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Volumen 42
Suplemento 1

Inmunología



Publicación oficial

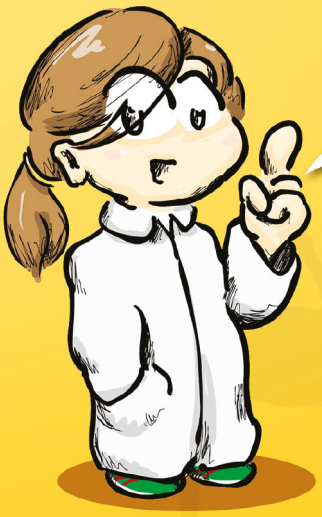
SEI
Sociedad Española
de Inmunología

Programa científico

Libro de abstracts

SEI
Sociedad Española de Inmunología

¿Por qué hacerse SOCIO de la SEI?



Estudiante Predoctoral

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

Para los que escogen la Especialidad de Inmunología, la SEI es la única asociación que defiende nuestros intereses y lucha para que se cree el ÁREA DE CAPACITACIÓN ESPECÍFICA y que nuestra especialidad tenga FUTURO.



Residente de Inmunología

Entra en www.inmunologia.org en la sección HACERSE SOCIO.
Si eres profesional de la Inmunología, introduce tus datos y el nombre e-mail de un socio en activo de la SEI que te avale. Nosotros contactaremos con el socio de referencia. Si no conoces socios en activo, introduce como socio "SEI" y el e-mail "gestionSEI@inmunologia.org" y nosotros te buscaremos referencia.

Inmunología

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1. *Descarga las instrucciones a los autores aquí.*
2. *Puedes contactar directamente con los coordinadores pulsando en el enlace en su nombre al inicio de cada sección o subsección.*
3. *Para comentarios, sugerencias o información sobre cómo anunciarse, contacta con la Secretaría Técnica aquí.*

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PATROCINADORES

















































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CARTA DE BIENVENIDA

Kide agurgarriak

Estimados compañeros/as

Es un verdadero placer daros la bienvenida al 44 Congreso de la Sociedad Española de Inmunología (SEI) que se celebrará en Bilbao del 10 al 13 Mayo del 2023, en el Palacio Euskalduna. Estamos trabajando con una gran ilusión y mucho esmero con el objetivo de tener un gran encuentro científico, en el que estamos dando un valor especial a las generaciones más jóvenes que vienen empujando fuerte. En el Congreso se abordarán los aspectos de investