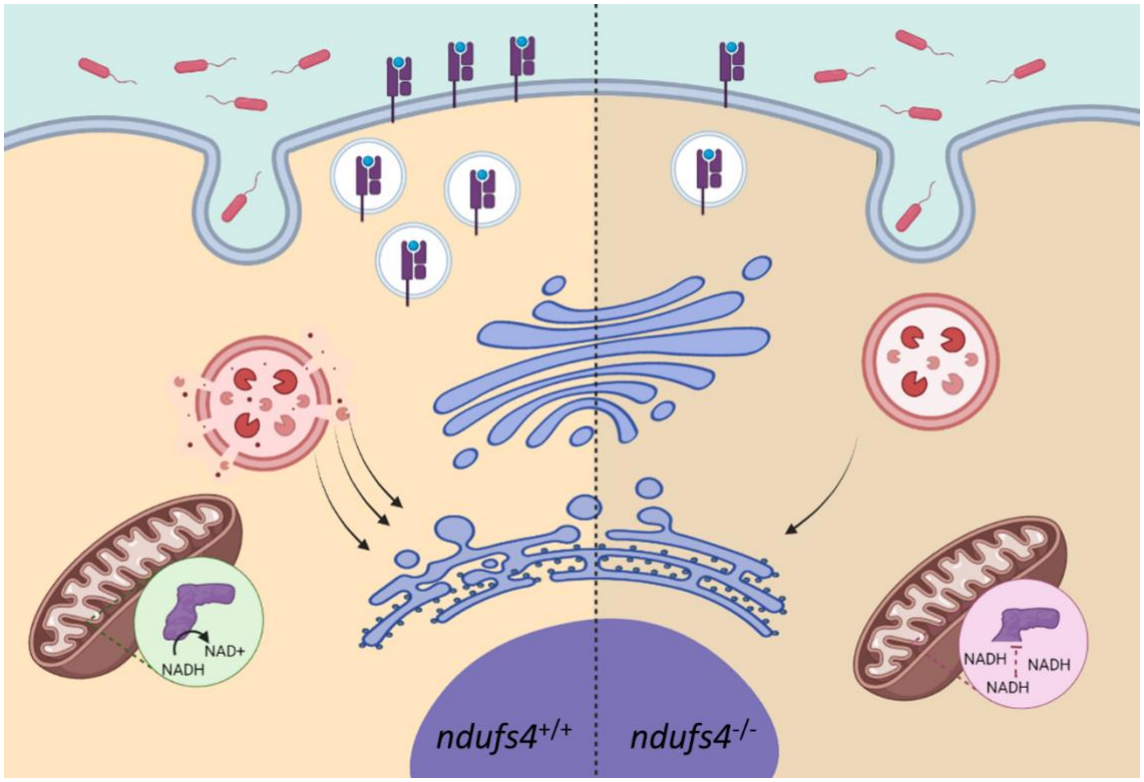
CO-39

MITOCHONDRIAL COMPLEX I REGULATES ANTIGEN CROSS-PRESENTATION IN DENDRITIC CELLS

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Mitochondrial metabolism regulates the inflammatory responses induced by dendritic cells. However, how the individual mitochondrial complexes impact the immunobiology of the dendritic cells remain largely unknown. Using a mouse model deficient for mitochondrial complex I in dendritic cells, we found an impairment of antigen cross-presentation but not direct presentation. The reduced ability to cross-present antigens to CD8 T cells was not associated with perturbations in cytokine production or phagocytosis. Notably, complex I deficiency induced a reduction in oxidative phosphorylation without compromising the mitochondrial membrane potential or extracellular acidification. Mechanistically, the reduction in lipid peroxidation decreases antigen leakage out of the phagosome, limiting antigen availability for cross-presentation. Together, this work reveals how the individual mitochondrial complex activity is repurposed to shape dendritic cell immunobiology and opens new approaches to target dendritic cells in vaccine development and other immunotherapies.


NAD+
NADH
NADH
NADH
NADH
ndufs4+/+
ndufs4-/-



CO-40

ASOCIACIÓN DE LOS NONAPÉPTIDOS PRESENTADOS POR HLA-E CON LA ENFERMEDAD DE BEHÇET

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Introducción

Las moléculas HLA clase I tienen una asociación bien establecida con la enfermedad de Behçet (EB), aunque la causa de esta asociación es desconocida. HLA-E presenta un polimorfismo limitado que podría calificarse de dimórfico, ya que los alelos más comunes, HLA-E*01:01 y *01:03, representan el 99% del total. Las moléculas HLA-E presentan un conjunto limitado de péptidos los cuales son nonapéptidos derivados de la secuencia líder de las moléculas HLA-I clásicas.

Objetivo

Estudiar la relación entre el dimorfismo alélico de HLA-E y el conjunto de nonapéptidos que presenta esta molécula con la predisposición a la EB.

Material y métodos

El estudio de alelos HLA-E incluyó 273 pacientes y 273 controles. Para el genotipado se utilizó PCR en tiempo real con sondas Taqman.

El estudio de péptidos incluyó a 435 pacientes y 444 controles previamente genotipados en HLA-I por PCR-SSOP, codificando muestras de acuerdo con sus secuencias de péptido líder HLA-A, B y C.

Código	Péptido	HLA
1	VMAPRTLLL	A*01,*03,*11,*29,*30,*31,*32,*33,*36; C*02,*15
2	VMAPRTLVL	A*02,*23,*24,*25,*26,*66,*69
5	VMAPRTVLL	B*07,*08,*14,*38,*39,*42,*48
6	VTAPRTLLL	B*13,*18,*27,*37,*40,*44,*47*54,*55,*56
7	VTAPRTVLL	B*15,*35,*40,*41,*45,*46,*49,*50,*51,*52,*53,*57,*58
8	VMAPRTLIL	C*01,*03,*04,*05,*06,*08,*12,*14,*16
9	VMAPRALLL	C*07,*18



Resultados

No hubo diferencias en la distribución de los alelos HLA-E*01:01 y *01:03 entre pacientes y controles (*01:01: 0,54 frente a 0,52; *01:03: 0,46 frente a 0,48). El análisis univariante de los péptidos líder reveló asociación de riesgo con los péptidos 2 y 7, y de protección con el péptido 5. En el análisis multivariante, la asociación de riesgo con el péptido 2 se mantuvo incluso después de condicionar con B51 ($p<0,05$, OR=1,47, 95%CI 1.05-2.06).

Conclusión

El nonapéptido VMAPRTLVL puede explicar la asociación con la predisposición a padecer EB descrita en la región HLA-A. La unión de este péptido a moléculas HLA-E podría modificar la expresión en la superficie de estas moléculas e influir en la regulación de la actividad citotóxica.



CO-41

HEK293F CELL LINE SHOWS DIFFERENT ANTIGEN PRESENTATION FEATURES THAN HEK293

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HEK-293 is a cell line of human embryonic kidney origin with several variants deriving from these. Cells of one of these variants, known as HEK-293F, grow in suspension rather than being adherent cells, which is the case of HEK-293 cells. This is an advantage when there is a need of high number of cells in culture. Both cell lines have been used as equivalent.

We planned to use HEK-293F cells to express different variants of the SARS-CoV-2 Spike protein and to find HLA-I ligands derived from them in the corresponding immunopeptidomes. To confirm that HEK-293F and HEK-293 cells present similar HLA-I peptide repertoires, these peptidomes were purified from non-transfected cells and analyzed by mass spectrometry. The resulting peptidomes showed that there was a lack of HLA-A*03:01 ligands among the peptides from HEK293F cells, while the peptidome from HEK-293 cells contained HLA-A*03:01 peptide ligands. The analysis of the HLA-I allotypes at the cell surface confirmed that there is no expression of HLA-A*03:01 molecules in the cell membrane of HEK-293F cells. The transcriptional expression of the different HLA-I alleles presented in HEK-293 and HEK-293F was studied by qPCR. Results showed that mRNAs corresponding to HLA-A3 were detected, but the ratio HLA-A3/HLA-A2 was lower in HEK-293F cells, partially explaining the absence of HLA-A*03:01.

Some Spike-derived peptides have the features to be ligands of HLA-A*03:01, but they would be lost in HEK-293F cells.

In conclusion, HEK-293F do not have the same antigen presentation features than the parental HEK-293.



CO-42

HLA-G EN UVEÍTIS: MENOS ES MÁS

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HLA-G es un gen HLA de clase I no clásico que codifica una molécula con propiedades tolerogénicas. Su expresión está regulada, en parte, por algunos polimorfismos de la región 3'UTR del gen: 14bp INS/DEL, +3142C/G, +3187A/G y +3196C/G. Altos niveles de HLA-G se han correlacionado con algunos tipos de cáncer, mientras que bajos niveles se han asociado con el riesgo a desarrollar enfermedades inflamatorias, como la uveítis.

Los polimorfismos de la región 3'UTR y los haplotipos que forman fueron analizados en una cohorte de 149 pacientes españoles con uveítis idiopática, caracterizada por inflamación de la úvea. Los pacientes fueron clasificados según criterios anatómicos (anterior, intermedia y posterior), y se compararon con 117 controles de la misma nacionalidad, para establecer una correlación entre los polimorfismos y el riesgo de desarrollar uveítis.

Se obtuvieron muestras de saliva de pacientes procedentes del Servicio de Oftalmología del Hospital Universitario Príncipe de Asturias (Alcalá de Henares – Madrid) y de voluntarios sanos. Se extrajo DNA genómico, y se analizaron los polimorfismos mediante PCR, PCR-RFLP y secuenciación de Sanger. Los datos obtenidos fueron analizados utilizando SNPStats y GraphPad Prism.

No se observaron diferencias significativas entre pacientes y controles al analizar la frecuencia de cada uno de los polimorfismos de forma individual. Sin embargo, estos polimorfismos se encuentran en desequilibrio de ligamiento, formando distintos haplotipos ($D'<1$, $p<0,05$). El análisis de haplotipos mostró que el haplotipo HLA-G UTR-2, asociado a bajos niveles de HLA-G, estaba aumentado de forma significativa en pacientes en comparación con los controles (29,2% vs 22,4%; OR IC (95%) = 2,1 (1,1-4,2); $p = 0,037$).

Estos resultados sugieren que los mecanismos que conducen a la inflamación ocular en la uveítis idiopática están asociados a una baja expresión de HLA-G, y establecen el haplotipo UTR-2 como un nuevo marcador genético de susceptibilidad a la enfermedad.



	+3142C/G	+3187A/G	+3196C/G
14bp INS/DEL	D' = 0,82 p < 0,001	D' = 0,87 p < 0,001	D' = 0,90 p < 0,001
+3142C/G		D' = 0,88 p < 0,001	D' = 0,93 p < 0,001
+3187A/G			D' = 0,99 p < 0,001

A) Desequilibrio de ligamiento entre pares de polimorfismos de la región 3'UTR del gen HLA-G. Las áreas coloreadas muestran desequilibrio de ligamiento (D'<1, p<0,05) entre los polimorfismos analizados, lo que indica que forman distintos haplotipos. D'= coeficiente de desequilibrio normalizado (valores próximos a 1 indican mayor desequilibrio de ligamiento).

14pb INS/DEL	3142 C/G	+ 3187 A/G	+ 3196 C/G	Pacientes	Controles	OR (95% IC)	p-valor
D	G	A	C	0.0998	0.1524	1	---
I	**G**	**A**	**G**	**0.2923**	**0.2237**	**2.1009 (1.0513 - 4.1982)**	**0.0366***
D	C	G	C	0.2548	0.2437	1.6268 (0.8101 - 3.2670)	0.1725
D	C	A	C	0.1733	0.161	1.5153 (0.6948 - 3.3044)	0.2971
I	G	A	C	0.1458	0.1241	1.9927 (0.8654 - 4.5884)	0.1064
I	C	A	C	0.0144	0.0144	1.9961 (0.3605 - 11.0513)	0.4293
D	G	A	G	0.0202	0	---	0.0000

B) Frecuencia de los haplotipos 3'UTR del gen HLA-G en pacientes con uveítis y en controles. El haplotipo formado por las variantes 14bp INS, +3142G, +3187A y +3196G (UTR-2) aumenta de forma significativa en los pacientes en comparación con los controles (0.29 vs. 0.22, OR (95% IC)=2.10 (1.05-4.20), p=0,0366). Esto indica que el haplotipo UTR-2, asociado a bajos niveles de HLA-G, es un factor de susceptibilidad a la enfermedad. Concretamente, este haplotipo genera el doble de riesgo de padecer uveítis en comparación con el resto de los haplotipos formados. *p-valor<0,05



Sesión Comunicaciones Orales 8. Inmunidad frente a las infecciones

CO-43

S PROTEIN VACCINE-INDUCED IMMUNE IMPRINTING REDUCES N PROTEIN ANTIBODY RESPONSE IN SARS-COV-2 INFECTION

Delgado De La Poza, Juan Francisco[1]; Vidal, Mónica[1]; Moya, Maria Carmen[2]; Espasa, Mateu[3]; Casabella, Antonio[3]; Seda, Manel[2]; Serrano, Rosa María[2]; Peña, Pilar[2].

[1]Immunology Unit, Laboratory Service. Parc Taulí Hospital Universitari. Institut d'Investigació i Innovació Parc Taulí (I3PT). Universitat Autònoma de Barcelona, Departament de Medicina., Sabadell; [2]Occupational Health Department. Parc Taulí Hospital Universitari. Institut d'Investigació i Innovació Parc Taulí (I3PT). Universitat Autònoma de Barcelona., Sabadell; [3]Microbiology Unit, Laboratory Service. Parc Taulí Hospital Universitari. Institut d'Investigació i Innovació Parc Taulí (I3PT). Universitat Autònoma de Barcelona. Sabadell. Spain., Sabadell.

Immune imprinting or original antigenic sin (OAS) refers to the immune system's preference to activate existing memory cells when an individual encounters a new but closely related antigen rather than stimulate de novo responses to the new epitopes. According to OAS, in a second entry of the same virus, the secondary response can inhibit a primary response against new epitope antigens if the virus partially modifies its antigen.

The present study analyzes whether the memory antibody B cell response generated by mRNA vaccines against S protein can inhibit the primary antibody immune response to other SARS-CoV-2 antigens, such as nucleocapsid (N) protein. Therefore, antibody response against N protein in both healthcare workers (HCWs) infected by SARS-CoV-2 after vaccination (n=51) and non-vaccinated HCWs (n=188) was analyzed and compared.

All HCWs in the vaccinated group after infection have increased antibody titers against S protein (> 34 fold-change, $p<0.001$). The increase in antibody titers against the S protein observed is mainly due to the memory response induced by vaccination. Subsequently, we have analyzed the N protein antibody response in HCWs from the vaccinated group and from the non-vaccinated group to evaluate the influence of the S protein memory antibody response in another SARS-CoV-2 antigen. N protein response is greater in the HCWs from the non-vaccinated group compared to the vaccinated group: 12.2 (IQR 4.2-32.0) versus 5.7 (IQR 2.3-15.2) AU/mL, respectively. This 2.1 fold-change between groups was statistically significant, $p=0.005$.

Further studies are needed to elucidate whether this reduced response could also occur with the new epitopes on S protein generated by SARS-CoV-2 variants. This phenomenon should be



taken into account in the development of vaccines against these new variants.

In conclusion, our results show that the vaccine-induced immune imprinting against the S protein partially inhibits the response against N protein after SARS-CoV-2 infection.



CO-44

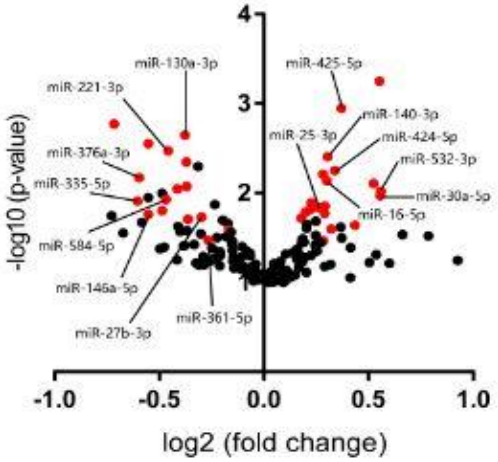
A DIFFERENTIAL SIGNATURE OF CIRCULATING MIRNAS AND CYTOKINES BETWEEN COVID-19 AND COMMUNITY-ACQUIRED PNEUMONIA UNCOVERS NOVEL PHYSIOPATHOLOGICAL MECHANISMS OF COVID-19

Martínez Fleta, Pedro; Vera Tomé, Paula; Jiménez Fernández, María; Requena, Silvia; Roy Vallejo, Emilia; Sanz García, Ancor; Lozano Prieto, Marta; López Sanz, Celia; Vara, Alicia; Lancho Sánchez, Ángel; Martín Gayo, Enrique; Muñoz Calleja, Cecilia; Alfranca, Arantzazu; González Álvaro, Isidoro; Galván Román, José María; Aspa, Javier; De La Fuente, Hortensia; Sánchez Madrid, Francisco.

HU de la Princesa, Madrid.

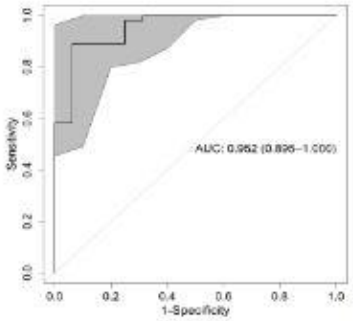
Despite enormous efforts to understand underlying etiopathogenic mechanisms of COVID-19, many of them remain elusive. The study of miRNAs as master regulators of gene expression could reveal new insights into the pathophysiology of this disease. miRNA profile was evaluated with RT-qPCR panels comprising 179 miRNAs commonly found in human plasma. The screening cohort consisted of 38 COVID-19 and 9 community-acquired pneumonia patients. Validation assays were carried out in a second cohort of 85 COVID-19 and 24 community-acquired pneumonia patients by customised PCR panels. Targets of differentially expressed microRNAs were identified via miRTarBase. Cytokines and soluble factors were measured by ELISA. Fifteen dysregulated miRNAs between COVID-19 and community-acquired pneumonia patients were detected. Additionally, multivariate analysis displayed a combination of 4 miRNAs (miR-106b-5p, miR-221-3p, miR-25-3p and miR-30a-5p) that significantly discriminated between both pathologies. The study of target molecules identified a differential plasma proteins signature between COVID-19 and community-acquired pneumonia that included EGFR, CXCL12 and IL-10. Significant differences were also detected in plasma levels of CXCL12, IL-17, TIMP-2 and IL-21R between mild and severe COVID-19 patients. This differential profile of plasma microRNAs and soluble factors in COVID-19 versus community-acquired pneumonia highlights the implication of inflammatory pathways and vascular related processes during the clinical course of COVID-19. These findings provide new insights into the etiopathological mechanisms underlying COVID-19.

A)

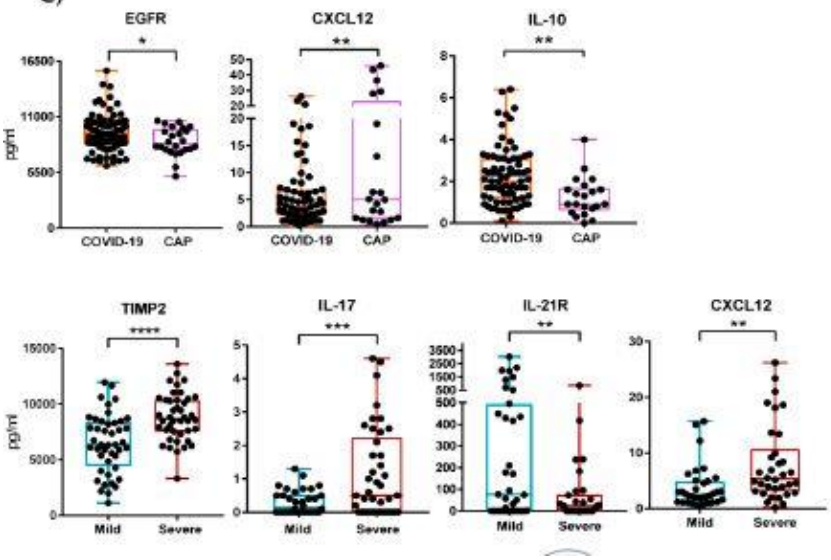


B)

miRNA	OR	CI 95%		p-value
Hemolysis	3.5155	1.1353	18.062	0.1200
hsa-miR-106b-5p	1000.01	8.8843	7.2608×10^{7}	0.0364
hsa-miR-221-3p	2.5533×10^{-5}	8.2152×10^{-9}	0.0031	0.0043
hsa-miR-25-3p	0.20365	0.042140	0.55820	0.0322
hsa-miR-30a-5p	3.9557×10^{7}	793.34	8.7356×10^{14}	0.0313



C)





CO-46

KIR-LIGAND POLYMORPHISM IMPROVED A MORTALITY PREDICTION MODEL INCLUDING CLINICAL DATA OF KNOWN COMORBIDITY IN PATIENTS WITH SARS-COV-2 INFECTION

Niño Ramírez, Jairo Eduardo; Gutierrez Zufiaurre, Maria Nieves; Marcos, Miguel; Bartol Sánchez, Mr; Eiros, Rocío; Alcoceba Sanchez, Miguel; García Alvarez, María; Terradillos Sánchez, Pilar; Presa, Daniel; Muñoz, Juan Luis; López Bernús, A; López Sánchez, E; González Calle, D; Sánchez, Pedro L; Compán Fernández, Olga; González, Marcos; García Sanz, Ramón; Boix, Francisco.

Hospital Universitario de Salamanca, Salamanca.

Background:

Natural Killer (NK) cells play an important role controlling the course of infections as key modulators of both innate and adaptive immune responses against viruses. Their cytotoxic activity is modulated by a series of membrane-bound receptors such as the immunoglobulin-like receptors (KIR) that trigger activating or inhibitory signals.

Aim:

The present study focused on analysing the impact of the KIR-ligand (A*03/*11, -Bw4, -Bw6, -C1, -C2) polymorphism with mortality in patients infected with SARS-CoV-2 from the University Hospital of Salamanca (n=459).

Material and methods:

Patients were grouped according to their clinical severity criteria. KIR typing of infected and healthy controls (n=667) was performed by PCR-SSOP (LIFECODES kit, Immucor®). KIR frequencies, haplotypes, centromeric and telomeric motifs, and functional combinations with ligands was calculated using IBM SPSS Statistics 26. Two predictive models were developed using the Hosmer-Lemeshow multivariate logistic regression and sensitivity and specificity from both were compared with the ROC curve analysis. A p-value <0.05 was considered statistically significant.

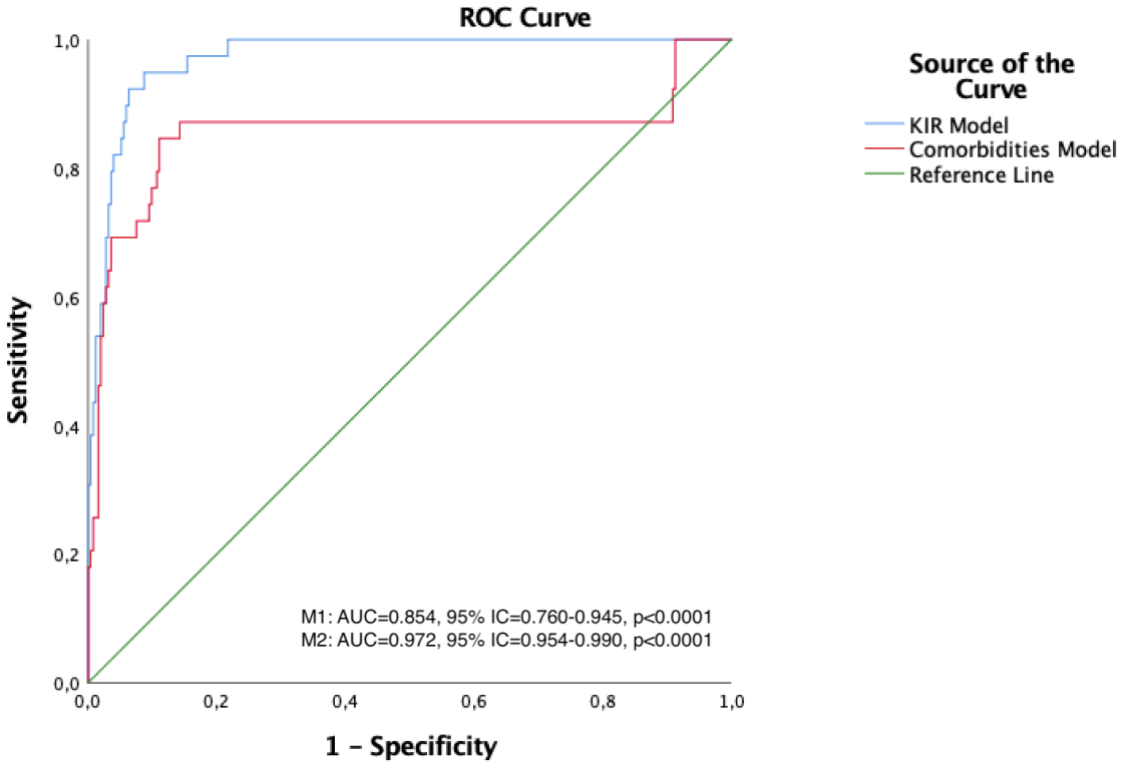
Results:

Mortality was the end-point of this study. The first model (M1) included hypertension, age, sex, diabetes, C-reactive protein, dyslipidemia, smoking, ferritin, and fibrinogen as clinical variable of known comorbidity in COVID-19 (AUC=0.854, $p<0.0001$), whereas in the second model (M2) *KIR*-ligand polymorphism was additionally added (AUC=0.972, $p<0.0001$). KIR2DL2/C1, KIR2DS2/C1 and KIR2DS3/C1 significantly impacted the progression of

patients to a more severe infection. KIR2DL2/C1 [OR=15.2 (95% CI 1.5-147), P= 0.018] showed the strongest association with mortality in our cohort of patients.

Discussion and Conclusion:

When KIR was included into the model, it increased its sensitivity predicting a more severe infection. We believe that inclusion of KIR genotype along with clinical data in patients with COVID-19 could improve their clinical stratification, allowing a more personalised therapeutical approach. These preliminary results should be validated in multicentre studies to ascertain the model's powerfulness predicting mortality.





CO-47

DIFFERENTIAL EXPRESSION OF CHEMOKINE RECEPTORS IN SARS-COV2-SPECIFIC CD4+ T LYMPHOCYTES.

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Servicio de Inmunología, Hospital de la Princesa, Madrid.

Chemokine receptors regulate immune cell migration in many situations, including autoimmune conditions**,** inflammation and infectious diseases. GWAS studies have identified several genetic loci, including those for CCR9 and CXCR6, associated with worse prognosis in COVID-19 infection. There are few studies about chemokine receptor expression in SARS CoV-2 specific immune cells, so it is very relevant to analyze this issue in deep.

Using activation-induced marker assays (AIM) we detected an increase in CCR9 and CXCR6 expression in cells activated with different pools of SARS-CoV 2 peptides. Furthermore, when cells were activated with staphylococcal enterotoxin B (SEB) (positive control), CXCR6 expression also increased but, on the contrary, CCR9 expression decreased. Chemokine receptor expression didn't change when cells were activated with actin-derived peptide pools (negative control). AIM assays with CMV peptides showed similar results as with SARS-CoV-2 peptides.

In order to rule out a general activation-dependent effect on chemokine receptor expression, we used SARS-CoV-2 class II tetramers to select specific CD4+ cells (tetramer+ cells); and found that this population selectively express higher levels of CCR9 than non-specific CD4+ lymphocytes (tetramer-).

CD4+ functional profile was assessed through the analysis of IFNg, TNFa, IL-17 and IL-4 by flow cytometry, both in tetramer+/tetramer- cells, and in CCR9+/CCR9- tetramer+ populations. Overall, cytokine profile was similar in the populations analyzed, with the exception of IL-4 and IL-17, whose levels were higher in SARS-CoV-2 specific cells. In addition, CCR9+ tetramer+ CD4+ lymphocytes showed increased frequency of IFNg+TNFa double positive cells.

In summary, there is an enrichment of CCR9+ expression in the specific SARS-CoV 2 CD4+ population, which show a pro-inflammatory profile.



CO-48

NATURAL KILLER CELL DEGRANULATION: A DETERMINANT FACTOR IN COVID-19 EVOLUTION

Garcinuño, Sara; Gil Etayo, Francisco Javier; Mancebo, Esther; Lopez Nevado, Marta; Lalueza, Antonio; Diaz Simón, Raquel; Pleguezuelo, Daniel Enrique; Serrano, Manuel; Cabrera Marante, Óscar; Allende, Luis M; Paz Artal, Estela; Serrano, Antonio.

Hospital Universitario 12 de Octubre, Madrid.

Background:

NK-degranulation plays an important role in the cytotoxic activity of the innate immunity in the clearance of intracellular infections and is an important factor in the outcome of the disease. This work has studied NK-degranulation and innate immunological profile and functionality in COVID-19 patients and its association with the illness severity.

Methods:

A prospective observational study enrolling a final number of 99 COVID-19 patients was conducted. Patients were grouped according to hospital requirements and severity. Innate immunity cells subpopulations and functionality were analyzed by flow cytometry and the measurement of extracellular granzyme A and B was assessed by ELISA.

Results:

The profile and functionality of innate immunity cells differs between healthy controls and severe patients, $CD56^{dim}$ NK cells being increased and MAIT cells and NK-degranulation rates decreased in COVID-19 subjects. High degranulation rates were observed in non-severe patients and in healthy controls compared to severe patients. Evaluation of the extracellular granzymes showed that benign forms of the disease had a higher GranzymeA/GranzymeB ratio than those with complex forms of the disease. In a multivariate analysis, degranulation capacity resulted a protective factor against severe forms of the disease (OR: 0.86), whereas permanent expression of NKG2D in NKT cells was an independent risk factor (OR: 3.81), all of this with an AUC: 0.84.

Conclusion:

An early and efficient degranulation functionality of the innate immune cells at the early stages of the infection could be used as a tool to identify patients who will have a better evolution. In addition, the examination of granzyme ratio in some infection diseases could be used as a potent prognostic biomarker.



Sesión Comunicaciones Orales 9. Trasplante

CO-49

THE IMPACT OF CD160 DEFICIENCY ON ALLOREACTIVE CD8 T CELL RESPONSES AND ALLOGRAFT REJECTION

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University of Leon, Leon.

CD160 is a member of the immunoglobulin superfamily with a pattern of expression mainly restricted to cytotoxic cells. To assess the functional relevance of the HVEM/ CD160 signaling pathway in allogeneic cytotoxic responses, exon 2 of the CD160 gene was targeted by CRISPR/Cas9 to generate CD160 deficient mice. Next, we evaluated the impact of CD160 deficiency in the course of an alloreactive response. To that aim, parental donor WT (wild-type) or CD160 KO (knock-out) T cells were intravenously adoptively transferred into non-irradiated semiallogeneic F1 recipients. In this setting, donor alloreactive CD160 KO CD4 T cells and CD8 T cells clonally expanded less vigorously than in WT T cell counterparts. This differential proliferative response rate at the early phase of T cell expansion influenced the course of CD8 T cell differentiation and the composition of the effector T cell pool that led to a significant decreased of the memory precursor effector cells (MPECs) / short-lived effector cells (SLECs) ratio in CD160 KO CD8 T cells compared to WT CD8 T cells. Despite these differences in T cell proliferation and differentiation, allogeneic MHC class I mismatched (bm1) skin allograft survival in CD160 KO recipients was comparable to that of WT recipients. However, the administration of CTLA-4.Ig showed an enhanced survival trend of bm1 skin allografts in CD160 KO with respect to WT recipients. Finally, CD160 deficient NK cells were as proficient as CD160 WT NK cells in rejecting allogeneic cellular allografts or MHC class I deficient tumor cells. CD160 may represent a CD28 alternative costimulatory molecule for the modulation of allogeneic CD8 T cell responses either in combination with costimulation blockade or by direct targeting of alloreactive CD8 T cells that upregulate CD160 expression in response to alloantigen stimulation



CO-50

NK CELL TRANSCRIPTOME AND PHENOTYPICAL CHANGES AFTER AUTOLOGOUS HSCT: TRANSIENT EXPANSION OF A CD9+ DECIDUAL-LIKE NK CELL SUBSET.

Astarloa-Pando, Gabirel[1]; Orrantia, Ane[1]; Terrén, Iñigo[1]; Amarilla-Irusta, Ainhoa[1]; Pérez-Fernandez, Silvia[1]; Astigarraga, Itziar[1]; Mateos-Mazón, Juan J.[1]; García-Ruiz, Juan C.[1]; Riñón, Marta[1]; González, Carmen[2]; Uranga, Alasne[2]; Uriz, José J.[2]; Rey, Mercedes[2]; Carrascosa, Tomás[3]; Vázquez-De Luis, Enrique[4]; Callejas, Sergio[4]; Quintas, Ana[4]; Dopazo, Ana[4]; Zenarruzabeitia, Olatz[1]; Borrego, Francisco[1].

[1]Biocruces Bizkaia Health Research Institute – Cruces University Hospital, Barakaldo; [2]Biodonostia Health Research Institute – Donostia University Hospital, Donostia; [3]Biocruces Bizkaia Health Research Institute – Galdakao-Usansolo Hospital, Galdakao; [4]Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid.

Rationale and objective:

Autologous hematopoietic stem cell transplantation (autoHSCT) is a worldwide-established treatment option for a diverse group of hematological malignancies, including multiple myeloma (MM), and for solid tumors in pediatric patients. Natural killer (NK) cells are the first lymphocyte subset to recover during immune reconstitution after transplantation. The aim of this project was to study NK cells reconstitution after autoHSCT to uncover changes in the NK cell repertoire.

Patients and methods:

Peripheral blood mononuclear cells from adult MM patients and pediatric patients with different solid tumors that underwent autoHSCT were studied by multiparametric flow cytometry. Samples collected before and after autoHSCT over one-year period were analyzed from each patient. Samples from MM patients were used to investigate the NK cell transcriptome using RNA-seq. Functional assays were performed to determine NK cell effector functions.

Results:

In adult patients, we found that the phenotype and gene expression profile of circulating NK cells changed shortly after autoHSCT. RNA-seq analysis revealed that several biological processes, including DNA replication and the mevalonate pathway, were enriched. In addition, we also observed that following autoHSCT, NK cells acquired a CD9+ decidual-like gene expression profile. CD9+ decidual-like NK cells exhibited lower degranulation besides higher granzyme B and perforin expression than CD9- NK cells.

RNA-seq results were validated by flow cytometry in the cohort of pediatric patients. They



showed altered NK cell markers repertoire following autoHSCT, characterized among others, by an expansion of the decidual-like NK cells.

Conclusion:

Transcriptome and phenotype of NK cells significantly changed after autoHSCT. There is a higher expression of markers associated with decidual-like NK cells, such as CD9. CD9+ decidual-like NK cells exhibit different effector functions.



CO-51

INMUNIDAD ENTRENADA EN PACIENTES CON ENFERMEDAD RENAL CRÓNICA AVANZADA (ERCA) Y EN RECEPTORES DE TRASPLANTE RENAL (RTR): NANOINMUNOTERAPIA PARA SU MODULACIÓN

Chivite Lacaba, Marta[1]; González Cuadrado, Cecilia[1]; Berges Buxeda, Marcos J[2]; Almendro Vázquez, Patricia[1]; Caro Espada, Jara[3]; González, Esther[3]; Utrero Rico, Alberto[4]; Laguna Goya, Rocío[3]; Mancebo Sierra, Esther[3]; Andrés, Amado[3]; Ochando, Jordi[2]; Paz Artal, Estela[3].

[1]Fundación para la Investigación Biomédica Hospital 12 de Octubre, Madrid; [2]Centro Nacional de Microbiología, Instituto de Salud Carlos III, Madrid; [3]Hospital 12 de Octubre, Madrid; [4]Icahn School of Medicine at Mount Sinai, Nueva York.

Introducción:

La respuesta inmune aumentada que se produce en células del sistema inmune innato sometidas a segundos estímulos es conocida como inmunidad entrenada (IE). La terapia renal sustitutiva (hemodiálisis o diálisis peritoneal) y/o el trasplante podrían tener un efecto de entrenamiento que podría obstaculizar la tolerancia del trasplante. La modulación de la IE mediante nanoinmunoterapia podría mejorar la supervivencia y funcionalidad del injerto.

Objetivos:

(1) Identificar IE en pacientes dializados o RTR. (2) Evaluar IE en monocitos humanos y en la línea monocitaria THP-1 *in vitro* y analizar la eficacia de nanopartículas lipoprotéicas unidas a rapamicina (mTORi-HDL) para su posible uso en modulación terapéutica de la IE.

Metodología:

En monocitos de pacientes dializados y RTR, tras activar con LPS, se estudiaron marcadores de inflamación sugerentes de IE. La inducción de IE in vitro se realizó en monocitos circulantes de controles sanos (CS) y células THP-1 diferenciadas a macrófagos con PMA, que se cultivaron con β-glucano (1er estímulo) y con LPS (2º estímulo), y se trataron con mTORi-HDL.

Resultados:

El trasplante no parece inducir IE, ya que monocitos de RTR no mostraron un aumento de TNFα en relación al pre-trasplante. En pacientes dializados se observó mayor producción de TNFα en monocitos de forma proporcional al tiempo en diálisis, en comparación con los no dializados. En monocitos de CS cultivados en condiciones de entrenamiento se detectó un tamaño celular superior, mayor expresión de CCR2 y CD86 y mayor producción de TNFα, que se redujo significativamente con mTORi-HDL de forma dosis-dependiente. El cultivo con β-



glucano (1er. estímulo) y LPS (2do. estímulo) entrenaba a los macrófagos THP-1 asociándose a una mayor producción de IL6 y TNFα.

Conclusiones:

La diálisis podría inducir IE. La nanoterapia con mTORi-HDL inhibe la inducción de IE *in vitro* en monocitos humanos.

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CO-52

COMPARISON OF IMMUNE RECONSTITUTION BETWEEN POST-TRANSPLANT CYCLOPHOSPHAMIDE AND CONVENTIONAL GRAFT-VERSUS-HOST DISEASE PROPHYLAXIS IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

Bolaños Gómez, Jennifer[1]; Gómez Hernando, Martha[2]; Caro, Jose Luis[1]; Serra, Carles[1]; Palou, Eduard[1]; Queralt Salas, María[2]; Pedraza, Alexandra[2]; Rovira, Monserrat[2]; Martínez Muñoz, Carmen[2]; Torres Canizales, Juan[1].

[1]Transplant Immunology Section, Immunology Department, Hospital Clínic de Barcelona, Barcelona; [2]Hematopoietic Stem Cell Transplantation Unit, Hematology Department, Hospital Clínic de Barcelona, IDIBAPS, Barcelona.

Introduction:

Graft versus host disease (GVHD) is every day less frequent in patients with allogeneic hematopoietic cell transplantation (allo-HCT) due to prophylaxis regiment applied. Post-transplantation high-dose cyclophosphamide (PTCy) is being increasingly used for GVHD prophylaxis across various donor types. However, immune reconstitution (IR) comparison with conventional GVHD prophylaxis has not been well studied. The aim of this study is to determine how PTCy influence IR after allo-HCT and its potential impact on clinical outcomes.

Materials and methods:

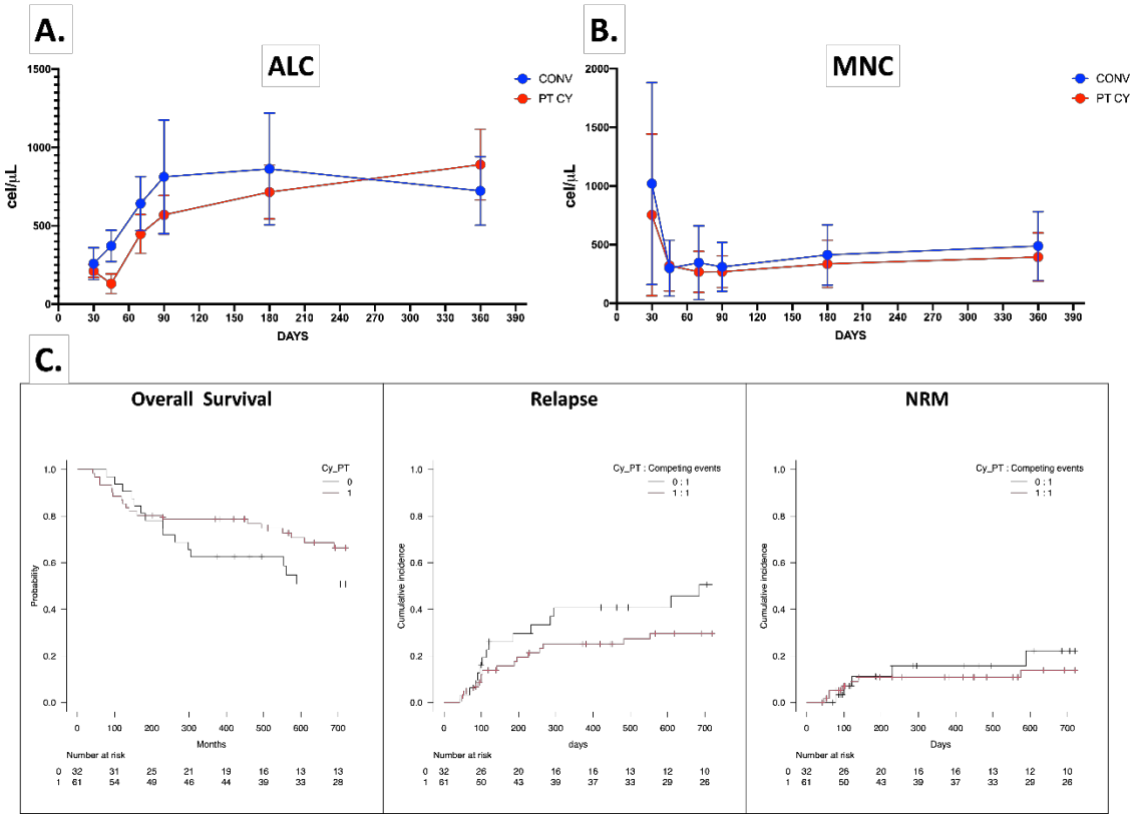
100 adult diagnosed with malignant haematological diseases, treated with allo-HCT from 2018-2020 were included. Dynamics of absolute lymphocytes (ALC), monocytes cell counts (MNC), CD3+, CD8+, CD4+ were collected, and overall survival (OS), relapse, non-relapse mortality (NRM), acute and chronic GVHD were analysed during the first year after transplant.

Results:

PTCy was used in 67 patients. Main differences in the patterns of immune cell dynamics between groups were detected in the first 100 days post-transplantation (Figure 1, A and B). Patients who received PTCy had low persistent ALC at day +45 (131vs.371cells/µL, $p<0.001$), d+70 (447vs.641cells/µL, $p<0.001$), and d+90 (570vs.817 cells/µL, $p>0.05$), respectively. PTCy treated patients had less chronic GVHD (23%vs.45%, $p=0.039$) and better 1 year OS (71%vs.55%, $p=0.05$) without difference in the cumulated incidence of NRM or relapse (Figure1,C). Preliminary analysis showed an increase in ALC at d+30 with more NRM and. On the other hand decreased MNC at d+70 was related to lower NRM and decreased MNC at day+90 to better OS. There were no differences in CD3, CD4 or CD8 at 6, 12 and 24 months between the groups.

Conclusions:

PTCy as GVHD prophylaxis influences peripheral lymphocyte expansion, favouring lower allorecognition, which manifests as less GVHD disease and better clinical outcomes. Analysis of early IR could provide useful information for post-transplant immunosuppressive treatment or adaptive cell therapy to optimize clinical outcomes.





CO-53

EVALUACIÓN DEL PERFIL DE SENSIBILIDAD A FÁRMACOS INMUNOSUPRESORES EN PACIENTES CON TRASPLANTE RENAL, MEDIANTE EL ENSAYO IMMUNOBIOGRAM® (IMBG) Y MARCADORES DE ACTIVACIÓN LINFOCITARIA.

González Cuadrado, Cecilia[1]; Chivite Lacaba, Marta[1]; Almendro Vázquez, Patricia[1]; Ortega, Alvaro[2]; Diez, Teresa[2]; Potero, Isabel[2]; Andrés, Amado[3]; Pascual, Julio[3]; Paz Artal, Estela[3]; Mancebo Sierra, Esther[3].

[1]Fundación para la Investigación Biomédica Hospital 12 octubre, Madrid; [2]Biohope Scientific Solutions for Human Health SL, Tres Cantos; [3]Hospital Universitario 12 de Octubre, Madrid.

Introducción:

Immunobiogram® es un nuevo inmunoensayo farmacodinámico *in vitro* que determina la sensibilidad del paciente a diferentes inmunosupresores.

Objetivos:

Analizar en trasplantados renales (TR) el IMBG y correlacionar los datos obtenidos del IMBG frente tacrolimus con marcadores de activación linfocitaria medidos por citometría de flujo (CF).

Metodología:

Reclutamos 30 pacientes TR estables (>1 año post-trasplante). En el IMBG, PBMCs aisladas y activadas con anti-CD3/CD28, se dispensan sobre hidrogeles con gradientes de concentración de everolimus, tacrolimus, micofenolato o metilpednisolona. Por fluorimetría se evalúa la inhibición de la activación linfocitaria en el gradiente, obteniéndose parámetros de máxima respuesta inhibitoria (RiMax) e ID25, 50, 75 (distancia al inmunosupresor donde la actividad celular es del 75%, 50% y 25%, respectivamente), valores altos determinan alta sensibilidad al inmunosupresor. Por CF se analizan los marcadores de activación linfocitaria (IL-2, IFN-γ), en PBMCs estimuladas con PMA/Ionomiciona, tras el tratamiento *in vitro* con tacrolimus.

Resultados:

En nuestra cohorte, el 13% y 26% de los pacientes resultaron poco sensibles a tacrolimus y micofenolato (en el punto de RiMax), respectivamente. La metilprednisolona fue el inmunosupresor con mayor tasa de sensibilidad con solo 1 paciente (3%) con baja inhibición en el punto de RiMax y valores de ID25/50/75 más altos que el resto de inmunosupresores. La sensibilidad a tacrolimus, everolimus y metilprednisolona correlacionó positivamente.
El tratamiento con tacrolimus disminuyó significativamente el porcentaje de linfocitos

$CD4^+IL2^+$, $IFN\gamma^+$ e $IL2^+IFN\gamma^+$ ($p<0.01$), evaluado por CF. La correlación entre los parámetros del IMBG y el *fold change* de la producción de IL-2 e IFN-γ tras el tratamiento con tacrolimus fue variable.

Conclusiones:

Es posible cuantificar la sensibilidad de cada paciente a la terapia inmunosupresora mediante el IMBG y la CF. El IMBG parece recapitular mejor la sensibilidad global de cada paciente a tacrolimus en comparación con la citometría de flujo.



CO-54

MODULATION OF THE INNATE IMMUNE RESPONSE IN TRASPLANTATION BY TARGETED DELIVERY OF MYCOPHENOLIC ACID BY MUSSEL-INSPIRED NANOPARTICLES

Nieto Jiménez, Celia[1]; Vega Moreno, Milena A.[2]; Martín Del Valle, Eva M.[2]; Del Río, María Luisa[3]; Rodríguez Barbosa, José Ignacio[1].

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Today, effective prevention of acute transplant rejection is possible thanks to the development of efficacious immunosuppressive agents (IAs). However, prevention of transplant rejection in the long-term is more complicated because IAs must be chronically administered, causing undesirable side effects that may lead to the gradual deterioration of the transplanted organ.

In this scenario, the novel use of targeted polydopamine nanoparticles (PDA NPs) transporting mycophenolic acid (MPA) has been assessed with the purpose of improving the efficacy while reducing the side effects of MPA. Taking advantage of the observation that myeloid innate cells avidly uptake foreign NPs, these phagocytic cells were targeted with 190 nm-in size PDA NPs, which were synthesized, characterized, and loaded with MPA (12-18 µg/mg NP) and an anti-CD11b receptor antibody (11-12 µg/mg NP).

According to our findings, MPA release profile from the developed nanosystem was sustained overtime and pH-dependent, which could be of interest as it may prevent early MPA release from PDA NPs in the systemic circulation. Besides, as demonstrated by confocal microscopy, NP uptake by myeloid cells (specifically by a mouse macrophage cell line (J774.A1)) was enhanced by the incorporation of the anti-CD11b antibody to the PDA NPs. Moreover, conjugated PDA NPs reduced *in vitro* the viability of J774.A1 cells and primary peritoneal macrophages in a dose-dependent manner *(*to 26.5-2% after 72h exposure).

Finally, *in vivo*, intraperitoneal administration of safe doses of both, naked and loaded PDA NPs, increased the absolute number of T cells, B1 and B2 cells, and granulocytes in this cavity, thus promoting an inflammation process that could enhance phagocytic cell targeting.

Thereby, based on these preliminary results, ongoing work is being performed to further evaluate the immunosuppressive activity of the developed nanosystem, which could represent



an innovative tool to modulate the innate immune response for the prevention of allograft rejection.



Sesión Comunicaciones Orales 10. Vacunas

CO-55

MESOPOROUS SILICON MICROPARTICLES (MSMPS) ENHANCE CELLULAR AND HUMORAL IMMUNE RESPONSE TO SARS-COV-2

López Gómez, Ana[1]; Real Arévalo, Irene[1]; Mayol Hornero, Elsa[1]; Juárez, Ignacio[1]; Martín Adrados, Beatriz[1]; González García, Raquel[1]; Martín Palma, Raúl[2]; Martínez Naves, Eduardo[1]; Gómez Del Moral, Manuel[3].

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Objectives

Mesoporous silicon Microparticles (MSMPs) show a high immunostimulatory capacity, which makes them potential adjuvants. The high transmissibility and mortality of Covid-19 have led to the development of different vaccines. Even though, the vaccine challenge is still present and research to find new adjuvants is essential.

This project studies the immunostimulatory capacity of MSMPs against SARS-CoV-2 on human PBMCs and BALB/c mice.

Methods

Activation of human PBMCs was analyzed after stimulation with and without MSMPs conjugated with a pool of Spike-peptides, measured as IFNγ production by flow cytometry (FCM).

The humoral and cellular immune response was analyzed in Spike (RBD) SARS-CoV-2 immunized mice, with and without MSMPs. Humoral response (IgG, IgG1, IgG2a) and neutralizing (ACE2-RBD) antibodies was studied by competitive ELISA assay and cellular response was measured as IFNγ production and memory B cells (CD19+ CD27+ CD138-) phenotyping in splenocytes by FCM

In addition, we study how MSMPs stimulate innate immune cells such as neutrophils, dendritic cells and macrophages in human and mice.

Results.

In vitro we observed that IFNγ production is higher in PBMCs stimulated with MSMPs-Spike-peptides (6.36 $p<0.05$) and this increase is greater when PBMCs are co-cultured with dendritic cells, previously stimulated with MSMPs- Spike-peptides (16.2 $p<0.05$).

In vivo, we observed that MSMPs-Spike immunized mice show a greater humoral response IgG, IgG1 and IgG2a (8.12; 2.71; 3.98 $p<0.05$) than mice immunized exclusively with Spike (0.71;

0.89; 1.55 $p<0.05$). Also, we observed higher neutralization (70% $p<0.05$) and a higher correlation (R^2=0.067 $p<0.0001$) between IgG and neutralizing antibodies in MSMPs-Spike mice. In addition, these mice have a better cellular response and a higher percentage of memory B cells.

Conclusion.

The results show that MSMPs, conjugated with spike, can generate a better humoral and cellular response than isolated antigen. This suggests that MSMPs could be used as adjuvants.

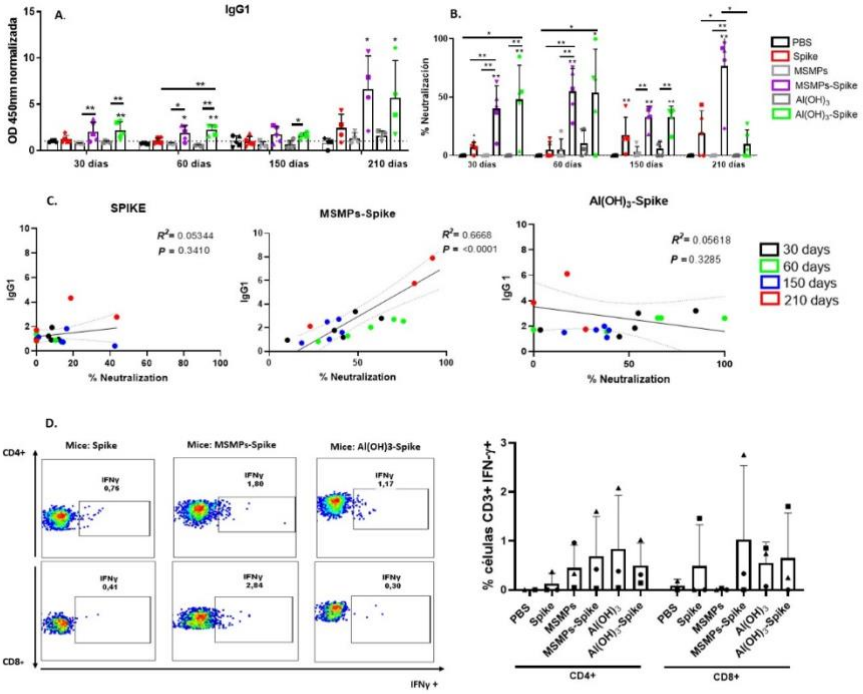


Figure: Shows the humoral and cellular response in immunized mice. **A)** Variation of the IgG1 response in mice immunized with Spike (red), MSMPs-Spike (purple) and Al(OH)3-Spike (green) over six months. **B)** Percentage of neutralizing antibodies in mice immunized. **C)** Percentage of neutralizing antibodies in relation to specific IgG1 antibodies 30 days (black), 60 days (green) and 150 days (blue) after the first dose and after the boost (red). **D)** Cellular response measured as percentage of IFNγ + cells in immunized mice.



CO-56

PREDICTIVE FACTORS OF THE MAGNITUDE AND LOSS OF ANTI-S ANTIBODY TITERS AFTER TWO DOSES OF BNT162B2 VACCINE IN OLDER PEOPLE: AN EIGHT MONTHS FOLLOW-UP

Pozo Balado, María Del Mar[1]; Bulnes Ramos, Ángel[1]; Garrido Rodríguez, Vanesa[1]; Olivas Fernández, Israel[1]; Lozano, Carmen[2]; Álvarez Ríos, Ana Isabel[2]; González González, Berta[2]; Lara, José Manuel[2]; Maldonado Calzado, Isabel[2]; Sánchez Bejarano, Encarnación[2]; Santamaría, Juan Antonio[3]; González Escribano, María Francisca[2]; Leal, Manuel[4]; Pacheco, Yolanda M.[1].

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Older adults achieve lower antibody titers than younger populations after Covid-19 vaccination, showing a marked waning of humoral immunity over time, likely due to immunosenescence.

We aimede to identify age-related factors potentially associated to the magnitude of the response to the Covid-19 vaccine in older people, as well as predictive factors of the loss of anti-S antibody titers after the second dose of vaccine.

Residents and health workers from the “Santa Caridad Elderly Home” receiving two doses of BNT162b2 were recruited (n=143). Blood samples were collected one month (T1) after the second dose, and four and eight months later (T4 and T8, respectively). IgG-antibodies against trimeric SARS-CoV-2 Spike protein were quantified by chemiluminescence (LIAISON®SARS-CoV-2 TrimericS IgG), considering seroconversion ≥33.8 BAU/mL. At T1, inflammatory markers and immune cellular subsets were determined by standard procedures. We also quantified telomere relative length (TRL) and ratio δ/β TREC on peripheral mononuclear cells by digital PCR and serum α1-thymosin levels by ELISA.

Participants were subdivided into three groups: Young <50 years-old (n=41) (38[30-44]; 24.4% men), Middle-Age 50-65 years (n=31) (61[54-64]; 55.8% men) and Elderly ≥65 years (n=71) (77[71-82]; 100% men). Although all participants seroconverted at T1, antibody titers were significantly lower in Elderly than in Young (Figure 1A). Age, homocysteine, β-2 microglobulin, D-dimers, and percentages of NK cells negatively correlated with anti-S titers at T1, while CD3, CD4, CD8, B cells, lymphocytes and δ/β TREC positively correlated (Fig.1B). Elderly also experienced a significantly higher decrease at short-term (T1-T4) (percentage of loss 75[67-82] vs. 69[59-72] respectively; p=0.011) (Fig.1C). Levels of α1-thymosin negatively

correlated with the loss of anti-S titers at both short- and long-term (Fig.1B).

Vaccination protocols including α1-thymosin as adjuvant could improve the durability of the humoral response to the Covid-19 vaccine in older people, possibly increasing the time between booster doses

Figure 1.

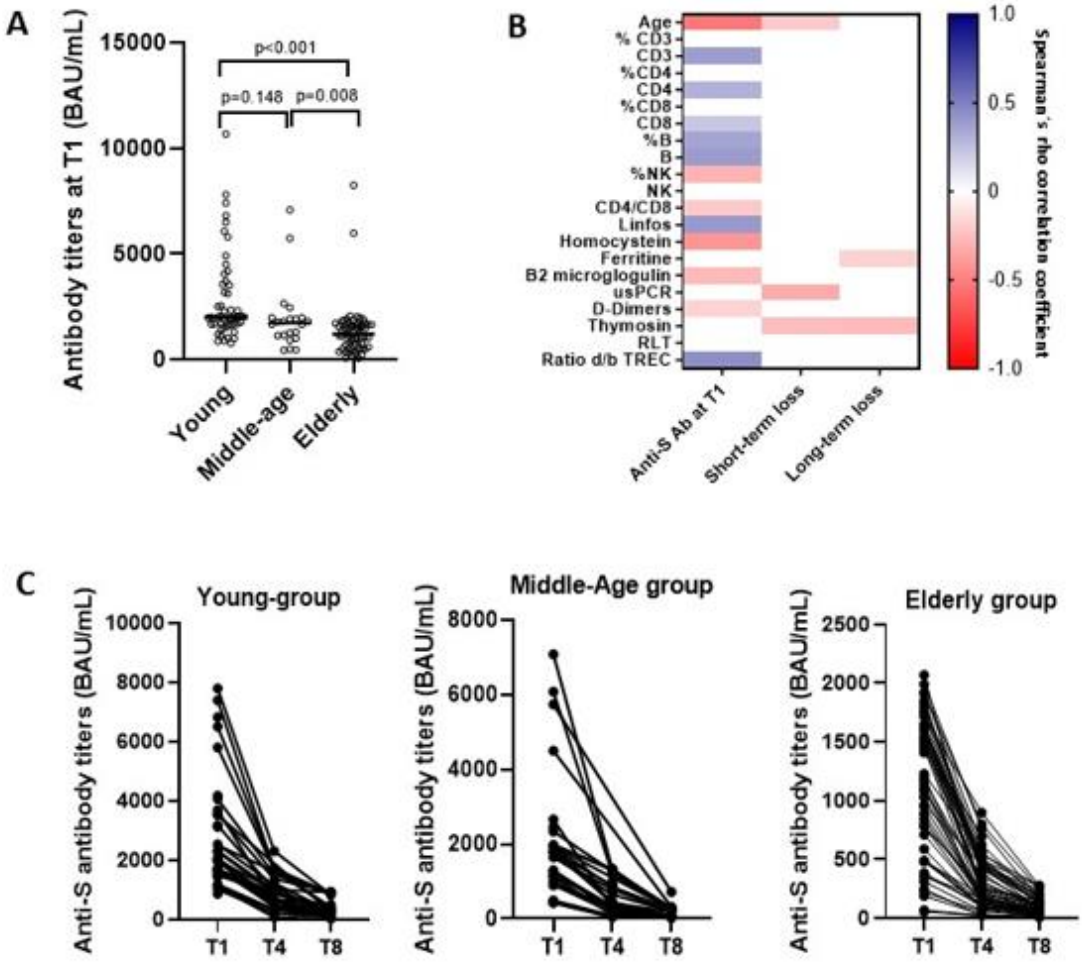


Figure 1. (A) Comparison of anti-S antibody titers between study groups at T1 (one month after the second dose). **(B)** Spearman's correlation analysis between potential age-related associated factors to the magnitude and loss of anti-S antibody titers. anti-S antibody titers at T1 (one month after the second dose). **(C)** Longitudinal follow-up of anti-S antibody titers at T1, one month after the second dose; T4, four months after the second dose; T8, eight months after the second dose .



CO-57

INMUNOTERAPIA INDUCIDA POR LA VACUNA MV130 EN UN MODELO EXPERIMENTAL DE NEUMONÍA NEUMOCÓCICA

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Streptococcus pneumoniae (SPN) causa infecciones respiratorias con una creciente resistencia a antibióticos. Existen varias vacunas antineumocócicas aunque su efectividad es nula frente a serotipos no incluidos en su formulación. El objetivo de este estudio ha sido la respuesta inmunitaria y protección que genera la vacuna bacteriana de mucosas MV130 (Inmunotek) en un modelo experimental de ratón. MV130 induce inmunidad entrenada y está compuesta por una mezcla de bacterias enteras inactivadas que incluye un 60% de SPN de 9 serotipos distintos (1, 3, 5, 6B, 9V, 14, 19F, 20, 23F).

Ratones C57BL6/J recibieron tres dosis de MV130 durante dos semanas intranasalmente y tras una semana de descanso, se infectaron con una dosis subóptima de SPN serotipo 3. A los tres días se sacrificaron y analizaron los pulmones para cuantificar y fenotipar diferentes poblaciones inmunitarias y el contenido de citoquinas.

Los animales tratados con MV130 e infectados con SPN: a) sobreviven más (curvas de Kaplan-Meier, $p<0,01$); b) pierden menos peso; c) presentan un incremento en el reclutamiento de neutrófilos y de monocitos clásicos respecto a no clásicos (ratios de 2,2 en MV130 y de 1,1 en no tratados); d) tienen una mayor proporción de células dendríticas derivadas de monocitos respecto a macrófagos derivados de monocitos (ratios de 3,7 en MV130 y 1,6 en no tratados); e) hay una mayor concentración de quimioquinas y citoquinas: CXCL1, CCL2 e IFNγ y menores niveles de IL1β y TNFα en los pulmones de los animales vacunados e infectados, comparado con los controles infectados, favoreciendo el reclutamiento temprano de las células mieloides en el pulmón e induciendo una respuesta inflamatoria moderada. Resultados similares se han obtenido con MV130X, que carece del serotipo 3 de SPN.

En conclusión, los resultados demuestran que MV130 induce una respuesta inmunitaria innata controlada y protege frente a SPN de forma serotipo independiente.



CO-58

CELLULAR AND HUMORAL RESPONSES FOLLOW-UP FOR 8 MONTHS AFTER VACCINATION WITH MRNA-BASED ANTI-SARS-COV-2 VACCINES

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Because there is no specific treatment against COVID-19, vaccination against SARS-CoV-2 has become the main method of reducing mortality and severity of the disease. mRNA-based vaccines have emerged as a new type of preparation, used for the first time worldwide against SARS-CoV-2. Since they are a novel type of vaccine, several questions have been raised about their efficacy and their ability to confer immunity against the novel coronavirus. For this reason, this work aims to study the evolution of the specific anti-SARS-CoV-2 cellular and humoral responses conferred by the two mRNA vaccines after the first two doses and potential factors that may be implicated in these responses. On days 30 and 240 after the 2nd dose of both vaccines, the anti-S antibodies in plasma were evaluated from 82 volunteers vaccinated with BNT162b2 (BioNTech/Pfizer) and 68 vaccinated with mRNA-1273 (Moderna). IgG Anti-S were measured directly in the plasma, while peripheral blood was stimulated with a mixture of peptides encompassing the entire SARS-CoV-2 protein Spike sequence to measure the cellular-specific memory response. Then, the inflammatory cytokines' levels (IFN-γ, IL-10, IL-2, and IL-4) were measured after 16 hours post-stimulation, corresponding to the level of cellular response. Stratifying the volunteers by age (< or ≥ 48 years old) and gender, differences between vaccines were observed mainly in women under 48 and men over 48 years old. Therefore, this work could help understand the evolution of the specific immune response over time post-vaccination, and identify potential cohort groups where a specific vaccination strategy could be applied to confer maximum immunity.



CO-59

DIFFERENCES BETWEEN B-CELL SUBPOPULATIONS AND NEUTRALIZING ANTIBODIES FOLLOWING BNT162B2 (PFIZER-BIONTECH) VACCINE IN PERSONS WITH AND WITHOUT HISTORY INFECTION OF SARS-COV-2

Morales Núñez, José Javier[1]; Díaz Pérez, Saúl Alberto[1]; García Chagollán, Mariel[1]; Muñoz Valle, José Francisco[1]; Torres Hernández, Paola Carolina[1]; Rodríguez Reyes, Saraí Citlalic[1]; Santoscoy Ascencio, Guillermo[2]; Sierra García De Quevedo, José Julio[2]; Hernández Bello, Jorge[1].

[1]Universidad de Guadalajara - Centro Universitario de Ciencias de la Salud, Guadalajara; [2]Unidad de Patología Clinica - UPC, Guadalajara.

Background:

The immunological mechanisms of immunization of novel COVID-19 vaccines still have specific questions, one of which is related to humoral immunity. The production of neutralizing antibodies is a parameter to measure the effectiveness of a vaccine, and the B cells are the origin of their production.

Objective:

To determine the dynamics of the subpopulations of B lymphocytes in response to the BTN162b2 mRNA vaccine (Pfizer-BioNTech) in persons with and without a history of COVID-19.

Methods:

Thirty-nine immunized persons (22 with prior COVID-19 and 17 without prior COVID-19) and ten subjects not vaccinated against SARS-CoV-2 (control group) were included. B cell subpopulations (transitional, mature, naïve, memory, and plasmablasts) and neutralizing antibody levels were analyzed and quantified by flow cytometry and ELISA, respectively.

Results:

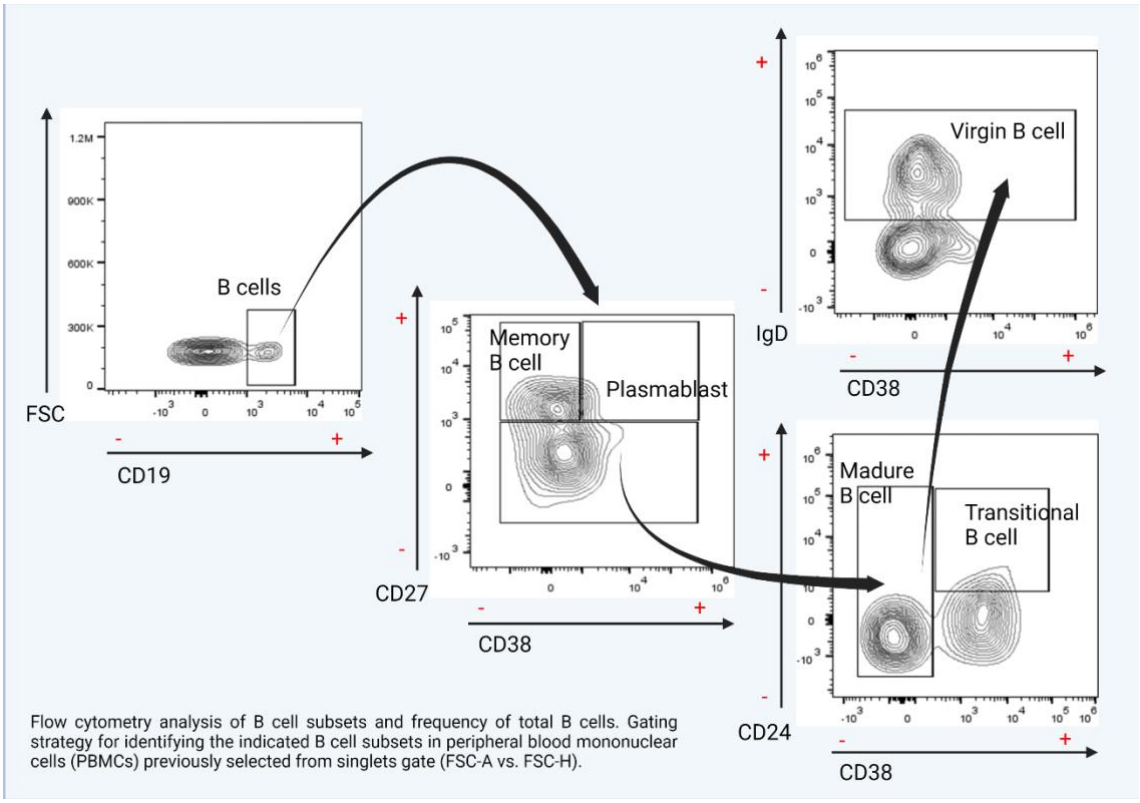
The dynamics of the B cells subpopulations after vaccination showed the following pattern: the percentage of transitional B cells was higher in the prior COVID-19 group ($p < 0.05$), whereas virgin B cells were more prevalent in the group without prior COVID-19 ($p < 0.05$), mature B cells predominated in both vaccinated groups ($p < 0.01$), and memory B cells and plasmablasts were higher in the not vaccinated group ($p < 0.05$).

Conclusions:

BNT162b2 vaccine induces changes in B cell subpopulations, especially generating plasma cells and producing neutralizing antibodies against SARS-CoV-2. However, the previous

infection with SARS-CoV-2 does not alter the dynamics of these subpopulations significantly but induces more rapid and optimal antibody production.

Keywords: B cell, BNT162b2, SARS-CoV-2, Immunophenotype, Vaccine



Flow cytometry analysis of B cell subsets and frequency of total B cells. Gating strategy for identifying the indicated B cell subsets in peripheral blood mononuclear cells (PBMCs) previously selected from singlets gate (FSC-A vs. FSC-H).



CO-60

MYCOPHENOLATE & MTOR INHIBITORS TREATMENT AFFECTS THE RESPONSE TO MRNA-1273 VACCINATION IN PATIENTS WITH RENAL TRANSPLANTATION

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Immunocompromised patients have an increased risk of developing severe COVID disease, as well as a tendency to suboptimal responses to vaccines. Patients undergoing kidney transplantation have pharmacological immunosuppression, limiting their potential response to vaccination, being one of the groups at a higher risk.

Objectives

To study the cellular and humoral response to SARS-CoV-2 after mRNA-1273 (Moderna-Biotech) vaccination in a cohort of kidney transplant patients.

Material and methods.

We recruited 221 patients from the Hospital Clínico San Carlos (Madrid). Blood was obtained prior to vaccination and at 3 time-points after the administration of the different doses. Anti-RBD IgG and the neutralization capacity of the sera was analyzed by ELISA. Cellular response (CD4 and CD8 T-cells) against Spike-protein peptides was analyzed by flow cytometry.

Results

Kidney transplant (KT) patients have lower CD8 (64.5%) and CD4 (58.3%) T-cell response-rates after three doses compared to controls (90.5% and 80.9%, respectively, $p<0.001$).
KT patients have lower humoral response-rates (56% after 2nd-dose and 75% after 3rd-dose, N=149) after vaccination compared to controls ($p<0.001$). IgG production is lower in KT patients, with a delay in antibody production after the second dose. Anti-RBD IgG antibody titers were correlated to the neutralizing capacity of the sera ($p<0.0001$, R-square=0.27).

Mycophenolate(MMF)-treatment reduces the response-rate to vaccination (50% vs 82% after 2nd-dose and 72% vs 89% after 3rd-dose, compared to non-MMF-treated patients). mTOR inhibitors(imTOR)-treatment increases the response-rate (85% vs 46% after 2nd-dose and 95%



vs 68% after 3rd-dose, compared to non-imTOR-treated KT patients). imTOR-treated patients have a higher neutralization capacity after three doses.

Conclusions

The response-rate of mRNA-1273 vaccine in KT patients is lower compared to the general population. MMF treatment deeply reduces the humoral response of patients, whereas imTOR-treatment may recover the effect of pharmacological immunosuppression in these patients. Administration of an additional dose to patients lacking response might be necessary.



POSTERS

Sesión Poster. Inmunología Básica. Pantalla 1

P-001

PTP4A2 GENE PERTURBATION BY CRISPR-CAS9 GENOME EDITING AFFECTS IS ASSEMBLY AND INTERLEUKIN-2 SECRETION IN JURKAT CD4+ T CELLS.

Carrasco Padilla, Carlos[1]; Aguilar Sopeña, Óscar[1]; Alegre Gómez, Sergio[1]; Fernández Boraita, Julia[1]; Torres Ruiz, Raúl[2]; Rodríguez Perales, Sandra[2]; Roda Navarro, Pedro[1].

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Phosphatases of regenerating liver (PRLs; PRL-1, PRL-2 and PRL-3 encoded by *PTP4A1*, *PTP4A2* and *PTP4A3* genes) are dual-specificity phosphatases (DSPs) with an important role in cancer. To further studied the role of these DSPs in T cell activation, IS assembly and effector function, CRISPR/Cas9 genome editing was here employed to make knockouts (KO) of PRL-1 and PRL-2 encoding genes. The loss of PRL-1 and PRL-2 in KO cells was corroborated by Western blot, in which a compensation of the expression between these two phosphatases was uncovered. We then characterized the antigen-induced activation and effector function of KO and control JK cells by measuring the early activation marker CD69 and the overnight secretion of IL-2 in response to decreasing concentrations of superantigen. PRL-1 and PRL-2 deficiency resulted in a reduced expression of IL-2, however, only PRL-2 deficiency caused increased expression levels of CD69 at the cell surface. The IS assembly was then investigated in PRL-2 KO cells interacting with superantigen loaded Raji B cells. An increased filamentous (F)-actin polarization and a smaller central F-actin clearance was found at the IS of PRL-2 KO cells in comparison to control cells. This phenotype was accompanied by a higher localization of CD3ε-containing microclusters at the F-actin rich peripheral area of the IS. We propose that PRL-2 is critical for earlier events of T cell activation and IS assembly and can compensate the loss of PRL-1 at this step. PRL-1, however, seems to be also critical for IL-2 production suggesting that different molecular mechanisms are controlled by these PRLs during early and late activation events. Thus, the presented data contribute more evidences supporting a regulatory role of PRLs in T cell immune responses. Further research in this line will help us to understand the role of these DSPs in healthy and cancer-related T cell biology.

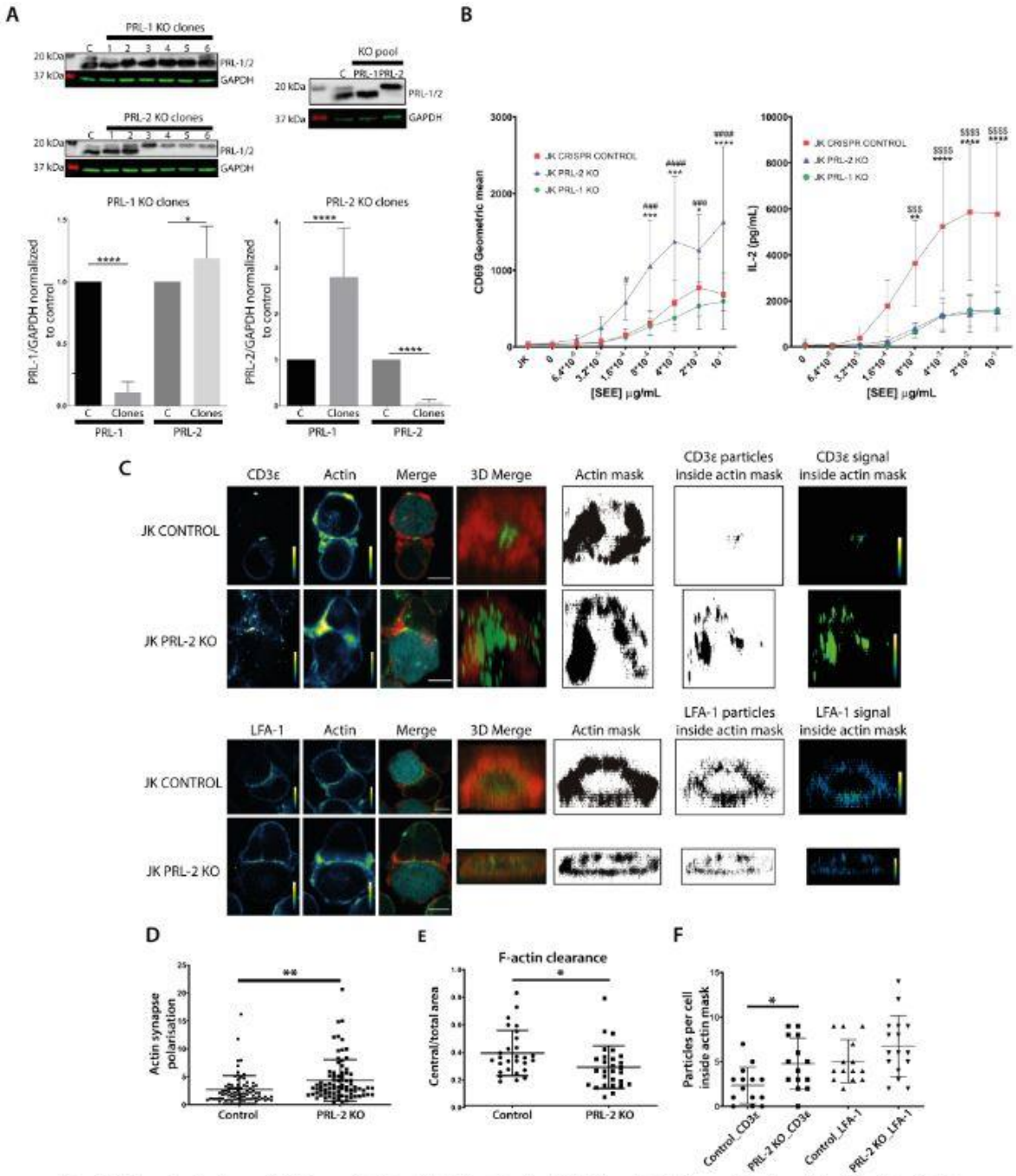


Figure 1. (A) Expression of endogenous PRL-1/2 assessed by Western blot of JK control cells and KOs of clones obtained from a polyclonal population of JK cells transfected via CRISPR/Cas9. Right Western blot shows endogenous expression of PRL-1/2 in JK control cells and pools of 14 PRL-1 or 13 PRL-2 KO clones. Molecular weights and protein bands are indicated on the sides. Graphs show expression of PRL-1 and PRL-2 normalized to GAPDH and, in turn, normalized to these levels in JK control. Means ± standard deviations (SD) of the analysis of 14 clones in JK PRL-1 KO and 13 clones in JK PRL-2 KO are represented. Samples were compared with one-sample t-test. *p<0,05 and ****p<0,0001. (B) Expression of early activation marker CD69 evaluated by flow cytometry in JK control, JK PRL-1 KO and JK PRL-2 KO stimulated during 24 hours with decreasing concentrations of SEE. Graph shows geometric means of CD69 fluorescence values ± standard deviations (SD) of n=3 independent experiments. IL-2 production measured by ELISA from the supernatants obtained from the same experiments in which we analysed CD69 expression. Graph displays means of IL-2 concentration (pg/mL) ± standard deviations (SD) of n=3 independent experiments. Samples are compared with two-way ANOVA test. * represents comparison between JK control and JK PRL-2 KO, $ between JK control and JK PRL-1 KO and # between JK PRL-1 KO and JK PRL-2 KO. For every symbol, *p<0,05, **p<0,01, *** p<0,001 and **** p<0,0001. (C) Representative cell conjugates of JK control and JK PRL-2 KO cells interacting with SEE-loaded and CMAC (blue) labelled Raji cells. The green and red channels (pseudocolour) and the merged channels are shown. The stained molecules are indicated. Calibration bar of the pseudocolour is indicated. Scale bar: 5 µm. The IS face obtained from the 3D reconstructions is shown. Actin masks employed to calculate CD3ε and LFA-1 particles inside actin area are represented. CD3ε and LFA-1 particles and signal are also displayed. Calibration bar of the pseudocolour is indicated. (D) Synapse polarisation of F-actin assessed from the increment in fluorescence at the IS in comparison with the rest of the cell. Dots indicate individual cells analysed and the black line the average value. (E) F-actin clearance from central region at the IS, obtained through ratio between central and total actin ring area. Dots indicate individual cells from 3D reconstructions analysed, and the black line, the average value. (F) Analysis of CD3ε and LFA-1 particles inside actin mask. Dots show the number of particles in individual cells, and the black line, the average value. Samples were compared with an unpaired t-test. *p<0,05 and **p<0,01.



P-002

TCR CROSSTALK WITH SURFACE COMPLEMENT REGULATORS (CREG) IN AB T LYMPHOCYTES

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The B cell receptor (BCR) and complement receptors such as CR2 (CD21) crosstalk when engaged by C3-opsonized antigen by reducing BCR activation threshold. We are exploring similar interactions in T cells by genetic analyses. Here we studied HTVL-1-immortalized αβ $CD4^+$ T cell lines, either normal or with different defects in TCR invariant chains ($CD247^{+/-}$, $CD247^{-/-}$, $CD3D^{\Delta Ex2/\Delta Ex2}$) derived from patients with such mutations, which caused increasing TCR signaling deficiency. Cells were phenotyped for CR (CR1, CR2, CR3, CR4, C5aR1, C3aR) and Creg (CD46, CD55, CD59), stimulated with Dynabeads® T-Activator CD3/CD28 for 24h and analyzed for induction of CR and Creg by flow cytometry. CD69 was used as positive surface stimulation control. PMA+ionomycin for 24 h was used as a TCR-independent stimulus.

The basal CR/Creg phenotype was similar in the different T cell lines ($CR1^{-}CR2^{-}CR3^{+}CR4^{-}C5aR1^{-}C3aR^{-}CD46^{+}CD55^{+}CD59^{+}CD69^{+}$). After TCR engagement, normal T cells showed increased levels of only CD46, CD55, CD59 and CD69, suggesting crosstalk between the TCR and all CReg expressed. $CD247^{+/-}$ T cells also induced CD69 expression, but, in contrast, did not induce any Creg, supporting TCR-dependence of expressed CReg induction. $CD247^{-/-}$ and $CD3D^{\Delta ex2/\Delta ex2}$ T cells were unable to induce CD69 or Creg. PMA+Ionomycin stimulation showed that normal, $CD247^{+/-}$ and $CD247^{-/-}$, but not $CD3D^{\Delta ex2/\Delta ex2}$, T cells, were capable of TCR-independent CD69, CD55 and CD59 induction.

Therefore, our data provide evidence of specific TCR-to-CReg signaling which is dependent on TCR signaling capacity by genetic analyses, suggesting that T cells may receive CReg-dependent information of extracellular complement levels after antigen engagement.



P-004

A CASE OF CD157 NEGATIVE NON-PAROXYSMAL NOCTURNAL HEMOGLOBINURIA WITH THE CLONE SY11B

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Paroxysmal nocturnal hemoglobinuria (PNH) is caused by somatic mutations in the PIGA gene (Xp22.1), which encodes a protein involved in the biosynthesis of the glycosylphosphatidylinositol (GPI) anchor. When a patient lacks GPI and suffers from PNH, some proteins are not correctly anchored to the membrane, such as CD55 or CD59, (which are involved in the regulation of hemolysis due to complement), that inactive complement activation avoiding hemolysis, or CD157 (BST1 gene), which is a useful flow cytometric marker.

It has been described that monoclonal antibody anti-CD157 clone SY11B5, used in (the) routine clinical practice, fails to detect the single nucleotide polymorphism (SNP) of BST1 (p.Arg145Gln). Furthermore, other factors can cause false negatives, such as interference of certain drugs with monoclonal antibodies anti-CD157.

We present a patient with suspected PNH, that had a false negative result in a flow cytometry test using the monoclonal antibody anti-CD157 clone SY11B5. This result was confirmed subsequently with a new blood sample. Then, another clone of anti-CD157 antibody, called RF3, was used, being the flow test positive for the expression of CD157 in the surface of monocytes and neutrophils. The explanation for this phenomenon is that RF3 (recognize also) also recognizes the SNP p.Arg145Gln of the gene BST1. The presence of the former SNP was confirmed by Sanger sequencing.

These types of cases highlight the importance of having different monoclonal antibodies directed against the same marker (CD157 in our case). This could be important to explain discordant results in immunological and hematological tests performed by flow cytometry.

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P-005

GERMINAL CENTER B CELL SELECTION MECHANISMS CANNOT BE STUDIED USING AS READOUT THE KINETICS OF ANTIBODY-ANTIGEN INTERACTIONS IN SOLUTION

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Antigens (Ag) form complexes with antibodies (Ab) and complement (*C'*) fragments. These complexes bind to Fc and *C'* receptors on the membrane of follicular dendritic cells (FDC), forming on them depots of complexes where Ag is maintained intact for months. Follicular B cells bind cognate Ag through BCRs during B cell-FDC interactions. Therefore, in contrast to Ab-Ag interactions, which take place with at least one component in solution and hence in 3 dimensional (3D) conditions, BCR-Ag interactions take place in 2D conditions. Importantly, it has been shown recently that the effective kinetics of BCR-Ag 2D binding cannot be predicted from Ab-Ag 3D kinetics due to qualitative differences in translational and rotational diffusion processes in 3D *vs* 2D conditions.

Similarly to TCRs, BCRs bound to membrane-tethered Ags are submitted to shear and tensile forces of the order of 10-20 pN. These forces affect in an unpredictable and substantial way the mean bond duration (inverse of the k_{off} rate constant). Moreover, two different BCRs specific for the same Ag may respond to force with very different increments of their intrinsic k_{off} rates. Therefore, the 2D kinetics of BCR-Ag interactions under zero force is also an inadequate proxy for BCR-Ag interactions under naturally generated forces.

Last, the shear forces generated during an immunological synapse formation act on BCR-Ag bonds laterally and, hence, differently than the pulling or tensile forces generated during the B-cell synapse retraction into an endosomal vesicle, which act normal to the cell membrane. This introduces an additional layer of complexity into the effective 2D kinetics of BCR-Ag interactions, in which BCR-Ag bonds are submitted to two different, consecutive force regimes. All of the above indicates that the paradigm of effective kinetic rates of Ab-Ag 3D interactions is totally inadequate to help uncover any B-cell selective mechanism in germinal centers.



P-006

INVOLVEMENT OF TYROSINE KINASE TYK2 IN THE ESTABLISHMENT AND FUNCTION OF SPLENIC B CELLS

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TYK2 is a member of the JAK family class of kinases that is responsible for mediating the response to IL-12, IL-23 and type I interferon (IFN). As a consequence, certain alterations in this kinase have been related to an increased susceptibility to viral and encapsulated bacterial infections, as well as hematological malignancies. Our group has previously described a significantly reduced expression of TYK2 in a cohort of patients with B-cell acute lymphoblastic leukemia, accompanied, in some cases, by the presence of loss-of-function variants. These data suggest that alterations in this gene may be involved in the appearance or development of this neoplasm.

Our working hypothesis consists of studying the role of Tyk2 in the splenic B cell subpopulations. Using a Tyk2-deficient mouse model, we analyzed the effect of Tyk2 loss on: 1) the establishment of B cells; 2) the gene expression profile of these subpopulations in homeostasis; and 3) the response of B cells to different Tlr-dependent stimuli. Flow cytometry studies determined that splenic B cell distribution was altered showing a decrease in marginal zone (MZ) B cells in Tyk2-/-. RNA-seq transcriptome studies in sorted B-cell subpopulations (MZ and follicular splenic B cells) identified several genes whose expression was altered, such as type I IFN pathway genes and a marked decrease in Tlr7 ~~receptor~~ expression. In addition, B-cell culture assays showed that Tyk2-/-B cells proliferated and differentiated into plasmablasts to a lesser extent than WT B cells in the presence of different Tlr stimuli (TLR7/8-CL097, TLR7-Imiquimod). Analysis of culture supernatants revealed a significant reduction of immunoglobulins and cytokines secreted by Tyk2-/-B cells (ELISA and CBA techniques). Taken together, these data demonstrate the involvement of Tyk2 in the establishment of mouse marginal zone B lymphocytes and in the functionality of splenic B cells.



P-007

SECRETOME FROM PORCINE CARDIOSPHERE-DERIVED CELLS REPROGRAMS PORCINE M1 MACROPHAGES TOWARD A MORE ANGIOGENIC AND ANTI-INFLAMMATORY PROFILE IN VITRO.

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[1]Centro de Cirugía de Minima Invasión Jesús Usón, Cáceres;[2]Universidad de Extremadura, Cáceres;

Activated macrophages are usually divided into M1 (pro-inflammatory profile) and M2 (anti-inflammatory profile), depending on the expression of surface markers and cytokine secretion. Both are related to inflammatory diseases such as myocardial infarction (MI), being key in both the initial inflammatory response and subsequent wound healing.

Cardiosphere-derived cells (CDCs) delivery has been suggested as a promising therapeutic approach for inflammatory diseases, as they appear to improve the inflammatory environment by modulating M1/M2 polarization.

Pigs are widely used as animal models in the field of cardiovascular diseases due to their similarity to the human heart. Therefore, we improved the therapeutic potential of CDCs by priming them with IFNγ and TNFα and evaluated the effect of their secretome (S-CDCs*) on macrophage polarization in vitro.

Porcine macrophages were isolated from the peripheral blood of healthy pigs and polarized to M1 by adding hGM-CSF to the medium. On day 7 of culture, surface receptor expression and cytokine expression were measured by flow cytometry and qPCR in response to 24 hours of stimulation with LPS + IFN and treatment with S-CDCs*.

M1-polarized macrophages with hGM-CSF and stimulated with LPS + IFNg, and M2-polarized macrophages with hM-CSF and stimulated with IL-4 were used as controls.

After treatment with S-CDCs*, there was an increase in the expression of anti-inflammatory genes such as Arg-1 and IL-10 and a decrease in the proinflammatory gene TNFa, as well as an increase in the expression of VEFGa and MMP9, both related to neovascularization.

In conclusion, administration of S-CDCs* may have a beneficial effect on both the inflammatory and reparative phases of AMI.



P-009

LA EXPRESIÓN DE TRAF1 EN LINFOCITOS T CAUSA LA EXPANSIÓN DE TIMOCITOS Y PREVIENE LA INVOLUCIÓN TÍMICA SIN ALTERAR EL NÚMERO DE LINFOCITOS T PERIFÉRICOS

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TRAF1 es el único miembro de la familia TRAF sin dominio RING y, consecuentemente, sin actividad E3-ubiquitina-ligasa. TRAF1 no se expresa en linfocitos T en reposo, pero se induce tras activación, pudiendo regular a distintos TNFRs. Hemos generado 2 líneas de ratones transgénicos (CBA-TRAF1-T y FvbN-TRAF1-T) que sobreexpresan TRAF1 específicamente en linfocitos T. Los niveles de expresión del TRAF1 transgénico son similares a los de TRAF1 en linfocitos T activados y pueden considerarse fisiológicos.

La expresión de TRAF1 causa un aumento del tamaño del timo ya observable al mes de edad que correlaciona con un aumento de la celularidad. Además, en ratones TRAF1-T de edad superior a 7 meses el timo no se atrofia y presenta un tamaño superior al de los ratones jóvenes TRAF1-T en ambas líneas de ratones debido a la acumulación de timocitos. Esta acumulación no altera los porcentajes de las poblaciones tímicas CD4+CD8+, CD4+CD8- y CD4-CD8+. Sin embargo, el número de linfocitos T en el bazo de los ratones salvajes y TRAF1-T permanece similar en ratones de 2 a 14 meses de edad.

Un análisis transcriptómico diferencial en timocitos de ratones CBA-TRAF1-T y salvajes demostró que el FGF-10 (un factor de crecimiento del epitelio tímico) se encontraba sobreexpresado (~9 veces) en los timocitos TRAF1-T, diferencias que fueron aún mayores en análisis por QPCR. Estudios inmunohistoquímicos mostraron que mientras que los timos de ratones salvajes de 1 año de edad eran atróficos, los de los ratones TRAF1-T no mostraban evidencias de atrofia y tenían áreas corticales y medulares engrandecidas y un epitelio tímico cortical con mayor densidad y con morfología alterada.

Estos resultados sugieren que la sobreexpresión de FGF10 mediada por TRAF1 en timocitos potenciaría el crecimiento del epitelio tímico que, a su vez, permitiría la acumulación de timocitos y prevendría la involución del timo.



P-010

ACUTE HYPOXIA EFFECT ON MENSTRUAL BLOOD-DERIVED MESENCHYMAL STROMAL CELLS (MENSCS) IMMUNOMODULATORY RESPONSE

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Menstrual blood-derived mesenchymal stromal cells (MenSCs) are a new source of stromal cells that stand out for being easy to isolate non-invasively, having a high rate of proliferation, and without ethical concerns. Currently, their therapeutic use is arousing interest due to their immunomodulatory and regenerative properties. New strategies are being developed in order to increase the likelihood of successful therapies. The aim of this work was to expose MenSCs to severe hypoxia to determine possible beneficial changes in their immunomodulatory properties. For that, five primary MenSCs cultures under basal condition or after 48h of incubation at acute hypoxia (0.1-1%) were compared in terms of surface receptors and gene expression. A total of 21 surface markers related to migration, cell adhesion, and immune response were analyzed by flow cytometry, and their gene expression profile (25 genes) was examined by qPCR. In addition, the secretion of two immunomodulatory molecules (IDO-1 and TGF-β) was determined by ELISA. Our results showed positive alterations in the expression of surface markers such as a significant increase in CD274, CD279, and other cell adhesion markers which enhance the MenSC immunomodulatory properties. The gene ontology (GO) analysis of the differences in the gene expression profile between the studied conditions showed an enrichment in the immune and inflammatory response GO categories. However, the migration capacity seems to be diminished. Moreover, an increase in IDO-1 and TGF-β expression was also detected under severe hypoxia. For all of this, short exposure of MenSCs to acute hypoxia should be considered as a method to modulate the immune response in patients treated with MenSCs.



P-011

MULTIPLE MIELOMA IGD. A REPORT OF 13 CASES.

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Introduction:

Multiple Mieloma (MM) IgD is a rare entity, it represents only 2% of MM. It is more often found in men and, compared with other isotypes, it associates a more aggressive development and lower survival (Revised International Staging System, (R-ISS III).

Nowadays there are few cohorts described, probably because it is an underdiagnosed entity, since serum IgD levels are very low (0.7-13 mg/dL) and routine immunofixations (IF) do not include IgD, making it difficult to identify monoclonal IgD proteins.

Methods:

Our review includes 13 patients of MM IgD identified in our laboratory from 2016 to 2022. Serum and urine IF (Hydrasys 2®, Sebia) and proteinograms (Capillarys®, Sebia) were made in all of them, as well as immunoglobulin (G, A , M, D and E) and light chain quantification (Optilite®, Binding Site).

Results:

12 out of 13 patients were men with a mean age of 66 years (range 47-75) and only one presented kappa-type IgD MM. Mean IgD quantification was 689 mg/dL (range 9-2162) and Lambda Free Light Chain 913 mg/L (5.7-26.3) with anemia in 46% and hypercalcaemia in 2 of them.

The mean concentration of the monoclonal component was 0.47 g/dL at diagnosis, showing all of them an altered ratio Kappa/Lambda. High rates of B2- microglobulin was found in 55% of the patients. Only one case did not present Bence Jones (BJ) and bone involvement was found in 31% and kidney involvement in 76.9%.

Use of free Kappa and Lambda antiserum help us to discard free light chain MM.

Discussion:

IF with specific antiserum against IgD and IgD quantification are tests that must be included to avoid misdiagnoses of MM IgD diagnoses.



P-012

EFFECT OF MESENCHYMAL STEM CELL CONDITIONED MEDIA (MSC-CM) ON THE EXPRESSION OF KILLER-CELL IMMUNOGLOBULIN-LIKE RECEPTORS (KIR) KIR3DL1, KIR2DS4 Y KIR2DL2/DL3 OF PERIPHERAL BLOOD NK CELLS.

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Introduction:

Mesenchymal stem cells (MSC) are a broadly used population in cell therapy for their ability to modulate the immune system responses. MSCs and their conditioned media (CM) can decrease the degranulation of NK cells among these responses, but there are only a few studies focusing on the modulation of Killer-cell immunoglobulin-like receptors (KIR) expression. Here we describe the effect of MSC-CM on the expression of Killer-cell immunoglobulin-like receptors (KIR) KIR3DL1, KIR2DS4 y KIR2DL2/DL3 of peripheral blood NK cells.

Materials and Methods:

First, CM were obtained from MSC cultures in 25 cm^2 flasks with different cell numbers (50000 (CM1), 100000 (CM2) and 200000 (CM3)) for 3 days in a volume of 5 ml of DMEM. Afterwards, PBMCs were isolated with Ficoll-Hypaque and cultured in 96-well plates at a final concentration of 10^6 cells/ml in a final volume of 200 uL of RPMI, supplemented with different concentrations of CM (10%, 25% and 50%) with and without PHA for 4 days at 37°C. After culture, PBMC were labeled with monoclonal antibodies (anti CD16/56, anti-KIR3DL1, anti-KIR2DS4 and anti-KIR2DL2/DL3) and analyzed by flow cytometry.

Results:

First, PHA caused an increase in the expression of the three analyzed markers in all cases. Regarding the effect of the different concentrations of CM, to a greater or lesser extent, the three markers followed the same pattern, increasing their expression for concentrations 10% and 25%, but decreasing for concentrations of 50%, observing an hormetic effect. No differences were observed between the different conditioned media (CM1, CM2 and CM3).

Conclusions:

MSC-derived conditioned medium increased the expression of KIR receptors KIR3DL1, KIR2DS4 and KIR2DL2/DL3 at low concentrations (10% and 25%) but decreased their expression at higher concentrations (50%).



P-013

A MOUSE MODEL WITH PI3 KINASE P110A INACTIVATION IN T CELLS. EFFECTS ON THE DEVELOPMENT OF FUNCTIONAL SUBPOPULATIONS.

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The enzymes phosphatidyl-inositol-3 kinases (PI3K) regulate essential cellular processes such as metabolism, activation, proliferation, motility, angiogenesis, inflammation and carcinogenesis. However, the specific involvement of certain catalytic Class I PI3K isoforms is poorly understood in immune processes. P110α is an ubiquitous catalytic isoform in body cells, with involvement in ICOS-mediated signals and a high rate of mutation in cancer. To study the role of p110α in T lymphocytes, we have developed a new mouse model with functional inactivation of p110α (Kinase dead) in T lymphocytes (p110αKD-T) linked to the Cre-lox system.

P110αKD-T mice show an increase in the number of cells in the thymus and spleen with a slight decrease in the proportion of single positive $CD4^+$ in the thymus and naive $CD8^+$ T ($CD44^{low}$ $CD62L^{high}$) in the spleen. No differences in "in vitro" proliferation or IFNγ production were observed in $CD4^+$ cultures stimulated with antiCD3±antiCD28 or ±antiICOS; nor in the "in vitro" differentiation of Th1 or Th17 subpopulations. However, "in vitro" differentiation of follicular Th subpopulations (Tfh) showed a lower yield of Tfh cell number and IL-21 production in p110αKD-T cultures versus control. "In vitro" ICOS-induced cell elongation assays showed defects in this response in Tfh-KD cells. Control Tfh cells showed inhibition of this elongation response in the presence of p110α(A66)- or p110δ(IC87114)-inhibitor. These results demonstrate, by genetic modification (p110αKD-T) and specific inhibition, the involvement of both p110α and δ catalytic chains in the elongation response mediated by ICOS in Tfh cells. Additional "in vitro" and "in vivo" tests will make it possible to characterize the effect produced by the inactivation of p110α in T lymphocytes in this mouse model.



P-014

BEYOND THE BIOMARKER: IMMUNOLOGIC AND METABOLIC PHENOTYPING OF THE MIR-721 KO MICE

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MicroRNAs (miRNAs) are small non-coding RNAs, which act as negative regulators of the gene expression at a post-transcriptional level. The focus of this study is mmu-miR-721, which was first described in mouse embryonic nervous tissue but is also related to other processes due to the activity of some of its target genes: Meox2, a negative regulator of angiogenesis; Ppargamma, a regulator of insulin metabolism; and Nos2, an enzyme involved in nitric oxide metabolism. In our laboratory, this miRNA has been found to be related to the biology of Th17 lymphocytes, which synthesise it during autoimmune myocarditis. To study the role of this miRNA and its mechanisms of action, we have generated the mmu-miR-721 knockout mouse. Hence, our objective is the immunological and metabolic characterisation of this strain.

The immunologic profiling of the mmu-miR-721 KO strain revealed a hypercellular thymus with an aberrant intrathymic T cell selection process, compared to WT mice measured by FACS. Particularly, intermediate thymocytes were enriched, while resultant CD4+ and CD8+ T cells were reduced, suggesting an arrest in the negative selection. This is in line with decreased Th17 responses observed in the model of myocarditis.

Furthermore, the metabolic phenotyping revealed an increased abdominal fat weight and lipidic plasma levels in adult miR-721 KO female mice, compared to WT. Nevertheless, this phenotype is not observed in pre-fertile females nor males. Moreover, male KO mice even present lower body weight and decreased abdominal fact when compared to WT littermates. This sex-dependant differences and the change upon fertility in this strain suggest miR-721 is involved in the sex hormone homeostasis.

These data make the miR-721 KO model a powerful tool to understand the role of this molecule in the development of the immune cells, as well as to illustrate the influence that lymphocytes can have in the lipidic metabolism.



P-015

EVALUATION OF DIFFERENT PRESERVATION METHODS FOR CEREBROSPINAL FLUID MONONUCLEAR CELLS.

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Background:

Characterization of changes in the different cerebrospinal fluid (CSF) leukocyte subsets in multiple sclerosis patients receiving a particular treatment often need to be done in a reduced number or reference laboratories. Thus, working with fresh cells is not always possible and cell preservation has to be done.

Objective:

To study two different methods of cell preservation to compare them with fresh cells.

Methods:

Eight patients diagnosed with different neurological diseases at Ramón y Cajal University Hospital (Madrid, Spain). CSF samples were always obtained for clinical purposes. CSF cells obtained from Fresh, TransFix and Frozen methods were analyzed by flow cytometry, using BD FACSDiva Software V.8.0. Statistical analyses were made using GraphPad Prism 8.0 software. Friedman ANOVA test for paired data was used to compare results obtained in fresh and preserved samples. Post-hoc analysis were done to evaluate differences between groups.

Results:

Statistical differences in absolute numbers of total mononuclear cells obtained in Fresh and preserved CSF samples were found ($p=0.0024$), due to lower counts found in Frozen samples compared to Fresh ones ($p<0.01$). No differences were found between total mononuclear cell numbers obtained in TransFix compared to Fresh samples. However, we found a relative increase in B cell percentages in Frozen samples compared to TransFix and Fresh ones ($p=0.0003$) and a clear decrease in the percentages of CD4+ T cells in CSF Frozen samples compared to the other two conditions ($p=0.018$).

Conclusions:

Cryopreservation of CSF mononuclear cells induced a significant reduction in absolute CSF cell numbers that affected mainly to CD4+ T cells. A relative increase of B cells was probably due



to CD4+ T cell reduction. These changes were not observed in transfixed cells. These results show that TransFix method could be useful for studying CSF leukocyte subpopulations by flow cytometry up to two days after lumbar puncture.



P-016

DESIGN AND VALIDATION OF NOVEL FLOW CYTOMETRY PANELS TO ANALYZE A COMPREHENSIVE RANGE OF PERIPHERAL IMMUNE CELLS IN C57BL/6J MICE.

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Introduction:

Flow cytometry is a widely used technique to diagnose several immune-related diseases in humans in peripheral blood (PB). However, in murine models, it presents several limitations, such as the meager amount of PB available, narrow variety of commercial antibodies and fluorochromes and high inter-subject variability in immune populations. We have defined novel cytometry panels to analyze extensively immune cell populations in mice from 100uL of blood. The panles have been tested in lipopolysaccharide (LPS) lung inflammation model.

M&M:

Twelve C57BL/6J mice were randomized into two groups: LPS, (n=6) undergoing intratraqueal inoculation of LPS (5mg/kg) in 100 µL of saline; and control (n=6). Fresh blood was collected from the maxillary venous sinus on day 1 pre-inoculation, and days 1 and 3 post-inoculation (p.i). Whole blood was labeled with 27 antibodies distributed in two panels, and acquired in a 16 channels Flow Cytometer. Peripheral populations were assessed overtime, overlapping innate and adaptive immune subsets.

Results:

LPS-induced inflammation was rapidly observed at day 1 p.i, by an increase of granulocytes frequency and numbers, correlated with the mice's weight loss. Interestingly, a decline in the pro-inflammatory Th1 and NK subsets was observed in the same group. Rare cellular populations have shown high variability, likely due to very low cells/µL.

Conclusions:

Our panels allowed us to analyze most physiological peripheral immune cellular subsets from a minimal amount of whole blood and can permit us to detect immune changes due to rapid inflammation induction. This longitudinal study could help in determining the best timing for future experimental designs, using minimal blood and with a minimal impact on the animal status, avoiding unnecessary animal sacrifices and thus, reducing sample size.

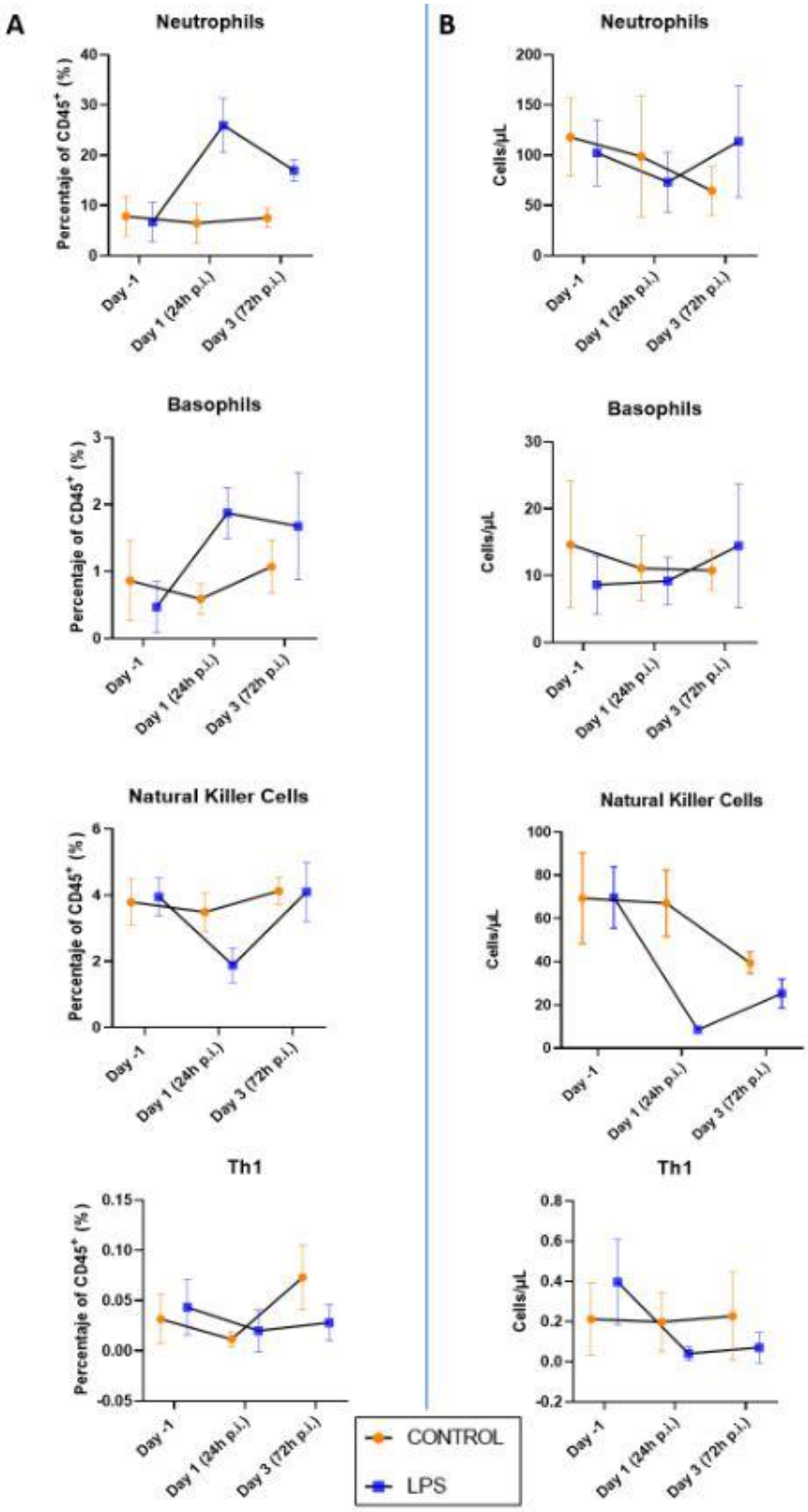


Figure 1: Flow cytometric characterization of leucocytes cells (CD45+) in fresh peripheral blood after LPS inoculation.
A: Percentage of basophils, neutrophils, natural killers and Th1 cells at 24 and 72 hours post inoculation (p.i.).
B Absolute counts of these populations at 24h and 72h p.i.



Sesión Poster. Inmunidad Innata. Pantalla 2

P-018

LACK OF TYPE 1 CONVENTIONAL DENDRITIC CELLS REDUCES ADIPOKINE PRODUCTION IN ADIPOSE TISSUE

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Our group has previously seen that type 1 conventional dendritic cells (cDC1) are necessary for a healthy adipose tissue. Batf3KO mice, lacking cDC1, become obese with age. On the other hand, mice that have more dendritic cells do not gain as much weight when put on a high fat diet as their control counterparts. The latter effect is lost in Batf3KO mice. To understand the mechanisms by which cDC1 are able to decrease white adipose tissue weight we looked at the production of adipokines in Batf3KO mice. Batf3KO mice have a reduced production of adiponectin, leptin and Fgf21. These hormones are important regulators of satiety and can explain why young Batf3KO mice eat more than WT mice. Forcefully increasing the energy expenditure in these mice is able to counteract age-induced obesity and promotes adiponectin production by the adipose tissue. However, Fgf21 production is still lower in Batf3KO mice compared to WT mice. Fgf21 is also implicated in the preference for sweet foods. Young Batf3ko mice have a disrupted eating pattern, waking during the resting period to feed. On a ketogenic diet, with very low concentrations of carbohydrate, Batf3KO mice correct this behaviour. Fgf21 has previously been shown to be required in order to survive LPS-induced endotoxemia. Batf3KO mice, producing less Fgf21, have reduced survival rates in this model. In conclusion, mice lacking cDC1 have a disrupted adipokine production that affects their eating habits and their weight gain, but also affects their response to a sterile inflammatory response.



P-019

CANDIDA ALBICANS PCA2 INFECTION PROGRAMS HEMATOPOIETIC STEM AND PROGENITOR CELLS IN THE BONE MARROW AND MOBILIZES THEM TO THE SPLEEN.

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Universidad de Valencia, Burjasot.

Hematopoietic stem and progenitor cells (HSPCs) are increasingly being appreciated to be important players in the fight against infection. In this study, we show that a mild infection with the low-virulent strain of *Candida albicans* PCA2, expands and reprograms HSPCs early during infection and mobilize them transiently to the spleen to produce trained macrophages.

HSPC populations in the bone marrow and the spleen were analyzed by flow cytometry during infection. Bone marrow LKS$^+$ (Lin$^-$ c-Kit$^+$ Sca-1$^+$) cells expand progressively in response to the PCA2 infection, with a general increase in all MPP (multipotent progenitors) populations. In the spleen, a significant increase in LKS$^+$ cells and all MPPs is observed at day 7 post-infection. To find out whether infection recruits HSPCs to the spleen or expands them from the few residing in the spleen, we used two different adoptive transfer models. By transferring HSPCs from uninfected mice to PCA2 infected mice, we demonstrate that infection enhances the ability of the spleen to recruit bone marrow HSPCs. Moreover, adoptive transfer of HSPCs from PCA2 infected mice into uninfected mice shows an increased ability of HSPCs from infected mice to home the spleen. Furthermore, we assessed the ability of *ex-vivo* HSPC-derived macrophages to produce proinflammatory cytokines at different time points post-infection. We found that bone marrow HSPCs are programed to produce trained macrophages early during infection (day 3), while day 7 HSPCs produce macrophages with an unaltered or tolerized phenotype. Interestingly, splenic HSPCs from 7-day PCA2-infected mice produce trained macrophages. Taken together, these data show that *C. albicans* PCA2 infection expands and reprograms HSPCs early in the bone marrow and mobilize them to the spleen to produce trained macrophages to fight against infection.

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P-020

MITOCHONDRIAL DYNAMICS OF HUMAN CYTOKINE-INDUCED MEMORY-LIKE NK CELLS

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Natural Killer (NK) cells represent a valuable tool in cancer immunotherapy, and mitochondria represent an attractive target that could further improve current therapeutic strategies. Indeed, recent findings suggested that NK cell-based immunotherapies could be successfully combined with mitochondrial-targeting strategies. A promising therapy for cancer patients is based on the adoptive transfer of IL-12/15/18-stimulated NK cells, also known as cytokine-induced memory-like (CIML) NK cells. In this work, we focused on the mitochondrial changes that happen in human CIML NK cells both after stimulation with IL-12, IL-15 and IL-18, and after a resting period. We have studied mitochondrial fitness by measuring changes in mitochondrial membrane potential and accumulation of mitochondrial reactive oxygen species. Additionally, we measured the autophagic and mitophagic activity of CIML NK cells. To understand the relevance of these results, we have analyzed changes in cell viability through different experimental approaches. Additionally, we studied mitochondrial dynamics by measuring the expression of mitochondrial shaping proteins and analyzing mitochondrial morphology. Altogether, our results suggest that CIML NK cells could progressively accumulate dysfunctional mitochondria, which can induce cell death and ultimately curtail the success of therapies based on the adoptive transfer of CIML NK cells.



P-021

THE GSK3B-MAFB AXIS CONTROLS THE PRO-FIBROTIC GENE PROFILE OF PATHOGENIC MONOCYTE-DERIVED MACROPHAGES IN SEVERE COVID-19

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Macrophages are responsible for orchestrating the immune response. They are fundamental effectors of the innate immune system and connect it to the acquired immune system. Macrophages are involved in a wide range of processes. They can stimulate and resolve inflammatory responses but are also involved in tissue development and homeostasis. Their functional diversity is reflected by the huge spectrum of polarization states acquired by macrophages in response to cues from the tissue environment. Of note, macrophage malfunction can give rise to clinically relevant pathologies such as tumors, COVID-19, and autoimmune and inflammatory disorders.

Macrophages can acquire a wide spectrum of activation states, although two opposite extremes of the spectrum have been defined for simplicity. Pro-inflammatory macrophages appear in the presence of IFNγ or GM-CSF, they express high levels of co-stimulatory molecules, and produce pro-inflammatory cytokines. On the other hand, IL-4 or M-CSF prompt the acquisition of an anti-inflammatory functional profile in macrophages, in which they secrete anti-inflammatory cytokines. The study of the transcriptional signature of anti-inflammatory macrophages, and their responses towards pathogenic stimuli constitutes the central axis of this work.

MAF and MAFB are two transcription factors that mediate tissue development, regulate immune responses, and are required for the acquisition and maintenance of the transcriptional and functional profile of anti-inflammatory human macrophages. We have identified MAF-dependent genes (such as *FOLR2*), MAFB-dependent genes (such as *IL10*), and MAF/MAFB-dependent genes (such as *IGF or CD163*). In addition, GSK3β has been stablished as a major regulator of the MAFB-dependent transcriptome. Furthermore, a direct link between GSK3β, MAFB-dependent genes and the transcriptional profile of pathogenic alveolar macrophages genes in severe COVID-19 has been found. Besides, we have identified macrophage effector functionalities that are differently regulated by MAFB and MAF, namely the production of



monocyte-recruiting chemokines and the ability to stimulate T lymphocyte proliferation.



P-022

DIFFERENTIAL ENGRAFTMENT OF A20 PD-L1 WT AND PD-L1 KO LEUKEMIA CELLS IN SEMIALLOGENEIC RECIPIENTS IN THE CONTEXT OF PD-L1/PD-1 INTERACTION AND NK MEDIATED HYBRID RESISTANCE

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The contribution of NK cells to tumor rejection in the context of PD-L1/PD-1 blockade is a matter of intense debate. To elucidate the role of PD-L1 expression on tumor cells and the functional consequences of engaging PD-1 receptor on cytotoxic cells, PD-L1 expression was genetically inactivated and WT or PD-L1 deficient parental tumor cells were adoptively transferred intravenously into F1 recipients. The engraftment of PD-L1 deficient A20 tumor cells in spleen and liver of F1 recipients was impaired compared with A20 PD-L1 WT tumor counterparts. To elucidate the mechanism responsible for this differential tumor engraftment and determine the relevance of the role of PD-L1 / PD-1 pathway in the interplay tumor cells / NK cells, a short-term competitive tumor implantation assay in the peritoneal cavity of semiallogeneic F1 recipients was designed. The results presented herein showed that NK cells killed target tumor cells with similar efficiency regardless of PD-L1 expression, whereas PD-L1 expression on A20 tumor cells conferred a significant tumor protection against rejection by CD8 T cells confirming the role of the co-inhibitory receptor PD-1 in the modulation of their cytotoxic activity. In summary, PD-L1 expression on A20 leukemia tumor cells modulates CD8 T cell-mediated responses to tumor-specific antigens, but does not contribute to inhibit NK cell-mediated hybrid resistance, which correlates with the inability to detect PD-1 expression on NK cells neither under steady-state conditions nor under inflammatory conditions.



P-023

SIDT1 IS REQUIRED FOR IFN-I RESPONSES TO NUCLEIC ACIDS BY ENDOSOMAL TOLL-LIKE RECEPTORS IN PLASMACYTOID DENDRITIC CELLS, AND MEDIATES THE PATHOGENESIS OF IFN-MEDIATED DISEASES

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Type I IFN (IFN-I) is involved in the pathogenesis of autoimmune and autoinflammatory diseases such as psoriasis and lupus. SIDT1 is an ER-resident protein expressed in the lymphoid lineage, and involved in anti-viral IFN-I responses *in vivo*, through an unclear mechanism. Herein we have dissected the role of SIDT1 in the natural IFN-producing cells, the plasmacytoid dendritic cells (pDC). The function of SIDT1 in pDC was determined by silencing its expression in human primary pDC and in GEN2.2 cell line. SIDT1 role *in vivo* was assessed using the imiquimod-induced psoriasis model and the TLR7.tg lupus model crossed with SIDT1-deficient mice (*sidt1*$^{-/-}$).

Silencing of SIDT1 in primary human pDC and GEN2.2 cells led to a blockade of the IFN-I response after stimulation of TLR7 and TLR9, without affecting the pro-inflammatory responses or upregulation of maturation markers. We found that SIDT1 migrates from the ER to the endosomal and lysosomal compartments together with TLR9 after CpG stimulation. Thus, SIDT1 participates in the access of the TLR9-CpG complexes to lysosome-related vesicles, and therefore mediates the activation of TBK1 and the nuclear migration of IRF7, but not of NF-kB. *sidt1*$^{-/-}$ mice showed a significant decrease in severity parameters of the imiquimod-induced acute psoriasis-like and spontaneous TLR7.tg lupus models, associated with a decrease in the production of IFN-I and IFN-dependent chemokines.

Our findings indicate that SIDT1 is at the cross-road between the IFN-I and the proinflammatory pathways and constitutes a promising drug target for autoinflammatory diseases mediated by IFN-I responses.



P-024

RESIDUAL VIRAL REPLICATION AND BACTERIAL TRANSLOCATION MAY DIFFERENTIALLY REGULATE LIGANDS FOR CHECKPOINT RECEPTORS ON MYELOID CELLS FROM PEOPLE LIVING WITH HIV-1

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Dendritic cell-based immunotherapies are a promising tool against HIV-1 infection to increase activation and antiviral function of CD8+ T cells. However, differential expression of checkpoint inhibitory receptors (CIR) in CD8+ T cells from people living with HIV (PLWH) limit their ability to respond to stimulation. Whether distinct chronic inflammatory processes differentially regulate the potential of myeloid cells remains understudied. Myeloid cells from HIV-negative (HC; n=13) and PLWH (n=6) were exposed to Pathogen-Associated Molecular Patterns (PAMP) mimicking bacterial translocation (LPS and Flagellin) or viral replication (CL097, Poly I:C and HIV-1 Gag peptides). Expression of CD64, CD40, ligands for CIR (PDL1, CD155 and Galectin-9), and IL1β levels were analyzed by flow cytometry, qPCR and ELISA.

Viral PAMPS preferentially induced higher levels of PDL1 and CD155 expression on myeloid cells more significantly in PLWH than HC. Interestingly, Poly I:C and CL097 induced both protein and mRNA levels of CD155 and PDL1, while HIV-1 Gag exclusively induced an increase of protein levels of these molecules. In contrast, LPS and flagellin were not able to induce protein expression of these ligands both in HC and PLWH. In contrast, Galectin-9 expression, ligand of TIM3, was significantly reduced by Gag peptides and Poly I:C specifically in Mo from PLWH and not in HC, suggesting negative regulation of Galectin-9 expression by viremia specifically in PLWH but not in not-infected individuals and negatively correlating with CD155 expression. Such trained state correlated with higher expression and secretion of IL1β at baseline and after viral PAMP stimulation, and with the induction of CD64+ CD40+ cells in Mo from PLWH compared to cells from HC.

In conclusion, residual viral replication rather than bacterial translocation, induce phenotypic changes on myeloid cells from PLWH that may potentiate CD8+ T cell dysfunctionality, underlying the need to introduce modulation of myeloid cells for effective HIV immunotherapies.



P-025

FH DESIALILATION ALTERS COMPLEMENT REGULATION ON CELLULAR SURFACES, AND COULD CONTRIBUTE TO ATYPICAL HAEMOLYTIC URAEMIC SYNDROME.

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Introduction

The plasma glycoprotein Factor H (FH) is essential to prevent autologous damage by the Complement system. FH mutations or autoantibodies favour disease conditions such as the Haemolytic Uraemic Syndrome (aHUS), where microvascular endothelial cells are injured by the host Complement. We recently observed transient desialylation of FH in aHUS patients with *Streptococcus Pneumoniae* infections, and proposed that desialilated FH could contribute to disease pathogenesis.

Objectives

To explore the role of FH sialic acids on its capacity to control Complement activation on different cellular surfaces.

Methods

Desialilated FH (dFH) was generated "in vitro" with *C. perfringens* neuraminidase. The binding of FH and dFH to C3b deposited on a surface was tested by a competition assay using fluorescently labelled-FH (FH^{A488}) on opsonized sheep erythrocytes (ShE). The ability of FH/dFH to regulate complement was assayed on Human Microvascular Endothelial Cells (HMEC-1), which were incubated with normal human serum (NHS) or with an aHUS serum carrying the FH_{W1183L} mutation, and with increasing amounts of exogenous FH/dFH. C3b deposition on the endothelial cells was then analyzed by immunofluorescence using a FITC-conjugated anti-human C3c antibody, and quantified with ImageJ Software.

Results

Experiments on ShE showed a decreased capacity of dFH compared with native FH to compete the binding of FH^{A488} to the opsonized erythrocyte surface, suggesting that dFH has a lower affinity to interact with C3b on a surface.



The experiments with HMEC-1 cells revealed that native FH decreased C3b deposition in a dose-dependent manner, while dFH barely show inhibitory activity. These findings were observed when using the NHS or the aHUS serum as a source for C3b.

Conclusions

We conclude that removal of FH sialic acids alters its capacity to efficiently control complement activation on cellular surfaces, although additional experiments will be required to determine the precise mechanism.



P-026

ANÁLISIS FUNCIONAL Y PERFIL DE EXPRESIÓN DE RECEPTORES ACTIVADORES E INHIBIDORES EN CÉLULAS NK TIPO-MEMORIA INDUCIDAS POR IL-12/IL-15/IL-18

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Las células NK son un componente principal de la inmunidad innata, con capacidad para lisar células infectadas o transformadas a través de una respuesta rápida y robusta que ha sido objeto de intensos estudios en los últimos años. Uno de ellos es la generación de subpoblaciones de células NK con características similares a memoria inducidas por citoquinas, denominadas células NK CIML (*cytokine-induced memory-like*). En este trabajo, describimos los cambios en el fenotipo y función de las células NK CIML generadas por la preactivación con las citoquinas IL-12, IL-15 e IL-18 en comparación con células NK control cultivadas en presencia de IL-15. A las 16 horas de preactivación con citoquinas, se observó un aumento de la expresión de los receptores NKG2A, CD25, y CD69 y un descenso del receptor CD16 en las células NK CIML con respecto a las células NK control, diferencias que se mantuvieron a los 7 días de cultivo a excepción de CD69. Los receptores activadores DNAM-1, NKp30, y NKp46 aumentaron en células NK CIML respecto al control, tanto a los 7 como a los 14 días. En cuanto a la expresión de los receptores inhibidores PAN-KIR y TIGIT disminuyeron en las células NK CIML. El análisis funcional reveló, en el día 7, un aumento de la degranulación de las células NK CIML en respuesta a las células diana K562 y MaMel-56. Tanto el TNF-α como el IFN-γ presentaron niveles bajos de expresión a los días 7 y 14.

En conclusión, los resultados muestran que las células NK CIML presentan un potencial citotóxico mayor probablemente debido al aumento de los receptores activadores lo que sugiere profundos cambios en términos transcriptómicos, proteómicos, metabolómicos o epigenéticos que deben ser analizados para la optimización de los protocolos de expansión de las células NK CIML para su uso en inmunoterapia adoptiva.



P-027

CARDIAC MACROPHAGE-DERIVED CIRCULATING MIRNAS AFTER ACUTE MYOCARDIAL INFARCTION

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Objectives

The aim of this work is the identification of specific circulating miRNAs of cardiac macrophages after acute myocardial infarction (AMI).

Material and Methods

An analysis was performed by means of a miRNA-microarray in cardiac pro-inflammatory macrophages and a small RNAseq in plasma, from different mouse models after AMI and myocarditis - as control of non-ischemic cardiomyopathy -. Next, the miRNAs of interest were validated by qPCR in mouse macrophages and plasma after AMI. To assess the expression of these miRNAs in circulation and within extracellular vesicles, *ex-vivo* cultures of cardiac macrophages isolated from mice after AMI were made. Finally, a translational study was carried out in swine model and in patients, for the validation of these miRNAs in cardiac macrophages after AMI (small-RNAseq) and plasma (qPCR), respectively.

Results

We have identified miR-185-5p, miR-222-3p and miR-1306-3p as miRNAs specific to cardiac pro-inflammatory macrophages after ischemic cardiomyopathy. These miRNAs, secreted into the circulation within extracellular vesicles, are specific to AMI and are not expressed in others non-ischemic cardiomyopathies such as myocarditis. The murine miRNAs miR-185-5p, miR-222-3p, and miR-1306-3p are conserved and expressed in cardiac macrophages and plasma, from swine and human, respectively. These miRNAs are involved in the regulation of processes such as inflammation, metabolism, and circadian cycles.

Conclusions

In this study we have identified a signature of miRNAs: miR-185-5p, miR-222-3p and miR-1306- 3p, which are specifically expressed in pro-inflammatory macrophages and are secreted into plasma within extracellular vesicles after AMI. These miRNAs are conserved in swine model and in AMI patients. Our work suggests that the expression of these miRNAs in circulation could be a tool to study the pro-inflammatory state in myocardium during AMI.

Funding

Ministerio de Educación y Formación Profesional (FPU19/03941); Ministerio de Economía y Competitividad (RTI2018-095928-B-I00); Comunidad de Madrid (S2017/BMD-3684); Instituto



de Salud Carlos III (PI19/00545).



P-028

OVERLAP CASE BETWEEN ACQUIRED ANGIOEDEMA AND SJÖGREN SYNDROME SUCCESSFULLY TREATED

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Hospital Universitario Reina Sofia, Córdoba.

Background.

Acquired angioedema is a life-threatening non-common disorder whose clinical presentation is characterized by recurrent episodes of edema and gastrointestinal symptoms. Sjögren syndrome is an autoimmune disease with a clinical presentation of ocular and oral dryness and salivary gland swelling, which commonly overlaps with other diseases. We aimed to describe the algorithm followed to diagnose an overlap case between acquired angioedema and Sjögren syndrome.

Material and Methods.

A 62-year-old female with no medical history of interest consulted Allergology Department with 15 days of daily facial edema distributed in eyelids and lips but no other skin lesions or systemic symptoms. In every episode, the patient needed to attend emergency room and was treated with corticoids iv and antihistaminic with temporary response and no need of hospitalization. Laboratory tests were performed, including hemogram, skin prick test, complement, immunoglobulin and autoantibody detection.

Results.

Blood analyses revealed low levels of C1INH 10 mg/dL [41-95], C1q 6,00 mg/dL [21,0-38,0], C3 51,80 mg/dL [75,00-120,00] and C4 8,21 mg/dL [12,00-50,00]. Acquired angioedema was diagnosed and treatment was initiated with intravenous C1-inhibitor weekly. During the followed-up, the patient referred partial improvement, but joint pain with metacarpal inflammation appeared in the absence of dry syndrome. Under these new symptoms, autoimmunity tests were performed, reporting positive anti-nuclear antibodies at 1/640 title with homogeneous pattern (AC-1) by indirect immunofluorescence and anti-SSA/Ro 8 U/L (<7,0) and anti-SSB/La antibodies 9 U/L (<7,0). Sjögren syndrome was diagnosed after the result of a minor salivary gland biopsy, and treatment was initiated with hydroxychloroquine and low corticoid dose. After 3-weeks of the treatment onset, the patient was asymptomatic with no new episodes of angioedema until now.

Conclusion.

Physicians must know the atypical presentation of a possible overlap between a complement disease and autoimmune disease, its correlation with analytical findings and its remission with



specific treatment.



P-029

LAMIN A/C IN ANTIGEN PRESENTING CELLS AND ITS ROLE IN INFLAMMATORY BOWEL DISEASE.

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The intermediate filament, lamin A/C, is in the nuclear envelope. Antigen presenting cells (APCs) stimulate naïve T cells, triggering T cell activation and adaptive immune responses. Several pathological processes are linked to abnormal APC function altering the subsequence T cell-mediated response. Lamin A/C in CD4+ T cells enhances T cell activation and differentiation towards Th1 phenotype but its role in APC has not been defined.
Inflammatory bowel disease (IBD) is a heterogeneous state of chronic intestinal inflammation of unknown causes. IBD has been linked to genetic and environmental factors, microbiota dysbiosis, innate and adaptive immunity exacerbation and epithelial intestinal barrier disfunction. IBD pathogenesis is classically associated to gut accumulation of proinflammatory Th1 and Th17 cells while insufficient Tregs suppression.
Our objective is to analyze the role of lamin A/C in APC capacity to trigger adaptive immune responses in IBD. To achieve this, we studied a mouse lacking lamin A/C ($Lmna^{-/-}$) in the myeloid compartment (LysM) as a model DSS induced-IBD, and analyzed GM-CSF bone marrow derived-DCs (BM-DCs) from these mice for *in vitro* studies.

In vitro, $Lmna^{-/-}$ GM-CSF BM-DCs showed less production of proinflammatory cytokines mRNA. $Lmna^{-/-}$ GM-CSF BMDCs showed a diminished capacity to form conjugates with naïve CD4+ T cells and to promote CD4+ T cells activation and proliferation compared to WT BM-DCs. $Lmna^{-/-}$ BM-DCs induced less Th1 differentiation as observed by a decrease in Th1-specific transcription factor T-bet and IFNγ production in CD4+ T cells when compared to WT BM-DCs. The adoptive transfer of $Lmna^{-/-}$ BMDC to WT mice before the treatment with DSS ameliorates colitis induction when compared to WT GMSCF-BMDC transfer.

In conclusion, the absence of lamin A/C in APCs protects from IBD maybe by a reduction in proinflammatory cytokine production and by the decrease of Th1-mediated response.



P-030

ELABORACIÓN DE PÍLDORAS FORMATIVAS POR ALUMNADO DE CIENCIAS DE LA SALUD, DE FORMA MULTIDISCIPLINAR Y COLABORATIVA.

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Universitat De València, Valencia.

En el presente proyecto hemos planteado un proceso de actualización e innovación docente basado en el uso del microaprendizaje entre profesorado y alumnado de la asignatura Inmunología e Inmunopatología (IEI) del grado de Bioquímica y Ciencias Biomédicas (BCB) y de varias asignaturas del grado de Enfermería de la Universitat de València.
La asignatura IEI contempla una práctica de laboratorio en la que se analiza la capacidad fagocítica y microbicida de leucocitos de sangre humana. Esta es una oportunidad para el alumnado del grado de BCB de manipular una muestra real, tal y como se desarrollaría en un laboratorio de hospital. La manipulación de estas muestras requiere una serie de medidas de bioseguridad, así como estar preparado para actuar ante alguna contingencia que pudiera producirse en el momento de la extracción. El alumnado del grado de Enfermería posee los conocimientos necesarios en cuanto a las prácticas en bioseguridad para la extracción y manipulación de muestras humanas, pero no están tan familiarizados con la metodología y las técnicas de algunos ensayos que se llevan a cabo en los laboratorios clínicos.
El alumnado de ambos grados interaccionó durante las prácticas (en la extracción y en la práctica posterior) y se generaron píldoras formativas en forma de vídeos cortos de cinco minutos, como herramienta educativa y como resultado del aprendizaje colaborativo interdisciplinar. El material generado se ofreció al alumnado de ambos grados junto con una encuesta de opinión y valoración. Los resultados de las encuestas fueron positivos en cuanto al nivel de conocimientos transmitidos, la utilidad y la calidad de los vídeos.
Este proyecto ha iniciado una colaboración interdepartamental en innovación docente y ha generado objetos de microaprendizaje que pretenden completar la formación del alumnado y generar cohesión entre diferentes disciplinas afines.
Proyecto de innovación docente UV-SFPIE_PID-1638203, Universitat de València.



P-031

LOCALIZACIÓN SUBCELULAR DE TRIPARTITE MOTIF (TRIM)-37 EN RESPUESTA A LPS E INTERFERÓN E IDENTIFICACIÓN DE LOS COMPONENTES DE SU SIGNALOSOMA

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Cada vez existen más evidencias del papel de determinadas especies componentes de la microbiota en la regulación de determinados procesos oncogénicos. En este trabajo, hemos estudiado en distintas líneas tumorales y no tumorales el efecto del lipopolisacárido (LPS) y del interferón (IFN)-γ en la localización subcelular de TRIM37, una E3 ubiquitina ligasa que contiene un dominio TRAF.

En el citosol de células HepG2 (y en otros tipos celulares) TRIM37 se asocia a peroxisomas (colocaliza con la catalasa). En respuesta a IFNγ se induce la expresión de las proteínas NMI e IFP35, las cuales colocalizan con TRIM37 en los peroxisomas. Sin embargo, en otros tipos celulares, como PC3 y EA.hy926, TRIM37 se encuentra preferentemente en el núcleo, donde colocaliza parcialmente con los cuerpos nucleares PML (PML-NBs). En respuesta a IFNγ, el número de PML-NBs aumenta, así como su colocalización con TRIM37. En PC3 y EA.hy926, NMI e IFP35 también se inducen con IFNγ, pero mientras que NMI se moviliza al núcleo y colocaliza con TRIM37 en los PML-NBs, IFP35 permanece mayoritariamente en el citosol y no colocaliza en los PML-NBs.

Cuando las células PC3 y EA.hy926 se tratan con LPS, se observa también la inducción de NMI e IFP35 y colocalizaciones similares a las anteriores, pero con una cinética diferente. Así, se observa que TRIM37 y PML-NBs colocalizan en el núcleo antes de la inducción de la expresión y la movilización al núcleo de NMI. Estos resultados indican que TRIM37 es un componente de los PML-NBs, a los que más tarde se asociaría NMI. También sugiere que TRIM37, una ubiquitina ligasa con un dominio homólogo a la histona deacetilasa-1 (aunque su funcionalidad no ha sido comprobada), podría ser un componente regulador de la actividad transcripcional de PML y participar en la regulación de PML por los receptores de reconocimiento de patrones.



P-032

ANÁLISIS DE GENES DE LA RUTA DE LA AUTOFAGIA EN ENFERMEDAD DE BEHÇET

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Introducción:

La Enfermedad de Behcet (BD) es una vasculitis sistémica. Inmunológicamente se considera una enfermedad de patrón mixto, en cuya predisposición influyen desajustes en ambas ramas del sistema inmunitario. Además de su relación bien establecida con HLA-B51, se ha descrito asociación de la enfermedda con genes relacionados con el sistema inmunitario innato como los del inflamasoma. Independientemente de otras funciones, la autofagia es un proceso que regula la inflamación, de heho, se ha descrito asociación entre genes relacionados con la autofagia y la predisposición a enfermedades con componente inflamatorio como la enfermedad de Crohn.

Objetivo:

Investigar la posible asociación de la ruta de la autofagia con susceptibilidad a BD.

Materiales y métodos:

Genotipado mediante secuenciación masiva del exoma de 17 genes relacionados con autofagia: ULK1, ATG13, RB1CC1, ATG101, PI3KC3, BECN1, ATG14, PIK3R4, NRBF2, RUBCN, UVRAG, WIPI1, WIPI2, ATG2A, ATG2B, ATG16L1, ATG5, ATG12, ATG3, ATG4A, ATG4B, ATG4D, ATG7 y ATG10. El estudio incluye una cohorte de 58 pacientes con BD. Las frecuencias alélicas de la población control se obtuvieron de la población IBS del proyecto 1000 genoma que incluye 107 controles. Las frecuencias alélicas en pacientes y controles se compararon mediante la prueba χ^2.

Resultados:

Se identificaron una media de 25 variantes por muestra. Se detectó diferente distribución alélica de la variante c.4147C>G (rs3759601), p.Gln1383Glu en el gen ATG2B en pacientes y controles (MAF G 0.32 en pacientes Vs. 0.42 en controles p=0.03, OR=0.59, 95% IC=0,36-0,96).

Conclusiones:

La autofagia y concretamente, variantes en el gen ATG2B pueden estar relacionada con la predisposición a desarrollar BD.



P-033

LA INMUNIDAD CELULAR: ¿LA GRAN OLVIDADA EN LA PANDEMIA DE COVID-19?

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La pandemia de SARS CoV2 a día de hoy, según el Jonhs Hopkins ha producido más de 535 millones de casos y cerca de 6,3 millones de muertes. Cifras que seguramente serán inferiores a las reales. Tras los primeros meses de incertidumbre y preocupación, quizás los medios de comunicación han influido en mirar única y exclusivamente a la inmunidad humoral quizás de forma obsesiva sobre el "nivel protector de anticuerpos neutralizantes" y quizás olvidando a la inmunidad celular, las células T de memoria.

A día de hoy quizás no hay una cifra cuantitativa que pudiéramos considerar como "de seguridad" de anticuerpos y que en la génesis de la Tras la infección, las células T se diferencian en una población heterogénea de células T efectoras que pueden mediar en la eliminación del patógeno, aparece un subconjunto de estas células T efectoras posee la capacidad de sobrevivir a largo plazo y madurar hasta convertirse en células T de memoria que pueden proporcionar inmunidad a largo plazo.

Creemos que las técnicas de medición y contaje de células T son caras y complicadas, pero nos ayudarían a comprender las señales que regulan el desarrollo de las células T de memoria es crucial para diseñar vacunas capaces de provocar una inmunidad basada en células T.

Las células T CD4(+) son esenciales para la formación de células T de memoria protectoras CD8(+) tras una infección o inmunización. Sin embargo, hasta hace poco, no estaban claros los mecanismos por los que las células T CD4(+) actúan para favorecer el desarrollo de células T CD8(+) de memoria tras la infección.



Sesión Poster. Inmunodeficiencias: primarias y secundarias.

P-034

DIAGNÓSTICO DE DEFICIENCIA DE NFKB2 A RAÍZ DE INFECCIÓN GRAVE POR COVID-19

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Hospital Universitario Virgen del Rocío, Sevilla.

Varón de 4 años sin antecedentes familiares, con dos episodios de ingreso en la Unidad de Cuidados Intensivos: un primer ingreso a los 2 años por meningoencefalitis por enterovirus y un segundo ingreso un año después por una neumonía causada por sobreinfección bacteriana en el contexto de gripe A. En los siguientes años el paciente estuvo en seguimiento por la unidad de neurología por un trastorno del lenguaje y sufrió diversas infecciones de las vías respiratorias altas que no requirieron ingreso hospitalario.

En enero de 2022 ingresa por infección por COVID-19 con neumonía con características de etiología bacteriana. Al inicio presentó una evolución clínica favorable pero el sexto día reapareció la fiebre acompañada de un marcado aumento de los reactantes de fase aguda y una bajada de la saturación de oxígeno al 93-94% que precisó del inicio de oxigenoterapia. El estudio inmunológico reveló una hipogammaglobulinemia pronunciada acompañada de niveles bajos de células NK y elevados de CD25 soluble.

Material y métodos:

Secuenciación del exoma con análisis dirigido de 461 genes asociados a Errores Congénitos del Sistema Inmunológico. La secuenciación se realizó utilizando la plataforma NextSeq 500 Sequencing System (Illumina). Para el análisis bioinformático se utilizó la plataforma SOPHiA DDM.

Resultados:

Se detectó una variante genética en el gen NFKB2 (c.2554delC) en heterocigosis, no descrita previamente. Esta deleción de un nucleótido implica el cambio de la pauta de lectura y la aparición de un codón de parada prematuro. Se valora, por tanto, como patogénica. Se utilizó la secuenciación Sanger para confirmar el resultado y realizar el estudio familiar, el cual reveló el carácter de novo de la mutación.

Conclusiones:

En base a la mutación presentada, la clínica y los datos inmunológicos (hipogammaglobulinemia); el paciente fue diagnosticado de deficiencia de NFKB2, de herencia



autosómica dominante y adscrita a la Inmunodeficiencia Común Variable.



P-035

NOVEL GERMLINE *TET2* MUTATIONS IN TWO UNRELATED PATIENTS WITH AUTOIMMUNE LYMPHOPROLIFERATIVE SYNDROME-LIKE PHENOTYPE AND HEMATOLOGIC MALIGNANCY

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[1]Immunology Department, University Hospital 12 de Octubre, Madrid; [2]Hematology Department, University Hospital La Princesa, Madrid; [3]Immunology Department, University Hospital Fundación Jiménez Díaz, Madrid; [4]Hematology Department, University Hospital Fundación Jiménez Díaz, Madrid; [5]Pathology Department, Research Institute Fundación Jiménez Díaz, Madrid; [6]Genetics Department, University Hospital 12 de Octubre, Madrid; [7]Immunology Department, University Hospital La Princesa, Madrid.

Somatic mutations in the ten-eleven translocation methylcytosine dioxygenase 2 gene (*TET2)* have been associated to hematologic malignancies. More recently, biallelic and monoallelic germinal mutations conferring susceptibility to lymphoid and myeloid cancer have been described. We report two unrelated autoimmune lymphoproliferative syndrome-like patients who presented with T-cell lymphoma associated to novel germinal biallelic or monoallelic mutations in the *TET2* gene. Both patients presented a history of chronic lymphoproliferation with lymphadenopathies and splenomegaly, cytopenias and immune dysregulation. We identified the first compound heterozygous patient for *TET2* mutations (P1) and the first ALPS-like patient with a monoallelic *TET2* mutation (P2). P1 had the most severe form of autosomal recessive disease due to TET2 loss of function resulting in absent TET2 expression and profound increase in DNA methylation. The immunophenotype showed some alterations in innate and adaptive immune system as inverted myeloid/plasmacytoid dendritic cells ratio, elevated terminally differentiated effector memory CD8 T-cells re-expressing CD45RA (CD8 TEMRA), regulatory T-cells and Th2 circulating follicular T-cells. According to the ALPS-like suspicion, double negative T-cells, vitamin B12 and IL-10 were elevated. Interestingly, the healthy brother of P1 carried one of the *TET2* mutations and presented some biomarkers of immune dysregulation, such as elevated IL-10 levels, decreased HDL levels, inverted mDCs/pDCs ratio, expanded CD8 TEMRA and increased DNA methylation in isolated CD8 subpopulations. P2 presented a later debut of the disease, with less severe immune alteration. According to ALPS-like suspicion, she showed elevated vitamin B12, hypergammaglobulinemia and decreased HDL levels. Therefore, novel molecular defects in TET2 confirm and expand both clinical and immunological phenotype, contributing to the better



knowledge of the bridge between cancer and immunity.



P-036

DEVELOPMENT OF ARTIFICIAL THYMIC ORGANOIDS AS A MODEL OF T-LYMPHOCYTE DIFFERENTIATION TO STUDY T-CELL IMMUNODEFICIENCIES

Cela Rodríguez, Carmela[1]; González García, Sara[1]; Alcain Sánchez, Juan[1]; García Torres, Enrique[2]; Arias Dachary, Francisco Javier[3]; Boni, Lorenzo[2]; Pastor Senovilla, Ángela[3]; González Granados, Luis Ignacio[3]; Toribio García, María Luisa[1].

[1]Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Madrid; [2]Cirugía Cardíaca Infantil. Instituto Pediátrico del Corazón. Hospital Universitario 12 de Octubre, Madrid; [3]Instituto de Investigación Hospital 12 de Octubre (i+12). Hospital Universitario 12 de Octubre, Madrid.

Introduction

Next Generation Sequencing has greatly improved the diagnosis of genetic defects underlying different types of immunodeficiencies. However, T-cell lymphopenia can originate from hematopoietic stem cell-extrinsic defects associated with the thymus microenvironment, for which alternative diagnostic methods are required. Identification of the patient's specific T-cell differentiation blockage can help to characterize disease severity and guide diagnosis and treatment. To this end, *in vitro* T-cell differentiation systems based on artificial thymic organoids (ATOs) have been developed.

Objectives

1.Generation and functional validation of ATOs for the differentiation of human early thymic progenitors (ETP) and hematopoietic stem cells from umbilical cord blood (CB) into mature T lymphocytes.

2.Application of ATOs to study T-cell differentiation from hematopoietic progenitors of patients with T lymphopenia in order to determine their maturational defect.

M&M:

CD34+ progenitors were obtained by immunomagnetic selection from thymus and CB from healthy donors, or from peripheral blood of immunodeficient patients. ATOs generated with the stromal cell line MS5 transduced with Notch ligand DLL4 (MS5-hDLL4) were seeded with CD34+ progenitors and cultured in vitro for 6-12 weeks in the presence of IL-7 and Flt3-L. T-cell differentiation was analyzed by flow cytometry.

Results:

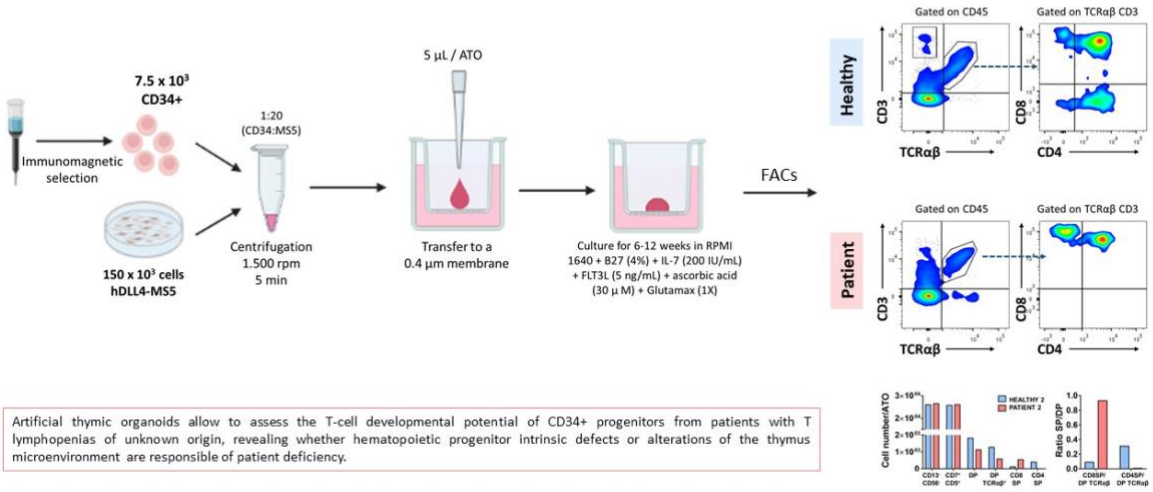
We provide evidence of the reliability of the ATO system for supporting the differentiation of human hematopoietic progenitors along the sequential intrathymic maturing stages and their

efficient generation of mature T lymphocytes. Comparative studies using CD34+ progenitors from patients with severe combined immunodeficiency (SCID) revealed specific developmental defects at particular T-cell maturation stages.

Conclusions:

ATOs allow to study T-cell maturation of patients with T -lymphopenia of unknown origin and to determine whether their defect relies on intrinsic alterations of hematopoietic progenitors or extrinsic cues due to thymic microenvironment defects. Therefore, ATOs represent a useful tool for diagnosis and treatment orientation of immunodeficient patients, opening a new door toward personalized medicine.

Artificial thymic organoids to study T-cell immunodeficiencies



Artificial thymic organoids allow to assess the T-cell developmental potential of CD34+ progenitors from patients with T lymphopenias of unknown origin, revealing whether hematopoietic progenitor intrinsic defects or alterations of the thymus microenvironment are responsible of patient deficiency.



P-037

ANTICUERPOS ANTI-SARS-COV-2 EN PACIENTES CON INMUNODEFICIENCIAS PRIMARIAS TRATADOS CON INMUNOGLOBULINAS

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Introducción: La pandemia de la COVID-19 ha originado una alta comorbilidad en pacientes con inmunodeficiencias primarias, contrarrestada con las vacunas frente al SARS-CoV-2.

Objetivos: Se determinó la respuesta inmune vacunal en 19 pacientes con déficit primario de anticuerpos (DPA), 15 de ellos tratados con inmunoglobulinas (Igs) de sustitución.

Material y métodos: La respuesta humoral se evaluó mediante la determinación de anticuerpos anti-spike (AAS) y anti-nucleocápside (AAN) y la respuesta celular mediante el ensayo de liberación de interferón gamma. Ambas respuestas se evaluaron antes de la vacuna y al mes de la segunda dosis y los resultados se compararon con los obtenidos en un grupo de 50 individuos sanos.

Resultados: Los valores medios de ASS fueron de 170 [20-800] AU/mL y 734 [532-1149] AU/mL en pacientes y controles, respectivamente. Se detectó respuesta celular en el 58% de los pacientes. El 44% de los pacientes tenían AAN prevacunales, sin evidencias de haber pasado la enfermedad, por lo que se analizaron diferentes lotes de Igs administradas, objetivándose la presencia de AAN.

Conclusiones: Se demostró una menor respuesta humoral y celular en pacientes con DPA tras la vacunación, en comparación con los controles. Se constató la presencia de AAN en las Igs administradas y su transferencia a los pacientes con DPA, pudiendo proporcionar un nivel adicional de protección frente al SARS-CoV-2.



Niveles de AAN en lotes de Igs administradas

Lote	Producto	Muestras	AAN (positivo ≥ 1.0)	
1	Flebogamma	1	Negativo	0.25
2	Flebogamma	1	Positivo	1.69
3	Flebogamma	1	Positivo	5.72
4	Flebogamma	1	Positivo	1.77
5	Flebogamma	1	Positivo	14.02 +/- 0.55
6	Flebogamma	1	Positivo	3.85 +/- 0.06
7	Flebogamma	1	Positivo	13.60
8	HyQvia	1	Positivo	6.06 +/- 0.13
9	Plangamma	1	Negativo	0.24 +/- 0.12
10	Plangamma	1	Positivo	57.70
11	Plangamma	1	Positivo	58.80
12	Flebogamma	1	Positivo	5.59



P-038

IMMUNE RECONSTITUTION AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION IN A PATIENT WITH AUTOIMMUNE LYMPHOPROLIFERATIVE SYNDROME-LIKE PHENOTYPE DUE TO ADA2 DEFICIENCY

López -Nevado, Marta[1]; López-Godino, Oriana[2]; Fernández-López, Ángel[3]; Quesada-Espinosa, Juan F[4]; Delmiro, Aitor[5]; Allende, Luis M[1].

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Adenosine deaminase 2 deficiency (DADA2) is an autosomal recessive auto-inflammatory disorder that presents in some cases with benign lymphoproliferation and cytopenias mimicking an autoimmune lymphoproliferative syndrome-like (ALPS-like) phenotype. Here we described an ALPS-like 40-year-old man with DADA2 who underwent hematopoietic stem cell transplantation (HSCT). The patient presented lymphadenopathies and splenomegaly with no history of infections in childhood. Biopsy of lymph node was consistent with an EBV-associated Hodking lymphoma (HL). After treatment, he achieved complete remission. He presented persistent splenomegaly and cytopenias, so screening for ALPS was indicated. Immunologic evaluation revealed T lymphopenia, decreased levels of naïve, increased TEMRA and activated CD8 T-cells, B lymphopenia, low levels of class-switched memory B-cells and elevation of transitional and CD21low B-cells. ALPS-biomarkers were normal except to elevated vitamin B12. Molecular studies revealed a previously unreported homozygous variant in ADA2 (c.1348G>C; p.Gly450Arg) that lead to a deficient ADA2 enzymatic activity. He was candidate for an autologous HSCT from her healthy carrier sister as haploidentical donor. Complete quimerism was presented since day +28 but immune reconstitution 2 months after transplant was partial, with severely decreased naïve and increased activated effector T-cells. Elevation of transitional B-cells suggesting an immature B compartment was also observed. Six months after transplant, an improvement was observed but not achieving total reconstitution. ADA2 enzymatic activity was already recovered at 2 months post-transplant. Nowadays, he has no history of graft versus host disease and/or complications after transplant. In conclusion, allogeneic HSCT was successfully performed in the patient reported here, without significant toxicity and it rescued ADA2 functional activity with complete remission of the associated HL in the reevaluation after HSCT.



P-039

TRATAMIENTO DE INMUNODEFICIENCIAS: PRODUCCIÓN DE MEDICAMENTOS HEMODERIVADOS EN UNA COMUNIDAD

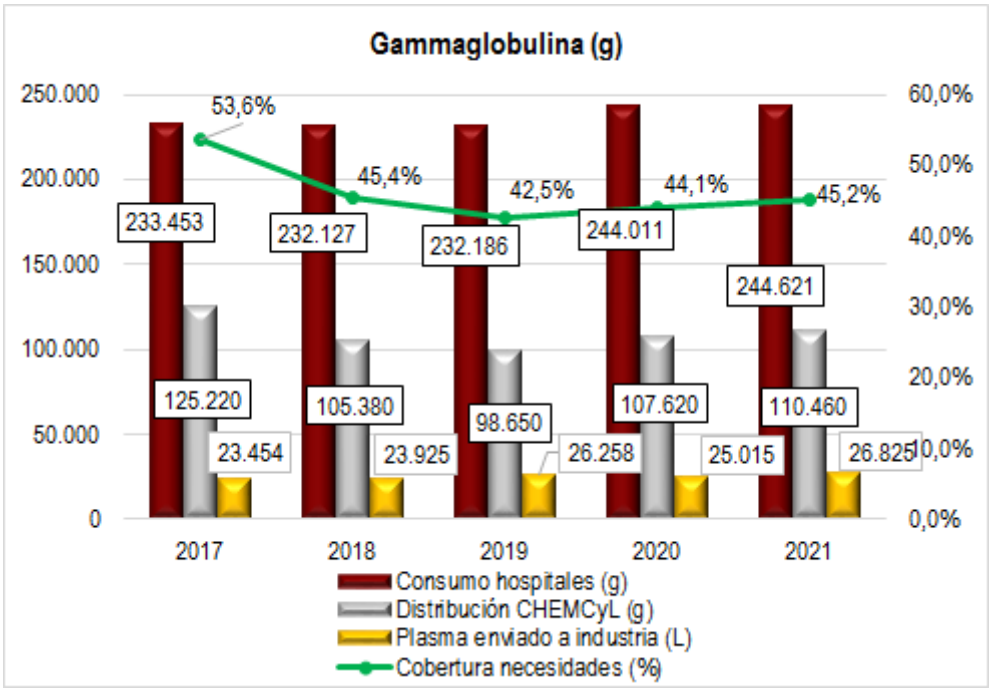
Martín, M Carmen; Gonzalez, M Isabel; Santamaría, Jennifer; Yáñez, Marta; Blanco-Peris, Lydia.

Centro de Hemoterapia y Hemodonación de Castilla y León, Valladolid.

El único tratamiento disponible para muchas inmunodeficiencias: la gammaglobulina, se obtiene exclusivamente del plasma de origen humano. Estos enfermos requerirán generalmente tratamiento de por vida y no es posible fabricar o producir gammaglobulinas, si no es a partir de plasma procedente de donantes.

Los datos analizados en el presente estudio se han obtenido de las bases de datos de nuestro centro de trasfusión (número de donantes y litros plasma), los registros de la industria fraccionadora (hemoderivados obtenidos) y el informe anual de actividad de centros y servicios de trasfusión (consumo de hemoderivados en nuestra comunidad).

En los últimos 5 años se ha obtenido una media de 25.095 L/año (23.824-26.367) de plasma (IC95%) procedentes de un mínimo de 41.825 donaciones. Por cada aféresis se obtienen 600 mL de plasma, mientras que de una donación de sangre total, solo se consiguen unos 200 mL. Desde 2017 se han obtenido 125.477 L totales de plasma, a partir de los cuales se han fraccionado 513.870 g de gammaglobulina (4.09 g/L de plasma) y 3.162.120 g de albúmina. Aunque la cantidad es variable, un paciente adulto podría necesitar unos 300 g de gammaglobulina al año, así que las donaciones de plasma estarían permitiendo tratar más de 300 casos cada año: cada uno de ellos requeriría unas 125 donaciones de plasma anuales. Con los medicamentos obtenidos de la industria fraccionadora se han cubierto el 46.1% de las necesidades de gammaglobulina de la comunidad, sin que este porcentaje de cobertura disminuyera con la pandemia, ni durante las campañas de plasma convaleciente. El Centro de Hemoterapia y Hemodonación de nuestra comunidad es líder en España en cuanto a litros de plasma donados por habitante pero, dado el elevado número de donaciones necesarias para tratar cada caso de inmunodeficiencias, es necesario continuar fomentando los programas de donación de plasma.


Gammaglobulina (g)
250.000
200.000
150.000
100.000
50.000
0
60,0%
50,0%
40,0%
30,0%
20,0%
10,0%
0,0%
53,6%
45,4%
42,5%
44,1%
45,2%
233.453
232.127
232.186
244.011
244.621
125.220
105.380
98.650
107.620
110.460
23.454
23.925
26.258
25.015
26.825
2017
2018
2019
2020
2021
Consumo hospitales (g)
Distribución CHEMCyL (g)
Plasma enviado a industria (L)
Cobertura necesidades (%)



P-040

MANEJO Y DETECCIÓN PRECOZ DE REACCIÓN ADVERSA GRAVE A GAMMAGLOBULINA INTRAVENOSA: EL PAPEL DE LA ENFERMERÍA ESPECIALIZADA

Escobar Palazón, María Ángeles; Díaz Luna, Mercedes; Balastegui Martín, Héctor; Moncayo Muñoz, Ana Paulina; Carbone Campoverde, Javier; Gil Herrera, Juana; Fernández Cruz, Eduardo.

HGU Gregorio Marañon, Madrid.

El TRALI *(*acrónimo de *transfusion related acute lung injury o reacción aguda pulmonar en relación a transfusión)* es una complicación rara tras uso de gammaglobulinas IV (GGIV). Presentamos el caso clínico de un varón de 86 años con Inmunodeficiencia Variable Común (IDVC), hiperesplenismo secundario y trombopenia asociada, en tratamiento sustitutivo con GGIV 10% a dosis de 20g cada 4 semanas. Durante una de sus infusiones mensuales en Hospital de Día de Inmunología, desarrolló un cuadro clínico de sensación distérmica, disnea y desaturación de O2 de hasta 82%, con cifras tensionales y frecuencia cardiaca normales. La infusión se detuvo y se trasladó al paciente al Servicio de Urgencias. En ningún momento presentó sintomatología cutánea ni gastrointestinal, dolor torácico o pérdida de consciencia, descartándose anafilaxia. En una radiografía de tórax se objetivaban múltiples opacidades bilaterales en campos medios con sospecha de infección respiratoria COVID-19, por lo que se realizó PCR para SARS-CoV2, resultando negativa. Analíticamente presentaba leucocitosis con linfopenia asociada, con elevación de reactantes de fase aguda (PCR y fibrinógeno) así como de dímero D. La evaluación reunió criterios clínicos y analíticos de *TRALI.* Tras análisis del lote administrado de GGIV, así como suero del paciente en el Centro de Transfusiones, se detectaron anticuerpos IgG antiHNA 1a, 1b, 1c, 3a y 3b en el preparado utilizado. Tras este episodio, se decidió cambiar la terapia de reemplazo de GGIV a gammaglobulina subcutánea (GGSC) facilitada, prescribiéndose a dosis de 20g cada 4 semanas. El paciente fue entrenado por parte de enfermería especializada en el hospital de día para el aprendizaje de la autoadministración de la GGSC en domicilio. Actualmente, ha acumulado varias sesiones de terapia sin complicaciones. Como conclusión, hay que estar atentos ante esta posible complicación grave del uso de GGIV. En caso de TRALI una opción es la terapia con GGSC facilitada.



P-041

ALTERNATIVAS TERAPÉUTICAS EN PACIENTE CON MUTACION EN *STAT1* Y CANDIDIASIS MUCOCUTÁNEA CRÓNICA

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Introducción

La proteína STAT1 (Signal Transducer and Activator of Transcription 1) media la expresión de una gran variedad de genes importantes en la respuesta a patógenos.

Presentación del Caso

Mujer de 37 años en seguimiento por la Unidad de Inmunología Clínica del Hospital Universitario Gregorio Marañón y con diagnóstico de candidiasis mucocutánea crónica (CMC) de predominio orofaríngeo con debut a los 8 meses. Ingresos por múltiples cuadro de candidiasis esofágica severa y con resistencia a numerosas líneas de tratamiento, incluido antifúngicos.

Inicio tratamiento con autovacuna de mucosas Candida al 100% en 2015 con mejoría de los cuadros llegando a desaparecer las lesiones y con empeoramiento clínico tras las temporadas de suspensión.

En el estudio inmunológico se objetiva defecto de IgG4, linfopenia B e hipocomplementemia C3. Además de anticuerpos de celiaquía positivos sin asociar clínica gastrointestinal.

Métodos

Se aisló DNA genómico a partir de sangre periférica de la paciente y se ha procedió a la construcción de una librería enriquecida en 200 genes relacionados con la respuesta inmune.

Resultados

En el análisis inmunogenético, se identifica una mutación missense patogénica de ganancia de función en heterocigosis en *STAT1*: p.Cys174Arg compatible con el diagnóstico molecular de CMC.

Conclusiones

El diagnóstico molecular de la paciente abre la posibilidad de nuevas alternativas terapéuticas entre las que destacan los inhibidores de JAK1/JAK2. No obstante, estos tratamientos llevan consigo una serie de riesgos y la recurrencia de los síntomas tras su interrupción. La paciente tuvo una buena respuesta con la autovacuna de mucosas con composición Candida al 100%. Por todo ello, y como consecuencia de tratarse de un tratamiento más inocuo, debería contemplarse dentro del arsenal terapéutico de la paciente. Se hipotetiza que la autovacuna podría asociarse a cambios epigenéticos o metabólicos englobados dentro del concepto “inmunidad entrenada” a

nivel del sistema inmune innato de mucosas.

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P-042

EXPLORING POTENTIAL MOLECULAR MECHANISMS OF B CELL DEREGULATION IN SECONDARY ANTIBODY DEFICIENCIES.

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Background:

Secondary antibody deficiencies (SADs) are becoming more prevalent as increasing numbers of patients are treated for autoimmune diseases or cancer with diverse B-cell targeted therapies (BCTT), in particular Rituximab. Here, we report the major immunological characteristics of a large cohort of SADs patients after BCTT. B cell recovery upon treatment discontinuation is quite heterogeneous.

Methods:

We conducted a retrospective study of a cohort of 90 patients with SAD upon Rituximab treatment, referred to our clinic between 2006 and 2021. Clinical, epidemiologic and general laboratory data were collected from clinical records. Initially, we analysed the integrity of NFkB activation and Ca2+ upregulation upon BCR stimulation in replenished naïve B cells several years after B cell targeted therapy by intracellular flow cytometry.

Results:

In our cohort 62% of patients were treated with Rituximab due to a hemato-oncologic process, and 38% of them for autoimmune conditions. 37% were males and 63% were females, with a median age at diagnosis of 57 years. Immunological assessment include immunoglobulin levels, specific antibodies, major lymphocyte subpopulations and detailed B cell phenotype: reduced total B cell numbers or severely decreased percentages of memory B cells are observed in most patients.

Preliminary results revealed conserved IKBA degradation and induced $Ca2^{+}$ levels upon several stimuli in recovered naïve B cells from patients with secondary hypogammaglobulinemia.



Conclusion:

Broad heterogeneity in the reconstitution of the B-cell compartment is observed in patients treated with Rituximab. Our preliminary results do not suggest a grossly impaired activating response in naïve B cells that re-emerged in peripheral blood after B cell depletion. A better physiological understanding of the impact of this therapy on the replenished B-cell compartment is mandatory. This will aid in the individual prognosis and clinical management of secondary antibody deficiency in patients.



P-043

KINETICS OF T CELL IMMUNE RESPONSES ELICITED AFTER THREE MRNA COVID-19 VACCINE DOSES IN PREDOMINANTLY ANTIBODY-DEFICIENT INDIVIDUALS

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Mass vaccination campaigns reduced COVID-19 incidence and severity. Recently, an additional dose has been recommended, particularly to those individuals that remain vulnerable. Here, we evaluated the cellular responses developed in Predominantly Antibody-Deficiency (PAD) individuals after three mRNA-1273 vaccine doses. Our PAD cohort includes 23 SARS-CoV-2-naïve individuals grouped as follows: Unclassified Primary Antibody-Deficiency (unPAD, n=9); Common Variable Immunodeficiency (CVID, n=12); Combined Immunodeficiency (CID, n=1) and Thymoma with Immunodeficiency (TID, n=1). Ten SARS-CoV-2 naïve healthy controls (HCs) were also included, matched by sex and age. PAD and HC groups were immunized at week 0 (w0) and w4. PAD individuals received an additional dose at w24. Cellular responses were evaluated by IFN-γ ELISpot and flow cytometry. UnPAD and CVID groups showed lower levels of Spike-specific IFN-g-producing T-cells compared to HCs, even after the third vaccine boost. However, the frequency of Spike-specific CD4+ T-cells after two vaccine doses (determined by the frequency of activation-induced markers) was similar between PAD patients and HCs. The third vaccine dose did not increase the frequency of these cells in PAD individuals. Anti-Spike CD8+ T-cell responses were comparable between unPAD and HCs. However, we observed reduced levels of these cells in CVID individuals, that still remained lower after the third immunization. CID and TID patients developed cellular responses after two immunizations.

In conclusion, we show that PAD individuals and HCs developed comparable frequency of Spike-specific CD4+ T cells responses after mRNA-1273 vaccination. However, CVID individuals showed reduced levels of CD8+ T-cell responses compared to unPAD and HCs. Despite that, PAD individuals showed reduced levels of IFN-γ-producing T-cells. Interestingly, we reported that the third vaccine dose had little effect on boosting T-cells responses. mRNA-



1273 vaccine-induced immune responses in PAD individuals are heterogeneous, depend on the type and degree of antibody-deficiency, and should be immunomonitored to define a personalized vaccination strategy.



P-044

CHARACTERIZATION OF VACCINE-INDUCED HUMORAL RESPONSES ELICITED AFTER THREE MRNA COVID-19 DOSES IN PATIENTS WITH PREDOMINANTLY ANTIBODY-DEFICIENCIES

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Mass vaccination has reduced incidence and severity of COVID-19 illness. However, little is known about how three COVID-19 vaccine doses work in patients with predominantly antibody-deficiencies (PAD). In this study, we characterized the humoral responses developed in SARS-CoV-2-uninfected PAD individuals (n=23) after mRNA-1273 vaccination.

We identified that patients with unclassified primary antibody-deficiencies (unPAD, n=9) developed comparable levels of binding antibodies after only two COVID-19 mRNA vaccine doses compared to healthy controls (HCs, n=10). Conversely, common variable immunodeficiency (CVID, n=12) patients showed lower levels of Spike-specific binding antibodies after two doses. After an additional dose, this group was able to reach similar levels to those elicited in HCs. We then characterized the breadth and potency of the neutralizing antibodies developed by our cohort of PAD patients against the original SARS-CoV-2 Wuhan strain, Delta and Omicron variants of concern. Again, while CVID patients elicited significantly lower titers against all three strains than the HCs, unPAD individuals were able to mount similar neutralizing responses against these strains. The third administration boosted these responses only in unPAD individuals. Last, we determined Spike-specific IgG avidity of our PAD cohort. Overall, we observed a gradual increase overtime in PAD and HC groups. Combined immunodeficiency (CID, n=1) and thymoma with immunodeficiency (TID, n=1) patients were not able to mount antigen-specific humoral responses after two immunizations. However, CID individual developed binding and neutralizing antibody titers against Wuhan and Delta after the third vaccine dose.

Thus, mRNA-1273-induced humoral responses in PAD individuals are heterogeneous, depend on the type and degree of antibody-deficiency, and should be immunomonitored to define a personalized vaccination strategy.



P-046

RELACIÓN ENTRE LAS SUBPOBLACIONES DE MEMORIA Y NAÏVE EN LA RESPUESTA A LA VACUNACIÓN FRENTE AL SARS-COV-2 EN PACIENTES CON INSUFICIENCIA RENAL CRÓNICA

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Los pacientes con Insuficiencia Renal Crónica (IRC) se caracterizan por un proceso de inmunosenescencia que incluye una disminución en sus valores absolutos de linfocitos T naïve, lo cual puede suponer una respuesta reducida o defectuosa frente a nuevas vacunas o patógenos. En este trabajo estudiamos la capacidad de respuesta inmune a la vacunación frente al SARS-CoV-2, así como la posible relación entre dicha respuesta y los niveles de linfocitos T $CD4^+$ y $CD8^+$ de memoria y naïve en pacientes con IRC.

Se reclutaron 40 pacientes con IRC y 40 controles sanos con la misma media de edad (68 años). La proporción de las subpoblaciones de linfocitos T $CD4^+$ y $CD8^+$ se determinó mediante marcajes superficiales de sangre periférica evaluados por citometría de flujo. La respuesta de memoria generada por los linfocitos T frente a la vacuna del SARS-CoV-2 fue determinada mediante ELISpots específicos para IFN-γ. Para ello, se aislaron células mononucleares de sangre periférica y se cultivaron en presencia de un pool de péptidos de las proteínas S1 y S2. Encontramos una menor respuesta frente a la vacuna en los pacientes con IR (mediana=60 células x 10^6 PBMCs, RI: 1528 células x 10^6 PBMCs) que en el grupo control (mediana=280 células x 10^6 PBMCs, RI: 1656 células x 10^6 PBMCs) ($p=0,001$). Además, hallamos una correlación negativa entre la respuesta celular frente a la vacuna y los linfocitos $CD4^+CD28^{null}$ y $CD8^+CD28^{null}$ ($p<0,05$). Finalmente, tanto los linfocitos T $CD4^+$ naïve, incluyendo los $CD4^+CD31^+$, como los $CD8^+$ naïve se correlacionaron positivamente con la respuesta a la vacuna frente al SARS-CoV-2 ($p<0,05$). Estos resultados sugieren que la proporción de linfocitos T naïve es clave para desarrollar una buena respuesta frente a nuevas vacunas y que un alto grado de diferenciación de los linfocitos T puede influir en una peor respuesta en pacientes con IRC.



P-047

VACUNACIÓN FRENTE A SARS-COV-2 EN PACIENTES CON INMUNODEFICIENCIA VARIABLE COMÚN: INMUNOGENICIDAD Y REPERCUSIÓN CLÍNICA

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Objetivo

La inmunodeficiencia variable común (IDVC) produce hipogammaglobulinemia y/o un defecto a la hora de producir anticuerpos frente antígenos T dependientes e independientes. Buscamos estudiar en una cohorte de pacientes con IDVC de forma prospectiva la inmunogenicidad producida por las vacunas anti-SARS-CoV-2 y su relación con la protección frente a COVID-19.

Métodos

Mediante técnica ELISA y Fluorospot, se determinó la producción de anticuerpos anti-S1 y la respuesta de células T IFN-γ específicas de S1 respectivamente de una cohorte de 18 pacientes de IDVC. Se compararon con los datos de 50 controles sanos. Se realizó un seguimiento durante un año en el que se registraron las infecciones por COVID-19 confirmadas por PCR y el curso de la enfermedad.

Resultados

Observamos que la presencia de respuesta humoral y celular fue más lenta en pacientes con IDVC en comparación con los controles. Tras la segunda dosis, los pacientes mejoraron su tasa de respuesta vacunal, tanto a nivel humoral como celular (83% ambas), pero seguían por debajo de los niveles de la población sana (100% y 98% respectivamente), así como en intensidad.

Con respecto al seguimiento clínico, 7 padecieron COVID-19 tras la vacunación. De esos, 6 pacientes presentaron un curso clínico leve y 1 grave.

De los 6 pacientes que presentaron un curso leve de la enfermedad (no requirieron hospitalización), 5 presentaban respuesta humoral y celular y 1 presentaba exclusivamente respuesta humoral.

La paciente con COVID-19 grave requirió de hospitalización durante 22 días, aporte oxigénico y corticoterapia. No había respondido ni a nivel humoral ni celular.

Conclusión

Una respuesta adecuada a la vacunación contra SARS-CoV-2 se asoció a menor riesgo de padecer COVID-19 grave comparado con quien no ha respondido a la vacunación. Se requiere más investigación para comprender si la respuesta a la vacunación en pacientes con IDCV es



suficientemente protectora.



P-048

THE ROLE OF THE DIFFERENT CD3Γ DOMAINS IN TCR EXPRESSION AND SIGNALING

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The CD3 subunits of the T-cell antigen receptor (TCR) play a central role in regulation of surface TCR expression levels. Humans who lack CD3g (g⁻) show reduced surface TCR expression levels and abolished phorbol ester (PMA)-induced TCR down-regulation. The response to PMA is mediated by a di-leucine motif in the intracellular (IC) domain of CD3γ. However, the molecular cause of the reduced TCR surface expression in g⁻ lymphocytes is still not known. Our aim was to understand the roles of CD3 chains domains in TCR surface expression and function.

Retroviral vectors carrying wild type CD3γ or CD3d or the following chimeras (EC-extracellular, TM-transmembrane and IC): $d_{EC}g_{TM}g_{IC}$ (dgg for short), ggd, gdd and gg- were expressed in g⁻ primary or Jurkat (JGN) T cells. Basal surface TCR expression, PMA-induced TCR down-regulation and TCR-dependent functional events (induction of CD69, IL-2 or TNFα) were measured by flow cytometry.

Expression of ggg, ggd, gdd or gg- in JGN demonstrated that cell surface TCR levels were dependent on the EC domain of CD3g, which could not be replaced by that of CD3d. In JGN and primary g⁻ T cells, the tested chimeras confirmed that the response to PMA maps to the IC domain of CD3g. In JGN cells all chimeric TCRs were signalling competent. However, an IC domain at CD3g was required for TCR-dependent CD69, IL-2 or TNF-α induction.

Our data confirm that TCR surface exportation depends on the CD3g EC domain, while PMA-induced TCR down-modulation and TCR-dependent CD69, IL-2 and TNF-α induction depends on the CD3g IC domain.



P-049

FACTORES ASOCIADOS A LA DISMINUCIÓN DE LINFOCITOS T NAÏVE EN PACIENTES CON INSUFICIENCIA CARIDACA CRÓNICA

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Los pacientes con Insuficiencia Cardíaca Crónica (ICC) presentan niveles disminuidos de linfocitos T naïve (N), además de niveles incrementados de memoria (M) y un elevado estatus inflamatorio asociado a la infección crónica por CMV. Analizamos el posible efecto de la inflamación sistémica sobre los niveles de linfocitos T N y su capacidad intrínseca de proliferación homeostática en respuesta a la IL-7.

Se reclutaron 40 pacientes con ICC (edad media=55 años). Los niveles de citocinas proinflamatorias séricas IL-1β, IL-6, TNF, IFN, IL-12p70 e IL-17A se determinaron mediante tecnología xMAP de Luminex y los niveles de anticuerpos anti-CMV se cuantificaron por ELISA. Las subpoblaciones de linfocitos $CD4^+$ y $CD8^+$ se evaluaron mediante citometría de flujo en sangre periférica y células mononucleares aisladas de sangre periférica se cultivaron en presencia de IL-7 para cuantificar la respuesta proliferativa (Ki67).

La capacidad proliferativa en respuesta a la IL-7 se encontró conservada en los linfocitos T N de los pacientes con ICC. La proliferación fue significativamente más elevada en los linfocitos $CD4^+$M que en los $CD4^+$N ($p=0,03$). Sin embargo, los linfocitos $CD8^+$N proliferaron más que los $CD8^+$M en presencia de IL-7 ($p=0,04$).

En cuanto a las citocinas, se encontró correlación negativa entre los valores absolutos de los linfocitos T $CD4^+$N y $CD8^+$N y los niveles séricos de las citocinas IL-1β, IL-6, TNF e IL-17A ($p<0,05$). Sin embargo, al realizar un análisis de regresión lineal solo se encontró correlación entre la IL-1 β y los linfocitos $CD4^+$N ($p=0,01$). Finalmente, los valores absolutos de $CD8^+$N correlacionaron negativamente con el título de anticuerpos anti-CMV ($p<0,05$). Nuestros resultados sugieren que, aunque la capacidad proliferativa de los linfocitos T N está conservada, el estatus inflamatorio de los pacientes con ICC así como su historia de infección por CMV condicionan los niveles de estas subpoblaciones.



P-050

NEW CRITERIA FOR A MORE ACCURATE DIAGNOSIS OF LATE-ONSET COMBINED IMMUNODEFICIENCY BASED ON AGE-REFERENCE NAÏVE CD4+ T-CELLS VALUES

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Background and aims

Several reports demonstrate that a subset of common variable immunodeficiency (CVID) patients actually suffers from a more severe disease, called late-onset combined



immunodeficiency (LOCID). Naïve $CD4^+$ T-cell counts have been proposed as the most powerful parameter for LOCID diagnosis. However, previously proposed classifications did not include age-related reference ranges for absolute counts.

Methods

97 CVID patients from eleven hospitals were studied by flow cytometry. Absolute naïve $CD4^+$ T-cell counts were normalized against the lower limit of normality (LLN) obtained from 262 healthy donors (4-9y:359; 10-17y:249; 18-39y:127; 40-59y:112 and >60y:31 cells/µL). This new criterion was compared to the DEFI (<20 naïve $CD4^+$T-cells/µL) and Freiburg (<10% naïve $CD4^+$ T-cells) classifications.

Results

A higher frequency of CVID patients was identified as LOCID with the new criteria (30%), compared to the DEFI (19%) and Freiburg (24%) classifications ($p<0.001$). The age-based criteria was particularly efficient to identify LOCID patients, among the 18-39y CVID patients (33% *vs.* 15% *vs.* 24%; $p<0.01$) and the 40-59y age-group (43% *vs.* 26% and 29%; $p<0.001$). In addition, the age-reference criteria discriminated a higher percentage of patients presenting with non-infectious complications typically associated with LOCID (autoimmune cytopenias, enteropathy and granulomas), compared to patients with normal naïve $CD4^+$ T-cell counts (83% *vs.* 46% of patients; $p=0.001$), as compared to the DEFI (78% *vs.* 52%; $p=0.06$) and Freiburg (78% *vs.* 50%; $p=0.03$) classifications. Furthermore, the presence of reduced naïve $CD4^+$ T-cell counts was also associated with higher rates of other complications, including organomegalies (83% *vs.* 47%, $p=0.001$; 83% *vs.* 52%, $p=0.02$; 87% *vs.* 49%, $p=0.001$) and lymphadenopathies (57% *vs.* 23%, $p=0.002$; 59% *vs.* 27%, $p=0.02$; 64% *vs.* 24%, $p=0.001$).

Conclusions

Age-matched reference values for the identification of decreased naïve $CD4^+$ T-cell counts in CVID patients, results in a more accurate diagnosis of LOCID and identifies patients with severe clinical phenotypes.



P-051

IMMUNE CELL PROFILE ANALYSIS IN FIVE PATIENTS WITH GOOD'S SYNDROME

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Background and Aims

Classically, Good's syndrome (GS) was defined as a rare association of thymoma and hypogammaglobulinemia, suggesting that it might be a subset of Common Variable Immunodeficiency. However, the knowledge of the disease is hampered by the incomplete and inconsistent reports about the disease due to low prevalence of the disease (1.5 cases per million). A more detailed dissection of the immune cells in a significant set of patients would contribute to understand the pathophysiology of the disease.

Methods

Up to 350 immune subpopulations were analyzed in five GS patients (53-72 years) from 3 different hospitals and 39 age-matched controls using next-generation flow cytometry.

Results

All patients consistently presented with lack of B-cells (<0.02 cells/μL). In addition, alterations



were also found in other immune subsets including significantly reduced count of total $CD4^+$ T-cells, NK-cells, eosinophils, $CD141^+$ and plasmacytoid dendritic cells (DCs) ($p \leq 0.05$), as compared to age-matched controls. Decreased of total $CD4^+$ T-cell counts was due to reduced numbers of naïve cells and, in addition, lower Treg, TFH, Th2, Th17, Th22, Th1/Th17 and Th1/Th2 cells ($p \leq 0.01$). However, number of Th1 cells was not different from age-matched controls ($p > 0.05$). Interestingly, counts of naïve $CD8^+$ and $TCRgd^+$ T-cells were normal, meanwhile total $CD8^+$ and $TCRgd^+$ T-cells were statistically significant increased ($p \leq 0.01$) due to statistically significant expanded effector memory $CD8^+$ T-cells and terminally differentiated $CD8^+$ and $TCRgd^+$ T-cells ($p \leq 0.01$). All the other immune subsets analyzed were within the normal range.

Conclusions

In addition to the lack of B-cells, a complex profile of alterations was observed in GS patients including but an also large spectrum of defects in the adaptative ($CD4^+$ T-cells) and innate cells (NK-cells, eosinophils, DCs). In contrast, the cytotoxic response was observed, including normal counts of $CD8^+$ and $TCRgd^+$ T-cells, and Th1 $CD4^+$ T-cells.



P-052

CD4$^+$ T-CELL COMPARTMENT ALTERATIONS ARE ASSOCIATED WITH CLINICAL COMPLICATIONS IN LOCID AND CVID PATIENTS

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Background and aims

Alterations in the CD4$^+$ T-cell compartment in common variable immunodeficiency (CIVD) patients have been reported earlier. However, most studies did not report on patients with a naïve CD4$^+$ T-cell defect who met the criteria for late-onset combined immunodeficiency (LOCID).



Methods

CD4$^+$T-cell subsets were analyzed in 48 CVID and 19 LOCID patients, in parallel to 156 healthy donors (4-88 years) using in depth EuroFlow-based flow cytometry methods.

Results

Higher frequencies of decreased T-cell subset counts were observed in LOCID *vs.* CVID patients, including Treg (84% *vs.* 60% of patients; p=0.09), Th2 (100% *vs.* 52%; p<0.001), Th17 (95% *vs.* 48%, p<0.001), and Th1/Th2 (63% *vs.* 33%; p=0.03), as compared to age-reference values. However, in both LOCID and CVID, decreased TFH (11% and 2%), Th1 (21% and 21%) and Th1/Th17 (16% and 15%) (p>0.05) counts were found in only a few patients. Multivariate analysis based on all age-matched CD4$^+$ T-cell compartments, showed two subgroups of LOCID cases presenting with clearly distinct clinical phenotypes: those with higher Th1 counts presented with a greater frequency of autoimmune cytopenias (89% *vs.* 19%; p=0.003) and interstitial lung disease (78% *vs.* 9%; p=0.003), together with lower percentage of non-respiratory infections (57% *vs.* 100%; p=0.03). Three subgroups of patients (CVID1, CVID2, and CVID3) were identified within those CVID cases without a T-cell production defect, based on Th1 and TFH cells, and a significantly higher frequency of autoimmune cytopenia (63% *vs.* 9% *vs.* 22%; p≤0.05) was observed in CVID cases with higher Th1 counts.

Conclusions

LOCID patients show more severe T-cell defects (Treg, Th2, Th17 and Th1/Th2) than CVID patients. Increased counts of Th1 CD4$^+$T-cells in blood, is strongly associated with the presence of autoimmune cytopenias in both LOCID and CVID cases, together with interstitial lung disease in LOCID (but not CVID) patients.



P-053

DEMOGRAFÍA DE LA INMUNODEFICIENCIA COMÚN VARIABLE EN LA REGIÓN DE MURCIA.

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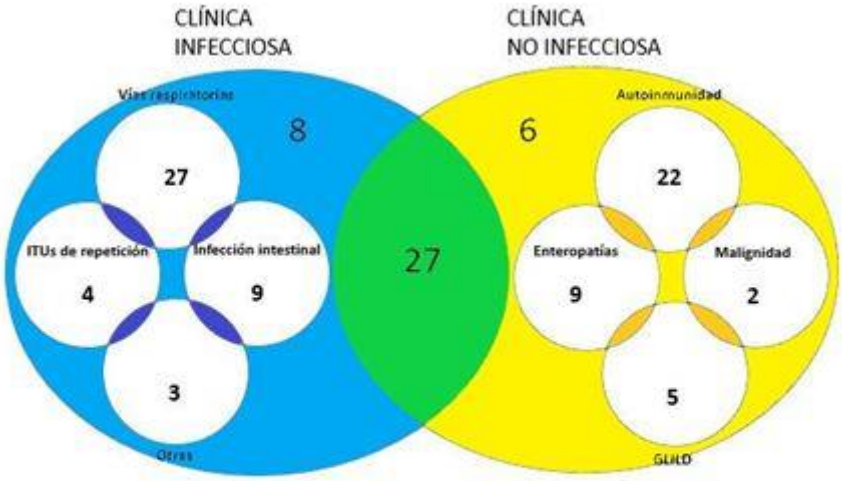
Hospital Clínico universitario Virgen de La Arrixaca IMIB, Murcia.

La IDCV (Inmunodeficiencia Variable Común) incluye un grupo heterogéneo de pacientes mayores de 4 años, con manifestaciones clínicas variables desde infecciones graves y recurrentes por patógenos comunes y oportunistas con compromiso de los tractos respiratorio y gastrointestinal, en cuyo contexto se establece el diagnóstico de hipogammaglobulinemia, deficiente producción de anticuerpos, y recuentos variables precursores B en sangre periférica. La variabilidad clínica e inmunológica de la enfermedad es tal, que pueden solaparse enfermedades inmunomediadas, fenómenos de linfoproliferación, desregulación inmune, complicaciones infecciosas y algunas neoplasias.

La prevalencia estimada es de 1 por cada 25.000 habitantes, afectando por igual ambos sexos. La región de Murcia con una población total de 1.518.486 habitantes (INE 2021), tiene actualmente 41 pacientes con IDCV entre los 9 a los 71 años (edad al diagnóstico), derivados de las 9 áreas de salud de la Región a la Consulta de Inmunología del Hospital Virgen de la Arrixaca y sometidos a cribado inmunológico mediante los criterios ESID, seguimiento, diagnóstico y manejo multidisciplinar.

La manifestación clínica más prevalente al debút han sido las infecciones respiratorias, bronquiectasias (*H. influenzae y S. Neumoniae)*, y la diarrea recurrente (*C. jejuni, C. difficile, Giardia lambdia*) edad de consulta 20 a 45 años.

Se detectaron 2 pacientes con autoinmunidad al debut, y progresión a linfomas, portadores de la Variante p.Ile87Asn en el gen TNFRSF13B. Dos hermanos adultos al debut con PTI (Púrpura Trombocitopénica idiopática), portadores p.Met860Asnfs*5 heterocigosis en NFKB1.





Variante génica según área de salud

Deleción de los últimos tres exones codificantes de TTC37 (variante monoalélica) (41-43) con fenotipo de THES-1	1
Variante p.Ile87Asn en el gen TNFRSF13B (que codifica la proteína TACI), variante de significado clínico incierto en el gen AICDA.	2
Variante patogénica NM_003998.3 c.2578dup; p.Met860Asnfs*5, en heterocigosis, en el gen NFKB1. inmunodeficiencia variable común tipo 12 de herencia autosómica dominante	3
Variante patogénica NM_003998.3 c.2578dup; p.Met860Asnfs*5, en heterocigosis, en el gen NFKB1. inmunodeficiencia variable común tipo 12 de herencia autosómica dominante	3
Variante intrónica en el gen IKZF1 de significado clínico incierto (NGS) asociada a inmunodeficiencia común variable (IDCV) tipo 13.	4
Variante p.Cys104Arg en gen TNFRSF13B (TACI) asociada a inmunodeficiencia común variable tipo 2.	6
Variante rara en el gen PIK3CD, NM_005026.3:c.2426+16C>A, en heterocigosis probablemente NO asociada a enfermedad.	6
Variante en heterocigosis c.454C>T (p.Arg152Trp) en el gen STAT3 (OMIM *102582) clasificada como variante patogénica	6



P-054

EVOLUTIONARY DYNAMICS OF SOMATIC PATHOGENIC VARIANTS IN ALPS: IMPLICATIONS FOR DIAGNOSTICS.

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

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

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

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



P-055

DESCRIPCIÓN DE DOS NUEVAS MUTACIONES EN EL GEN WAS

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Las alteraciones en el gen *WAS* dan lugar a 3 entidades clínicas bien diferenciadas: el síndrome de Wiskott-Aldrich (WAS), la trombocitopenia ligada al X (XLT) y la neutropenia ligada al X (XLN). El WAS está causado por mutaciones que implican una pérdida completa de la función de la proteína WASp; la XLT por alteraciones que dan una pérdida parcial de su funcionalidad; y la XLN por mutaciones que implican una ganancia de función de WASp.

El primer caso que presentamos es un niño hispanoamericano de 2 años que desde el nacimiento presentó la tríada clásica de WAS: trombocitopenia microtrombocítica, eczema e infecciones recurrentes. La secuenciación de *WAS* reveló una deleción en el exón 2 (c.176del) que provocaba un desplazamiento en la pauta de lectura originando un codón *Stop* 17 aminoácidos después (p.Pro59Leufs*17). Se trata de una mutación no descrita que implica seguro una pérdida de función ya que la proteína se reduce a tan solo 57 aminoácidos.

El segundo caso es un niño caucásico de 11 años que presentó neutropenia desde el nacimiento, inicialmente orientada como aloinmune. Tras descartar otros defectos centrales de neutropenia, se estableció la sospecha diagnóstica de XLN. El estudio del exón 9 del gen *WAS* reveló una mutación *missense* (c.895G>A, p.G299R) no descrita previamente, pero en una posición muy próxima a las ya asociadas a XLN. Los estudios de viabilidad, migración, fagocitosis y capacidad oxidativa en los neutrófilos del paciente realizados hasta la fecha parecen indicar que se trata de una mutación que implica una ganancia de función de WASp. La madre es portadora de la mutación y presenta un fenotipo parcial de XLN.

Como conclusión, hemos identificado dos nuevas mutaciones del gen *WAS*: una compatible con WAS y otra en la región asociada a XLN que parece dar lugar a una ganancia de función



P-056

A CHARACTERIZATION OF CHRONIC CAMPYLOBACTER INFECTION IN COMMON VARIABLE IMMUNODEFICIENCY PATIENTS.

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Background

Campylobacter infection usually causes a self-limited clinical illness lasting 5-7 days, resolving without antimicrobial treatment in immunocompetent subjects. However, an inadequate immune response can lead to a prolonged and severe disease requiring antibiotics and more aggressive therapeutic approaches.

Methods

A retrospective cohort of 17 common variable immunodeficiency (CVID) patients with Campylobacter infection and 109 CVID controls attending the Immunology clinic at the Royal Free Hospital was assessed. Immunological, clinical and microbiological parameters were measured. Patients were treated according to a novel algorithm for Campylobacter in antibody deficient patients.

Results

Immunological parameters: Flow cytometry evaluation of peripheral B lymphocytes demonstrated a lower long-term average CD19+ count with a higher proportion of $CD21^{LOW}CD38^{LOW}$ B cells in Campylobacter patients versus controls. Furthermore, a reduction in CD4+ T cell and NK cell counts over time was observed in Campylobacter patients.

Treatment response: Patients were treated according to our novel algorithm. Clinical improvement and bacterial clearance were obtained after a median of 21 and 169 days for acute Campylobacter (ACP) and chronic Campylobacter (CCP) infections respectively. Ten received first-line of treatment [Azithromycin or Chloramphenicol], four second-line [Neomycin], and three received third-line [Tigecycline, Chloramphenicol and Ertapenem (one received Gentamicin due to resistance to carbapenems)].

Conclusions

Our study highlights immunological and clinical characteristics of recurrent Campylobacter

infections in patients with CVID. Our treatment algorithm was successful and should be evaluated in a larger cohort.

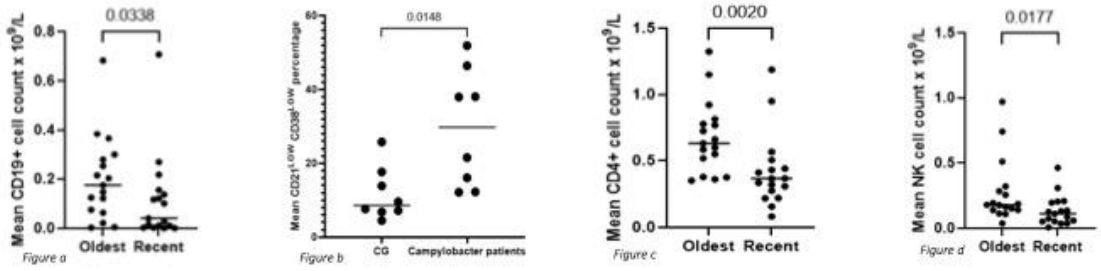


Figure 1. Oldest and most recent absolute CD19+ B cell lymphocyte counts (Figure a). Values for the long term average $CD21^{+LOW}CD38^{+LOW}$ B cell percentage (from 2000 [or date of CVID diagnosis, if later] to 2022). Control group, CG (Figure b). Values for most recent and the oldest available for CD4+ T cell counts (Figure c), and Natural killer (NK) cell counts (Figure d). Lines represent medians. p-values were obtained using Mann-Whitney tests



P-057

EVALUACIÓN DE LA INMUNIZACIÓN CON TYPHIM VI EN EL DIAGNÓSTICO DE INMUNODEFICIENCIA SECUNDARIA EN SÍNDROMES LINFOPROLIFERATIVOS B

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Los síndromes linfoproliferativos B (SLPB) se asocian a una inmunodeficiencia secundaria (SID) caracterizada por hipogammaglobulinemia o déficit de producción de anticuerpos e infecciones graves y recurrentes, siendo la causa más importante de morbilidad en estos pacientes. El objetivo principal del estudio fue determinar la utilidad de la respuesta frente al antígeno polisacárido puro de *Salmonella Typhi* (Thyphim Vi) en el diagnóstico y tratamiento de SID, determinando los rangos de respuesta normal en pacientes con SLPB.

Se estudió un total de 84 pacientes con SLPB, de los cuales 34 con infecciones recurrentes o graves, 20 sin infecciones recurrentes o graves y 30 controles sanos. En función del SLPB se han reclutado 16 pacientes con leucemia linfocítica crónica (LLC), 37 pacientes con linfomas no Hodgkin (LNH) y 11 pacientes con gammapatía monoclonal de significado incierto (GMSI), en los cuales se compararon los niveles de anticuerpos prevacunales y postvacunales.

Hubo niveles significativamente menores de respuesta a Typhim Vi en los pacientes con LLC, LNH, MGUS respecto a sanos ($p<0.001$, $p<0,001$ y $p=0,005$, respectivamente). En la respuesta frente a neumococo, los pacientes con LLC y LNH fueron significativamente menores que los controles sanos ($p=0,003$ y $p=0,001$, respectivamente), sin diferencias entre el grupo GMSI y sanos. La respuesta a la vacunación con Typhim Vi permitió la identificación de crecimiento tumoral en pacientes en etapas pre-malignas, como lo es la GMSI.

La vacunación con polisacárido puro Typhim Vi permite diferenciar individuos sanos de pacientes con SLPB, además de presentar la capacidad de discriminar pacientes con GMSI con infecciones recurrentes o graves, por lo que podría utilizarse como test funcional predictivo de infecciones, además del crecimiento tumoral y progresión de la etapa pre-maligna. El uso



combinado de las tres vacunas (Typhim Vi, neumococo y toxoide tetánico) puede aportar un mayor conocimiento de la situación inmunológica del paciente.



P-058

DESCRIPCIÓN DE 3 NUEVAS VARIANTES MONOGÉNICAS EN EL GEN IRF2BP2 EN COHORTE DE PACIENTES CON INMUNODEFICIENCIA COMÚN VARIABLE

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La inmunodeficiencia común variable (IDCV) es la inmunodeficiencia primaria que con más frecuencia encontramos en la práctica clínica. Clínicamente se define como la presencia de infecciones recurrentes y una reducción en suero de IgG (al menos 2 desviaciones estándar por debajo de los valores de referencia para la edad) y al menos otra de las inmunoglobulinas (IgA o IgM) y una reducción o ausencia de producción de anticuerpos.
La clínica que presentan estos pacientes es variada, incluyendo infecciones pulmonares, bacterianas sistémicas y complicaciones gastrointestinales.
Aproximadamente el 5% de los casos son familiares, y la mayor disponibilidad de tecnología de secuenciación masiva ha permitido la identificación de un número creciente de genes asociados a IDCV.
Uno de estos genes es IRF2BP2, cuya variante en heterocigosis (herencia autosómica dominante) estaba presente en 3 miembros de una familia.
A través de estudios de secuenciación masiva, identificamos 3 nuevas variantes genómicas en heterocigosis del gen de IRF2BP2, no descritas previamente, en pacientes con clínica compatible de IDCV. Las variantes son:

- p.Cys37Phe: Mujer de 57 años de edad con infecciones respiratorias de repetición y síndrome bronquiectásico.
- p.Pro25Leu: Mujer de 55 años con bronquitis a repetición.
- p.E253Cfs*8: Mujer de 46 años con clínica de infecciones recurrentes ORL y respiratorias, candidiasis esofágica/vaginal, enfermedad de Crohn y artritis seronegativa.

A falta de los estudios funcionales que valoren la patogenicidad y estudios familiares para analizar su penetrancia, hemos encontramos nuevas variantes heterocigóticas de IRF2BP2, lo que nos lleva a concluir que mutaciones monoalélicas en IRF2BP2 pueden causar IDCV monogénica con diferentes manifestaciones clínicas e inicio de síntomas, apoyando lo que se ha descrito recientemente en la literatura.



P-059

EFECTOS DE LAS GAMMAGLOBULINAS POLICLONALES SOBRE LAS POBLACIONES MIELOIDES CIRCULANTES. ESTUDIO BIRTH

Peixoto Rodríguez, Alejandro; Bolaños, Estefanía; Ramiro Ponce, Vanessa; Mayol, Elsa; Bailey Maldonado, Rodrigo; Ochoa-Gruñón, Juliana; Jimenez García, Carlos; Rodríguez De La Peña, Antonia; De La Fuente, Eduardo; Villegas, Ángela; Mansilla, Mariló; Perez Lopez, Cristina; Peña Cortijo, Ascensión; Mateo Morales, Marta; Anguita Mandley, Eduardo; Fernández-Arquero, Miguel; Benavente Cuesta, Celina; Sánchez-Ramón, Silvia.

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Los pacientes afectos de síndromes linfoproliferativos B (SLPB), en concreto, mieloma múltiple (MM) y leucemia linfocítica crónica (LLC), presentan habitualmente un déficit de producción de anticuerpos y/o hipogammaglobulinemia que les hace susceptibles a infecciones recurrentes o graves, estando indicada la terapia de remplazo de inmunoglobulinas TRIg. Hasta la fecha no existen estudios sobre el uso de TRIg sobre la evolución del SLPB ni biomarcadores de seguimiento de la inmunodeficiencia secundaria (SID).

Se ha demostrado que la TRIg induce una reprogramación de los macrófagos hacia un estado más pro-inflamatorio, lo que podría frenar procesos infecciosos recurrentes. Se ha estudiado un total de 16 pacientes (4 pacientes con LLC, 11 con linfoma no Hodking (LNH) y 1 paciente con gammapatia monoclonal de significado incierto (MGUS). El desarrollo experimental constará de cuatro procesos analíticos por cada paciente, en primer lugar al inicio del TRig, posteriormente a los siete días, a los seis meses y por último, transcurrido un año.

En 16 pacientes estudiados, se observó un aumento significativo de la población de M-MDSCs tras la infusión de IgIV a las 6 horas (13,37±13,17 vs 26,62±16.52; p= 0,008). Además, en 3 de ellos se mantuvo este aumento a los siete días. También se ha observado una disminución significativa de los monocitos intermedios (13,60±10,63 vs 8,07±14,08; p=0,008 y no clásicos (4,53±2.46 vs 1,79±2.72; p=0.001). Asimismo, se aprecia que este tratamiento produce un cambio de fenotipo hacia M2 mediante la disminución de citoquinas antinflamatorias (CXCL10 e IL-10) y aumento de las proinflamatorias (IL-12 y TNF-alfa).

Conclusiones: Nuestros datos preliminares sugieren que la infusión de IVIg induce un efecto proinflamatorio mediante la reprogramación de macrófagos hacia el fenotipo M2 permitiendo una reducción de las infecciones y de la progresión tumoral. Pero también tienen efecto antiinflamatorio mediante la expansión de MDSCs circulantes y la disminución de monocitos proinflamatorios.



P-060

NEW STAT3 GAIN-OF-FUNCTION MUTATION (P.MET162ARG) IN A PAEDIATRIC PATIENT

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Gain of function mutations in STAT3 (signal transducer and activator of transcription 3) gene cause “infantile-onset multisystem autoimmune disease-1”. Insulin dependent diabetes mellitus, autoimmune enteropathy or coeliac diseases are the most common manifestations together with hematologic autoimmune alterations. Patients usually are of short stature and present non-specific dermatitis.

We present a 2 years old boy admitted at 18 days of age with respiratory syncytial virus bronchiolitis that required PICU admission. At 6 weeks of age he presented severe Salmonella and Rotavirus acute gastroenteritis (AGE) accompanied by dehydration again requiring admission to Paediatric Intensive Care Unit (PICU). Episodes of AGE caused several hospital admissions in the first months of live. He presented failure to thrive and atopic eczema well controlled with topical treatment in the second year of live. Laboratory findings included normal percentage and absolute values of lymphocyte subpopulations CD3, CD4, CD8, CD19 and NK cells with a slight decrease of switch memory (CD27+ IgD-) B cells and a decrease of serum IgG (440 mg/dl). He had a normal response to *Streptococcus pneumoniae, Haemophilus influenzae*, tetanus and hepatitis B virus vaccination. The genetic study identified a non-previously described heterozigous “de novo” variant in STAT3 gene (p.Met162Arg;c.485T>G). “In vitro” functional characterization by STAT3 luciferase studies revealed an increase in STAT3 activity in cells transfected of M162R mutant STAT3 plasmid.

Due to the heterogeneous manifestation of the disease and the progressive onset of symptoms, genetic study is essential for the early diagnosis and follows up of “infantile-onset multisystem autoimmune disease-1”

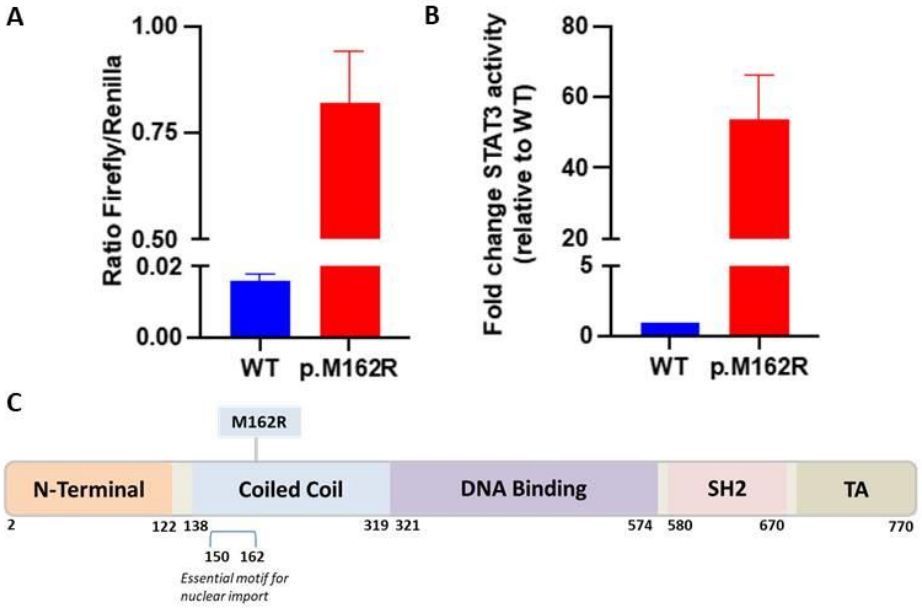


FIGURE. STAT3 M162R mutation confers a GOF. A-B) STAT3 binding activity measured by luciferase assay. A4 cells were transfected with WT or STAT3 M162R mutant and STAT3-driven firefly luciferase and control renilla reporters. A) Luciferase was assayed at 48 hours and STAT3 activity is shown as a ratio of firefly/control. M162R has significantly increased activity compared with WT. B) STAT3 activity STAT3 activity following 12-hour activation with IFNα. Results shown represent fold-change vs WT. C) Schematic STAT3 human protein showing the location of STAT3 M162R mutation. The numbers represent the amino acid location. WT: Wild type. TA, transactivation domain.



P-061

DIAGNÓSTICO NEONATAL EN SCID DEBIDO A CD3 DELTA EN POBLACIÓN AUTÓCTONA DE MANABÍ (ECUADOR): PUESTA A PUNTO Y PROPUESTA.

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Debido a su sintomatología poco específica, las Inmunodeficiencias Severas Combinadas (SCID) constituyen un grupo de enfermedades subdiagnosticadas. Diferentes estudios moleculares van ampliando el conocimiento y la capacidad de detección de las mismas, incluyendo el estudio perinatal de la prueba del talón. Una de ellas es el SCID asociado a CD3-Delta (CD3D). Esta enfermedad ha sido asociada a 3 variantes alélicas, todas con herencia autosómica recesiva, diagnosticándose un total de 11 casos: 7 relacionados con la mutación R68X, 1 con la mutación C93X y 4 relacionados con IVS2DSG-A+5. En los dos primeros tipos de mutación todos los casos refieren consanguinidad, mientras que la última variante alélica se ha descrito en pacientes no consanguíneos de etnia precolombina, procedentes de una única región, Manabí. Con el objetivo de determinar la frecuencia de la mutación IVS2DSG-A+5, hemos llevado a cabo un muestreo sobre 396 individuos sanos originarios de esta zona de la costa ecuatoriana. A partir de sangre periférica recolectada en papeles FTA se realizó la extracción de ADN total que fue sometido a un ensayo de genotipificación mediante PCR en tiempo real utilizando sondas TaqMan® capaces de detectar el SNP asociado a esta mutación (rs730880296). Para validar esta metodología, 50 de las muestras fueron también analizadas mediante RFLP y secuenciación. Como controles se utilizaron 1000 muestras de individuos sanos de otras regiones de Ecuador, incluyendo diferentes orígenes étnicos, familiares portadores de pacientes con SCID debido a CD3-Delta y los mismos pacientes. Los resultados del ensayo de genotipificación mostraron un total de 18 individuos heterocigotos (porcentaje de portadores = 4,55 %), obteniendo una frecuencia del alelo mutado de 2,27.

La elevada frecuencia de dicha mutación en la región de Manabí indica la necesidad de un diagnóstico poblacional específico basado en la detección de esta variante mediante la prueba del talón.



P-062

INSUFICIENCIA DE SUBCLASE IGG2 E INFECCIÓN BACTERIANA. UNA NUEVA MANERA DE IDENTIFICAR EL RIESGO DE INFECCIÓN.

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El rol de la subclase IgG2 para el control de infecciones bacterianas es bien reconocido. Se utiliza el punto de corte establecido por el laboratorio como nivel definitorio de deficiencia de IgG2. La hipótesis de este estudio es que los pacientes que desarrollan infecciones bacterianas tienen menores concentraciones séricas de IgG2 siendo posible identificar un punto de corte de asociación que entra dentro del rango considerado como normal que podría definirse como insuficiencia.

En un estudio retrospectivo se incluyeron 223 pacientes con infecciones bacterianas y 117 sin antecedente de infección bacteriana, con distintas patologías de base inmunológica (controles). Se define infección bacteriana a la presencia de 3 o más procesos infecciosos anuales tratados con antibioterapia o al menos un ingreso hospitalario por infección bacteriana. Se recogió información de los siguientes parámetros: IgG, IgA, IgM, subclases IgG, C3, C4, anticuerpos basales anti-polisacárido de neumococo, recuentos absolutos de subpoblaciones linfocitarias. Los niveles de IgG2 se testaron mediante turbidimetría, analizador Optilite®.

En la comparación de medias entre grupos se detectó un nivel significativamente más bajo de IgG total (999.8 vs 1124.9 mg/dL, p=0.016) a expensas de la subclase IgG2 (2.79 vs 3.61 g/L, p<0.001). El nivel basal de anticuerpos anti-polisacárido de neumococo fue significativamente más bajo en los pacientes con infecciones bacterianas (7.28 vs 8.91 mg/dL, p=0.0022). No se detectaron diferencias significativas en los otros parámetros estudiados. Utilizando los niveles normales del reactivo (1,47-6,29g/L) y curvas ROC se definieron los siguientes valores: IgG2 < 1.47 g/L deficiencia; 1.47-3.7 insuficiencia; > 3.71 suficiencia. Los niveles de insuficiencia se asociaron significativamente al riesgo de infección bacteriana (p<0.001).

Concluimos que es posible identificar un nivel de insuficiencia de IgG2 como factor de riesgo de infección bacteriana. Si se validan estos resultados en un estudio multicéntrico, podrían identificar pacientes en riesgo inmunológico.



P-063

SINDROME DE KABUKI: UN DIAGNOSTICO QUE NO DEBES IGNORAR

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Caso 1. Mujer de 51 años con antecedente de panhipogammaglobulinemia, luxación congénita de caderas bilateral, sordera neurosensorial, diarrea crónica por criptosporidium, enfermedad pulmonar intersticial linfocitica granulomatosa compatible (biopsia pulmonar), gastritis crónica atrófica, hiperplasia nodular linfoide ileal, hipertransaminasemia a estudio, pancitopenia a estudio, diabetes mellitus tipo 2. A los 40 años tras estudio inmunológico completo se diagnostica de Inmunodeficiencia Variable Común (IDVC) y se inicia tratamiento sustitutivo con inmunoglobulinas.

Caso 2. Mujer de 51 años con antecedente de hipogamaglobulinemia, retraso psicomotor, glaucoma, hipoacusia conductiva bilateral, hipotiroidismo primario, pancitopenia, arteria subclavia derecha aberrante, enfermedad pulmonar intersticial linfocítica granulomatosa e hipertransaminasemia. Se realizó estudio inmunológico que revelo una deficiencia de producción de anticuerpos y alteraciones compatibles con IDVC.

Ambos casos se caracterizaban por una combinación de manifestaciones clínicas similares y rasgos faciales característicos (Figura 1), por lo que se realizó estudio genético dirigido para descartar síndrome de Kabuki. En el Caso 1 se detecta la variante c.13884dupC en el gen KMT2D en heterocigosis (NGS-MiSeq, Agilent) y en el Caso 2 se identifica la variante probablemente patogénica c.1613del en el gen KMT2D en heterocigosis (Next Seq 500, Agilent).

Nuestros casos muestran que el Sindrome de Kabuki (SK) (OMIM 147920 y 300867) es una forma de IDVC monogénica, potencialmente grave (con elevada frecuencia de complicaciones no infecciosas), que debemos sospechar ante la presencia rasgos faciales característicos (fisuras palpebrales largas con eversión del tercio lateral del párpado inferior, cejas arqueadas y anchas con muescas o escasez en el tercio lateral, columela corta con punta nasal deprimida, orejas grandes, prominentes o ahuecadas y dermatoglifos fetales persistentes) y alteraciones clínicas compatibles.

A



B C

Fig.1 Caso 1. Panel A: perdida de la cola de las cejas y eversion leve de parpados inferiores. Panel B: dermatoglifos fetales. Panel C: columna corta con punta nasal deprimido



P-064

FENOTIPO HIPER IG-E LIKE ASOCIADO A MUTACION HETEROCIGOTA COMPUESTAS DE LA FOSFOGLUCOMUTASA 3 (PGM3) EN GEMELOS MONOCIGÓTICOS

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La deficiencia de fosfoglucomutasa 3 (PGM3) (Inmunodeficiencia 23, OMIM: 615816) es trastorno congénito raro de la glicosilación que puede llevar a los pacientes a un síndrome de hiper-IgE (HIES). La PGM3 es una enzima responsable de la conversión de N-acetil-glucosamina (GlcNAc)-6-fosfato en GlcNAc-1-fosfato, esta conversión es necesaria para la producción de uridina difosfato (UDP)-GlcNAc, que es un sustrato común de múltiples vías de glicosilación y es un precursor esencial de la glicosilación.

Nuestro objetivo es caracterizar el fenotipo clínico, el perfil inmunológico y el resultado genético de unos gemelos monocigóticos de 37 años con deficiencia de PGM3.

Seleccionamos el gemelo con la presentación clínica más grave como caso índice, realizando una secuenciación del exoma completo. La secuenciación Sanger de PGM 3 se realizó en el otro gemelo y en ambos padres. Los pacientes fueron estudiados con un panel de pruebas inmunológicas que incluían la detección de poblaciones TH17, estudio de lectinas por citometría de flujo (L-PHA) y otras pruebas orientadas al HIES.

Ambos gemelos presentaban rasgos faciales dismórficos, micrognatia, enfermedad atópica severa, abscesos cutáneos recurrentes, e infecciones respiratorias severas (virales y bacterianas) y deterioro cognitivo de diferente grado de severidad.

La secuenciación del exoma identificó una variante heterocigota compuesta de PGM3 c.1438_1442del y c.1475C>T. La secuenciación Sanger de PGM3 realizada al otro gemelo, identificó la misma variante genética. Cada uno de los progenitores era portador de una de las variantes confirmando el patrón de herencia.

El análisis de L-PHA de las células mononucleares de la sangre periférica mostró una menor unión de la lectina en comparación con los controles sanos. Ambos pacientes mostraron una mayor expresión de la población Th17.

Nuestros pacientes presentan un síndrome similar al de Job, consideramos que la mutación del gen PGM3 debe ser considerada entre los posibles diagnósticos en individuos con HIES.



P-065

FALTA DE ESTANDARIZACIÓN EN LA CUANTIFICACIÓN DE LA IGG Y DE SUS SUBCLASES

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Hosìtal General Dr. Balmis, Alicante.

La IgG es dominante en el suero y existen cuatro subclases (IgGSc) cuya abundancia, funciones y afinidad varían dependiendo de la edad. Pueden existir desequilibrios particularmente en inmunodeficiencias primarias. Los ensayos comerciales para su cuantifiación utilizan los calibradores ERM-DA470k y Sanquin-M1590. En nuestro laboratorio observamos diferencias en la suma de IgGSc y la IgG.

Se comparó la suma de IgGSc e IgG, así como la IgG y IgGSc cuantificadas usando ambos calibradores.

La IgG y IgGSc se cuantificaron en 185 muestras de suero siguiendo las instrucciones del fabricante:

-IgG (OSAS15) y IgGSc (OQXI09, OQXK09, OPAV03, OPAU03) usando Sanquin-M1590 en el autoanalizador BNII (Siemens).

-IgG (NK009.OPT) y IgGSc (NK006.OPT, NK007.OPT, LK008.OPT, NK004.OPT) usando ERM-DA470k en el autoanalizador Optilite (Binding Site).

Se descartaron los valores atípcos con fines estadísticos. Se determinó la correlación lineal (Test Pearson) y compararon la IgG con la suma de IgGSc para cada calibrador, así como los valores de IgG y IgGSc entre calibradores (T Student de muestras pareadas).

Los valores de IgG fueron similares a la suma de IgGSc con Sanquin-M1590 ($p=0,223$), pero significativamente distintos con ERM-DA470k (IC 95% IgG-suma IgGSc= 20,4 a 43,0; $p<0.001$). Existió correlación lineal entre la IgG y IgGSc entre ambos calibradores ($p<0,001$). Pero la cuantificación de IgG fue diferente entre calibadores (IC 95% IgG Sanquin-M1590 - IgG ERM-DA470k= -88,4 a -15,2; $p=0.006$). Las IgGSc fueron distinas entre calibradores (IgG1: IC 95% Sanquin-M1590 - ERM-DA470k= 80,4 a 113,7; $p<0,001$; IgG2: IC 95% Sanquin-M1590 - ERM-DA470k= -75,4 a -50,8; $p<0,001$; IgG3: IC 95% Sanquin-M1590 - ERM-DA470k= -39,0 a -32,3; $p<0,001$; IgG4: IC 95% Sanquin-M1590 - ERM-DA470k= 7,1 a 12,5; $p<0,001$).

Es necesaria la estandarización de la cuantificación de IgG y IgGSc, pues su cuantificación es diferente entre calibradores. La suma de IgGSc sólo fue similar a la IgG determinadas con el calibrador Sanquin-M1590.



Sesión Poster. Miscelánea. (Inflamación / Alergias y reacciones de hipersensibilidad / Inmunidad anti-tumoral). Pantalla 4

P-066

ANGIOPOIETIN-LIKE PROTEIN 2 LEVELS AS A MARKER OF INFLAMMATION IN PATIENTS WITH HIDRADENITIS SUPPURATIVA

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Introduction

Hidradenitis suppurativa (HS) is a chronic inflammatory disorder characterized by nodular lesions, abscesses and fistulae that affects skin regions. It has been described that high levels of TNFα in skin lesions as well as IL-17 in serum of HS patients correlate with disease severity, together with an inflammatory profile and a predominance of Th1 and Th17 lymphocytes. Angiopoietin-like protein 2 (ANGPTL2) signaling functions in angiogenesis and tissue repair, whereas an excess of ANGPTL2 signaling has been associated with chronic inflammation and subsequent pathological irreversible tissue remodeling.

Since ANGPTL2 is involved in tissue homeostasis by inflammation and regeneration, the aim of this study is to evaluate if ANGPTL2 serum levels could be involved in HS pathogenesis.

Methods

This was a cross-sectional study including 94 patients with HS and 60 controls, recruited from our Dermatology outpatient clinic at the Marqués de Valdecilla University Hospital (Santander, Spain). Patients were all ≥18 years and fulfilled the diagnostic criteria for HS. The control group was recruited among hospital medical staff and subjects with non-inflammatory cutaneous disorders. Determination of ANGPTL2 serum was carried out by ELISA by following manufacturer's instructions

Statistical analysis was performed using Graph Pad Prism software 6.0 version. Comparisons were based in U-Mann-Whitney test

Results

Differences between HS patients and controls ANGPTL2 levels were statistically significant ($p<0.0001$). Medians and interquartile ranges of controls and patients were calculated: 0.37 (0.0925-0.9200); 42.79 (26.99-53.69), respectively (Figure 1).

Conclusion

The precise inflammatory mechanism underlying HS is still not fully elucidated. High serum levels of ANGPTL2 have been associated with inflammatory processes and autoimmune diseases. Here we demonstrated that ANGPTL2 elevation in HS patients could serve as a biomarker that would indicate an alteration of tissue homeostasis. Further and larger studies are needed in order to know the exact role of this protein.

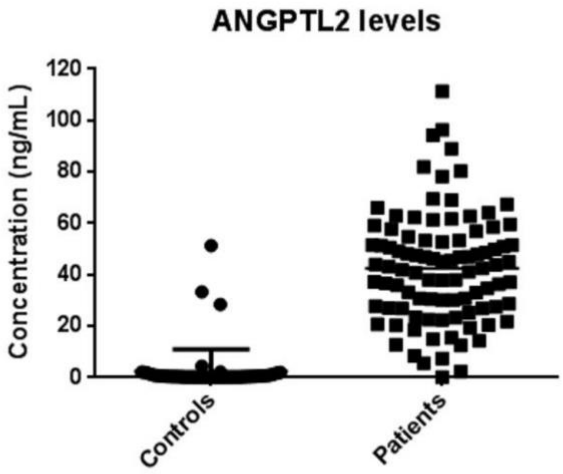


Figure 1. ANGPTL2 levels (ng/mL) in controls and HS patients.



P-067

ANALYSIS OF THE EXPRESSION OF FOUR POTENTIAL ASTHMA TRIGGERS IN LUNG TISSUES OF ASTHMATIC PATIENTS

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[1]Departamento de Inmunología, IIS-Fundación Jiménez Díaz-UAM, Madrid; [2]Unidad de Bioestadística y Epidemiología, Fundación Jiménez Díaz, Madrid; [3]Departamento de Alergia, Hospital Universitario Fundación Jiménez Díaz / CIBER de Enfermedades Respiratorias, CIBERES, Madrid; [4]Departamento de Inmunología, IIS-Fundación Jiménez Díaz-UAM / CIBER de Enfermedades Respiratorias, CIBERES, Madrid.

Background

Asthma is a chronic inflammatory disease, with high heterogeneity, making its management difficult sometimes. Besides, treatments are symptomatic and needed for long periods. We previously defined and theoretically prioritize, using systems biology, a group of potential biomarkers with the ability to differentiate asthma phenotypes in peripheral blood samples; and identified potential triggers: AKT1, MAPK13, STAT1 and TLR4.

Objective

Analyze the expression of the theoretically defined asthma triggers in lung samples of asthmatic patients with the aim of find new possible therapeutic targets.

Methods

Lung tissue samples from 10 healthy controls (HC) and 19 asthmatic patients were obtained from the CIBERES (CIBER of respiratory diseases) biobank. After mechanic homogenization of tissue samples, RNA was extracted trough TRIzol method and gene expression was analyzed trough RT-qPCR. Differential gene expression was analyzed with InStat program; and a study of their sensitivity and specificity to discriminate groups was performed through ROC curve analysis.

Results

All genes showed a lower expression in the asthma group compared to HC, with statistically significant differences for MAPK13, STAT1 and TLR4. ROC curve analysis showed the best AUC values for TLR4 and MAPK13.Then, we analyzed separately the expression in patients with asthma only (n=9), and observed that all genes showed statistically significant differences with the HC group, while only MAPK13 and TLR4 were significantly different in the asthmatic patients presenting other respiratory diseases (n=10). The best AUC values were found for the discrimination between the HC and the patients with asthma only. Finally, the comparison between both asthma subgroups showed differences in the expression of the four genes, without statistical significance, and the higher AUC value for MAPK13.

Conclusion



AKT1, MAPK13, STAT1 and TLR4 gene expression in lung tissue is differential between patients with asthma and healthy subjects, opening new possibilities in the search of asthma treatments.



P-068

TRANSVERSAL STUDY OF NK-CELL SUBSETS AND T-CELL ACTIVATION PATTERNS IN ALCOHOL USE DISORDER PATIENTS ACCORDING TO LIVER FIBROSIS DEGREE

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[1]Fundació Institut d'Investigació en Ciències de la Salut Germans Trias i Pujol (IGTP), Badalona; [2]Hospital Universitari Germans Trias i Pujol, Badalona.

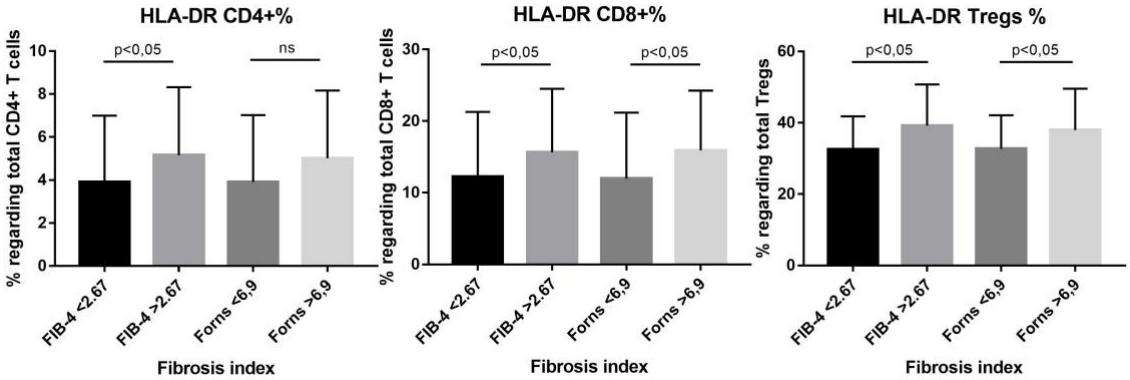
Introduction: Cellular immunity in alcohol-related liver diseases (ARLD) is currently being characterized. $CD8^+$T-cell activation is known to be detrimental enhancing liver damage, whereas Natural Killer (NK)-cells and NKT-cells play an important role in ARLD development having subsets with opposing roles. Here, we aimed to evaluate the phenotype of NK and NKT-cells and the T-cell activation profile in patients with alcohol use disorder (AUD) according to their liver fibrosis degree.

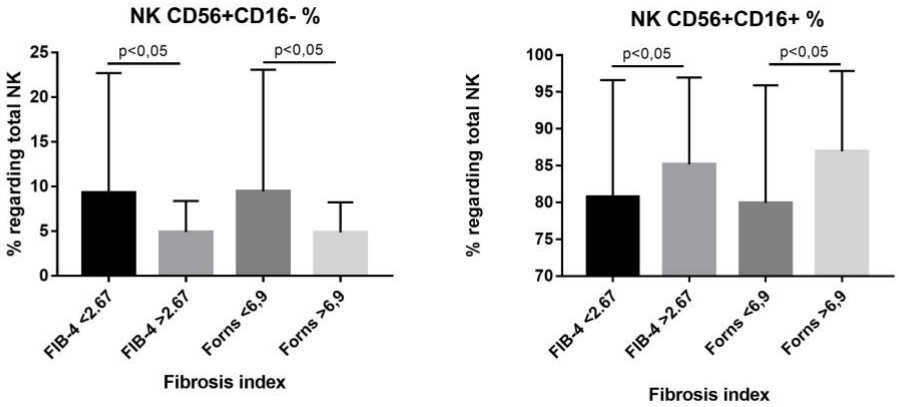
Methods: Patients were admitted to treatment of severe AUD (2019-2021). Advanced liver fibrosis (ALF) was defined when: FIB-4 score >2.67 (n=19), or Forns score >6.9 (n=21). Patients with HCV-infection, HIV-infection and history of liver cirrhosis were excluded. We analyzed NK-cell subsets ($CD3^-CD56^+CD16^+$, $CD3^-CD56^+CD16^-$, $CD3^-CD56^-CD16^+$), NKT-cells ($CD3^+CD56^+$), and the activation status of $CD4^+$, $CD8^+$ and regulatory T-cells (Tregs) according to HLA-DR expression.

Results: 79 patients (51 years, 71% males), with a mean AUD duration of 18±11 years and a daily alcohol consumption of 155±77gr/day, were included. $CD3^-CD56^+CD16^-$NK-cells in ALF patients were significantly lower (4.8±3.3; 4.9±3.4) compared to non-ALF patients (7.8±6.2; 7.7±6.2) according to Forns and FIB-4, respectively. $CD3^-CD16^+CD56^+$NK-cells were significantly higher in ALF patients (87±11) compared to non-ALF patients (80±16) according to Forns. No significant differences were observed in $CD3^-CD56^-CD16^+$NK-cells and NKT-cells. ALF patients also presented increased $HLA\text{-}DR^+$-expressing Tregs (38±11.6; 39.2±11.5) and $CD8^+$ (16±8.3; 15.6±8.8) compared to Tregs (32.7±9.4; 32.5±9.3) and $CD8^+$ (12±9.1; 12.2±9) in non-ALF patients according to Forns and FIB-4, respectively. $HLA\text{-}DR^+CD4^+$cells were higher in ALF patients (5.1±3.1) than in non-ALF patients (3.9±3) according to FIB-4 ($p<0.05$) (**Fig.1**).

Conclusions: Severe AUD patients with ALF presented an increased NK cytotoxic phenotype

concomitant with a decreased cytokine-secreting phenotype, possibly as a compensating mechanism of liver fibrosis. Meanwhile, the increased activation pattern observed in some T-cell subsets of ALF patients could be a mechanism that favors the development and maintenance of liver fibrosis.







P-069

ROLE OF JNK KINASES IN CHRONIC INTESTINAL INFLAMMATION AND ITS RELATIONSHIP WITH EPITHELIAL MALIGNANCY

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Introduction

The c-Jun N-terminal kinases (JNKs) are conserved proteins which regulate a wide range of cellular responses, including cell proliferation, differentiation or survival. Hyperactivation of the JNK signaling pathway is observed in many inflammatory diseases, such as colitis, and this increased activation of JNK frequently results in pathology via the regulation of the transcription of numerous inflammation-related genes. The general objective of this work is to identify specific mechanisms of the JNK signaling pathway involved in injury-derived chronic inflammation.

Methods

Mice with conditional deletion of *Jnk1* and *Jnk2* in the intestinal epithelial cells ($JNK^{\Delta IEC}$) were generated by crossing $Jnk1/2^{flox/flox}$ mice with VillinCre-transgenic mice. We then analyzed samples of small and large intestine using histological techniques and RNAseq. Moreover, we analyzed the phenotype of intraepithelial lymphocytes by flow cytometry.

Results

10 weeks old $JNK^{\Delta IEC}$ mice develop spontaneous mild inflammation in the duodenum and colon. Preliminary data shows that in both small intestine and colon may be a reduction of Tαβ lymphocytes and an increase of Tγδ and iNKTs in $JNK^{\Delta IEC}$ mice compared with controls. 52 weeks old $JNK^{\Delta IEC}$ mice present aberrant architecture and epithelial-dysplasia together with cellular infiltrates in the duodenum. At this age we observed significant changes in the intestinal transcriptome. Interestingly, we found a dramatic downregulation of the complement pathway in colon samples, while this is highly enriched in duodenum samples compared with controls. Moreover, we found that 72 weeks old $JNK^{\Delta IEC}$ mice develop numerous tumors in the duodenum.

Conclusions

Jnk1 and *Jnk2* deletion in intestinal epithelial cells is associated with a deregulation of the immune system that produces spontaneous intestinal inflammation in young $JNK^{\Delta IEC}$ mice, which may be the cause of the progression to epithelial-dysplasia and the tumors observed in the duodenum of older mice. The complement system might be a key factor in this process.

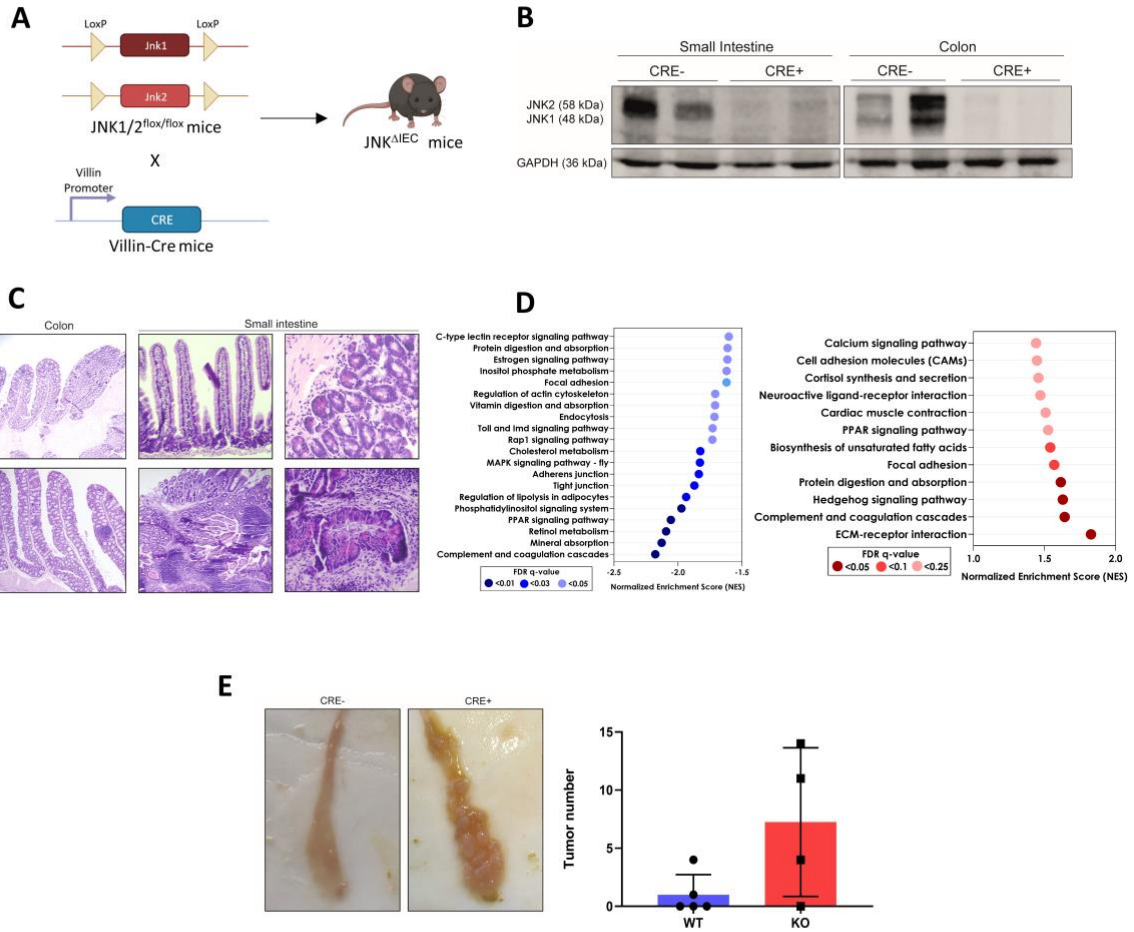


Figure. A) Generation of mice with conditional deletion of *Jnk1* and *Jnk2* in intestinal epithelial cells ($JNK^{\Delta IEC}$) using VillinCre-Loxp system. **B)** Western Blot analysis to demostrate the deletion of JNK1 and JNK2 in small intestine and colon of CRE+ mice. **C)** Hematoxylin and eosin staining of colon and duodenum of 52 weeks old $JNK^{flox/flox}$ and $JNK^{\Delta IEC}$ mice. **D)** Gene Set Enrichment Analysis of colon (left) and duodenum (right) of $JNK^{\Delta IEC}$ mice compared with $JNK^{flox/flox}$ mice. **E)** Macroscopic appearance of the duodenum of $JNK^{flox/flox}$ and $JNK^{\Delta IEC}$ mice and number of tumors.



P-070

INCREASED MYELOIDE-DERIVED SUPRESSOR CELLS IN PATIENTS WITH RECURRENT MISCARRIAGE AND RECURRENT IMPLANTATION FAILURE

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[1]Fundación para la Investigación Biomédica del Hospital Clínico San Carlos, Madrid; [2]Hospital Clinico San Carlos, Madrid; [3]Hospital Clínico San Carlos, Madrid.

Recurrent miscarriage (RM) and recurrent implantation failure (RIF) are two distinct disorders that can be associated with baseline pro-inflammatory state. Different surrogate inflammatory biomarkers have been associated with these disorders, including expanded cytotoxic NK cells, increased pro inflammatory cytokines or reduced regulatory T cells (TReg) in a subgroup of these patients. Myeloid-derived supressor cells (MDSCs) are a heterogeneous population of leukocytes, characterized by their ability to modulate immune responses. The immunosuppressive function of MDSC in cancer has been well established, while a controversial role in chronic inflammatory diseases has been reported. Typically, there are two major groups of MDSCs in humans: polymorphonuclear MDSCs (PMN-MDSCs) and monocytic MDSCs (M-MDSCs).

Freshly collected blood samples were analyzed by multiparametric flow cytometry using a BD FACS CANTO II. Peripheral blood M-MDSCs were characterized based on HLA-DR, CD14, CD33 and CD45 expression. A group of healthy non-pregnant women up to 45 years old with two or more children (proven fertility) and no spontaneous miscarriage was used as control group (CS).

In this study, we evaluated 65 women, 33 that fulfilled RM criteria and 32 with RIF. In addition, a group of 28 non-pregnant fertile women was included as a control group. We characterized MDSCs as CD14+CD33+CD45+HLA-DR-/LOW, according to previous reports. We observed a significant expansion of peripheral blood MDSCs in RM group (7,9 [6,75-10,55]; p= 0.002) and RIF (8,6 [6,15-12,15]; p=0.003) compared to CS group (6,35 [4,78-7,6]). We performed ROC curve analysis to evaluate the predictive power. The area under the curve was 0.72. We established an optimal cut-off level of 7.65% for both groups, with a sensitivity of 58.5% and a specificity of 77.8%.

MDSCs might be a good predictor for RM and RIF patients and with other biomarkers may classify a subgroup of patients with pro-inflammatory profile that can benefit from personalized therapies.



P-071

EVALUATION OF BASAL "EX VIVO" INFLAMMASOME ACTIVATION IN PERIPHERAL BLOOD MONOCYTES SUBSETS FROM HEALTHY DONORS BY FLOW CYTOMETRY

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HCUVA, Murcia.

Introduction

Human peripheral blood monocytes are classified in three subsets namely classical (CD14++CD16−), intermediate (CD14++CD16+), and non-classical (CD14+CD16+) . CD16 (+) monocytes have an inflammatory function and are expanded in inflammatory conditions. Inflammasomes are cytosolic multiprotein complexes composed by a NLR, the adaptor protein ASC and caspase-1 that proteolytically cleave pro-inflammatory cytokines like IL-1β and IL-18. In the present work we investigate "ex vivo" the activation of the inflammasome by human monocyte subsets in basal conditions measuring ASC protein oligomerization and caspase-1 activation by flow cytometry.

Methods

Human PBMCs obtained from 18 healthy donors were stained with anti-CD3, CD56, CD19, HLA-DR, CD14 and CD16 fluorescent monoclonal antibodies to isolate CD14++CD16-, CD14++CD16+ and CD14+CD16+ monocytes by cell sorting. $1x10^5$cells of each subset were cultured in RPMI media 10% FBS during 18 hours. CD14++CD16- were also cultured in the presence of LPS (200ng/ml) during 18h as positive control for inflammasome activation. Cells were then stained with FAM FLICA caspase-1 kit (Bio-Rad) following manufacturer instructions, fixed, permeabilized, stained with anti-ASC-PE antibody and adquired in a FACS Lyric flow cytometer to measure intracellular ASC aggregates and active caspase-1 as markers of inflammasome activation. Statistical analysis was performed using prism software v5.

Results

We found a significantly higher percentage of CD14++CD16+ and CD14+CD16+ monocytes forming intracellular ASC aggregates (Figures 1a-c) and with intracellular active caspase-1 (Figures 1b-c) compared to CD14++CD16- monocytes after 18 hours of cell culture in resting conditions, suggesting a higher basal inflammasome activation in CD16+ monocytes subsets.

Conclusions

In the present work we report an increased basal "ex vivo" activation of inflammasome by



CD14++CD16+ and CD14+CD16+ monocytes compared to CD14++CD16- monocytes. This result needs further investigation to identify the mechanism of inflammasome activation in monocytes subsets and whether inflammasome activation in CD16+ monocytes could be involved in the development of inflammatory diseases.



P-072

PLASMATIC DETERMINATION OF CYTOKINE INDUCTION TO ASSESS THE INFLAMMATORY RESPONSE AGAINST VIRAL ANTIGENS

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Hospital Universitario Central de Asturias, Oviedo.

During the SARS-CoV-2 pandemic, the “cytokine storm” phenomenon has been widely described in severe cases of COVID-19, being established as the main biomarker of severity. This fact makes relevant to be able to predict those individuals who are going to develop an exaggerated innate response to viral antigens.

We developed a protocol to measure the intrinsic capacity of cytokine production against viral antigens in whole blood cultures. Thirty-nine patients who had overcome COVID-19 (mean time after infection= 5.5 months) without requiring hospitalization (mean age=67.9, N=14), requiring hospitalization on the ward (mean age=71.7, N=13) or requiring ICU admission (mean age=66.8, N=12) was studied to evaluate the test and to compare their ability to produce proinflammatory cytokines.

Peripheral blood samples anticoagulated with heparin were cultured for 18 hours both without stimulation and stimulated with R848, an agonist to TLR7 and TLR8. After incubation, plasma was obtained and the inflammatory cytokine profile was measured using the kit cytokine 9-Plex ProcartaPlex Panel (ThermoFisher) and LuminexxMAPtechnology. This kit includes the cytokines IL-1β, IL-2, IL-6, IL-8, IL-10, IFN-α, IFN-β, IFN-γ and TNF-α.

Cytokine levels were undetectable or very low in unstimulated cultures. Only IL-8 was detected in all patients, and TNF-α, IL-6 and IL-1β were at very low levels in more than half patients. R848 induced the production of all cytokines in all patients and a significant increase in those in which it was already detected without stimulation. IL-8, IL-1β, IL-6 and TNF-α reached the highest levels. When individuals who had different degrees of SARS-CoV-2 severity were compared, we found that those admitted to ICU produced higher levels of IL-2, IL-10 and TNF-α ($p<0.05$).

We conclude that whole blood cultures stimulated with R848 are a useful system to evaluate response to viral antigens and it may predict individuals with a higher risk of exaggerated inflammatory response.



P-073

EFFECTOR AND REGULATORY CD4+ T CELL SUBSETS IN NEWBORNS WITH LEUKEMOID REACTION

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Introduction: Neonatal leukemoid reaction (LR) is a rare disease that reaches 17% incidence among preterm neonates with high morbidity and mortality. Its etiology is unknown although it has been related to an excessive inflammatory response. Perinatal immune system is tightly regulated to avoid an allogenic response and an excessive reaction against new antigens. Different subsets of effector and regulatory lymphocytes are involved in these regulatory mechanisms. The role of T regulatory (Treg) and T CD4+ T helper effector (Th) cell subsets has not been evaluated in LR.

Aim: To study the contribution of different peripheral lymphocyte subsets in preterm neonates with leukemoid reaction (LR): n=8, compared to healthy preterm neonates (PT): n=12, healthy full-term neonates (T): n=20 and healthy adults (HA): (n=10).

Methods: 2 ml of peripheral whole blood were collected. In LR and their paired controls (PT) two samples were taken, at the disease's debut and one week later. Cell surface markers were analysed by flow cytometry using BD FACSLyric, data evaluation was done with the BD FACSuite Software (Becton Dickinson).
We quantified: Treg (CD4+CD25+CD127-), Th: CD4+CD45RA-CXCR5- and circulating follicular Th (cTfh): CD4+ CD45RA-CXCR5+, expressing CXCR3 (Th1/cTfh1), CCR6 (Th17/cTfh17), CXCR3 and CCR6 (Th17.1/cTfh17.1) and CXCR3-/CCR6- (Th2/cTfh2).

Results: The contribution of Treg was found in the order PT>T>HA, as has already been reported, without differences between LR and PT. The main finding was that we detected a statistically significant reduction of TH17 percentages in PT (7.2±6) and T (5.5±3.8) compared to HA group (17.86±4.1): $p<0.01$ and $p<0.001$, respectively, that was not significant in LR (8.8±4.9). The remaining cell subsets did not show relevant results (Figure1).

Conclusions: Th17 CD4+ effector cells are increased in LR newborns. Functional studies will

discern whether they have a role in their inflammatory response and could be targets of specific biological therapies.

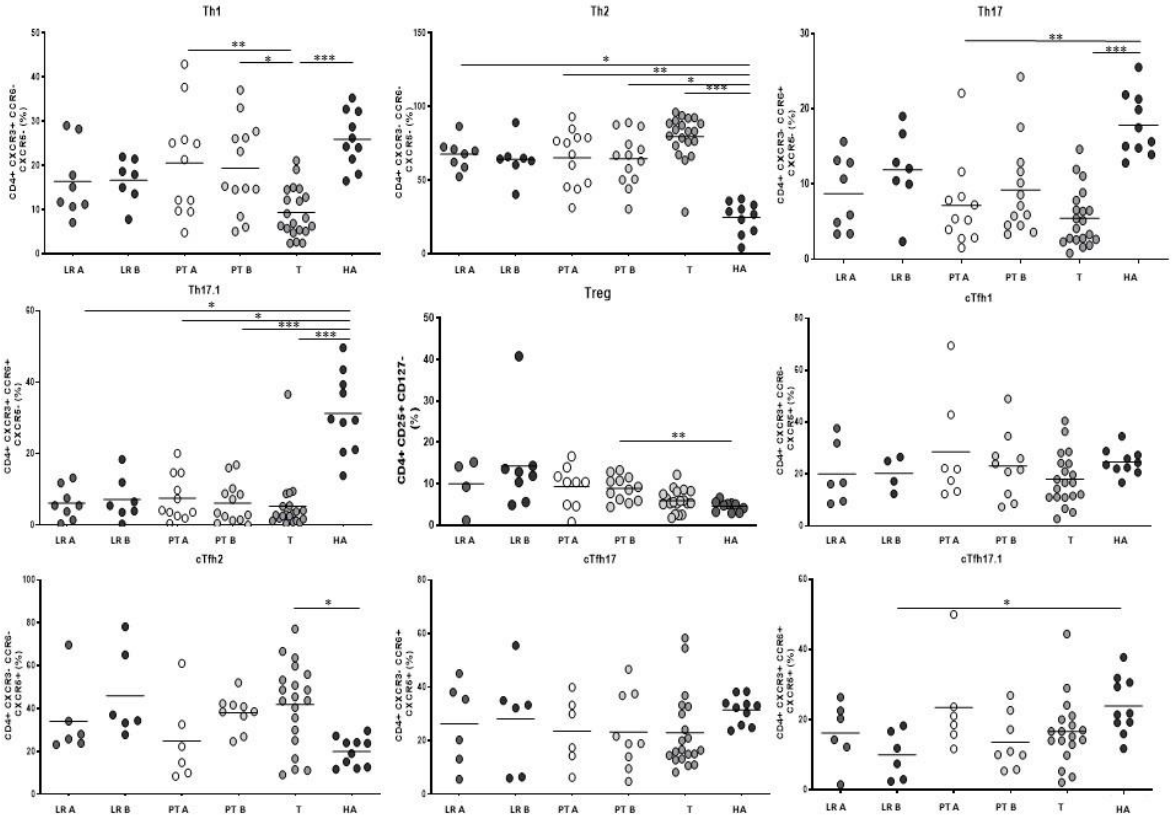


Fig1. Percentage of CD4+Thelper effector (Th) and circulating follicular Th (cTfh) in CD4+CD45RA- and T regulatory (Treg) in CD4+ T cells. A: Sample taken at the disease's debut. B: Sample taken one week later. One way ANOVA statistic test was performed (GraphPad Prism 5.01). *p<0.05, **p<0.01, ***p<0.001

P-074

DEFICIENCIA DE LA CONTRIBUCION DE LA POBLACIÓN DE MONOCITOS CD16+ CD14-/+ EN EL DIAGNÓSTICO DE PACIENTES EN LOS QUE COEXISTE UN PROCESO INFLAMATORIO Y TUMORAL.

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Hospital Universitario Ramón y Cajal, Madrid.

A través de un caso clínico, se expone el reto diagnóstico que acompaña a las entidades que cursan con expansión de monocitos, dada la dificultad de distinguir, mediante las tres técnicas diagnósticas habituales (citología, citometría de flujo e histología) procesos/patologías tan dispares como las monocitosis reactivas, la leucemia mielomonocítica crónica (LMMC) o las leucemias mieloides agudas (LMA) con componente monocítico. En un intento de esclarecer este problema, recientemente se describió la práctica desaparición de monocitos CD16+ en la LMMC como un excelente biomarcador. Sin embargo, la coexistencia de un proceso inflamatorio/infeccioso, caracterizado por un aumento de la población de monocitos CD16+ invalida parcialmente la utilidad del mismo.

El caso se centra en una mujer con una insuficiencia respiratoria aguda que debuta con monocitosis, objetivándose en el frotis un alto porcentaje de promonocitos, sin rasgos displásicos en ninguna de las tres series. Con la sospecha inicial de LMMC, se realizó el estudio inmunofenotípico por citometría en sangre periférica, detectándose una marcada expansión de monocitos maduros no clásicos (CD14-/dim CD16+; 24% respecto a monocitos totales) e intermedios (CD14+ CD16+; 41%), sin evidente expresión aberrante de antígenos y no detectándose formas inmaduras de monocitos (figura A).
Con estos datos contradictorios, se realizó el estudio en médula ósea, obteniéndose resultados muy dispares en las tres técnicas diagnósticas antes referidas, hasta el punto que se propusieron hasta tres posibilidades diagnósticas distintas: LMA, LMMC (figura B) y proceso reactivo. El caso se resolvió gracias a los resultados de la biología molecular, que detectó el gen NPM1 mutado lo que decantó la balanza hacia un proceso agudo.

Estos hallazgos demuestran que la elección de este biomarcador para el diagnóstico diferencial de estos procesos no es completamente fiable y abre la posibilidad de que distintas técnicas denominen al mismo monocito de diferente forma.

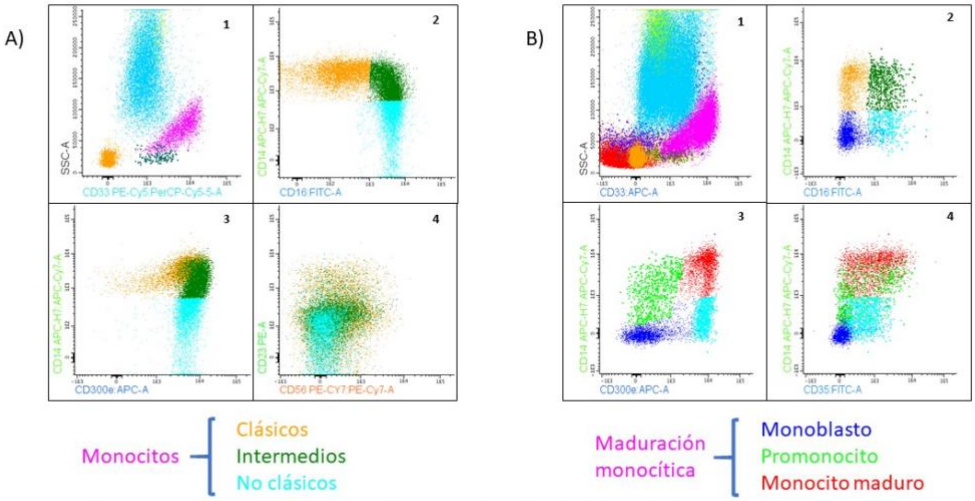


Figura A): Estudio de citometría realizado en sangre periférica, donde se aprecia un aumento de monocitos (rosa)[1]; su distribución por subpoblaciones atendiendo a la expresión de CD14 y CD16[2]: clásicos (naranja), intermedios (verde oscuro) y no clásicos (azul claro); la práctica totalidad de la población se correspondió con monocito maduro[3] y no se detectó expresión significativa de antígenos aberrantes[4].
B): Estudio de citometría realizado en aspirado de médula ósea, donde se sigue apreciando el aumento de la serie monocítica[1]; su distribución por subpoblaciones atendiendo a la expresión de CD14 y CD16[2] o atendiendo a su estadio madurativo: monoblasto (azul oscuro), promonocito (verde claro), monocito maduro (rojo) [3 y 4].



P-075

SÍNDROME AUTOINFLAMATORIO CON MUTACIÓN EN LOS GENES *PSTPIP1 Y WAS:* RETO DIAGNÓSTICO EN PACIENTE CON ESPONDILOARTROPATÍA REFRACTARIA A MÚLTIPLES TERAPIAS BIOLÓGICAS.

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Hospital General Universitario Gregorio Marañón, Madrid.

Nuestro objetivo es presentar el caso de una paciente mujer de 40 años referida a nuestra Unidad de Inmunología Clínica por cuadro de infecciones respiratorias recurrentes, espondiloartropatía axial HLA-B27+, brotes recurrentes de herpes zóster y artropatía psoriásica. Asociaba picos febriles, astenia y artromialgias con limitación para actividades básicas de la vida diaria.

A su llegada a consultas, la paciente ya había recibido tratamiento con Adalimumab, Etanercept, Golimumab y Ustekinumab y gammaglobulina intravenosa, sin mejoría clínica.

En el estudio inmunológico inicial, únicamente se detectó un ANA positivo (1/80) de patrón homogéneo e hipocomplementemia C3 (68.3mg/dL). Se realizó estudio genético mediante la secuenciación de nueva generación (NGS) utilizando un panel de 200 genes asociados a inmunodeficiencias.

Dicho análisis genético reveló dos variantes heterocigotas en el gen *PSTPIP1* (p.Arg405Cys) y el gen *WAS* (p.His180Asn) respectivamente. Ambas mutaciones implican interpretaciones conflictivas en cuanto a patogenicidad según las bases de datos genómicas revisadas. La primera de ellas sugiere una alteración en la interacción de dicha proteína con el citoesqueleto y otras moléculas de sus vías de actuación, como la IL-1β, cuya producción también es regulada por la interacción de esta proteína con la pirina. También se encontró evidencia de expresión reducida de la proteína WASP en asociación con la segunda mutación descrita.

En relación con la sospecha de posible enfermedad autoinflamatoria con desregulación de producción de la IL-1β, se inició tratamiento con Anakinra.

La paciente mostró buena tolerancia inicial con mejoría del cuadro clínico permitiéndole reincorporarse a sus actividades y mantuvo el tratamiento durante 3 meses, con reaparición progresiva de la sintomatología.

Como conclusión, en estos casos complejos el estudio genético puede brindar luz en cuanto a las vías moleculares alteradas, lo que puede brindar una opción terapéutica eficiente como en el caso descrito.



P-076

REFERENCE RANGES OF INFLAMMATORY CYTOKINES IN HEALTHY POPULATION

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Background:

Inflammatory cytokines are signalling molecules involved in the immune system homeostasis, but also in many pathological processes. Due to their diagnostic, prognostic and predictive values, determining their serum concentration has become a routine practice in specialised laboratories, despite the heterogeneity in quantitation methods. The aim of this study was to determine inflammatory cytokine levels by flow cytometry in a healthy population according to age in order to stablish normal ranges.

Materials and methods:

Samples were obtained from 152 healthy volunteers, who were stratified into 3 or 6 groups of age. Those with chronic diseases, immunological alterations, infections, cancer or metabolic syndrome or under immunomodulation drugs treatment were excluded, except when they were >70 y.o. Circulating IL-1β, IL-6, IL-8, IL-10, IL-12p70, TNF-α, IFN-γ, MCP-1 and IP-10 were measured in 1:4 diluted serum samples using Cytometry Bead Array (CBA) on a BD FacsCanto II and analysed with FCAP Array.

Results:

IL-1β, IL-6, IL-10, IL-12p70, TNF-α and IFN-γ were undetectable. Results did not show significant differences considering gender, allergy or smoke habits. Correlation analyses revealed a decreased in IL-8 ($r=-0.44$) and an increased in IP-10 levels ($r=0.37$) with age ($p<0.05$). In addition, MCP-1 slightly increased with weight ($p<0.05$; $r=0.16$). IL-8, MCP-1 and IP-10 levels were affected by diabetes, hypertension, hypercholesterolemia and cardiopathy ($p<0.05$).



Cytokine	Group 1 (19-29 y.o.)	Group 2 (30-39 y.o.)	Group 3 (40-49 y.o.)	Group 4 (50-59 y.o.)	Group 5 (60-69 y.o.)	Group 6 (>70 y.o.)
IL-8	0.00-2587.92	0.00-643.38	0.00-798.08	1.69-211.63	0.00-447.39	0.00-10.37
MCP-1	0.00-259.48	7.40-395.38	8.33-263.83	7.63-371.05	14.15-427.84	11.35-83.97
IP-10	21.35-218.93	22.09-397.89	11.01-215.24	67.27-200.97	55.16-584.00	83.35-1339.49

Cytokine	Group A (<40 y.o.)	Group B (40-59 y.o.)	Group C (>60 y.o.)
IL-8	0.00-2319.76	0.00-616.66	0.00-447.39
MCP-1	4.07-320.64	7.86-345.91	11.35-427.84
IP-10	21.76-371.76	16.85-210.60	55.16-1339.49

Conclusion:
In conclusion, inflammatory cytokine levels in healthy individuals change with age and the presence of chronic diseases.



P-122

CHARACTERIZATION OF HLA-DR IMMUNOPEPTIDOME PRESENTED BY DENDRITIC CELLS PULSED WITH THE BREAST CANCER TUMORAL CELL LINE MCF-7 LYSATES

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

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

The objectives of this study were: (i) to determine whether there are differences between the peptidome presented by pulsed and non-pulsed moDCs, both at peptide and protein levels; (ii) to determine the percentage of peptide and protein overlap between moDCs with different HLA-DR alleles; (iii) to evaluate the influence of the allele combination on antigenic presentation. The results showed that: (i) the pulse displaces part of the proteins of the moDCs ligandome, since the non-pulsed samples share most of the proteins with the rest of the pulsed moDCs, but not the other way around; (ii) there is a positive correlation between the abundance of an allele in the samples studied and the level of peptide and protein overlap presented by them but not with the sample size; (iii) the immunopeptidome of the pulsed moDCs is modified by different HLA-DR alleles.

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



P-124

COMPARATIVE ANALYSIS AND CHARACTERIZATION OF TCR REPERTOIRES IN BREAST CANCER TIL

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The characterization of T cell receptors (TCR) expressed by tumor-infiltrating lymphocytes (TIL) is gaining interest since TCR can be used as a tool of immunotherapy but also as a biomarker. Thus, it is necessary to understand the TCR features and their differences in the CD4+ and CD8+ infiltrating subsets.

Biopsy fragments from 7 breast cancer patients were cultured as explants to analyze the TCR in the initial biopsy-derived T cells. Later, expanded cells were sorted by their CD4 and CD8 expression. TCR HTS was performed to analyze the clonality, the sequence overlapping and different properties of the TRA and TRB CDR3, as well as to find motifs in the TRB sequences. Significant differences in the biochemical properties of the TRB CDR3 amino acid sequences were found, i.e., TRB CDR3 sequences presented shorter N(D)Nnt regions in the CD8+ set and more positively charged sequences in the CD4+ set. We also found a higher diversity in the CD8+ compared to the CD4+ TIL subsets – both in the TRA and TRB sequences – and a higher sequence overlap, as well as a higher number of motifs, shared between biopsies in the CD4+ subset.

Overall, this data indicates different characteristics of the TIL's TCR in each subset independently of the biopsy. Interestingly, the CDR3 sequences of the CD4+ cells show a higher similarity between biopsies than that of the CD8+ cells, suggesting that these are more restricted in the tumor environment.



P-125

MELANOMA SPHEROIDS AND MEMORY-LIKE NK CELLS: OPTIMIZATION OF AN *IN VITRO* ASSAY TO EVALUATE TUMOUR-IMMUNE CELL SYNAPSIS

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NK cells are a key component of the innate immune system, essential not only in host defence against virus-infected cells, but also in tumour immune surveillance. Their function is regulated by a balance of activating and inhibitory signals. The *in vitro* stimulation of NK cells using cytokine cocktails (IL-12/15/18) induces a memory-like phenotype. These cytokine-activated memory-like (CIML) NK cells are strongly cytotoxic and have a longer half-life, which makes them ideal for immunotherapy.

Although NK cell cytotoxicity can be studied through monolayer cultures, 3D cultures have turned into a state-of-the-art model for studying molecular interactions between immune and tumour cells.

The purpose of this study was to establish a protocol to obtain memory-like NK cells and to characterize them in terms of phenotype and function. Moreover, we aimed to develop a 3D culture method of melanoma cell lines to evaluate NK cell-tumour interactions.

Briefly, NK cells were isolated from peripheral blood and analysed through flow cytometry to identify phenotypic and functional differences between control and CIML NK cells at different time points of culture.

Significant differences were found in the expression of activating and inhibitory receptors, as well as in intracellular perforin and granzyme. Moreover, functional differences were also observed in terms of TNF-α/INF-γ expression and degranulation against target cell lines.

Regarding, 3D cultures of tumour cells, spheroids from the melanoma cell line MaMel56 were established and co-cultured at day 7 with *in vitro*-expanded NK cells and monitored with different microscopy techniques. After 24h of co-culture, several changes in spheroid morphology could be observed.

In conclusion, the obtention of highly cytotoxic and longer-lived NK cells, together with the optimization of a co-culture method using tumour spheroids, could give a better understanding for modelling the crosstalk between tumour and immune cells and may help for the development of new immunotherapies.



P-126

ORAL SQUAMOUS CARCINOMA INDUCES IMMUNOSUPRESSIVE MONOCYTES

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Introduction

Oral squamous carcinoma (OSC) is one of the top 10 cancers in prevalence and mortality. The tumor microenvironment is highly important in OSC, where tumor-associated macrophages (TAMs) are the most abundant cell type. Most of these TAMs derive from circulating monocytes and differentiate in situ. TAMs can be M2-like macrophages possessing pro-tumoral functions, enabling immunosuppression, vasculogenesis, tumor progression and metastasis, but can also be M1 macrophages, which have been related to anti-tumoral effects and better prognosis.

Objectives

Study the effect of OSC cells (OSCCs) on monocytes differentiation.

Materials and Methods

We have used the H413 and TR146 OSCC lines and its supernatant for co-culture assays with primary monocytes from healthy donors' peripheral blood during different times (16 hours or 5 days). Also, we have co-cultured conditioned-monocytes with allogeneic total T cells. The complement-mediated phagocytic capacity of those monocytes was also tested using opsonized labelled sheep red blood cells. Cell markers and cytokine production were studied by flow cytometry and ELISA.

Results

OSCCs induce early activation of monocytes, as pro-inflammatory cytokines (TNFα, IL-6, IL-12, IL-1β) are increased. However, at longer times, OSCCs imprint an immunosuppressive phenotype on monocytes related to M2 macrophages, judged by the lower expression of HLA-DR, CD86, CD11c and increase of PD-L1, IDO and IL-10. In addition, supernatant-conditioned monocytes were unable to activate allogeneic T cells, indicated by the expression of IFNγ and Tbet. Finally, the phagocytic ability of these monocytes was decrease when they were co-culture with OSCCs.

Conclusion

Our data indicate that OSCCs promote the differentiation of immunosuppressive monocytes into an M2-like phenotype, which may facilitate tumor progression.



P-127

LEUCEMIA LINFOBLASTICA B CD2+ CON TRANSDIFERENCIACIÓN A ESTIRPE MONOCÍTICA: CARACTERÍSTICAS DE PROLIFERACIÓN Y APOPTOSIS DE SUS DIFERENTES SUBPOBLACIONES

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En las Leucemias Linfoblasticas Agudas B (LLA-B) el cambio de linaje de los precursores B aberrantes hacia linaje mieloide durante el tratamiento ocurre en raras ocasiones y puede provocar dudas sobre el diagnóstico inicial y la continuación del tratamiento. Recientemente se ha descrito un subgrupo de pacientes cuyos blastos al diagnóstico expresan total o parcialmente el antígeno CD2, y tras inicio del tratamiento presentan mayor probabilidad de transdiferenciación hacia células de estirpe monocitoide. Se expone a continuación un caso clínico.

Paciente pediátrico diagnosticado de LLA-B común en aspirado de médula ósea (MO) (95% de blastos linfoides), con expresión aberrante del antígeno CD2. Se inició quimioterapia según la guía Pethema en rama de riesgo intermedio.

En día +15 tras tratamiento, se realiza estudio de control, objetivándose un 28% de precursores aberrantes, con clara diferenciación de una fracción de linfoblastos originales hacia células monocitoides, pudiéndose establecer hasta 3 poblaciones claramente diferenciadas.

Se realizó un cultivo celular con el aspirado de MO del paciente en el día +15 post-tratamiento, determinándose a días +2 y +5 del cultivo el porcentaje de cada población, viabilidad y capacidad de proliferación. Los resultados demostraron mayor capacidad de proliferación en las células con diferenciación monocitoide. Además, tras 5 días de cultivo se apreció un notable incremento de mortalidad en la fracción de blastos linfoides, mientras que las subpoblaciones con diferenciación monocítica permanecían viables en su práctica totalidad. En el día +30 post-tratamiento, no se detectaron blastos aberrantes de ningún tipo de linaje.

Este caso demuestra que una evidente transdiferenciación del blasto linfoide CD2+ original no cambia el diagnóstico ni su tratamiento, los pacientes consiguen respuestas completas aunque más lentamente. El estudio *in vitro* revela que durante el tratamiento, las células con clara diferenciación monocitoide mostraron una mayor capacidad proliferativa y supervivencia, explicando el comportamiento de las diferentes poblaciones.



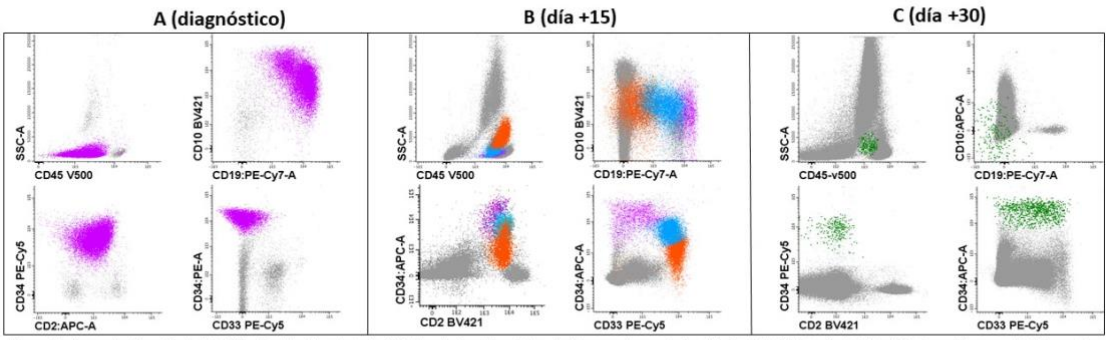


Figura A: Imágenes de citometría de flujo (CF) obtenidas del aspirado de MO del paciente al diagnóstico, donde se puede apreciar la población de linfoblastos B originales CD2+ (morado), con positividad para los antígenos CD19, CD10 y CD34 y negatividad para antígenos mieloides/monocíticos como el CD33. Figura B: Imágenes de CF del día +15 post-tratamiento donde se pueden identificar tres poblaciones de blastos tumorales, la población de linfoblastos original (morado), una población intermedia (azul), que empieza a perder características linfoides y a adquirir características monocíticas y una población monocítica (naranja) en la que prácticamente han desaparecido muchas de las características linfoides iniciales. Se observa que la población intermedia y la población monocítica mantienen la expresión aberrante de CD2. Figura C: Imágenes de CF del día +30 post-tratamiento en la que únicamente se observa una población muy minoritaria de blastos normales de estirpe mieloide (verde), sin expresión aberrante de CD2.



P-128

STUDY OF THE THERAPEUTIC APPLICATIONS OF BACTERIORUBERIN AND ITS DERIVATIVES AGAINST CANCER.

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Because high levels of ROS are generated during the early stages of tumour development, the cancer cells with the highest antioxidant capacity will be those that will initially determine the tumour phenotype. However, given that throughout tumour development the concentrations of ROS that will be reached will usually be higher than those reached in normal cells, cancer cells, in order to avoid ending up experiencing selective pressure with respect to normal cells, increase their antioxidant molecules. On the basis of this premise, it has been suggested, on the one hand, that cancer cells may be more sensitive than normal cells to increases in ROS levels. Furthemore, with regard to the use of carotenoids as antioxidant substances, it should be noted that their use on tumour cells with high levels of ROS may end up oxidising these molecules, in such a way that when the amount of oxidised antioxidant exceeds certain levels, a pro-oxidant effect of these molecules predominates. This is why it is currently thought that carotenoids could act selectively against tumour cells, showing prooxidant activity on them and, in turn, protecting normal cells from oxidative stress due to their antioxidant activity under physiological conditions. On the other hand, we also assume that many other mechanisms have been described by which carotenoids suppress the onset and progression of cancer. Finally, it is important to mention that bacteriorruberin, the subject of this project, is a carotenoid pigment with a much higher antioxidant capacity than conventional pigments and whose biomedical applications are still largely unexplored. All this suggests that this compound could have a greater anti-tumour capacity than conventional pigments. Preliminary data from the research group suggest that bacteriorruberin could increase cell death and decrease the rate of proliferation in the K562 tumour cell line.



P-129

IMPORTANCIA DE LOS RECEPTORES KIR Y SUS LIGANDOS COMO FACTOR PRONÓSTICO TRAS TRATAMIENTO CONVENCIONAL EN PACIENTES CON CÁNCER DE OVARIO

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El tumor de ovario es el de mayor mortalidad a nivel ginecológico. Su abordaje terapeutico convencional con cirugía y quimioterapia no ofrece buenos resultados por lo que nuevas terapias están siendo analizadas. En este sentido, nuestro grupo trabaja en un proyecto en etapa experimental preclínica que emplea linfocitos TCAR multidianas. Los receptores KIR regulan en parte la función de las células NK y linfocitos T en la respuesta antitumoral. Sin embargo, se desconoce si su amplio polimorfismo podría intervenir en el curso de estas pacientes tras los tratamiento clásicos.

Objetivo: Analizar los polimorfismos KIR/HLA en estas pacientes para establecer posibles biomarcadores pronóstico tras tratamiento convencional y de indicación de tratamiento precoz con nuevas terapias.

Pacientes y métodos. Se han incluido pacientes con cáncer de ovario intervenidas en los últimos 5 años, analizando tiempo hasta recaida y supervivencia. Se aislará ADN conservado en parafina y se analizarán los genes KIR (Genotipaje KIR de Innotrain Diagnostik) y sus ligandos HLA (Lifecodes HLA-A,B SSO Typing Kit y Lifecodes HLA-C eRES SSO Typing Kit, Immucor)

Resultados: Se estudia la influencia de las combinaciones KIR/HLA en los pacientes que han sido estratificadas según su diferente respuesta a los tratamientos convencionales. Se presentará la influencia de forma independiente de 2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 3DL1, 3DL2, 3DL3 y 3DS1, 2DP1 y 3DP1 y las variantes de KIRDL5, KIR2DS4 y KIR3DP1. También los datos referentes al análisis de los diferentes haplotipos y semihaplotipos centroméricos y teloméricos con diferente potencial inhibidor o activador. Se incluye también los resultados de los ligandos HLA (Bw4 y del dimorfismo de HLA-C). Entre los alotipos Bw4, se consideran HLA-A*24:02, A*32:01 y A*23:01, pero no HLA-A*25:01. Los resultados previos no muestran diferencias entre las pacientes incluidas sin tener en cuenta las variables estudiadas y el grupo control



P-130

LA VÍA HLA-G/ILT2 MODULA LA FUNCIÓN EFECTORA DE LOS LINFOCITOS T CD8 EN CÁNCER GÁSTRICO

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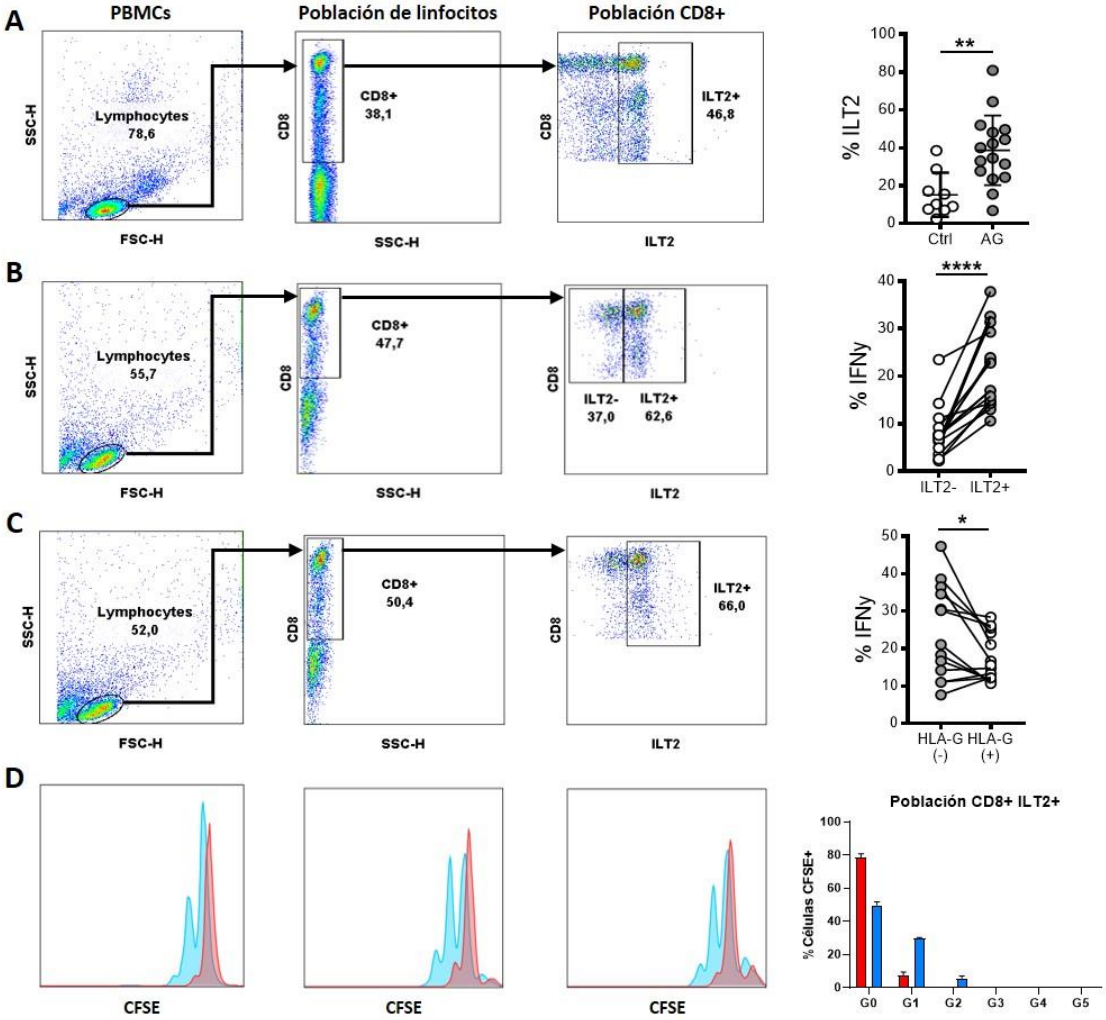
Introducción: Las terapias actuales con inhibidores de puntos del control del sistema inmunitario (ICI; PD1/PDL1), han supuesto un gran avance en el tratamiento del cáncer gástrico. Sin embargo, no todos los pacientes pueden beneficiarse, lo que lleva a la necesidad de buscar nuevas dianas. HLA-G es una molécula HLA no clásica, capaz de inducir tolerancia a través de su receptor ILT2 en células T, lo que podría establecer HLA-G como una nueva diana para ICI.

Objetivos: Estudiar el fenotipo y función de los linfocitos ILT2+, modulados por HLA-G, en cáncer gástrico.

MyM: Se aislaron PBMCs de 16 pacientes y 9 controles. Se realizaron ensayos de activación con αCD3/CD28/CD2 y de proliferación con CFSE en presencia o ausencia de HLA-G, mediante citometría de flujo con anticuerpos αCD8, CD3, PD1, ILT2 e IFNy.

Reultados: Encontramos un aumento de la expresión de ILT2 en linfocitos CD8+ de pacientes (38,7%) con respecto a los controles (15,1%, p=0,002). Parte de la población ILT2+ era PD1-, lo que indica una regulación independiente.
El 21,9% de los linfocitos CD8+ ILT2+ producen IFNy en comparación con los CD8+ ILT2- (7,9%, p<0,0001).
Cocultivando PBMCs en presencia de HLA-G, las células CD8+ILT2+ producen una cantidad significativamente menor de IFNy en comparación con los cocultivos sin HLA-G (p=0,018).
En estos cocultivos también se observa una reducción de la proliferación de células ILT2+ en presencia de HLA-G, ya sea de membrana o soluble.
Conclusiones: Existe un aumento significativo en la frecuencia de células CD8+ ILT2+ en PBMCs de pacientes con cáncer gástrico. Además, las células ILT2+ tienen una mayor capacidad de producir IFNy tras la activación mediada por el TCR. HLA-G es capaz de bloquear la activación y proliferación, de los linfocitos de estos pacientes. La vía HLA-G/ILT2

puede convertirse en una nueva vía para el tratamiento del cáncer gástrico.



A: *Fenotipado de la población ILT2 en pacientes (AG) y controles (Ctrl).* Los pacientes presentan un número significativamente mayor de células CD8+ILT2+ (38,7%) en comparación con los controles (15,1%, p=0,002). **B:** *Capacidad de activación (producción de IFNγ) por parte de las células ILT2.* Al ser estimuladas vía TCR, las células CD8+ILT2+ producen una cantidad mayor de IFNγ (21,9%) en comparación con las CD8+ILT2- (7,9%, p<0,0001). **C:** *Efecto de HLA-G en la activación de las células ILT2.* La producción de IFNγ por parte de las células CD8+ILT2+ es menor cuando se cocultivan con células que expresan HLA-G (17,5%) que cuando se cocultivan con células que no lo expresan (24,2%, p=0,018). **D:** *Efecto de HLA-G en la proliferación de las células ILT2.* En estos cocultivos se observa una reducción de la proliferación de células ILT2+ en presencia de HLA-G. *p<0,05; **p<0,01; ***p<0,005; ****p<0,001.



P-131

CROSS-REACTIVITY STUDY BETWEEN OLE E 7 AND PRU P 3 NSLTPS

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Background: Non-specific Lipid Transfer Proteins (nsLTPs) are small proteins thoroughly represented in plants and responsible for severe forms of allergy. Pru p 3 is a common primary sensitizer in southern Europe, while Ole e 7 is a very powerful sensitizer particularly in Andalusian area, where the existence of co-sensitization to both of them is usually described. The aim of this study was to identify a possible cross-reactivity between Pru p 3 and Ole e 7 underlying this co-sensitization.

Materials and methods: We measured specific IgE (sIgE) to Ole e 7 and Pru p 3 by ImmunoCAP 250 (sIgE>0.35 kUI/L positive cut-off) in sera samples of 9 patients, who were classified into: Ole e 7-monosensitized (MONOLE; n=3), Pru p 3-monosensitized (MONPRU; n=3) and bisensitized (BI; n=3). Basophil Activation Test (BAT) was performed using Ole e 7 and Pru p 3 recombinant proteins at 10 µg/ml. For epitope mapping, MALDI-TOF was performed after protein digestion with trypsin and separately incubation with a serum mix from each group.

Results: As expected, BI patients presented a positive BAT to both allergens, while MONPRU basophils only degranulated with Pru p 3. Surprisingly, BAT of MONOLE patients was positive to both nsLTPs. The epitope mapping identified one Pru p 3 peptide, “ISASTNCATVK”, able to be recognized by the three groups of mixed sera, including MONOLE. Likewise, the digestion of Ole e 7 provided one peptide “KSALALVGNKV” also recognized by all the groups. No linear sequence homology was detected between the above peptides.

Conclusion: A possible cross-reactivity between different epitopes from Ole e 7 and Pru p 3 could be the underlying cause of the co-sensitization observed in the south Mediterranean. The epitope sequence disparity suggests two distinct pathways of sensitization, in which Pru p 3 or Ole e 7 could act as primary sensitizers.

P-132

SYSTEMIC MASTOCYTOSIS. A RETROSPECTIVE EVALUATION OF CASES IN OUR CENTER

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Introduction

Mastocytosis is a heterogeneous group of diseases defined by an unusual accumulation of pathological and clonal mastocytes in various tissues. Systemic mastocytosis (SM) is normally diagnosed based on bone marrow (BM) histopathology and there are major and minor diagnostic criteria.

Objectives

To determine the diagnostic efficiency of the study of the D816V mutation in C-KIT, study of aberrant mast cells by flow cytometry (FCM) and serum tryptase levels in a cohort of patients with suspected SM, as well as to describe the characteristics of the cohort.

Methods

The cohort consisted of 34 patients from the province of Cádiz with suspected SM from January 2020 to February 2022. Tests results that form part of diagnostic criteria, clinical characteristics, study of hymenoptera venom allergy and other data of analytical interest were collected.

Results

Of the 34 patients, 11 (32.4%), were diagnosed with SM. Sensitivity and specificity values of the 3 tests analysed are shown in table 1. Both serum tryptase levels and the percentage of aberrant mastocytes in BM are higher in patients diagnosed with SM. In addition, 8 out of 11 patients with SM (73%) had a history of cutaneous mastocytosis (CM). There are two cases (18%) with sensitivity to hymenoptera venom.

Table 1.

	Sensitivity (%)	Specificity (%)
C-KIT mutation	90.9	91.3
Aberrant mastocytes FCM	100	89.5
Serum tryptase >20 ng/ml	90.9	69.5

Conclusion

The evaluated tests have a high sensibility for the diagnosis of SM in our cohort of patients. According to the bibliography, more than the 70% of the SM cases have a history of CM. SM is underdiagnosed in cases of allergic reactions caused by hymenoptera venom and its diagnosis in

our cohort would help to take appropriate preventive measures in case of such reactions.



P-133

THERAPEUTIC EFFICACY OF ENDOVENOUS LIVE TUBERCULOSIS VACCINES AGAINST ESTABLISHED EXPERIMENTAL ASTHMA BY SYSTEMIC DESENSITIZATION

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Asthma is one of the most common pulmonary diseases in the world, and its incidence continues increasing, generating a huge economic burden in public health. Asthma is often associated with an exacerbated type 2 inflammation. Allergen-specific Th2 lymphocytes produce cytokines which are responsible of some of the clinical symptoms. Thus, therapies targeting asthma-associated type 2 responses have demonstrated to be highly promising. MTBVAC is a novel live attenuated tuberculosis vaccine currently under clinical evaluation as tuberculosis vaccine. Intradermal MTBVAC has been widely tested in preclinical (mice to non-human primates) and clinical (phase I and II) trials, and it is expected to start efficacy trials in newborns during 2022. Due to its Th1 response-promoting activity, the pulmonary administration of the MTBVAC has been shown to have strong therapeutic efficacy.
Here, considering the systemic character of endovenous administration of vaccines, we explore this route with MTBVAC to study how its mechanism against allergies works. Results show that the endovenous administration of MTBVAC is capable of desensitizing (reduction of antigen specific Th2 cytokines) in spleen, also in absence of stablished symptoms, such as eosinophilia. In addition, MTBVAC produces IFNγ, which is not necessary for reducing eosinophils in BAL and lung. According to efficacy studies, MTBVAC has a long-term protection against asthma, as therapeutic effect is maintained six months post vaccination. Our data showed strong therapeutic efficacy of MTBVAC endovenous administration in a scenario of established disease.



P-134

SPECIFIC IGE ALLERGIC ADJUSTED CUT-OFFS IN THE DIAGNOSIS AND INDICATION OF ALLERGENIC IMMUNOTHERAPY

Bernardo Serrano, Raquel; Manzanares, Bárbara; González, Rafael; Moreno, Carmen; Jurado, Aurora.

Hospital Universitario Reina Sofía, Córdoba.

Background. Specific IgE (sIgE) quantification plays a crucial role in the diagnosis of allergy. Given the heterogeneity observed between techniques, standardized cut-offs must be established to improve the accuracy of the diagnosis and the administered treatment. Since the importance of Olive and Grass pollens throughout the Mediterranean area, the objective of this study was to establish an effective and efficient sIgE cut-off supporting the diagnosis and the indication of allergen immunotherapy (AIT).

Material and methods. The clinical history of 1172 patients previously diagnosed with seasonal rhino-conjunctivitis and with the suspicion of allergy to olive and/or grass was retrospectively reviewed, including data of symptoms, sIgE to the whole extract and to main allergenic components (Ole e 1, Ole e 7, Ole e 9 and Phl p 1+5). ROC curves were performed considering final diagnosis and AIT as primary outcomes to obtain the best cut-offs.

Results. At univariate ROC analysis, sIgE to Lolium and sIgE to Olea showed the best AUC (0.957; 95% CI 0.945-0.968 and 0.950; 95% CI 0.936-0.963, respectively). Thus, sIgE to Lolium of 1.79 kU/l (sensitivity 91.60%) was appropriated to discard and of 5.62 kU/l (specificity 92.27%) was appropriated to confirm grass allergy, whereas 8.83 kU/l was optimal for the indication of AIT (AUC 0.872; 95% CI 0.849-0.896). Furthermore, sIgE to Olea of 2.41 kU/l (sensitivity 91.52%) and of 6.49 kU/l (specificity 91.75%) were appropriated to discard or confirm olive allergy, respectively. Considering the indication of AIT, Ole e 1 showed the best AUC (0.86; 95% CI 0.83-0.88) with a sIgE cut-off of 14.05 kU/l (AUC 0.86; 95% CI 0.83-0.88).

Conclusions. Results show differences between the standard cut-off (0.35 kU/L) and those obtained by us. These calculated cut-offs would allow shortening the gap between sensitization and clinical diagnosis of seasonal allergic rhinitis and the indication of AIT.



Sesión Poster. Inmunoterapia (I). Pantalla 5

P-077

GENERACIÓN DE LINFOCITOS INFILTRANTES DE TUMOR CD3+/PD1+ PARA TERAPIA CELULAR ADOPTIVA EN CÁNCER DE MAMA AVANZADO O TRIPLE NEGATIVO.

González, E. Azucena[1]; Español-Rego, Marta[1]; Heredia, Libertad[1]; De Guzman, Catherine[2]; Olesti, Eulalia[3]; Calvo, Gonzalo[3]; Chic, Nuria[4]; Juan, Manel[5]; Prat, Aleix[6]; Pascal, Mariona[7].

[1]Immunology department. Hospital Clínic, Barcelona. Institut d'investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona; [2]Immunology Department. Hospital Clínic, Barcelona; [3]Advanced Therapies Unit. Hospital Clínic, Barcelona; [4]Medical Oncology Department. Hospital Clínic.Translational Genomics and Targeted Therpeutics in Solid Tumors Group (IDIBAPS), Barcelona; [5]Immunology Department. Hospital Clínic. Institut d'investigacions Biomèdiques August Pi i Sunyer (IDIBAPS) University of Barcelona, Barcelona; [6]Medical Oncology Department.Hospital Clinic. Translation Genomics and Targeted Therapeutics in Solid Tumpors Group (IDIBAPS). University of Barcelona, Barcelona; [7]Immunology Department. Hospital Clínic. Translational Genomics and Targated Therapeutics in Solid Tumors Group) IDIBAPS). University of Barcelona., Barcelona.

El cáncer de mama es el segundo tipo tumoral más frecuente en España, con más de 32.000 nuevos casos al año, siendo la primera causa de muerte relacionada con cáncer en mujeres (*Globocan 2018*), lo que pone de manifiesto la necesidad de encontrar alternativas terapéuticas más eficaces. La terapia celular adoptiva (TCA), que consiste en aislar células del sistema inmunitario del propio paciente y manipularlas en el laboratorio para su posterior infusión, ha logrado resultados esperanzadores en algunos tumores avanzados politratados (especialmente melanoma) presentándose como una terapia prometedora en estos pacientes, ya sea en monoterapia como en combinación con otros tipos de inmunoterapia (como los *checkpoint inhibitrs*). Sin embargo, a día de hoy, pocos datos existen de esta terapia en pacientes con cáncer de mama avanzado/metastásico.

Nuestro objetivo es determinar la factibilidad de seleccionar TILs PD1+ y su posterior expansión para producir TCA a partir de biopsias de pacientes con cáncer de mama avanzado. Hasta la fecha, se han conseguido aislar y expandir TILs en un 76% de las muestras tumorales de mama procesadas (22 de 29). Además, se han llevado a cabo tres expansiones celulares completas en un biorreactor celular (*Xuri Cell Expansion System*) bajo condiciones GMP, consiguiendo en todas ellas un producto final de más de $1x10^9$ TILs (mediana $15x10^9$ TILs) con más de un 90% de CD3+.



P-078

IMMUNOMODULATORY AND ANTIOXIDANT CAPACITY OF BACTERIORRUBERIN ON MESENCHYMAL STEM CELLS AND PERIPHERAL BLOOD MONONUCLEAR CELLS.

Medina García, Miguel; Baeza Morales, Andrés; Martinez Peinado, Pascual; Pascual García, Sandra; López Jaén, Ana Belén; Sempere Ortells, Jose Miguel.

Universidad de Alicante, Alicante.

The immunomodulatory and toxic effects of various pigments have been observed in different studies and how they can affect different cells of the immune system and mesenchymal cells (MSCs), with promising results. Because of this, MSCs have been proposed as a therapy for various autoimmune diseases. However, there is no study at present that focuses on the use of bacterioruberin as an immunomodulatory or therapeutic agent and what its effect on these cell populations might be.

In the present study, physiological response studies have been carried out in MSC and in different cell populations of the immune system, with the aim of determining the antioxidant and immunomodulatory capacity of bacterioruberin. For this purpose, both MSC and peripheral blood mononuclear cells were cultured with different concentrations of our pigment of interest. To observe the direct effect of bacterioruberin on the different cell groups, measurements of various factors were made at different incubation times, such as cell division and proliferation, apoptosis, or modulation of regulatory T lymphocytes. In addition, to observe the immunomodulatory capacity, we intend to co-culture peripheral blood mononuclear cells with MSCs, with the goal of being able to test this possible immunomodulatory effect in cell lines of autoimmune diseases, such as rheumatoid arthritis or lupus erythematosus.

The results obtained in the first phase of the study show that the use of the pigment in the different cell cultures together with peripheral blood mononuclear cells does not induce apoptosis at any of the concentrations used, which are between 150 µl/ml and 1.25 µl/ml.



P-079

THE ACTIVATION OF TLRS BY THEIR LIGANDS POLARIZES MACROPHAGES TOWARDS M1 AND ACTIVATES ITS CYTOCIDAL FUNCTION AGAINST GLIOBLASTOMA STEM CELLS IN *IN VITRO* GLIOBLASTOMA MODELS

Fraga-Lodeiro, María; San-Miguel, Teresa; Muñoz-Hidalgo, Lisandra; Gozalbo, Daniel; Cerdá-Nicolás, Miguel; López-Ginés, Concha; Gil, María Luisa; Monleón, Daniel; Cabrera-Pastor, Andrea; Megías, Javier.

Universidad de Valencia, Valencia.

Glioblastoma (GBM) is the most frequent and most malignant primary brain tumor. The median survival rate of treated patients will not exceed two years, so new and more effective therapies should be investigated. GBM stem cells (GSC) have been linked to resistance to treatments, therefore one of the most promising lines of research against GBM is based on inducing the differentiation of GSC into mature GBM cells, which are more susceptible to treatments. Recently, the induction of GSC differentiation through Toll-like Receptors (TLR) signaling has been studied. In the present work, two *in vitro* models have been developed for the study of GSCs: one of glioblastoma cells enriched in GSC and a co-culture of GSC and macrophages, a cell type highly relevant in the tumor microenvironment. In these models we studied the effect of TLR activation by three of their ligands (Pam_3CSK_4, LPS and imiquimod) on: GSC differentiation, cell viability and macrophage phenotype polarization towards M1 (inflammatory) or M2 (protumoral). Our results show that Pam_3CSK_4 and LPS ligands reduced the percentage of GSC and increased the proportion of mature cells in both models, making the cells more vulnerable to treatment with temozolomide (main chemotherapeutic drug used against this tumor). In addition, both ligands induced the differentiation of macrophages to their M1 phenotype with antitumor function, which reduced the viability of glioblastoma cells, especially in the presence of temozolomide. Imiquimod was shown to have a role in the differentiation and elimination of glioblastoma cells by decreasing cell viability in the presence of temozolomide in the *in vitro* glioblastoma model and reducing the percentage of GSC and mature cells in the co-culture model. These findings enhance TLR ligands as potential drugs in immunotherapy against GBM.

Trabajo realizado en el marco del Proyecto VLC-Bioclínic AP-2021-001, LIGAM1.



P-080

EVALUATION OF A CHEMILUMINESCENT IMMUNOASSAY FOR THERAPEUTIC DRUG MONITORING OF INFLIXIMAB

Morandeira Rego, Francisco[1]; García Serrano, Lydia[1]; Padullès Zamora, Núria[2]; Santacana Juncosa, Eugènia[2]; Pol Pol, Elisabet[3]; Segura Guerrero, Marina[3]; Julià Benique, Maria Rosa[3].

[1]Department of Immunology, Hospital de Bellvitge, L'Hospitalet de Llobregat; [2]Department of Pharmacy, Hospital de Bellvitge, L'Hospitalet de Llobregat; [3]Department of Immunology, Hospital Universitari Son Espases, Institut d'Investigació Sanitària Illes Balears (IdISBa), Palma.

Introduction

Therapeutic drug monitoring of Infliximab (IFX) is useful for optimizing therapy of inflammatory diseases. ELISA is the most commonly used technique to measure IFX through levels, nevertheless, it is time-consuming and requires analysis of multiple samples at once to reduce costs. The chemiluminescent analyzer i-Track10 is a fully automated random acces system that could be a good alternative to ELISA.

Objective

To evaluate the performance of the CLIA analyzer i-Track10 (Theradiag) to measure Infliximab concentration in serum by comparing it with the ELISA kit Promonitor (Grifols).

Methods

117 serum samples: 65 from Hospital Son Espases, 52 from Hospital de Bellvitge, were obtained from patients treated with IFX (mean age: 45±15 years, 66% men) and analyzed by ELISA and CLIA. Diagnoses are shown in table 1. Results were compared using Pearson's correlation and Passing-Bablock regression.

Results

2 out of the 117 pair of results were excluded from the statistic analysis because its diferences were considered outliers. The values of the 115 remaining samples showed a Pearson's correlation of r=0.97. Passing-Bablock regression showed this equation: y=1.03x + 0.67
The analysis did not show a proportional difference (slope: 1.026; 95% CI: 0.977-1.075) but it showed a constant difference given that the 95% confidence interval (CI) of the intercept did not contain 0 (intercept: 0.678; 95% CI: 0.037-1.312).

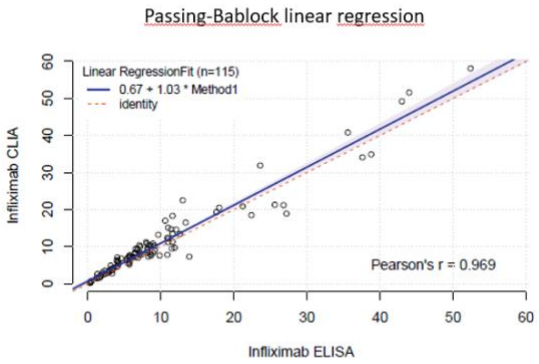
Diagnosis	Frequency
Crohn's disease	50.4%
Ulcerative colitis	31.3%
Ankylosing spondylitis	4.3%
Crohn+ rheumatoid arthritis	1.7%
Ulcerative colitis+arthritis	1.7%
Rheumatoid arthritis	0.9%
Crohn+ spondylitis	0.9%
Ulcerative colitis+ spondylitis	0.9%
Other diagnoses	7.7%

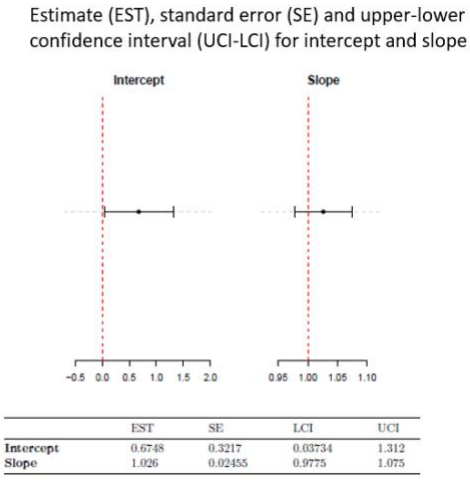
Table 1

Conclusions

There is a strong correlation between the results of Promonitor and i-Track10, but even though the values are very similar, according to Passing-Bablock regression the 2 methods are not interchangeable.

The substitution of the ELISA method for the CLIA method would involve a period of adaptation to the new values.





	EST	SE	LCI	UCI
Intercept	0.6748	0.3217	0.03734	1.312
Slope	1.026	0.02455	0.9775	1.075



P-081

EVALUATION OF A CHEMILUMINESCENT IMMUNOASSAY FOR THERAPEUTIC DRUG MONITORING OF ADALIMUMAB

García Serrano, Lydia[1]; Morandeira Rego, Francisco[1]; Padullès Zamora, Núria[2]; Santacana Juncosa, Eugènia[2]; Pol Pol, Elisabet[3]; Segura Guerrero, Marina[3]; Julià Benique, Maria Rosa[3].

[1]Department of Immunology, Hospital de Bellvitge, L'Hospitalet de Llobregat; [2]Department of Pharmacy, Hospital de Bellvitge, L'Hospitalet de Llobregat; [3]Department of Immunology, Hospital Universitari Son Espases, Institut d'Investigació Sanitària Illes Balears (IdISBa), Palma.

Introduction

Therapeutic drug monitoring of Adalimumab (ADL) is useful for optimizing therapy of inflammatory diseases. ELISA is the most commonly used technique to measure ADL through levels, nevertheless, it is time-consuming and requires analysis of multiple samples at once to reduce costs. The chemiluminescent immunoassay (CLIA) analyzer i-Track10 is a fully automated random acces system that could be a good alternative to ELISA.

Objective

To evaluate the performance of the CLIA analyzer i-Track10 (Theradiag) to measure Adalimumab concentration in serum by comparing it with the ELISA kit Promonitor (Grifols).

Methods

71 serum samples: 26 from Hospital Son Espases, 45 from Hospital de Bellvitge, were obtained from patients treated with ADL (mean age: 46±16 years, 56% women) and analyzed by ELISA and CLIA. Diagnoses are shown in table 1. Results were compared using Pearson's correlation and Passing-Bablock regression.

Results

3 out of the 71 pair of results were excluded from the statistic analysis because its diferences were considered outliers. The values of the 68 remaining samples showed a Pearson's correlation of r=0.98. Passing-Bablock regression showed this equation: y=0.89x + 0.4.
The analysis did not show a constant difference (intercept: 0.405; 95% CI: -0.1861-0.9961), but it showed a proportional difference given that the 95% confidence interval (CI) of the slope did not contain 1 (slope: 0.889; 95% CI: 0.843-0.935).

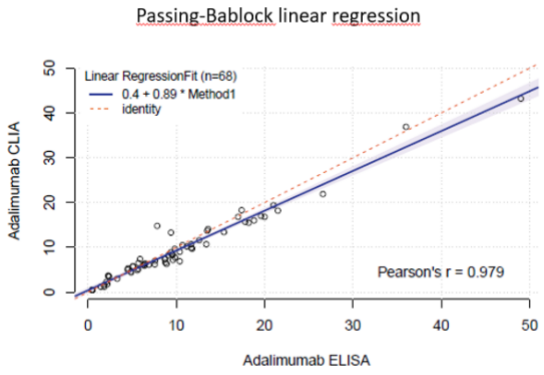
Diagnosis	Frequency
Crohn's disease	59.2%
Ulcerative colitis	14.1%
Rheumatoid arthritis	9.9%
Psoriasis	4.2%
Hidradenitis	4.2%
Ankylosing spondylitis	1.4%
Other diagnoses	7.0%

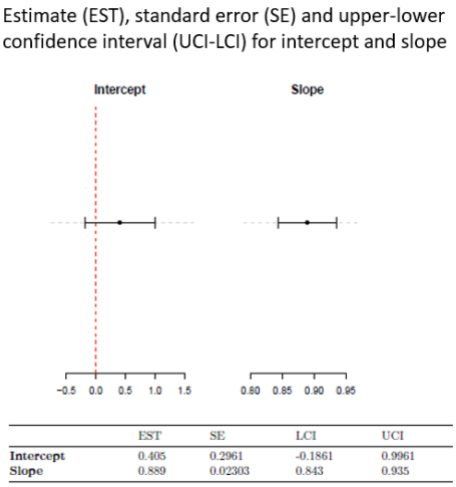
Table 1

Conclusions

There is a strong correlation between the results of Promonitor and i-Track10, but even though the values are very similar, according to Passing-Bablock regression the 2 methods are not interchangeable.

The substitution of the ELISA method for the CLIA method would involve a period of adaptation to the new values.





	EST	SE	LCI	UCI
Intercept	0.405	0.2961	-0.1861	0.9961
Slope	0.889	0.02303	0.843	0.935



P-083

STUDY OF THE EFFICACY OF INTRAVENOUS BCG IN A METASTATIC LUNG ADENOCARCINOMA MOUSE MODEL.

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Universidad de Zaragoza, Zaragoza.

Bacillus Calmette-Guérin (BCG) is a live attenuated mycobacterium vaccine and it is at present the only tuberculosis vaccine used in humans. Since more than four decades ago, intravesical BCG represents a gold standard therapy for high-risk non-muscular invasive bladder cancer. Recently, systemic delivery of BCG has recently been reported to drive profound changes in bone marrow (BM) myelopoiesis providing a more efficient capacity of response against a subsequent tuberculosis infection. However, i.v. BCG has never been studied in the context of orthotopic lung tumors. Here a metastatic lung adenocarcinoma mouse model was generated to study the antitumoral response of intravenous (i.v.) BCG.

Mice inoculated with i.v. BCG prior to tumor cells inoculation showed higher survival compared to non-treated mice. However, i.v. BCG delivery after tumor challenge did not provide any therapeutic advantage, suggesting that tumor might be influencing i.v. BCG efficacy. A congenic BM transplant mouse model suggested the ability of i.v. BCG to re-educate haematopoietic progenitors to overcome tumor influence. Moreover, prior administration of i.v. BCG avoided myeloid-derived supressor cells (MDSCs) accumulation in lungs of tumor-bearing mice, in contrast to i.v. BCG after tumor challenge leading to an immunosuppresive ambience. Finally, therapeutic combination of i.v. BCG with gemcitabine, which reduce MDSCs at low doses, futher increased mouse survival compared to gemcitabine in monotherapy.

As a result, combination of i.v. BCG with chemotherapy could represent an attractive therapeutic approach for advanced lung cancer.



P-084

LA ADICIÓN DE CÉLULAS TRONCALES MESENQUIMALES *EX VIVO* DISMINUYE LA EXPRESIÓN DE MARCADORES DE DEGRADACIÓN DE LA MATRIZ EXTRACELULAR Y DE LINFOCITOS T *NAÏVE*

Pascual García, Sandra; Pujalte Satorre, Carolina; Martínez Peinado, Pascual; López Jaén, Ana Belén; Baeza Morales, Andrés; Medina García, Miguel; Sempere Ortells, José Miguel.

Universidad de Alicante, San Vicente del Raspeig.

Las células troncales mesenquimales (MSC) son células multipotentes que, debido a la liberación de factores solubles o al contacto directo con otras células, presentan una gran capacidad inmunomoduladora e inmunosupresora. Esto las ha convertido en una potencial herramienta terapéutica para diversas enfermedades inflamatorias.

El objetivo del presente estudio es analizar la expresión de marcadores de degradación de la matriz extracelular, metaloproteinasa de matriz (MMP) 9, y de linfocitos T *naïve*, CD45RA, en células mononucleares de sangre periférica (PBMC) tras el cocultivo con MSC derivadas de tejido adiposo.

Las PBMC se aislaron de muestras de sangre anticoagulada de voluntarios sanos (n=5) mediante centrifugación en gradiente de densidad. Posteriormente, se realizó un cocultivo con MSC:PBMC (10^4:10^5 células) en medio RPMI-1640 suplementado con 10% suero de ternera fetal, 1% antibióticos y 1% glutamina. Las células fueron estimuladas con 20 ng/ml PMA y se incubaron durante 24h a 37ºC y 5% CO_2. Se añadió a las PBMC, 3 µg/ml brefeldina A y se volvieron a incubar durante 3h. Finalmente, se fijaron, se permeabilizaron y se marcaron con los anticuerpos anti-MMP-9-Alexa Fluor-488 y anti-CD45RA-PE. El análisis se realizó con el citómetro de flujo EPICS XL.

Las PBMC mostraron una expresión de MMP-9 y de CD45RA en torno al 70-80%, tanto en condiciones sin estimular como estimuladas. La adición de MSC a las PBMC produjo una disminución en la expresión de MMP-9 tanto en condiciones controles, como estimuladas (p<0,05). El cocultivo de ambas poblaciones celulares causó una ligera disminución de CD45RA en las PBMC en condiciones sin estimular y estimuladas.

En conclusión, estos resultados muestran que las MSC producen una disminución en la expresión de MMP-9 y de CD45RA, confirmando su papel antiinflamatorio e inmunosupresor, lo que abriría la posibilidad a utilizarlas como terapia celular frente a enfermedades autoinmunes, como la artritis reumatoide.



P-085

EFICACIA DE ANAKINRA EN UNA PACIENTE CON PERICARDITIS RECURRENTE REFRACTARIA

Moncayo Muñoz, Ana Paulina; Díaz Luna, María De Las Mercedes; Mejía, María Alejandra; Fernandez-Cruz, Eduardo; Carbone, Javier.

Hospital General Universitario Gregorio Marañon, Madrid.

Introducción

La pericarditis refractaria se asocia con una importante afectación de la calidad de vida de los pacientes y a riesgos de complicaciones graves. Describimos una paciente con pericarditis de larga evolución tratada con Anakinra por esta indicación.

Métodos

Recogida de datos de la historia clínica personal electrónica. Seguimiento prospectivo. Datos obtenidos en visitas clínicas protocolizadas realizadas.

Resultados

Mujer de 34 años que en 2016 presentó el primer episodio de pericarditis. Como antecedentes refiere una picadura de garrapata en 2008 con serología positiva frente a *Borrelia Burgdorferi*, presentó eritema migrans recibiendo tratamiento con Tetraciclina. Los estudios de autoinmunidad fueron negativos. RMN cardiaca en 2019 presenta datos de lesión pericárdica leve y fibrosis incipiente. Ha tenido entre 3-4 episodios por año de pericarditis consistentes en dolor retroesternal de moderada-grave intensidad, disnea que exacerba con las actividades físicas, limitación de tareas de vida diaria, sueño no reparador por el dolor. El tratamiento inicial fue: AAS, colchicina, ciclos de glucocortioides a altas dosis con reducción gradual de dosis, hidroxicloroquina, y azatioprina; sin cambios a nivel clínico ni radiológico de la pericarditis. La paciente fue remitida a nuestro servicio en 2020, presentaba hipogamaglobulinemia leve con déficit de subclases IgG3 y consumo de complemento C3 y C4, también se realizó un panel de secuenciación genética siendo negativo. Inició el tratamiento con Anakinra 100 mg SC autoadministrados diarios en mayo del 2020 con respuesta clínica favorable, refería dolor torácico leve, deambulación sin limitaciones y sueño reparador durante 6 meses. No hubo complicaciones infecciosas.

Conclusión

En un caso de pericarditis recurrente se observa respuesta favorable inicial frente al uso de Anakinra sin complicaciones aparentes, siendo esta una opción para mantener una aceptable



calidad de vida en una paciente previamente muy sintomática y largamente tratada con otras opciones de terapia convencionales.



P-086

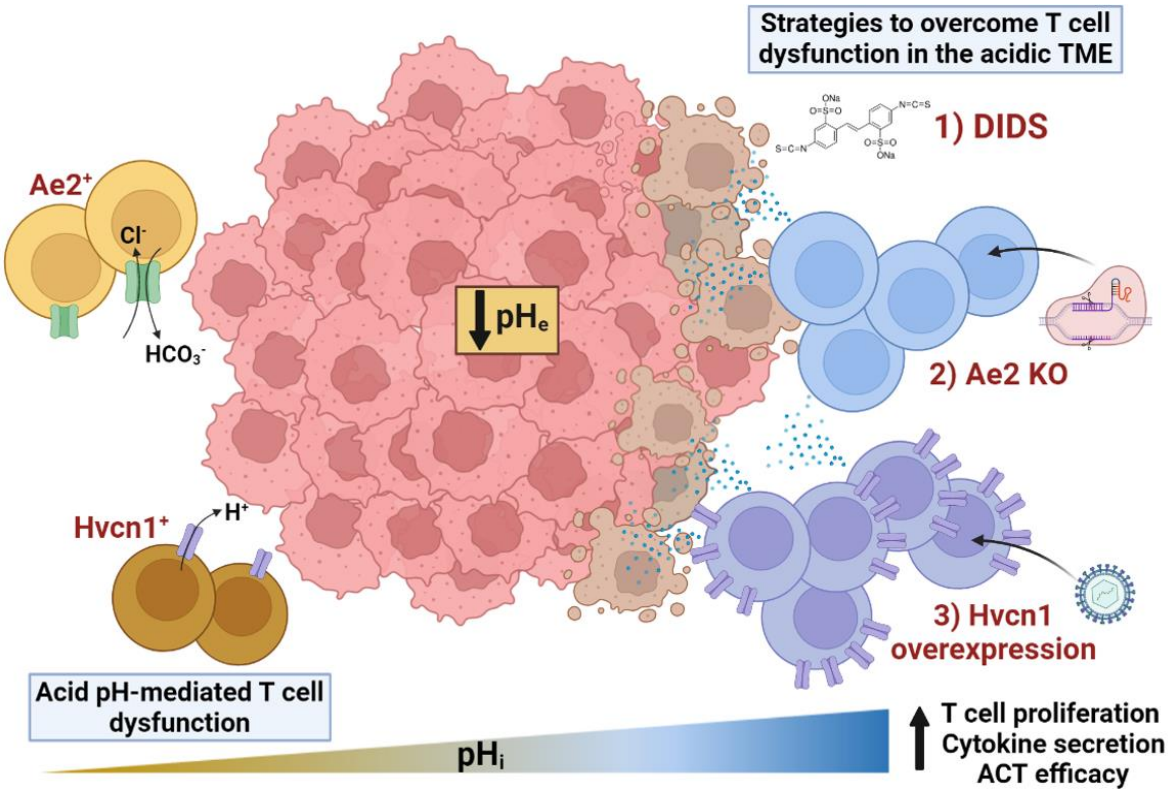
OVERCOMING T CELL DYSFUNCTION IN THE TUMOR MICROENVIRONMENT'S ACIDIC PH TO ENHANCE ADOPTIVE T CELL TRANSFER IMMUNOTHERAPY

Navarro Negredo, Flor[1]; Casares, Noelia[2]; Martin-Otal, Celia[2]; Lasarte-Cia, Aritz[2]; Covo, Angela[3]; Gorraiz, Marta[2]; Sarrion, Patricia[2]; Llopiz, Diana[2]; Reparaz, David[2]; Varo, Nerea[4]; Rodriguez-Madoz, Juan Roberto[2]; Prosper, Felipe[2]; Hervas-Stubbs, Sandra[2]; Lozano, Teresa[2]; Lasarte, Juan Jose[2].

[1]Centro de Investigación Medica Aplicada (CIMA), Pamplona; [2]CIMA, Pamplona; [3]Universidad de Navarra, Pamplona; [4]Clínica Universitaria de Navarra, Pamplona.

The high metabolic activity and insufficient perfusion of tumors leads to the acidification of the tumor microenvironment (TME). The acidified intracellular pH (pH_i) of tumor infiltrating T cells resuls in inhibited T cell function and anti-tumor activity. We found that pharmacological inhibition of the acid loader chloride/bicarbonate anion exchanger 2 (Ae2) with 4,4'-diisothiocyanatostilbene-2,2'-disulfonicacid (DIDS) alkalinized the pH_i of $CD4^+$ and $CD8^+$ T cells and enhanced their function upon TCR activation *in vitro*, especially under acid pH conditions. *In vivo*, DIDS administration delayed B16OVA tumor growth in immunocompetent mice as monotherapy or when combined with adoptive T cell transfer immunotherapy using OVA-specific T cells. Notably, the genetic silencing of Ae2 in OVA-specific T cells alkalinized the pH_i of $CD4^+$ and $CD8^+$ T cells and improved their function *in vitro* as well as their anti-tumor activity *in vivo*. B16OVA-challenged mice treated with Ae2 KO OVA-specific T cells had significantly better overall survival than those treated with control T cells. Similarly, genetic modification of OVA-specific T cells to overexpress the voltage gated hydrogen channel 1 (Hv1), a selective H^+ extruder that prevents T cell acidification, significantly improved T cell function *in vitro*, even at acid pH conditions. B16OVA tumor growth was significantly delayed when mice were given adoptive T cell transfer immunotherapy of Hv1-overexpressing OVA-specific T cells indicating that the overexpression of Hv1 results in a better anti-tumor T cell response. Hv1 overexpression also improved the anti-tumor activity of chimeric antigen receptor (CAR) T cells specific for Glypican 3 (GPC3) in mice bearing PM299L-GPC3 hepatocellular carcinoma tumors. Our results demonstrate that preventing intracellular acidification by regulating the expression of acidifier ion channels such as Ae2 or alkalinizer channels like Hv1 in tumor-specific lymphocytes enhances their antitumor response by making them more resistant to the acidic TME.




Strategies to overcome T cell dysfunction in the acidic TME
1) DIDS
Ae2+
Cl-
HCO3-
pHe
2) Ae2 KO
Hvcn1+
H+
3) Hvcn1 overexpression
Acid pH-mediated T cell dysfunction
pHi
T cell proliferation
Cytokine secretion
ACT efficacy



P-087

LIPOSOMES CONJUGATED WITH THE 9 KDA ISOFORM OF GRANULYSIN POTENTIATES ITS CYTOTOXICITY AGAINST TUMOR CELLS

Soler, Ruth[1]; Guerrero, Patricia[1]; Marco, Joaquín[1]; Ibáñez, Raquel[1]; Marzo, Isabel[1]; Martínez, Luis[2]; Anel, Alberto[1].

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9 kDa granulysin (GRNLY) is a human cytolytic protein secreted by cytotoxic T lymphocyte CTL and NK cells of the immune system whose demonstrated physiological function is the elimination of bacteria and parasites. In previous studies of our group it was demonstrated the anti-tumoral capacity of recombinant granulysin, both *in vitro* and *in vivo*. In the present work we have developed lipid nanoparticles whose surface can bind recombinant granulysin through the formation of a complex of coordination between the histidine tail of the protein and Ni^{2+} provided by a chelating lipid in the liposome composition and termed them LUV-GRNLY, for granulysin-bound large unilamellar vesicles. The objective of this formulation is to increase granulysin concentration at the site of contact with the target cell and to increase the cytotoxicity of the administered dose. The results obtained indicate that recombinant granulysin binds to the surface of the liposome with high efficiency and that its cytotoxicity is significantly increased in association with liposomes. In addition, we also demonstrate that the main mechanism of death induced by both granulysin and LUV-GRNLY is apoptosis. Jurkat-shBak cells are resistant to GRNLY and also to LUV-GRNLY, showing that LUV-GRNLY uses the mitochondrial apopototic pathway to induce cell death. On the other hand, we show that LUV-GRNLY induces the expression of the pro-apoptotic members of the Bcl-2 family Bim and especially Puma, although it also induced the expression of anti-apoptotic Bcl-X_L.



P-088

INFLAMMATORY RESPONSE IN BLADDER FIBROBLASTS INDUCED BY BACILLUS CALMETTE-GUÉRIN-IMMUNOTHERAPY FOR NON-MUSCLE INVASIVE BLADDER CANCER

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Transurethral instillations of *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) has been used for decades as immunotherapy for the treatment of high risk non-muscle invasive bladder cancer (NMIBC) patients. While relatively effective, 43% of BCG-treated patients will recur within 5 years and we do not know why. BCG-induced innate and adaptive responses are key for the desired anti-cancer immunity. But how these two are generated and connected is still not well understood. Fibroblast exert important immunological roles in many tissues. But their role in the BCG-treatment scenario is not known. Here we show that repeated instillations of BCG in healthy mice induce formation of lymphoid aggregates reminiscent of early granuloma structures, formed by leukocytes and fibroblasts. Furthermore, these fibroblasts appear activated as determined by flow cytometry. In vitro studies using mouse primary bladder resident fibroblasts (BFs) show that these cells respond directly to the attenuated pathogen. We also observed a similar inflammatory response against BCG in cancer-associated fibroblasts. We performed RNAseq analysis in FACS-sorted BFs, finding that BFs upregulate genes that control leukocyte migration. Specifically, we found that BFs and no other cell types, present a robust increased the expression of Ccl2, Cxcl1 and Cxcl2, among other chemotactic factors. Transwell experiments show that BCG-treated BFs attract monocytes and neutrophils. Besides, instillation of BCG in Ccr2-KO mice shows reduced inflammation in the bladder, which further supports



the importance of the CCR2/CCL2 axis to mount an adequate organ response against BCG. Finally, statistical analysis suggests that the presence of fibroblasts in the tumour tissue is detrimental for the efficacy of BCG therapy. In summary, we found that BFs directly respond to BCG and acquire an inflammatory phenotype likely controlling immune cell recruitment. Association of high expression of fibroblast markers with poor prognosis suggests that fibroblast actions may reduce efficacy of BCG-induced immunotherapy.



P-090

ARTIFICIAL INTELLIGENCE-DRIVEN, DECENTRALIZED PRODUCTION FOR ADVANCED THERAPIES IN THE HOSPITAL

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Artificial intelligence (AI) is a very broad concept and encompasses many disciplines that have emerged with the advancement of computing and data science. We could define AI as any technique that allows a computer to mimic human intelligence. But within AI we have different disciplines that support it. One of them and the most important is Machine learning, it is a discipline within AI that includes those techniques that allow machines to improve tasks with experience. And within machine learning we would have its main base, which is deep learning, which is a system based on neural networks that allow any machine to train itself to perform a task.

Artificial intelligence has many potential applications, such as business, commerce, transport or health services. In the health system, the appearance of a large amount of data has allowed us to improve and implement systems for diagnosis, prognosis, treatments, etc. These factors have created the need to improve the multidisciplinary relationship between the world of engineering and the world of health, allowing the creation of collaborative consortiums at different levels to improve the quality of life of patients.

Faced with these needs, the AIDPATH (Artificial Intelligence-driven, Decentralized Production for Advanced Therapies in the Hospital) EU consortium was born. Its principal aim is to facilitate and improve the next generation of personalized medicine with genetically engineered T-lymphocytes, modified to express a synthetic chimeric antigen-specific receptor (CART), in EU hospitals. AIDPATH will use AI technology to integrate patient-specific data and biomarkers into CART therapy and optimize manufacturing, scheduling and resource planning to reduce costs and hospital resource utilization. Our task in the consortium is the recruitment of CART manufacturing, patient follow-up and resource management data to improve the AI system.



P-091

ASSESSMENT OF MESENCHYMAL STEM/STROMAL CELL-BASED THERAPY IN K/BXN SERUM TRANSFER-INDUCED ARTHRITIS

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Rheumatoid arthritis (RA) is an autoimmune disease characterized by systemic and chronic inflammation in synovial tissues together with cartilage/bone destruction. Despite the advances in understanding the aetiology and mechanisms of RA and the existence of numerous anti-rheumatic drugs, significant number of individuals with RA do not respond or are intolerant to current treatments. Mesenchymal stem cells (MSCs) have emerged as a very promising treatment for different autoimmune and inflammatory diseases such as rheumatoid arthritis (RA) due to their immunosuppressive properties. Collagen-induced arthritis (CIA) mouse model is the most commonly used model in preclinical studies in MSC-based therapy. However, due to its low incidence, the mouse strain restriction and the prolonged induction phase, alternative experimental models of RA have been developed. In this study, we evaluated whether the K/BxN serum transfer-induced arthritis (STIA) could be used as an alternative model to study the MSC-based therapy. Surprisingly, our data suggest that allogeneic adipose-derived MSC therapy was unable to modulate the course of systemic joint inflammation in arthritic mice despite the various experimental conditions and protocols tested. These results suggest that the discrepancies in the efficacy of MSC-based therapy among the arthritic models may be based on the differences in the pathogenesis of inflammation according to the different immune statuses and monocytic/macrophage balance in the inflamed mice among the different preclinical models of arthritis. These observations could help to identify those RA patients most likely to respond to MSC-based therapy that will clearly accelerate the clinical translation of MSC.



P-092

IMPROVED FUNCTIONALITY OF GENETICALLY ENGINEERED HUMAN MESENCHYMAL STEM/STROMAL CELLS EXPRESSING CXCR4 AND IL10 IN EXPERIMENTAL COLITIS

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Mesenchymal stem/stromal cell (MSCs)-based therapy represents a potential alternative treatment for immune-mediated disorders due to their immunomodulatory and regenerative properties. In this context, preclinical data from animal models and clinical trials have demonstrated that MSC therapy is safe, although a significant heterogeneity exists in terms of therapeutic efficacy in the clinic. Inflammatory bowel diseases (IBD) consist on a chronic inflammation of intestinal mucosa, thus, the reported heterogeneity in the therapeutic efficacy of MSC-based therapy may be due to a limited trafficking of infused MSCs to the damaged intestinal tissue. To improve the efficacy of MSC-based therapy, we tested genetically engineered adipose-derived MSCs carrying the CXCR4 to enhance their trafficking to inflamed tissues. Additionally, interleukin 10 gene was used to improve the anti-inflammatory potential of the MSCs. The therapeutic efficacy of CXCR4/IL10-expressing MSCs was analysed using the dextran suphate sodium (DSS)-induced colitis model. A single dose of CXCR4/IL10-expressing MSCs reduced the disease activity index, total body weight losses and the incidence of colitis together with increased survival compared to unmodified MSCs suggesting that CXCR4/IL10-expressing MSCs represent a promising and more efficacious therapeutic approach for the treatment of inflammatory bowel diseases.



P-094

IN SILICO LYNCH SYNDROME-RELATED NEOANTIGENS PREDICTION AND VALIDATION FOR A DENDRITIC-CELL BASED CANCER PREVENTION VACCINE

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Introduction:

Lynch syndrome (LS), caused by germline mutations on DNA mismatch-repair genes (MMR) predisposes individuals to colorectal and endometrial cancer (CRC, EC) amongst other tumors. Although CCR prevention methods are effective, no preventive strategies exist for the majority of LS-related tumors. Ex-vivo generated and tumor-antigen-loaded dendritic cell (DC) vaccines have mainly been used in advanced cancers therapy; however their full therapeutic potential would likely be as a preventive approach in high-risk cancer patients. LS is a paradigmatic model for its limited and predictive mutational spectrum in repetitive DNA sequences termed microsatellites (MS). We have identified the main frame-shift derived neopeptides (FSDN) that are shared among cancers from LS patients to develop a cancer preventive DC-based neoepitope vaccine.

Methods:

Selection of LS-related coding MS and FSDN published in literature and in public databases (Seltar Database; The Cancer Genome Atlas, TCGA). Application of neoepitope prediction pipelines to prioritize FSDN with high coverage on the most frequent HLA-I and HLA-II haplotypes (pVACbind; pVACtools v2.0.1). Validation of the selected FSDN in colorectal adenomas (CrAD), EC and CRC samples from LS patients and non-LS MS instability (MSI) tumors through mutational signature analysis, HLA-typing and neoantigens prediction (pVACseq).

Results:

98 FSDN restricted to HLA-I and HLA-II were prioritized. Validation analysis determined that selected FSDN A) virtually bind up to 36 HLA-II and 60 HLA-I alleles, B) Have sequence similarity to known pathogens and no significant self-similarity, C) Are virtually presented in CrAD, EC and CRC LS samples, and C) 29 FSDN-deriving mutations in coding-MS have high



presence in non-LS MSI-h tumors.

Conclusions:

Our predicted neoepitope set has an optimal coverage among LS patients in terms of HLA alleles, associated cancers and prevalence and is highly represented in non-LS MSI tumors. *In vitro* immunogenicity studies are ongoing to consolidate an optimal FSDN set.



P-095

EXTINCTION CATASTROPHE OF ANTIVIRAL DRUGS MOLNIPIRAVIR (MK-4482), REMDESIVIR AND 4 -FLUOR URIDINE (4FIU) ACTING AS A NUCLEOSIDE ANALOGUES AGAINST PANDEMIC HUMAN CORONAVIRUSES.

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Error catastrophe could be defined as a cumulative loss of genetic information in alineage of organisms due to high mutation rates. The process leading to viral extinctionthrough the accumulation of errors is known as lethal mutagenesis. Error catastropheoccurs when the mutation rate exceeds an error threshold. Viruses and bacteria haveevolved to mainto maintain a characteristic error rate. RNA viruses have very high mutation rates and replicate near the error threshold for the maintenance of genetic information .

.Error catastrophe occurs when the mutation rate exceeds an error threshold. Viruses and bacteria haveevolved to maintain a characteristic error rate. RNA viruses have very high mutation ratesand replicate near the error threshold for themaintenance of genetic information Molnupiravir (a prodrugof β-D-N4-hydroxycytidine) has been recently approved for the treatment of SARS-CoV-2 infection. Its triphosphate derivative can be incorporated into viral RNA and extended by the coronavirus RNApolymerase. Incorrect base pairing and inefficient extension by the polymerase promote mutagenesisby increasing the G→A and C→U transition frequencies. Molnupiravir is the isopropylester prodrug of the ribonucleoside **analogue β-D-N4-hydroxycytidine (NHC). NHC** is abroad-spectrum antiviral compound that inhibits the replicationof multiple viruses in cell culture (e.g., Chikungunya virus, Venezuela equine encephalitis virus, respiratory syncytial virus, hepatitis C virus, norovirus, influenza A and B viruses, Ebola virus, and human coronaviruses) (3). The triphosphorylated derivative of NHC is a substrate for viral RNA polymerases andinterferes with viral replication. In cell culture assays, molnupiravir was found to be a potent inhibitor of SARS-CoV-2replication with an EC50 in the submicromolar range.

Nucleotide analogues **Remdesivir, Molnipiravir and Paxlovid (Ritonavir +Nirmatrelvir)** remains as the only drugs approved by regulatory agencies to treat COVID-19.

A new drug such as **4-FIU** also an analogue of Uracil; have a “terminal chain effect” that must be carefully studied.



P-096

RECRUITMENT OF CCR2^{+} MONOCYTES IS NOT ESSENTIAL FOR THE BENEFICIAL EFFECT OF MESENCHYMAL STEM/STROMAL CELL-BASED THERAPY IN EXPERIMENTAL COLITIS

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Inflammatory bowel diseases (IBD) are characterized by persistent and relapsing inflammatory processes of the intestinal mucosa related to genetic background, environmental factors and commensal microbiota. In spite of recent advances in clinical treatments leading to decrease inflammation, significant numbers of IBD patients experienced severe side effects or loss of response to current treatments. Thus, new effective alternatives are highly needed. In this sense, mesenchymal stem/stromal cells (MSCs) have emerged as a new therapeutic strategy for IBD thanks to their immunomodulatory and regenerative properties. Although numerous immune responses and mechanisms of action of MSCs have been described, nowadays the precise mechanisms remain to be fully defined. We have previously reported a transient increase in Ly6C^{++} monocyte populations in peripheral blood following the infusion of MSCs in a model of collagen-induced arthritis. Additionally, an increase of regulatory IL10-expressing macrophages were also observed in the draining lymph nodes that paralleled to a decline in the clinical score of arthritis. Based on these data, in this study, we evaluated whether the CCR2-mediated trafficking of myeloid populations is involved in the beneficial effects of MSC-based therapy using an experimental model of colitis induced by treatment with dextran sulphate sodium (DSS). In contrast to our initial hypothesis and previously reported studies, a single dose of allogeneic adipose-derived MSCs infused in DSS-induced colitic CCR2-deficient mice was as effective as in WT mice. These results suggest that the CCR2-mediated trafficking of immune cells is not involved in the mechanism of action mediated by MSC-based therapy at least in DSS-induced colitis.



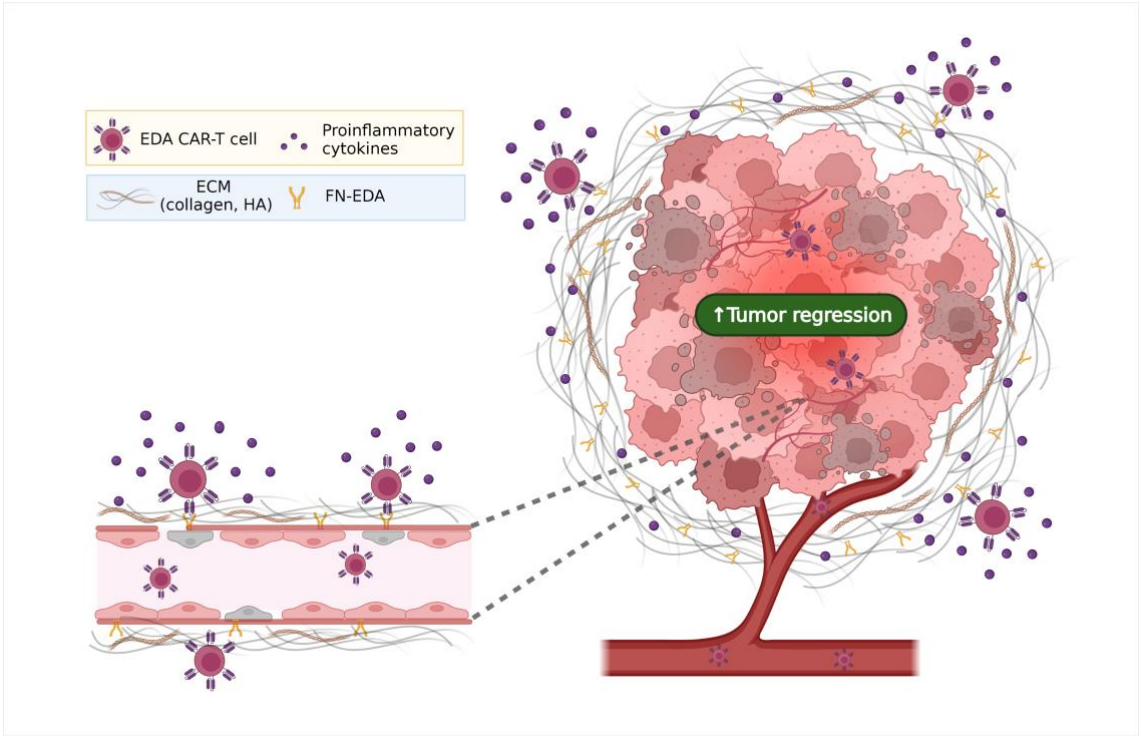
P-098

CÉLULAS EDA CAR-T FRENTE AL MICROAMBIENTE TUMORAL COMO ALTERNATIVA AL TRATAMIENTO DE TUMORES SÓLIDOS

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El microambiente tumoral (TME) de los tumores sólidos constituye una importante barrera que dificulta la eficacia antitumoral de las células CAR-T. La generación de CAR específicos de antígenos del TME podría considerarse una alternativa para superar esta limitación y mejorar así su eficacia. Dentro del TME destaca la función de la matriz extracelular en la progresión e invasión tumoral. Uno de los componentes principales de esta matriz es la fibronectina. Hemos generado un CAR específico para el *splicing* alternativo EDA de la fibronectina, que se encuentra altamente expresado en muchos tipos de tumores (hepatocarcinoma, colangiocarcinoma o cáncer de páncreas) y sin embargo, no se detecta expresión en tejidos sanos. Las células EDA CAR-T fueron capaces de reconocer y eliminar células tumorales que expresan EDA en la membrana. Interesantemente, la transferencia adoptiva de células EDA CAR-T retrasó el crecimiento tumoral en el modelo de teratocarcinoma F9, donde la expresión de EDA está asociada al endotelio del tumor y no a la propia célula tumoral. El efecto anti-tumoral observado en este modelo se acompañó de una reducción significativa de la vasculatura tumoral. Sorprendentemente, la versión humana de EDA CAR, incluyendo los endodominios humanos 41BB y CD3ζ, ejerció actividad antitumoral en el modelo de hepatocarcinoma humano PLC implantado en ratones NSG, que expresa altamente EDA en la matriz extracelular y la vasculatura endotelial. Las células EDA CAR-T exhibieron un tropismo por los tumores que expresaban EDA y no se observó toxicidad en ratones con tumor ni en ratones sanos tras la terapia. Estos resultados sugieren que la terapia celular adoptiva basada en células CAR-T dirigidas a la variante específica de tumor EDA de la fibronectina es factible y ofrece una opción terapéutica ampliamente aplicable a diferentes tipos de cáncer.


EDA CAR-T cell
Proinflammatory cytokines
ECM (collagen, HA)
FN-EDA
↑Tumor regression



P-099

POTENTIAL USE OF TREG CELLS OBTAINED FROM PEDIATRIC THYMIC TISSUE (THYTREG) AS ALLOGENEIC CELL THERAPY FOR EXACERBATED IMMUNE RESPONSES

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Due to its immunomodulatory potential, regulatory T cell (Treg) therapies have acquired great interest in the treatment of diseases where restoring immune homeostasis is crucial. Until now, autologous Treg cell therapy has proven its safety, but employing blood as the source of Treg presents limitations regarding recovery and quality. Our group has developed a protocol to produce massive amounts of GMP Treg derived from thymic tissue (thyTreg) that could overcome the main obstacles found in the current Treg therapies. We are employing thyTreg cells with success in a clinical trial as autologous cell therapy in heart transplanted children. Given the large amounts of thyTreg obtained from a single thymus, the main objective is to evaluate the possibility of the allogeneic use of thyTreg, in terms of immunogenicity and functionality.

ThyTreg obtained in our laboratory exhibit high viability (>90%) and purity (>80% CD25+FoxP3+ expression) and does not overexpress immunogenicity markers. Moreover, *in vitro* co-culture models of thyTreg with allogeneic peripheral blood mononuclear cells (alloPBMC) from healthy donors have been performed to i) determine if thyTreg trigger an immunogenic response on alloPBMC, and ii) evaluate the capacity of thyTreg to suppress the alloPBMC proliferation. Although HLA disparity in the co-cultures between thyTreg and alloPBMC was high (13 of the 21 typed pairs had HLA <4/12 concordance), thyTreg did not induce the expression of activation markers nor the proliferation or the production of pro-inflammatory cytokines (IFN-g) by alloPBMCs, even when alloPBMC were pre-stimulated (mirroring an exacerbated immune response). Moreover, thyTreg greatly inhibit allogeneic CD4 and CD8 T cells proliferation.

The results suggest that thyTreg are not immunogenic and can exert their suppressive function in an allogeneic context, indicating their possible off-the-shelf use as treatment for transplant

rejection, graft-versus-host disease, autoimmune diseases, or the cytokine release syndrome characteristic of severe COVID-19 patients.



Sesión Poster. Inmunoterapia (II). Pantalla 6

P-100

INFLUENCE OF MULTIPLE SCLEROSIS INFLAMMATORY CONTEXT IN THE DEVELOPMENT OF A TOLEROGENIC DENDRITIC CELL THERAPY

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Introduction:

Multiple Sclerosis (MS) is a chronic inflammatory disease affecting the central nervous system where autoreactive lymphocytes induce demyelination and neurodegeneration. Current treatments reduce inflammation but can't cure the disease. In this context, antigen-specific immunotherapies represent a potentially curative approach, able to re-educate immunity toward homeostasis. Our group developed an autologous tolerogenic dendritic cell product (VitD3-TolDCs) produced from peripheral blood monocytes in presence of Vitamin D3, which showed safety/tolerability in a Phase I clinical trial in patients with MS and for which we are starting a Phase II trial. However, given MS systemic inflammation, monocytes could present a proinflammatory phenotype in comparison to Healthy Donors (HD), leading to less-than-ideal VitD3-TolDCs.

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

Methods:

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

Results:

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

Conclusions:



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



P-101

A QUASI-MONOCLONAL APPROACH TO STUDY ATHEROPROTECTION BY A12 ANTIBODY.

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Atherosclerosis is the main underlying cause of **cardiovascular diseases**, the leading cause of death worldwide, and can remain asymptomatic for long periods of time. Thus, there is a **critical need for new therapeutic and diagnostic avenues in this area**. Both the innate and adaptive arms of the immune response are important in atherosclerosis. However, our knowledge on the antigenic triggers of the adaptive immune response in atherosclerosis is very limited. Recently, our lab has identified the self-protein ALDH4A1 as a novel atherosclerosis antigen targeted by the atheroprotective antibody A12, revealing ALDH4A1 and A12 as potential therapeutic targets.

To study the molecular mechanisms underlying the atheroprotective role of the A12 antibody we have developed a quasi-monoclonal mouse model that will allow both isotype switching and somatic hypermutation of the A12 antibody in vivo. We used a cassette that harbors a prerearranged Igκ VJ segment, a human Igκ constant (hukappa) region (for tracing purposes), a P2A cleavage site and a prerearranged IgH VDJ segment and inserted it into the Igh endogenous locus (IghA12/+mice). We have generated 3 independent founders.

To characterize IghA12/+ mice we first performed FACS analysis of peripheral blood samples from IghA12/+ founders and their offspring and found a subset (3-7% of B cells) of hukappa positive cells, confirming the presence of the transgenic construct. In addition, the proportion of peripheral B cells was reduced in IghA12/+ mice compared to control Igh+/+ littermates. FACS analysis of the bone marrow showed a severe reduction of hukappa+ immature B cells, strongly suggesting that hukappa+ cells are subject to strong, yet incomplete, **negative selection**.

Together IghA12/+ model provides additional evidence that A12 recognizes a self-antigen and provides a valuable tool to study A12 function in atherosclerosis.



P-102

CORRELATIVE BIOLOGICAL STUDIES RELATED TO THE RESPONSE, PEAK AND PERSISTENCE OF ARI0002H, AN ACADEMIC BCMA-DIRECTED-CAR-T CELL FOR PATIENTS WITH RELAPSED AND/OR REFRACTORY MULTIPLE MYELOMA (RRMM)

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Background

ARI0002h is an academic humanized anti-BCMA CAR T-cell, evaluated in CARTBCMA-HCB-01 clinical trial against RRMM. Here, we present the correlative biological studies performed related to disease response.

Methods

ARI0002h manufacturing data were analyzed, including the percentage of CAR expression in the final product (FP) by flow cytometry (FC). Peripheral blood (PB) samples were collected at inclusion and sequentially after infusion. T-cell subpopulations were analyzed in apheresis, FP, and PB samples at day +28. ARI0002h persistence was evaluated in PB after infusion by qPCR. Plasmatic soluble BCMA (sBCMA) and human anti-human antibodies (HAHAs) were determined prior to infusion, at day +28 and +100, and after a booster dose (starting 4 months post first infusion).

Results

Thirty-three apheresis collections were transduced, with a median manufacturing time of 11 days (range 9-14). The mean CAR expression in FP was 56% (SD ±25). A higher CD4/CD8 ratio in the apheresis (but not in the FP) was correlated with the achievement of stringent complete response (sCR) ($p=0.003$). T-cell subpopulations in the FP did not correlate with response. A significant increase of exhaustion markers was observed between the FP and at day +28 PB paired samples.



ARI0002h cells showed a maximum PB expansion at day +14. Median persistence of ARI0002h cells in PB was 5 months without correlation with RRMM response. 85% of eligible patients received a booster dose, with a subsequent CART-cell expansion in 50% patients.
All patients showed a deep decrease of sBCMA after CAR T-cell infusion.
HAHAs were detected in only 4% and 11% patients at day +100 and +6 months, respectively.

Conclusion

ARI0002h manufacturing is fast and feasible. The peak of CAR T-cell expansion occurs 14 days after the infusion, and also in some patients after the booster dose. Responses obtained are deep and construct immunogenicity seems to be low.



P-103

DASATINIB-INDUCED SPLEEN CONTRACTION LEADS TO TRANSIENT LYMPHOCYTOSIS

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Dasatinib is a tyrosine kinase inhibitor (TKI) approved for the treatment of Philadelphia-positive chronic myeloid leukemia (CML) and acute lymphoblastic leukemia. Although effective and well tolerated, patients usually exhibit a transient lymphocytosis 2 hours after the oral intake that resolves spontaneously around 4 hours later. Despite this lymphocytosis has been associated to better response to treatment, the underlying mechanism has remained elusive to date. Following the hypothesis that dasatinib could interfere with lymphocyte recirculation to and from secondary lymphoid organs (SLO), we used a murine model to investigate the effect of this drug on lymphocyte homing and egress.

We did not observe any effects on lymphocyte homing to lymph nodes and spleen. In contrast, dasatinib induced a significant lymphocyte egress from the spleen, which was independent of the canonical sphingosine-1-phosphate-mediated egress signaling. Dasatinib also resulted in a significant reduction of spleen size, while preserving its microarchitecture, and was able to promote myosin light chain phosphorylation in splenic stromal cells, supporting a dasatinib-induced spleen contractility. Accordingly, pharmacological inhibition of the contraction-promoting factor ROCK partially reversed dasatinib-induced lymphocytosis and egress *in vivo*.

Interestingly, we have uncovered the reduction of spleen size in CML patients immediately after dasatinib intake, as measured by ultrasound. This reduction paralleled the increase of lymphocytes in peripheral blood and was proportional to dasatinib plasma levels.

In conclusion, our results suggest that dasatinib triggers the contraction of splenic stromal cells, leading to a mechanical egress of lymphocytes from the spleen to peripheral blood, likely contributing to the lymphocytosis associated to its intake. Thus, we have described a novel effect of dasatinib, which opens up new potential clinical applications for this drug.

P-104

CARACTERIZACIÓN FENOTÍPICA Y FUNCIONAL DE LINFOCITOS T MEMORIA PRODUCTORES DE IFN-Γ ESPECÍFICOS DE SARS-COV-2, PROVENIENTES DE DONANTES CONVALECIENTES DE COVID-19

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Introducción

La pandemia de la COVID-19 ha sido responsable de millones de fallecimientos en todo el mundo. Ello ha motivado la búsqueda de tratamientos para pacientes hospitalizados por infección por SARS-CoV-2. Con el objetivo de validar su utilización como Inmunoterapia Adoptiva en pacientes con neumonía asociada a SARS-CoV-2, se analizó el fenotipo y la funcionalidad de los linfocitos T memoria específicos de SARS-CoV-2 (LT-SC2), existentes en sujetos convalecientes de una infección moderada.

Material y Métodos

Se reclutaron 12 donantes convalecientes de infección por SARS-CoV-2. La identificación, cuantificación y análisis fenotípico de los LT-SC2 se realizó mediante ensayos de secreción de IFN-γ y tinción de citoquinas intracelulares tras estimulación con péptidos específicos. Posteriormente, se realizó una expansión policlonal *in vitro* de LT-SC2, seguida de su selección inmunomagnética a día 14. Con estas células se desarrollaron ensayos de liberación de calceína y de dilución de CFSE para determinar su potencial citotóxico y proliferativo.

Resultados

El 75% de los sujetos se consideraron aptos, y presentaron una frecuencia media de 0.019±0.010%, con predominio de $CD3^+CD4^+IFN\text{-}\gamma^+$ frente a $CD3^+CD8^+IFN\text{-}\gamma^+$. Estos LT-SC2 mostraron mayormente fenotipo T_{EM}, con mayor expresión de $CD8^+$Granzima B^+ y $CD8^+$Perforina, frecuencia destacable de $CD4^+PD\text{-}1^+$, y ausencia de FasL y marcadores de activación (CD38, Ki-67; Figura 1A). La expansión provocó un aumento de $CD3^+CD45^+IFN\text{-}\gamma^+$ hasta 4.16±2.37%. Tras su selección, la pureza alcanzó 75.5±11.0% $CD3^+CD45^+IFN\text{-}\gamma^+$, con recuperación de 65.9±22.7%. Estas células mostraron *in vitro* capacidad citotóxica dependiente de la frecuencia relativa de $CD8^+$ y de la proporción diana:efectora (Figura 1B); así como potencial proliferativo al diluirse el CFSE tras ser co-cultivadas con feeders pulsados con péptidos específicos (Figura 1C).



Conclusiones

Realizada su caracterización fenotípica, y demostrada su capacidad funcional *in vitro,* los LT-SC2 provenientes de individuos convalecientes por infección por SARS-CoV-2 podrían utilizarse como producto de Inmunoterapia Adoptiva para tratar pacientes graves por COVID-19.



P-105

METASTATIC MELANOMA (MM) PATIENTS WITH STABLE DISEASE DISPLAY REDUCED MO-MDSCS LEVELS IN PERIPHERAL BLOOD

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

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

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

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



P-106

SERUM MASS SPECTROMETRY: AN ALTERNATIVE TECHNIQUE TO ANALYZE DISEASE RESPONSE IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA TREATED WITH ANTI-BCMA CAR T-CELL THERAPY

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Background:

ARI0002h is an academic BCMA-directed CAR T-cell, effective against relapsed/refractory multiple myeloma (RRMM). Here, we explore EXENT Quantitative Immunoprecipitation Mass Spectrometry (QIP-MS) as an alternative monitoring technique to serum Immunofixation (IFE) or to next generation flow cytometry (NGF) bone marrow (BM) in RRMM patients, and able to identify interferences.

Methods:

Thirty-three RRMM patients with measurable disease were treated with ARI0002h. The M-protein (MP) was analyzed in serum by QIP-MS at three time points: before ARI0002h infusion, and 28 and 100 days post-infusion. The presence of disease was also investigated by IFE and NGF at 28 and 100 days post-infusion.

Results:

Concordant and discordant results between QIP-MS and IFE (A), or QIP-MS and NGF (B) are summarized in Table 1.

Interferences due to daratumumab and tocilizumab were successfully detected. Before ARI0002h, daratumumab was identified in 10 patients (30.3%) and persisted at day +100 in 5 of them (15.2%). Tocilizumab was detected at day +28 in 15 (71.4%) patients out of the 21 receiving it, persisting at day +100 in 3 of them (14.3%).

Regarding to clinical outcome, the achievement of QIP-MS(-) at day +100 post ARI0002h, was associated with a prolonged overall survival (OS) (Fig. 1).

Conclusions:

In this study, serum QIP-MS allowed the identification of the MP in all cases with high sensitivity.



QIP-MS was also able to differentiate between the MP and t-mAb, allowing a correct labeling of treatment response.

In our cohort, QIP-MS provided valuable information of patient´s clinical outcome in terms of OS.

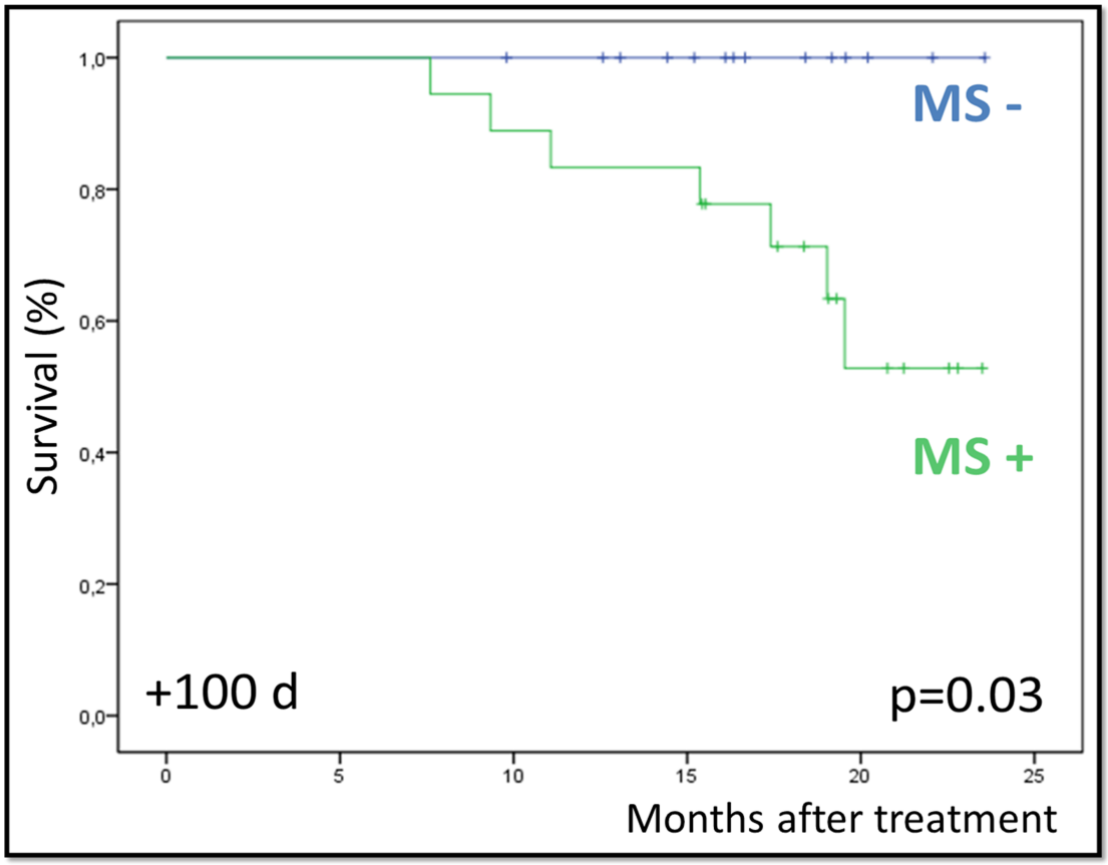
Table 1.

A)	+28d ARI0002h		+100d ARI0002h	
	QIP-MS(-)	QIP-MS(+)	QIP-MS(-)	QIP-MS(+)
IFE(+)	1*	15	0	10
IFE(-)	8	6	14	6

* False positive (daratumumab interference).

B)	+28d ARI0002h		+100d ARI0002h	
	QIP-MS(-)	QIP-MS(+)	QIP-MS(-)	QIP-MS(+)
NGF(+)	0	1	0	2
NGF(-)	7	17	13	14
NGF(NE)	2	6	1	2

NE: Not evaluable


Survival (%)
1,0
0,8
0,6
0,4
0,2
0,0
MS -
MS +
+100 d
p=0.03
0
5
10
15
20
25
Months after treatment



P-107

PRE-CLINICAL DEVELOPMENT OF AN ACADEMIC FRATRICIDE-RESISTANT ANTI-CD7 CAR T-CELL THERAPY FOR T-CELL HEMATOLOGIC MALIGNANCIES

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Background:

T-cells modified to express a CD7-directed chimeric antigen receptor (CAR T-cell) are showing effective results against T-cell malignancies. However, disrupting CD7 membrane expression in CAR T-cells is required to prevent fratricide. Our objective is the development of a lentiviral vector that allows the co-expression of a CD7trap, to avoid CD7 membrane trafficking, and an effective anti-CD7 CAR (CARCD7).

Methods:

Three anti-CD7 antibody-secreting hybridoma (named as A, B and C) were sequenced to obtain their corresponding single chain variable fragments (scFv). Each scFv was fused to an intracytoplasmic retention domain to obtain CD7trap constructs. The most effective CD7trap was fused to each CARCD7 candidate. The expansion and CAR expression capacity of T-cells modified with the resulting constructs were evaluated. Their anti-tumor effect was analyzed in co-cultures against Jurkat-GFP and MOLT4-GFP cell lines at different effector-target rates. IFNγ, TNFα and granzyme-B levels were measured from the co-culture supernatants by ELISA.

Results:

The percentage of membrane CD7 decrease from A, B and C-derived CD7traps was 50-90%, 40% and 10-40%; respectively. Thus, A-derived CD7trap (trap) was fused to B- or C-derived anti-CD7 CARs, obtaining CARCD7B-trap and CARCD7C-trap, respectively.

T-cells modified with CARCD7B-trap showed a population doubling of 3.5, while this value reached 4.5 and 5 for CARCD7C-trap and untransduced control T-cells, respectively. The CAR expression was 10% for CARCD7B-trap and 30% for CARCD7C-trap. However, CARCD7B-trap eliminated the 90-100% of Jurkat-GFP and the 80-90% of MOLT4-GFP target cells, while the CARCD7C-trap was ineffective. These data agreed with ELISA results.

Conclusions:

We have developed a CD7trap, derived from A scFv, that avoids CD7 trafficking to the



membrane and prevents fratricide.

CARCD7B-trap seemed to be the best candidate for future approaches to develop an anti-CD7 CAR T-cell therapy against T-cell malignancies due to its anti-tumor capacity and fratricide resistance.



P-108

PRE-TCR-SPECIFIC CAR-PTA T CELLS AS A NOVEL IMMUNOTHERAPY FOR T-ALL

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Introduction: T-ALL is an aggressive haematological malignancy mostly affecting children. Still, high rates of relapse with ineffective options for refractory disease and poor clinical outcomes are associated with T-ALL. Therefore, there is an urgent need for developing novel, effective and safe therapies to improve life expectancy of T-ALL patients. T-cells engineered to express chimeric antigen receptors (CARs) against tumours have emerged as a powerful tool against refractory haematopoietic malignancies. However, CAR-T therapy for T-ALL has been puzzling due to the shared antigen expression between malignant and healthy T-cells, which can lead to CAR T-cell fratricide and T-cell aplasia, resulting in impaired anti-tumour efficacy and life-threatening immunodeficiency. Therefore, identification of antigens exclusively expressed in T-ALL blasts is critical for CAR-T cell development against T-ALL.

Objective: We aimed at developing and preclinically validating a CAR-T immunotherapy, based on a surface antigen specifically expressed on distinct T-ALL subgroups, the pre-TCR complex. As we showed that the pre-TCR is involved in human T-ALL pathogenesis and progression, our anti-pre-TCR strategy may represent a specific, safe and efficacious CAR-T immunotherapy for T-ALL treatment.

Results: Taking advantage of a mAb developed in our lab against the pTα-chain of the human pre-TCR, we produced a second-generation CAR-pTα (CD28TM-CD28IC-CD3ζIC). After validating its specificity, cytotoxic potential and pro-inflammatory cytokines production by CARpTα primary T-cells was confirmed in *in vitro* functional assays. The efficacy of this novel immunotherapy was also validated *in vivo* in preclinical assays, showing that administration of CAR-pTα T-cells hampers tumour progression of both pre-TCR+ T-ALL cell lines and patient-derived leukaemias, resulting in increased overall mice survival. Moreover, CAR-pTα T-cells persisted *in vivo* for long periods in the infused animals.

Collectively, we provide proof of concept that our CAR-T therapy against the pre-TCR can be an effective strategy for the treatment of a significant number of T-ALL patients.



P-109

DEVELOPMENT OF GENE-MODIFIED T-CELLS TO EXPRESS SPECIFIC T-CELL RECEPTORS (TTCR) FOR THE RECOGNITION OF INTRACELLULAR TUMOR ANTIGENS PRESENTED ON CLASS II MAJOR HISTOCOMPATIBILITY COMPLEX MOLECULES.

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



P-110

SYNAPSE TOPOLOGY AND DOWNMODULATION EVENTS DETERMINE THE FUNCTIONAL OUTCOME OF ANTI-CD19 T CELL-REDIRECTING STRATEGIES

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Immunotherapeutic approaches focused on the redirection of T cell activity toward tumor cells are actively being investigated. Strategies based on the endogenous secretion of T cell redirecting bispecific antibodies by engineered T lymphocytes (STAb-T) are emerging as alternative or complementary approaches to those based on chimeric antigen receptors (CAR-T). The anti-tumor efficacy of bispecific anti-CD19 x anti-CD3 (CD19xCD3) T cell engager (BiTE)-secreting STAb-T cells has been demonstrated in several mouse models of B cell acute leukemia.

Here, we have investigated the spatial topology and downstream signaling of the artificial immunological synapses (IS) that are formed by CAR-T or STAb-T cells. Upon interaction with CD19-positive target cells, STAb-T cells form IS with structure and signal transduction which more closely resemble those of physiological cognate IS, compared to IS formed by CAR-T cells expressing a second-generation CAR bearing the same CD19-single-chain variable fragment. Importantly, while CD3 is maintained at detectable levels on the surface of STAb-T cells, indicating sustained activation mediated by the secreted BiTE, the anti-CD19 CAR was rapidly downmodulated, which correlated with a more transient downstream signaling. Furthermore, CAR-T cells, but not STAb-T cells, provoked an acute loss of CD19 in target cells. Such differences might represent advantages of the STAb-T strategy over the CAR-T approach and should be carefully considered in order to develop more effective and safer treatments for hematological malignancies.



P-111

DEVELOPMENT OF A NOVEL IMMUNOTHERAPY BASED ON NANOBODIES TARGETING FUNGAL GLUCANOSYLTRANSFERASES FOR THE TREATMENT OF INVASIVE ASPERGILLOSIS AND CRYPTOCOCCOSIS

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The incidence of opportunistic fungal infections has significantly increased over the last two decades due to a growing number of immunocompromised patients, being *Aspergillus spp* and *Cryptococcus spp* the main responsible for these infections and the cause of greatest number of deaths. Elevated mortality rates mainly due to late non-specific diagnosis and emerging antifungal resistance highlight the importance of finding new therapeutic alternatives. Here we have developed a new nanobody (Nb3) targeting a β-1,3-glucanosyltransferase, Gel4, a key fungal enzyme involved in cell wall remodeling that has been previously shown to be essential for fungal survival. We have found out that Nb3 is able to cross the fungal cell wall reaching its target, Gel4, located at the cell membrane, exerting fungistatic in vitro activity against several strains from *Aspergillus fumigatus* and *Cryptococcus neorformans* including azole-resistant strains. In addition, using in vivo mouse models of *Aspergillus* and *Cryptococcus* infections we show that therapeutic administration of Nb3 in infected animals is highly effective and significantly increases animal survival, correlating with a reduction of fungal burden in lung and brain. Importantly, in vivo biodistribution analysis shows that Nb3 is able to cross the blood-brain barrier reaching the brain which is the main organ affected during *Cryptococcus infections*. Our results present a novel class of compounds to treat lethal fungal infections and provide the basis to further develop other immunotherapy treatments based on Nb3 (i.e. Nb3-based CAR cells) to effectively treat invasive fungal infections. Nb3 and related treatments present a safe and efficient alternative to overcome increasing antifungal drug resistance and reduce morbidity and mortality of a high number of patients at risk of these infections including cancer, UCI and transplantation.



P-112

CART CELL DETECTION: COMPARISON, PROS AND CONS OF THE TWO MOST COMMONLY USED TECHNIQUES.

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The genetic modification of autologous T lymphocytes to express a chimeric membrane protein directed against a specific cell antigen, currently known as CAR-T cell therapy, is revolutionizing the treatment of many hematologic malignancies and is expected to be approved for so many more, as solid tumors or autoimmune diseases.

We present a comparison between the two most used flow cytometry techniques for CAR-T cell therapy monitorization and characterization. The first one (protocol A) is based on the use of anti-mouse IgG-Fab antibody, which binds the chimeric portion of the CAR-T construction. The second one (protocol B) consists in a human CD19 extracellular domain and a mutated human IgG1-Fc region that constitutes a biotinylated antigen-based fusion protein, which can be detected by an anti-biotin antibody conjugated to a fluorochrome. Viral load(copies/□L) was determined by qPCR in peripheral blood.

We analyzed a negative control, 3 infusion products(IP) and 5 peripheral blood(PB) from infused patients(median days post-infusion 12 IQR 9-16). As expected, in both protocols median %CD3+CART+ was higher in the IP compared to PB, nevertheless protocol B showed higher %CD3+CART+ for both sample types (protocol A, IP 8.33 IQR 4.17-9.95 vs PB 0.77 IQR 0.50-1.09; protocol B, IP 18.28 IQR 15.98-19.15 vs PB 7.85 IQR 0.15-88.17). Negative control rendered higher background when using protocol A vs B (2.19 vs 0.20 %CD3+CART+). Interestingly, peripheral blood samples with viral load under 10^4 copies/□L



showed a %CD3+CART+ below the background.

In conclusion, protocol B was selected for a more sensitive follow-up of CAR-T therapy by flow cytometry in our laboratory. Despite this is just a modest observation, the wide range of options and the rapid implementation of new therapies makes it clear that it is necessary the standardization across the health care system, in order to magnify the evidence, which may help us to understand and improve patient outcomes.



P-113

A NEW CAR-T ANTI-IL13RA2 AS AN OPTION FOR TREATING GLIOBLASTOMA

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Background: Gliomas are the most common type of primary neoplasms in the central nervous system, being glioblastoma (GBM) the most frequent and malignant form. With the current treatment, only 7% of patients survive more than 5 years. New therapeutical approaches based on immunotherapy have shown promising results, particularly CAR-T cells directed against the Interleukin 13 receptor alpha2 (IL13Rα2), a marker that has been described to be overexpressed in GBM.

Methods: (A) After obtaining several anti-IL13Ra hybridomas, to determine our single chain variable fragment sequence from these hybridoma cells, we amplified and sequenced their DNA sequences by PCR and via Sanger method, respectively. (B) Cloning determined sequences into plasmid expression vectors to generate lentiviral vectors for the different CAR constructs. (C) Gene engineering by transduction of donor human T-cells to assess proliferation upon CD3/CD28 stimulation and CAR surface expression (D) Testing of our CAR-T cytotoxic capacity and specificity against cell lines with different IL13Ra2 expression using the XCelligence Real Time Analyzer®.

Results: A total of 3 different donors were used to assess CAR expression and proliferation, obtaining proliferation results within the accepted range and proper CAR surface expression after 9 days of expansion. These same 3 donors were used to assess cytotoxic capacity, CAR-T cells showed a good killing capacity when confronted with U87MG cell line. When confronted against different cell lines that do not express the IL13Ra2, CAR-T cells did not show any cytotoxic effect.

Conclusions: Our engineered CAR T cells demonstrated to have a good T cell proliferation fitness and CAR surface expression. Our CAR-T cells demonstrated a promising and specific cytotoxic capacity against a IL13Rα2 glioblastoma-derived cell line.



P-115

CLINICAL DEVELOPMENT OF TRANSPOSON-BASED CAR T THERAPY FOR LYMPHOMA TREATMENT.

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In the TranspoCART project we have developed an innovative CAR T cell product (TranspoCART19 cells) based on a viral vector-free gene-transfer strategy using the *Sleeping Beauty* (SB) transposon system, that presents high transduction efficiencies with a safe integration profile and a significantly reduced production cost compared to conventional protocols based on viral vectors. For TranspoCART19 cell generation, T cells are engineered with SB minicircles carrying a second generation CAR construct targeting CD19 with a 41BB□E endodomain and a truncated human epidermal growth factor receptor (EGFRt) as a safety switch. Our optimized manufacturing protocol showed transduction efficiency ranged between 30 and 70% in both healthy donors and patients' samples, with great expansion rates (between 15.2x and 26x), compatible with clinical applications. Extensive *in vitro* characterization of TranspoCART19 cells showed an enriched stem-cell memory/central memory phenotype, no signs of cell exhaustion and high level of specific cytotoxicity activity against tumoral cell lines and primary tumor cells expressing CD19. Dilution of SB minicircle



and transposase protein during cell manufacturing process was confirmed, with no detectable levels of both components at the end of the expansion period. *In vivo* antitumoral efficacy of TranspoCART19 cells was evaluated using xenograft mice model using immunodeficient NSG mice. Animals treated with TranspoCART19 cells showed improved survival compared to untreated mice, similar to the one observed in mice treated with lentivirus-produced CAR T cells. Biodistribution and toxicity studies showed that TranspoCART19 cells administration is safe. Moreover, *in vivo* functionality of the safety switch with EGFRt was demonstrated with the complete depletion of TranspoCART19 cells after cetuximab administration. Finally, scale-up GMP manufacturing was validated in two independent GMP facilities, demonstrating that our protocol is highly efficient and reproducible. These encouraging results will support our clinical trial with TranspoCART19 cells for the treatment of lymphoma.



P-116

SEMLIKI FOREST VIRUS-BASED VACCINES ENCODING CAR ANTIGENS TO FOSTER THE ACTIVATION, EXPANSION AND ANTITUMOR EFFICACY OF CAR-T CELLS

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Chimeric antigen receptor (CAR) T (CAR-T) cells represents a revolutionary treatment for hematological tumors but this success has not been extrapolated to solid tumors. The activation and expansion of transferred cells *in vivo* is key for the success of CAR-T therapy, with both phenomena being highly dependent on the recognition of the target antigen (Ag). In the case of solid tumors, target Ag recognition occurs exclusively in the tumor, but the immunosuppressive microenvironment prevents the efficient activation and expansion of CAR-T cells. Furthermore, CAR-T cells require a relatively high level of Ag expression to be adequately activated and this makes their activation inefficient when tumor cells express low Ag levels. We believe that the promotion of CAR-T-cell activation through off-tumor recognition of the target Ag may substantially improve the efficacy of CAR-T therapy in solid tumors. This may be safely achieved using vaccines able to deliver the target Ag. To test our hypothesis, we chose a CAR tumor Ag with low surface density expression, such as gp75 expressed in B16 melanoma cells. In an immunocompetent mouse model we studied the role of a Semliki Forest virus (SFV)-based vaccine encoding a mutant gp75 protein (gp75mut), which exhibits enhanced surface expression, to foster the antitumor efficacy of CAR-T cells. We demonstrate that SFVgp75mut vectors increased the activation and expansion of cognate CAR-T cells *in vitro*. Moreover, intratumoral (IT) administration of SFVgp75mut vaccine strongly increased the number of gp75CAR-T cells within the tumor. More importantly, SFVgp75mut in combination with gp75CAR-T cells delayed tumor growth and enhanced overall survival. Interestingly, the CAR-T/SFVgp75mut combination also exerted antitumoral effect on distal, non-SFVgp75mut-treated tumors. Our data show that the administration of SFV-based vaccines increase the therapeutic efficacy of CAR-T therapy in solid tumors, with the local administration of these vaccines being even able to exert abscopal effects



P-117

GALECTIN-1 AS A NOVEL MODULATOR OF REGULATORY T CELL FUNCTION IN TREG CELL-BASED THERAPY IN CHRONIC COLITIS

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Cell therapy with regulatory T cells (Tregs) is under intense development thanks to their potential to maintain peripheral tolerance and their capacity to modulate unwanted immune responses. Galectin-1 (Gal-1) is a β-galactoside-binding protein that has broad anti-inflammatory and pro-resolving activities by targeting glycoproteins expressed by immune cells. We have previously demonstrated that Gal-1 is expressed by regulatory T cells and contributes to their immunosuppressive function *in vitro*. In this study, we analyzed the ability of galectin-1 deficient $CD4^+CD25^+$Foxp3 Tregs (*Lgals1*$^{-/-}$ Tregs) to suppress gastrointestinal immune responses using a T-cell transfer model of experimental colitis to study their functionality *in vivo*. *Lgals1*$^{-/-}$Tregs showed reduced immunomodulatory capacity compared to wild type Tregs, as suggested by the increase number of *Lgals1*$^{-/-}$ Tregs required to modulate intestinal inflammation induced by naïve $CD4^+$ T cells into *Rag-1*$^{-/-}$ mice. In addition, long-term survival of colitic mice treated with *Lgals1*$^{-/-}$ Tregs was compromised with respect to colitic mice treated with wild type Tregs mainly due to a systemic decline in *Lgals1*$^{-/-}$ Tregs, which paralleled to an increase in Th17 T cells frequencies in colon lamina propria and in spleen. These results indicate that galectin-1 is a key effector molecule in Tregs that is also required for long-term protection in chronic colitis. In summary, an adequate expression of galectin-1 should be analyzed in future cell therapy protocols with Tregs in inflammatory bowel disease.



P-118

A PHASE I/II STUDY TO EVALUATE A POINT-OF-CARE MANUFACTURED ANTI-CD19 CAR T IN RELAPSED OR REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) OR SMALL LYMPHOCYTIC LYMPHOMA (SLL)

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Introduction

CLL/SLL are similar types of B-cell non-Hodgkin lymphoma (NHL) usually with an indolent course and today incurable. Patients who develop relapsed/refractory disease and become resistant have a poor prognosis and new therapeutic strategies are needed. Adoptive cell therapy with T-cells expressing chimeric antigen receptors (CAR) targeting CD19 is a well-established approach for the treatment of B-cell malignancies. Despite there are anti-CD19 CAR T-cell products currently available, none has been approved for CLL/SLL yet.

We present a clinical trial with a new CART-CD19 product for CLL/SLL (BCN-CP01), a follow-on product of CARTCD19-ARI0001 developed by our institution. Both have the same CAR design but differ in manufacturing process. BCN-CP01 is manufactured with the Cocoon® bioreactor (Lonza), a closed automated instrument suitable for decentralized, point-of-care manufacturing, optimized towards fresh administration with a short vein-to-vein time (1 week).

Methods

The study is a multicenter, non-randomized, open-label study with a Dose escalation phase (Phase I) and Dose expansion phase (Phase II). The main objective of phase I is to evaluate safety, preliminary efficacy and to establish the recommended phase II-dose of BCN-CP01. Dose escalation will follow the Bayesian Optimal interval (BOIN) design, based on the occurrence of dose-limiting toxicities evaluated during a 28-day period. Three dose levels are evaluated in 15 patients: low, medium and high. The primary objective of phase II is to evaluate efficacy, measured by the objective response rate (ORR). Secondary objectives include further



assessment of the safety profile and efficacy endpoints such as duration of response, PFS, OS, as well as the feasibility of point-of-care BCN-CP01 manufacturing with Cocoon® from patient-derived starting material.

Conclusion

Recruiment of patients on-track and awaiting clinical results. Cocoon manufacturing successful with a 7 day vein-to-vein time, offering a fast alternative process for CAR-T manufacturing. (Sponsored by CellPoint, a Galapagos Company; Euplagia-1/CP0101-CLL, EU registration number 2021-003815-25).



P-119

STUDY OF TCR DIVERSITY IN BREAST CANCER PATIENTS TREATED WITH ACTIVE IMMUNOTHERAPY

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Introduction

Breast cancer is the most common oncology disease in women. Lack of therapeutic efficacy in some subtypes makes it necessary to develop new strategies. We design a clinical trial to evaluate the clinical efficacy of the combination of standard treatment with monocyte-derived dendritic cells vaccines (DCV) preloaded with autologous tumour lysate in patients with BC without HER2 overexpression. T lymphocytes mediate the cellular immune response and their function is an essential component in the humoral immune response. T cells recognize the antigen through their surface receptor (TCR) and this is a powerful tool to rapidly expand and activate functional properties of the specific clon of T cells that has recognized the antigen. The study of the TCR repertoire could be a useful tool to detect specific activation of the immune system after treatment with DCV.

Objectives

To determine whether DCV induces changes in the TCR repertoire that may indicate activation of a specific response by flow cytometry and sequencing, and to compare both techniques.

Methods

Peripheral blood mononuclear cells were collected from 12 patients before and after treatment and TCR diversity was evaluated by flow cytometry and next generation sequencing. Various diversity index were calculated to determine the diversity/clonality of the samples.

Results

Baseline samples from patients with very good clinical response (VGCR) (pT4/5 with Miller&Payne classification; pN0) showed greater TCR diversity that no-VGCR. Moreover, the change in diversity indices for both flow cytometry and sequencing suggests that there is a decrease in diversity and an increase in clonality of T cells.

Conclusion

Our data provide strong support that vaccines induce an oligoclonal expansion of T cell. Since

the results of both techniques are similar, it can be concluded that cytometry, which is a simpler technique available in most laboratories, is a useful tool for studying the TCR repertoire.



P-120

AIDPATH: AI METHODS FOR PRODUCTION SCHEDULING AND RESOURCE MANAGEMENT

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The EU consortium AIDPATH is dedicated to enable and to augment the next-generation of personalised medicine with gene-engineered immune cells at EU hospitals through the use of AI technology. The exemplary embodiment of gene-engineered immune cells in AIDPATH will be T cells, modified to express a synthetic chimeric antigen receptor (CAR-T), which are already a revolutionary novel treatment in haematology and oncology.

AIDPATH will apply AI technology to integrate personnel, hospital sites, materials and patient-specific data to optimise production scheduling and resource planning, to reduce costs and to ensure the effective utilisation of hospital resources. In the case of resource management for personalized CAR T-cell therapies, tasks are the medical procedures which have a strict order of precedence. The resources are the medical equipment that is used for a given procedure and the medical staff, doctors and nurses, who perform these procedures. We have determined several possible optimization objectives for the CAR T-cell therapy, such as minimizing the number of protocol deviations or the number of patients who drop out from the therapy. The main feature of the resource management software will be to generate a close-to-optimal patient and medical staff schedule every time a new schedule is needed.

As the high-level overview of the resource management, the simulation model highly relies on real life datasets about ongoing CAR-T therapies. These datasets are used for building, fine-tuning, and validating the simulation model, in order to best describe the real process. During later stages of the project, a reinforcement learning (RL) based resource management strategy will be developed, which will be trained on the simulation model of the hospital environment.



P-275

INFECTIOUS BURSAL DISEASE VIRUS AS A POTENTIAL ONCOLYTIC VIROTHERAPY AGAINST GLIOBLASTOMA AND OTHER CANCER CELL TUMOR MODELS

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Glioblastoma is considered the most aggressive type of central nervous system (CNS) cancer. Current therapies such as temozolomide (TMZ) do not offer a life expectancy greater than 15 months, while other cancers such as colon carcinoma still do not have a consolidated therapy. The use of oncolytic viruses (OVs) is an immune-related therapeutic strategy based on the selective destruction of cancer cells lacking or with limited anti-viral innate immune mechanisms, leaving non-tumoral cells as barrier controlling virus growth without cell damage. In this work, we propose the potential use of infectious bursitis disease virus (IBDV) as a novel virotherapy. IBDV is a bird pathogen causing the Gumboro disease and is not pathogenic in humans. We have tested and compared the oncolytic effect of IBDV with the reference Newcastle disease virus (NDV) in several tumor models in vitro and in vivo. Results indicate a potential oncolytic activity of IBDV in several cancer types such as glioblastoma (CT-2A and GL261), melanoma (B16-F10), colon carcinoma (CT26) and B cell lymphoma (A20). In all cell lines tested, IBDV has the ability to infect, replicate and kill cancer cells as well as stimulate pro-inflammatory cytokines that may potentially trigger the activation of the immune system against both, the virus and the tumor. Finally, we have used Flow Cytometry to study in more detail the innate and adaptative immune responses of Glioblastoma after IBDV infection in vivo, characterizing the immune profile within the Tumor Microenvironment (TME).



Sesión Poster. Autoinmunidad (I). Pantalla 1

P-135

A DIFFERENT CONSULTATION WITH THE CLINICAL IMMUNOLOGIST: SUSPICION OF HETEROPHILIC ANTIBODIES AS A CAUSE OF FALSE POSITIVE IN AN IMMUNOASSAY

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The case of consultation referred from the oncology service is described, in which a 38-year-old patient with a history of stage IIA embryonal testicular carcinoma in remission and sarcoidosis, was requested to be evaluated due to suspicion of a false-positive serum beta-human chorionic gonadotropin (BhCG) determination.

BhCG is a sensitive and specific marker for tumors of germ cell origin. Thus, treatment of recurrent testicular carcinoma is often initiated on the basis of rising BhCG levels, even in the absence of clinical, radiological, or histological evidence of a relapse.

Several causes for false-positive serum hCG levels have been reported in the literature and Heterophilic antibodies are mentioned as the most frequent. Heterophile antibodies can be IgG, IgM, and IgA isotypes that are poorly defined and react with a wide spectrum of antigens. These antibodies may develop after infections or contact with animal tissues or constituents. They may bind the animal antibodies used in an immunoassay and thus produce erroneous results. Such interference has been increasingly recognized as a diagnostic problem.

To study its possible presence, we carry out different analyzes in collaboration with the Clinical Biochemistry service: heterophile antibody assays based on erythrocyte agglutination, Indirect immunofluorescence assays for detection of autoantibodies AMA, ASMA, LKM, or APCA in rat tissues and use of commercial blocking solutions.

Finally, the presence of heterophile antibodies was confirmed as a cause of false positives by incubating the serum samples with solutions composed of immunoglobulins and other antigens that allow the fixing of heterophile antibodies.

Thus, it is important to note that although the frequency of heterophilic antibody interference is not very significant, recently reported cases to have a large magnitude of interference, affecting the clinical interpretation of test results. Therefore, the multidisciplinary management of these patients in reference centers is essential.



P-136

CHARACTERIZATION AND CLINICAL ASSOCIATION OF AUTOANTIBODIES AGAINST PERILIPIN 1 IN PATIENTS WITH ACQUIRED GENERALIZED LIPODYSTROPHY

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Introduction: Acquired generalized lipodystrophy (AGL) is a rare condition characterized by massive loss of adipose tissue through the body, causing severe metabolic complications. Autoimmune destruction of adipocytes is strongly suspected based on the frequent association of AGL with autoimmune disorders. In 2018 autoantibodies against Perilipin 1 (PLIN1) were identified in three patients with autoimmune-associated AGL. However, the pathogenic mechanism and clinical impact of anti-PLIN1 remain unsolved.

Objective: To characterize anti-PLIN1 autoantibodies and assess its clinical association in patients with AGL.

Results: The prevalence of anti-PLIN1 autoantibodies in an AGL cohort of 40 patients was 50% (20/40). Among positive patients, ten had the autoimmune variety and ten had panniculitis-associated AGL. The IgG isotype was predominant although some IgM antibodies were detected. Epitope mapping studies did not identify a single, major epitope. Instead, autoantibodies typically bound to several different peptides, among which the central (233-405) domain was detected in all antibody positive patients, both for IgG and IgM autoantibodies. In-depth epitope mapping indicated that anti-PLIN1 autoantibodies predominantly recognize the ABHD5 binding site (383-405). Autoantibodies dose-dependently blocked the binding of PLIN1 to ABHD5 and caused a dislocation of ABHD5 towards cytosol, leading to an increase in lipolysis and lipase activities. Finally, anti-PLIN1 titers significantly correlated with the amount of fat loss, metabolic control impairment, and severity of the liver injury.

Conclusions: Our data strongly support that anti-PLIN1 autoantibodies are a diagnostic biomarker and a cause of lipodystrophy in patients with AGL.



P-138

EVALUATION OF INDIRECT IMMUNOFLUORESCENT ASSAYS FOR LGI-1 ANTIBODY DETECTION IN PATIENTS WITH LGI1-ASSOCIATED ENCEPHALITIS

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LGI1 autoantibodies are probably the most common cause of autoimmune limbic encephalitis and the second most common cause of autoimmune encephalitis. Detection of antibodies to neuronal surface proteins and synaptic receptors is important to establish a definitive diagnosis of autoimmune encephalitis. Herein, we assess the clinical performance of two antigen-specific indirect immunofluorescent assays (IIFA) for LGI-1 antibody detection in serum and CSF: 1) commercial IIFA with cells transfected with a GPI-LGI1 construct and 2) *in-house* IIFA with HEK293 cells co-transfected with LGI1 and ADAM23 constructs. We also evaluated whether CSF or serum is the most suitable sample to detect LGI1 antibodies by IIFA and Indirect Immuno Histochemistry (IIHC), employed as screening assay.

We retrospectively examined 70 patients with serum and CSF paired samples and a positive IIHC for LGI1 classic staining pattern in at least one of the two samples. From the 70 analyzed sera, 69 (98.5%) were positive by IIHQ, 66/70 (94.3%) were positive by commercial IIFA and 58/70 (82.8%) were positive by *in-house* IIFA assay. Regarding CSF samples analysis, 68/70 (97.1%) resulted positive by IIHC, 58/70 (82.8%) were positive by commercial IIFA while 70/70 (100%) CSF resulted positive when tested by *in-house* IIFA.
All patients were positive for LGI-1 antibodies in CSF. The only way to detect all patients by antigen-specific test was by testing CSF by in-house IIFA (LGI-1 co-transfected with ADAM23). As screening method, the sensitivity of IHC (gold standard method) was similar when comparing sera (69/70) and CSF (68/70) testing.



P-139

RHEUMATOID ARTHRITIS ASSOCIATED INTERSTITIAL LUNG DISEASE: THE ROLE OF NON-CRITERIA AUTOANTIBODIES

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Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic joint inflammation. RA-associated interstitial lung disease (ILD) is the most frequent and severe extra-articular manifestation and is associated with an increase of mortality. Rheumatoid Factor (RF-IgM) and anti-citrullinated peptide antibodies (ACPA-IgG) are important diagnostic tools included in the classification criteria (ACR/EULAR 2010). However, new biomarkers are still needed, to diagnose and predict patient's disease subsets and prognosis.

The aim of this study was to analyse the prevalence and the association with RA-ILD of non-criteria isotypes of RF, ACPA and anti-hnRNP-A2/B1 (RA33) in Hospital-Clínic Arthritis-Unit RA cohort.

Methods

This cross-sectional study included 229 patients with definite RA (ACR/EULAR 2010 criteria). ILD was diagnosed after clinical suspicion and confirmed by high resolution computed tomography. Serum samples from all RA patients and 50 healthy controls were tested for IgM/IgG/IgA of RF, ACPA (CCP2 antigen) and RA33 using EliA Platform. Cut-offs values were established with 95%-specificity for healthy controls.

Results

ILD was confirmed in 57 patients (RA-ILD). IgA isotype for ACPA (OR: 2.1 [1.1-4.0]), IgA-RF (OR: 2.8 [1.2-6.5]), as well as IgG-RF (OR: 2.8 [1.5-5.3]) were associated with ILD in RA. Combinations of non-criteria autoantibodies showed a wide sensitivity (70.2% - 36.8%) and specificity (84.3% - 50.0%) range for ILD in RA. Particularly, IgA-ACPA + IgA-RF + IgG-RF showed 52.6% sensitivity and 74.4% specificity.

Conclusions

This study confirms the association of IgA-ACPA antibodies with RA-ILD, but no association with IgG-ACPA was observed. Moreover, both IgA-RF and IgG-RF are associated with RA-



ILD. The results of the present study support the role of mucosal immunity in the development of ILD in patients with RA. Combinations of non-criteria autoantibodies could be useful to identify RA-ILD patients.

More prospective studies to confirm the non-criteria autoantibodies predictive value for RA-ILD stratification are warranted.



P-140

LA ACTIVIDAD DESMIELINIZANTE EN LOS PACIENTES CON EM SE ASOCIA A LA PRESENCIA DE LINFOCITOS EN EL SISTEMA NERVIOSO CENTRAL.

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Introducción: Los linfocitos B pueden jugar un papel fundamental en el desarrollo de la esclerosis múltiple (EM). Nosotros nos planteamos analizar la relación entre el número de linfocitos B y la actividad de las lesiones.

Métodos: 71 necropsias obtenidas de pacientes con EM (n=14). Análisis inmunohistoquímico utilizando los anticuerpos anti-CD68 (actividad de la lesión), anti-CD79 (linfocitos B), anti-CD138 (células plasmáticas y anti-IgG y anti-IgM.

Resultados: El número de linfocitos B era mayor en los espacios perivasculares de las lesiones agudas (2,32□0,99) y activas (1,09±0,31) que en las crónicas inactivas (C.I, 0,21□0,06) y la sustancia blanca aparentemente normal (NAWM) (0,04□0,020). En las meninges también se observó un incremento de linfocitos B en las agudas (19,63□10,49) en comparación con las lesiones C.I (2,16□0,58) y NAWM (2,55□2,49). Sin embargo, la proporción de células plasmáticas era mayor en los espacios perivasculares de las lesiones crónicas activas (0,54□0,16) y las lesiones C.I (0,50□0,020) que en las lesiones agudas (0,13□0,05) y la NAWM (0,02□0,01). Las lesiones con depósitos de IgG tenían mayor número de linfocitos en el espacio perivascular que las que no presentaban (1,24□0,35 vs 0,36□0,57) y también en el parénquima (0,45□0,13 vs 0,06□0,05). Por el contrario, en las lesiones que había depósitos de IgM se observó un mayor número de células plasmáticas en el espacio perivascular (0,78□0,21 vs 0,13□0,04) y meninges (6,76□2,28 vs 2,28□0,093) en comparación con las que no tenían.

Conclusiones: La actividad desmielinizante aguda está relacionada con el número de linfocitos B y la crónica con las células plasmáticas. Es necesario el desarrollo de nuevos fármacos para regular a estas células residentes en el sistema nervioso central.



P-141

ANTIPHOSPHOLIPID ANTIBODIES ARE CO-STARS IN COVID-19 THROMBOSIS, NOT MERE STONE GUESTS

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Background: Patients with COVID-19 present thrombophilia reminiscent of the antiphospholipid syndrome (APS), a disease characterized by vascular thrombosis, or gestational morbidity in carriers of antiphospholipid antibodies (aPL). The relationship between aPL-presence and the appearance of thrombi as well as the transience of aPL in COVID-19 has been extensively studied but with small patient samples and contradictory results.

Methods: A group of 360 COVID-19 patients were recruited in the first 24 hours after diagnosis and followed-up for 6 months. Sidney´s Classic aPL (anti-cardiolipin or anti-B2GP1 IgG/M), as well as anti-phosphatidylserine/prothrombin IgG/M, anti-B2GPI IgA and anti-SARS-CoV-2 IgG antibodies were evaluated at diagnosis and >12 weeks later. A reference control group was created with 143 healthy volunteers of the same age-range distribution.

Results: Prevalence of aPL in COVID-19 patients did not show significant differences with that observed in reference population, however COVID-19 patients positive for criteria aPL or IgA anti-B2GP1 had a higher incidence of thrombosis in the first weeks after infection (OR: 2.33, 1.03–5.29, p=0.043 and 3.71, 1.71–8.05, p=0.001).

Thrombosis non-aPL-associated occurred earlier after hospital admission (median 4 days, IQR: 3-7), however the aPL-associated appears later (median: 17 days, IQR:6-28, p=0.016). A strong agreement in aPL positivity at diagnosis and >12 weeks was observed for classic aPL and anti-B2GPI IgA (Weighted kappa: 0.92). COVID-19 did not seem to induce aPL *de novo*, since anti-SARS-CoV-2 antibodies levels increased during convalescence, aPL hardly changed.

Conclusions: In COVID-19 thrombosis at least two different mechanism could co-exist, an early immune-dysregulation-mediated thrombosis after infection and belated-aPL-mediated thrombosis, with SARS-CoV-2 behaving as a second hit. The presence of aPL in COVID-19 patients is not associated with the infection, they were pre-formed. The 27% of aPL carriers could develop thrombotic events, once infected of SARS-CoV-2.



P-142

USO DE *LIKELIHOOD RATIO* EN LA INTERPRETACIÓN DE LOS RESULTADOS DE ANTICUERPOS ANTI-RACH PARA EL DIAGNÓSTICO DE MIASTENIA GRAVIS.

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Introducción: Los anticuerpos anti-receptor de acetilcolina (anti-RACh) son criterio diagnóstico de Miastenia Gravis (MG). La técnica RIA es el *gold standard* para su detección pero requiere equipamiento y personal cualificado. Una alternativa es el ELISA, pero presenta una menor especificidad. Nuestro objetivo es valorar si la razón de verosimilitud (likelihood ratio, LR) puede mejorar la interpretación del ELISA de anticuerpos anti-RACh para el diagnóstico de MG.

Metodología: Se analizaron 666 peticiones de anticuerpos anti-RACh recibidas durante los años 2019-2022 (abril), que se categorizaron en pacientes (n=526) o controles (n=140) en función de si tenían diagnóstico clínico de MG. Se calculó la curva ROC, puntos de corte y el LR utilizando el programa estadístico GraphPad Prism v9.

Resultados: La sensibilidad y especificidad obtenida para el punto de corte establecido por el kit (0,45 nmol/L) fue 81% y 74%, respectivamente. El percentil 95% de los controles fue 0,69 nmol/L, muy similar al punto de corte de la zona gris establecida por el laboratorio previamente (0,45-0,68 nmol/L). El área bajo la curva fue 0,85 (IC95% 0,80-0,89). El LR por intervalos fue: LR=0,26 (Ac.Anti-RACh<0,45 nmol/L), LR=0,79 (Ac.Anti-RACh = 0,45-0,68 nmol/L), LR=1,25 (Ac.Anti-RACh = 0,68-1 nmol/L) y LR=102,75 (Ac.Anti-RACh>1 nmol/L).

Conclusión: El uso del LR para informar resultados de tests de anticuerpos ha sido propuesto como un método válido para transformar datos numéricos en información clínica. Nuestros resultados reflejan que el LR se aproxima más a la realidad de la práctica clínica que la interpretación de los resultados mediante un punto de corte para los anticuerpos anti-RACh medidos por ELISA.



P-143

ANTICUERPOS ANTI-TH/TO: CLAVES PARA AFRONTAR RESULTADOS DESCONCERTANTES.

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Los anticuerpos anti-Th/To son marcadores de esclerosis sistémica (ES) con afectación pulmonar. Se han descrito discrepancias entre técnicas comercializadas debido a la diferente representación antigénica [hPop (Euroimmun) y Rpp25 (DTEK)].

Analizar nuestra serie de muestras positivas para anti-Th/To comparando los ensayos comerciales disponibles.

Revisión retrospectiva de pacientes anti-Th/To positivo por al menos un inmunoblot disponible (SCL10D24-DTEK, lectura subjetiva y EurolineSSc-Euroimmun, sistema de lectura EuroblotOne) desde 01/2020-04/2022. Se recopilaron datos clínicos y la presencia de otros anticuerpos, dividiéndolos en criterioES, no criterioES y otras especificidades asociadas a EAI sistémicas. Se recogió el resultado de inmunofluorescencia indirecta (IFI).

Obtuvimos 71 muestras positivas por alguna de las dos técnicas (65 pacientes). $\bar{x}$edad 57 años (21-86), 66%mujeres.

En 34/65 pacientes se estudiaron los dos inmunoblots. De estos, 8 tuvieron positivo en ambos, el resto discordantes (Tabla1).

En la asociación con otros anticuerpos de ES, lo más frecuente fue la coexistencia con anticuerpos no criterio ES (18/28). En todos los grupos asociados (criterioES, no criterioES y otras especificidades) lo más frecuente fue encontrar anti-Th/To a título débil (9/10, 12/18 y 4/4, respectivamente).

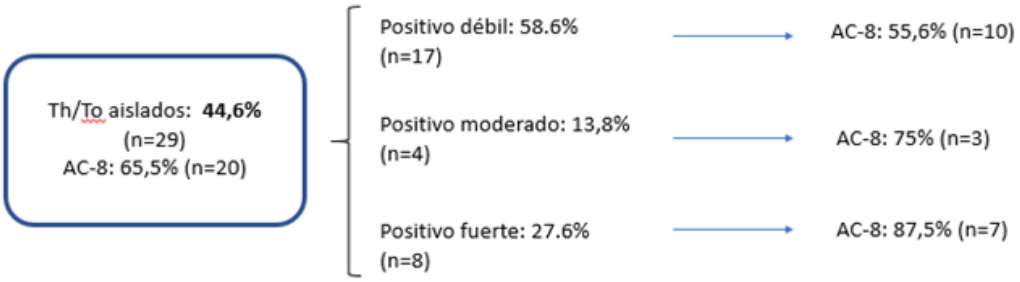
Un 66% de anti-Th/To aislados presentaban patrón AC-8, un 41% en no aislados. La menor frecuencia de patrón AC-8 se dio en los positivos débiles de ambos grupos (Imagen 1).

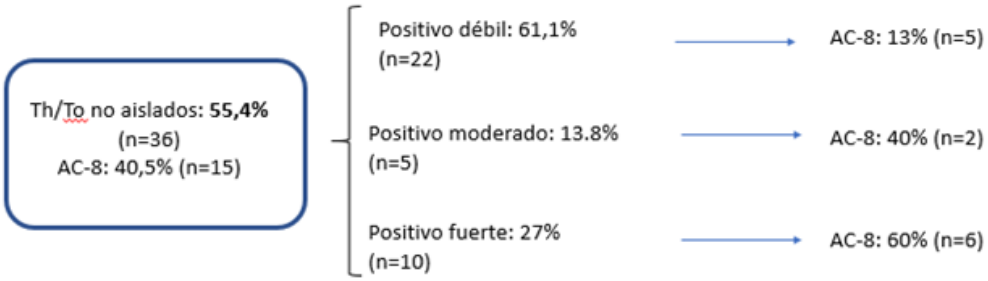
Disponíamos de información clínica en 38/65 pacientes. De estos, 15/38 tenían ES (60% monoespecíficos Th/To): 8 positivos por Euroimmun (38% sólo realizado blot Euroimmun), 6 por DTEK y 1 positivo por ambos blots. 7/15 presentaban positividad débil.

Las positividades solapadas hPop+ Rpp25+ son infrecuentes. Los casos de ES se dan en ambas especificidades, lo que enfatiza la importancia de abordar la búsqueda de anti-Th/To con los dos inmunoblots.

Tabla1

		DTEK				
		ND	NEG	+	++	+++
Euroimmun	ND					
	NEG			3	2	7
	+	25	10			2
	++	4	2		1	
	+++	2	2			5







P-144

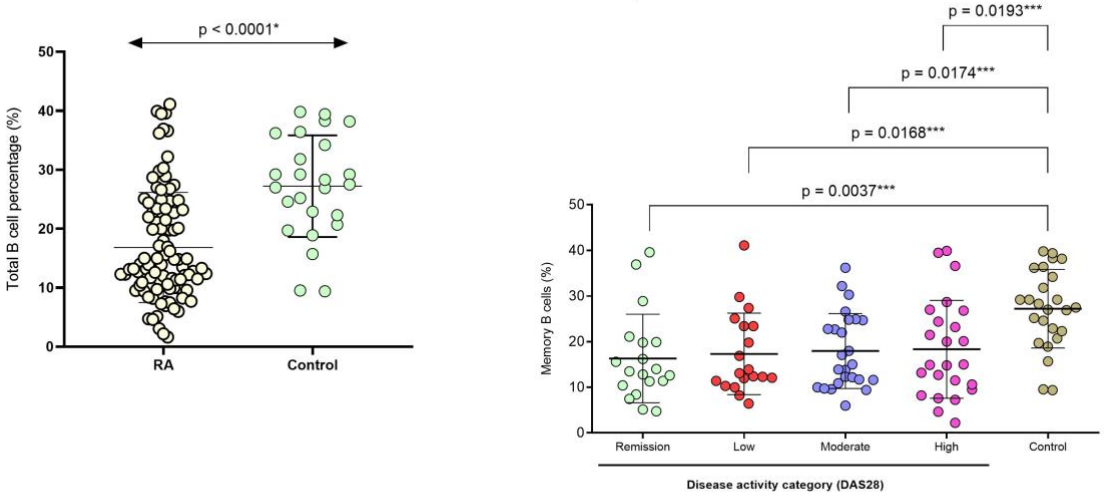
ASSOCIATION OF IL10 AND IL17 RECEPTOR EXPRESSION IN B-CELL SUBPOPULATIONS WITH CELL ACTIVATION, ANTI-CPP LEVELS, AND CLINICAL DISEASE ACTIVITY IN RHEUMATOID ARTHRITIS PATIENTS

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Background: The pathogenesis of rheumatoid arthritis (RA) depends on cytokine deregulation and autoantibody secretion; B cells play a predominant role in both processes. Some studies suggest that IL-10 and IL-17, as well as their receptors, can be key molecules in the activation and differentiation of B cell subpopulations of, which can be altered in autoimmune and inflammatory pathologies such as RA and contribute to the inflammatory process, production of autoantibodies and clinical disease activity. **Objective:** To determine the association of IL-10 and IL-17 receptor expression in B cell subpopulations with cell activation, autoantibody levels, and clinical disease activity in patients with RA. **Methods:** 87 RA patients classified according to the DAS28 index and 25 control subjects (CS) were included. Flow cytometry was used to determine peripheral B cell subpopulations' frequencies (%), CD69, IL-10R, and IL-17R markers. Erythrocyte sedimentation rate (ESR) was measured by the Westergren method. Anti-CCPs were measured by an ELISA test. **Results:** Total B cells did not differ between patients and CS (p>0.05). Memory B cells were decreased (p=0.0013), and mature B cells increased (p=0.0186) in RA compared to CS. A higher percentage of total B cells in patients were activated (CD69+, p=0.0026) than in CS, patients with high activity showing the highest activation (p=0.0373). IL-10R and IL-17R receptors were expressed more in plasmablasts (p<0.0001), but their expression does not differ by clinical activity (p>0.05). IL-17R is expressed more in B cells than IL-10R (p<0.0001). **Conclusions:** IL-10R and IL17RA may be associated with the differentiation of B cells due to their high expression in plasmablasts. In AR, B cells are more activated, and memory B cells are probably a cellular subpopulation involved in the pathogenesis of RA.

Keywords: Rheumatoid arthritis, B cell, IL-10R, IL-17R, DAS28



Total expression of B cells in patients with RA and CS. Further categorization by disease activity. Differences were calculated using the Mann-Whitney U* and Kruskal-Wallis tests, followed by Dunn's multiple comparison test***. Values are provided as medians and interquartile ranges.**



P-145

FROM ALLO- TO AUTOANTIBODIES AGAINST BILE CANALICULI

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Cholestatic liver injury can be caused by primary (genetic) or secondary (e.g. alcohol) defects of bile canaliculi proteins such as membrane proteins, tight junction proteins (TJ) and/or hepatic transporters of bile salt. The bile salt export pump protein (BSEP) is in the canalicular membrane of the hepatocyte and its deficiency due to congenital mutations leads to progressive familial intrahepatic cholestasis (PFIC). Curative treatment is a liver transplant. However, some patients relapse as a result of blocking alloantibodies against the native BSEP of the donor organ.

We included 4 patients, 3 pediatric liver transplanted patients due to PFIC disease and one adult male with alcoholic cirrhosis. All of them presented a bile canaliculi pattern by Indirect Immunofluorescence Assay (IFA) on rat liver sections (Fig.1A). We confirmed anti-BSEP specificity by western blot (WB) in the 3 pediatric patients (Fig.1B). During follow up, one clinically recovered patient presented a negative seroconversion of the anti-BSEP alloantibodies by IFA and WB (Fig.1B). Next, we performed WB with extracted proteins of 3 human healthy tissues (liver, stomach and thyroid). Immunoblotting with the serum of the alcoholic cirrhotic patient revealed two specific bands, one of 55kDa in the stomach and one of 30kDa in the liver and stomach (Fig.1C).

In conclusion, the IFA pattern in bile canaliculi is caused by alloantibodies against BSEP. Moreover, in patients clinically recovered is possible to observe a negative seroconversion. However, BSEP is not the only specificity for this pattern. We have presented an alcoholic cirrhotic patient with this pattern as a result of *de novo* autoantibody formation against other proteins of the canaliculi. Molecular weight of the WB bands and the location in more than one tissue (liver and stomach) suggested that the specificity of the autoantibodies anti-canaliculi in this patient could be a TJ protein. However, further studies are needed.

Figure 1.

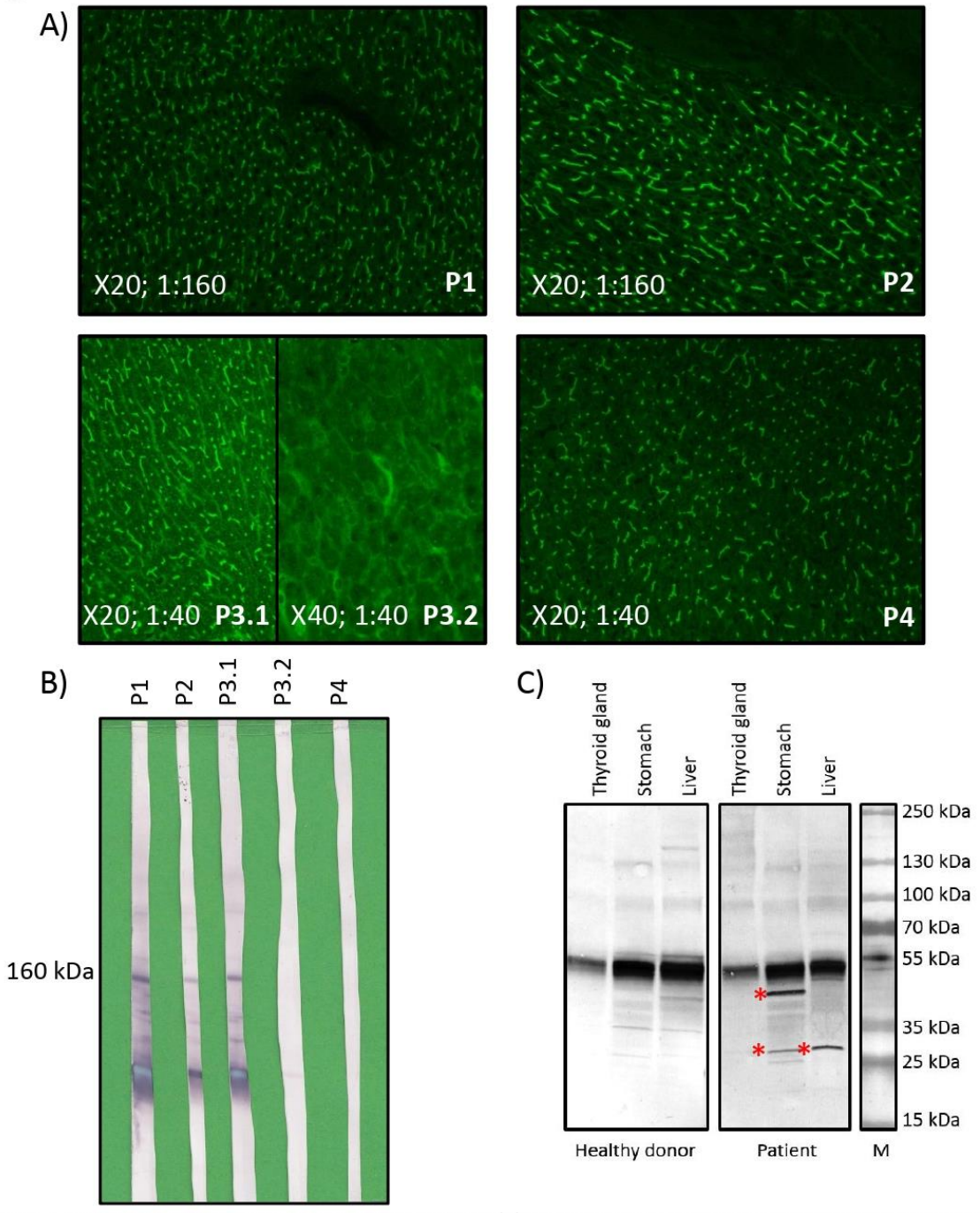


Figure. 1. Presence of antibodies against bile canaliculi proteins. (A) Bile canaliculi pattern by Indirect Immunofluorescence Assay on rat liver section in 2 different concentrations 1:40 and 1:160; P1-3 PFIC pediatric patients, P4 adult with alcoholic cirrhosis. Magnification: ×20 and ×40. (B) Anti-BSEP specificity by Western Blot (WB); P1-3.1 PFIC pediatric patients, P3.2 seroconverted patient, P4 adult with alcoholic cirrhosis. (C) Antibody detection from an adult alcoholic cirrhotic patient by WB with extracted proteins of 3 human healthy tissues.



P-146

COMPARATIVA ENTRE UN ELISA DE RUTINA Y UNA TÉCNICA *POINT-OF-CARE* PARA LA DETERMINACIÓN DE NIVELES DE FÁRMACO Y ANTICUERPOS ANTI-FÁRMACO.

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Las técnicas *point-of-care* (POC) permiten monitorizar niveles de anti-TNF y anticuerpos anti-fármaco de manera rápida.

Comparar las concentraciones de infliximab(IFX), adalimumab(ADA) y anticuerpos anti-fármaco obtenidas por ELISA y POC.

Estudio prospectivo en el que se analizaron muestras de rutina por ELISA (Promonitor-Grifols) y POC (Quantum Blue-Bühlmann) entre 11/2021 y 04/2022. En muestras con fármaco indetectable se estudiaron anticuerpos anti-fármaco por ambas técnicas. Se valoró la correlación global y en 4 grupos de concentración de fármaco (0-4,99ug/ml, 5-9,99ug/ml, 10-14,99ug/ml y ≥15ug/ml). Para la comparativa se utilizó índice de correlación intraclase (ICC)(fármaco) y kappa cohen (anticuerpos).

Se estudiaron 280 muestras (180 IFX y 100 ADA). En IFX [$\bar{x}$edad 45(rango 12-85), 46,7%mujeres]: niveles medios IFX-ELISA 7.77(±9.96) y POC 13.86(±22.37). En ADA [$\bar{x}$48(4-81), 48,5%mujeres]: niveles medios de ADA-ELISA 8.33 (±6.24) y POC 9.78 (±7.18). Globalmente obtuvimos un ICC=0,66(IC95% 0,57-0,73) para IFX y 0,84(IC95% 0,77-0,89) para ADA.
En la comparativa por rangos observamos que la correlación es buena para valores inferiores a 5ug/ml para IFX y ADA (ICC=0,75 y 0,80, respectivamente), pero no para concentraciones superiores (Tabla 1).
Los anticuerpos se valoraron en 44 muestras (31 IFX, 13 ADA): índice Kappa=0,73(IC95% 0,50-0,97) y 0,57(IC95% 0,03-1) respectivamente. En anti-IFX la sensibilidad del POC fue 71.43%(IC95% 45-88), con 2/4 resultados POC negativo y ELISA positivo en niveles>150 UA/ml.

La utilidad del POC radica en la posibilidad de identificar de manera rápida concentraciones <5 ug/mL. Sin embargo, su correlación con ELISA se pierde para concentraciones altas.



En el estudio de anticuerpos llama la atención la limitada sensibilidad del POC que obliga a confirmar todos los resultados por ELISA.

	IFX			ADA	
Rangos (ug/ml)	Muestras	ICC(IC95%)		Muestras	ICC(IC 95%)
0-4,99	93	**0,74**(0,65-0,83)		26	**0,8**(0,6-0,9)
5-9,99	44	0,21(0-0,47)		45	0,31(0,02-0,55)
10-14,99	15	0(0-0,43)		13	0,14(0,-0,55)
≥15	28	0,20(0-0,53)		16	0,61(0,19-0,84)

Tabla 1. ICC y $\bar{x}$ para IFX y ADA por rangos.



P-147

ANTIBODY RE-FORMATTING, ASSISTED BY PHAGE DISPLAY, A NOVEL METHODOLOGY FOR DEVELOPING A HIGH-AFFINITY FAB AGAINST GLUTEN.

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Gluten-related disorders refer to a group of heterogeneous conditions related to the consumption of gluten proteins (found in wheat, rye, and barley) by sensitized persons. These disorders are classified by their etiopathology in three main groups: allergic, autoimmune, and non-allergic and non-autoimmune diseases.

Celiac disease is the best known and most prevalent condition. Although many pharmacological approaches have been tried to settle a therapy (anti-inflammatory drugs, monoclonal antibodies, intestinal permeability modulators…), a gluten-free diet is still the most effective.

Epidemiological evidence suggests that most individuals with celiac disease can tolerate the ingestion of 20 ppm of gluten, so this is the limit used in most countries to label a product as "gluten-free". The main objective of this work is to develop new antibody-based tools (in Fab format) for gluten traceability, enabling food safety assurance for all intolerant patients.

A new strategy for developing new Fabs has been implemented for the first time for this application: merging chains of different origins, a semisynthetic heavy chain, and an immune light chain. A pre-existing dAb (selected from a semi-synthetic human VH3-23 based library) was transformed into a subset of IgGs heavy chains that were cloned into a celiac patient-derived light chain immune repertoire, resulting in a novel Fab library. Then, phage-displayed Fabs were screened against gliadin to select the best gluten binding candidates. Once the most suitable candidate was found, it was expressed and purified from an *E.coli* bio-factory as soluble molecules. In addition, structural and antigen-antibody interactions *in silico* analysis were performed.

The generated functional molecules will be the main reactant to develop new gluten immunodetection systems. Furthermore, this protein evolution methodology is proven to be efficient for antibody re-formatting and led to an affinity and stability increase of pre-existing molecules.



P-148

THE UNEXPECTED ROLE OF MACROPHAGES AND AUTOIMMUNITY IN THE HOMEOSTASIS OF RED BLOOD CELLS

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The number of red blood cells (RBCs) in circulation emerges from an exquisite balance between cell production and destruction. RBC production is regulated by erythropoietin, a hormone secreted by kidney fibroblasts in response to the levels of oxygen in the body. The destruction of senescent RBCs relies on hepatic and splenic macrophages. Mammalian RBCs are devoid of cytoplasmic structures (including the nucleus and the mitochondria), which hinders their ability to undergo apoptosis. Instead, they use two membrane molecules to control phagocytosis. Phosphatidylserine acts as an "eat-me" signal and promotes RBC destruction by macrophages. On the other hand, CD47 (a universal marker of self in mammals) conveys "don't-eat-me" signals that inhibit the phagocytosis of the RBC. The profile of membrane signals determines the fate of RBCs when they pass through the liver and the spleen. According to this model of RBC destruction, widely accepted in the literature, the behavior of macrophages would be determined by "eat-me" and "don't-eat-me" signals on the membrane of passing RBCs. Therefore, macrophages would be passive agents of RBC homeostasis. In this talk, we argue that this approach is overly simplistic and misses key aspects of the biology of RBCs. We suggest that macrophages actively regulate RBC lifespan depending on the levels of erythropoietin, allowing the organism to adjust the number of cells in response to internal or external perturbations (e.g. hemorrhages or infections). Moreover, macrophages can trigger autoimmune responses that result in the production of auto-antibodies against circulating RBCs. This would be a physiological mechanism intended to rapidly remove RBCs from the blood when they are no longer needed. The frequent detection of anti-RBC antibodies in the serum under normal conditions (and with no clinical manifestation) would respond to this new role of macrophages and autoimmunity in RBC homeostasis.



P-149

ENSAYOS PARA ANTI-MI2, ¿SON INTERCAMBIABLES?

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Anti-Mi2 son marcadores específicos de dermatomiositis. Para su identificación existen diferentes ensayos comercializados.

El objetivo fue evaluar el comportamiento de las principales técnicas disponibles para la detección de anti-Mi2.

Se revisaron retrospectivamente las muestras anti-Mi2 positivo por ELiA(Thermo–Positivo>7 U/mL) entre 01/2013-05/2022 y se compararon con los resultados obtenidos por inmunoblot-Euroimmun® (BlotA) (MYO7G-Lectura automatizada EuroblotOne) e inmunoblot-Dtek® (BlotB)(PMS12D-24). Se registró patrón de fluorescencia Hep2, otros anticuerpos asociados a EAIs y datos clínicos.
Para evaluar la especificidad de BlotA, en una segunda fase se analizaron retrospectivamente las muestras detectadas en rutina como positivas para anti-Mi2 alfa y/o beta entre 01/2020-05/2022.
De 1997 muestras analizadas por ELiA, el 4.9% fueron positivas (98muestras/40pacientes) edad$\bar{x}$=45años(5-88), 75%mujeres, niveles$\bar{x}$=174,54[7.4-2520U/mL]. En el 40% de los pacientes se observó reactividad para al menos otro anticuerpo.
En 23 muestras se realizaron ambos blot:

- 18/23 coincidieron: 13 positivas (10/13 perfilα+β en BlotA) y 5 negativas.
- En 5/23 se obtuvo un resultado discordante: 3/5BlotA+/BlotB- ($\bar{x}$=28U/mL) y 2/5BlotA-/BlotB+ ($\bar{x}$=77U/mL).

En las que sólo se había estudiado un blot:

- BlotA: 6/10 fueron positivos (2/6perfilα+β) ($\bar{x}$Negativo:136.9U/mL/$\bar{x}$Positivo:193.3U/mL, p=0.5303)
- BlotB: 9/17 positivos ($\bar{x}$Negativo:25.5U/mL/$\bar{x}$Positivo:133.6U/mL p=0.0325).

En un segundo paso analizamos 1259 determinaciones realizadas por BlotA. El 93% fue negativo para anti-Mi2α y anti-Mi2β. De las 88 positivas: 6 fueron positivas para α+β (5/6ELiA Positivo), 16 fueron positivas aisladas para anti-Mi2α y 66 para anti-Mi2β. De las aisladas, 4/69

determinadas por ELiA fueron positivas, especificidad Mi2α=8% y Mi2*β*=5%. Comparando las dos series observamos que el perfil α y β de BlotA cambia cuali y cuantitativamente cuando es positivo por todas las técnicas versus positivos aislados (Figura1).

La probabilidad de confirmar anti-Mi2 por inmunoblot depende del fabricante empleado. Esta limitación en BlotA se observa incluso en pacientes con niveles altos. Describimos además inespecificidades del BlotA, donde el perfil α+*β* es el más especifico. Estos resultados implican una variable capacidad diagnóstica.

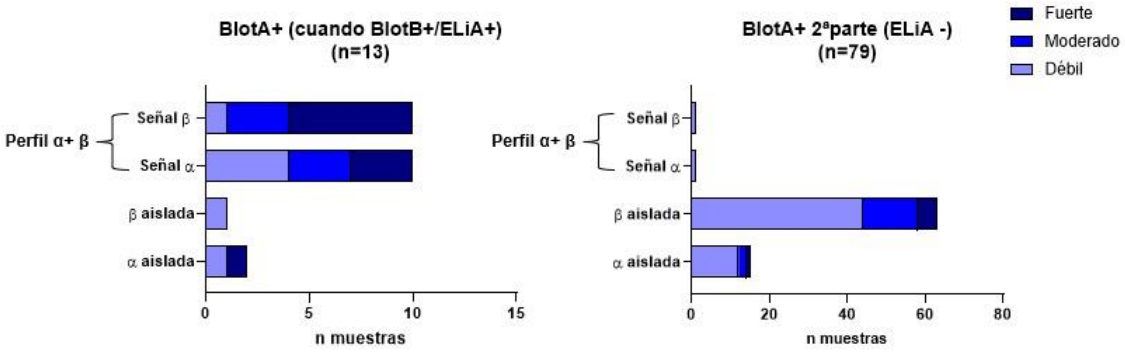


Figura1



P-150

PERFILES DE ALTERACIÓN HEPÁTICA EN PACIENTES CON AUTOANTICUERPOS ANTINUCLEARES ASOCIADOS A COLANGITIS BILIAR PRIMARIA

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Introducción

Los anticuerpos antinucleares (ANA), anti-SP100 y anti-GP210, y antimitocondriales (AMA), son claves junto con la elevación de fosfatasa alcalina (FA) en el diagnóstico de colangitis biliar primaria (CBP). Últimamente la elevación de gamma-glutamil transferasa (GGT) está siendo considerada.

Objetivo

Evaluar la asociación entre anti-SP100 y/o anti-GP210 con o sin AMA con diferentes perfiles de alteración colestásica y diagnóstico de CBP.

Métodos

Estudio observacional retrospectivo de 289 pacientes anti-sp100/anti-GP210+ (2008-2021). ANA y AMA (M2) fueron detectados mediante inmunofluorescencia indirecta en células Hep2000 y triple tejido de rata respectivamente (*A.* Menarini Diagnostics) y confirmados por Line-dot (Euroline *Autoimmune liver disease*, Euroimmun). Se recopiló de la historia clínica: alteración de FA, GGT y diagnóstico.

Resultados

Del grupo estudiado 36,84% presentaban ANA y AMA y 64,56% sólo anti-SP100/GP210. 34,6% fue diagnosticado de CBP.

En AMA+ se observó con más frecuencia la alteración enzimática de FA y GGT que en AMA-, así como más CBP (p-valor <0.05). De 105 pacientes AMA+, 68 (64,76%) presentaron alteración de GGT y FA, 3 (2,85%) de FA aislada y 11 (10,47%) de GGT. La presencia de CBP fue mayor en elevación enzimática conjunta (85,29%) respecto a FA aislada (66.6%) o GGT (27.27%).

En AMA- (184 casos) la distribución fue diferente: 48 (26,08%) elevaron FA y GGT, 10 (5.43%) FA y 43 (23.36%) GGT. Además, la elevación de dos enzimas colestásicas no asoció



más CBP (50%) que sólo FA (40%). En alteración GGT aislada hubo un 7.5% de CBP.

Conclusiones

En pacientes con anti-SP100 y/o anti-GP210, un AMA+ aumenta la frecuencia de alteración enzimática y el diagnóstico de CBP. Además, en este grupo la elevación de GGT aumenta la probabilidad diagnóstica respecto a FA aislada. En AMA- la alteración de GGT no incrementa la probabilidad de CBP respecto a FA aislada en nuestra muestra y tiempo de estudio.



P-151

EFFECTS OF BAMBI DEFICIENCY IN THE DEVELOPMENT OF SYSTEMIC LUPUS ERYTHEMATOSUS.

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Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the production of autoantibodies and immune complexes deposition in various organs. Follicular $CD4^+$ T cells play a crucial role in this autoimmune syndrome by activating autoreactive germinal center B cells. The transforming growth factor β (TGBβ) inhibitor BAMBI (**B**one Morphogenetic Protein and **A**ctivin **M**embrane-**B**ound **I**nhibitor) is known to control the differentiation of $CD4^+$ T lymphocytes into either tolerogenic Tregs or pathogenic Th17 cells. BAMBI absence in mutant mice or its pharmacological inhibition with the B101.37 monoclonal antibody promotes an increase in Treg differentiation and activity and at the same time, a reduction in Th17 cell differentiation, resulting in the inhibition of autoimmune disease development.
Based on these findings, in the present study we have analysed the effects of BAMBI deficiency in the development of SLE. We have employed the chronic graft-versus-host disease lupus model induced after the transference of 40-60 × 10^6 lymphocytes from B6 or B6-BAMBI-KO mice into either Bm12 or Bm12-BAMBI-KO recipients. As reported previously, Bm12 mice receiving lymphocytes from B6 mice produce high levels of circulating IgG anti-DNA and anti-nucleosomal autoantibodies and exhibit linear glomerular IgG deposits in their kidneys. In contrast, both Bm12 mice injected with allogeneic B6-BAMBI-KO lymphocytes and Bm12-BAMBI-KO recipients receiving lymphocytes from B6 donors do not produce significant levels of circulating autoantibodies. Taken together, these results indicate that the absence of BAMBI in either donor or recipient lymphocytes protects mice against the development of SLE.



P-152

EL EFECTO DE LAS CÉLULAS DECIDUALES ESTROMALES HUMANAS EN LA MIGRACIÓN DE CÉLULAS T ACTIVADAS CXCR3+ (TH1, TC1). PAPEL EN LA TOLERANCIA INMUNOLÓGICA MATERNO-FETAL

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Las células deciduales estromales (DSC) juegan un papel clave en la tolerancia inmunológica materno-fetal. En modelo murino se ha observado que las DSC inhiben la expresión de quimiocinas que atraen células Th1 y Tc1, de actividad abortígena. No obstante, no hay estudios equivalentes en humanos. Por ello, el objetivo del trabajo es determinar la expresión de quimiocinas en DSC humanas no decidualizadas (preDSC) y DSC decidualizadas (dDSC), así como estudiar su actividad quimiotáctica sobre células T activadas.

Las líneas de DSC se decidualizan con AMPc y progesterona y se recogen medios condicionados (MC) del sobrenadante del cultivo de preDSC y dDSC. Se compara, mediante RT-PCR, la expresión de CXCL9, CXCL10 y CXCL11. Además, se estudia la migración, en Cámaras Transwell, de linfocitos de sangre periférica activados con PHA+anti-CD28+IL-2, en contacto con MC. Se emplean membranas de diálisis MWCO 6-8kD para caracterizar moléculas implicadas en la migración.

Los resultados indican que preDSC y dDSC no expresan CXCL9 y la expresión de CXCL10 y CXCL11 disminuye significativamente con la decidualización. Los MC de preDSC incrementan significativamente la migración de células T activadas, pero esta se inhibe significativamente con los MC de dDSC. La diálisis abolió el efecto inhibitorio de la migración del MC de dDSC.

Concluimos que la decidualización inhibe la expresión en DSC de quimiocinas que atraen células Th1 y Tc1. Además, este proceso induce la secreción de factores inhibidores de la migración. Así se evita la llegada de células T abortígenas a la decidua.



P-153

THE IMMUNOMODULATORY FUNCTION OF ENDOGENOUS GALECTIN-1 IN DSS-INDUCED COLITIS

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Galectin-1 is a member of a family of carbohydrate-binding proteins, the so-called lectins, with high affinity for β-galactosides. Galectin-1 is widely expressed in many tissues and has immunomodulatory activity over different immune cell subsets that has been associated to induction of apoptosis in the target cells. Although, the immunosuppressive function of recombinant galectin-1 has been reported in many studies, the role of endogenous galectin-1 remains to be fully defined. In this study, galectin-1 deficient mice (*Lgals1*$^{-/-}$ mice) were used to investigate the *in vivo* function of galectin-1 in experimental colitis. *Lgals1*$^{-/-}$ mice showed enhanced susceptibility to experimental colitis with respect to WT mice as shown by the increased body weight losses and higher DAI compared to colitic WT mice. An increased plasticity of Th17 cells towards Th1 profile in DSS-induced colitic Lgals1$^{-/-}$ mice were determined. Strikingly, the exacerbated intestinal inflammatory responses in colitic Lgals1$^{-/-}$ mice was accompanied with increased frequencies of regulatory T cells in cLP. In addition, our results show that in the absence of endogenous galectin-1 an enhanced conversion of Foxp3^{+}CD4^{+} Treg cells into pathogenic T-Bet-expressing Foxp3^{+}CD4^{+} T cells was determined. Adoptive transfer of wild-type Foxp3^{+}CD4^{+} regulatory T cells into Lgals1$^{-/-}$ mice during DSS-induced colitis reduces the severity of the disease, achieving a similar intestinal inflammation as to colitic WT mice. Altogether, our results highlight the relevance of the expression of endogenous galectin-1 to prevent the development of aberrant pathogenic Treg cells during the course of an intestinal inflammatory response.



P-154

CHARACTERIZATION OF INTERSTITIAL LUNG DISEASE PRESENT IN P-SELECTIN KO MICE (LUPUS-LIKE SYNDROME) AND PSGL-1 KO MICE (SCLERODERMA-LIKE SYNDROME)

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Systemic lupus erythematosus (SLE) and Systemic sclerosis (SSc) are multiorganic autoimmune diseases with significant mortality and morbidity. One of most severe clinical manifestation in both diseases is interstitial lung disease (ILD), a parenchymal lung disorder with a pattern of inflammation and fibrosis. The interaction of P-Selectin glycoprotein ligand-1 (PSGL-1)/P-selectin plays an important role in immune system homeostasis. Indeed, the absence of PSGL-1 develop SSc-like syndrome and P-Selectin absence develop SLE-like syndrome in mice.

C57BL/6 WT, PSGL-1 KO and P-Selectin KO mice (n=8/group), aged 12-24 month, were used in this study. Bronchoalveolar lavage (BAL) and lungs were obtained and analyzed by flow cytometry to characterize the ILD present in these animals.

P-selectin and PSGL-1 KO mice presented an increase number of CD45(+) cells in BAL, including alveolar and interstitial macrophages, eosinophils and granulocytes, with respect to WT mice. Also, lung analyse in P-Selectin KO mice showed increased number of eosinophils, granulocytes, T cells and B cells compared to WT, coinciding with PSGL-1 KO mice. Secondly the study of CD45(-) population revealed a higher total number of cells in BAL and lung of PSGL-1 KO mice. Further analyses showed increased CD31(+) cells in lung and increased CD90(+) cells in BAL. However, P-Selectin KO mice just showed increased number of cells in CD45(-) population of BAL compared to WT.
The study of apoptosis in lymphoid and non-lymphoid populations showed a higher number of apoptotic CD45(+) and CD45(-) cells in both P-Selectin and PSGL-1 KO mice in lung tissue. Regarding BAL, PSGL-1 KO but not P-Selectin KO mice, showed increased number of apoptotic CD45(+) cells.

These results indicate that P-Selectin KO-ILD and PSGL-1 KO-ILD show a similar lung



infiltration pattern regarding CD45(+) population although B cells were only increased in P-Selectin KO mice. Moreover, high level of apoptotic cells was found in both groups.



P-155

EVOLUCIÓN DEL LINFOGRAMA DUODENAL EN PACIENTES CELIACOS ADULTOS TRAS RETIRAR EL GLUTEN DE LA DIETA

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Introducción: La enteropatía celiaca (EC) se caracteriza por cambios en las subpobaciones de linfocitos intraepiteliales (LIE), su determinación mediante citometría de flujo (CMF) es el linfograma duodenal. Nuestro grupo ha establecido los puntos de corte que definen un linfograma celiaco: %LIE TCR□□+ >14% y %LIE sCD3- <4%.

Objetivo: Conocer cómo evoluciona el linfograma en pacientes adultos con EC activa (ECA) al iniciar dieta sin gluten (DSG).

Pacientes y Métodos: Estudio retrospectivo sobre una cohorte de 59 pacientes ECA. Disponemos de los resultados de anatomía patológica y linfograma antes y después de la retirada de gluten de la dieta (tiempo mediano de DSG 24 meses), y de las determinaciones de anticuerpos anti-trasglutaminasa (AcTG2). Como grupo control se incluyeron 410 pacientes a los que se descartó EC (ver tabla).

Resultados: Tras la DSG el 90% de los pacientes mantiene un %LIE TCR□□ superior al 14% y el 56% mantiene un %LIE sCD3- inferior al 4% sin poder establecer una relación clara con el grado de atrofia vellositaria. El 52,4% mantienen el linfograma celiaco completo, un 45,6% linfogramas parciales y solo el 6,8% presenta un linfograma no celiaco.

CONCLUSIONES: La mayoría de los pacientes mantienen alteraciones en el linfograma a pesar de una correcta adherencia a la DSG, con negativización de AcTG2 y recuperación de la integridad de la mucosa (ver tabla). El linfograma celiaco constituye un marcador de EC, tanto en el estadío inflamatorio como tras tratamiento cuando se ha revertido la inflamación y la lesión histológica.



	Control	ECA	DSG
%IEL mediana Q1/Q3	8,1 Q1:5,1/Q3:11,2	18,2 Q1:14,3/Q3:23,7	10,5 Q1:6,8/Q3:14,8
%TCR□□+ mediana Q1/Q3	4,9 Q1:2,5/Q3:9,4	21,2 Q1:16,2/Q3:31,1	27,3 Q1:20,5/Q3:34,4
%sCD3- mediana Q1/Q3	14,4 Q1:8,6/Q3:24,2	0,8 Q1:0,4/Q3:1,4	3,2 Q1:1,2/Q3:6,7
Pacientes con AcTG + (%)	0	91,5	3,4
Pacientes con atrofia vellositaria grado MIII (%)	0,5	100	18,6

Q1: cuartil 1, Q3: Cuartil 3



P-156

PSGL-1/P-SELECTIN AXIS IN THE HOMEOSTASIS OF MONOCYTES FROM HEALTHY DONORS, LUPUS AND SCLERODERMA PATIENTS.

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Background:

PSGL-1, a transmembrane protein found in all leukocytes, is involved in the immune system homeostasis regulation through interaction with P-selectin. One of the pathways activated after P-selectin/PSGL-1 interaction is AKT-mTOR, a signaling pathway implicated in autophagy. P-selectin and PSGL-1 KO mice develop autoimmune syndromes similar to lupus and scleroderma, respectively. Interestingly, these autoimmune diseases have alterations in autophagy that prevent the correct elimination of cell fragments, thus contributing to their pathogenesis.

Objectives:

1- To study the basal levels of autophagy (LC3B-II expression), pAKT and p-mTOR in monocytes from HD, lupus and scleroderma patients.

2- To determine whether PSGL-1/P-selectin interaction regulates the expression of LC3B-II, pAKT and p-mTOR in monocytes from HD, lupus and scleroderma patients.

Materials and Methods:

Human monocytes were isolated from healthy donors (HD), lupus and scleroderma patients. After incubation with BSA or BSA+P-selectin in rolling-like conditions (shaking at 60 rpm) for 15 min, cells were left to adhere at 37ºC for 1 hour. Other cells were incubated without rolling in BSA at 37ºC for 1 hour. The measurement of LC3B-II, pAKT and p-mTOR values was performed by flow cytometry.

Results:

Circulating monocytes from lupus and scleroderma patients show increased LC3B-II and pAKT levels compared to HD. In addition, scleroderma patients present higher p-mTOR expression than HD.

PSGL-1/P-selectin interaction in rolling-like conditions induces a decrease in LC3B-II levels in monocytes from HD and scleroderma patients, with respect to rolling on BSA. Moreover, PSGL-1/P-selectin interaction reduces pAKT values in HD and lupus patients. P-selectin interaction also reduces p-mTOR level in lupus patients but not in HD.



Conclusions:

Lupus and scleroderma patients show alterations in the levels of LC3B-II, pAKT and p-mTOR. PSGL-1/P-selectin interaction reduces LC3B-II and pAKT expression in monocytes from HD. However, monocytes from lupus and scleroderma patients have altered the response to P-selectin engagement.



P-157

PHENOTYPE CLUSTERS OF ANTI-MDA5 POSITIVE DERMATOMYOSITIS PATIENTS IN ASTURIAS

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Anti-Melanoma Differentiation-Associated gene 5 (MDA5) is a Dermatomyositis (DM) specific antibody (Ab) initially described in amyopathic patients with rapidly progressive interstitial lung disease (RP-ILD). Its widespread determination by commercial methods has allowed the description of other clinical associations. In 2020, by applying a decisional algorithm, a French multicenter study of 83 anti-MDA5 DM patients established three well differentiated phenotype clusters: a RP-ILD cluster (15/83, 18.1%), a rheumatoid cluster (46/83, 55.4%) and a vasculopathic cluster (22/83, 26.5%). The RP-ILD cluster showed a high mortality rate while the rheumatoid cluster had an overall better outcome. This study applies the proposed algorithm to a cohort of anti-MDA5 Asturian DM patients.

We reviewed relevant clinical information of 10 anti-MDA5 positive DM patients detected between 2008 and 2022 in the Immunology Department of the Hospital Universitario Central de Asturias (HUCA) which is the reference center for the autoimmune serologic diagnosis in the autonomous region of Asturias, Spain. In all patients, the presence of anti-MDA5 was confirmed by two commercial immunoblots.

Six patients were female (60%) and the age at DM diagnosis was 44-77 years. All the patients exhibited dermatologic manifestations; 7 patients showed respiratory symptoms, 6 of them suffering from ILD; 6 patients had muscle involvement; 8 developed rheumatologic manifestations and 4 of them presented Raynaud phenomenon. By applying the decisional algorithm, 2 of the studied patients would be grouped in the RP-ILD cluster (20%), 5 fit in the rheumatoid cluster (50%) and 3 could be included in the vasculopathic cluster (30%). Two patients died, both belonging to the RP-IDL cluster.

These results show that the Asturian anti-MDA5 DM cohort reproduces the phenotype cluster distribution observed in the French cohort, with most of the patients in the rheumatoid cluster.



Sesión Poster. Autoinmunidad (II). Pantalla 2

P-159

ACETATE CORRELATES WITH OPTIMAL RESPONSE TO OCRELIZUMAB IN LOW INFLAMMATORY PRIMARY PROGRESSIVE MULTIPLE SCLEROSIS PATIENTS.

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Background: Short-chain fatty acids (SCFA) are compounds derived from gut microbiota metabolism that have a role in gut-brain axis and have been related to multiple sclerosis (MS) etiopathogenesis.

Objective: To ascertain the role of plasma SCFA levels in the response to ocrelizumab in primary-progressive multiple sclerosis (PPMS) patients.

Methods: Multicentre prospective study including 69 patients with PPMS who initiated ocrelizumab treatment, classified according to baseline presence [Gd+, n=16] or absence [Gd-, n=53] of gadolinium-enhancing lesions in brain MRI. Serum SCFA and immunoglobulin levels were measured at baseline and 6 months after ocrelizumab initiation by liquid chromatography-mass spectrometry and nephelometry, respectively.



Results: Ten (62.5%) Gd+ and 41 (77.4%) Gd- patients reached an optimal response (NEDA) defined as no disability progression or new MRI lesions after 1 year of ocrelizumab treatment. NEDA status associated, only in low-inflammatory Gd- patients, with an increase of plasma acetate levels 6 months after the first ocrelizumab dose ($p=0.0013$) and this correlates with an elevation in serum IgA levels ($p=0.006$) and IgA/IgG ratio ($p=0.0011$). No differences were found in Gd+ groups.

Conclusions: NEDA associated in Gd- patients with increased plasma acetate levels, thus strongly suggesting an association between gut microbiota and response to ocrelizumab in low-inflammatory PPMS patients. Future studies will further demonstrate the correlation between microbiota composition and response to ocrelizumab in these patients.



P-160

NORMALIZACIÓN DE ANTICUERPOS ANTITRANSGLUTAMINASA EN PACIENTES CON DÉFICIT DE IGA Y ENFERMEDAD CELÍACA: EXPERIENCIA DE UN CENTRO

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Introducción y Objetivos

La enfermedad celíaca (EC) es una enteropatía autoinmune frecuente, desencadenada por el consumo de gluten en individuos con predisposición genética a la misma. Los pacientes con déficit de IgA tienen mayor riesgo, en comparación con población sana, de desarrollar patologías autoinmunes como la EC.

El objetivo de este estudio es determinar si hay diferencias significativas en el tiempo de persistencia de anticuerpos antitransglutaminasa IgG (TGG) o IgA (TGA) en pacientes celíacos con o sin deficiencia de IgA, respectivamente, desde el inicio de una dieta sin gluten (DSG).

Métodos

Se realizó un estudio retrospectivo mediante la revisión de historias clínicas de los 1342 pacientes con deficiencia de IgA seguidos en la Unidad de Inmunología del Hospital Universitario La Paz: desde 2009, 78 de ellos se han diagnosticado de EC, de los cuales 36 han tenido un seguimiento regular y fueron seleccionados para este estudio. Se eligieron 36 pacientes celíacos de edades similares sin deficiencia de IgA como grupo control. Se realizó un análisis de supervivencia utilizando el test de Mantel-Coz para comparar el tiempo de normalización de los títulos de anticuerpos antitransglutaminasa desde el inicio de DSG en ambos grupos.

Resultados

Se encontraron diferencias significativas entre ambos grupos: la mediana de tiempo necesario para aclarar TGG en pacientes con EC y deficiencia de IgA fue de 19.47 meses, mientras que la necesaria para aclarar TGA en pacientes con solo EC fue de 9.45 meses (P=0,0002)

Conclusiones

Los pacientes con EC y deficiencia de IgA tardan más tiempo en alcanzar valores normales de anticuerpos antitranglutaminasa desde el inicio de DG que los celíacos que no presentan deficiencia de IgA.



P-161

EXPRESIÓN DE RECEPTORES DE BAFF Y APRIL EN PACIENTES CON LUPUS ERITEMATOSO SISTÉMICO

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Los desajustes en los mecanismos de control de las células B autorreactivas son importantes en la etiopatogenia del lupus eritematoso sistémico (LES). BAFF (B cell activating factor belonging to the TNF family) es una citocina miembro de la familia TNF, clave en la regulación de la homeostasis de los linfocitos B, ya que controla el número total de células B periféricas mediante la interacción con sus receptores. APRIL (A proliferation-inducing ligand) es una citocina relacionada con BAFF con la que comparte dos receptores, TACI y BCMA, mientras que, un tercero, BAFF-R, es exclusivo de BAFF. Estos receptores se expresan en la superficie de linfocitos B dependiendo de su grado de maduración y pueden encontrase en otros tipos celulares, como BCMA que se expresa en células plasmáticas. Con el objetivo de mejorar el conocimiento de la implicación del sistema BAFF/APRIL y sus receptores en el desarrollo del LES reclutamos una cohorte de mujeres entre 18 y 60 años compuesta por 42 pacientes (18 con nefritis y 24 sin nefritis) y 26 controles sanos de las que se obtuvo muestra de sangre total y se determinaron, a través de citometría de flujo, los porcentajes de expresión de BAFF-R y TACI con respecto a los linfocitos CD19+ y porcentaje de expresión de BCMA respecto a los linfocitos CD19+/CD27+/CD38+ (células plasmáticas), junto a sus intensidades de fluorescencia media (MFI). Como resultado, no encontramos diferencias en los porcentajes de expresión entre pacientes y controles (94,5% vs. 94,8% en BAFF-R; 27,0% vs. 31,1% en TACI y 3,9% vs. 3,6% en BCMA respectivamente, $P>0.05$). Tampoco encontramos diferencias significativas en las MFI, ni al comparar estas variables entre los grupos de pacientes con y sin nefritis. En conclusión, nuestros resultados sugieren que la expresión de receptores tanto BAFF como APRIL no estarían implicados en la fisiopatogenia del LES.



P-162

DETECTION OF M2-TYPE ANTI-MITOCHONDRIAL ANTIBODIES SUBTYPES IN THE DIAGNOSIS OF PRIMARY BILIARY CHOLANGITIS IN PATIENTS WITH DISCORDANT RESULTS

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



P-163

AUTOANTIBODIES DETECTION BY MULTIPARAMETRIC ASSAY IN PATIENTS WITH SYSTEMIC AUTOIMMUNE DISEASES

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Introduction

Systemic autoimmune diseases (SAID) are a heterogeneous group of diseases characterized by an immune response against self-antigens. Autoantibodies (Abs) are a hallmark of most of the SAID being some of them part of the classificatory criteria. In the last years, multiparametric technologies for Abs detection have been proposed to improve the accuracy of the immunological diagnosis of SAID. Particle-based multi-analyte technology (PMAT-Aptiva) is a multiplexed system based on the coupling of different antigens to paramagnetic particles with unique signatures. It allows us to obtain results from different Abs simultaneously.

The aim of this study was to evaluate the CTD-Essential (PMAT-Aptiva) diagnostic performance in a cohort of patients with SAID from the Hospital Clínic.

Patients and Methods

Study cohort includes patients with Systemic Sclerosis (n=99), Systemic Lupus Erythematosus (n=63), Sjögren Syndrome (n=39), Mixed Connective Tissue Disease (n=12), anti-Synthetase Syndrome (n=5), other SAID (n=32) and other patients without SAID (n=70).
Anti-dsDNA, -Sm, -Ribo-P, -Ro/SSA, -La/SSB, -Ro52, -U1-RNP, -Jo1, -Cenp-B, -Scl70, -DFS70 Abs were measured by the classical method employed by our laboratory (EIA, CIA, FEIA or Immunoblot) and PMAT-Aptiva.

Results

A good correlation (Cohen kappa >0.7) was observed for all Abs, except for anti-Sm (CIA results) and Jo1 (Immunoblot results). CIA results for anti-Jo1 Abs show good correlation. Using PMAT-technology, positive-likelihood ratio for each biomarker ranges from 1.93 to >50.00 being the best (>10) for anti-Sm, -Ribo-P, -Jo1 and -Cenp-B. Specificities range between 72.9%-100% for all biomarkers whereas sensitivities range between 43.2%-80.0% excluding anti-Sm (15.9%), -Ribo-P (15.9%) and -Ro52 (33.9%) Abs.



Conclusions

In relation to anti-Sm and -Jo1 Abs, the difference observed can be explained due to the different nature of the antigen used for each method.

However, multiparametric Abs testing using PMAT-Aptiva is a good option for Abs detection and to improve SAID diagnosis, showing a good sensitivity and specificity.



P-164

ASOCIACIÓN ENTRE BIOMARCADORES "NO CRITERIO" Y COMPONENTES DEL COMPLEMENTO EN PACIENTES CON SÍNDROME CORONARIO AGUDO Y SÍNDROME ANTIFOSFOLÍPIDO SUBYACENTE.

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Antecedentes y Objetivos

El síndrome antifosfolípido (SAF) es una enfermedad autoinmune sistémica definida por la aparición de abortos recurrentes y trombosis en presencia de anticuerpos antifosfolípidos. Investigaciones recientes muestran el papel del perfil inmunológico en el infarto agudo de miocardio (IAM) en pacientes con APS. En este ensayo comparamos dos grupos (pacientes de APS con SCA frente a control) para comprobar la contribución de estos anticuerpos.

Métodos

Para comparar ambos grupos de pacientes estudiamos los siguientes parámetros:

Citocinas (IL-1β, IL-17, TNF-α e IL-6) analizadas con Luminex® Multiplex Assays.

Componentes del complemento e inmunoglobulinas analizadas por nefelometría.

Autoanticuerpos analizados por ELIA y ELISA.

Resultados

Se observaron diferencias estadísticamente significativas, en los valores de ACA IgA, IL-6, IL-17, MPO, PR3 y antiCCP. Así como en las determinaciones de C3, C4, PCR y VSG.

No se encontró diferencias estadísticas en los niveles de inmunoglobulinas.

Conclusión

Nuestro estudio confirma la existencia de alteraciones en el perfil inmunológico (autoanticuerpos "no criterio" y componentes del complemento) en los pacientes con SAF que sufren SCA

Se deben seguir haciendo estudios que confirmen estos resultados, así como recomendar la determinación de un perfil inmunológico en pacientes con predisposición de sufrir con SCA dada su relevancia clínica y terapéutica.

P-165

SAFO NO CRITERIO, ¿REALMENTE NO CRITERIO?

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Las nuevas técnicas de laboratorio para anticuerpos antifosfolípido (AAF) y el concepto de morbilidad obstétrica "no criterio" señalan la necesidad de revisar los criterios de clasificación del Síndrome antifosfolípido obstétrico (SAFO).

Valorar si la ampliación del panel de AAF y de los criterios de morbilidad obstétrica permite clasificar como SAFO a mujeres hasta ahora fuera de esta clasificación y analizar la eficacia del tratamiento.

Estudio retrospectivo y descriptivo que incluyó mujeres <45 años, remitidas a la consulta de hematología e inmunología de la gestación de nuestro centro (2020-2021) en las que no existía otra causa que justificara morbilidad obstétrica. Se les realizó estudio de AAF clásicos por triple técnica: ELISA-QUANTALite® (Inova Diagnostics), CLIA-BIO FLASH® (Inova Diagnostics) y ELiA-ImmunoCap® (Thermo Fisher Scientific); y de AAF "no clásicos": aPS/PT IgG/IgM ELISA-QUANTALite® (Inova Diagnostics), EliA-Cardiolipin IgA® y EliA-β2GlycoproteinI IgA® (Thermo Fisher Scientific).

La morbilidad obstétrica "no criterio" se definió según Arachchillage 2015. Se analizó la variable "embarazo a término" y su relación con el tratamiento.

73 de las 278 pacientes cumplieron criterios de inclusión (Figura 1). La aplicación de la triple técnica para AAF clásicos permitió incluir a un 69.23% de las pacientes en criterios de Sídney respecto al 30.77% clasificadas utilizando una sola técnica.

13.70% de las pacientes presentaban únicamente AAF "no clásicos" (presentes en un 38.36% del total).

En un 67.12% se identificó morbilidad obstétrica "no criterio". No se encontraron diferencias entre las pacientes SAFO "criterio" y SAFO "no criterio" respecto a la variable "embarazo a término" (p=1), ni entre los tratamientos recibidos.

El uso de diferentes ensayos para la detección de AAF clásicos permitió reclasificar como SAFO a un grupo de mujeres susceptibles de tratamiento. Esto y la consideración de la morbilidad obstétrica "no criterio" podría aportar beneficio clínico a un subgrupo de pacientes actualmente no contemplado.



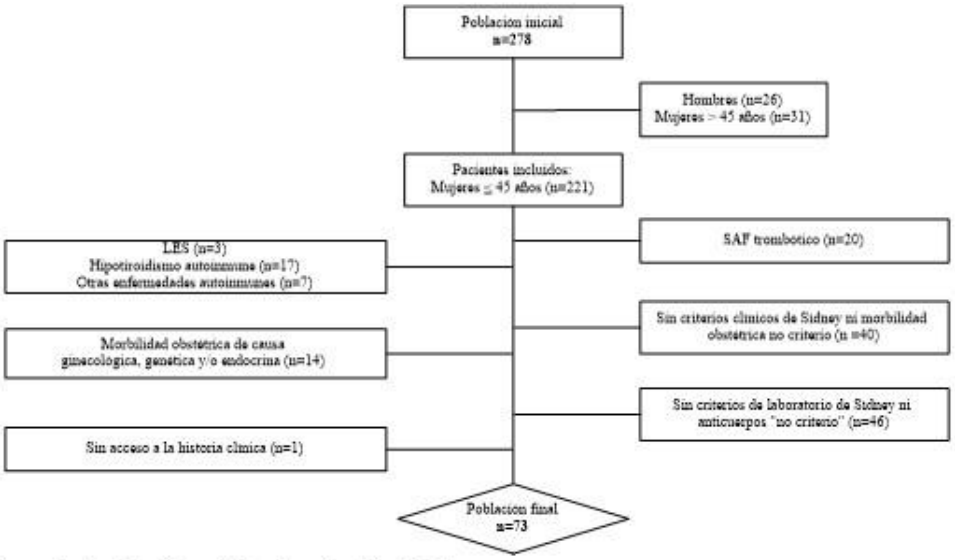


Figura 1. Población sujeta de estudio SAFO



P-166

RECUPERACIÓN DE PACIENTES CON SOSPECHA DE COLANGITIS BILIAR PRIMARIA PERDIDOS POR EL SISTEMA DE SALUD.

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Introducción.

La Colangitis Biliar Primaria (CBP) es una enfermedad autoinmune caracterizada por la destrucción de los canalículos biliares intrahepáticos, lo que produce colestasis y daño hepático. Se estima una prevalencia en España de 19,5/100000 habitantes y una incidencia de 1,7/100000 habitantes. La identificación de estos pacientes es importante para un correcto seguimiento y tratamiento. Al tratarse de una enfermedad infrecuente, la sospecha de CBP es baja, existiendo el riesgo de "perder" pacientes en el transcurso de la atención sanitaria. El objetivo de este estudio es identificar, según la literatura, los pacientes con CBP que se han perdido por el sistema de salud en el área de Gipuzkoa.

Materiales y métodos.

Estudio retrospectivo de bases de datos de resultados analíticos de inmunología y bioquímica, y revisión de historias médicas de los sistemas informáticos del Área de Salud de Gipuzkoa, entre el 01/01/2019 y el 31/12/2021. El diagnóstico de CBP se basa en un título de anticuerpos anti-mitocondriales (AMA) ≥ 1/80, elevación crónica de la fosfatasa alcalina (FA) y ausencia de otras patologías hepáticas. Los pacientes fueron identificados como "perdidos por el sistema" en ausencia de diagnóstico médico y/o seguimiento y tratamiento médico específicos.

Resultados.

Pacientes con FA y AMA positivos: 178

Pacientes diagnosticados de CBP, HAI, síndromes *overlap* o patologías relacionadas: 164 (92,1%)

Pacientes perdidos por el sistema: 14 (7,9%)

Conclusiones.

Un 7,9% de los pacientes que cumplían con los criterios diagnósticos de CBP han sido perdidos por el sistema.



Estos pacientes no se están beneficiando de tratamientos específicos efectivos (principalmente ácido ursodesoxicólico) para modificar la progresión a cirrosis y complicaciones asociadas. El screening de AMA con título ≥ 1/80 junto con elevación crónica de FA se muestra como criterio eficaz para el diagnóstico de CBP. Junto con la historia clínica, permite detectar "pérdidas de pacientes" por el sistema de salud.



P-167

ANTI-OJ ANTIBODY NOT DETECTED BY COMMERCIAL LINE IMMUNOASSAY

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Introduction:

The anti-synthetase syndrome (ASS) is a rare autoimmune disease defined by antibodies directed against an aminoacyl tRNA synthetase along with clinical features that can include interstitial lung disease (ILD), myositis, Raynaud's phenomenon, arthritis, unexplained fever, and mechanic's hands. The anti-synthetase antibodies are particularly useful in the identification of patients with ILD, since many times the distinctive muscle weakness is not present.

Clinical case:

We report a 65 years old male who debuted in February 2021 with a low exertion dyspnoea, with cough without expectoration that required oxigenotherapy. Radiography was performed showing bilateral infiltrates in the lungs, and it was evaluated as a bilateral pneumonia, highly suspicious of infection by SARS-COV-2. The result of the PCR was negative and the CT scan showed findings consistent with bilateral organized pneumonia.

Treatment with 80 mg prednisone was started but despite that, due to increased O2 requirements, he was transferred to the ICU where he required invasive ventilation. In the differential diagnosis of organized pneumonia, antinuclear antibodies (ANA) and commercial myositis profile were tested.

Results:

The ANA testing revealed a 1:640 cytoplasmic dense fine speckled (AC-19) pattern that strongly suggested an anti-synthetase antibody. But the myositis profile turned out to be negative. Because the clinical suspicion matched the ANA pattern, an RNA immunoprecipitation was performed and we observed a pattern compatible with anti-synthetase antibody. Then, we performed a protein immunoprecipitation-western blot. The result revealed that it was an anti-OJ antibody that recognizes conformational epitopes.



Conclusion:

In the case of a negative myositis immunoblot with high clinical suspicion for anti-synthetase syndrome and ANA pattern that supports it, the diagnosis should not be discarded. In these cases, it is recommended to perform an extended analysis including RNA and protein immunoprecipitations.



P-168

COMPARACIÓN DE TRES TÉCNICAS PARA LA DETERMINACIÓN DE ANTICUERPOS ANTI-DSDNA

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Introducción

Los anticuerpos anti-ácido desoxirribonucleico de cadena doble (anti-dsDNA por sus siglas en inglés) se asocian principalmente a lupus eritematoso sistémico (LES), siendo de utilidad para el diagnóstico y el seguimiento. No obstante, si existe una alta sospecha clínica, el LES no debe descartarse ante un valor negativo de anticuerpos anti-dsDNA, ya que existen otros criterios diagnósticos.

Objetivo

Comparar los resultados de tres técnicas para la determinación de anticuerpos anti-dsDNA: inmunoensayo multiplex (BioPlex 2200, Bio-Rad), inmunoblot (EUROBlotOne, Euroinmmun) e inmunofluorescencia indirecta (IFI) sobre *Crithidia luciliae* (Sprinter, Euroinmmun).

Material y Métodos

Se analizaron un total de 214 muestras séricas por estas tres técnicas. En el caso del inmunoensayo multiplex, se consideraron positivas aquellas muestras con un valor >10 IU/mL siguiendo las indicaciones del fabricante.

Resultados

Todas las muestras negativas por multiplex (≤10 IU/mL) fueron también negativas por los otros dos métodos con la excepción de una muestra que fue positiva por IFI y otra que fue positiva por inmunoblot. De los resultados positivos por multiplex con valores entre 11 y 20 IU/mL, el 22,99 % se confirmaron por IFI sobre *Crithidia*, el 10,34 % por inmunoblot y el 6,90 % por ambas técnicas.

El porcentaje de resultados concordantes entre multiplex e IFI aumentó hasta el 33,33 % en el rango de 21-30 IU/mL, hasta el 59,57 % para valores entre 31-90 IU/mL y hasta el 80,95 % en resultados >90 IU/mL.

Conclusión

La diferencia de sensibilidad y especificidad entre los distintos métodos hace necesaria la realización de al menos dos técnicas para confirmar los resultados positivos. La técnica por

multiplex demuestra una alta sensibilidad, pero es necesaria la confirmación por IFI, mucho más específica, para descartar los falsos positivos. El uso de una tercera técnica, como podría ser el inmunoblot, podría ser de utilidad en casos con resultados dudosos o discrepantes.



P-169

DISCREPANT RESULTS BETWEEN DSDNA ANTIBODY ASSAYS FOR DIAGNOSIS AND FOLLOW UP OF SLE

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Introduction

There are a variety of tests to identify anti-dsDNA autoantibodies, but EliA and Crithidia immune-fluorescence test (CLIFT) are the most commonly used. Due to their differences in specificity and sensitivity, it is frequent to obtain discrepant results between them. Despite the high sensitivity of EliA, CLIFT is still considered the gold standard for high avidity anti-dsDNA antibodies in SLE. This study aimed to outline the relevance of EliA in discrepant cases after 4-6 years of follow-up.

Materials and methods

ANA screening by indirect immunofluorescence was performed in 6954 samples and EliA anti-dsDNA antibodies were tested for those showing a related pattern. 1024 samples were classified as positive (anti-dsDNA >10 UI/ml) and CLIFT was only performed in 417 of them belonging to 225 patients. The diagnosis, evolution and clinical relevance of both tests were studied in patients with discrepant results.

Results

Among 225, 89 of patients were CLIFT and EliA positive and 127 were discrepant (EliA positive and CLIFT negative). 44 discordant patients were SLE diagnosed, 10 of them were new diagnoses and 34 were active flares in already diagnosed patients. Quantitative results for anti-dsDNA in each group are shown in Table 1.

Our results shown that 52% of positive sera for EliA and negative CLIFT belonged to SLE patients. Discrepant samples of SLE patients showed moderate titers (15-50 UI/ml), 10% of discrepant results belonged to individuals who were diagnosed between 4 months and 4 years after testing and 78% of SLE discrepant patients were already diagnosed.

Conclusions

Positive EliA with negative CLIFT cases and with no adhesion to EULAR/ACR 2019 criteria, should be followed up. Moreover, CLIFT can display negative results in patients suffering a flare that is detected by EliA, reinforcing the importance of quantitative assays as monitoring



P-170

LABORATORY DIAGNOSTIC APPROACH TO SUSPECTED AUTOIMMUNE ENCEPHALITIS IN A REGIONAL REFERRAL HOSPITAL: IS THE COST-BENEFIT RATIO BALANCED

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Autoimmune encephalitis (AE) comprises a group of immune-mediated inflammatory disorders involving central nervous system. In past years, the number of autoantibodies targeting neuronal surface or synaptic antigens has increased. We aimed to evaluate the performance of the diagnostic approach to AE in a regional referral hospital.

Materials and methods

469 samples from 415patients admitted to Neurology with clinical suspicion of AE or compatible symptoms between 2019 and 2022 were revised. These samples of serum, cerebrospinal fluid (CSF) or pairs sera-CSF were tested using a commercial biochip by Euroimmun. This kit detects autoantibodies by indirect immunofluorescence assay (IIFA) using human-embryonic-kidney cells transfected to express NMDAR, GAD65, LGI1, CASPR2, AMPAR1-2 and GABABR.

Results

9 (2.17%) patients showed a positive result: 5 (1.2%) were positive against NMDAR, 3 (0.72%) were anti-CASPR2, and 1 (0.24%) was anti-GABABR. Nevertheless, the initial suspicion of AE was maintained in some patients despite a negative result.

Discussion

We obtained similar ranges to published data (2.62-5.3%), but the incidence in our population was fewer than expected (0.35/100.000 person-years compared to 0.8-1/100.000 in published data). The presence of antibodies against antigens not included in the kit, or posible false-negative results, have been reported for anti-GABAR, LGl1 and AMPAR. The low number of tests requested and positive cases ratio, due to a non-restrictive patient selection, would be higher if suspicion were nearer to proposed guidelines. However, it could miss non-typical presentations, meaning the delay of the treatment onset.

Conclusions

It is advisable to carry out a fine selection of patients to be tested under the recommended guidelines.

In high suspicion of AE with negative result of the test, the study should be repeted in reference laboratories with different techniques.



The efficiency of the early treatment and the severe course without therapy offsets the potential cost of non-selected cases.

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P-171

LONGITUDINAL FOLLOW UP OF ILCS IN PERIPHERAL BLOOD OF CLADRIBINE-TREATED MS PATIENTS

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[1]Fundació Institut d'investigació en Ciències de la Salut Germans Trias i Pujol (IGTP), Badalona; [2]Hospital Universitari Germans Trias i Pujol, Badalona.

Introduction: Multiple Sclerosis (MS) is a disease characterized by the autoimmune response against the myelin of the central nervous system in which various cell types are involved. Cladribine is an oral MS treatment reported to induce a decrease in both $CD4^+$ and $CD8^+$T-cells, B-cells and Natural Killer cells. Alterations on the myeloid lineage such as in Dendritic Cells have not been observed after cladribine treatment.

Innate lymphoid cells (ILCs) are lymphocytes that lack the expression of an antigen-specific cell receptor. These represent a very small subset in peripheral blood and are divided in three immunoregulatory subsets; ILC1, ILC2 and ILC3, that mimic Th1, Th2 and Th17 $CD4^+$ T-cell subsets, respectively.

ILCs are involved in the pathogenesis of some autoimmune diseases. Considering their potential involvement in MS pathogenesis and the lack of data regarding the effect of cladribine on ILCs, we aimed to evaluate the long-term effects of cladribine on ILCs in a 3-year follow-up study.

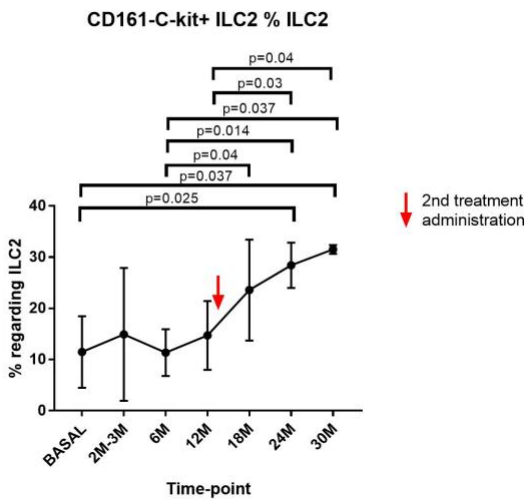
Methods: 11 MS patients were included. Samples were obtained at Baseline, 3, 6, 12, 18, 24 and 30 months after initial treatment. We performed flow cytometry analyses on samples obtained after bulk-lysis of 15ml of whole blood. Samples were acquired through FACSFortessa (BDBiosciences) and analysed through FlowJo Software. Statistical analyses and graphs were performed through SPSS and GraphPad softwares.

Results: No significant differences were observed in the main three ILC subsets throughout the follow-up. However, the C-kit^+CD161^-ILC2 subset was significantly increased after 12 months (***Fig.1a)***. Also, NCR^+CD161^-ILC3 cells increased throughout time and significantly decreased at the 12-month timepoint ***(Fig.1b).*** Finally, CD161 expression in ILC2s significantly increased throughout time. ***(Fig.1c)***.

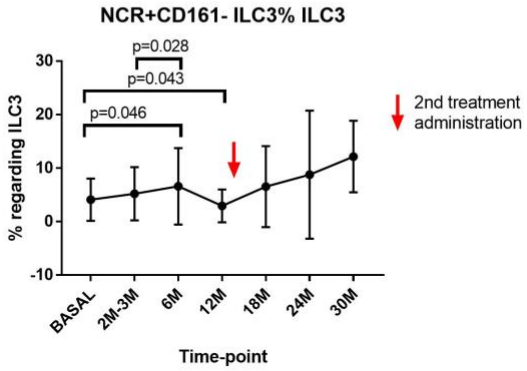
Conclusions: Results indicate that cladribine treatment induces changes in several ILC subsets, that could be possibly related with its therapeutical effect. An increased sample size might unravel other subset differences masked due to interindividual variability.



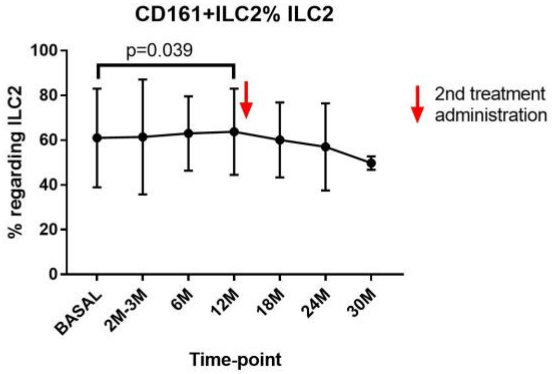
a



b



c



BASAL: N=8
2M-3M: N=7
6M: N=8
12M: N=7
18M: N=5
24M: N=3
30M: N=2



P-172

DETERMINACIÓN DE ANTICUERPOS ANTI-FIBRILARINA POR LINEDOT

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Indtrodución:

Los anticuerpos antifibrilarina/U3snRNP (AFA), específicos de la esclerosis sistémica (ES), presentan patrón de inmunofluorescencia indirecta (IFI) AC-9 sobre células hep-2. La confirmación de los mismos debe realizarse por otras técnicas como linedot o ELISA. Nuestro objetivo es comprobar la correlación, en nuestro medio, de patrón AC-9, antifibrilarina, y desarrollo de ES.

Material Métodos:

Estudio retrospectivo de pacientes con patrón AC-09 (IFI en células Hep-2000) a los que se realizó linedot específico de esclerosis (Euroimmun ©) (periodo de estudio 2018-2021). Se revisaron sus historias clínicas en base a los criterios clasificatorios de ACR/EULAR 2013.

Resultados:

Un total de 26 pacientes fueron analizados: 15 AFA+ (11 mujeres, 4 hombres. Mediana edad 57 años) y 11 AFA- (6 mujeres, 5 hombres. Mediana de edad 60 años).

Entre los pacientes AFA+, dos casos presentaban ES definida (>9 puntos), 8 presentaban algún ítem sin llegar a reunir criterios clasificatorios (fenómeno de Raynaud, edema de manos y/o enfermedad pulmonar intersticial), 5 pacientes no presentaban ningún criterio. En total 10 (66%) presentaban diagnóstico/criterios clasificatorios.

Entre los AFA- se detectó un paciente con ES definida. El resto (90%) no presentaba ningún criterio.

De un total de 11 casos con diagnóstico/síntomas compatibles, 10 (90%) son AFA+. De los 15 casos sin ningún criterio, 5(33%) son AFA+. Según nuestros datos, para pacientes con ES/síntomas de inicio, la sensibilidad de los AFA sería del 91%

Conclusiones:

Aunque la detección del patrón AC-9 no es frecuente, podría orientar hacia la detección de anticuerpos antifibrilarina por método específico como el linedot. La presencia de AFA en nuestro estudio se asocia a esclerosis sistémica definida o a síntomas de inicio. Según la literatura, dada la especificidad de este anticuerpo, el seguimiento de los pacientes AFA+ (con o sin síntomas) podría ser aconsejable para un diagnóstico y tratamiento precoz de ES.



P-173

INMUNOCROMATOGRAFÍA ES TAN EFICAZ COMO ELISA PARA LA DETERMINACIÓN DE FÁRMACOS ANTI-TNFA EN SANGRE

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Introducción: Los fármacos anti-TNFα son una de las principales líneas de tratamiento para enfermedades autoinmunes sistémicas, como la artritis reumatoide y las enfermedades inflamatorias intestinales. La monitorización de los niveles de fármaco en sangre permite la individualización de la posología ya que tiene buena correlación el grado de respuesta clínica. La técnica más utilizada para medir concentración de fármaco es el ELISA debido a su gran sensibilidad. La inmunocromatografía es una metodología muy rápida que permite obtener resultados en minutos, posibilitando una rápida modificación de la actitud terapéutica adaptada a las necesidades reales del paciente. La equivalencia de los resultados obtenidos por inmunocromatografía con los aportados por otros inmunoensayos más lentos no es suficientemente conocida.

Objetivo: Evaluar la correlación entre las concentraciones sanguíneas de fármacos determinadas por ELISA e inmunocromatografía.

Material y Métodos: Se estudiaron 87 muestras de pacientes en tratamiento con Infliximab y 83 con Adalimumab respectivamente. Los niveles de cada fármaco se evaluaron mediante ELISA (Promonitor, Grifols) e inmunocromatografía (QuantumBlue, Bühlmann).

Resultado: En la comparativa entre ambas técnicas se obtuvo una fuerte concordancia para Infliximab (Índice Kappa: 0.86) y Adalimumab (índice Kappa: 0.74). Además, se observó una correlación fuerte de los valores obtenidos por ambas técnicas tanto para Infliximab ($r=0.81$, $p<0.0001$) y muy fuerte para Adalimumab ($r=0.91$, $p<0.0001$).

Conclusión: La inmunocromatografía es una técnica que ha llegado a su madurez y actualmente proporciona resultados comparables a ELISA en un tiempo mucho más corto lo que posibilita realizar intervenciones terapéuticas rápidas que redundan en beneficio del paciente.



P-174

A CASE OF TRANSVERSE MYELITIS WITH MYELIN OLIGODENDROCYTE GLYCOPROTEIN ANTIBODIES AND COVID-19 INFECTION.

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Introduction

The inflammatory demyelinating diseases of the central nervous system has expanded with the identification of glial autoantibodies against aquaporin-4 (AQP4-IgG) and myelin oligodendrocyte glycoprotein (MOG-IgG). MOG-IgG antibodies have been established as a biomarker for MOG-antibody-associated disease (MOGAD). In this disease, myelitis is the second most common presentation in adults, followed by optic neuritis. MOG-IgG-associated myelitis features help to distinguish MOGAD from both multiple sclerosis and neuromyelitis optica spectrum disorder. It has long been known that viruses contribute to the production of autoimmune antibodies. Acute transversal myelitis has been reported in patients with COVID-19.

Objectives

To report a case of a patient with dysuria and subsequent anuria, fever, and weakness in the lower extremities, presenting antibodies to MOG and with COVID-19.

Material and Methods

MOG-IgG antibodies were determined using a cell-based assay: BIOCHIP KIT of EUROIMMUN AG (Lübeck, Germany) with employs human HEK293 cells expressing aquaporin-4 and MOG. Cerebrospinal fluid analysis and serological tests were performed. Information regarding patient´s image data, functional tests and other tests were available in the electronic medical history.

Results

We present a 51-year-old male patient with dysuria of four days of evolution and anuria, fever of up to 39 ºC, lumbar pain and weakness in the lower extremities, and testing positive for COVID-19. Laboratory results showed positivity for autoantibodies against MOG at serum dilution of 1/400. Immunosuppressive therapy significantly improved patient´s condition.



Conclusions

The availability of a quick and simple test for the determination of MOG antibodies provides the clinician with additional information for the diagnosis and treatment of MOGAD. This case adds to the growing literature describing a possible relationship between this autoimmune disease and COVID-19.



P-175

COMPARATIVE ANALYSIS OF TRANSGLUTAMINASE IGA AUTOANTIBODIES DETECTED BY QUANTA-FLASH® VERSUS APTIVA® IN THE DIAGNOSE OF CELIAC DISEASE

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Background: Celiac disease (CD) is a chronic enteropathy mediated by immune mechanisms and triggered by the exposure to diet gluten in genetically susceptible individuals, resulting in an atrophy of the small bowel mucosa and a malabsorption syndrome. The identification of sera autoantibodies (IgA) against tissular transglutaminase (tTG) is critical for its diagnosis, supported by other clinical, genetic and histologic findings. The aim of this study was to compare two semi-quantitative methods detecting serum tTG IgA: the recent launched Aptiva® (Werfen, Inova Diagnostics, Inc.) and the previous established QUANTA-Flash® (Werfen, Inova Diagnostics, Inc.).

Materials and methods: A total of 172 serum samples were routinely and duplicate tested for tTG IgA autoantibodies in patients with the suspicion of or having being diagnosed with CD, using QUANTA-Flash® and Aptiva®. According to the manufacturer, the positivity threshold for QUANTA-Flash® was 20 CU (Chemiluminescent Units), with a grey zone ranging from 20 to 30 CU; and 5 FLU (Fluorescent Light Units) for Aptiva®. Anti-endomysium antibody detection was performed as confirmatory test.

Results: Both methods equally identified 166 negative and 5 positive values. Exceptionally, one value was detected in the grey zone only by QUANTA-Flash®. This value belonged to a patient who exhibited uncertain CD symptomatology. The positive tTG samples also presented positive anti-endomysium autoantibodies, and all of them belonged to patients diagnosed with CD. The correlation analysis showed a Pearson coefficient of 0.976 ($p<0.01$). The difference between values obtained by each method was calculated and then analysed by Student T test, with no significant differences found ($p=0.13$), suggesting an adequate concordance.

Conclusion: Anti-transglutaminase autoantibody detection by QUANTA-Flash® and Aptiva® was highly reproducible. However, Aptiva system is able to simultaneously detect anti-tTG IgA antibodies, anti-deaminated gliadin peptide IgA antibodies and total IgA quantification in a single reaction thanks to the multi-analyte technology.



P-176

PACIENTE CON LUPUS ERITEMATOSO SISTÉMICO Y PRESENCIA DE ANTICUERPOS ANTI MI-2

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HUD Donostia, San Sebastián (Guipúzcoa).

Introducción:

El LES es una enfermedad autoinmune sistémica en la que multitud de órganos se ven dañados por el depósito de inmunocomplejos. Para su diagnóstico se deben cumplir los criterios diagnósticos del EULAR/Colegio Americano de Reumatología que combinan diferentes manifestaciones clínicas y criterios analíticos (ANAs e hipocomplementemia). Sin embargo, existen otros autoanticuerpos muy raramente asociados a LES como anti-Mi2, específico de miopatía inflamatoria.

Objetivo:

Presentamos el caso de una mujer de 35 años sin antecedentes médicos de interés ingresada en Medicina Interna tras cuadro de astenia severa. Asocia rápida pérdida ponderal, febrícula, vértigos, aftas orales, erupciones cutáneas simétricas en extremidades y espalda. En la exploración llama la atención la dificultad para subir escaleras. En la analítica sanguínea destacan trombopenia y linfopenia junto con test de Coombs directo positivo en ausencia de anemia hemolítica.

Materiales y métodos:

Análisis de ANAs y otras especificidades mediante IFI e IB por el Servicio de Inmunología tras valoración clínica en planta.



Resultados:

AUTOINMUNIDAD	
Nucleares anticuerpos (ANA) (IFI)	Positivo a título 1/1280. Patrón homogéneo
DNA doble cadena, Ac (IFI)	Positivo
DNA doble cadena, Ac	6.356,70
Antígeno nuclear extraíble (ENA), Ac	Positivo
Antígeno nuclear extraíble ENA, Ac (IB)	
Nucleosomas, Ac	Positivo++
DFS70, Ac	Positivo++
Histona, Ac	Positivo++
Ro-52, Ac	Positivo+++
SS-A, Ac	Positivo+++
Miopatías Inflamatorias, Ac (IB)	
Mi-2 alfa, Ac	Positivo+++
Ku, Ac	Positivo+++
Resto de especificidades negativas	

Conclusiones:

Existen muy pocos casos descritos en la literatura de pacientes diagnosticados de LES con presencia de anticuerpos anti-Mi-2, específicos de miopatía inflamatoria.

Resulta llamativa la combinación de manifestaciones clínicas y anticuerpos específicos de ambas entidades (LES y miopatía) simultáneamente.



P-177

COMPARATIVA ENTRE LA PCR-SSO Y LA PCR EN TIEMPO REAL EN EL ESTUDIO GENÉTICO DE LA ENFERMEDAD CELÍACA

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La enfermedad celíaca es una patología crónica autoinmune de afectación enteral causada por el gluten (presente en trigo, centeno y cebada) y prolaminas, que afecta a 1 de cada 100 personas a nivel mundial. La celiaquía se caracteriza por el desarrollo de una respuesta inmunitaria contra el intestino delgado que daña las vellosidades intestinales, produciendo inflamación y malabsorción. Aunque los síntomas clínicos son heterogéneos dependiendo de los pacientes, los más comunes son la diarrea, dolor y distensión abdominales. La enfermedad es una enfermedad poligénica que muestra una fuerte asociación genética con genes localizados en la región HLA (*Human leukocyte Antigen*) del cromosoma 6p21, desarrollándose en personas genéticamente susceptibles. Las moléculas HLA implicadas en el reconocimiento del gluten y presentación a los linfocitos T CD4+ son heterodímeros formados por una subunidad α y una β codificadas por los genes HLA-DQA1 y HLA-DQB1, respectivamente. La gran mayoría de los pacientes celíacos (90%-96%) presentan el heterodímero HLA-DQ2 (isoforma DQ2.5) y los restantes el HLA-DQ8. Además, hay un efecto de dosis en los genes HLA-DQB1*02 y HLA-DQB1*03, siendo recomendable su información. Para determinar los heterodímeros implicados en la enfermedad se pueden genotipar los genes HLA-DQA1 y HLA-DQB1, o bien determinar los alelos específicos que los codifican.

Se compararon los genotipos HLA-DQA1*03/05 y HLA-DQB1*02/03:02 determinados en 200 muestras mediante PCR-SSO (*single specific oligonucleotid*) y PCR en tiempo real.

Se observó una correlación cercana al 100% en la determinación de los genotipos anteriores mediante ambas técnicas. La PCR en tiempo real ofrece resultados en menor tiempo que la PCR-SSO.



P-178

REFERENCE VALUES OF ANTI-MYELOPEROXISASE AND ANTI-SERINE PROTEIN 3 ANTIBODIES

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Introduction

Antineutrophil cytoplasmic autoantibodies (ANCA) against myeloperoxidase (MPO) and serineprotease3 (PR3) are useful in the diagnosis of small vessel vasculitis. Their early detection is crucial due to the rapid progression of the pathology.

Objective

Review of reference values for anti-MPO and anti-PR3.

Material and Methods

Establishment of anti-MPO and anti-PR3 reference values in 40 presumably healthy individuals (age:0.58-86 years, 60%men) by non-parametric test.

Retrospective review of 66 patients (age:59.2 (9-91) years, 61%women). We analised anti-MPO and anti-PR3 by chemiluminescence (AcuStar. Werfen) over 10 years. Demographic and clinical variables were collected. Sensitivity (Se), specificity (Sp) and AUC were calculated by ROC curves, the risk (OR) of vasculitis by logistic regression and the difference of antibodies by Student's t-test in different diseases using Stata 14.0.

Results

With the manufacturer's cut-off (19.9IU/mL), 16.7% had anti-PR3 (46% vasculitis, 55% gastric disorders, 27% rheumatic), 13.6% anti-MPO (44% vasculitis, 56% bronchial alteration and 22 % rheumatoid arthritis (RA)) and 4.5% (n:3) anti-MPO+anti-PR3 (1. Vasculitis and RA, 2. neurological and gastric disorders, 3. rheumatological disorders). For vasculitis, anti-PR3 showed Se:42%, Sp:85%, AUC: 0.56 and anti-MPO Se:42%, Sp:85%, AUC:0.58 and OR:4(p:0.043). The cut-off obtained in our healthy population presented: anti-PR3 <3.2IU/mL (Se:58%, Sp:43%, OR:4.8(p:0.020)) and anti-MPO <4.1IU/mL(Se:58% and Sp:36%). The optimal Se-Sp cut-off presented: anti-PR3<12.7IU/mL(Se:42%, Sp:83%, OR:4(p:0.031)) and anti-MPO<9.2IU/mL(Se:58%, Sp:74%). Anti-PR3 and anti-MPO showed differences in vasculitis (p:0.025 and p<0.001, respectively) and anti-MPO in RA (p:0.004) and bronchial alteration (p:0.002).



Conclusions

-We suggest to decrease the anti-PR3 cut-off (12.7IU/mL) and to maintain the anti-MPO cutoff (19.9IU/mL). Both cut-offs obtained in our healthy population have low diagnostic performance. Lower anti-PR3 cut-off(12.7IU/mL) supposes a significant increase in the vasculitis diagnostic capacity with similar Se-Sp. Lower anti-MPO cut-off (9.2IU/mL) decreases Sp and loses the diagnostic capacity.

-Anti-PR3 and anti-MPO increases significantly in vasculitis and anti-MPO in RA and bronchial alteration.



P-179

EVALUACION DE LA DEMANDA DE ANTICUERPOS ANTINUCLEARES EN EL LABORATORIO DE INMUNOLOGÍA DE UN HOSPITAL TERCIARIO

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Hospital Universitario de Donosti, Donosti.

Introducción: Una gestión apropiada del análisis de los anticuerpos antinucleares (ANAs) es necesaria ya que suponen importantes cargas de trabajo en el laboratorio de inmunología, un aumento del coste en el sistema de salud, influyen en la seguridad del paciente y tienen un impacto negativo en la eficiencia de los facultativos.

Objetivo: Evaluar la demanda de ANAs en el laboratorio de Inmunología de un hospital terciario durante dos meses, provenientes de atención especializada y primaria en el área sanitaria de Gipuzkoa. Otro objetivo es evaluar el porcentaje de resultados positivos.

Materiales y métodos: Análisis retrospectivo de ANAs solicitados desde atención primaria y especializada. Se evalúan los resultados totales y en % sobre el total de las solicitudes de las 10 especialidades más demandantes. Se evalúa el porcentaje de resultados positivos.

Resultados:

En dos meses se analizaron 4324 sueros, fueron positivos 1442 (33%).

1. Resultados del TOP 10 de especialidades con más solicitudes y % sobre el total solicitados.

	Total	%
AP	1939	44,8
Digestivo	525	12,14
Reumatología	416	9,6
Neurología	206	4,75
Crónicos	168	3,9
Hematología	140	3,23
Neumología	113	2,6
MI	109	2,5
Nefrología	101	2,33
Dermatología	101	2,33



2.- TOP 10 por porcentaje de resultados positivos.

	Total	Positivos	%
Reumatología	416	273	65,6
Nefrología	101	41	40
Digestivo	525	186	35,4
Hematología	140	48	34,2
Crónico	168	57	33,9
Dermatología	101	34	33,5
Neumología	113	37	32,7
AP	1939	523	26,56
MI	109	27	24,7
Neurología	206	50	24,27
Media			35,08

Conclusiones:

1.- El área de AP presenta una solicitud de ANAs excesiva (44.8% del total), con un porcentaje de positivos muy bajo (26.56%). MI y Neurología tienen porcentajes positivos inferiores a la media.

2.- En la especialidad de AP son necesarias medidas de adecuación de la demanda para disminuir costes y mejorar la eficiencia del sistema.



P-180

ANTICUERPOS ANTI-BETA-2-GLICOPROTEÍNA I DE ISOTIPO IGA EN PACIENTE CON LIVEDO RETICULARIS

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Mujer, 74 años, derivada para estudio de livedo reticularis de año de evolución. También disestesias y parestesias en manos. Niega artromialgias, xerostomía, xeroftalmía, aftosis, fenómeno Raynaud. AP: HTA, insuficiencia venosa, no abortos o trombosis. Tratamiento: Aspirina, Losartan, Atenolol, HCTZ.

Exploración: livedo reticularis severa, rizartrosis del pulgar, acrocianosis, pulsos simétricos, pobreza capilar en capilaroscopia. Laboratorio: hemograma, coagulación, Coombs directo, crioglobulinas, complemento, factor reumatoide, Ac anti-péptido cíclico citrulinado, inmunoglobulinas, proteinograma y PCR normales. Hematuria y leucocituria. IFI Hep-2 patrón nuclear homogéneo (AC-1) > 1/320, Ac anti-DNAdc 51 U/mL [0-15] confirmados por IFI Crithidia. Anticoagulante lúpico negativo, Ac anti-beta-2-Glicoproteína I IgM 10 U/mL [0-10], anti-beta-2-Glicoproteína I IgG 1,9 U/mL [0-10], anti-beta-2-Glicoproteína I IgA 29 U/mL [0-10], anti-cardiolipina IgM 5.5 MPL/mL [0-40], anti-cardiolipina IgG 1.3 GPL/mL [0-20], anti-cardiolipina IgA 7 APL/mL [0-20]. A las 12 semanas, confirma positividad anti-beta-2-Glicoproteína I IgA 33 U/mL.

El síndrome antifosfolípido (SAF) es un trastorno autoinmune sistémico caracterizado por fenómenos trombóticos y/o morbilidad gestacional en pacientes con anticuerpos antifosfolípidos (aPL) altos y mantenidos, concretamente anticoagulante lúpico, y los isotipos IgG e IgM anti-beta-2-Glicoproteína I y anti-cardiolipina. Puede darse como cuadro primario o asociado a otras enfermedades autoinmunes, principalmente lupus eritematoso sistémico (LES). Para su diagnóstico se emplean los criterios de Sydney, sin embargo, han sido sujeto de debate por la existencia de pacientes con clínica de SAF y ‘’aPL criterio’’ negativos, o pacientes con manifestaciones "extracriterio", destacando livedo reticularis, trombocitopenia, enfermedades valvulares cardíacas o nefropatía. La presentación de livedo reticularis en la paciente y positividad anti-beta-2-glicoproteína I IgA reflejan SAF seronegativo, y ANA y Ac anti-DNAdc positivos sugieren SAF asociado a LES que aún no cumple criterios, planteando inicio con hidroxicloroquina. El tratamiento con aspirina podría estar enmascarando fenómenos



trombóticos, siendo imprescindible el seguimiento de la paciente, recalcando la importancia de aPL ‘’no criterio’’ en la patogenia del SAF.

aPL	Primera medición	A las 12 semanas
Ac IgM anti-beta-2-Glicoproteína I [0-10]	10 U/mL	11 U/mL
Ac IgG anti-beta-2-Glicoproteína I [0-10]	1.9 U/mL	1.7 U/mL
Ac IgA anti-beta-2-Glicoproteína I [0-10]	29 U/mL	33 U/mL
Ac IgM anti-cardiolipina [0-40]	5.5 MPL/mL	5 MPL/mL
Ac IgG anti-cardiolipina [0-20]	1.3 GPL/mL	1.2 GPL/mL
Ac IgA anti-cardiolipina [0-20]	7 APL/mL	7.9 APL/mL

Tabla 1. Medición Ac anti-fosfolípidos en la paciente en la primera consulta y a las 12 semanas.



Sesión Poster. Inmunogenética. Pantalla 3

P-182

ESTUDIO DE LOS GENES HLA-C Y KIR EN PACIENTES CON ABORTO RECURRENTE

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Fundamento y objetivos: Durante el embarazo en el ser humano, la decidua está en su mayoría poblada por linfocitos NK los cuales expresan receptores de tipo inmunoglobulina (KIR) que reconocen a sus ligandos HLA-C de las células del trofoblasto. El objetivo de este trabajo fue analizar las frecuencias de fenotipos KIR de mujeres estériles (E) o con abortos recurrentes (AR) y su ligando HLA-C expresado por el feto, para evaluar si existe una susceptibilidad genética en comparación con controles fértiles.

Métodos del estudio: Se estudiaron las frecuencias de los genes KIR y HLA-C de 50 mujeres de AR y 96 de E; los genes HLA-C se analizaron en las parejas de cada grupo; total 119 donantes. Las frecuencias se compararon con los datos de los controles; 33 donantes.

Resultados: HLA-C1 aparecía con mayor frecuencia en el grupo control en comparación con el grupo AR y mujeres estériles (0, 504, 0,.193; respectivamente), además las mujeres control tenían una frecuencia de C1C1 combinada con KIR AA más alta que el resto de grupos. Al contrario, las mujeres del E y AR, tenían mayor frecuencia de C2C2 en comparación con el grupo control (mirar esta frecuencia tiene que ser menos de 1 5, .120, 5,647, respectivamente), además, las mujeres del grupo AR con un KIR AA tuvieron mayor frecuencia de C2C2 (centromérico: $P<0,05$, telomérico: $P<0,05$). La frecuencia KIR BB presentó mayor frecuencia de C1C1 en mujeres controles en comparación con grupo de esterilidad (centromérico no significativo, telomérico: $P<0,05$).

Conclusiones: Sugiere susceptibilidad a desarrollar aborto cuando aumenta la frecuencia de C2C2, así como protección frente a éste cuando C1C1 está aumentado. Las combinaciones C1C1/AA y C1C1/BB podrían favorecer el embarazo natural, mientras que C2C2/AA tiene efecto directo en la inhibición de las células NK y la generación de abortos.



P-183

RETOS EN EL DIAGNÓSTICO GENÉTICO EN EL ESPECTRO DE PATOLOGÍAS QUE CURSAN CON ELEVACIÓN DE LA IGE Y MANIFESTACIONES ALÉRGICAS

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Introducción

La elevación de IgE se observa en el contexto de numerosas enfermedades: desde asma y atopia hasta ser una primera manifestación de inmunodeficiencias primarias complejas. Presentamos 4 pacientes con fenotipo clínico semejante cuyo análisis genético resultó crucial en el pronóstico y el manejo de los mismos.

Materiales y Métodos

Se aisló DNA genómico a partir de sangre de los pacientes y se procedió a la construcción de una librería enriquecida en 200 genes relacionados al Sistema Inmune.

Resultados

Caso 1: Varón de 1 año con antecedentes de asma, placas eritematosas congénitas e infecciones respiratorias. Asociaba fenotipo facial característico con incremento de IgE. Se detectó una deleción de 6 aminoácidos inframe en el dominio linker de la proteína STAT3 compatible con diagnóstico molecular de Síndrome HiperIgE.

Caso 2: Varón de 7 años con dermatitis atópica grave, asma bronquial y elevación de IgE. El análisis genético detecta 2 mutaciones en el gen *NLRP12* (p.Leu859Ile y p.Ala523Gly) y una mutación en *NLRP3* (p. Arg556Ter) que podrían estar en relación a la desregulación inmune del paciente.

Caso 3: Niño de 8 años con múltiples otitis medias agudas y neumonías con elevación persistente de la IgE donde se detecta mutación en *SPINK5* (p.Glu748Gly) en heterocigosis.

Caso 4: Niño de 2 años con elevación de la IgE, múltiples alergias alimentarias, infecciones de repetición y dermatitis atópica grave. Se detecta mutación en *CASP10*. Se hipotetiza el compromiso en la apoptosis de la vía extrínseca y de células plasmáticas aberrantes secretoras de IgE.

Conclusiones

La elevación de la IgE y presencia de eccemas puede ser una manifestación presente en patologías con síntomas solapantes. La detección de variantes en genes relacionados con el Sistema Inmune puede ayudar a predecir el curso de la enfermedad mediante la monitorización exhaustiva de síntomas o parámetros de laboratorio.



P-184

NATURAL KILLER RECEPTOR VARIANTS ROLE IN THE EVOLUTION OF MYELODYSPLASTIC SYNDROMES PATIENTS

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Myelodysplastic Syndromes (MDS), a complex group of haematological malignancies, are characterized by ineffective haematopoiesis, bone marrow dysplasia, cytopenia and risk of progression to secondary Acute Myeloid Leukemia (sAML).

The aim of this retrospective study was to decipher the role in disease development and evolution of activating and inhibitory KIR genes as well as the activating NKG2D receptor, all them present in NK cells, and also their respective ligands, HLA-A, -B, -C, -G, -F, MICA and MICB. For this purpose, 89 DNA samples from MDS patients from Institut Català d'Oncologia (ICO) Badalona were collected and their immune target genes were studied by Next Generation Sequencing with an in-house designed gene panel. As controls, 445 healthy donors for KIR-HLA class I and 96 for non-classic HLA and NKG2D genes were analysed. Moreover, 76 AML de novo DNA samples were compared with sAML.

In the first 5 years, 18 patients (20.22%) developed sAML in an average of 37.8 months (range= 3.48-60 months). The presence of the *KIR2DL3*/HLA-C1 pair was associated with the onset of the disease [(n=70/89, 78.65%) vs (n=283/445, 63.59%), p-value=0.0089]. Moreover, genes *KIR2DL2* and *KIR2DS2* were found to present a protective role of sAML and AML de novo [(n=8/18, 44.4%) and (n=35/76, 46.05%) vs (n=266/445, 59.77%), p-value=0.1 and p-value=0.02]. Furthermore, MICA129met, which enhances a stronger biding with NKG2D activating receptor, was statistically associated with protection against MDS [(n=37/89, 41.57%) vs (n=57/96, 59.38%), p-value=0.01]. No differences were found between MDS patients who underwent to sAML and AML de novo. Finally, presenting a high number (4 or more) activating KIR genes appeared to be a protective factor against sAML progression [(HighAct= 3/40, 7.5% vs LowAct=15/49, 30.61%), p-value = 0.005].

In summary, our results showed that activating NK receptor genotypes might protect against MDS, as well as the progression from MDS to sAML.



P-185

HLA-DQA1*05 EN PACIENTES CON ENFERMEDAD INFLAMATORIA INTESTINAL EN TRATAMIENTO CON FÁRMACOS ANTI-TNF

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Introducción: La mitad de los pacientes con enfermedad inflamatoria intestinal (EII) presentan pérdida de respuesta al tratamiento con fármacos anti-TNF. Estudios recientes han demostrado una relación entre la presencia del alelo HLA-DQA1*05 con un aumento de la inmunogenicidad asociada a los fármacos anti-TNF en la EII medida por medio de la aparición de anticuerpos contra el fármaco (Sazonovs et al. Gastroenterology, 2020).
El objetivo de nuestro estudio es evaluar si la presencia del alelo HLA-DQA1*05 se asocia al desarrollo de anticuerpos contra los fármacos biológicos en pacientes con EII en una serie de pacientes de nuestro hospital.

Métodos: Estudio de cohortes retrospectivo de pacientes con EII tratados con fármacos anti-TNF a los que se les había realizado determinación de niveles del fármaco. La determinación de anticuerpos anti-fármaco por Elisa (positivo >10 AU/mL) se realiza únicamente en pacientes con niveles del fármaco menores de 0,5 µg/mL. Se incluyeron en el estudio un total de 259 pacientes en los que se realizó el tipaje HLA-DQA1 por técnica de PCR-SSOP con kits comerciales de la casa One Lambda.

Resultados: El 42,1% de los pacientes estudiados eran portadores del alelo HLA-DQA1*05. Únicamente en 28 pacientes se detectó la presencia de anticuerpos anti-fármaco (10,8%), siendo 16 de ellos portadores del alelo HLA-DQA1*05 (53,3%). De los 28 pacientes con anticuerpos positivos, 9 de ellos estaban tratados con adalimumab y 19 con infliximab. Aunque en los pacientes con anticuerpos anti-fármaco es más frecuente la presencia del alelo DQA1*05, esta diferencia no alcanza significación estadística (p=0.08).

Conclusión:
No podemos confirmar los estudios previos que asocian la presencia del alelo HLA-DQA1*05 con la aparición de anticuerpos. Sin embargo, nuestros resultados muestran una tendencia que podría llegar a la significación con un número mayor de pacientes.



P-186

HETEROGENEIDAD GENÉTICO-CLÍNICA EN EL ANGIOEDEMA HEREDITARIO: EXPERIENCIA EN EL LABORATORIO DE INMUNOGENÉTICA Y REVISIÓN DE CASOS.

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Introducción

Mutaciones en el gen *SERPING1,* que codifica para la proteína C1-Inhibidor (C1-INH), se asocian al desarrollo de Angioedema hereditario (AEH) de tipo I si hay deficiencia cuantitativa, y de tipo II si se produce una alteración funcional con niveles normales. El AEH es una patología con herencia dominante de baja prevalencia y potencialmente grave que se manifiesta clínicamente con edemas cutáneos y en mucosas, destacando como mecanismo molecular el aumento de bradicinina y la alteración de proteínas reguladoras asociadas como C1-INH. Variantes en otros genes que afectan a estas vías se relacionan con AEH-nC1INH (AEH con C1-INH normal).

Serie de casos

Caso-1: Identificación de variante en heterocigosis en exón 8 de *SERPING1*:p.Pro475Leu, no descrita previamente, en tres generaciones de familia con niveles reducidos de C1-INH (AEH-I) y clínica similar asociada, solo con tratamiento sintomático.

Caso-2: Variante en heterocigosis en *SERPING1*: p.pro498ser, ya descrita, en familia con AEH-I (aparición *de novo* en progenitor) y mal control de la clínica, en tratamiento mantenido.

Caso-3: Identificación de dos variantes complejas monoalélicas en *SERPING1:* p.Glu451_Thr452insAlaAlaGly y p.Gly453delinsAspArg en familia con AEH-II, penetrancia completa pero expresión clínica muy variable.

Caso-4: Paciente sin antecedentes familiares con diagnóstico de AEH-I y sintomatología intestinal. Variante monoalélica en exón 5 de *SERPING1*:p.Leu287His, no descrita previamente.

Caso-5: Paciente con antecedentes de carcinoma de colón y mama, en seguimiento por Inmunología por hipogammaglobulinemia IgM, disminución células IgM de memoria y escasa producción tímica. Mutación monoalélica en *SERPING1:*p.Arg537Gln considerada como probable patogénica. Sin antecedentes familiares ni clínica actual sugestiva de angioedema.

Caso-6: Tres familias no relacionadas con variante en *FXII*:p.Thr328Lys y AEH-III: una familia con clara asociación a estrógenos (embarazos) y otras dos con débil/sin asociación.



Conclusión

El diagnóstico molecular de AEH se ve dificultado por la heterogeneidad alélica de los genes implicados y un amplio espectro de manifestaciones clínicas asociadas.



P-187

SEQUENCING OF TWO NEW *HLA-B* ALLELES, *B*44:517 AND B*45:01:01:09*, IDENTIFIED IN TWO HAEMATOLOGICAL PATIENTS.

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Introduction

The usual HLA typing protocol for patients with an indication for hematopoietic stem cell transplantation includes high-resolution typing of at least the *HLA-A, -B, -C, -DRB1* and *-DQB1* loci. Currently, HLA typing by Next Generation Sequencing (NGS) is the one with the highest level of resolution.

Objective

Molecular characterisation of two novel *HLA-B* alleles by NGS.

Material and Methods

The new allele *HLA-B*44:517* was identified in a patient diagnosed of acute myelogenous leukemia. Family samples were not available to confirm the new allele, therefore verification was performed with a second patient's sample obtained from epithelial buccal cells.The new *HLA-B*45:01:01:09* allele was identified in a patient diagnosed with myelodysplastic syndrome with excess blasts (MDS-EB1) and in his daughter. The NGS procedure was performed using commercially available reagents (NGSgo®-MX6-1, GenDX) and a MiniSeq platform (Illumina™). Data were analysed using the NGSengine® Software (GenDX) and the 3.43.1 version of the IPD-IMGT/HLA Database.

Results

The new *HLA-B*44:517* differs from its most closely related *B*44:02:01:01* allele by one nucleotide substitution at genomic position 1704 G>A, codon 229 GAG>AAG, within exon 4. The nucleotide change results in a substitution at amino acid 229E>K. Complete HLA typing was: *A*01:01:01,*02:01:01; B*37:01:01,*44:517; C*05:01:01,*06:02:01; DRB1*10:01,*13:01:01; DQB1*05:01:01,*06:03:01; DPB1*04:01:01,*14:01:01.*
NGS sequencing of the MDS patient´s sample for *HLA-B* locus provided a *B*14:02:01:01, B*45:01var* assignment. The daughter´s sample gave a *B*45:01var, B*51:01:01:01* assignment. The new *HLA-B*45:01:01:09* allele showed a 10 base pairs deletion within intron 2, positions 677-686, when compared with its closest allele *B*45:01:01:01*. Family haplotype analysis demonstrated that this allele is included in the haplotype:



*A*02:01:01~C*16:01:01:01~B*45:01:01:09~DRB1*10:01~DQB1*05:01:01~DPB1*02:01:02*.

The new *HLA-B* alleles were officially named as *B*44:517* and *B*45:01:01:09* by the WHO Nomenclature Committee for Factors of the HLA System in May 2021.

Conclusions

NGS technology is revealing greater polymorphism in the HLA system and facilitates the detection of new alleles.



P-188

CHARACTERISATION OF TWO NEW HLA ALLELES, *HLA-A*68:02:01:14 AND -DPB1*1153:01*, IDENTIFIED IN TWO SPANISH BONE MARROW DONORS BY NEXT GENERATION SEQUENCING.

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Introduction

In order to optimize the Spanish registry of bone marrow donors, in recent years an effort has been made to type new donors to loci *HLA-A, -B, -C, -DRB1 –DQB1* and *-DPB1* by Next Generation Sequencing (NGS).

Objective

Molecular and serological characterisation of two novel HLA alleles: *A*68:02:01:04* and *DPB1*1153:01*.

Material and Methods

The new alleles were identified in two Spanish voluntary bone marrow donors. The NGS procedure was performed using commercially available reagents (NGSgo®-MX6-1, GenDX) and a MiniSeq platform (Illumina™). Data were analysed using the NGSengine® Software (GenDX). Serologic typing analysis of cell including the new *A*68:02:01:14* was performed using monoclonal and polyclonal serological commercial trays (Innotrain).

Results and Discussion

The new *HLA-A*68:02:01:04* has a nucleotide mismatch, T>C, in the position -102 in the 5'UT region of the *A*68:02:01:01* allele. Sample showed the following typing: *A*01:01:01,*68:02:01:14; B*14:02:01,*57:01:01; C*07:01:01,*08:02:01; DRB1*01:02:01,*07:01:01; DQB1*03:03:02,*05:01:01; DPB1*04:01:01,*04:02:01*.

Nucleotide position -102 is placed within the enhancer B region which has been involved in the expression level of HLA class I molecules. In order to determine a possible weak serological reaction pattern for the A*68 molecule, serologic typing analysis of cell was performed, but similar result was obtained to that for a wild type A68 molecule. This suggests that the point mutation observed does not has any effect on the expression of the HLA class I molecules.

The new *HLA-DPB1*1153:01* differs from its most closely related *DPB1*04:02:01:02* allele by one nucleotide substitution at genomic position 9784 A>G, codon 222 AAG>GAG, within exon 4. This nucleotide change results in a substitution at amino acid 222K>E. Complete HLA typing was: *A*25:01:01,*30:02:01; B*15:01:01,*18:01:01; C*03:03:01,*05:01:01;*



*DRB1*01:01:01,*15:01:01; DQB1*05:01:01,*06:02:01; DPB1*04:01:01,*1153:01.*

The new HLA alleles were officially named as *A*68:02:01:14* (HWS10061027), and *DPB1*1153:01* (HWS10060569), by the WHO Nomenclature Committee for Factors of the HLA System in January 2021 and October 2020, respectively.



P-189

SEARCH PROGNOSIS ALGORITHM TO DETERMINE SIRE PROBABILITY IN CORD BLOOD UNITS FROM VALENCIAN COMMUNITY (SPAIN) DEPENDING ON *HLA-A*, *-B* AND *-DRB1* FREQUENCIES

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Background

The development of different search algorithms for the prognosis of finding an adult unrelated donor (URD) is of great importance for transplant teams. These search tools provide the probability, according to patient's ethnicity as well as frequency of *HLA* genotype, of finding a match.

Aim

We aimed to determine the *HLA* genotype frequency from cord blood units (CBU) with low cellularity to stratify them as good, fair or poor prognosis groups.

Methods

We used the *Search Prognosis Application* to have genotype frequency and prognosis from 577 low-cellularity CBU collected from 1999 to 2011 from Valencian Community. CBU were categorized into 3 groups: good (likely to have 6/6 match), fair (likely to have a 5/6 match) and poor (unlikely to have a 6/6 and may not have a 5/6 match) for each ethnicity group. Data were analyzed using Chi-Square tests. P-values <0.05 were considered significant.

Results

Out of all CBU, 66.72% (N=385) had a good prognosis, 31.54% (N=182) had a fair prognosis and 1.73% (N=10) showed a poor prognosis. The Asian Pacific Islander (API) group showed the highest percentage of poor prognosis (N=161, 27.9%), whereas Hispanic (HIS) group showed the lowest percentage of poor prognosis (N=12, 2.08%).

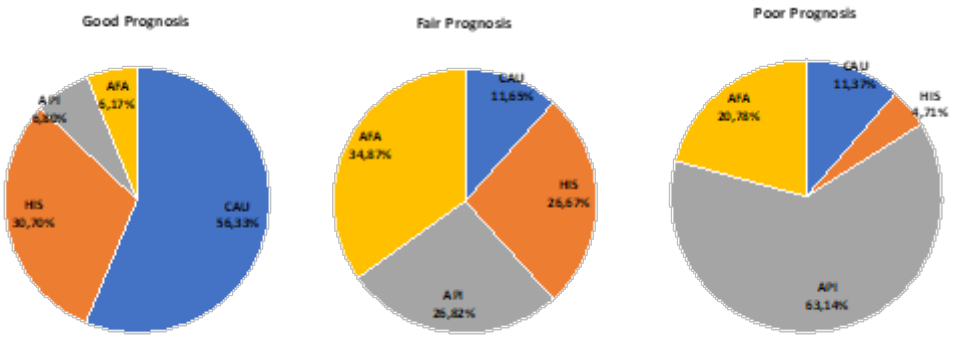
Table 1. *Prognosis Search from 577 CBU from Valencian Community with low cellularity*

	Good	Fair	Poor	P-value
CAU	66.90%	28.08%	**5.03%**	**0.0046**
HIS	33.62%	64.30%	2.08%	**<0.0001**
API	7.45%	64.64%	27.90%	**<0.0001**
AFA	6.76%	84.06%	9.19%	**<0.0001**

CAU: White; HIS: Hispanic; API: Asian Pacific Islander; AFA: African American

Conclusions

The probability to find a match, for each ethnicity group, using the *Search Prognosis Application* could help cord blood banks to optimize typing and selection strategies of their stored CBU suitable for allogeneic stem cell transplantation. We conclude that from our stored CBU, Caucasian will have the highest likelihood to find a 6/6 match.





P-190

ESTUDIO DEL POLIMORFISMO GENÉTICO DE *KIR3DL2* EN SÍNDROME DE SÉZARY Y MICOSIS FUNGOIDE AVANZADA.

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KIR3DL2 es un receptor con características estructurales y funcionales únicas dentro de su familia, los receptores KIR. Se expresa típicamente en células NK y en poblaciones minoritarias de células T. Además, la expresión de este receptor en células tumorales T CD4+ es una característica molecular de algunos linfomas cutáneos de células T como el síndrome de Sézary (SS) y la micosis fungoide en estadios avanzados (MFa), sin embargo, se desconoce su papel en la etiopatogenia de estas enfermedades. Existen muy pocos datos sobre la diversidad genética de *KIR3DL2* en SS/MFa, y nuestro objetivo es estudiar su posible implicación en estas patologías. Para ello, hemos realizado un estudio de frecuencias alélicas y de SNPs del gen en una cohorte de 37 individuos diagnosticados de SS/MFa procedentes de los Hospitales Universitarios Puerta de Hierro-Majadahonda y 12 de Octubre y en una serie de 40 individuos control de la misma área geográfica. Los resultados muestran que determinados alelos de *KIR3DL2* tienden a estar sobrerrepresentados en los pacientes con SS/MFa respecto a los controles. Estos alelos comparten un polimorfismo en la región que codifica para el dominio ITIM (*immunoreceptor tyrosine-based inhibition motifs*) de este receptor, cuya presencia aumenta en tres veces el riesgo de padecer SS/MFa (OR: 3.3, IC95%: 1.27-8.59).

Además, hemos analizado la expresión alélica de este polimorfismo de *KIR3DL2* en muestras de sangre y en biopsias parafinadas de piel de MF en estadios tempranos, en SS/MFa y en controles. Con ello, hemos querido estudiar la asociación de los diferentes patrones de expresión alélica de *KIR3DL2* con el curso clínico de estas enfermedades. En conclusión, nuestro estudio muestra que la variabilidad genética de *KIR3DL2* es un factor de riesgo asociado al desarrollo de SS/MFa. Estudios posteriores permitirán esclarecer el mecanismo de acción del polimorfismo genético de *KIR3DL2* en este grupo de linfomas.



P-191

GENERACIÓN DE MATERIAL AUDIOVISUAL PARA LA ENSEÑANZA DE LA INMUNOGENÉTICA EN GRADOS DE CIENCIAS BÁSICAS Y DE LA SALUD

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Universidad de Valencia, Valencia.

Como resultado de una colaboración entre los Departamentos de Patología, Microbiología y Fisiología de la Universidad de Valencia, se han producido dos vídeos que introducen al alumnado de los Grados en Ciencias Biológicas, Medicina, Odontología y Podología a la inmunogenética.

Los vídeos son breves, a modo de píldora educativa, y llevan por título: "Descripción, estructura y diversidad de las inmunoglobulinas" el primero, con una duración aproximada de cuatro minutos, y "Generación de diversidad de anticuerpos en los linfocitos B", el segundo, de seis minutos y medio de duración. La temática de estos vídeos, centrada en la inmunogenética humana, y el enfoque particular que se ha dado a su contenido, hace que sean de gran utilidad es asignaturas como: Inmunología, Genética Médica/Humana, Biología celular y Fisiología, ya que integra conocimientos de estas cuatro disciplinas en diferente medida:

A. Inmunología: descripción de las inmunoglobulinas, mecanismos de producción de diversidad de inmunoglobulinas, afinidad en la unión antígeno-anticuerpo.
B. Genética médica/humana: *loci* de las cadenas de inmunoglobulinas humanas, recombinación VDJ, hipermutación somática, exclusión alélica.
C. Biología celular: características de los linfocitos B, control de la expresión génica por modificaciones de la secuencia de ADN.
D. Fisiología: tejidos hematopoyéticos y sus tipos celulares, linfopoyesis.

Para alcanzar una mayor difusión entre el alumnado de la Universidad de Valencia, los vídeos se ofrecen en español, inglés y valenciano y se acompañan de una encuesta de evaluación de realización voluntaria.

La formación de equipos de trabajo multidisciplinares en innovación docente nos obliga a seguir innovando, a aprender y a esforzarnos por aportar algo más a la docencia. En este caso, los vídeos de inmunogenética, realizados por docentes de diferentes áreas permiten una difusión del conocimiento a un gran número de alumnos de diferentes grados.



Trabajo realizado en el marco de la convocatoria NOU-PID, UV-SFPIE_PID-1639302 de la Universitat de València.



P-192

SINGULAR EVOLUTION OF MAJOR HISTOCOMPATIBILITY COMPLEX (MHC)-G AND –C, AND NK RECEPTORS IN OLD WORLD AND NEW WORLD PRIMATES

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Major Histocompatibility Complex (MHC)-G and -C molecules bear ligands to natural killer immunoglobulin receptors (KIR). MHC-G evolution in primates shows some anomalies. In New World monkeys MHC-G molecules show a high polymorphism and most likely are classical antigen presenters; they also cluster closer to MHC-E in a relatedness dendrogram. Their genes lack intron 2 deletion, which is typical of all other primates in regard to MHC-G. Medium-sized Eurasian-African monkeys (*Cercopithecinae*) show stop codons in exon 3: only MHC-G isoforms without exon 3 are possible. Big apes such as the orangutan, gorilla, and chimpanzee as well as human beings show limited HLA-G polymorphism. HLA-C has not been found in medium-size Eurasian-African monkeys, but we have found MHC-C DNA sequences in more evolutionary ancient New World monkeys. Taking into account that the KIR inhibitory receptors signal is dominated by MHC-C in human beings, this suggests that both MHC-C molecules and their ligands within natural killer lymphocyte KIR also exist in the most evolutionary ancient apes (New World monkeys were present on Earth before 40 million years ago), as KIR receptors also appeared before 130 million years ago in evolution. Indeed, KIR receptor genes have recently been found in a New World monkey.



P-193

TWO NEW HLA-C ALLELES, *HLA-C*14:02:36* AND *–C*15:236*, IDENTIFIED BY NEXT GENERATION SEQUENCING IN THE SPANISH POPULATION.

Balas Pérez, Antonio[1]; Planelles Silvestre, María Dolores[2]; Rodríguez Cebriá, Manuel[2]; Granell, Rosa[2]; Mocholí, Carmen[2]; Vicario, José Luis[1].

[1]Centro de Transfusión de Madrid, Madrid; [2]Centro de Transfusión de la Comunidad Valenciana, Valencia.

Introduction

Mismatching for HLA-C between patients and unrelated donors generally leads to higher risk of graft-versus-host disease and mortality after hematopoietic stem cell transplantation (HSCT), so an effort has been made recently adding HLA-C typing to registered donors.

Objective

Molecular characterization of two novel HLA-C alleles by Next Generation Sequencing (NGS) technology.

Material and Methods

The new allele *HLA-C*14:02:36* was identified in a Spanish voluntary bone marrow donor. *HLA-C*15:236* was identified in a patient diagnosed of chronic myeloid leukemia; this new allele was confirmed in a second sample of DNA obtained from epithelial buccal cells. The NGS procedure was performed using commercially available reagents (NGSgo®-MX6-1, GenDX) and a MiniSeq platform (Illumina™). Data were analyzed using the NGSengine® Software (GenDX).

Results

The new *HLA-C*14:02:36* has one synonymous nucleotide change from *C*14:02:01:01* at nucleotide position 2570 G > A, codon 325 GCG>GCA, within exon 6, which does not lead to any amino acid change (325A>A). The complete HLA typing of this sample was: *A*02:01:01, --; B*07:02:01, *51:01:01; C*07:02:01, *14:02:36; DRB1*09:01:02G, *15:01P; DQB1*03:03:02:02, *06:02; DPB1*04:01P, *04:02P.*

The new *HLA-C*15:236* differs from its most closely related *C*15:04:01:01* allele by one nucleotide substitution, position 67 T>A, within exon 1, codon -2 TGG>AGG. The nucleotide change results in a substitution at amino acid residue -2 W>R. HLA typing of this sample was: *A*24:02:01,--; B*45:01:01, *51:01:01, C*15:236,*16:01:01:01; DRB1*10:01, *13:02:01; DQB1*05:01:01, *06:04; DPB1*14:01:01, *17:01:01:01.*

The new HLA-C alleles were officially named as *C*14:02:36* and *C*15:236* by the WHO Nomenclature Committee for Factors of the HLA System in March 2020 and May 2021, respectively.



Conclusions

HLA-C is now routinely typed both in patients with an indication for HSCT and in unrelated donors, which is allowing the detection of an increasingly growing number of alleles in this locus until now considered the least diverse of class I.



P-194

TWO NEW HLA-B ALLELES, *HLA-B*35:510* AND *–B*55:01:24*, IDENTIFIED IN TWO SPANISH BONE MARROW DONORS BY NEXT GENERATION SEQUENCING.

Balas Pérez, Antonio[1]; Planelles Silvestre, María Dolores[2]; Rodríguez Cebriá, Manuel[2]; Martínez, Francisco[2]; Laguarda, Encarna[2]; Vicario, José Luis[1].

[1]Centro de Transfusion de Madrid, Madrid; [2]Centro de Transfusion de la Comunidad Valenciana, Valencia.

Introduction

The application of NGS to routine HLA typing of donors from the Spanish voluntary marrow donor registry is allowing the detection of a large number of new HLA alleles.

Objective

Molecular characterisation of two novel HLA-B alleles by Next Generation Sequencing (NGS) technology.

Material and Methods

The new alleles *HLA-B*35:510* and *HLA-B*55:01:24* were identified in two Spanish voluntary bone marrow donors. The NGS procedure was performed using commercially available reagents (NGSgo®-MX6-1, GenDX, Utrecht, The Netherlands) and a MiniSeq platform (Illumina™, San Diego, California). Data were analysed using the NGSengine® Software (GenDX) and the 3.38 (*HLA-B*55:01:24*) and 3.41 (*HLA-B*35:510*) versions of the IPD-IMGT/HLA Database.

Results

The new *HLA-B*35:510* differs from its most closely related *B*35:03:01:01* allele by one nucleotide substitution at genomic position 968 G>A, codon 175 GGG>AGG within exon 3, resulting in coding change, Glycine 175 is replaced by Arginine. Complete HLA typing for this sample was the following: *A*29:01:01,*68:01:02; B*07:05:01,*35:510; C*04:01:01,*15:05:02; DRB1*10:01:01,*11:02:01; DQB1*03:01:01,*05:01:01; DPB1*04:01:01,*13:01:01.*

The new *HLA-B*55:01:24* has one synonymous nucleotide change from *B*55:01:01:01* at nucleotide position 449 C > T, codon 83 GGC>GGT, within exon 2, which does not lead to any amino acid change (83G>G). The complete HLA typing of this sample was: *A*29:02:01,*68:01:02; B*35:02:01, *55:01:24; C*03:03:01, *04:01:01; DRB1*11:01:01, *11:04:01; DQB1*03:01P,-; DPB1*04:01:01, -.*

The names *B*35:510* (HWS10061167) and *B*55:01:24* (HWS10059043) have been officially



assigned by the WHO Nomenclature Committee for Factors of the HLA System in February 2021 and March 2020, respectively.

Conclusions

Due to the extreme polymorphism of the HLA system, NGS typing is the suitable procedure for a complete and reliable HLA typing.



P-195

A NEW HLA-C ALLELE, C*12:358, DETECTED IN THE COURSE OF THE SPANISH EXTERNAL PROFICIENCY TESTING FOR HISTOCOMPATIBILITY & IMMUNOGENETICS (GECLID-SEI) PROGRAMME.

Planelles Silvestre, María Dolores[1]; Balas Pérez, Antonio[2]; De Juan Echávarri, María Dolores[3]; Muro Amador, Manuel[4]; Palau, Eduard[5]; Martín Alonso, Carmen[6].

[1]Centro de Transfusión de la Comunidad Valenciana, Valencia; [2]Centro de Transfusión de la Comunidad Madrid, Madrid; [3]Hospital Universitario de Donostia, San Sebastián; [4]Hospital Universitario Virgen de la Arrixaca, Murcia; [5]Hospital Clínic Universitari de Barcelona, Barcelona; [6]Centro de Hemoterapia y Hemodonación de Castilla y León, Valladolid.

Introduction

A new HLA allele, C*12:358, has been identified by Next Generation Sequencing (NGS) typing in the course of one of the HLA typing schemes within the Spanish External Proficiency Testing (EPT) for Histocompatibility & Immunogenetics (GECLID-SEI) Programme (https://www.geclid.es/).

Objective

Disambiguation of an HLA-C*12 typing.

Material and Methods

HLA-C low resolution typing of the EPT sample HLA21-5.06r2 by polymerase chain reaction-sequence specific oligonucleotide (PCR-SSO) method (Lifecodes HLA-C eRES SSO test, Immucor) reported inconclusive typing result with a suggestive C*04, C*06:76/*12 ambiguous assignment, with the most likely C*12 allele being C*12:03. Real time PCR typing using Olerup SSP® reagents (QType test, *Olerup* SSP AB) reported unambiguous C*04,*12 result, but the C*12 most probably assignment was *12:04. Confirmatory typing by NGS was performed by one laboratory (from the EPT Steering Commitee) using commercially available reagents (GenDX) and a MiniSeq platform (Illumina). Data were analyzed using the NGSengine Software (GenDX) and the 3.45.1 version of the IPD-IMGT/HLA Database.

Results

HLA-C sequencing gave a C*04:01:01, C*12:03:01:01var assignment after complete genomic HLA-C analysis in a single phase. The new allele showed one nucleotide difference from C*12:03:01:02 in exon 2, genomic position 435 (T>C) codon 78 (CTG>CCG), resulting in a coding change, 78L>78P.

This new HLA-C allele was officially named as C*12:358 (HWS10063929) by the WHO Nomenclature Committee for Factors of the HLA System in November 2021. EPT-assigned typing of sample including C*12:358 allele was the following: A*03,*26; B*35,*38; C*04:01:01,*12:358; DRB1*04,*11; DRB3*02, DRB4*01; DQA1*03,*05; DQB1*03,*03; DPA1*01,-.



Conclusions

The application of NGS to HLA typing is revealing that the HLA-C locus is almost as polymorphic as the HLA-A locus (7393 alleles vs 7452, respectively, in the latest release of the IPD-IMGT/HLA Database) and its diversity is very close to that of the HLA-B, the most polymorphic locus with 8849 alleles officially recognised as of June 2022.

Figure 1. Partial Exon 2 nucleotide sequence alignment of C*12:03:01:01 and C*12:358. Mismatch position E2-305 is shown.

```
cDNA                  280        290        300        310        320        330        340
 C*12:03:01:01  TACAAGCGCC AGGCACAGGC TGACCGAGTG AGCCTGCGGA ACCTGCGCGG CTACTACAAC CAGAGCGAGG CCG
 C*12:358       ---------- ---------- ---------- ----C----- ---------- ---------- ---------- ---
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P-196

NGS AND FLOW CYTOMETRY IN RICHTER'S SYNDROME CLASSIFICATION

López Pérez, Juan; Gallardo García, Alberto; García Cuesta, Daniel; De La Varga Martínez, Raquel.

Hospital Universitario Puerta del Mar, Cádiz.

Introduction: A 59-year-old man diagnosed with long-standing B-CLL had lymphocytosis, consisting of lymphocytes of different sizes and morphological appearance. In addition, the medulogram revealed 58% of blasts of lymphoid habitus with Burkitt morphology. The study was therefore extended to include a possible Richter's syndrome.

Objective: To examine and classify Richter's syndrome by means of flow cytometry analysis (FCM) and genetic study.

Material and Methods: The patient's peripheral blood was evaluated by FCM and the usage of the Euroflow LST tube, ALOT, BCP-ALL and B-CLPD tube 2. Bone marrow sample was used for genetic study by NGS sequencing and FISH, and also for an immunohistochemical analysis.

Results: The FCM study revealed an infiltration of clonal B-lymphoid blasts with surface expression of immunoglobulin kappa light chain with SSC/FSC+high CD45+low CD19+ CD20+ CD5- Kappa+ CD10+ CD38+ CD23- CD200- CD79b+ phenotype, representing 41% of the lymphoid population. In addition, a clone with a B-CLL phenotype (CD19+ CD20+low CD23+ CD5+ kappa+low) was observed, representing 7% of the lymphoid population. Immunohistochemistry revealed an infiltration by a B lymphoproliferative process compatible with Burkitt's lymphoma. The genetic study showed three mutations in the TP53 gene by NGS (c.314G>A, c.313G>C, c.701A>G), previously described in patients with Burkitt's lymphoma. The FISH study was negative for MYC rearrangement.

Conclusions: The study was suggestive of B-CLL in progression to leukaemic Burkitt's lymphoma by both flow cytometry and immunohistochemistry analysis. Richter syndrome affects 2-10% of patients with B-CLL and consists of transformation to a fast-growing lymphoma, most commonly to DLBCL (90%) and Hodgkin lymphoma. Although rarely, it may also progress to dendritic cell/histiocytic sarcoma, lymphoblastic lymphoma or other lymphomas (<1%). We therefore present a rare case of a patient diagnosed with long-progressing B-CLL who transformed into leukaemic Burkitt's lymphoma (LLAB-IV).



P-197

VARIANTES *MISSENSE* EN HETEROCIGOSIS EN LOS GENES *ELANE* Y *FPR1* ASOCIADAS A LA BIOLOGÍA DE LOS NEUTRÓFILOS EN PACIENTE CON INFECCIONES DE REPETICIÓN

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Paciente y Método:

Niña de 13 años en seguimiento por posible inmunodeficiencia primaria asociada a la aparición de abscesos axilares, infección por *Mycobacterium tuberculosis* y 4 episodios de neumonía que han requerido hospitalización. Otros aspectos clínicos relevantes son: broncoespasmos de repetición, dermatitis atópica, asimetría de miembros y alteraciones ortopédicas y taquicardia supraventricular intervenida.

El estudio genético de secuenciación del ADN genómico, extraído de sangre periférica, se ha llevado a cabo por secuenciación masiva de segunda generación (*NGS, Next Generation Sequencing)* a través de un panel de 200 genes asociados con inmunodeficiencias.

Resultados:

Los resultados de la secuenciación han permitido identificar dos variantes *missense* en heterocigosis: una en el gen *ELANE* (ENSP00000263621.1:p.Gly125Ala) que codifica la elastasa, una serín proteasa que se encuentra en los gránulos de los neutrófilos y monocitos; y otra en el gen *FPR1* (ENSP00000302707.3:p.Ile147Thr) que codifica el receptor de péptidos formilados tipo 1 que se localiza en las células fagocíticas y que interviene en la activación y migración de los neutrófilos. Ninguna de las variantes ha sido descrita en las bases de datos internacionales de mutaciones del genoma humano o de variantes con significación clínica. Sin embrago, otro cambio en el *gen ELANE* (p.Gly125Trp) que afecta al mismo codón que la variante que presenta nuestra paciente se ha asociado a neutropenia cíclica con herencia autosómica dominante.

Conclusión:

Aunque el diagnóstico genético no es definitivo debido a la falta de evidencia sobre las variantes encontradas en esta paciente, ambas alteraciones en los genes *ELANE* y *FPR1* están asociadas a defectos en la correcta funcionalidad de los neutrófilos pudiendo explicar el fenotipo de la paciente y la susceptibilidad a la infección.



P-198

THE ORIGIN OF MAPUCHE AMERINDIANS (CHILE) ACCORDING TO THEIR HLA PROFILE

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Universidad Complutense de Madrid, Madrid.

The peopling of the Americas is thought to have occurred from Siberia through Beringia. However, the oldest human activity is found in southernmost South America (Monte Verde, Chile), a Mapuche Amerindian area. We decided to compare the Mapuche HLA features with other North and South American Amerindians and other worldwide populations, particularly Pacific populations, since some authors have put forward a southern Pacific route for the peopling of southern South America. One hundred and ten unrelated and healthy Mapuche volunteers from an area close to Concepcion (Chile) were genetically analysed using a SSO (single specific oligonucleotide) standard Luminex HLA-typing technique. Allele and haplotype frequencies, genetic distances between populations, neighbour-joining (NJ) dendrograms and correspondence analyses were computed using ARLEQUIN, DISPAN and VISTA software. Results show how Mapuche Amerindians are genetically close to other Andean Amerindians populations (Quechuans, Aymaras) and also to North American Amerindian populations (Lakota-Sioux, Tarahumaras). This general phenomenon of lack of correlation between geography and genetics supports the idea that all Amerindians came to America in approximately the same time, and from the same genetic background. The peopling of the Americas through Beringia is supported by the present Mapuche HLA data.



P-199

ESTUDIO GENÉTICO EN EL DÉFICIT SELECTIVO DE IGA Y ENFERMEDAD CELÍACA

Cruz Quevedo, Mileydis[1]; Bodas Pinedo, Andrés[2]; Vecino López, Raquel[2]; Barrio Merino, Alfonso[3]; Fernández-De-La-Madrid Yagüe, María Eugenia[1]; Ochoa Grullón, Juliana[2]; Guzmán Fulgencio, María[2]; Sánchez Ramón, Silvia[2]; Fernández Arquero, Miguel[2].

[1]Universidad Complutense de Madrid, Madrid; [2]Hospital Clínico San Carlos, Madrid; [3]Hospital Fundación Alcorcón, Madrid.

Introducción: Se ha planteado una posible predisposición genética entre el déficit selectivo de IgA (DsIgA) y la enfermedad celíaca (EC) por sus prevalencias entre sujetos de una misma familia o en un mismo individuo (10-15%).

Objetivo: Estudiar los haplotipos HLA clase II compartidos entre enfermedad celíaca y déficit selectivo de IgA y su implicación entre ambas patologías.

Métodos: Se realizó un estudio multicéntrico descriptivo transversal en pacientes de los servicios de Inmunología y/o Gastroenterología del Hospital Clínico San Carlos de Madrid y Hospital Universitario Fundación Alcorcón, entre noviembre del 2021 y junio del 2022. Se estudiaron los genes HLA-DRB1, DQA1 y DQB1 mediante la tecnología Luminex.

Resultados: La muestra estuvo conformada por 213 pacientes, distribuidos en tres grupos: 50 pacientes con DsIgA, 149 con EC y 14 con EC asociada a DsIgA. Los resultados se compararon con una población sana (n=85) de origen y características similares. En pacientes con DsIgA se demostró una asociación estadísticamente significativa con el haplotipo HLA-DRB1*01:01-DQA1*01:01-DQB1*05:01 (68%; OR=13,99) y su homocigosis (12%; OR=7,13). En el estudio de los pacientes celíacos, se encontró una asociación estadísticamente significativa con el haplotipo DRB1*03:01-DQA1*05:01-DQB1*02:01 (62,4%; OR=11,78) y los heterocigotos DRB1*03:01-DQA1*05:01-DQB1*02:01 / DRB1*01:01-DQA1*01:01-DQB1*05:01 se encuentran aumentados (OR= 4,23). En el estudio de los pacientes afectados de EC y DsIgA se encontró el haplotipo DRB1*03:01-DQA1*05:01-DQB1*02:01: (50,0%; OR=13,17), y el DRB1*01:01-DQA1*01:01-DQB1*05:01: (37,5%; OR=7,32).

Conclusiones: Existe una inmunogenética compartida entre EC y DsIgA.



P-200

IDENTIFICATION OF THE NOVEL HLA-C*08:243 ALLELE IN A SPANISH BONE MARROW DONOR

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[1]Servicio de Inmunología. Hospital Universitario Universitario Marqués de Valdecilla, Santander; [2]Histocompatibilidad, Centro de Transfusión de Madrid, Madrid; [3]Banco de Sangre y Tejidos de Cantabria, Santander.

We report here the novel *HLA-C*08:243* allele found in a Spanish bone marrow donor. Routine HLA typing by Next Generation Sequencing (NGS), detected a variant *HLA-C*08* allele, closely related to the partially defined and unconfirmed *HLA-C*08:51 allele.* The NGS procedure was performed using commercially available reagents (GenDX, Utrecht, The Netherlands) and an iSeq100 platform (Illumina, San Diego, California). Data were analyzed using the NGSengine Software (GenDX) and the 3.45.1 version of the IPD-IMGT/ HLA Database (1).

The new allele showed one nucleotide difference from *C*08:51* in exon 3, genomic position 915 (G > T), codon 156 (CGG > CTG), producing an amino acid replacement 156R>L. Two additional mismatches can be found when compared to the most frequent *C*08* subtype in Caucasians, *C*08:02*, at nucleotide positions 959 (T>C) and 977 (A>G), giving an additional change at codon 171Y>H (Figure 1).

Amino acid positions 156 and 171 are placed at the H2 □-helix domain of the HLA class I molecule, and are part of the antigen binding pockets F and A respectively (2). Therefore, *C*08:243* would show relevant mismatches, from a functional point of view, compared to the most common *C*08:02* allele.

Complete HLA typing for this sample was the following: *A*11:01:01,23:01:01:01; B*14:02:01:01,51:01:01; C*08:243,14:02P; DRB1*01:02:01,12:01P; DQB1*03:01:01:05,05:01P; DPB1*03:01:01,04:01:01*. According to this, a tentative haplotype A*11:01:01~C*08:243~B*14:02:01~DRB1*01:02:01~DQB1*05:01P could be defined for this new HLA-C allele.

The name *C*08:243* has been officially assigned by the WHO Nomenclature Committee for Factors of the HLA System in March 2022. This follows the agreed policy that, subject to the conditions stated in the most recent Nomenclature Report (3) names will be assigned to new sequences as they are identified. Lists of such new names will be published in the following WHO Nomenclature Report.



Figure 1

Exon 3

```
AA
Codon                        95                  100                 105                 110                 115
 C*08:02:01:01    GG TCT CAC ACC CTC CAG AGG ATG TAT GGC TGC GAC CTG GGG CCC GAC GGG CGC CTC CTC CGC GGG TAT AAC CAG
 C*08:51          -- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 C*08:243         -- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

AA
Codon                       120                 125                 130                 135                 140
 C*08:02:01:01   TTC GCC TAC GAC GGC AAG GAT TAC ATC GCC CTG AAT GAG GAC CTG CGC TCC TGG ACC GCC GCG GAC AAG GCG GCT
 C*08:51         --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 C*08:243        --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

AA
Codon                       145                 150                 155                 160                 165
 C*08:02:01:01   CAG ATC ACC CAG CGC AAG TGG GAG GCG GCC CGT GAG GCG GAG CAG CGG AGA GCC TAC CTG GAG GGC ACG TGC GTG
 C*08:51         --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 C*08:243        --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -T- --- --- --- --- --- --- --- --- ---

AA
Codon                       170                 175                 180
 C*08:02:01:01   GAG TGG CTC CGC AGA TAC CTG GAG AAC GGG AAG AAG ACG CTG CAG CGC GCG G
 C*08:51         --- --- --- --- --- C-- --- --- --- --- --- G-- --- --- --- --- --- -
 C*08:243        --- --- --- --- --- C-- --- --- --- --- --- G-- --- --- --- --- --- -
```

Figure 1. Exon 3 nucleotide sequence alignment of the *C*08:02:01*, *C*08:51* and *C*08:243* alleles. Codon numbers are indicated above. Identity to the *C*08:02:01:01* allele sequence is shown by hyphens (—).



P-201

CHARACTERISATION OF A NOVEL *HLA-B* ALLELE, *B*45:29*, BY FULL-LENGTH PCR-NGS-BASED SEQUENCING IN A PATIENT WITH SICKLE CELL ANAEMIA DISEASE AND RELATED DONORS.

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[1]Centro de Transfusión de la Comunidad Valenciana, Valencia; [2]HOSPITAL INFANTIL LA FE, Valencia.

Introduction

Polymorphism is a defining feature of the HLA system, with *HLA-B* being the most polymorphic locus, currently with 8.849 alleles identified to date.

Objective

Molecular characterisation of a novel *HLA-B* allele.

Material and Methods

The new allele *B*45:29* was identified in a haematological patient, his father, and his identical sibling, of Moroccan origin, by Next Generation Sequencing (NGS) using commercially available reagents (NGSgo®-MX6-1, GenDX, Utrecht, The Netherlands) and a MiniSeq platform (Illumina™, San Diego, California). Data were analysed using the NGSengine® Software (GenDX) and the 3.45.1 version of the IPD-IMGT/HLA Database. Confirmatory typing was carried out by polymerase chain reaction-sequence specific oligonucleotide (PCR-SSO) method (Lifecodes® HLA-B SSO test, Immucor) reporting inconclusive typing results. A suggestive *B*45:02/03,*14*, type for the patient and his sister; and a *B*45,*50:14*, with the most likely *B*45* allele being *B*45:01*, in the case of the father.

Results

NGS sequencing of the patient and sister's samples for *HLA-B* locus provided a *B*14:02:01:01, B*45:02var* assignment after completing full-length genomic *HLA-B* analysis in a single phase. Furthermore, the father´s sample gave yet a *B*45:02var, B*50:01:01:01* assignment.

The new allele showed one single nucleotide difference from its closest allele *B*45:02* in exon 3, genomic position 792 (T>A), cDNA 419, codon 116 (TTC>TAC), resulting in an amino acid change, 116F>116Y (Figure 1). Family haplotype analysis demonstrated that this new *HLA-B* allele is included in the haplotype: *A*29:02:01:01~C*16:01:01:01~B*45:29~DRB1*13:02:01~DQB1*05:01~DPB1*02:01:19:01*.

This new *HLA-B* allele was officially named *B*45:29* (HWS10060520) by the WHO Nomenclature Committee for Factors of the HLA System in February 2022.



Conclusions

NGS technology allows simultaneous sequencing of multiple loci for HLA typing and facilitates the detection of new alleles, thus benefiting the process of selecting compatible donors for haematopoietic stem cell transplantation.

```
cDNA                350       360       370       380       390       400       410       420       430       440
B*45:02          GGTCTCA CACTTGGCAG AGGATGTATG GCTGCGACCT GGGGCCCGAC GGGCGCCTCC TCCGCGGGTA TAACCAGTTC GCCTACGACG GCAAGGATTA
B*45:29          ------- ---------- ---------- ---------- ---------- ---------- ---------- --------A- ---------- ----------

cDNA                450       460       470       480       490       500       510       520       530       540
B*45:02       CATCGCCCTG AACGAGGACC TGAGCTCCTG GACCGCGGCG GACACCGCGG CTCAGATCAC CCAGCGCAAG TGGGAGGCGG CCCGTGTGGC GGAGCAGGAC
B*45:29       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

cDNA                550       560       570       580       590       600       610
B*45:02       AGAGCCTACC TGGAGGGCCT GTGCGTGGAG TCGCTCCGCA GATACCTGGA GAACGGGAAG GAGACGCTGC AGCGCGCGG
B*45:29       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------
```



P-202

CONSERVED RESIDUES OF CLASS I MHC IN ALL VERTEBRATES ARE NOT PRESENT IN SONGBIRDS (PASSERIFORMES): FUNCTIONAL, STRUCTURAL AND EVOLUTIONARY CONSEQUENCES

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Birds are considered dinosaurs that passed the 65 million years ago bottleneck. Songbirds (*Passeriformes*) included about half extant bird species (about 5000) and are general the most air-thriving bird species, particularly in conjunction with their small size. Mayor Histocompatibility Complex (MHC) molecules stimulate immune responses against microbes and its class I molecules has seven conserved residues in all vertebrates from jawed-fishes, 300 million years ago, to humans, including chickens. All wild songbird species tested by us (n=18) and others (n= 2) differ in α1 domain residue 10 and α2 residue 96 from all other vertebrates. Amplification, cloning and sequencing were performed by standard methods. Sequences alignment were done by using PAUP and MEGA programs software. Crystallographic studies were performed by using mammal and bird MHC molecules from MPID database and other sources and showed that these changes did not significantly vary the MHC class I molecule stability in songbirds. Further α1 and α2 domain comparisons by simple Composition Distances and Bayesian Inference showed that songbirds overall MHC class I molecules are phylogenetically more separated from mammal and other birds molecules. In addition MHC class I introns from *Passeriformes* (songbirds) were found to be longer than humans, chicken introns having the shortest ones. These small mainly air-borne dinosaurs (*Passeriformes*) have undergone a different evolutionary pathway, regarding to MHC, than all other tested vertebrates and more terrestrial birds. This may have been originated by an altogether different dinosaurs linage origin or to adaptation to more aerial than terrestrial environment or other unknown cause. In any case, the specific changes observed in this work for class I molecules in songbirds have reached an entropic, stable solution similar to that reached by other vertebrates.



P-203

NARCOLEPSIA Y ENFERMEDADES AUTOINMUNES

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Introducción: La narcolepsia es un trastorno neurológico crónico poco frecuente en el que se produce una desregulación del ciclo vigilia-sueño. No ha sido muy estudiada su relación con otras enfermedades autoinmunes, sin embargo, se ha reportado comorbilidad con enfermedades inmunomediadas.

Objetivo: Estudiar la relación existente entre la narcolepsia y su comorbilidad con otras enfermedades autoinmunes.

Métodos: Se realizó un estudio descriptivo transversal y observacional en pacientes pertenecientes del Clínico San Carlos, en el período 2018-2020. La población de estudio consistió en una cohorte de 109 pacientes con narcolepsia y otras enfermedades inmunomediadas. Para su estudio se dividió la muestra en tres grupos: grupo A pacientes con narcolepsia (n= 87; 79,8%), grupo B pacientes con narcolepsia y una enfermedad autoinmune asociada (n=15; 13,76%) y grupo C pacientes con narcolepsia y dos enfermedades autoinmunes asociadas (n=6; 5,5%).

Resultados: Se encontró que la edad de comienzo de la enfermedad era menor en aquellos pacientes que con una manifestación más agresiva: Cateplejía y Enfermedad autoinmune . En cuanto al sexo, se observó predominio del sexo masculino en pacientes con narcolepsia sin otra patología (3:1) y del sexo femenino cuando esta se asocia a autoinmunidad (3:1).

Conclusiones: 1. la narcolepsia presenta una comorbilidad con la enfermedad autoinmune 2. Las formas más agresivas de la enfermedad están asociadas cuando existe enfermedad autoinmune 3. El sexo masculino en pacientes con narcolepsia sin otra patología (3:1) y el sexo femenino se asocia a autoinmunidad (3:1)



Sesión Poster. Trasplante. Pantalla 4

P-204

ESTADO INMUNOLÓGICO DE PACIENTES CON ENFERMEDAD RENAL CRÓNICA (ERC) INFECTADOS POR SARS-COV-2 SOMETIDOS A HEMODIÁLISIS (HD).

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Introducción: Se ha observado que los pacientes con ERC en HD infectados por SARS-CoV-2 experimentaban un empeoramiento de los síntomas, con reaparición de la fiebre y mal estar general, tras las sesiones de HD.

Objetivos: Evaluar cambios en el sistema inmunológico asociados al deterioro clínico durante la sesión de HD en pacientes con COVID-19.

Metodología: Estudiamos 14 pacientes en HD, 9 con COVID-19 confirmada por RT-PCR y 5 no infectados. En sangre periférica en el momento pre y post-diálisis se cuantificó IL2, IL7, IL6, IL10, IL17A, GM-CSF, IL1β, IL21, IFNγ, IL8, CCL3 y TNFα, subpoblaciones linfocitarias (CD4/CD8 MC, CD4/CD8MP, CD19, CD56) y de monocitos clásicos (CD14+CD16-), intermedios (CD14+CD16+) y no clásicos (CD14-CD16+), y marcadores de activación (HLA-DR, CD86) y migración (CCR2, CCR5). Aislamos células CD14+ que fueron estimuladas con LPS overnight y se cuantificó en el sobrenadante: GM-CSF, IL18, IL1β, IL1Ra, IL6, IL8, CXCL10, CCL2, CCL3, IL10 y TNFα.

Resultados: Los pacientes en HD infectados vs. no infectados, presentaron en pre-diálisis: (1) mayores niveles séricos de las citoquinas pro-inflamatorias IL6, TNFα e IL8; (2) linfopenia T CD4 y CD8; (3) mayor porcentaje de monocitos intermedios y menor de no-clásicos; (4) menor expresión en membrana de CCR2, HLA-DR y CD86 en monocitos; (5) mayor producción de GM-CSF, IL6, IL1β, IL1Ra, CCL3, CCL2 y TNFα por monocitos estimulados con LPS. Entre la pre- y la post-diálisis (fold change), los monocitos estimulados con LPS aumentaron la secreción de GM-CSF, IL6, IL1β, IL8, CCL2 y CCL3 en pacientes que desarrollaban deterioro clínico post-HD en relación con los pacientes sin COVID-19 ($p<0.05$ para CCL3 e IL6) o con pacientes sin empeoramiento ($p<0.05$ para todas las citoquinas).

Conclusiones: El deterioro clínico en pacientes con ERC infectados por SARS-CoV-2 tras la sesión de HD se acompaña de activación de monocitos y secreción de citoquinas pro-inflamatorias.



P-205

MONITORIZACION DE EXPRESIÓN GÉNICA DEL SISTEMA BAFF (BAFF/APRIL/R-BAFF/TACI/BCMA) Y ANTICUERPOS ANTI-DSA EN TRASPLANTE RENAL. META-ANALISIS DE ESTUDIOS TRANSCRIPTOMICOS DEL ÍNDICE CADI EN GEO

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La señalización del sistema del factor activador de células B (BAFF) es fundamental para la homeostasis de las células B, las funciones efectoras y el mantenimiento de la tolerancia en el trasplante, pero no se ha estudiado en los receptores de trasplante renal (KTR). El objetivo era analizar los cambios en la expresión del sistema BAFF en KTR con/sin rechazo agudo (AR/NAR). La expresión del sistema BAFF se analizó por qPCR en 40 KTRs. Se identificó también un metanálisis de la expresión del sistema BAFF y el daño renal histológico mediante el Índice de daño crónico del aloinjerto (CADI) y se realizó a partir de la base de datos GEO. La expresión del ligando inductor de proliferación (APRIL) aumentó a los tres y seis meses después del trasplante renal (p=0,014 y p<0,001). La expresión del antígeno de maduración de células B (BCMA) aumentó a los seis meses después del KT (p=0,038). La expresión de BAFF se mantuvo estable en NAR-KTR, pero aumentó en CADI con respecto al grupo No-CADI al año (p=0,008). La expresión de BCMA aumentó en el grupo CADI en uno (p = 0,001) y seis años después del trasplante renal (p=0,024). El gen Interactor del modulador de calcio y activador transmembrana (TACI) se elevó significativamente en receptores renales con DSA (p=0,034) a los tres meses. Los KTRs con DSA aumentan significativamente el receptor del factor activador de células B (R-BAFF; p=0,021) y TACI (p=0,018) entre pre-trasplante y tres meses después del implante. Los cambios en la expresión del sistema BAFF aumentan durante el post-trasplante renal en el desarrollo de AR y daño crónico del aloinjerto y podrían ser una herramienta patológica importante para detectar y prevenir el resultado del injerto renal.



P-206

INFLUENCE OF HLA-DPB1 MISMATCH ON ALLOGENEIC MATCHED RELATED HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: HLA-matched sibling donor allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the first curative option for some hematological disorders. Acute graft-versus-host disease (aGVHD) remains the leading cause of morbidity and mortality, even in HLA 10/10 identical sibling pairs. However, the occurrence of chromosomal crossing-over can cause disparities that affect HLA-DPB1 locus. Until recently, DPB1 locus was not included in studies to identify donor/recipient (D/R) compatibility. Therefore, the influence of DPB1 mismatch in allo-HSCT evolution is still unclear.

Objective: We aimed to assess the frequency of disparities in HLA-DPB1 and its association with the outcome of related HLA-identical allo-HSCT.

Material and methods: 131 Donor/Receptor (D/R) HLA-identical 10/10 (A, B, C, DRB1 and DQB1) sibling pairs from the University Hospital of Salamanca were retrospectively DPB1 typed by PCR-SSOP LIFECODES®. DPB1 mismatch status was classified as permissive or non-permissive according to T cell epitope algorithm V2.0 (IPD-IMGT/HLA). Statistical analyses were performed using IBM® SPSS 26 software.

Results: Of 131 D/R pairs, 11 (8.4%) were DPB1 mismatch (8 single mismatch and 3 double allelic mismatch). Of these, six (4.6%) were permissive and five (3.8%) were non-permissive. In non-permissive group, four (3.1%) pairs showed predicted immunogenicity in the Graft-versus-Host (GvH) direction and one in the Host-versus-Graft (HvG) direction. Three patients with GvH mismatch presented aGVHD, while the only patient with HvG mismatch presented graft failure. Non-permissive disparity augmented the risk of aGVHD development in univariate (80% vs. 68.3%, p=0.024) and multivariate analysis [HR: 3.5; 95%CI 1.08-11.42].

Conclusion: In our centre, the frequency of DPB1 mismatch in related HLA-identical allo-HSCT is 8.4%, although the frequency of DPB1 non-permissive discrepancy is lower (3.8%). Our data suggest that non-permissive DPB1 disparities could increase the risk of aGVHD development after allo-HSCT even in related HLA 10/10 identical sibling pairs.



P-207

EL EFECTO A LARGO PLAZO DE LA TERAPIA CON GAMMAGLOBULINA IV EN TRASPLANTE DE ÓRGANO SÓLIDO CON INFECCIÓN E HIPOGAMMAGLOBULINEMIA

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Introducción. Las infecciones, el rechazo y el cáncer siguen siendo barreras a largo plazo para buenos resultados tras el trasplante de órgano sólido. Las gammaglobulinas IV están indicadas en pacientes inmunosuprimidos con infecciones graves que tienen hipogammaglobulinemia. La hipótesis de este estudio es que el uso de GGIV puede impactar positivamente no sólo en el desarrollo de infecciones sino en otras complicaciones post trasplante.

Material y Métodos. Estudio prospectivo de seguimiento ampliado tras la última visita de un ensayo de GGIV para terapia de infección grave en presencia de hipogammaglobulinemia IgG < 600 mg/dL. El protocolo administrado consistió en 2 dosis de 15 gramos (intervalo 7-15 días) y otras 3 de 20 gramos (intervalo 15-30 días). De 22 pacientes incluidos en un centro en el ensayo aleatorizado, 9 fueron aleatorizados a GGIV y 13 a terapia antimicrobiana convencional. Durante un seguimiento mínimo de 2 y máximo de 7 años, adicional tras la última visita de ensayo se recogieron prospectivamente: eventos de infección, rechazo, cáncer y muerte y un evento compuesto por la presencia de al menos uno de los anteriores.

Resultados. La tasa de eventos de infección, rechazo, cáncer y muerte en los pacientes tratados con GGIV versus los no tratados con GGIV fue como sigue, respectivamente: 22 vs 84.6% (p=0.011), 11.1 vs 30.7% (p=0.279), 11.1 vs 15.38% (p=0.774), 11.1 vs 30.7% (p=0.279). La variable de resultado compuesta la desarrollaron un 44.4% de pacientes que usaron GGIV vs un 84.6% de pacientes no tratados con GGIV (p=0.047).

Conclusiones. El uso de GGIV en pacientes que desarrollan infecciones e hipogammaglobulinemia IgG moderada tras el trasplante de órgano sólido se asocia con menor prevalencia de distintas complicaciones y no solo con la disminución de infecciones. Es posible que haga falta redefinir el punto de corte de indicación de GGIV en trasplantados.



P-208

EFFECTIVE DESENSITIZATION APPROACH IN A CARRIER OF VENTRICULAR ASSIST DEVICE AS A STRATEGY TO URGENT CARDIAC TRANSPLANT.

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The risk of Antibody Mediated Rejection and allograft loss in sensitized patients is increased due to preformed donor specific antibodies (DSA) detected before transplantation. One in seven heart transplant candidates are sensitized.

Ventricular assist devices (VADs) are increasingly being used as a bridge to transplantation and have been implicated as a risk factor for allosensitization to human leukocyte antigens (HLA). Our aim is to describe the evolution of a 27-year-old heart recipient patient after been treated with a desensitization protocol.

His personal history included acute lymphocytic leukaemia in childhood with chemotherapy-induced cardiotoxicity that progressed to terminal heart failure and hepatitis C virus infection. At age 27, required VDA implantation as bridge to transplant, due to the need for prolonged pre-transplant HCV treatment.

Complement-dependent cytotoxicity (CDC), panel reactive antibody (PRA) levels and Luminex antibody profiles were followed and, after two months of the implantation of the VDA, anti-HLA class I antibodies were detected (A2,A9,A23,A24,A28,A68,A69,B17,B57,B58) with high titres (MFI 3000-11000, PRA 75%).

Elimination of circulating anti-HLA antibody was viewed as a critical strategy to successfully allow transplant. He received plasmapheresis along with IVIG (400 mg/kg) on days 1, 3 and 5. On day 7, after the MFI value decreased to 1000 – 3000, the patient was placed on the emergency 0 transplant list. A dose of Rituximab (375mg/m2sc) was added, as well as 3 more cycles of plasmapheresis and IVIG on alternate days, with subsequent complete negativization of cytotoxic antibodies, at which time the heart transplant could be performed with an induction protocol that included thymoglobulin instead of basiliximab.

After transplantation, anti-HLA antibodies have remained persistently negative and, five years after the transplant, he has not presented graft vascular disease or rejection episodes. Desensitization protocol was safe.



In high sensitized patients, desensitization may allow transplantation in well selected candidates for this intervention.



P-209

RELEVANCE OF THE BW4 AND BW6 EPITOPES DETERMINATION IN THE MANAGEMENT OF HYPERIMMUNIZED PATIENTS IN RENAL TRANSPLANT: ABOUT A CLINICAL CASE.

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The presence of anti-HLA Donor Specific Antibodies (DSA) and their specificities are determined by routine pre-transplant check-ups for all the potential recipients by solid phase techniques (LABScreen™, One Lambda), allowing to know in advanced the concrete specificity of these anti-HLA DSA.

In this case, the compatibility between a donor and a potential hyperimmunized recipient was thoroughly analyzed, in the context of a dead donor renal transplant virtual cross-match.

HLA typing was done by two different low-resolution molecular techniques: real time PCR (qPCR) with commercial LinkSeq™ panels (One Lambda) as well as with SSP-PCR commercial Fluogene® panels (Inno-Train). The obtained result in both cases was the same: A*02,*11; B*13,*27; C*02,*06; DRB1*01, 15; DQB1*05, 06.

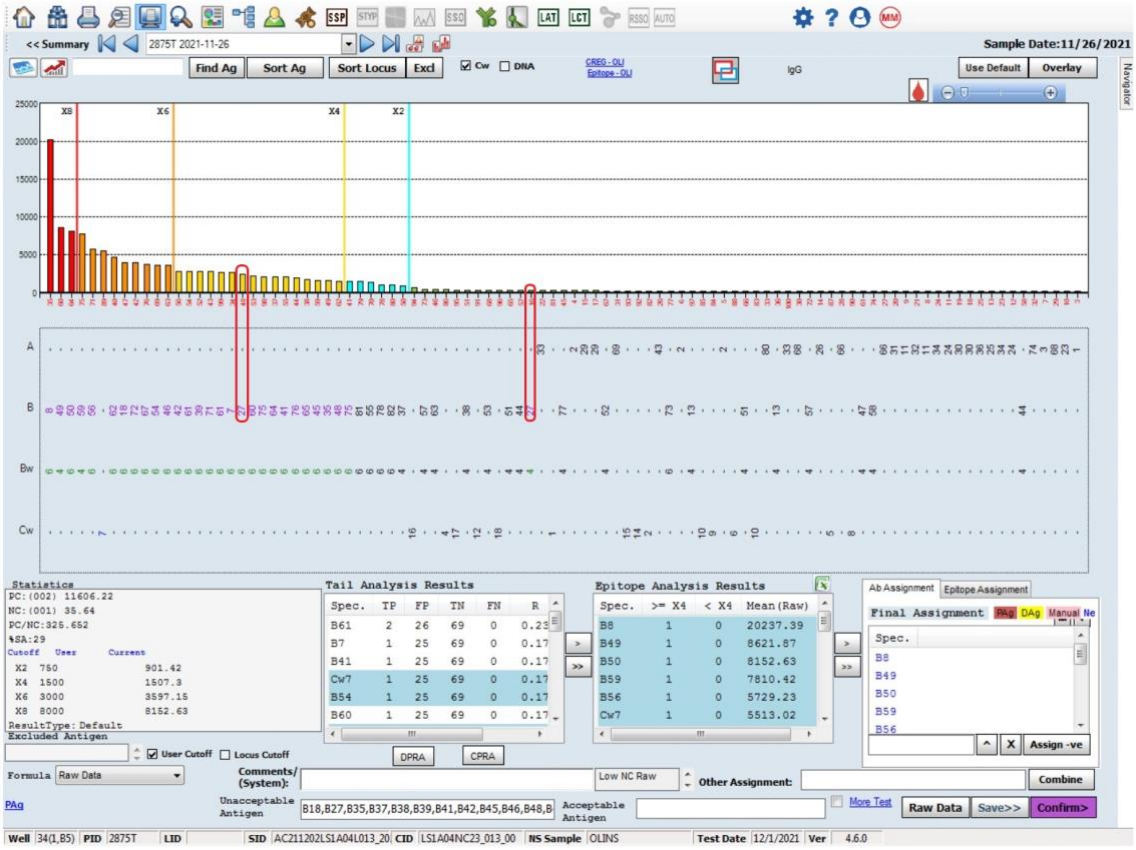
After an exhaustive analysis of hyperimmunized recipients in the virtual cross-match (vPRA > 95%), a recipient with a 99% vPRA was selected. This recipient presented anti-HLA DSA against HLA-B*27:08 allele (MFI ~ 2400), but not against HLA-B*27:05 (MFI ~ 195).

Not having a higher resolution HLA typing from the donor, Bw4 and Bw6 epitopes were of great importance to face this dilemma, as in the B*27 allelic group all the alleles share the Bw4 epitope, with the exception of B*27:08, which presents Bw6 specificity. Both of the HLA typing molecular techniques that were used do have specific probes to determine Bw4 and Bw6 individually, revealing this way that the potential donor was homozygous for Bw4. Thus, the presence of B*27:08 in the donor typing was excluded, and the transplant was carried through with no immunological complications.

Subsequently, donor high resolution typing was performed with SSO-PCR (Immucor), confirming that the allele in the donor was indeed B*27:05, dismissing this way the presence of HLA DSA in the recipient.

In summary, the independently determination of the Bw4/Bw6 epitopes allow to perform the transplant, without higher resolution HLA typing techniques.




Sample Date:11/26/2021
Find Ag
Sort Ag
Sort Locus
Excl
Use Default
Overlay
X8
X6
X4
X2
Statistics
PC:(002) 11606.22
NC:(001) 35.64
PC/NC:325.652
%SA:29
Cutoff User Current
X2 750 901.42
X4 1500 1507.3
X6 3000 3597.15
X8 8000 8152.63
ResultType: Default
Excluded Antigen
Tail Analysis Results
Spec. TP FP TN FN R
B61 2 26 69 0 0.23
B7 1 25 69 0 0.17
B41 1 25 69 0 0.17
Cw7 1 25 69 0 0.17
B54 1 25 69 0 0.17
B60 1 25 69 0 0.17
Epitope Analysis Results
Spec. >= X4 < X4 Mean(Raw)
B8 1 0 20237.39
B49 1 0 8621.87
B50 1 0 8152.63
B59 1 0 7810.42
B56 1 0 5729.23
Cw7 1 0 5513.02
Ab Assignment
Epitope Assignment
Final Assignment
Spec.
B8
B49
B50
B59
B56
Assign -ve
DPRA
CPRA
Formula
Raw Data
Comments/ (System):
Low NC Raw
Other Assignment:
Combine
Unacceptable Antigen
B18,B27,B35,B37,B38,B39,B41,B42,B45,B46,B48,B
Acceptable Antigen
More Test
Raw Data
Save>>
Confirm>
Well 34(1,B5) PID 2875T LID SID AC211202LS1A04L013_20 CID LS1A04NC23_013_00 NS Sample OLINS Test Date 12/1/2021 Ver 4.6.0



P-210

IMPACTO DEL GENOTIPO KIR E INCOMPATIBILIDAES KIR-LIGANDO EN LA EVOLUCIÓN DE LOS TPH CON DONANTE HAPLOIDÉNTICO

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El trasplante de progenitores hematopoyéticos (TPH) es un tratamiento curativo disponible para diferentes neoplasias de origen hematológico. El donante haploidéntico es una opción válida, por su disponibilidad rápida y económica y por sus resultados equiparables al donante no relacionado. La presencia de determinadas combinaciones KIR y de las incompatibilidades KIR/ligando han sido descritas como variables a tener en cuenta para la elección del mejor donante. Sin embargo, existe controversia para determinar que influencia tienen cada una de ellas.

Objetivos: Determinar si el genotipo y semihaplotipos KIR en el donante y la incompatibilidad KIR/ligando tiene importancia en la evolución de los TPH sin depleción de células T con donante haploidéntico

Pacientes y métodos: Análisis retrospectivo de TPH haploidéntico entre 2013-2021. Se han analizado los genes KIR (Genotipaje KIR de Innotrain Diagnostik) en el donante y sus ligandos HLA (Lifecodes HLA-A,B SSO Typing Kit y Lifecodes HLA-C eRES SSO Typing Kit, Immucor) en donante y receptor. Se estudia su influencia en la supervivencia global (SG), supervivencia libre de evento (SLE), probabilidad de recaída (PR) y EICH.

Resultados: la mitad de los pacientes fueron trasplantados por una LMA, siguiéndoles LLA y linfoma. El 21% de los donantes presentaban AA y el 79% Bx. En los pacientes con neoplasias mieloides la presencia de Bx se asocia con una disminución significativa de la PR en comparación con AA. Esta significación se pierde cuando no se dividieron a los pacientes por su patología previa, aunque seguía observándose cierta tendencia a favor de Bx. Los semihaplotipos CENB y TELB se asociaron a una PR significativamente menor tanto en la serie global como en el subgrupo mieloide. La SG fue mayor en el grupo con 2 o más semiaplotipos B. La incompatibilidad KIR-ligando no influyó ni en la SG, SLE, PR ni EICH.



P-211

FORMAS SOLUBLES DEL SISTEMA BAFF/BLYS Y ANÁLISIS DE CORRELACIÓN DE ANTICUERPOS ANTI-DSA EN LA MONITORIZACIÓN Y EVOLUCIÓN DEL TRASPLANTE RENAL

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Objetivo. El sistema BAFF juega un papel fundamental en la homeostasis y tolerancia de las células B, aunque no ha sido ampliamente probado en trasplante con resultados dudosos. El objetivo principal fue estudiar las formas solubles de BAFF y su correlación con el rechazo agudo (AR) y los anticuerpos específicos del donante (DSA).

Métodos. Se analizaron los niveles séricos de BAFF, APRIL y formas solubles de sus receptores en receptores renales (RR) con y sin aparición de rechazo agudo (RA/NAR). Todas las moléculas se evaluaron antes y después del trasplante. **Resultados**. sTACI mostró una correlación significativa con los niveles de BAFF y sR-BAFF, y sBCMA también mostró una correlación positiva con los niveles de sAPRIL. También se encontró un aumento significativo en los niveles de sAPRIL en pacientes con RA, y el análisis de las curvas ROC mostró un AUC=0,724, siendo una concentración de 6,05 ng/ml (sensibilidad=66,7%; especificidad=73,3%) el mejor punto de corte para predecir AR. En la dinámica posterior al trasplante de los niveles de sAPRIL en la cohorte longitudinal, observamos una disminución significativa a los tres y seis meses posteriores al trasplante en comparación con el estado previo al trasplante. También observamos que los receptores con niveles altos de sAPRIL pretrasplante generaron anticuerpos antes que estos pacientes con niveles más bajos de sAPRIL, aunque su postrasplante a largo plazo no fue diferente.

Conclusión. Nuestros resultados muestran que los niveles séricos elevados de APRIL pueden ser útiles como biomarcador para el diagnóstico de AR, aunque el estudio longitudinal muestra que no es útil como biomarcador pronóstico.



P-212

A COMPARATIVE STUDY OF CELL CULTURE CONDITIONS DURING CONVERSION FROM PRIMED TO NAIVE HUMAN PLURIPOTENT STEM CELLS

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The successful reprograming of human somatic cells into induced pluripotent stem cells (hiPSCs) represented a turning point in the stem cell research field owing to their ability to differentiate into any cell type and lack of ethical issues. In the mouse, PSCs are thought to exist in a naive state, the cell culture equivalent of the immature, pre-implantation embryo whereas in humans, PSCs are in a primed state, which is more committed pluripotent state than naive state. Recent studies have focused on capturing a similar cell state in human, since their earlier development stage and lack of cell-of-origin epigenetic memory make the better candidates for further re-differentiation and use in disease modelling, regenerative medicine and drug discovery. In this study, we described and evaluated the successful establishment and maintenance of primed hiPSC and human embryonic stem cell (hESC) lines with three different naive conversion media both feeder and feeder-free conditions. In addition, we compared the directed differentiation capacity of primed and naive cells in the three germ layers. We characterized these different cell states with commonly used pluripotent and lineage specific markers, and showed that, in general, naive culture medium 1 (in both feeder and feeder-free system) confers greater hiPSCs and hESCs viability and highest naive pluripotency markers expression. This medium also allows for better differentiation of cells toward endoderm. In conclusion, we developed a cell culture differentiating protocol with the aim of future potential applications in cell therapies.



P-213

ANÁLISIS DE REPRODUCTIBILIDAD DE PCR DIGITAL EN LA DETERMINACIÓN DE QUIMERISMO HEMATOPOYÉTICO

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Introducción:

La cuantificación de ADN de donante y receptor (análisis del quimerismo hematopoyético) se usa para monitorizar pacientes sometidos a trasplante alogénico de progenitores hematopoyéticos (aloTPH). Una de las metodologías más utilizada es el estudio de marcadores INDELs mediante PCR en tiempo real (qPCR). La PCR digital (dPCR) ha demostrado mayor precisión y menor susceptibilidad a inhibidores de la PCR, no necesitando curvas de calibración. Sin embargo, es una técnica larga y laboriosa. Actualmente se han desarrollado nuevos equipos que favorecen el uso de esta técnica.

Objetivo:

Comparar la precisión en los estudios de quimerismos de qPCR y dPCR realizada con el equipo "QuantStudio™ Absolute Q™ Digital PCR" de Thermo Fisher.

Materiales y métodos:

Se analizaron muestras post-trasplante de 3 pacientes varones sometidos a aloTPH de una mujer. Se estudió el marcador SRY (ChrY) mediante qPCR y dPCR. Se realizaron 5 repeticiones independientes con cada técnica. Para comparar las desviaciones estándar de ambos métodos se usó el test de ANOVA (análisis de varianzas).

Resultados:

En la **Tabla 1** se muestran los resultados de la media (μ) y desviación estándar (σ) obtenidos por qPCR y dPCR. En las tres muestras, la σ fue estadísticamente más pequeña en los estudios de dPCR en comparación con la qPCR ($p<0.05$).

	dPCR		qPCR		ANOVA
	μ	σ	μ	σ	p-valor
Paciente 1	0,135	0,186	0,176	0,021	<0,01
Paciente 2	0,357	0,368	0,765	0,039	<0,01
Paciente 3	0,723	0,270	1,15	0,078	0,03

Tabla 1. Media y desviación estándar de 5 determinaciones realizadas por ambos métodos de PCR en cada paciente. p-valor del Test Anova comparando los dos métodos para cada paciente.
μ: media; σ: desviación estándar

En la **Figura 1** se representa un gráfico de los resultados.

Conclusiones: Los resultados obtenidos por dPCR en los estudios de quimerismos hematopoyéticos son más exactos y reproducibles que mediante qPCR.



Figura 1. Estudio de quimerismo SRY (%) en 3 muestras, mediante qPCR y dPCR. Se observa una mayor agrupación de los datos obtenidos en las determinaciones realizadas mediante dPCR en los tres casos.



P-214

IMMUNOTHERAPY BASED IN QPCR ANALYSIS IN INTESTINAL TRANSPLANT RECIPIENTS AND CANDIDATES

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Introduction

Inflammatory bowel disease (IBD) in intestinal transplantation (ITx) recipients and candidates is a common complication due to the immunosuppression or the underlying disease. Conventional treatment uses to be effective, but in some patients the inflammation is refractory.

Aim

To determine the intestinal cytokine expression in order to establish the optimal therapy in ITx recipients and candidates with persistent enteritis.

Methods

Two multivisceral recipients with episodes of ulcerative enteritis refractory to conventional treatment were studied, as well as, two ITx candidates with intestinal failure and inflammatory colitis and enteropathy, respectively. Mucosal biopsies were taken from affected and healthy areas.

Expression of cytokine genes which are targeted by approved drugs (IL1B, IL6, IL12p70, IL17A, IL23 and TNF) was determined by qPCR.

Results

ITx recipients (P1 and P2): both patients (13 and 14 years old males) developed in the late posttransplant ulcerative ileitis and ulcerative colitis respectively. They were treated with metilprednisolone and infliximab without endoscopic improvement. Graft cytokine expression of healthy and inflamed area was performed, showing a significant increase of IL6 (Figure1A/1B); Tocilizumab was administered, getting clinical resolution in two months.

ITx candidates:

P3 (5 years old male) suffered from trichohepatoenteric syndrome with ulcerative colitis. He was tread with corticosteroids without improvement. Intestinal cytokine expression objectified elevated IL1 and IL6 levels. After Tocilizumab treatment, healing of the colitis was observed (Figure1C).

Enteropathy in P4 (1 year old male) was derived from an autoinflammatory disorder associated to MVK mutation. He was treated with Infliximab with only transient improvement. Intestinal cytokine expression showed elevated IL1, IL6 and TNF levels (Figure1D). He was then treated with Anakinra without clinical improvement, changing for Adalimumab achieving clinical



resolution.

Conclusions

In the context of mucosal inflammation, intestinal cytokine expression analysis by qPCR is a useful and affordable tool to guide the treatment in a personalized way.



P-215

DETECTION OF NON-HLA ANTIBODIES IN KIDNEY TRANSPLANT PATIENTS WITH GRAFT DYSFUNCTION

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Introduction: No donor-specific anti-HLA antibodies (DSAs) are detected in some patients with suspicion of antibody-mediated rejection (ABMR) in renal biopsies performed for renal function impairment. Non-HLA antibodies directed against self- or allo-antigens present pre-transplantation or produced de novo post-transplantation could play a relevant role.

Objective: To study the presence of non-HLA antibodies in pre- and post-transplant sera, coincident with biopsy, of kidney transplant patients with impaired renal function.

Materials and Methods: 22 kidney transplant recipients from 2014 to 2019 with renal graft function impairment were included. The mean time from transplantation of biopsies was 137 ± 275 days. The presence of anti-HLA DSA antibodies was ruled out by Single Antigen Luminex assay (Immucor). The study of non-HLA antibodies was performed with version 1 (32 antigens) and version 2 (60 antigens) of the Lifecodes Non-HLA Antibody kit (Immucor).

Results: In 17 patients the biopsy was reported with findings compatible with ABMR with positive C4d (10 patients) or negative C4d (7 patients), while in 5 patients there were no signs of ABMR (3 without morphological data of rejection, 2 with cellular rejection). All patients, except 3, still have a functioning graft. In 20/22 (91%) of the patients some non-HLA antibodies were detected, both in the pre-transplant and in the post-transplant samples. In some patients, non-HLA antibodies against only one antigen were detected, whereas other patients had antibodies against up to 11 different non-HLA antigens. There was no difference between patients with or without non-HLA antibodies and the diagnosis of ABMR.

Conclusions: Prospective studies and a larger cohort are needed to confirm or not the usefulness of such determination and the pathological role that these non-HLA antibodies may play, although in this small group of patients we do not find any clinical relevance.



P-216

EXPRESIÓN DE MIRNAS Y EFICACIA DIAGNÓSTICA DE MIR-150-5P COMO POTENCIAL BIOMARCADOR DE RECHAZO EN TRASPLANTE RENAL , PREDICCIÓN COMPUTACIONAL Y MODELO DE INTERACCIÓN CON MBD6

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Antecedentes: La biopsia de aloinjerto renal se considera el estándar de oro para el rechazo, pero es invasivo y podría ser indeterminado. Varias publicaciones apuntan al papel de los miARN expresión al sugerir su implicación en la aceptación o rechazo del trasplante de órganos. Este estudio tuvo como objetivo analizar microRNAs involucrados en la diferenciación y activación de B y Linfocitos T de leucocitos de sangre periférica de pacientes trasplantados de riñón (KT) para ser utilizados como biomarcadores de rechazo renal agudo (RA). Métodos: Un total de 15 pacientes KT con y sin agudo rechazo (AR/NAR) fueron analizados y cuantificados por miRNA PCR array. Un total de 84 miARN relacionados con la diferenciación y activación de linfocitos B y T. Las funciones y Se analizaron las vías biológicas para predecir los objetivos potenciales de los miARN expresados diferencialmente. Resultados: Se incrementaron seis miRNA en el grupo AR (miR-191-5p, miR-223-3p, miR-346, miR-423-5p, miR-574-3p y miR-181d) y miR-150-5p aumentaron en el grupo NAR. Los estudios in silico mostraron un total de 2603 genes diana para el aumento de miARN en AR, mientras que para la disminución de miARN, un total de Se encontraron 1107 genes de potencial diana. Conclusiones: Nuestros resultados muestran que KT con AR muestra una disminución en la expresión de miR-150-5p en comparación con NAR, lo que sugiere que la disminución en miR-150-5p podría estar relacionado con un aumento de MBD6 cuya desregulación podría tener consecuencias clínicas y evitar el rechazo.



P-217

IDENTIFICATION AND CHARACTERIZATION OF THE NOVEL HLA-B*49:78 ALLELE WITH LEADER SEQUENCE MODIFICATIONS

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Introduction: Allogeneic-Hematopoietic Stem Cell Transplantation (a-HSCT) is a curative treatment for several malignant and non-malignant diseases. HLA mismatches lead to higher risk of Graft-versus-host disease (GVHD) and mortality after a-HSCT. Nowadays, the degree of HLA compatibility is evaluated according to polymorphisms present within the peptide binding region. However, HLA-B leader sequence can influence a-HSCT outcome depending on the dimorphism (Methionine or Threonine) at position -21. Here, we describe a novel HLA-B allele (HLA-B*49:78) which is included in HLA-B*49:01P group, but does not have the same leader sequence as B*49:01:01:01.

Material and methods: HLA-B*49:78 allele was identified in a female bone marrow donor by next-generation sequencing (NGS).

Results*:* HLA sequencing gave a B*44:03:01 and B*49var assignment after complete genomic HLA-B analysis in a single phase. The HLA-B*49var showed six nucleotide differences from B*49:01:01:01: three in coding regions and the other three in non-coding regions. Coding regions substitutions were all detected in exon 1, while substitutions in non-coding regions were found in 5'UTR and intron 1. First change in exon 1 was located in genomic position 5 (G>T), codon -23 (CGG>CTG) producing an amino acid modification Leucine -23 to Arginine. Next change was placed in genomic position 11 (C>T), codon -21 (ACG>ATG) causing another amino acid change Methionine -21 to Threonine and the modification of the leader sequence genotype. Last variation in exon 1 was a synonymous substitution for the amino acid Alanine located in genomic position 15 (A>T), codon -20 (GCA>GCG). The three single nucleotide changes identified in non-coding regions, where located in genomic positions -61 (T>G), -18 (A>G) and 89 (C>G).

Conclusions: HLA-B*49:78 and HLA-B*49:01:01:01 have the same amino acid composition at peptide binding region, but does not share the same leader sequence. This change could influence a-HSCT outcome depending on patient's HLA-B matching and HLA-B leader variant.



P-218

COMPARISON OF NEXT-GENERATION SEQUENCING AND SHORT-TANDEM REPEATS TO MONITOR CHIMERISM ANALYSIS

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Introduction: Chimerism analysis is of great clinical utility to monitor engraftment and to predict relapse after allogeneic hematopoietic stem cell transplantation. More sensitive techniques such as next-generation sequencing (NGS) possible allow anticipating relapse in cases than the gold-standard short tandem repeats (STR) could not.

Aim: To compare the limit of detection (LOD) and the quantification ability of NGS using AlloSeq HCT kit (CareDx) and STRs with PowerPlex 16HS kit (Promega) in clinical samples and artificial mixtures.

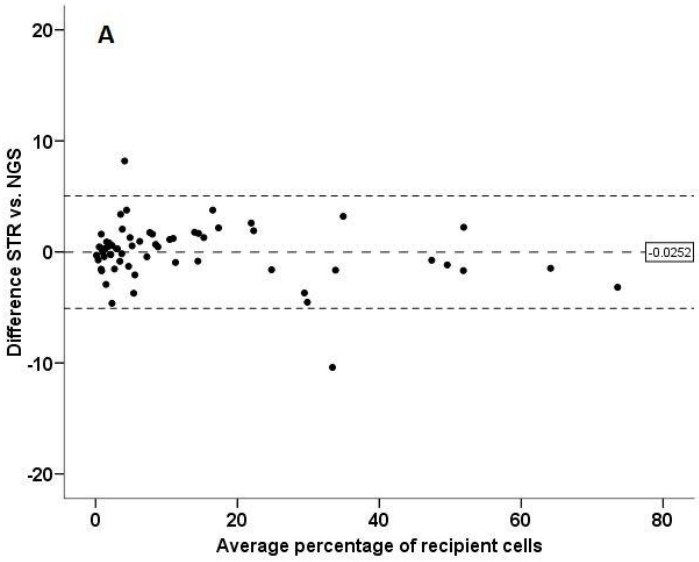
Methods: LOD accuracy was evaluated by 8 artificial mixtures (50% to 0.38%). Intra- and inter-experiment reproducibility was evaluated by duplicate samples (n=3). Informativeness was assessed in 21 donor-recipient pairs (D/R). Quantification correlation of both techniques was performed on 62 clinical samples from 19 patients applying a Bland and Altman analysis.

Results: The analysis of artificial mixtures showed better LOD in NGS (0,4%) than in STR (2%). There was an excellent correlation of the quantification between both techniques, with Bland and Altman analysis showing a small bias (-0,0252, Figure 1A). Informativeness was better for NGS than for STR in 21 D/R (100% vs. 95%). Reproducibility was similar for both techniques (SD □0,74% NGS vs. □0,42% STRs). A mixed chimerism was detected only by NGS in 14/64 samples (Figure 1B), all below the LOD of STR. This allows to detect increasing recipient cells by NGS in 2/19 patients with complete chimerism by STRs, at a median of 21 days prior to relapse. Regarding technical features, both techniques use the same quantity of DNA (10ng), but NGS is more time-consuming and expensive than STR.

Conclusions: NGS technology allows a reliable quantification of chimerism with better LOD and Informativeness than STR. However, it requires to collect higher number of samples, and time to results and costs are higher.

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B

		NGS	
		-	+
STR	-	4	14
	+	1	43



P-219

OPTIMIZATION OF FLOW CYTOMETRY-BASED METHOD FOR THE DETECTION OF BOTH CYTOTOXICITY AND IGG BINDING FOR HLA ANTIBODY CROSSMATCH ASSAY

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

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

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

Results: Successive experiments have established that the optimum protocol conditions required for the crossmatch were as follows: 1) Incubation (30 minutes) of 100000 donor cells in 25 µL with 50 µL of recipient serum. 2) Incubation (30 minutes) with 100 µL of complement. 3) Incubation (30 minutes) with a titrated mix of monoclonal antibodies and 7AAD. 4) Analysis by flow cytometry. Tests were performed with sera from HLA-sensitized patients, demonstrating that the technique is able to detect the cytotoxic capacity of IgG anti-HLA class I and/or II antibodies, as well as to discriminate them from IgM antibodies. (Figure 1)

Conclusion: We describe the optimization of a flow cytometry-based procedure for the determination of both cytotoxicity and IgG HLA antibodies binding to donor-derived lymphocytes, without the need of magnetic separation of T and B lymphocytes and lowering the

time spent in relation to the conventional CDC technique. This procedure could improve pre-transplant risk assessment and progress organ allocation efficiency.

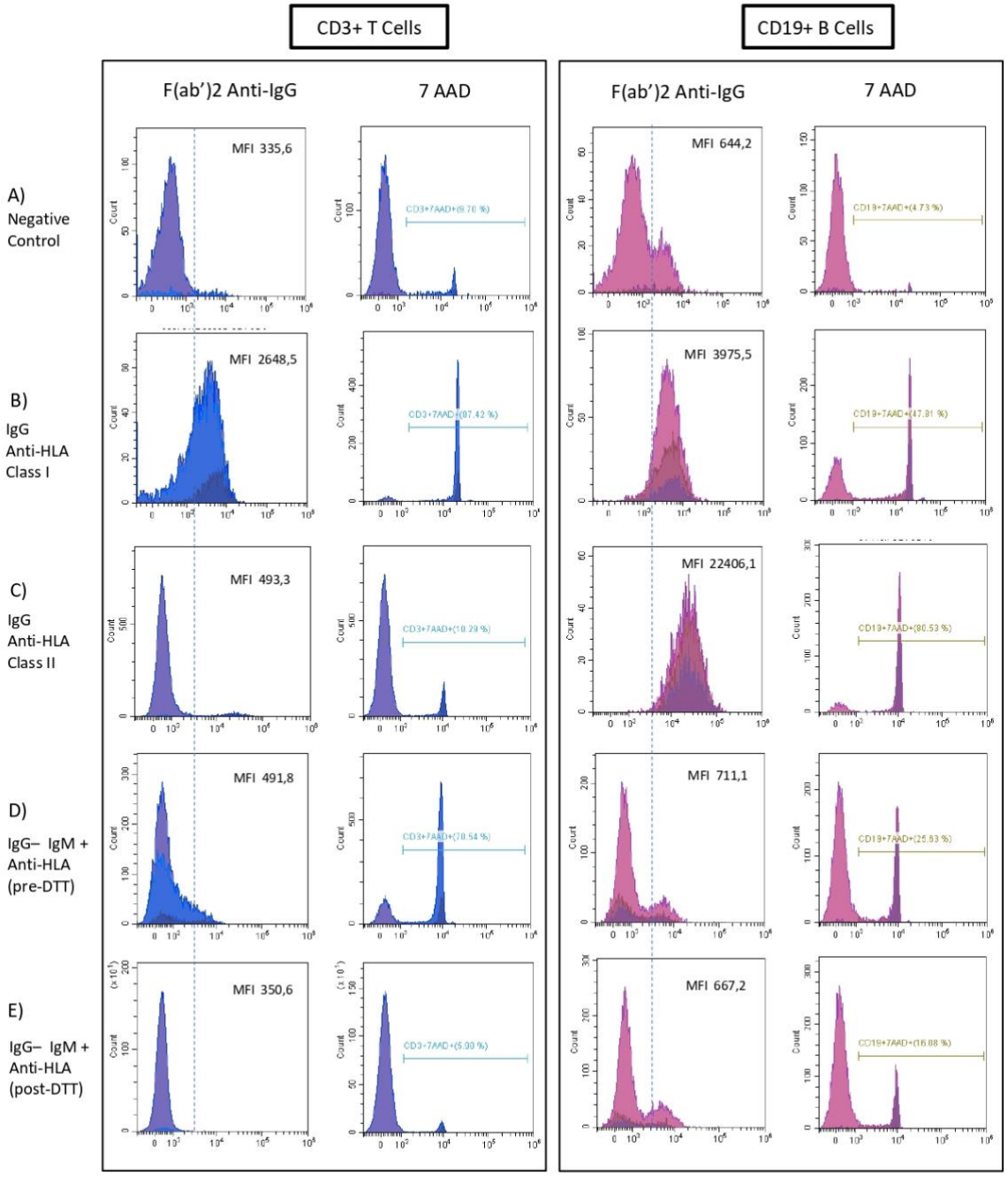


Fig 1. Representative data plots from CDC-FCXM.



P-220

ESTUDIO DE LA DEFICIENCIA DE FORMACIÓN DE ANTICUERPOS EN PERIODO DE LISTA DE ESPERA DE TRASPLANTE CARDIACO

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Introducción. En la última actualización de indicaciones de gammaglobulinas intravenosas (GGIV) de la EMA (Enero 2019) se estableció como criterio el fallo de formación de anticuerpos frente a antígenos polisacáridos definido como aumento menor a 2 veces sobre el nivel basal tras vacuna de neumococo. En los primeros 6 meses post-trasplante cardiaco (TC) esta vacuna no se suele indicar por lo que no se puede utilizar esta definición ante infecciones graves. La hipótesis de este trabajo es que existen pacientes con fallo de formación de anticuerpos en el periodo pre-TC y que estos pacientes podrían ser considerados como candidatos a GGIV si acontecen eventos infecciosos graves en el post-TC.

Material y Métodos. Estudio prospectivo de cuantificación de anticuerpos anti-polisacárido de neumococo pre y post vacuna de 23 serotipos de polisacárido de neumococo mientras el paciente esta en lista de espera para TC. El nivel post-vacunal se realizó 2-3 semanas tras vacuna. Se utilizó un ELISA de 23 serotipos de polisacárido de neumococo coincidentes con los 23 de la vacuna de neumococo (Binding Site, Birmingham, UK).

Resultados. Durante el estudio se hizo la evaluación pre y post-vacunal en 16 pacientes. En 6 de los 16 (37.5%) pacientes se pudo demostrar un fallo de formación de anticuerpos frente a polisacárido de neumococo tras 1 dosis de vacuna. El 66,6% de estos pacientes desarrollaron infecciones graves post-TC mientras que un 20% de pacientes sin deficiencia de formación de anticuerpos las tuvieron (p=0.062).

Conclusiones. Un importante porcentaje de pacientes con patología cardiaca terminal y en lista de espera para TC muestran datos de fallo de formación de anticuerpos frente a antígenos polisacáridos. Si estos pacientes desarrollan infecciones graves post-TC podrían evaluarse como candidatos a terapia con GGIV utilizando los criterios de indicación de la EMA.



P-221

PREDICCIÓN COMPUTACIONAL DE BIOMARCADORES, VÍAS Y NUEVOS FÁRMACOS DIANA EN LA PATOGENIA DE ENFERMEDADES DE BASE INMUNITARIA CON RESPECTO A RECHAZO DEL TRASPLANTE RENAL.

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Diagnóstico de rechazo en trasplantados renales (TR) es realizado por biopsia, pero la evolución de ciencias ómicas y bioinformáticas ha contribuido al avance en predicción de biomarcadores, vías y nuevos fármacos diana que permitan un diagnóstico preciso y menos invasivo. El objetivo era buscar genes diferencialmente expresados (DEG) en pacientes con/sin rechazo mediado por anticuerpos (AMR) y encontrar leucocitos infiltrantes en el injertoinvolucrados en AMR, nuevos fármacos diana, interacciones proteína-proteína (PPI) y conocer su análisis funcional y biológicocon herramientas bioinformáticas. Cuatro bases de datos GEO de biopsias renales se analizaron con/sin AMR. Nuestros resultados muestran los DEG y las poblaciones de leucocitos infiltrantes en el injerto presentaban un aumento en la expresión de genes relacionados con diferentes etapas de la activación inmunitaria, presentación antigénica, ADCC o migración de leucocitos durante AMR. La importancia de las vías de respuesta IRF/STAT1 al IFN en el control de la expresión de genes relacionados con AMR se postularon como posibles dianas terapéuticas y biomarcadores AMR. Estos procesos biológicos correlacionados mostraron la infiltración de células NK y monocitos hacia el aloinjerto, además del aumento en la maduración de las células dendríticas, juega un papel central en la mediación del daño sufrido por el injerto en AMR. Enfoques computacionales para la búsqueda de nuevos usos terapéuticos de diana aprobada. Los medicamentos también mostraron que imatinib teóricamente podría ser útil en TR para la prevención y/o tratamiento de AMR. Nuestros resultados sugieren la importancia de las vías IRF/STAT1 en AMR. Células NK y monocitos tienen un papel esencial en el daño del injerto durante rechazo, eimatinib mejora los resultados del TR. Nuestros resultados tendrán que ser validados para el uso potencial de genes sobreexpresados como biomarcadores de rechazo que pueden usarse como marcadores diagnósticos y pronósticos y como dianas terapéuticas para evitar el rechazo.



P-222

MUERTES POR SEPSIS E INSUFICIENCIA HEPÁTICA EN PACIENTES CON CIRROSIS ALCOHÓLICA Y TRASPLANTADOS DE HÍGADO. INFLUENCIA DE LOS RECEPTORES KIR.

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Los mecanismos clínicos y moleculares implicados en la causa y el momento de la muerte y la supervivencia de los pacientes con cirrosis alcohólica (CA) sometidos a trasplante hepático (TH) no se conocen del todo. Este estudio tuvo como objetivo determinar, en pacientes con CA sometidos a TH, la influencia de las características sociodemográficas, clínicas y genéticas de los genes KIR, principales causas/tiempo de muerte y supervivencia. Se analizaron un total de 122 pacientes CA fallecidos hasta tres años después del TH. El ADN genómico se extrajo de sangre periférica y el genotipado KIR se realizó mediante PCR-SSO con tecnología Luminex®. Se observó un 53,3% de muerte no súbita. La sepsis fue la principal causa de muerte súbita con mayor porcentaje (21,3%) de mortalidad, seguida del fracaso del injerto (FG) y el fallo multiorgánico (15,6%) a los diez años. La mortalidad por FG de los pacientes con AC sin encefalopatía parece estar influenciada por la presencia de KIR3DL1+ (P=0,045). Además, KIR2DS1+/S1+ (P=0,011) o KIR3DL1+ (P=0,012) parecen influir en la supervivencia de los pacientes con GF a los tres años. En conclusión, nuestros resultados muestran la vulnerabilidad del paciente AC sometido a TH en función de las características de los genes KIR. La estrecha vigilancia médica, y la búsqueda de biomarcadores que predigan una causa y momento concretos de la muerte, son fundamentales para mejorar, controlar y adaptar los tratamientos médicos para prolongar la supervivencia del paciente.



P-223

CAUSAS DE MUERTE Y SUPERVIVENCIA EN PACIENTES CON CIRROSIS ALCOHÓLICA SOMETIDOS A TRASPLANTE HEPÁTICO: INFLUENCIA DE VARIABLES CLÍNICAS, VULNERABILIDAD INMUNITARIA Y COMPLICACIONES EN EVOLUCION POST-TRASPLANTE.

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Los mecanismos clínicos y moleculares involucrados en la causa y el momento de la muerte de los receptores con cirrosis alcohólica (CA) y trasplante hepático (TH) no se conocen por completo. En los casos de muerte súbita, la práctica de la autopsia judicial es obligatoria para determinar la causa y circunstancias de la muerte. Los datos de la autopsia médico-legal son fundamentales para ayudar a las autoridades sanitarias a orientar las futuras actuaciones de salud pública y evaluar la eficacia de sistemas sanitarios y adoptar las medidas preventivas para mejorar y adaptar los tratamientos para aumentar la supervivencia de estos pacientes. Nuestro estudio tuvo como objetivo determinar las diferentes causas clínicas y sociodemográficas que influyen en las diferentes causas de muerte y supervivencia del receptor CA y TH. Se analizaron 122 receptores CA y TH en diferentes momentos postrasplante. Se analizaron las complicaciones pre- y postrasplante sobre la causa de muerte y sobrevida, y las causas y tiempo en que ocurrió la muerte. Se observó 53,3% de muerte no súbita. Gran parte de las muertes de pacientes CA y TH se debe a muerte no súbita, sepsis y fallo del injerto (FG), siendo similares en ambos sexos. En las muertes no súbitas, no existen diferencias significativas entre tasas de muerte relacionadas o no relacionadas con TH. La sepsis fue la principal causa con mayor porcentaje (21,3%) de mortalidad seguida de FG (18,9%) y fallo multiorgánico (15,6%) a diez años. Además, nuestros resultados muestran cómo las complicaciones clínicas pre-trasplante, como infecciones virales y encefalopatía, influyen en la edad a la que se produce el fallo multiorgánico del trasplantado. Conclusion: El fallo multiorgánico es la primera causa de muerte súbita, con mayor mortalidad durante el primer año post-trasplante, seguida de sepsis y FG, mostrando vulnerabilidad del paciente CA dentro y fuera del hospital.



P-224

UTILIDAD DEL TIPAJE HLA DE MEDIA/ALTA RESOLUCIÓN EN EL TRASPLANTE RENAL

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La respuesta inmunitaria frente a antígenos HLA representa la principal barrera al trasplante de órganos sólidos. Pequeños cambios en la secuencia de ADN de una molécula HLA pueden inducir la producción de anticuerpos donante específicos. En la mayoría de centros se realiza tipificación HLA de baja resolución para diferentes loci HLA, generalmente HLA-A, B, C DRB1 y DQB1. Presentamos tres casos de seguimiento de DSA postrasplante renal en los que los pacientes se sensibilizaron contra antígenos HLA del mismo grupo alélico de baja resolución que los suyos propios. La tipificación HLA se realizó utilizando PCR-SSOr (LabtypeTM SSO) y el estudio de anticuerpos se realizó con mediante screening y single antigen en plataforma Luminex (lifecodes® LSA y lifeScreen deluxe). Los tipajes de baja resolución de los pacientes eran los siguientes: paciente 1 (A*03,30; B*49,52; DRB1*03,15), paciente 2 (A*11,29; B*38,44; DRB1*01,11) y paciente 3 (A*02,03; B*07,35; DRB1*04,11). En el paciente 1 se detectaron anticuerpos frente a HLA-A*03:01P, en el paciente 2 frente a HLA-B*44:02P/B*44:03P y en el paciente 3 frente a HLA-B*35:01P. Al re-analizar el tipaje HLA de estos pacientes, el A*03 del paciente 1 era del grupo A*03:02P, el B*44 del paciente 2, B*44:04P y el B*35 del paciente 3, B*35:02P. Por tanto, pequeñas diferencias en las secuencias de proteínas pueden desencadenar sensibilización. Estos resultados demuestran que, al menos en algunos pacientes, es necesario realizar tipaje HLA con intermedia o alta resolución y la necesidad de contar con este nivel de resolución en los tipajes de los donantes para realizar el seguimiento postrasplante. Asimismo, la sensibilización frente a moléculas del mismo grupo alélico que las suyas propias dificultará las posibilidades de acceso a un posible retrasplante si se utilizan sistemas de tipaje de baja resolución.



P-225

DETECTION OF POSSIBLE FALSE ANTI-HLA ANTIBODIES AGAINST SELF ANTIGENS USING SINGLE ANTIGEN LUMINEX TECHNOLOGY

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Introduction: The accurate identification of anti-HLA antibodies is crucial in the context of transplantation since false positive results can decrease the chance of finding a suitable donor and undergo patients to unnecessary immunosupression. The aim of this study was to determine the prevalence of these false HLA autoantibodies in a cohort from recipients on kidney transplant waiting list.

Materials and methods: We analyzed the records from a total of 5570 Single Antigen (SAB) results performed from 2019 to 2020. First, results with calculated panel-reactive antibody ≠ 0 (n= 2560) were selected. Then, patients with at least two determinations in this period (n=1147) were filtered and finally those patients having a high-resolution HLA typing for class I and/or class II were analyzed in detail and the presence of a null allele in the recipient was discarded. The positivity criteria in the SAB were the recommended by the manufacturer (Immucor).

Results: None out of 253 patients showed anti-HLA class I antibodies against self allele beads. For class II, we detected potential auto-HLA antibodies in 14 out of 159 patients (10.7%): 6 DQ, 3 DRB3, 1 DRB4, 3 DPB1 and 1 DRB1. Median Fluorescence Intensity (MFI) was similar among each couple of sera except in one case. The MFI range was between 813 and 22423. We check the highest positive sera of each patient with the SAB kit from the other manufacturer (One Lambda) and in all cases except one the results were negative against these specific alleles.

Conclusions: The detection of false anti-HLA class II autoantibodies is not uncommon, whereas we did not detect any case in class I. In order to confirm that these antibodies are recognizing cryptic or denatured antigens, these positive results should be verified by the use of a cellular crossmatch and/or a different SAB kit.



P-226

PRESENCIA DE FALSOS POSITIVOS POR ANTICUERPOS ANTI-HLA NATURALES EN ENSAYOS DE FASE SOLIDA

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Introduccion

Los ensayos en fase sólida para el estudio de anticuerpos anti-HLA son importantes en estudios pre y post-trasplante de receptores renales. No obstante, por su alta sensibilidad y la presencia de antígenos HLA en diversos grados de desnaturalización, da lugar a la detección de falsos positivos así como anticuerpos anti-HLA no patogénicos.

Objetivo

Discriminación de anticuerpos anti-HLA patogénicos de no patogénicos.

Materiales y Metodos

Se realizan estudios de screening, especificidades de anticuerpos anti-HLA y estudio de anticuerpos fijadores de complemento mediante ensayos Luminex por bead arrays (Labscreen Mixed, Labscreen Single Antigen (SA) Clase I y II y C1qscreen, One Lambda, INC) en sueros de paciente en lista de espera de trasplante renal.

Resultados

El estudio de screening resultó negativo y el estudio de SA positivo para todos los beads con DR y varios con DQ. Se realizaron pruebas cruzadas por CDC y citometría de flujo frente a dianas celulares contra las que los sueros problema presentaban elevadas MFI.

El ensayo C1q resultó negativo en los sueros pre-trasplante.

Lo anterior sugiere la presencia de anticuerpos anti-HLA frente a epítopos ocultos (neoepítopos) que quedarían expuestos en las moléculas de HLA desnaturalizadas presentes en los beads de SA.

Tras el trasplante, con inmunosupresión estándar, el seguimiento de anticuerpos reveló un importante descenso de las MFI de los anticuerpos anti-HLA detectados anteriormente y la aparición de anticuerpos anti-HLA frente a incompatibilidades del injerto. Hallazgos que se confirman por ensayo C1q.

Conclusion

Los ensayos de SA, por su alta sensibilidad y presencia de moléculas HLA desnaturalizadas en los beads, pueden dar lugar a falsos positivos lo que dificulta su correcta interpretación. Es recomendable el uso de técnicas complementarias (ensayo C1q, pruebas cruzadas con dianas celulares), para confirmar la patogenicidad de los anticuerpos anti-HLA detectados por SA



P-227

NEED TO USE OF THE SINGLE ANTIGEN KIT IN POST-TRANSPLANT FOLLOW-UP OF B*45 AND DRB1*12 DONORS

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According to the Banff classification, the presence of donor-specific anti-HLA antibodies (DSA) is one of the diagnostic criteria for humoral rejection and the correct detection of anti-HLA antibodies is essential, as it is used in clinical practice to assess the risks of rejection and graft loss. Solid phase assays with the Luminex platform for the study of anti-HLA antibodies have become the "gold standard" technique and have been incorporated into the routine of all histocompatibility laboratories, allowing their study in a simple way. The usual practice is to use the screening kit and if it is completely negative, the non-detection of DSA is reported. However, it may happen that there are HLA antigens that are under represented in the screening beads and for their detection it is necessary to use the single antigen kit. We present two renal transplant recipients in a follow-up study of DSA in which patients were sensitized to donor HLA antigens. The antibody study was performed with the single antigen and screening kit on the Luminex platform (lifecodes® LSA and lifeScreen deluxe). Here we identified the presence of anti-B45 antibodies by single antigen and not by screening kit in a transplanted patient whose donor was B*45, as well as the presence of anti-DR12 antibodies in the other patient whose donor was DRB1*12. These antibodies are detected with median fluorescence intensity (MFI) of around 5,000. Currently, the patient with anti-B45 antibodies shows deterioration of renal function with increased creatinine and the patient with anti-DR12 antibodies remains stable. It is very important to report these cases to avoid errors in the diagnosis and consequent treatment that the patient may receive, so we recommend always using the single antigen kit when we are evaluating the presence of DSA with B*45 and DRB1*12 donors.



P-228

LIVER TRANSPLANT RECIPIENTS WITH ALTERED ELASTOGRAPHY DISPLAY A MORE PROINFLAMMATORY IMMUNE PROFILE.

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Introduction:

Non Alcoholic Fatty Liver Disease (NAFLD) involves hepatic alterations ranging from simple hepatic steatosis to Non Alcoholic Steatohepatitis (NASH), potentially leading to cirrhosis and hepatocellular carcinoma. NAFLD has been positioned as one of the main indications for liver transplantation in the last decade and, more recently, it has also been identified as a post-transplant complication. Transplant recipients might have enhanced risk for NASH due to, first, the proinflammatory response caused by the ischemia-reperfusion injury and the allogenic stimuli and, second, the use of immunosuppressive treatments which might predispose to metabolic syndrome, a well-known risk factor for NAFLD. The main objective of this work was to determine the immune cellular landscape of hepatic transplant recipients with altered Fibroscan values in comparison with healthy subjects.

Methods:

We studied a cohort of 36 hepatic transplant recipients and 7 healthy subjects similar in age and gender proportions. A Fibroscan test was performed to all patients and controls in order to look for hepatic fibrosis and steatosis, both characteristics of NASH. Multiparametric flow cytometry was conducted to assess a wide variety of immune cellular components. Mann Whitney U test was used to explore differences between groups.

Results:

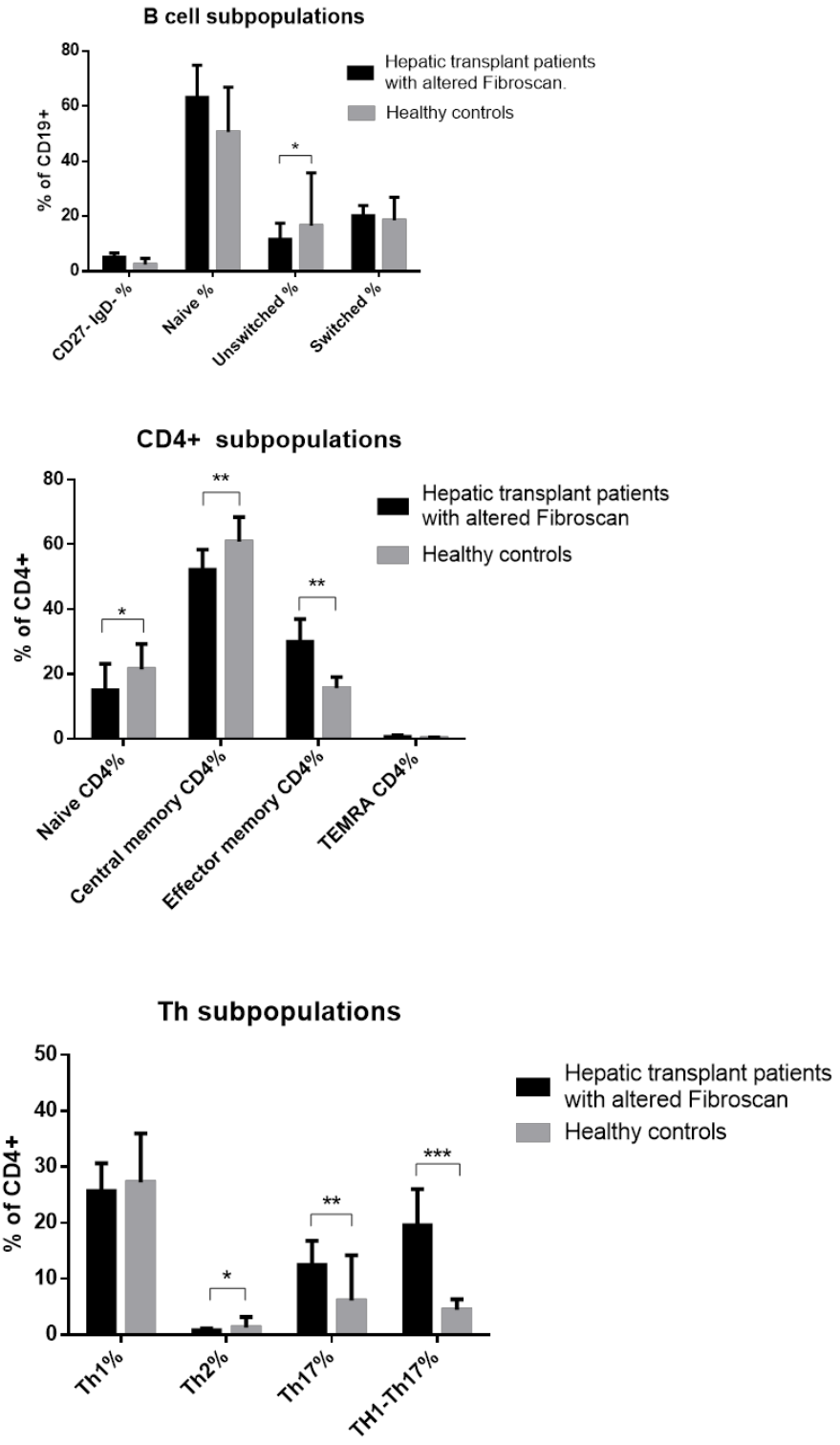
No significant differences were found in the main cellular components such as B cells, T cells (CD4+ or CD8+), NK cells or NKT cells. However, in transplant recipients with altered liver elastography CD4 subset of cells showed a more effector phenotype in transplant recipients, and a more proinflammatory profile with decreased Th2 (CCR6-CXCR3-CD294+) and increased Th17 (CCR6+CXCR3-) and Th1-Th17 mixed phenotype (CCR6+ CXCR3+). In addition, transplant recipients showed significantly lower proportion of unswitched B cells (CD27+IgD+).

Conclusion:

Liver transplant recipients with altered Fibroscan values show a more effector and

proinflammatory helper T cell phenotype than healthy controls despite immunosuppression. This might be associated to NASH development.

Figure: Differences in immune cell populations between healthy controls and hepatic transplant recipients with altered liver elastography.





P-229

OPTIMIZED NGS-BASED HLA TYPING FOR THE DETECTION OF HLA LOSS RELAPSE

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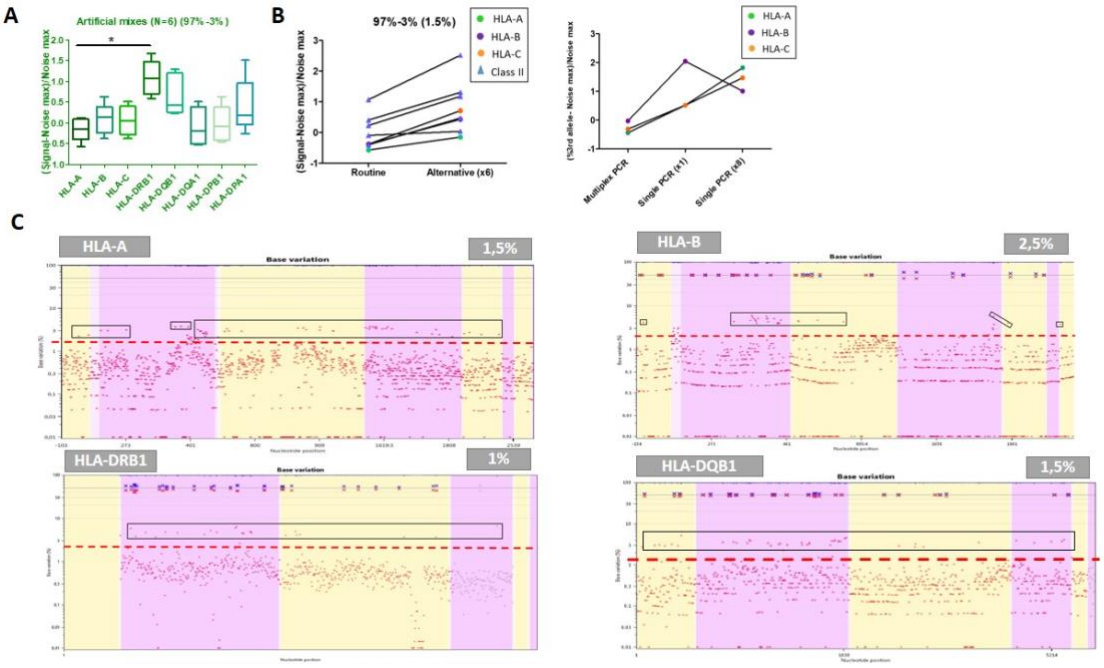
HLA loss relapse (HLA-LR) consists on genomic loss of mismatched HLA molecules in re-emerging leukemic cells. Prospective HLA-LR detection would require a sensitive and cost-effective technique implemented in routine laboratories. HLA-KMR® (GenDx) is the unique commercial kit specifically designed to detect HLA-LR. Although it has advantages intrinsic to the qPCR technique (high sensitivity and fast), allele and gene HLA coverage is limited. Assessing HLA-LR by analysing chimerism plus Next-generation sequencing (NGS)-based HLA typing not only could overcome this limitation but also involves high-throughput and flexibility (adjusting deep coverage according to the required sensitivity).

The aim of this study is to optimize our NGS-based HLA typing strategy to detect HLA loss. For this purpose, we assessed the behavior of this technique with artificial chimerism curves and the noise level and pattern among HLA genes. Then, some conditions of current NGS protocol were modified (such as increasing the deep coverage of these specific samples) in order to increase the sensitivity to detect low-represented alleles.

Analysing 6 artificial chimerim curves with the routine procedure, we observed that the capacity of detection of the low-represented allele varied among HLA genes being limited in class I genes due to the high noise level (Figure 1A). The alternative conditions in NGS protocol improved the discrimination between the low-represented allele signal and the noise (Figure 1B). With the optimized conditions, the obtained sensitivity in all class I and II HLA genes was comparable to HLA-KMR® kit based on qPCR (Figure 1C). Finally, samples from patients with classical and HLA-LR were used as validation samples for our optimized HLA loss detection strategy.

In conclusion, optimized HLA typing NGS strategy is a useful tool for HLA-LR detection considering its high sensitivity and complete HLA gene and allele coverage.




A
Artificial mixes (N=6) (97% -3%)
(Signal-Noise max)/Noise max
HLA-A
HLA-B
HLA-C
HLA-DRB1
HLA-DQB1
HLA-DQA1
HLA-DPB1
HLA-DPA1
B
97%-3% (1.5%)
(Signal-Noise max)/Noise max
Routine
Alternative (x6)
HLA-A
HLA-B
HLA-C
Class II
(%3rd allele- Noise max)/Noise max
Multiplex PCR
Single PCR (x1)
Single PCR (x8)
C
HLA-A
1,5%
Base variation
HLA-B
2,5%
HLA-DRB1
1%
HLA-DQB1
1,5%



Sesión Poster. Inmunidad frente a las infecciones. Pantalla 5

P-230

TH SUBSETS PLAY A KEY ROLE IN THE PROGNOSIS OF COVID-19 PROGRESSION

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Background

The coordination of the immune response by T-helper subsets is determinant in the evolution of infectious diseases. This fact is well known in HIV or Mycobacterium leprae/tuberculosis. This work has been aimed to evaluate the T-helper responses in the early infection in COVID-19 patients and to classify them according to disease evolution based on infection severity.

Methods

A randomized unicenter prospective study 184 COVID-19 patients diagnosed in the Hospital Universitario 12 de Octubre (Madrid). In the first 24 hours after diagnosis, T-helper subsets and the serum cytokine profile were evaluated by flow cytometry and Luminex, respectively and were followed-up until discharge/death. Patients were classified according the severity of the disease during the follow-up: 1) Asymptomatic 2) Symptomatic without hospital requirements 4) Hospitalized with mild-moderate disease 4) Severe patients who developed ADRS.

Results

COVID-19 patients showed lymphopenia, including proportions of Th1, Th2 and Th17 than healthy controls. Hospitalized patients (groups 3 and 4) presented a marked proinflammatory cytokine prolife related to their non-hospitalized counterparts (groups 1 and 2). Non-hospitalized patients showed a potent and robust Th1 immune response, whereas hospitalized patients, especially the severe ones exhibited hyper activated Th2 and Th17 responses. The multivariable analysis of every parameter associated with bad prognosis showed that early Th1 immunity behaved as an independent protective factor for the development of severe forms of the disease (OR: 0,11, IC 95%: 0,02-0,6, AUC: 0,874). The evaluation of the subsets showed that Th1 immunity is necessary to control in an efficient manner the infection, even avoiding the appearance of symptoms.

Conclusions

A predominant Th1 immunity in the acute phase of COVID-19 could be used as a tool to



identify patients with low risk for unfavorable prognosis. This finding is important for designing prophylactic and therapeutic strategies in which cellular immunity should be enhanced against antibody-based immunity.



P-231

A MACHINE LEARNING MODEL TO ASSOCIATE CELLULAR SUBSETS AND CLINICAL MARKERS WITH COVID-19 SEVERITY IN INFECTED INDIVIDUALS ATTENDING THEIR INGRESSION INTO THE EMERGENCY SERVICE

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SARS-CoV-2 was a novel coronavirus emerging in December 2019, responsible of the coronavirus disease 19 (COVID-19). Since December 2019, more than 511 million infection cases and 6.24 million deaths have been reported globally, the more significant pandemic in recent history, saturating healthcare systems worldwide. Because of its broad symptoms leading to organs' failure and lack of knowledge regarding the infection curse of this novel coronavirus, most patients went to the emergency services. Therefore, there is a need to stratify COVID-19 patients who need hospitalization or be treated in the intensive care unit. For that purpose, we performed a 4-flow cytometry panel distinguishing a high spectrum of T-, B- and innate cell subsets in 75 individuals on the day of their ingression into the emergency service. At the same time, their demographic and clinical characteristics were collected. Then, they were classified a posteriori for their infection severity, according to the WHO Severity Score. We developed a machine learning model that allowed us to automatically predict the Severity Score of each patient with a blood sample on their first day at the emergency service. Besides, this mathematical model allowed us to identify some immune populations highly correlated to the severity; for example, the percentage of regulatory T cells expressing the CD38 marker had a positive correlation regarding the severity, whereas the absolute number of plasmacytoid dendritic cells, NK 16^+ 56^- and NK 16^+ 56^- HLA-DR^+ had a negative correlation. Therefore, this model allows us to predict the severity of the infection in individuals at the beginning of the SARS-CoV-2 infection regarding specific immune populations and clinical markers. Those results could allow us to identify individuals who will have more severe COVID- to better optimize healthcare resources.



P-232

PROFILAXIS INFECCIOSA CON VACUNAS POLIBACTERIANAS SUBLINGUALES EN PACIENTES CON ENFERMEDADES AUTOINMUNES SISTÉMICAS E IMUNOSUPRESIÓN FARMACOLÓGICA ACTIVA

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Las infecciones en pacientes con enfermedades reumáticas autoinmunes sistémicas (EAs), se relacionan con disfunción inmunológica, actividad de la enfermedad e inmunosupresión.

Los fármacos modificadores de la enfermedad (FAMEs) sintéticos y biológicos, han mejorado el pronóstico estas. Se ha evidenciado que las vacunas polibacterianas sublinguales disminuyen la tasa de infecciones recurrentes del tracto urinario (ITU) y del tracto respiratorio (ITR), mejorando la morbimortalidad.

Se realizó un estudio observacional de pacientes con ERAs que habían recibido dichas vacunas entre 2014 – 2018. A partir de 2018 y hasta 2021, se analizó la incidencia de ITU e ITR y se comparó con incidencia anual previa a la administración y tras el año postvacuna.

Se observó un aumento del número de infecciones entre los años 2018 a 2021, comparado con el año postvacuna, para ITU (1,53±2,17 vs 0,63±1,13; p=0,005) y para ITR (1,63±2,32 vs 0,67±0,92; p=0,003).

Al categorizar por número de infecciones, observamos que a pesar del aumento del número medio de ITU e ITR, aproximadamente la mitad de los pacientes no tuvieron infección en el periodo 2018-2021 (51,2% ITU y 43,5% ITR).

La comparación con el año previo a la vacuna, a pesar del aumento del número de infecciones durante el periodo 2018-2021 respecto al postvacunal, el número de episodios de ITR seguía siendo menor (1,61±2,26 vs 2,76± 2,57; p= 0,002), al igual que el número de ITU (1,56±2,12 vs 2,69±3,07; p= 0,010).

El efecto de las vacunas polibacteranas sublinguales se mantuvo hasta 3 años, con disminución significativa de infecciones con respecto al año previo a la vacuna, lo que sugiere que estas vacunas podrían tener beneficio a largo plazo. El número de infecciones respiratorias y urinarias aumentó durante los años que no estuvo disponible la administración de la vacuna. Sin embargo, se observó ausencia de infecciones en la mitad de los pacientes.



P-233

IL11 AND WNT5A DYSREGULATION SIGNALING BY SARS-COV-2 ACCESSORY PROTEINS ARE INVOLVED IN INFLAMMATORY AND PRO-FIBROTIC DISEASES

Dies López-Ayllón, Blanca[1]; García García, Tránsito[2]; Mendoza, Laura[1]; De Lucas Rius, Ana[1]; Fernández Rodríguez, Raúl[2]; Redondo, Natalia[1]; Pedrucci, Federica[1]; Zaldivar López, Sara[2]; Jiménez Marín, Ángeles[2]; Garrido, Juan J[2]; Montoya González, María[1].

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SARS-CoV-2, the cause of the COVID-19 pandemic, possesses eleven accessory proteins encoded in its genome. Their roles during infection are still not completely understood and several of them have been mutating into the different variants of the virus. WNT5A dysregulation signaling has been implicated in the development of various pathological conditions in humans such as inflammation and fibrosis. Interleukin-6 (IL6) family members induce WNT5A expression in various cell types, highlighting a critical role for WNT5A in immune responses. Expression of Interleukin-11 (IL11), a member of IL6 cytokine family, correlates with the extent of fibrosis and its signaling induced fibroblast activation via TGFβ. In this study, A549 were transduced with lentivirus expressing individual viral accessory proteins ORF6, ORF8, ORF9b or ORF9c from SARS-CoV-2 (Wuhan-Hu-1 isolate) and their interaction with cellular responses were analyzed. Firstly, transcriptomic analysis revealed that both WNT5A and IL11 were significantly up-regulated in all transduced cells. Some IL11 signaling-related genes, such as STAT3 or TGFβ, were differentially expressed. IPA software analysis showed that both WNT5A and IL11 were involved in pulmonary fibrosis idiopathic disease. Subsequently, bioinformatics and functional assays revealed that these four accessory proteins were implicated in both inflammatory and fibrotic responses. While overexpression of ORF8 and ORF9c appear to trigger a STAT3-dependent cellular response mediated by IL11, ORF6 and ORF9b seem to provoke a cell profibrotic response mediated by TGFb through WNT5A. Our results suggest that ORF6, ORF8, ORF9b and ORF9c could be involved in inflammatory and fibrotic responses in SARS-CoV-2 infection. Thus, these accessory proteins could be targeted by new therapies for COVID-19 disease.



P-234

DINÁMICA DE LA RESPUESTA INMUNE ADAPTATIVA EN LA INFECCIÓN NATURAL POR SARS-COV-2

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[1]Instituto de Investigación Hospital 12 de Octubre, Madrid; [2]Hospital 12 de Octubre, Madrid; [3]Icahn School of Medicine at Mount Sinai, Nueva York; [4]Centro de Biologia Molecular Severo Ochoa, Consejo Superior de Investigaciones Cientificas (CSIC), Madrid.

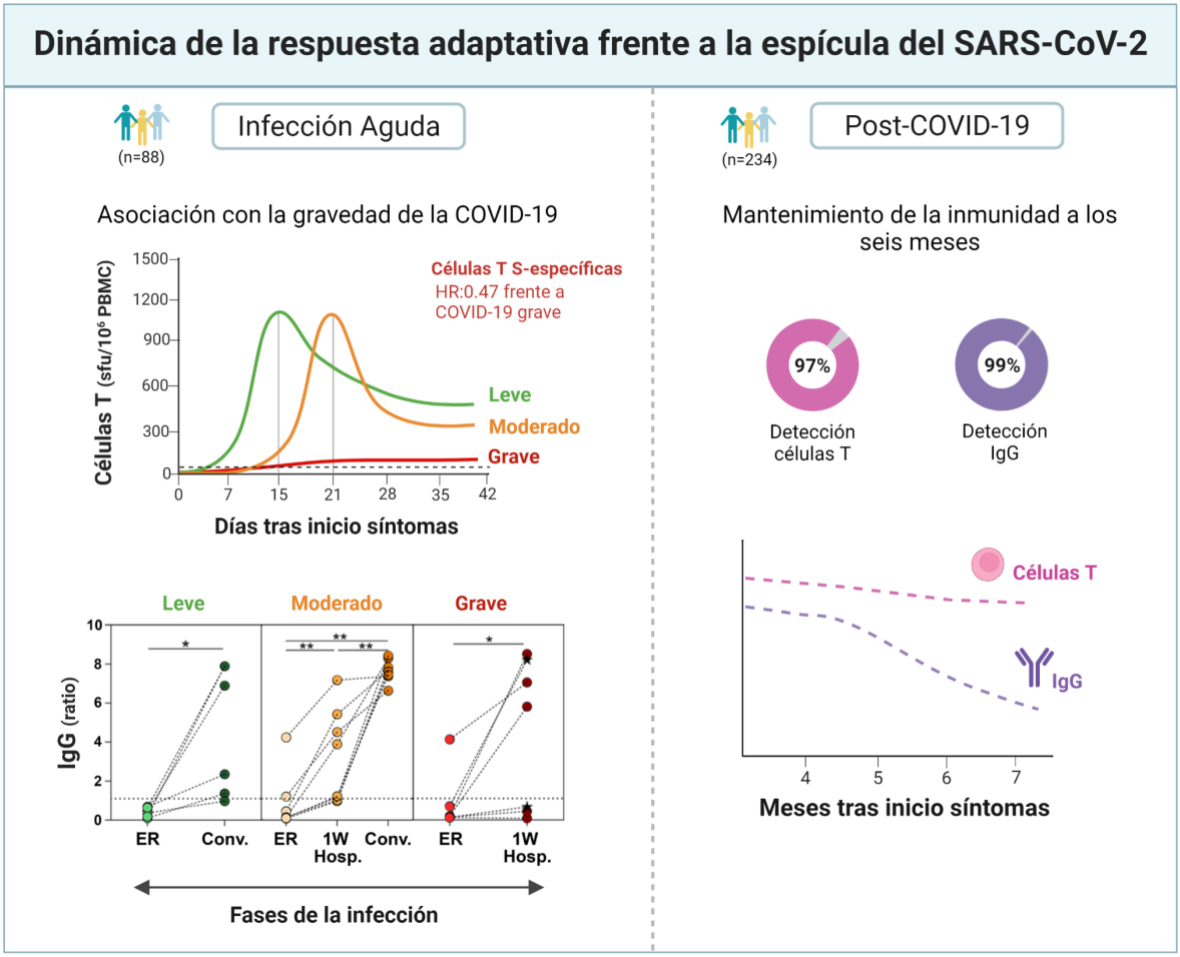
El objetivo del estudio fue comprender cómo se desarrolla la respuesta inmune adaptativa en la infección natural por SARS-CoV-2, su relación con los diferentes niveles de gravedad y su mantenimiento a largo plazo.

Se analizó longitudinalmente la respuesta celular (IFN-γ y/o IL-2) mediante FluoroSpot (*spot forming unit* (sfu)/10^6 PBMC) frente a la espícula (S), membrana y nucleocápside del SARS-CoV-2 y la respuesta de anticuerpos anti-S y su actividad neutralizante en dos cohortes: 88 pacientes con infección aguda clasificados en leves (seguimiento ambulatorio, n=32), moderados (ingreso corto sin complicaciones, n=34) y graves (UCI, ventilación mecánica y/o exitus, n=22); y 234 pacientes recuperados de COVID-19.

La enfermedad leve se caracterizó por el rápido desarrollo de células T altamente funcionales, alcanzando su pico 15 días tras el inicio de síntomas (DPSO), seguido de una coordinada respuesta humoral. Los pacientes moderados iniciaron más tempranamente la producción de anticuerpos y alcanzaron la máxima respuesta T aproximadamente 21DPSO. Los pacientes graves, sin embargo, no mostraron células T específicas dentro de los 21DPSO y produjeron altos niveles de anticuerpos desde el principio. En un análisis multivariante, la respuesta celular específica para S1 medida en urgencias permaneció como factor protector frente a COVID-19 grave, independientemente del sexo y la edad (OR por incremento de 100 IFN-γ-sfu/10^6 PBMC: 0.47, $p<0.05$).

Entre los pacientes recuperados, el 97% y el 99% mantenían, respectivamente, respuesta celular (IL-2 predominante) y humoral 4-7 meses tras la infección, observándose una disminución progresiva únicamente de anticuerpos y actividad neutralizante.

Estos resultados sugieren que los pacientes sin una pronta respuesta T progresan a formas graves de COVID-19, mientras que una respuesta celular temprana y coordinada con la producción de anticuerpos contiene la infección y favorece cuadros leves. La medición de ambas ramas de la inmunidad adaptativa proporciona información sobre el pronóstico y la protección frente a COVID-19.





P-235

SARS-COV-2 SEROPREVALENCE AND HAEMATOLOGICAL FEATURES IN BLOOD DONORS

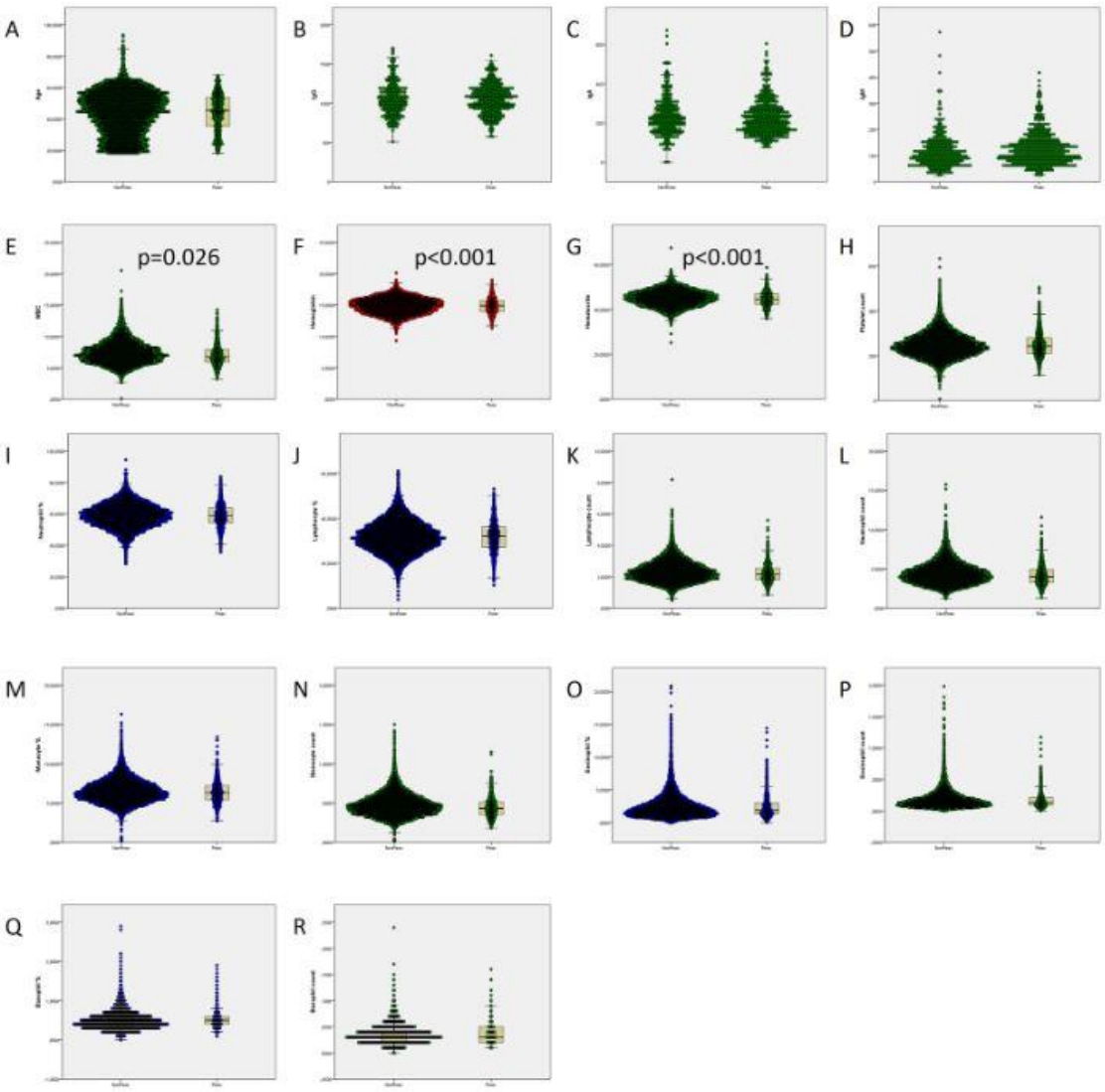
Martín, M Carmen; Gonzalez, M Isabel; Holgado, Nuria; Jimenez, Ana; Ortega, Nuria; Parrado, Alba; Page, Isabel; Perez, Maria; Blanco-Peris, Lydia.

Centro de Hemoterapia y Hemodonación de Castilla y León, Valladolid.

Background: The vast majority of COVID-19 cases both symptomatic and asymptomatic develop immunity after COVID-19 contagion. Whether lasting differences exist between infection and vaccination boosted immunity is yet to be known. The aim of this study was to determine how long total anti-SARS-CoV2 antibodies due to past infection persist in peripheral blood and whether sex, age or haematological features can influence their lasting.

Material and Methods: A series of 2421 donations either of SARS-CoV-2 convalescent plasma or whole blood from 1107 repeat donors from January 2020 to March 2021 was analysed. An automated chemiluminescence immunoassay for total antibodies recognizing the nucleocapsid protein of SARS-CoV-2 in human serum and plasma was performed. Sex, age, blood group, blood cell counts and percentages and immunoglobulin concentrations were extracted from electronic recordings. Blood donation is allowed after a minimum of one-month post symptom's relapse. Donors were 69.7% males and their average age was 46. The 250 donors who had later donations after a positive one underwent further analysis. Both qualitative (positivity) and quantitative (rise or decline of optical density regarding consecutive donations) outcomes were evaluated.

Results and discussion: In 97.6% of donors with follow-up, anti-SARS-CoV-2 protein N total antibodies remained positive at the end of a follow-up period of 12.4 weeks median time (1-46, SD=9.65) after the first positive determination. The blood group was not related to antibody waning. Lower lymphocyte counts and higher neutrophils would help predict future waning or decay of antibodies. Most recovered donors maintain their total anti-SARS-CoV-2 N protein antibodies for at least 16 weeks (at least one month must have been awaited from infection resolution to blood donation). The 10 individuals that could be followed up longer than 40 weeks (approximately 44 weeks after symptom's relapse) were all still positive.


A
B
C
D
E
p=0.026
F
p<0.001
G
p<0.001
H
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P-236

SINGLE-CELL PROFILING OF SUCCESSFULLY-TREATED HIV-INFECTED SUBJECTS WITH INVERTED CD4/CD8 RATIO

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Most HIV-infected subjects fail to normalize the CD4/CD8 ratio after successful antiretroviral therapy (ART), showing higher risk of clinical progression and death. We characterized the immune profile of chronic HIV-infected subjects according to their CD4/CD8 ratio.

We analysed samples from 25 HIV-infected patients achieving 500 CD4 T-cell counts after 3 years under ART (RIS-BioBank), grouped according to their CD4/CD8 ratio into LOW (<0.8) and HIGH (>1.2) groups. PBMCs were deeply phenotyped using Maxpar Direct Immune Profiling Assay (Fluidigm) by mass cytometry (CyTOF). Dimensionality reduction (tSNE) analysis was performed in FCS Express 7 and statistical analysis was performed using Mann-Whitney U test (SPSS).

A total of 100,000 cells and 32 markers were used to generate a representative t-SNE map of the immune system of HIV-infected patients with LOW (N=13) and HIGH (N=12) CD4/CD8 ratio (Figure 1). No differences were found regarding CD4 T-cells maturational subsets distribution, but a trend to higher Th1 frequency was observed in the LOW group (5.0[3.8-5.4] *vs*. 3.8[3.2-4.6], p=0.079). Moreover, the LOW group showed lower frequency of naïve CD8 T-cells (41.0[28.8-50.5] *vs*. 55.3[46.0-77.0], p=0.014) while a higher frequency of the effector memory phenotype (6.5[3.2-10.8] *vs*. 3.0[1.5-9.4], p=0.055). Interestingly, higher frequencies of dendritic cells (DCs) (15.2[10.2-18.4] *vs*. 10.3[8.0-13.3], p=0.026), myeloid-derived suppressor cells (MDSCs) (41.2[25.9-53.0] *vs*. 32.3[16.7-42.8], p=0.042) and $CD4^+CD8^+$ double positive cells (2.2[1.9-3.1] *vs*. 1.5[0.7-2.1], p=0.007), as well as a trend of higher frequencies of mucosal associated invariant T/invariant NKT (MAIT/iNKT) lymphocytes (1.2[1.0-2.2] *vs*. 0.7[0.5-2.2], p=0.074) were found in the LOW group. Total monocytes, B and NK lymphocytes and their maturational subsets showed no differences between groups.

Our results suggest that the distribution of certain populations is altered in HIV-infected subjects keeping an inverted CD4/CD8 ratio despite successful ART. This feature could be contributing to their immune dysfunction and clinical progression.

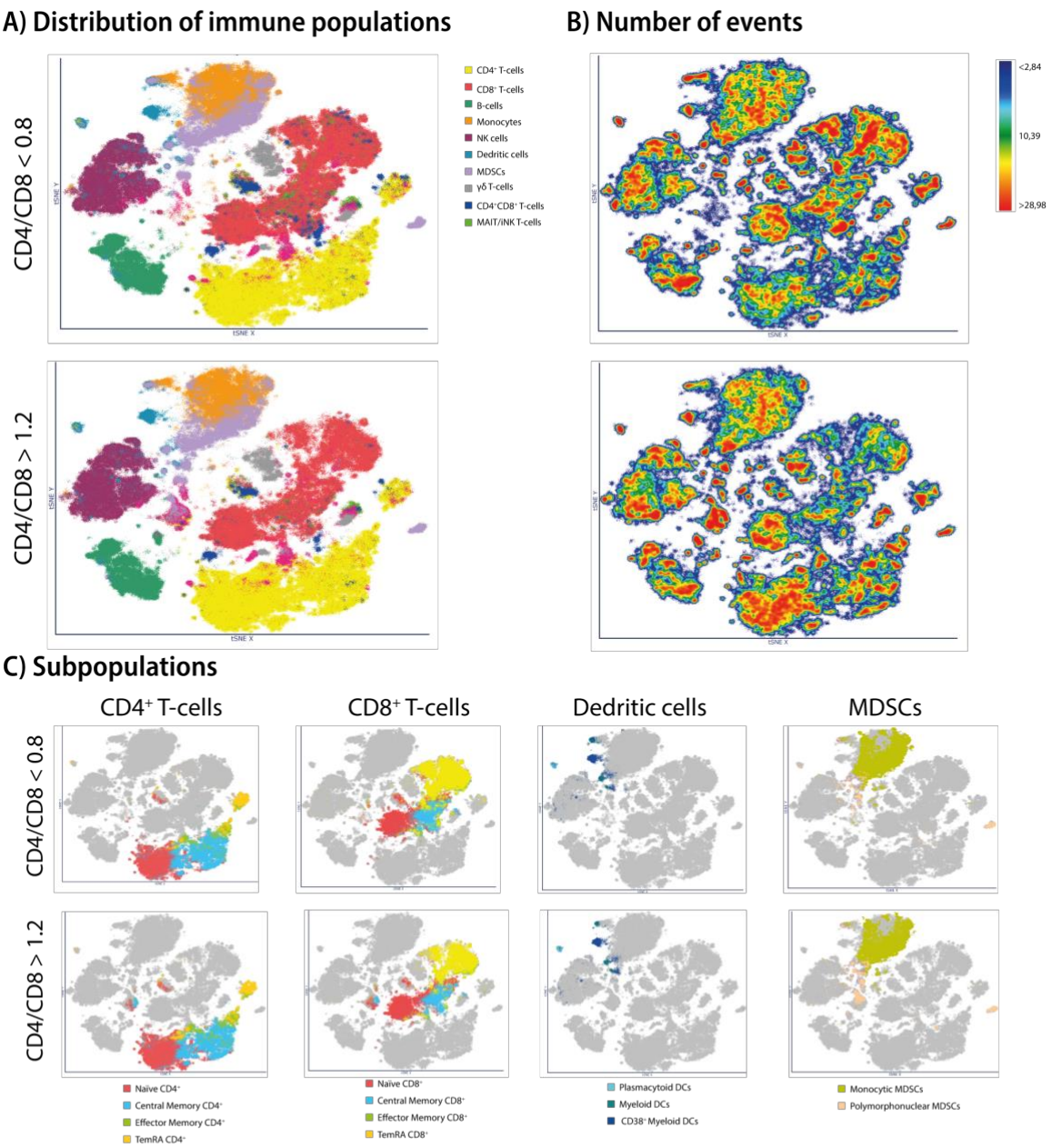


Figure 1. (A) Distribution of the main immune cell populations in HIV-infected patients acording to their CD4/CD8 ratio; (B) Number of events that integrate each population; (C) Maturational subsets of cell types found signficantly different between groups. Abbreviations: NK, Natural Killers; MDSCs, Myeloid-derived supressor cells; DCs, dendritic cells.

P-237

DIFFERENTIAL PATTERN OF IFNΓ AND IL-2 SECRETION BY T-CELLS IN CHRONIC HIV-INFECTED SUBJECTS KEEPING INVERTED CD4/CD8 RATIO

Garrido-Rodríguez, Vanesa[1]; Olivas-Martínez, Israel[1]; Bulnes-Ramos, Ángel[1]; Pozo-Balado, María Del Mar[1]; Viladés, Consuelo[2]; Rull, Anna[3]; Vidal, Francesc[3]; Pacheco, Yolanda[1].

[1]Laboratorio de Inmunología, Instituto de Biomedicina de Sevilla (IBiS), Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Sevilla; [2]Hospital Universitari Joan XXIII, IISPV, Universitat Rovira i Virgili, Sevilla; [3]Hospital Universitari Joan XXIII, IISPV, Universitat Rovira i Virgili, Tarragona.

Most HIV-infected subjects fail to normalize the CD4/CD8 ratio after successful antiretroviral therapy (ART), showing higher risk of clinical progression and death. We explored antigen-specific T-cell responses in patients depending on the CD4/CD8 ratio.

Samples from patients achieving 500 CD4 T-cell counts after 3 years under ART (RIS-BioBank) were classified according to their CD4/CD8 ratio into LOW (<0.8; N=18) and HIGH (>1.2; N=22) groups. PBMCs were stimulated by CEFTA (CD4-specific), CEF (CD8-specific) and Gag (HIV-specific) peptide pools by ELISPOT assay. Positive response was considered when Spots Forming Units (SFUs)>50 after subtracting SFUs from the negative control (unstimulated PBMCs). Comparisons between pie charts were made using a Permutation test (SPICE), while those between SFUs were made using a U Mann-Whitney's test (SPSS).

The frequencies of patients responding to one or several stimuli in both IFNγ and IL-2 secretion were different between study groups (p<0.0001; Figure). The proportion of patients responding to 3 stimuli was higher in LOW regarding both, IFNγ (56% *vs*. 43%) and IL-2 (50% *vs*. 27%) secretion. Interestingly, none of the LOW patients, but 14% of the HIGH, were absolute non-responders regarding IFNγ. Regarding individual stimulus, the simultaneous secretion of IFNγ+IL2 was similar between groups. Among responders, no differences were observed regarding IFNγ secretion in response to any stimuli, however, a trend to lower IL-2 production was found in LOW patients after CEF stimulation (148[94-810] *vs.* 1004[220-1640] SFUs, p=0.083). Regarding the total cytokine secretion per well, no differences were found in the spot volume of IFNγ, although a significant lower IL-2 spot volume was found in LOW patients after CEFTA stimulation (30144[10594-44419] *vs.* 84223[15266-127141], p=0.028).

Our results show a differential pattern of T-cell cytokine secretion in chronic patients maintaining an inverted CD4/CD8 ratio despite successful ART, which could be reflecting an immune dysfunction and ultimately, affecting their clinical progression.

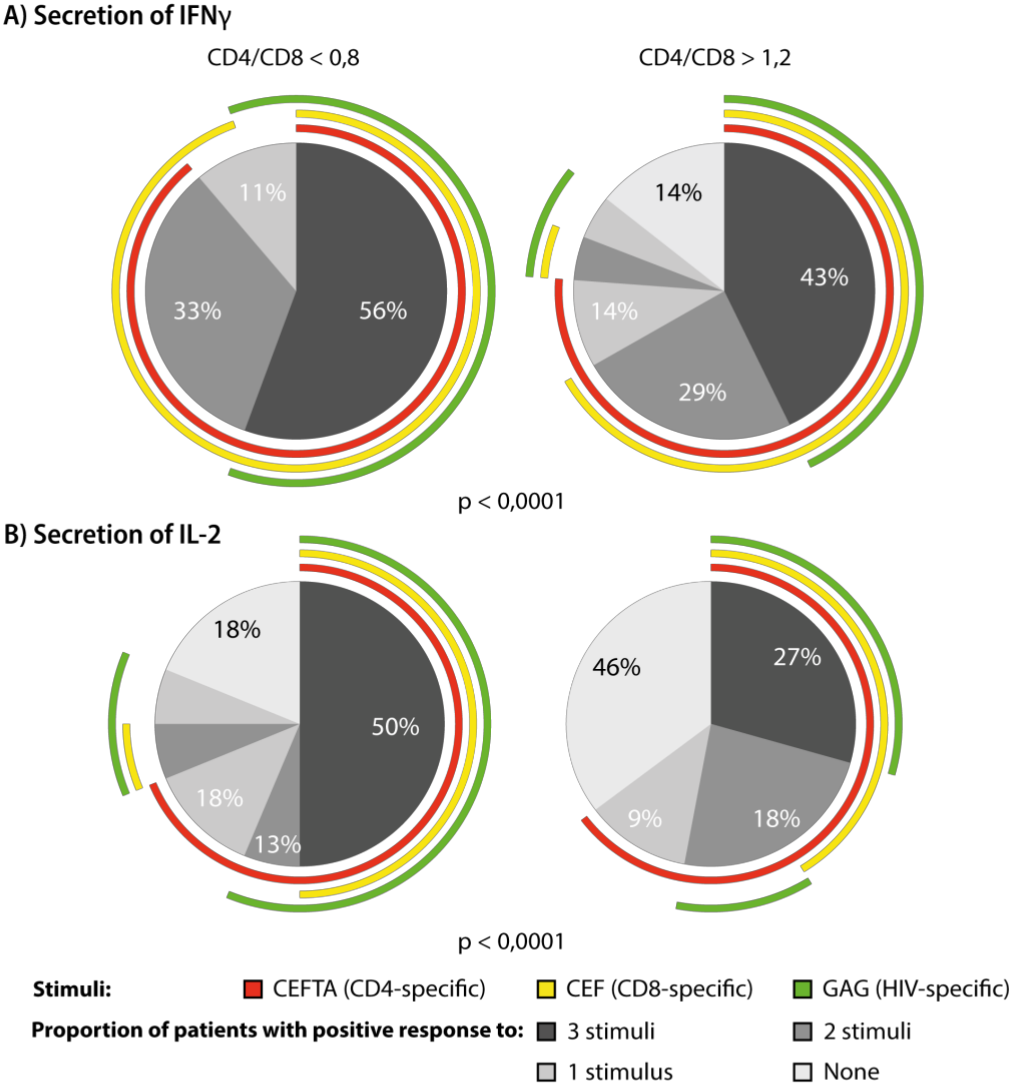


Figure. Proportion of patients secreting IFNγ (A) or IL-2 (B) in response to the pools of peptides CEFTA (CD4-specific), CEF (CD8-specific) or Gag (HIV-specific). A response is considered positive when the number of spot forming units (SFU) is above 50 after subtracting the number of SFU from the negative control (unstimulated). Pie charts represent the proportion of patients that respond to 0, 1, 2 or 3 stimuli, while arcs represent the specific stimulus. Abbreviations: IFNγ, interferon-γ ; IL2, interleukin-2.



P-238

INDIVIDUALS WITH POST-COVID SYNDROME SHOW REDUCED ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY AND EBV REACTIVATION, REGARDLESS OF WHETHER THEY WERE HOSPITALIZED OR NOT DURING COVID-19

Galán, Miguel[1]; Vigón, Lorena[1]; Nasarre-De-Letosa, Laura[1]; García-Pérez, Javier[2]; Mateos, Elena[1]; Aránzazu Murciano-Antón, María[3]; Torres, Montserrat[1]; Rodríguez-Mora, Sara[1]; Domínguez-Mateos, Susana[3]; Alcamí, José[2]; López-Huertas, María Rosa[1]; Pérez-Olmeda, Mayte[4]; Coiras, Mayte[1].

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Introduction: About 10% of individuals with SARS-CoV-2 infection suffer from Post-COVID syndrome. It is currently unknown why some individuals recover completely from COVID-19 whereas others not. The main objective of our study was to evaluate the effectiveness of the humoral immune response in individuals with Post-COVID syndrome, in comparison with individuals who completely recovered after COVID-19.

Methods: Individuals with Post-COVID were subdivided according to the COVID-19 severity in non-hospitalized (NH-PostCOVID, n=30) and hospitalized (H-PostCOVID, n=9). 20 individuals recovered completely for mild COVID-19 (Recovered) were also recruited as controls. PBMCs B cell subpopulations were analyzed by flow cytometry. Anti-SARS-CoV-2 IgG titers were analysed by ELISA and their neutralizing capability was measured using pseudotyped SARS-CoV-2. ADCC was analysed using rituximab-coated Raji cells as target. Herpesvirus reactivation (EBV, CMV) was analysed in blood by qPCR.

Results: 1) 86.6%, 88.9% and 55.50% participants were female in NH-PostCOVID, H-PostCOVID, and Recovered cohorts, respectively. Median age at diagnosis was 42 (IQR 37-46), 48 (IQR 44-54), and 45 years (IQR 28-57), respectively. 2) Levels of tissue-like memory B cells were reduced 3.0-fold in NH-PostCOVID and 5.5-fold in H-PostCOVID groups, in comparison with Recovered. 3) No significant differences were found in anti-SARS-CoV-2 IgG titers and their neutralizing capacity between all groups. 4) ADCC was decreased 1.6-fold in NH-PostCOVID, in comparison with Recovered. 5) reactivation of CMV was not detected in any participant, but EBV was reactivated in 33.3% of NH-PostCOVID participants and 44.4% of H-PostCOVID participants, in comparison with 5% of Recovered individuals.

Conclusion: Individuals with Post-COVID syndrome showed normal levels of neutralizing antibodies but ADCC was impaired, regardless of whether they were or not hospitalized during



COVID-19. This may indicate that there is a reduced immune response and an impairment in viral clearance in these individuals, which would contribute to the EBV reactivation observed in these individuals.



P-239

LA RESPUESTA INMUNE IGM FRENTE A LÍPIDOS DE LA ENVOLTURA DEL SARS-COV-2 ES FUNDAMENTAL EN LA DEFENSA FRENTE AL VIRUS.

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Antecedentes: la pandemia de Covid-19 ha supuesto una crisis de salud mundial. La progresión de la enfermedad es heterogénea, algunos de los pacientes son asintomáticos, otros desarrollan formas leves o graves. Esta última está relacionada con el trastorno metabólico de los lípidos y la coagulopatía intravascular diseminada, asociada a la presencia de anticuerpos contra los lípidos (aPL).

Nuestro **objetivo** fue establecer la relación entre el perfil lipídico, la presencia de aPL y la progresión de la enfermedad.

Métodos. Se analizaron muestras de suero de pacientes con COVID-19 con buena (n=40) y mala (n=40) evolución y grupo control (n=40). El perfil de lípidos se analizó mediante UHPLC-ESI-QTOF-MS. Se identificaron más de 200 fosfolípidos y glucolípidos. Se utilizó un ensayo ELISA de alta sensibilidad desarrollado en nuestro laboratorio para analizar IgG e IgM frente a fosfatidilcolina (PC), fosfatidiletanolamina, fosfatidilinositol, fosfatidilinositol, fosfatidilserina, esfingomielina, sulfátidos y cardiolipina.

Resultados: observamos que los pacientes con Covid-19 mostraron niveles elevados de lisofosfatidilcolina en comparación con el grupo control. Por el contrario, los pacientes con Covid-19 con mala evolución mostraron niveles de fosfatidilinositol más bajos que aquellos con buena evolución.

En esta línea, los pacientes con Covid-19 de buena (0,440±0,038) y mala (0,420±0,034; $p<0,0001$) evolución tenían niveles mayores de IgM frente a fosfatidilcolina que el grupo control (0,218±0,029; $p<0,0001$). Además, los pacientes con Covid-19 de buena evolución mostraron niveles séricos de IgM frente al fosfatidilinositol superiores (0,233±0,026; $p<0,0001$) que los pacientes de mala evolución (0,128±0,026) y el grupo control (0,103±0,028; $p<0,0001$).

Conclusiones. La respuesta inmune humoral está relacionada con el perfil lipídico en Covid19. La IgM a la fosfatidilcolina y al fosfatidilinositol es una respuesta inmune principal frente a SARS-CoV-2. Son necesarios estudios futuros para definir el papel de los mismos en la fisiopatología de la enfermedad y/o como biomarcadores.



P-240

EVALUACIÓN DE LA RESPUESTA T-ESPECÍFICA FRENTE AL CITOMEGALOVIRUS POR CITOMETRÍA DE FLUJO EN LA SELECCIÓN DE POSIBLES DONANTES PARA INFUSIÓN DE LINFOCITOS

Cuesta Martín De La Cámara, Ricardo; González Torbay, Andrea; Matas Pérez, Elisabeth; Miguel Berenguel, Laura; Rodríguez Pena, Rebeca; Martínez Feíto, Ana; López Granados, Eduardo; Sánchez Zapardiel, Elena.

Hospital Universitario La Paz, Madrid.

Una de las principales complicaciones post-trasplante de progenitores hematopoyéticos (TPH) son las infecciones virales, siendo la de citomegalovirus (CMV) una de las más frecuentes. Presentamos el caso de un varón diagnosticado con 2 años de enfermedad granulomatosa crónica (variante en *NCF2*) que, a los cuatro meses de recibir un TPH alogénico de donante no emparentado (marzo 2021), sufre una ventriculoencefalitis por CMV. Recibe antivirales, inmunoglobulinas (Ig) intratecales anti-CMV e intravenosas y una única infusión con linfocitos T citotóxicos (CTLs) anti-CMV. Las cargas virales en sangre y líquido cefalorraquídeo (LCR) disminuyen; aunque, tras su aumento en LCR a los 3 meses, se decide realizar una infusión de linfocitos de donante (DLI) emparentado (padre, madre o hermano), distinta fuente de las que recibe del donante TPH (CMV-negativo).

Mediante citometría de flujo se estudió la producción intracelular de interferón-gamma (IFNg) en respuesta a CMV, estimulando *in vitro* PBMCs con péptidos específicos (CMV-PT, Miltenyi Biotec) y con Dynabeads CD3/28 (Gibco), además del control sin estímulo. Tras 6 horas de incubación, se marcó la superficie celular (7-AAD, CD45-KO, CD3-ECD, CD4-APC-AF750 y CD8-APC-AF700, BC), se permeabilizó (IntraPrep Permeabilization, BC) y se marcó intracelularmente (IFNg-PB, BC). El nivel de competencia se calculó restando el porcentaje CD3+IFNg+ del control negativo al del estímulo CMV. Cut-off >0.10% CD3+IFNg+.

Los tres familiares presentaban serología IgG-positiva para el CMV, con diferente respuesta T específica: 0,66%, 0,58% y 1,61% CD3+IFNg+ en el padre, la madre y el hermano, respectivamente. Con estos resultados se realizaron 5 DLIs del hermano. Actualmente las cargas se mantienen indetectables, con clínica estable.

Las DLIs tras evaluación de la respuesta T específica al virus constituyen una alternativa en caso de no disponibilidad de CTLs específicos de CMV que puede contribuir al manejo del tratamiento de la infección en el contexto del post-TPH.



P-241

CD64 EXPRESSION ON NEUTROPHILS AS A BIOMARKER FOR SPONTANEOUS BACTERIAL PERITONITIS

González López, Elena[1]; Renuncio García, Mónica[1]; Gutiérrez Larrañaga, María[1]; Roa Bautista, Adriel[1]; Castro Hernández, Carolina[1]; Mikhalkovich, Dzmitry[1]; Mota Pérez, Nerea[1]; Comins Boo, Alejandra[1]; Irure Ventura, Juan[1]; Odriozola, Aitor[2]; San Segundo Arribas, David[1]; Fortea, Ignacio[2]; López Hoyos, Marcos[1].

[1]Servicio de Inmunología, Hospital Universitario Marqués de Valdecilla, Santander; [2]Servicio de Digestivo, Hospital Universitario Marqués de Valdecilla, Santander.

Introduction

The innate immune system is responsible for generating the first response against foreign agents. During bacterial infections, type II interferon induces CD64 expression on neutrophils (CD64Neu). The assessment of CD64Neu by flow cytometry in both peripheral blood and other biological fluids has been validated for bacterial infections. Ascites can be caused by several complications as spontaneous bacterial peritonitis (SBP) in patients with cirrhosis. For its diagnosis, manual counting of polymorphonuclear cells in ascitic fluid is essential. The rapid identification of a possible bacterial infection in this type of patient would be useful for therapeutic intervention. We aimed to validate the expression of CD64Neu in ascitic fluid and assess its potential usefulness in the identification of SBP.

Materials and methods

The expression of CD64 on neutrophils of 41 ascitic fluid samples, recruited from November 2021 to May 2022 in the Immunology Laboratory at Marqués de Valdecilla University Hospital, was analysed by flow cytometry.

Results

A total of 8 out of 41 (13.8%) samples were diagnosed as SPB (SPB+ group). The median of CD64Neu MFI was significantly increased in SPB+ group (2838.2 MFI [1756.5-5230.0] vs 1155.3 MFI [658.8-2096.1], p= 0.048). In order to assess the risk of SPB, a binary logistic regression analysis was conducted with those parameters involved in SPB risk. The increase of CD64Neu was independently associated with SPB risk (p=0.042). The area under the curve of the model was 0.902 with 75% sensitivity and 97% specificity.

Conclusion

The CD64Neu determined by flow cytometry could help in diagnosis of SPB. The role of CD64Neu by flow cytometry in the assessment of SPB should be validated in larger studies to confirm our results.



P-242

CORRELATION OF IMMUNE CELL COUNTING BASED ON FLOW CYTOMETRY IN ASCITIC FLUID SAMPLES WITH THE GOLD STANDARD FOR THE DIAGNOSIS OF SPONTANEOUS BACTERIAL PERITONITIS.

Renuncio-García, Mónica[1]; González-López, Elena[1]; Gutiérrez-Larrañaga, María[1]; Roa-Bautista, Adriel[1]; Castro-Hernández, Carolina[1]; Mikhalkovich, Dzmitry[1]; Mota-Pérez, Nerea[1]; Comins-Boo, Alejandra[1]; Irure-Ventura, Juan[1]; Odriozola, Aitor[2]; San Segundo, David[1]; Fortea, José Ignacio[2]; López Hoyos, Marcos[1].

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Introduction

Spontaneous bacterial peritonitis (SBP) is defined as the infection of ascitic fluid without the existence of a secondary intra-abdominal infectious focus. A polymorphonuclear count (PMN) ≥ 250/uL confirms the diagnosis irrespective of a positive culture result. The gold standard (GS) for PMN count is based on light microscopy. However, the higher sensitivity of flow cytometry (FC) opens new opportunities to clinical diagnosis. The aim was to evaluate the PMN count by flow cytometry and the potential role for clinical purposes.

Methods

The study includes 154 ascitic fluids from 94 patients with ascites of any etiology. The PMN cell count was performed both with an optical microscope in a Neubauer chamber and by FC. Final SBP diagnosis was assigned based on clinical records. For clinical assessment, those ascitic fluids of the same hospital income were excluded.

Results

A high-positive correlation between both methods was observed (r=0.939). Different cut-off points were calculated for SBP diagnosis and finally, 4 different groups were defined: 2 with concordant results: both Negative (NN) (n=81) or Positive (PP) (n=7) results and 2 with discordant results: GS Positive and FC Negative (n=1) and GS Negative and FC Positive (n=11) (NP group) (Figure1). The median (IQR) of hospital stay was significantly increased in PP compared with NN group (p=0.011) 11(10-20) vs 9 (6-13) whereas in NP group the median was 9 (7-14).

Conclusion

The PMN cell count in ascitic fluid by FC is highly correlated with the GS method based in optical microscopy. The higher sensitivity of FC could allow the identification of intermediate

clinical phenotypes with longer hospital stay.

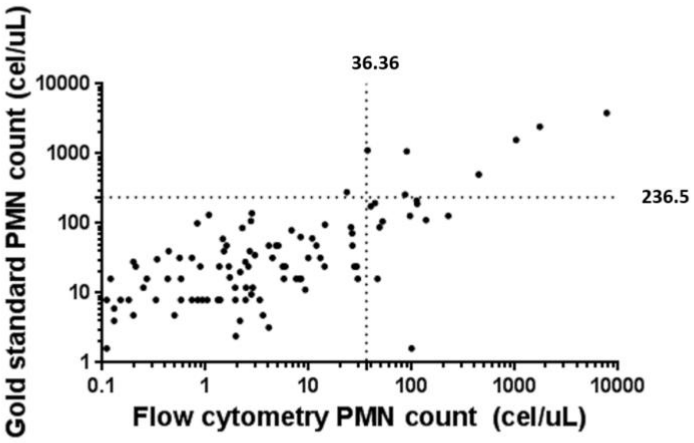


Figure 1. Correlation between gold standard PMN count and flow cytometry PMN count of ascitic fluids.



P-244

EFFECT OF LATENT CMV INFECTION AND AGING ON CD4 AND CD8 T-CELL FUNCTIONALITY

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Memory T cells undergo replicative senescence upon prolonged proliferation by chronic antigen stimulation. In humans, senescent T cells are marked in part by loss of CD28 expression and acquired expression of CD57. However, these senescent T cells can still produce large quantities of pro-inflammatory cytokines and cytotoxic mediators. CMV infection also induces the expansion of CD57+ T-cells, but whether this has a beneficial, detrimental, or neutral role for immunity is still debated. The increased functionality of CD57+ T-cells can be considered useful, but a growing body of evidence suggests that these cells may have pathogenic potential in conditions like chronic kidney rejection, cancer, atherosclerosis, and myocardial infarction, underscoring the detrimental roles of these cells in various chronic inflammatory responses. We have demonstrated that CMV-seropositivity is associated with the expansion of polyfunctional CD57+ T-cells in young and middle-aged individuals in response to different stimuli. Here, we expand our results on the effects of age and CMV infection on T-cell functionality in a cohort of healthy middle-aged and older individuals stratified by CMV serostatus. Specifically, we studied the polyfunctional responses (degranulation, IFN-γ and TNF-α production) of CD4+ and CD8+ T-cells according to CD57 expression in response to Staphylococcal Enterotoxin B (SEB). Results showed that CD57+ T-cells were mainly expanded in CMV-seropositive individuals and clearly demonstrate that in the absence of CMV, age has no effect on the size or polyfunctionality of both the CD4 and CD8 T-cell responses to SEB. The study shows that CD57+ T-cells are more polyfunctional than CD57− T-cells in any age group. Our results also show that CMV, but not aging, has a significant role in the expansion CD57+ T-cells. This data could be of clinical importance because CMV infection can be treated, and the expansion of such cytotoxic proinflammatory cells could potentially be prevented.



P-245

SPECIFIC CELLULAR RESPONSE AGAINST THREE DIFFERENT MODELS OF VIRAL ANTIGENS IN ELDERLY IMMUNOCOMPETENT INDIVIDUALS

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T-lymphocytes have a crucial role controlling viral infections. CMV chronic infection, seasonal H1N1 influenza and the novel SARS-COV2 infection represent three distinct models of inducing viral immune memory. The aim of this work was to characterise the specific cellular responses against these three different viruses in 59 individuals aged over 60 years. They had overcome SARS-CoV-2 infection asymptomatically or with very mild symptoms. We characterized T subpopulations by flow cytometry. Specific memory T-lymphocytes against these three viruses were measured by IFN-ɣ released by ELISpot. Responses to chronic CMV infection among CMV seropositive individuals showed negative correlation with naïve CD8+ and with naïve and the less differentiated effector memory EM1 CD4+ T-lymphocytes. Positive correlation was only observed with the highly differentiated subpopulation EMRA CD4+ ($p.value<0.05$). On the contrary, Influenza responses among individuals recently vaccinated, but with probable numerous encounters with the virus throughout life, correlated negatively with highly differentiated EM3 CD4+ and EMRA CD8+ and positively with EM1 CD4+ ($p.value<0.05$). Similarly, responses against the new and unique infection with SARS-COV-2 showed negative correlation with EMRA CD8+ and positive correlation with naïve CD8+ ($p.value<0.05$). In fact, the ability to develop specific cellular responses against influenza and SARS-CoV2 in the influenza vaccinated group correlated positively. There was no significant correlation between the responses against SARS-CoV-2 and CMV virus although SARS-CoV2 responses were lower in CMV seropositive than in CMV seronegative patients. Our results suggest that specific cellular response against novel pathogens resembles the memory response enhanced by repeated but not chronic virus encounters. Both may be favoured by a more naïve T-lymphocyte phenotype comparing with the immunosenescence induced by chronic CMV infection. Moreover, subpopulation distribution and antigen-specificity of T-lymphocyte subpopulations could be good biomarkers of the immunocompetent status in elderly individuals, reflecting their ability to generate specific memory responses against new pathogens.



P-246

ANALYSIS OF ANTIGEN-SPECIFIC CELLULAR AND HUMORAL IMMUNE RESPONSE IN COVID-19 PATIENTS.

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Background

The cellular and humoral immune responses are essential towards COVID-19 recovery and protection against SARS-CoV-2 reinfection. To this day, the evaluation of the immune protection to SARS-CoV-2 has mainly been focused on the detection of antibodies; However a large body of evidence suggest T lymphocytes have a greater role to resolve an established infection.

Objective

Compare the magnitude of the cellular and humoral immune response in 252 convalescent COVID-19 from mild to severe patients.

Methods

252 individuals with COVID-19 (200 mild, 20 moderate and 32 severe COVID-19) were enrolled in the study. The study inclusion criteria included individuals age ≥18 years diagnosed by SARS-CoV-2 positive real-time reverse transcriptase PCR (RT-PCR) tests from nasopharyngeal swabs. Blood samples were collected two weeks after the first RT-PCR positive test. The IgG, IgA and IgM were assessed by an in-house Luminex-based assay. The N- and S-reactive T cells were evaluated by in-house ELISpot and CoVITEST assay.

Results

SARS-CoV-2-specific T cells were identified in 100% of mild COVID-19, 95% of moderate COVID-19 and 84.3%% in severe COVID-19. On the other hand, anti-SARS-CoV-2 immunoglobulins were identified in 95% of mild COVID-19, 90% of moderate COVID-19 and



87.5% of severe COVID-19.

We observed a strong SARS-CoV-2-specific T cell responses in mild COVID individuals compared to moderate and severe COVID-19.

Conclusion

We show that the T-cell responses, unlike antibodies, are detected in practically all patients with COVID-19. Reinforcing the need of assessing cellular immunity, especially in vulnerable patients, instead of the humoral response.

P-247

CHANGES IN T-CELL DIFFERENTIATION PATTERNS AFTER ALCOHOL RELAPSE IN ALCOHOL USE DISORDER (AUD) PATIENTS

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[1]Fundació Institut d'investigació en Ciències de la Salut Germans Trias i Pujol (IGTP), Badalona; [2]Hospital Universitari Germans Trias i Pujol, Badalona.

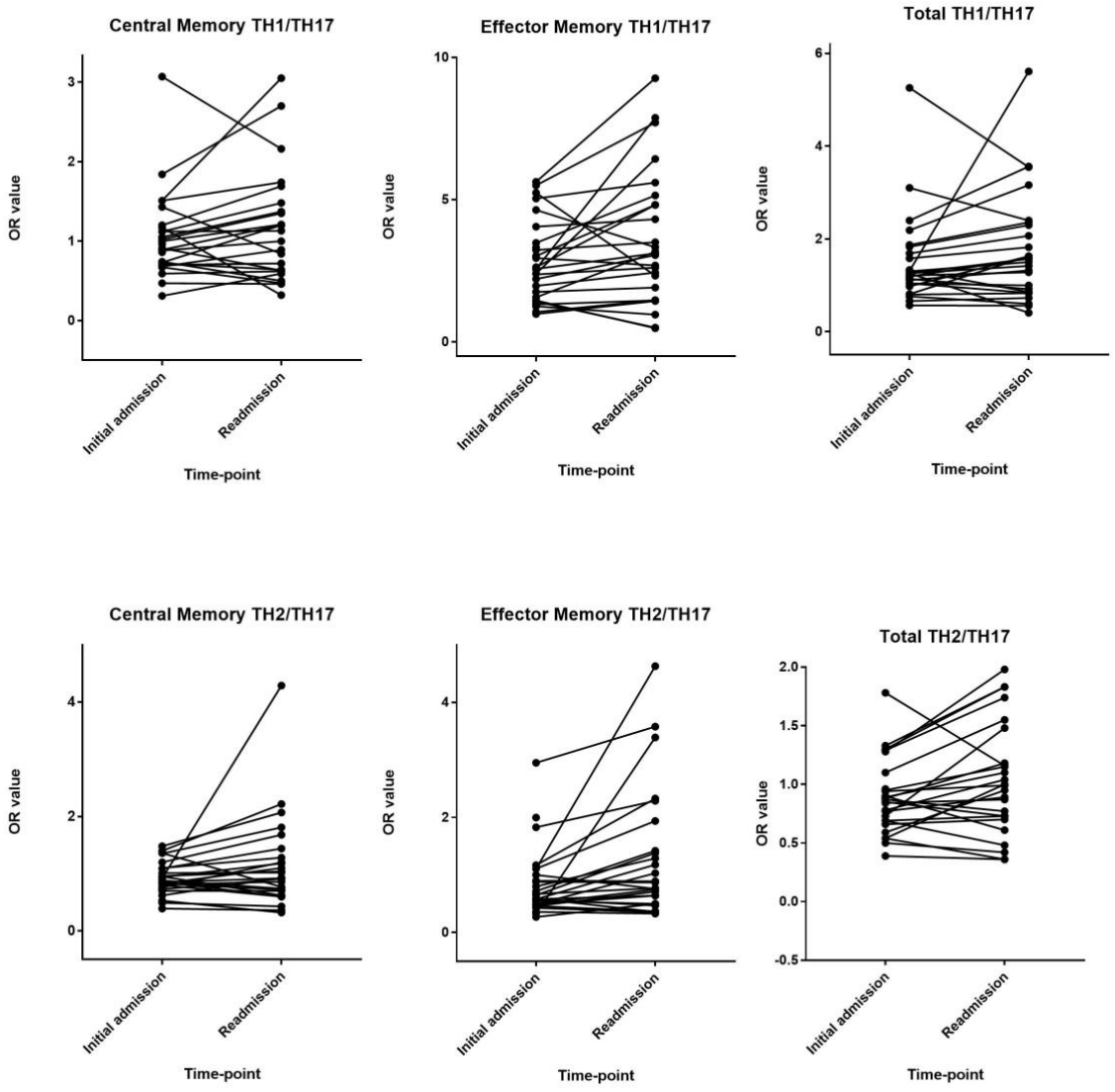
Introduction: Alcohol has a main immunosuppressive role conferring increased risk of infections or decreased vaccines effect in alcohol use disorder (AUD) patients. CD4+T-cells exert three main types of responses depending on the insult: Th1, Th2 and Th17. However, cellular differentiation is dynamic and can polarize towards a specific response. Here, we aim to evaluate the differences in $CD4^+$T-cell differentiation in AUD patients that need a second hospital admission for treatment after alcohol relapse.

Methods: Patients were initially admitted to treatment between 2013 and 2017 in Germans Trias I Pujol Hospital. Readmission occurred between 2014 and 2021. HCV-positivity, HIV-positivity and liver cirrhosis were exclusion criteria. T-cells Immunophenotyping defined four cellular differentiation profiles according to CCR7/CD45RA expression: naïve, central memory (T_{CM}), effector memory (T_{EM}) and terminally differentiated effector memory. Memory subsets were subdivided into Th1, Th2 and Th17 according to CXCR3/CCR6 expression. T-cell activation was measured according to HLA-DR/CD38 expression. Paired analyses were performed to compare these subsets between both time-points.

Results: 27 patients (46-y at first admission, 82% males) were included. AUD duration was 17,4±9 and 17,5±8 years and daily alcohol consumption was 186±112 and 157±66 gr/day at the first admission and readmission, respectively. No differences were observed regarding activation markers. However, we observed significantly increased T_{CM}Th2/T_{CM}Th17 (1,49±1,97 VS 0,9±0,28), T_{EM}Th2/T_{EM}Th17 (1,64±2,17 VS 0,83±0,59), total-Th2/total-Th17 (1,51±1,98 VS 0,88±0,31) and T_{EM}Th1/T_{EM}Th17 (4,89±5,11 VS 3,32±2,89) ratios in the readmission compared to the initial admission. We observed a tendency to increase of T_{CM}Th1/T_{CM}Th17 (1,63±2,01 VS 1,03±0,53) and total-Th1/total-Th17 (2,18±2,5 VS 1,49±0,94) ratios in the readmission compared to the initial admission. ***Fig1***

Conclusion: In AUD patients with alcohol relapse there is a change in the phenotype of CD4+T-cells observed in the increase of Th1 and Th2 phenotypes proportional to a Th17 decrease. These immune-related effects could explain the increased

susceptibility to infections of AUD patients.





P-248

POTENTIAL IMMUNOLOGICAL BIOMARKERS OF COVID-19 DISEASE PROGRESSION

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Background: Although the majority of Covid-19 disease clinical manifestations range from asymptomatic to mild respiratory infection, several patients undergo severe pneumonia requiring advanced medical support. A reliable prediction of the infection clinical course could be critical to improve outcome success by enabling an early therapy implementation in patients with high risk of progression towards severe disease and death. We aimed to find immune biomarkers capable of foreseeing the evolution of SARS-CoV-2 infection.

Material and methods. A total of 1008 patients hospitalized with SARS-CoV-2 positive PCR were included. Levels of peripheral-blood lymphocytes and serum inflammatory cytokines were measured at the moment of hospital admission and compared according to infection severity (graded 1-3 following local-guidelines), intensive care requirement and decease. Lymphocyte subsets were identified by BD Multitest™, whereas circulating IL-1β, IL-6, IL-8, IL-10, IL-12p70, TNF-α, IFN-γ, MCP-1 and IP-10 (pg/mL) were measured by Cytometry Bead Array on a BD FacsCantoII cytometer according to manufacturer instructions.

Results. In critical patients (group 3), IL-8, IL-6, IL-10 and IP-10 levels were higher ($p<0.005$), whereas T-cell populations were significantly lower ($p<0.005$) compared to patients with less severe disease (groups 1 and 2). Similarly, counts of helper and cytotoxic T-cells and NK cells were decreased in patients requiring ICU admission ($p<0.005$) and levels of IL-8, IL-6 and IL-10 were increased ($p<0.005$). Interestingly, patients displaying the triad of risk factors (age>65, T-cell lymphopenia and high-level of IL-6 and IL-8 proinflammatory cytokines) at hospital admission exhibited almost 60-times more risk of fatal outcome in contrast to patients without any risk feature ($p<0.001$).

Conclusions. T-lymphocyte populations (including CD4+ and CD8+) and levels of proinflammatory cytokines, mainly IL-6 and IL-8, measured at the moment of admission could be useful biomarkers to predict Covid-19 outcome addressing a rapid intervention to patients.



P-249

THE MIGRATORY PROFILE OF DIFFERENT IMMUNE CELLS DEFINES TWO GROUPS OF POST-COVID-19 PATIENTS

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Introduction: SARS-CoV-2 infection causes a mild to severe respiratory disease (COVID-19) in humans with a mortality rate of 2%. Clinical symptoms following resolution of the infection are known as long-COVID. The symptoms and clinical manifestations are clinically heterogeneous and suggest involvement of multi-organs/systems. The diagnostic criteria are mainly based on the narrative of the patients and general recurrent symptoms include fatigue, dyspnoea, joint pain, etc. Here, we study the immunological changes in a cohort of convalescent COVID-19 patients in order to characterize their potential relationship with persistent syndrome or acute disease severity.

Methods: We collected peripheral blood samples from patients who recovered from COVID-19, 3 months after they were discharged from hospital (n = 83). Immune phenotyping was performed using a 40-plex mass cytometry panel on PBMC, and cytokine levels were quantified in the plasma by Luminex. Results were compared to 17 pre-pandemic healthy donors and 25 post-influenza individuals.

Results: Several cytokines and immune cell subsets were altered in post-COVID patients, compared to both post-influenza individuals and pre-pandemic healthy donors, revealing a post-COVID-specific immune signature. Furthermore, immune cells of post-COVID patients showed a differential migratory phenotype. Using a logistic regression model, we were able to classify all individuals according to their diagnosis with good accuracy. Machine learning unsupervised algorithm K-means revealed four clusters of individuals when combining cytokine and mass cytometry data. Two of these clusters corresponded to post-COVID patients (figure 1), which did not correlate with post-COVID symptoms or acute severity but seemed to differ in age of individuals.

Conclusions: Our results showed immune abnormalities, both in cells and cytokines levels, in post-COVID patients three months after hospital discharge. We were able to divide post-COVID individuals into two groups through machine learning models. The immune alterations observed were independent of acute or post-syndrome symptoms.

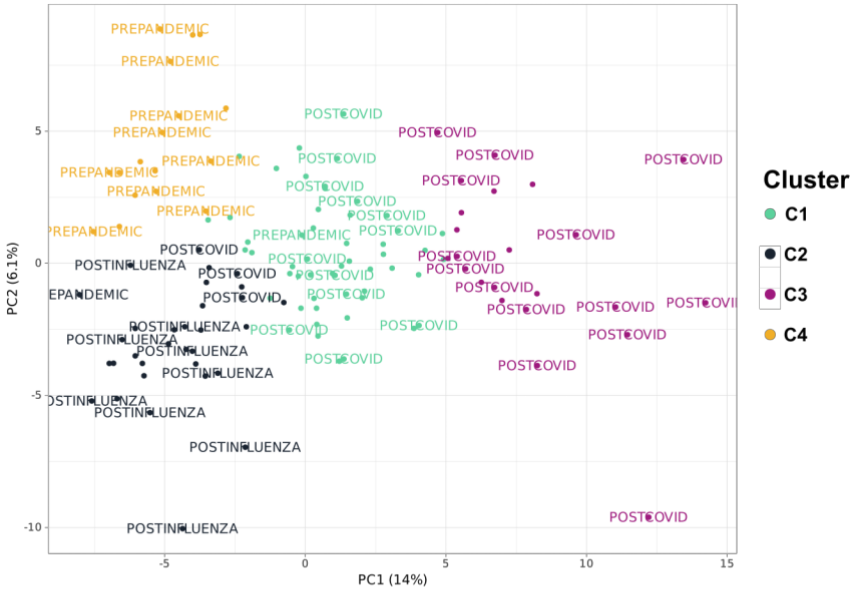


Figure 1. Individuals distributed in the dimension 1 and 2 of the Principal Component Analysis (PCA), calculated from mass cytometry and cytokine data, and color-coded according to K-means clusters. Cluster 1 (light green) consisted mainly of post-COVID patients (52 post-COVID individuals and 1 pre-pandemic healthy donor); cluster 2 (navy blue) were enriched in post-influenza patients, although it also contained 6 post-COVID patients and 1 pre-pandemic healthy donor. Cluster 3 (purple) consisted exclusively of post-COVID individuals, while cluster 4 (yellow) were mainly pre-pandemic healthy donors and 2 post-COVID patients.



P-250

SARS-COV-2 ORF7A AND ORF7B DIFFERENTIALLY CONTROL IMMUNE RESPONSES, CELLULAR METABOLISM AND CELL ADHESION

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The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of the ongoing coronavirus disease 2019 (COVID-19) pandemic that is a serious global health problem. There is an urgent need to better understand the molecular mechanisms governing SARS-CoV-2 pathogenesis, as this will have implications for the design of better treatments and vaccines. SARS-CoV-2 possesses eleven accessory proteins encoded in its genome and some of them have been involved in facilitating infection and pathogenesis by their interaction with cellular components. Among these proteins, accessory protein ORF7a and ORF7b functions have not been fully comprehended. In this study, A549 human epithelial cells were transduced to express ORF7a and ORF7b, respectively, to explore more in depth the role of each accessory protein in the pathological manifestation leading to COVID-19. Using transcriptomic combined with bioinformatic analysis and functional assays we showed that individually expressed ORF7a and ORF7b are sufficient to alter lung cells creating conditions more favorable for SARS-CoV-2. Overexpression of ORF7a or ORF7b induce alteration of metabolic cascades via UGT1A9, PTGS2 and CYP1A1; interferon responses via OASL, IFIT1 and IFIT2; inflammation via IL-8, IL-11 and CXCL1 and cell adhesion via ICAM-1, ZO-1 and gamma-catenin. Thus, in light of these results, it is plausible to speculate that ORF7a and ORF7b could be targeted by new therapies or used as future biomarkers in this pandemic.



P-251

IMMUNE CELL POPULATION AND CYTOKINE PROFILING SUGGEST AGE-DEPENDENT DIFFERENCES IN THE RESPONSE TO SARS-COV-2 INFECTION

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Introduction

Coronavirus disease 2019 (COVID-19) is an infectious disease produced by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Epidemiological studies have shown that older patients are at higher risk of developing severe COVID-19. To further understand the relation between host age-related factors, immunosenescence/exhaustion of the immune system and the response to the virus, we characterized immune cells and cytokine responses in COVID-19 patients and healthy controls (HCs) of different age ranges.

Materials and Methods

Immune cell populations were analyzed in blood samples of 58 COVID-19 patients and 40 HCs. Circulating cytokines in serum were evaluated in the same individuals and 29 extra patients. Five multicolor flow cytometry panels were used to analyze the lymphocyte populations. Cytokines were measured with the BD-CBA Human Inflammatory Cytokines Kit. A FACS Canto II flow cytometer was used.

Results

COVID-19 patients exhibited lower T lymphocytes, but increased B cells. Patients also present a higher proportion of Th2 and Th17 subtypes and a decrease in Tregs. Besides, exhausted (PD1+) cells are higher in patients than in HCs. A significant reduction in TNF and IL-1β levels in patients was reported.

The analysis per age ranges showed that the greatest number of significant differences between patients and HCs was found in the youngest age range (30-39 years). In this group, among other changes, IL-8, Th and naïve Th cells are decreased while Th2 cells are increased in COVID-19 patients. On the contrary, there are some variables, which are more affected in older patients (60-69 years) – increased IL-12, IL-10 and IL-6 and decreased total lymphocytes.

Conclusions

Our findings suggest that there are age-dependent differences in the immunological response to SARS-CoV-2 infection. Further studies are needed to know whether these differences are the



cause or consequence of the increased severity of the SARS-CoV-2 infection in older individuals.



P-252

ELDERLY PEOPLE RESPOND TO SARS-COV-2-VACCINATION BUT REQUIRE MORE IMMUNIZATIONS THAN YOUNG DONORS TO GENERATE IMMUNOLOGICAL MEMORY AND DO NOT REACH THE LEVELS ACQUIRED AFTER INFECTION

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Taking advantage of the COVID-19 pandemic and the vaccination campaign against SARS-CoV-2, we have taken this vaccination program as a model to study the development of immunological memory against new antigens.

To study the ability of elderly people to respond to neoantigens, we analysed the immunological memory of 35 elderly donors (ED; age: 75.5, IR:6) and 57 young donors (YD; age: 50, IR:34) a month after receiving the second dose RNA-based SARS-CoV-2 vaccine, and 34 elderly patients who were studied six months after their recovering from SARS-CoV-2 infection in 2020 (EP; age: 71.5, IR:16). In 25 ED we also studied the immunological memory a month after receiving the third dose of the SARS-CoV-2 vaccine.

Specific memory T-lymphocytes to SARS-CoV-2 were quantified using IFN-□-Enzyme-Linked ImmunoSpot Assay (ELISpot) after their stimulation with viral peptide pools (S1, S2, N). We found IFN-□□producing T-lymphocytes in 28/35 and 56/57 patients in ED and YD, respectively. The median number of specific IFN-□□producing T-lymphocytes was 116 (IR:340) and 252 (IR:292) cells/10^6PBMC, respectively ($p<0.05$). ED had also less specific IFN-□□producing T-lymphocytes than EP (median: 526, IR:797) ($p<0.001$).

Although no significative differences were found between the number of specific memory T-lymphocytes in ED after the third dose of the vaccine (median: 168, IR:290) and YD after the second one, levels were still lower than in EP ($p<0.01$).

We compared ED individuals with higher and lower cellular immune responses and we observed a higher percentage of naïve B-lymphocytes and higher absolute number of Th2 and follicular helper memory $CD4^+$ T-lymphocytes in those with higher cellular memory ($p<0.05$).

Our results suggest that, despite their aged immune system, elderly people can respond to SARS-CoV-2-vaccination, although they require more immunizations than young donors to generate the same level of immunological memory and their responses are lower than the ones generated after SARS-CoV-2 infection.



P-253

CHARACTERIZING THE HUMORAL RESPONSE AGAINST SARS-COV-2 INFECTION IN RELATION TO BIOCHEMICAL AND THYMIC FUNCTION-RELATED PARAMETERS

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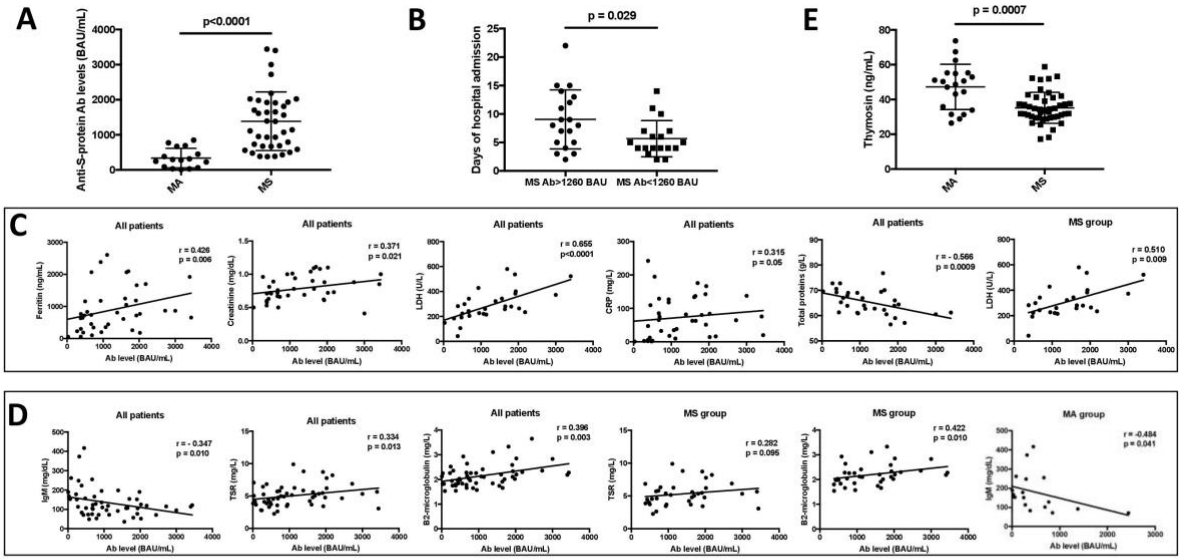
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SARS-CoV-2 infection shows variable humoral responses and outcomes. We aimed to analyze predictive factors of humoral response and potential relationships with biochemical and immune parameters, including thymic function.

During the first wave of COVID-19 pandemic, unvaccinated SARS-CoV-2-infected subjects were recruited, including moderate-severe symptomatology, with hospital admission and different grades of pneumonia (MS, n=50) and mild-asymptomatic without hospital admission (MA, n=20). Different biochemical markers were measured at diagnosis in all hospital admitted subjects and in seven MA. One month after, blood samples were obtained from all subjects for biochemical analysis, and determination of leukocyte populations and thymic function-related parameters (δ/β TRECs ratio, PBMCs' relative–telomere-length_RTL and thymosin levels). IgG-antibodies (Ab) against trimeric SARS-CoV-2 Spike protein were quantified by chemiluminescence at this point.

Our cohort was 56 [46-64] years-old, 41.4% females and had 790 [384-1700] anti-S Ab levels. A significantly higher level of specific Ab was observed in MS respect to MA (1260[674-1923] *vs*. 287[68-614], $p<0.0001$) (Figure 1A), not being affected by age or comorbidities. Among MS patients, those with higher Ab levels spent more days at hospital (Figure 1B). Globally, Ab response positively correlated with ferritin, CRP, creatinine and LDH (the last association being kept in MS), but negatively with protein levels at diagnosis (Figure 1C). In multivariable analysis, only LDH independently predicted Ab levels (beta coefficient=5.13 [2.29-7.97 95%CI], $p=0.001$). Ab levels negatively correlated with IgM (also inside MA group) whereas positively with □2-microglobulin and transferrin soluble receptor values one month after diagnosis (both also inside MS group) (Figure 1D). No associations were found with leukocyte subpopulations or thymic-function related parameters. However, MA showed significantly higher levels of thymosin than MS (Figure 1E).

Inflammation and damage-related levels, especially LDH, at diagnosis seem to impact the humoral response against SARS-CoV-2 infection. A potential role of thymic function in infection's severity deserves study.



Comparison of anti-S-protein Ab levels between the two studied groups showing different severity of symptoms after SARS-CoV-2 infection (A). Comparison of days of admission at hospital after coronavirus infection between MS group patients according to higher or lower values respect to the median of Ab levels (B). Significant correlations found between SARS-CoV-2 anti-protein-S Ab levels in the different groups of infected patients studied and some of the biochemical and immune markers determined at the two timepoints analyzed, diagnosis (C) and one month after infection (D). Comparison of thymosin hormone levels between MA and MS groups (E). Note: MS, moderate-severe group; MA, mild-asymtomatic group; CRP, C-reactive protein; LDH, lactate dehydrogenase; TSR, transferrin soluble receptor; IgM, immunoglobulin M; BAU, Binding Arbitrary Units; Ab, antibodies; r, Spearman's rho.



P-254

CLOSE LONGITUDINAL IMMUNE-MONITORING ALLOWS THE IDENTIFICATION OF IMMUNE PROFILES ASSOCIATED WITH THE SEVERITY OF INFECTION BY SARS-COV-2

Fuentes Herrero, Blanca[1]; Oliva Ariza, Guillermo[1]; Carbonell, Cristina[2]; Lecrevisse, Quentin[1]; Pérez Pons, Alba[1]; Torres Valle, Alba[1]; Pozo, Julio[1]; Martín Oterino, Jose Ángel[2]; González López, Óscar[1]; López Bernús, Amparo[2]; Bernal Ribes, Marta[1]; Belhassen García, Moncef[2]; Pérez Escurza, Oihane[1]; Pérez Andrés, Martín[1]; Vazquez, Lourdes[2]; Hernández Pérez, Guillermo[2]; García Palomo, Francisco Javier[1]; Leoz, Pilar[1]; Costa Alba, Pilar[2]; Pérez Losada, Elena[2]; Yeguas, Ana[2]; Santos Sánchez, Miryam[1]; García Blázquez, Marta[2]; Morán Plata, F. Javier[1]; Damasceno, Daniela[1]; Botafogo, Vitor[1]; Muñoz García, Noemí[1]; Fluxa, Rafael[3]; Contreras Sanfeliciano, Teresa[2]; Almeida, Julia[1]; Marcos, Miguel[2]; Orfao, Alberto[1].

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Introduction. COVID-19 pandemic has resulted in high morbidity and mortality. Although most cases are mild/asymptomatic, 20% develop severe forms. Still, the relationship between the (variable) response of the immune system and the clinical heterogeneity of the disease remains to be fully elucidated.

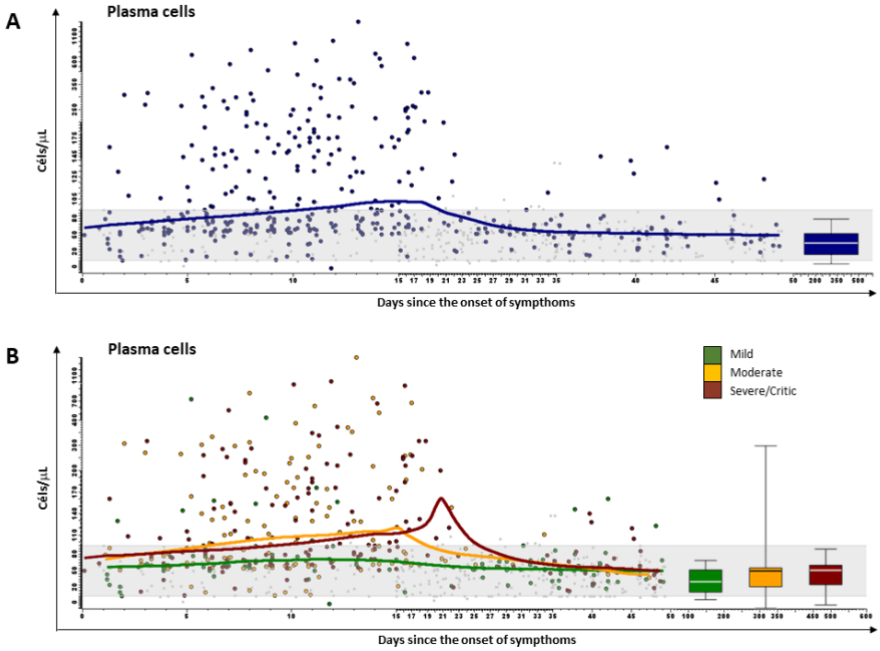
Objective. To analyse in blood the kinetics of leukocyte populations and anti-SARS-CoV-2 antibodies, and their relationship with disease severity, through close monitoring along the infection.

Material and Methods. A total of 802 samples from 345 patients (median 56y, from 20-99y) were analysed, including 28 cases (n=161 samples) studied every 24h-48h during the acute phase. The distribution of cell populations was analysed by flow-cytometry; in all cases, plasma levels of anti-virus N-protein antibodies were determined by ELISA. Results were relativised from the day of symptom onset (d0) and adjusted by age with healthy donors (n=960).

Results. Eosinopenia, lymphopenia (mainly T-lymphocytes - all subpopulations) and neutrophilia were evident early (d+2), followed by an increase in plasma cells (d+10-d+15), returning to normal from d+50 onwards (Fig.1A); most patients had detectable plasma antibodies (IgM, IgG and IgA) from d0. Eosinopenia, lymphopenia and neutrophilia were more pronounced in more severe patients, with a later recovery, while the plasma cell peak was higher (albeit later) vs. milder patients (Fig.1B). Also, increased levels of specific IgG and IgA were found in more severe cases.

Conclusions. There are unique immunological profiles of each leukocyte population, particularly during the acute phase of infection, that follow different patterns depending on severity, with more severe patients showing a greater increase/decrease in affected populations and antibody levels, but with a delay in reaching peak or returning to normal. Precise

knowledge of the kinetics of the immune response and the profiles associated with severity will contribute to a more accurate prognostic assessment in COVID-19 patients.





P-256

IRON METABOLISM IN CHRONIC HIV: IMPAIRED HEPCIDIN-SOLUBLE TRANSFERRIN RECEPTOR AXIS

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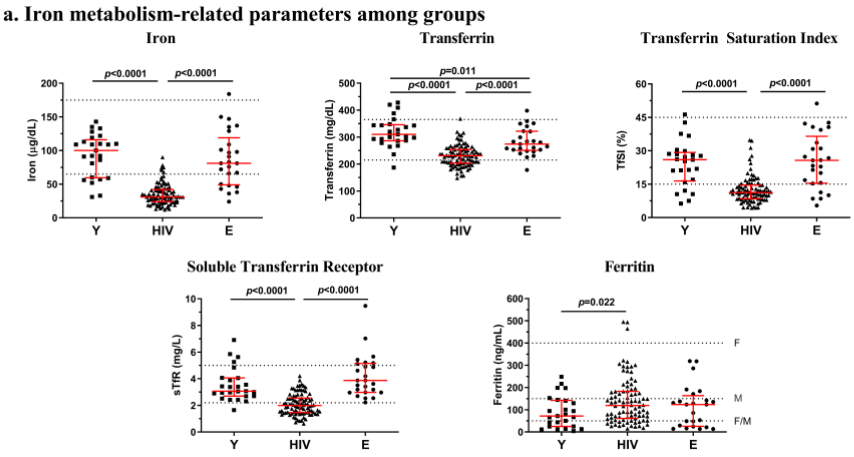
Chronic HIV-infected subjects under effective antiretroviral treatment (ART) exhibit an immune dysfunction leading to comorbidities. Since iron metabolism plays an essential role in immune cell function, its regulation may be relevant in this context.

We included 92 asymptomatic chronic HIV-infected subjects under suppressive treatment (HIV), 25 age-matched non-HIV healthy donors (<65 years-old; Young, Y) and 25 non-HIV elderly subjects (>65 years-old; Elderly, E). For partial analysis, 84 patients with iron deficiency and ferritin<50 ng/mL were also included (Ferropenic, F). Traditional biomarkers of iron metabolism, as well as soluble transferrin receptor (sTfR), hepcidin, and inflammatory markers were determined in plasma.

Compared to both healthy (Young and Elderly) controls, HIV subjects exhibited decreased iron (Y, 100[60-116]; HIV, 31[24-42]; E, 81[49-119] µg/dL), transferrin saturation (Y, 26[16-29]; HIV, 11[8-15]; E, 26[15-37] %) and sTfR (Y, 3.1[2.7-4.1]; HIV, 2.0[1.5-2.6]; E, 3.9[3.0-5.2] mg/L), although increased ferritin (Y, 72[25-142]; HIV, 119[61-183] ng/mL), but similar hepcidin levels (Figure 1A and B). As expected, ferropenic subjects showed the lowest levels of hepcidin (HIV, 9.4[6.1-14.4]; F, 1.9[1.6-3.0] ng/mL). Interestingly, HIV showed altered relationships between iron parameters and their regulators unlike young, elderly or even the ferropenic group (Figure 1C). Specifically, associations between sTfR and iron or transferrin saturation index were negative in healthy and ferropenic groups, while positive in HIV. Moreover, the expected negative correlation between hepcidin and sTfR, observed in healthy and ferropenic groups, was absent in HIV. Interestingly, the HIV inflammatory profile differed from the Elderly one (with an inflammaging-related profile), showing high levels of homocysteine (HIV, 3.2[2.4-4.3] vs. Y, 1.8[1.6-2.2] mg/L, p<0.0001) and β2-microglobulin (HIV, 2.1[1.9-2.5] vs. Y, 1.8[1.6-2.3] mg/L, p=0.018) compared to young.

Chronic HIV-infected patients under ART exhibit an imbalanced regulation of iron metabolism-related components, including hepcidin and sTfR, suggesting a complex functional iron deficiency that could contribute to their persisting immune dysfunction.





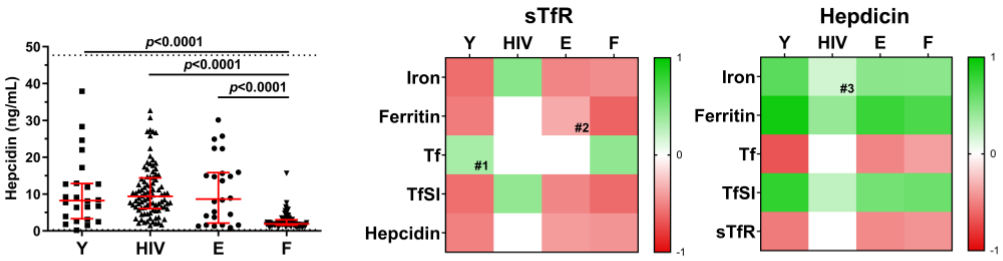


Figure 1. Iron-related parameters. a) Levels of iron metabolism-related parameters (iron, transferrin, TfSI, sTfR and ferritin) in Young non-HIV (Y), HIV, and Elderly non-HIV (E) groups. b) Hepcidin levels in Y, HIV, E and F (ferropenia with ferritin <50 ng/mL) groups. Comparisons were assessed using nonparametric Kruskal-Wallis/Mann–Whitney U tests. Variables with a p-value <0.05 were considered statistically significant. Grid lines represent minimum and maximum standard values. c) Associations between sTfR/Hepcidin and the rest of iron metabolism parameters in Y, HIV, E and F groups. Color intensity of boxes represents Spearman's rank correlation coefficient value as indicated in the color legend. All colored boxes represent statistically significant correlations, excepting for those noted as # ($0.05 \leqslant p \leqslant 0.1$). White boxes represent correlations with *p*-values >0.1. #1, p=0.092; #2, p=0.100; #3, p=0.096. Abbreviations: Tf, transferrin; TfSI, transferrin saturation index; sTfR, soluble transferrin receptor.



P-258

CMV IN AORTIC STENOSIS

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Immunosenescence is not always a consequence of chronological ageing, but it can also be accelerated by persistent antigens inducing chronic stimulation of the immune system. Regarding this, our group has demonstrated that the expansion of proatherogenic and proinflammatory CD28^{null} T cells is driven by cytomegalovirus (CMV) and not an effect of age. In the past years, CMV latent infection has also been associated with several autoimmune conditions, and an increased risk of cardiovascular death (up to 20%). Recent studies suggest that aortic stenosis (AS) is an active inflammatory atherosclerotic-like process. Furthermore, the expansion of peripheral CD28^{null} T cells has been observed in AS patients, suggesting that CMV could have a role in the degeneration of the aortic valve. Thus, we performed a detailed phenotypical/functional characterization of peripheral and valvular immune cells from AS patients, and healthy volunteers (HD) by multiparametric flow cytometry. This analysis included the identification of valve infiltrating CMV-specific T cells and their response to CMV peptides. CMV-associated pro-atherosclerotic and highly cytotoxic T cells were increased in peripheral blood and valvular tissue in AS patients compared with HD. Furthermore, we found CMV-specific T cells (tetramer positive) infiltrating the aortic valve tissue in AS patients. This was further corroborated by valvular T cell response to CMV peptides. Our results show for the first time an association of ASwith CMV chronic infection supporting its role in the calcification of the aortic valve. This will be mediated, partly, by CMV-specific T cells and other CMV-driven proinflammatory cells. Moreover, we successfully developed a novel protocol for the characterization of immune cells implicated in cardiovascular disease immunopathology.

Funding: This research was funded by Instituto de Salud Carlos III Ministerio de Salud, grants PI19/00075 (to Pera A.) and FI20/00194 (to Alvarez-Heredia P.).



P-259

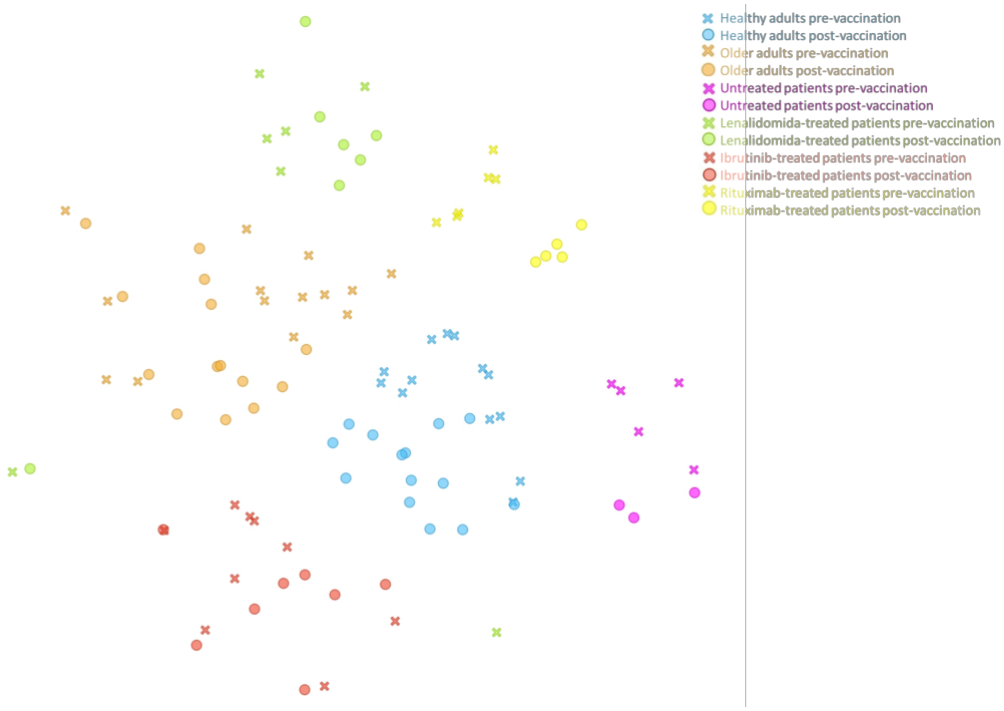
IMMUNE RESPONSE OF MRNA COVID-19 VACCINES IN ELDERLY ADULTS AND PATIENTS WITH LYMPHOID MALIGNANCIES.

Hernández Vázquez, Juan[1]; De La Cal Sabater, Paloma[1]; Arribas, Elisa[1]; Fiz Lopez, Aida[1]; Perez Segurado, Candido[1]; Martin Muñoz, Alvaro[2]; Gutierrez, Sara[3]; Telleria, Pablo[3]; Novoa, Cristina[3]; Rojo, Silvia[4]; De Prado, Angel[1]; Garcia Blesa, Antonio[1]; Sedano, Rosa[3]; Martinez Garcia, Ana Maria[4]; Garcinuño Perez, Sonsoles[4]; Dominguez Gil, Marta[4]; Hernan Garcia, Cristina[5]; Guerra, Maria Mercedes[5]; Muñoz Sanchez, Eduardo[6]; Barragán, Cristina[6]; Diez Morales, Soraya[6]; Cassazza, Oriana[6]; Ramos Pollo, Daniel[6]; Santamarta Solla, Natalia[6]; Alvarez Manzanares, Paula M[6]; De La Fuente Graciani, Ignacio[7]; Perez Gonzalez, Sonia[7]; Bravo, Sara[7]; Garcia Alonso, Cristina[7]; Tesedo Nieto, Angel[8]; Lopez Moreno, Elisabet Carmen[8]; Cabrera Sanz, Maria Esther[8]; Borge Olmedo, Sara[8]; De Paula Ortiz, Miguel[8]; Castellanos Asenjo, Alberto[8]; Gay Alonso, Jenifer[9]; Garrote, Jose A[1]; Arranz, Eduardo[1]; Eiros, Jose Maria[10]; Rescalvo Santiago, Fernando[8]; Quevedo Villegas, Carolina[8]; Tamayo, Eduardo[11]; Orduña, Antonio[4]; Dueñas, Carlos[3]; Peñarrubia, Maria Jesus[7]; Bernardo Ordiz, David[1]; Cuesta Sancho, Sara[1].

[1]Mucosal Immunology Lab, Unit of Excellence Institute of Biology and Molecular Genetics (IBGM), University of Valladolid and CSIC, Valladolid, Spain., Valladolid; [2]Flow Cytometry facility. Unit of Excellence Institute of Biology and Molecular Genetics (IBGM), University of Valladolid-CSIC, Spain., Valladolid; [3]Internal Medicine Department, Hospital Clínico Universitario de Valladolid, Valladolid, Spain., Valladolid; [4]Microbiology and Inmunology Unit, Hospital Clínico Universitario de Valladolid, Valladolid, Spain., Valladolid; [5]Servicio de Medicina Preventiva, Hospital Clínico Universitario de Valladolid, Valladolid, Spain., Valladolid; [6]Unidad de Cuidados Paliativos del Hospital Universitario Rio Hortega, Valladolid, Spain., Valladolid; [7]Department of Hematology, Hospital Clínico Universitario de Valladolid, Valladolid, Spain., Valladolid; [8]Servicio de Prevención de Riesgos Laborales, Hospital Clínico Universitario de Valladolid, Valladolid, Spain., Valladolid; [9]Residencia y Centro de Día ORPEA Valladolid, Valladolid, Spain., Valladolid; [10]Microbiology Department, Hospital Universitario Rio Hortega, Valladolid, Spain., Valladolid; [11]Unidad Investigación, Hospital Clínico Universitario de Valladolid, Valladolid, Spain., Valladolid.

COVID-19 affects the population unequally with a higher impact on aged and immunosuppressed people. Hence, we characterized the immune response triggered by mRNA vaccines in 25 healthy adults (under 70); 28 institutionalized elderlies (over 70 years); and 48 oncohematologic patients untreated or treated with B-cells antiproliferative drugs (lenalidomide, ibrutinib and rituximab). Humoral and cellular memory towards SARS-CoV-2 was determined before and after vaccination, together with the phenotype of peripheral blood mononuclear cells by spectral cytometry (40-plex spectral cytometry panel) and the plasma cytokine profile (13-plex panel). While healthy adults and institutionalized elderlies developed a serologic response, that was much lower in the hematooncologic cohorts (untreated: <70%; lenalidomide-treated: <90%; ibrutinib-treated: 60%; rituximab-treated: <10% response). Nevertheless, all cohorts (excluding patients treated with ibrutinib) developed a T-cell mediated immune response. T-SNE analysis of the spectral cytometry characterization revealed that all cohorts grouped independently of each other (Figure 1). Indeed, while healthy adults, together with untreated and

rituximab patients changed their immune profile following vaccination, elderly adults and patients treated with lenalidomide or ibrutinib were not affected. Moreover, Post/Pre vaccination ratio for every immune subset was revealed that untreated hematologic patients had a significant increase in monocytes, NK, $CD4^+$ and $CD4^+CD8^+$ T-cells and T regs coupled with a decrease on ILCs. On the other hand, elderly adults had an increase in NK, and $CD8^+$ T-cells. In addition, the NK subset was reduced on rituximab patients. Cytokine analysis showed significant increased CXCL10 levels in older adults and untreated patients, as well as increased CCL2 in patients treated with rituximab and elderly prior to immunization. No differences were nevertheless observed due vaccination. In summary, here we have shown that these cohorts, especially oncohematologic patients treated with ibrutinib, display a reduced immune response to mRNA COVID vaccines, suggesting that they may be less protected against infection.





P-260

LA INFECCIÓN POR SARS-COV-2 AUMENTA EL RIESGO CARDIOVASCULAR ASOCIADO A CMV

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[1]Instituto Maimónides de Investigación Biomédica de Córdoba, Córdoba; [2]Hospital Universitario Reina Sofía, Córdoba.

La severidad de la infección por SARS-CoV-2 aumenta con la edad asociándose a inmunosenescencia. El citomegalovirus (CMV) induce inmunosenescencia acelerada acompañada de una expansión de células T $CD28^{null}$ proinflamatorias y ateroménicas. Además, la seropositividad a CMV se asocia con un aumento del riesgo de muerte por enfermedad cardiovascular (ECV). La infección por SARS-CoV-2 también se asocia a ECV. Las células T $CD28^{null}$ expresan CD57 y CX3CR1, y son capaces de infiltrar la vasculatura atraídas por la fractalkina producida por células endoteliales vasculares en respuesta a inflamación. Así, realizamos un análisis de la frecuencia de células T $CD28^{null}$CD57+CX3CR1+ como marcador de riesgo cardiovascular en pacientes COVID-19 leves/asintomáticos a los 3-6 meses y al año post-infección; pacientes con ECV; y controles sanos (CS), estratificados según seropositividad a CMV. Observamos un aumento de estas células en pacientes COVID-19 comparados con CS, solo en individuos CMV-seropositivos. Esta expansión se observó incluso un año después de la infección por SARS-CoV-2, siendo la frecuencia de estas células proaterogénicas similar a pacientes con ECV. Nuestros resultados sugieren que, tras infección leve/asintomática por SARS-CoV-2, se acelera el riesgo cardiovascular asociado a CMV. Este efecto se observó en individuos 20 años (edad media) más jóvenes que pacientes con ECV, alertando de un aumento del riesgo potencial de desarrollar eventos cardiovasculares a edades más tempranas. Dada la elevada prevalencia mundial de infección por CMV y SARS-CoV-2, esto puede suponer un grave problema de salud mundial en un futuro próximo. **Financiación:** Proyecto PI1900075, Proyectos I+D+i de Investigación en Salud 2019, Instituto de Salud Carlos III (Pera, A.); proyecto PE-COVID-0053-2020, Subvenciones para la financiación de la I+D+i biomédica y en ciencias de la salud en Andalucía 2021, co-financiado por la Unción Europea, Consejería de Salud y Familias de la Junta de Andalucía (Pera, A. y Juan Molina); contrato PFIS FI20/00194 (Álvarez-Heredia, P.)



Sesión Poster. Vacunas. Pantalla 6

P-261

IMPLICATIONS OF THE PREVIOUS SARS-COV-2 INFECTION IN THE CELLULAR AND HUMORAL RESPONSES BEFORE AND AFTER THE MRNA-1273 BOOSTER DOSE IN TWO-DOSE BNT162B2 VACCINATION SCHEDULE

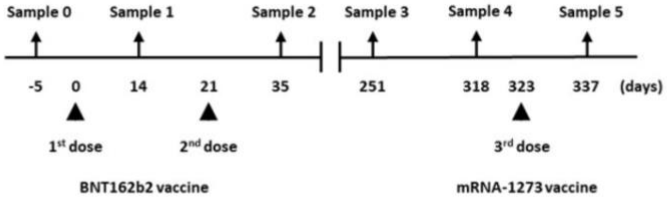
Lozano-Rodríguez, Roberto[1]; Avendaño-Ortiz, José[2]; Terrón-Arcos, Verónica[1]; Montalbán-Hernández, Karla[1]; Casalvilla-Dueñas, José Carlos[1]; Bergón, Marta[1]; Del Fresno, Carlos[1]; López-Collazo, Eduardo[1].

[1]Instituto de Investigación Hospital Universitario La Paz (IdiPAZ), Madrid; [2]Instituto de Investigación Hospital Ramón y Cajal, Madrid.

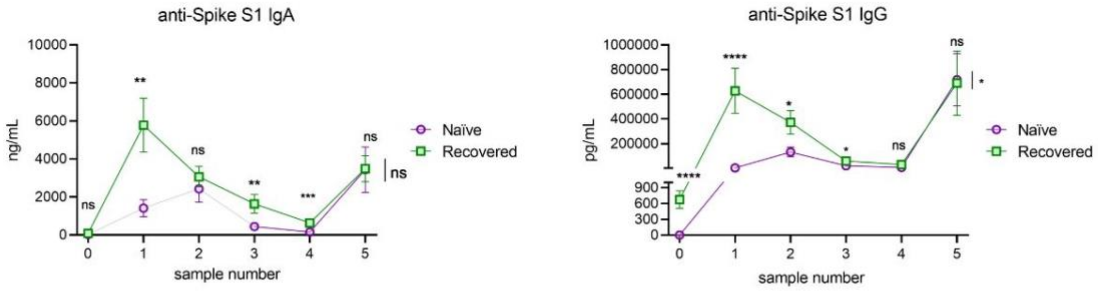
We have assessed both humoral and cellular immune responses induced by the vaccination schedule of two-dose BNT162b2 (Pfizer mRNA vaccine) plus booster mRNA-1273 (Moderna mRNA vaccine) in a longitudinal cohort of naïve and recovered individuals from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Regarding the humoral response, we have observed a disparate pattern in the increment of the plasmatic anti-Spike IgA and IgG antibodies levels after the first and second doses between naïve and recovered individuals that were abolished both along the time and after the boost vaccination. In addition, after the boost dose, both individuals reached similar anti-Spike IgA antibodies as after the second dose and comparable anti-Spike IgG antibodies levels as recovered individuals after the first dose. In relation to the cellular response, we have evaluated the SARS-CoV-2 Spike-specific T lymphocyte proliferation capacity before and after the boost dose using 15-mer peptides of Spike protein with 11 overlapped amino acids of the wild type (WT), delta (B.1.617.2) and Omicron (B.1.1.529) variants. We have discerned a robust T cell proliferation, especially in $CD4^+$ T cells, after the boost dose against all spike peptide pools used. Furthermore, when we broke down between naïve and recovered individuals, both responded in a similar way against WT, delta and Omicron peptide pools after the boost dose with non-significant differences between them in the T $CD4^+$ cell proliferation. Our data indicate that in both naïve and COVID-19-recovered individuals, both humoral and cellular responses balance between them after the third dose, losing the differential pattern observed with the two-dose typical schedule of vaccination.



A



B



(A) Experimental schedule indicating the blood sample collection along the time. **(B)** Concentrations of plasmatic anti-Spike S1 IgA (lef panel) and IgG (right panel) antibodies of naïve (violet dots, n=16) and COVID-19-recovered (green squares, n=11) individuals along the time.



P-263

ANÁLISIS LONGITUDINAL DE LA INMUNIDAD ADAPTATIVA TRAS 2 Y 3 DOSIS DE LAS VACUNAS DE ARNM ANTI-SARS-COV-2 EN INDIVIDUOS NAÏVE Y RECUPERADOS DE COVID-19

Almendro Vázquez, Patricia[1]; Chivite Lacaba, Marta[1]; Utrero Rico, Alberto[2]; González Cuadrado, Cecilia[1]; Laguna Goya, Rocío[3]; Moreno Batanero, Miguel[1]; Sánchez Paz, Laura[4]; Luczkowiak, Joanna[1]; Labiod, Nuria[1]; Folgueira, María Dolores[3]; Delgado, Rafael[3]; Paz Artal, Estela[3].

[1]Instituto de Investigación Hospital 12 de Octubre, Madrid; [2]Icahn School of Medicine at Mount Sinai, Nueva York; [3]Hospital 12 de Octubre, Madrid; [4]Hospital Infanta Leonor, Madrid.

En este estudio caracterizamos longitudinalmente, a lo largo de 12 meses, la respuesta de células T mediante FluoroSpot (spot forming unit(sfu)/10^6 PBMC), IgG y anticuerpos neutralizantes (Nabs) inducidos por la vacunación BNT162b2 de dos dosis más una tercera de mRNA-1273 en sujetos sanos: 17 con COVID-19 previo a la vacunación (recuperados) y 60 naïve.

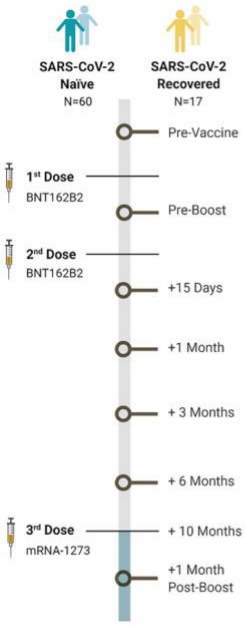
La respuesta máxima de células T e IgG inducida por dos dosis fue similar en ambos grupos y se alcanzó a los 15 días de la segunda dosis. Sin embargo, la neutralización fue menor en los naïve. Tras el pico, la respuesta T se mantuvo estable 3-6 meses, mientras que los niveles de IgG y Nabs disminuyeron progresivamente. En comparación con los individuos naïve, los recuperados mantuvieron niveles significativamente superiores de células T, IgG y Nabs 1-6 meses tras la vacunación. La tercera dosis potenció la neutralización únicamente en los naïve, mientras que la potenciación de la respuesta T fue superior en recuperados.

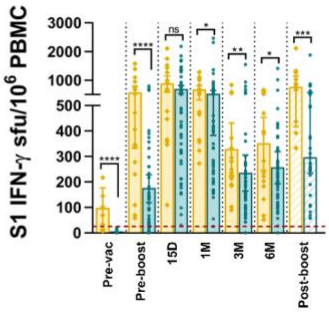
Durante los 12 meses de seguimiento se reportaron 17 infecciones por SARS-CoV-2, asintomáticas o leves, todas en individuos naïve: cinco (8,3%) 3-5 meses tras la segunda dosis y doce (21,8%) 15-30 días tras la tercera (ola Ómicron). Esta exposición al virus potenció la respuesta adaptativa e indujo el desarrollo de células T frente a la nucleocápside y la membrana. Finalmente, un análisis de clusters demostró que el desarrollo de una robusta respuesta T y neutralizante a los 15 días de la segunda dosis se asocia con protección frente a infección tras la vacunación.

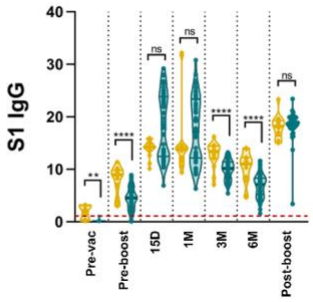
En conclusión, la respuesta celular se mantiene tras la vacunación y la tercera dosis fue especialmente útil para incrementar la neutralización en individuos naïve. Haber sufrido COVID-19 previamente y el perfil de protección detectado con células T y Nabs elevados podrían tenerse en cuenta para personalizar próximas estrategias de vacunación.

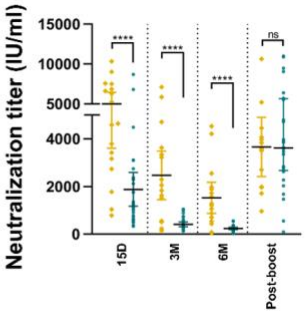
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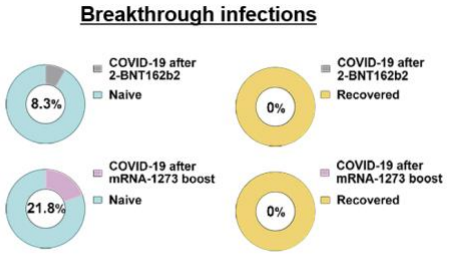












P-264

AVIDITY STUDY OF THE HUMORAL RESPONSE AFTER ONE AND TWO DOSES OF MRNA COVID-19 VACCINES

Barrios Del Pino, Yvelise[1]; Placer Díaz, M José[1]; Álava Cruz, Cristina[2]; Franco Maside, Andrés[1]; Matheu Delgado, Víctos[2].

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Introduction.

mRNA vaccines induce good immunogenicity and excellent effectiveness in real-world population. However, a detailed profile of immunological properties of the humoral response achieved after one or two doses of the vaccine is needed to guide recomendations about vaccine schedule in the future.

Methods.

After informed consent was obtained, serum samples from 30 vaccinated and healthy HCW were studied 3 weeks after first and second dose. Avidity index was determined by their antibody-antigen binding resistance to 8 M urea. Serum samples were prediluted to give an OD at 450 nm readout of between 1.0 and 1.5 (ELISA) and were added to plates coated with RBD antigen (Euroimmun, Lübeck, GE). Plates were then washed with either PBS-T (1% BSA–0.05% Tween in PBS) or 8 M urea in PBS-T, before incubation with the conjugate. Samples were developed and the avidity index was calculated as the percentage of the OD of urea-treated samples/OD of PBS-T-treated samples. The study was approved by Ethics Committee of Hospital Universitario de Canarias (CHUC_2021_04).

Results.

The mRNA vaccines used were Pfizer (n=27) and Moderna (n=3). After 1D, avidity index was 8,7+/-4,0% vs 11,2+/-5,7% in the female (f): male (m) participants. Avidity index after 2D was 27,4+/-16,3% vs 28,7+/-19,1% (f:m). The avidity index results comparing the two mRNA vaccines administered were as follows: 9,0+/-4,5% (1D) to 23,9+/-14,1% (2D) for the Pfizer group and 13,2+/-4,4% (1D) to 56+/-13,3% (2D) in the Moderna group. A summary with these results is provided in figure 1.



Discussion.

Avidity is used to describe the total strenght of the interaction exhibited by the Ab-Ag binding. As expected, there is a generalised increase in the avidity index after the second dose of the vaccine, a biological expression of the humoral immunological improvement achieved after two doses of these mRNA vaccines in these healthy HCW.

Figure 1. IgG SARS-Cov-2 avidity after 1st and 2nd doses of mRNA vaccine in healthy subjects.

			IgG SARS-Cov-2 Avidity (%)	
Gender	**N**	**Age:years (Mean±SD)**	**1st Dose (Mean±SD)**	**2nd Dose (Mean±SD)**
Female	21	50,3±8,3	8.7%±4.0%	27.4%±16.3%
Male	9	49,8±6,2	11.2%±5.7%	28.7%±19.1%
mRNA vaccine				
Pfizer	27	49,7±7,7	9.0%±4.5%	23.9%±14.1%
Moderna	3	55,0±7,1	13.2%±4.4%	56.0%±13.3%
Total	30	50,1±7,7	9.4%±4.6%	27.1%±16.9%



P-265

RESPUESTA IMMUNE Y SEGURIDAD DE LA VACUNA MRNA-1273 CONTRA SARS-COV-2 EN PACIENTES CON MIASTENIA GRAVIS.

Lynton Pons, Elionor[1]; Reyes Leiva, David[1]; Bravo Gómez, Andrea Carolina[1]; Gallardo, Eduard[2]; Albert Jarés, Daniel[1]; López-Contreras, Joaquín[1]; Plà Juncà, Francesc[2]; Cortés Vicente, Elena[1]; Moga Naranjo, Esther[1].

[1]Hospital de la Santa Creu i Sant Pau, Barcelona; [2]Institut de recerca de l'Hospital de la Santa Creu i Sant Pau, Barcelona.

La Miastenia Gravis (MG) es una enfermedad autoinmune que, en el 85% de los casos, cursa con anticuerpos anti-receptor de acetilcolina, causando debilidad muscular. La fluctuación de los síntomas es característica de la MG aunque diferentes factores como las infecciones pueden inducir exacerbaciones de la enfermedad. La pandemia causada por el SARS-CoV-2 ha propiciado el desarrollo de vacunas basadas en la tecnología del ARN mensajero (ARNm). En el contexto de la necesidad de una vacunación masiva de la población, se abrió el debate sobre su efectividad en pacientes con enfermedades autoinmunes tratados con inmunosupresores y la seguridad de estas vacunas para los pacientes con MG.

En este estudio longitudinal se evaluó la eficacia y seguridad de la vacuna mRNA-1273 en un grupo de 100 pacientes con MG tratados con diferentes terapias inmunosupresoras. Los pacientes recibieron dos dosis de la vacuna en concordancia con el protocolo de administración del *Hospital de la Santa Creu i Sant Pau* (Barcelona). Se recogieron muestras de los pacientes antes de la primera y después de la segunda dosis, determinando la respuesta inmune humoral y celular en ambos tiempos. Igualmente, se reportaron los cambios clínicos mediante la puntuación MG-ADL (*Myasthenia Gravis activities of daily life score*) antes de la primera dosis y también después de la primera y de la segunda dosis.

Después de la segunda dosis, el 88.77% de los pacientes presentaron anticuerpos IgG anti-spike y el 73.47% mostraron un test de liberación de interferón-gamma positivo, sin asociación con empeoramiento sintomático de relevancia clínica. Los pacientes con terapia combinada (prednisona con otro inmunosupresor) reportaron menores niveles estadísticamente significativos de seroconversión y de respuesta celular T.

En conclusión, la vacuna mRNA-1273 es segura en pacientes con MG y no afecta negativamente al curso de la enfermedad, logrando además niveles altos de respuesta inmune humoral y celular.



P-266

RESPUESTAS CELULAR Y HUMORAL A LA VACUNA MRNA-1273 FRENTE AL SARS-COV-2 EN PACIENTES ONCOLÓGICOS

Mohamed Mohamed, Kauzar; Alonso Arenilla, Barbara; Benitez Fuentes, Javier David; De Luna Aguilar, Alicia; Jiménez García, Carlos; Guevara Hoyer, Kissy; García Bravo, Laura; Rodríguez De La Peña, Antonia; Jiménez Ortega, Alejandro Francisco; Mayol Hornero, Elsa; Pereiro Rodríguez, Alejandro; Perez Segura, Pedro; Sánchez-Ramón, Silvia.

Hospital Clínico San Carlos, Madrid.

Objetivos

El objetivo del estudio fue evaluar las respuestas celular y humoral en pacientes con cáncer sólido vacunados con mRNA-1273 frente al SARS-CoV-2.

Material y método

Se determinó la producción basal de interferón-gamma (IFN-γ) y de anticuerpos frente a la subunidad S1 del SARS-CoV-2 y dos meses después de la segunda y tercera dosis de la vacuna mRNA-1273 en 36 pacientes oncológicos. Además, se evaluaron los linfocitos T exhaustos mediante citometría de flujo en una selección de pacientes oncológicos y controles sanos.

Resultados

Se obtuvieron resultados basales positivos de inmunidad celular en 13 de 36 (36.1%) pacientes oncológicos y en 33 de los 36 (91,7%) tras la segunda dosis, observándose diferencias significativas con los niveles basales (p<0.001). Tras la tercera dosis, se observó un comportamiento dual: 24 pacientes (66.7%) mostraron una respuesta celular parcial tras la segunda dosis que se incrementó tras la tercera dosis vacunal (p=0.002); mientras que 11 pacientes (30,5%) presentaron una respuesta celular óptima tras la segunda dosis y una marcada reducción en los niveles de INF-γ tras la tercera dosis (p=0.010). La seroconversión ocurrió en todos los pacientes tras la segunda dosis vacunal. Los pacientes que presentaron una bajada en la respuesta celular tras la tercera dosis vacunal mostraron una elevada expresión de linfocitos T exhaustos CD8 + CD57hiPD1hi y CD8+CD57 medPD1hi en comparación con los pacientes oncológicos que aumentaron su respuesta celular y los controles sanos.

Conclusiones

Estos resultados muestran que la vacunación frente al SARS-CoV-2 con dos dosis resulta beneficiosa en todos los pacientes oncológicos. Una dosis adicional puede ser útil en los respondedores parciales mientras que los que presentan una respuesta máxima puede presentar linfocitos T exhaustos por exposición antigénica repetitiva. Nuestros datos podrían respaldar la relevancia de las pruebas inmunológicas para evaluar la necesidad de dosis de refuerzo.



P-267

MAINTENANCE OF A GOOD ANTI-S ANTIBODY TITER AFTER THE COVID-19 VACCINE. EIGHT-MONTH FOLLOW-UP STUDY IN A HOME FOR THE ELDERLY.

Pozo Balado, María Del Mar[1]; Bulnes Ramos, Ángel[1]; Olivas Fernández, Israel[1]; Garrido Rodríguez, Vanesa[1]; Lozano, Carmen[2]; Álvarez Ríos, Ana Isabel[2]; Sánchez Sánchez, Berta[2]; Lara, José Manuel[2]; Maldonado Calzado, Isabel[2]; Sánchez Bejarano, Encarnación[2]; Santamaría, Juan[3]; Bernal, Rafael[3]; González Escribano, María Francisca[2]; Leal Noval, Manuel[4]; Pacheco, Yolada M.[1].

[1]Instituto de Biomedicina de Sevilla (HUVR), Sevilla; [2]Hospital Universitario Virgen del Rocío (HUVR)), Sevilla; [3]Residencia Hogar de la Santa Caridad, Sevilla; [4]Hospital Viamed Santa Ángela de la Cruz/Residencia Hogar de la Santa Caridad, Sevilla.

Neutralizing anti-S antibody levels are highly predictive of clinical protection against symptomatic Covid-19.

We aimed to characterize immune predictors for the maintenance of a good anti-S antibody titer and to determine the primary antibody cut-off (after the two-dose vaccine protocol) that guarantees such maintenance along time.

Residents and health workers from the “Santa Caridad Elderly Home” without previous Covid-19, receiving two doses of BNT162b2 vaccine were recruited (n=143). Blood was collected one (T1), four (T4) and eight (T8) months after second-dose. IgG-antibodies against trimeric SARS-CoV-2 Spike protein were quantified by chemiluminescence (LIAISON®SARS-CoV-2 TrimericS IgG, Diasorin). Seroconversion was ≥33.8 BAU/mL and good response (GR) was≥520 BAU/mL (1:80 median neutralization titer). Inflammatory markers and immune cellular subsets were determined by standard procedures. Telomere relative length (TRL) and ratio δ/β TRECs were quantified on PBMC by digital PCR and serum α1-thymosin levels by ELISA. Cut-off antibody values at T1 to maintain GR at T4 and T8 were obtained from ROC curves using Youden index.
Our cohort was 64 [46-77] years-old (with 49.7% people over 65) and 66.9% men. All participants seroconverted at T1, showing most of them GR (86.5%) (**Fig.1A**). GR rate decayed while NR rate increased along time. At T4, subjects with GR significantly differed from those with NR or PR regarding immune parameters measured at T1 (**Fig.1B**). Thus, subjects maintaining GR were younger and had higher anti-S antibody titers as well as higher levels of T-and B-cells and δ/β TRECs ratios but lower levels of NK cells, homocysteine, beta-2-microglobuline, CRP and d-dimers. Cut-offs of 1645 BAU/mL (AUC 0.914(0.864-0.965); 87.5% Sensitivity and 81.4% Specificity; PPV=91.8, NPV=72.3) and 1995 BAU/mL (AUC

0.912(0.848-0.975); 100% Sensitivity and 79.4% Specificity; PPV=100%, NPV=74.1%), were obtained for T4 and T8, respectively.

Our model could help to develop personalized strategies of vaccination based on the duration of humoral responses.

Figure 1

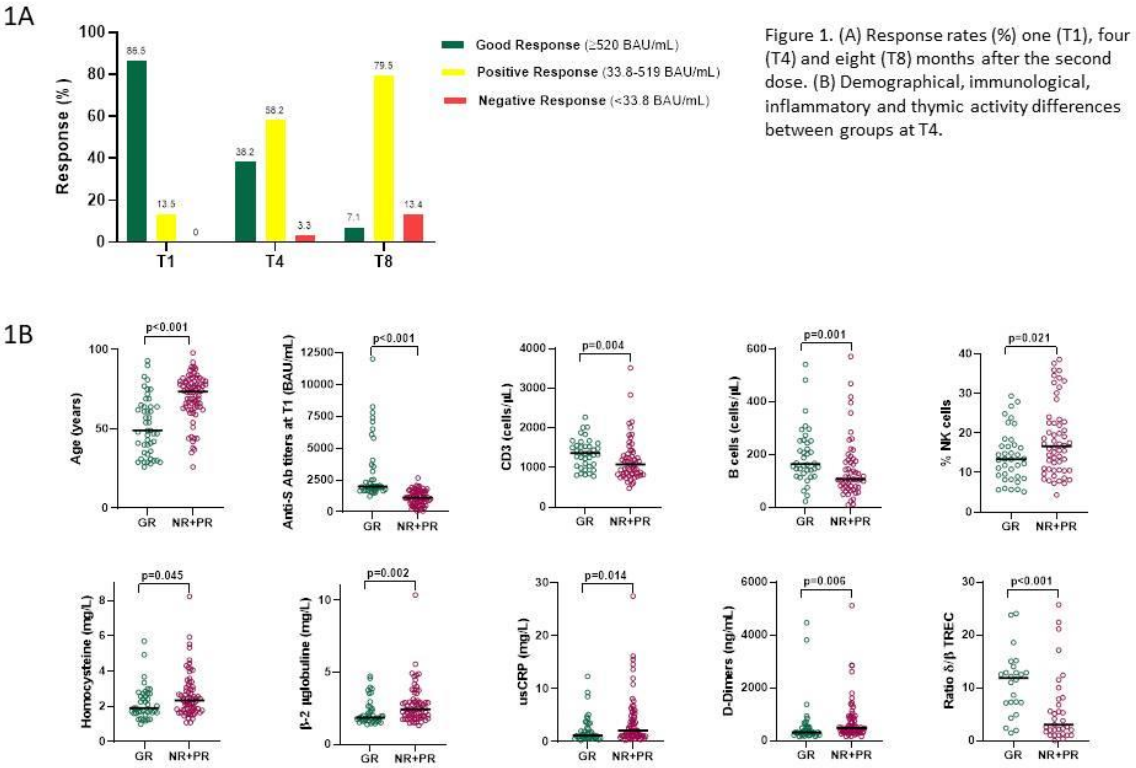


Figure 1. (A) Response rates (%) one (T1), four (T4) and eight (T8) months after the second dose. (B) Demographical, immunological, inflammatory and thymic activity differences between groups at T4.



P-268

PRODUCTION OF NEUTRALIZING ANTIBODIES AGAINST SARS-COV-2 AFTER CORONAVAC IMMUNIZATION IN YOUNG INDIVIDUALS WITH AND WITHOUT PRIOR COVID-19

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Universidad de Guadalajara - Centro Universitario de Ciencias de la Salud, Guadalajara.

Background: Neutralizing antibody levels can determine the effectiveness of a vaccine. CoronaVac is a vaccine against SARS-CoV-2 with an efficacy of 50.7% and higher effectiveness against severe diseases. However, breakthrough infections leading to severe disease and death in adults vaccinated with CoronaVac have been reported. Thus, it is essential to clear the immunological mechanisms induced by this vaccine.

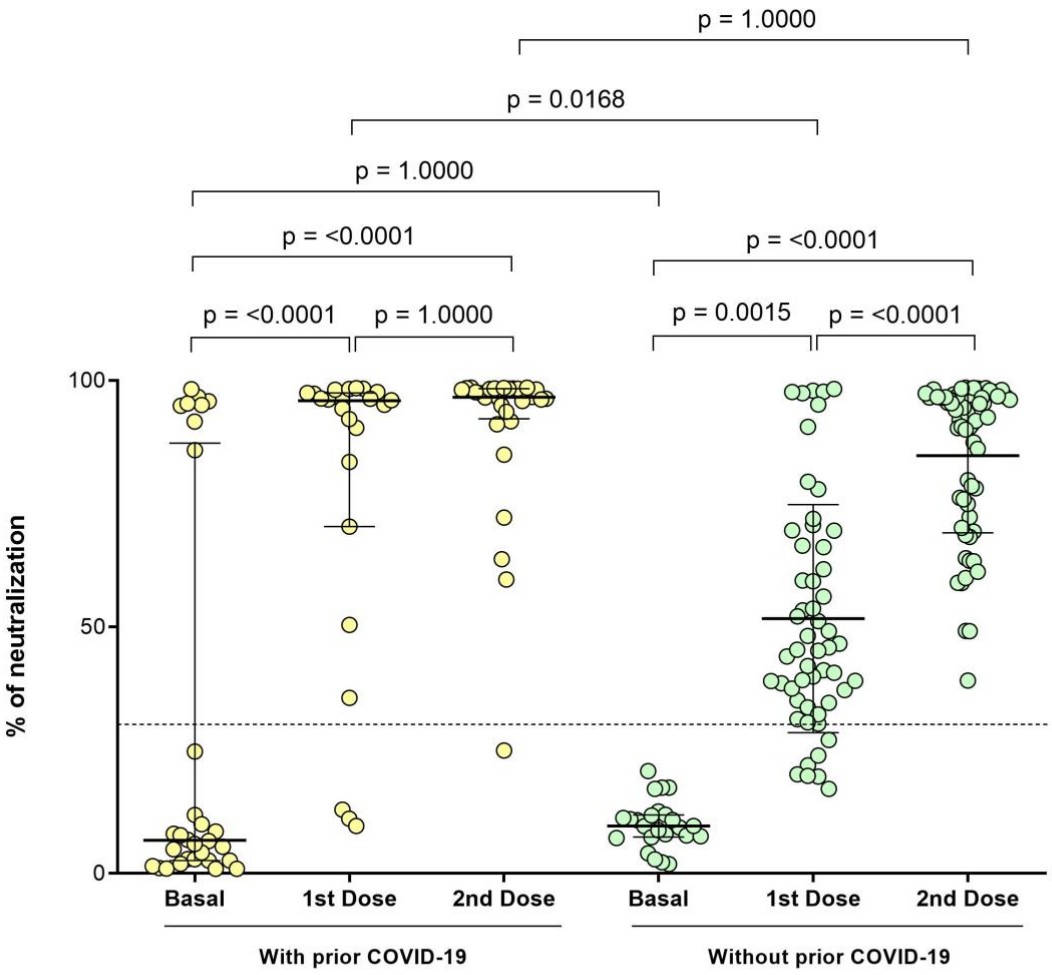
Objective: To determine the production dynamics of neutralizing antibodies against SARS-CoV-2 and side effects in response to immunization with the CoronaVac vaccine.

Methods: We included 112 individuals immunized with CoronaVac, with COVID-19 (n=37), and without prior COVID-19 (n=75). The presence of IgG and IgM antibodies against protein S was determined by lateral flow assays and the presence of neutralizing antibodies and their inhibitory capacity by a competitive ELISA.

Results: After the first dose of CoronaVac, there was an increase in neutralizing antibodies in individuals with and without prior COVID-19 ($p < 0.001$); however, a higher inhibitory capacity was observed in individuals with prior COVID-19 ($p = 0.0168$). For the group without prior COVID-19, after the second dose, antibody values were optimized ($p < 0.0001$), and all generated neutralizing antibodies. Based on the complete scheme, there was no difference between the groups ($p<0.05$). The most frequent side effects of the first and second vaccine doses were headache, asthenia, myalgia, and irritability, without significant difference by groups ($p > 0.05$).

Conclusions: Two doses of the CoronaVac vaccine efficiently stimulate the neutralizing antibody production in individuals with and without a history of COVID-19. However, in individuals with prior COVID-19 infection, it induces a faster production of antibodies and since the first dose induces an optimal inhibitory capacity. This vaccine did not induce severe side effects in any study group.

Keywords: CoronaVac, neutralizing antibodies, vaccine, SARS-CoV-2



Dynamics of the generation of neutralizing antibodies in CoronaVac in the groups with and without prior COVID-19. Differences were calculated using the Kruskal-Wallis test. Values are provided as medians and interquartile ranges.



P-269

EFFICACY AND SAFETY OF MRNA SARS-COV-2 VACCINATION IN HEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS: SINGLE CENTRE EXPERIENCE.

Boigues-Pons, Marc[1]; Huguet, Maria[2]; Iglesias Escudero, María[3]; Martínez-Cáceres, Eva[1]; Morgades, Mireia[2]; Felip, Eudald[4]; Ferrà, Christelle[2]; Quirant, Bibiana[1].

[1]Division of Immunology, LCMN, Germans Trias i Pujol University Hospital and Research Institute, Barcelona, Spain.; Departament of Cellular Biology, Physiology and Immunology, Universitat Autònoma de Barcelona, Barcelona, Spain., Badalona; [2]Hematology Department, Institut Català d'Oncologia de Badalona, Hospital Germans Trias i Pujol, Josep Carreras Research Institute, Badalona. Universitat Autonòma de Barcelona, Spain., Badalona; [3]Division of Immunology, LCMN, Germans Trias i Pujol University Hospital and Research Institute. FOCIS Center of Excellence- UAB, Barcelona; [4]Universitat de Vic – Universitat Central de Catalunya, Spain., Badalona.

Background

Hematopoietic stem cell transplantation (HCST) recipients present high risk of severe SARS-CoV-2 infection and have been considered for priority vaccination. However, as they usually remain immunosuppressed for months after HSCT, vaccine efficacy might be compromised. First reports on serological response after vaccination in hematological patients confirm the lower antibody response rate, compared to the general population (>90%). The characterization of the humoral and cellular response of transplanted patients could help to design more efficacious vaccination programs.

Methods

SARS-CoV-2 vaccination with mRNA1273 vaccine (Moderna) was prospectively evaluated in haematological patients after HSCT and serological response was assessed after complete vaccination. In patients without specific anti-SARS-CoV-2 antibodies, we reassessed humoral and cellular response after receiving a third booster.

Results

Eighty-seven patients (56 auto-HSCT and 31 allo-HSCT) received the complete vaccination regimen with two doses between March and May 2021. At 2-4 months, 86% of patients presented seroconversion. Median antibody response rate was higher for the allo-HSCT group (2578 UI/mL) compared to auto-HSCT group (1675.5 UI/mL). Twelve patients presented a persistent negative serological status after vaccination. We found an association between negative serological response and low B-lymphocyte count (<113.5 cells/uL), low IgG (<700 mg/dL) and treatment with anti-CD20 antibodies in the last year before vaccination. 11/12 patients without seroconversion received a third dose of the vaccine after



which 5/11 patients achieved seroconversion and 6/11 presented cellular response. At 6 months follow-up, no cases of COVID-19 infection have been reported in this series.

Conclusions

Seroconversion rate after mRNA SARS-CoV-2 vaccination in HSCT recipients is elevated although lower than in the general population (>90%). Anti-CD20 therapy, low B-lymphocyte
count and hypogammaglobulinemia are associated with no serological response after vaccination. Specific cellular and humoral response monitoring after SARS-CoV-2 vaccination is indicated to identify booster dose candidates.



P-270

HUMORAL AND CELLULAR RESPONSES TO BNT162B2 VACCINATION AFTER HAPLOIDENTICAL ALLOGENIC BONE MARROW TRANSPLANT IN A PATIENT WITH SEZARY SYNDROME

López Gómez, Ana[1]; Juárez, Ignacio[1]; Rossi, Nineth[1]; Horndler, Lydia[2]; González García, Raquel[3]; Martinez-Quiles, Narcisa[3]; Alarcón Sánchez, Balbino[4]; Gómez Del Moral, Manuel[5]; Martínez-Naves, Eduardo[3]; Regueiro, José Ramón[3].

[1]Departamento de Inmunología, Oftalmología y ORL, Facultad de Medicina, Universidad Complutense de Madrid., Madrid; [2]Centro de Biología Molecular Severo Ochoa CBMSO, Madrid; [3]Departamento de Inmunología, Oftalmología y ORL, Facultad de Medicina, Universidad Complutense de Madrid, Madrid; [4]Centro de Biología Molecular Severo Ochoa, CBMSO, Madrid; [5]Departamento de Biología Celular, Facultad de Medicina, Universidad Complutense de Madrid, Madrid.

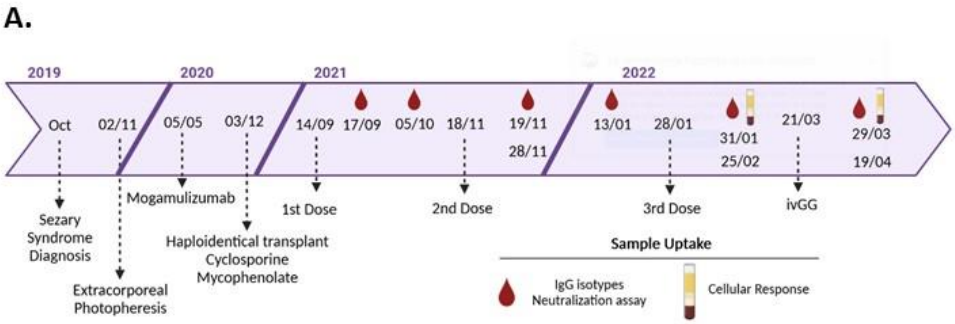
We studied the response to three doses of BNT162b2 vaccine (Pfizer-BioNTech, September 2021 to January 2022) in a 63-year-old male with Sezary syndrome after receiving an haploidentical allogenic bone marrow transplant, when under cyclosporine and mycophenolate treatment. We analyzed serum and PBMC from the patient to determine the humoral and cellular response to vaccines.

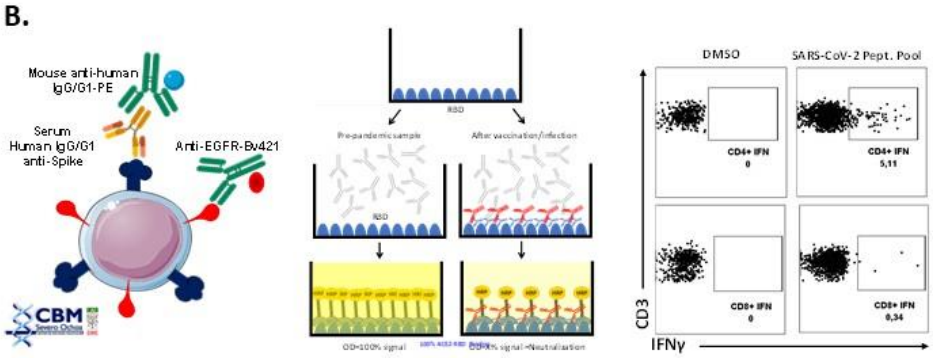
Anti-Spike SARS-CoV-2 IgG antibody isotypes were quantified by flow cytometry (FCM) of Jurkat cells transfected with Spike and EGFR. Total IgG and IgG1 were detected, particularly after the first and third dose. The sera neutralized SARS-CoV-2 ACE2-RBD binding with similar kinetics to IgG levels, as determined by a competitive ELISA assay.

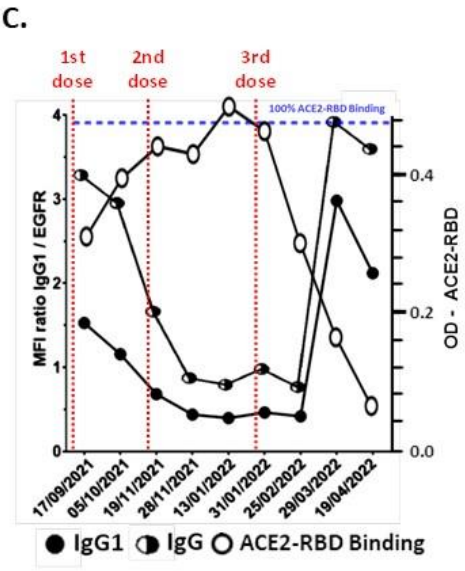
The cellular response was analyzed by FCM in PBMC as IFNγ production in CD8 and CD4 T-cells after 7 days expansion and restimulation with SARS-CoV-2-specific Spike protein peptides. We observed a high production of IFNγ (5.11% in CD4 and 0.34% in CD8 T-cells) after the third dose that weaned in the next two months (0.73% in CD4 and 0.086% in CD8 T-cells).

These data suggest that three doses of BNT162b2 can induce strong SARS-CoV-2-neutralizing cellular and humoral responses in patients with an haploidentical allogenic bone marrow transplant and immunosuppression, but further vaccines may be required to maintain protection.









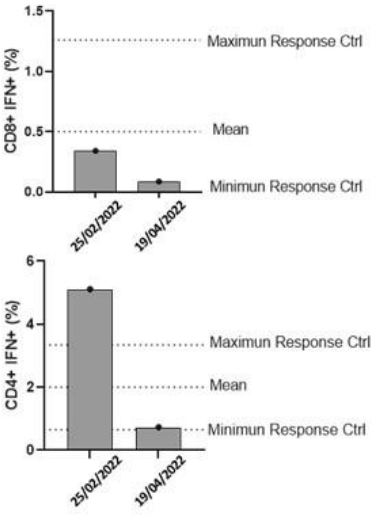


Figure. Response to BNT162b1 in a patient with Sezary syndrome after haploidentical bone marrow transplantation. A) Clinical evolution of the patient (timeline) B) Methodology. Left: Image shows Jurkat-Spike FCM to detect IgG isotypes. Middle: Image shows the ACE2-RBD neutralization assay. Right: Dot plots shows IFNγ production by T cells after a 7-day expansion with Spike peptide-pool. C) Graph shows the evolution of total IgG and IgG1 response and ACE2-RBD neutralization capacity of the sera according to vaccination and sample dates. D) Cellular response to Spike-specific peptides. Graphs show CD8 and CD4 T cell responses after the third dose and after ivGG administration



P-271

VACUNAS QUE (QUIZÁS) DEBERIAN ADMINISTRARSE EN LOS CENTROS DE VACUNACIÓN INTERNACIONAL.

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[1]Subdelegacion del Gobierno, Alicante; [2]Hospital San Juan Alicante, Alicante.

En los centros de Vacunacion Interncional de l Minsiterio de sanidad se dan las recomendaciones de viaje, profilaxis conra enfermedades tropicales , fundamentalmente malaria y se recomiendan y administran determinadas vacunas:

Vacuna anti hepatitis A (RNA virus, dos dosis espaciadas 2-3 semanas). para personal de riesgo, generalmente joven (menos de 45 años) que viajan a zona endémica.

Vacunas contra la Encefalitis japonesa ; 3 dosis, 0,7 y 28 dias.

Vacuna contra la febre amarilla (cepa 17 D del flavivirus atenuado en pases en células de huevo.

Vacuna oral contra Fiebre tifoidea, oral, administrada, tres dias alternativos.

Vacuna intramuscular contra Fiebre tifoidea, una sola dosis.

Vacunacion Tétanos . Normalmente a prescripcion facultativa segun el estado inmunológicvo del viajero.

Vacuna Encefalitis Europea (garrapatas del género ixodes).Tres dosis, 0,7 y 28 días.

Quizás sepodría ampliar la lista de vacunas a:

Vacuna contra el Ébola . Ervebo y Zabdeno + Mvabea; el Zabdeno es (Ad26.ZEBOV-GP), suspensión inyectable del adenovirus 26 (si, el mismo virus vector que usa el instituto Gamaleya para la vacuna "Spuknik" contra el SARs CoV 29). A las 8 semanas se administra la Mvabea.Vacunación de refuerzo con Zabdeno (personas que hayan recibido previamente la pauta de primovacunación de 2 dosis con Zabdeno y Mvabea) Las personas que hayan completado previamente la pauta de primovacunación de 2 dosis, pueden recibir una dosis de refuerzo de Zabdeno.

Hay otra vacuna ,Erbevo,con una proteina del virus Ebola.

Vacuna contra el dengue. Hay una vacuna EMA/688282/2021 EMEA/H/C/004171 llamada Dengvaxia (vacuna tetravalente contra el dengue), es atenuada y se prescribe para personas de entre 6 y 45 años que han padecido una infección previa por el virus del dengue, por que la segunda vez que se pasa la enfermedad es mucho más grave.



P-272

THYMIC FUNCTION AND THE HUMORAL RESPONSE TO THREE DOSES OF COVID-19 VACCINATION IN KIDNEY TRASPLANT RECIPIENTS

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Kidney transplant recipients (KTR) achieve lower seroprotection rates than the general population after two-doses of COVID-19 mRNA vaccine.

To analyse humoral response to the third dose of COVID-19 vaccine in KTR and potential predictive factors including thymic function.

Methods: KTR receiving three doses of mRNA-1273 vaccine and healthy controls (HD) receiving two doses of BNT162b2 vaccine were included. Humoral response to vaccine, as SARS-CoV-2 Trimeric-S-IgG antibodies (BAU/mL) was analysed before vaccination (T0), 3-4 weeks after the first dose (T1), one and four months after the second dose (T2 and T3, respectively) and one month after the third dose (T4). Seroconversion was defined as BAU/mL≥33.8. Immune cellular subsets, kidney function-related parameters, inflammatory status, thymic output and thymosin-α1 (T□1) were measured at T0.

Seventy-four KTR were included (T1 and T2) and 54 KTR remained at T3 and T4. Sixteen HD were included. KTR showed a lower seroconversion rate than HD after two doses of vaccine (44.3% *vs.* 100%, $p<0.001$), improving after the third dose (64.8%). After the full vaccination schedule, baseline immunosuppression therapy, kidney function, inflammatory status and age were negatively related to the response in KTR while higher counts of lymphocytes, T-CD3, T-CD4, B-Cells, T□1 plasma concentration and thymic output were related to a better humoral response (Figure 1). T□1 was independently associated to seroconversion (OR (95%CI): 1.1(1.0,1.2), $p=0.037$) whereas a trend to independent association was observed with baseline blood concentration of mycophenolate (OR (95%CI): 0.3(0.1,1.0); $p=0.059$). Antibody titers at T4 were independently associated with age (βI (95%CI): -132(-239.2-25.7), $p=0.017$) and baseline mycophenolate blood level (βI (95%CI): -705(-1392.5-18.3), $p=0.045$) and β2-microglobuline (βI (95%CI): 656(90.4-1222), $p=0.025$).

Immune cellular subsets, thymic function, inflammatory status, immunosuppression therapy and age before vaccination could be relevant for the design of future trials aiming to optimize the COVID-19 vaccination protocol with mRNA vaccines in KTR.

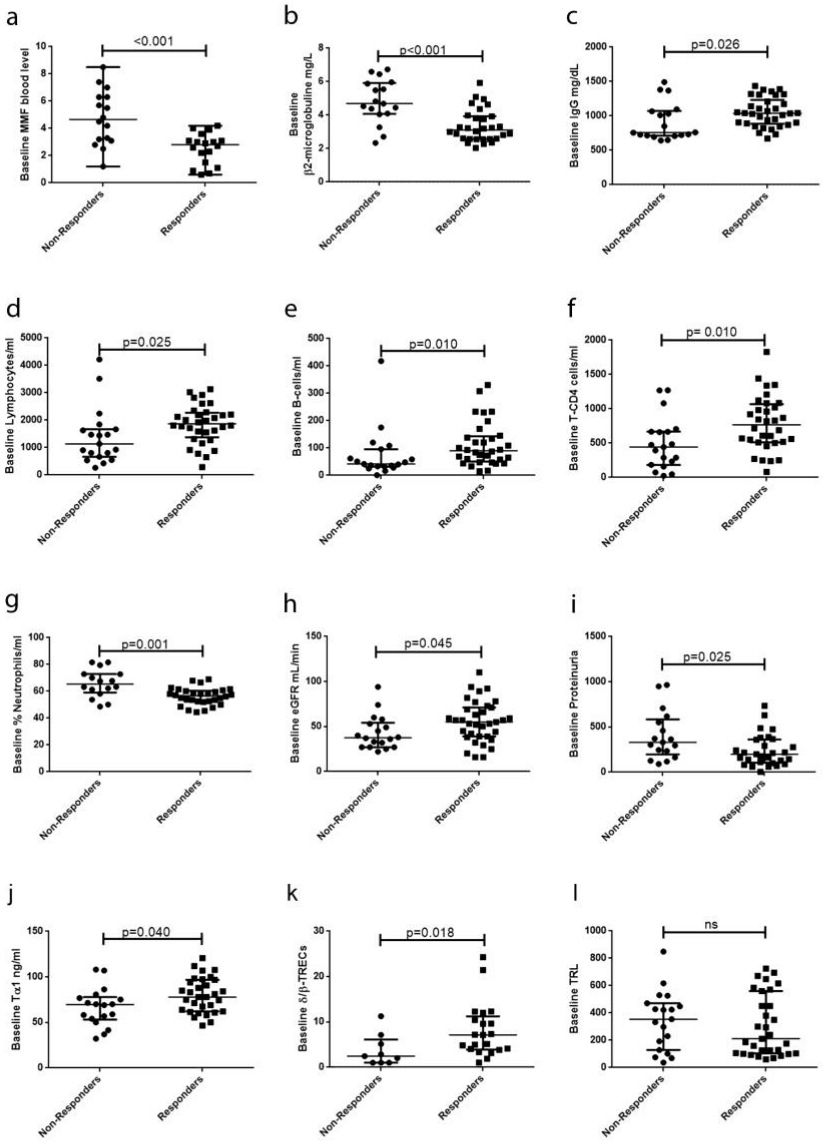


Figure 1: Analysis of predictive factors between responder and non-responde KTR.
Plots show comparisons of baseline levels of a) mycophenolate, b) β-2 microglobulin, c) IgG, d) lymphocytes, e) B-cells, f) T-CD4 cells, g) Neutrophils, h) eGFR, i) Proteinuria, j) Thymosine-α-1, k) δ/β-TRECs, l) TLR, between positive vs. negative KTR for SARS-CoV-2 Trimeric-S IgG detection. Parameters were compared using a U-Mann Whitney test and those with a p-value <0.050 were considered statistically significant.
eGFR: estimated glomerular filtration rate, TLR: telomere relative length.



P-274

RESPUESTA HUMORAL A VACUNAS EN PACIENTES CON INMUNODEFICIENCIAS. COMPARACIÓN DE VACUNAS FRENTE A SARS-COV-2, DIFTERIA, TÉTANOS Y PNEUMOCOCO

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Objetivos:

Valorar la respuesta humoral a vacunas frente SARS-CoV-2 en comparación con la respuesta a otras vacunas en pacientes de la consulta de inmunodeficiencias mediante un estudio observacional retrospectivo descriptivo.

Materiales y métodos:

Han sido estudiados 129 pacientes, divididos en 6 grupos diagnósticos: Déficit de IgA (1), Déficit de IgA y subclases de IgG (2), Disminución de IgG y subclases (3), Hipogammaglobulinemia (4), Hipogammaglobulinemia secundaria a Rituximab (5) e Inmunodeficiencia Combinada Variable (IDCV) (6). Todos han sido vacunados con Ditanrix frente a antígenos proteicos de Difteria y Tétanos, Pneumovax®23 y/o Prevenar-13® frente a Pneumococo y entre 1 y 4 dosis de las vacunas: Comirnaty y/o COVID-19 Vaccine Moderna de ARNm, y/o COVID-19 Vaccine AstraZeneca suspension inyectable (multidosis) de ADN frente a SARS-CoV-2. El estudio serológico de respuesta para las vacunas de Difteria, Tétanos y Pneumococo fue realizado a los 28 días tras la administración, mientras que para el SARS-CoV-2 se realizó entre 10 y 180 días tras la vacunación. Para el diagnóstico serológico se utilizaron kits de cuantificación de IgG específica en cada caso.

Resultados:

Los pacientes pertenecientes a los grupos 1-4 presentaron una adecuada respuesta humoral tras la administración de vacunas conjugadas, proteicas, y SARS-CoV-2. En cambio, los pacientes de los grupos 5 y 6 presentan respuesta humoral nula o insuficiente ante vacunas conjugadas o proteicas en el 70-75% de los casos, sin embargo, tras la vacunación con ARNm y/o ADN frente a SARS-CoV-2, el 70-75% de los pacientes han desarrollado una respuesta adecuada.

Conclusiones:

Gran parte de los pacientes que no responden a vacunas conjugadas o proteicas, han respondido adecuadamente a vacunas frente a SARS-CoV-2 (de ARNm y/o ADN). Esto podría ser debido al tipo de vacuna, lo que sugeriría una posible nueva vía de inmunización para este tipo de pacientes.



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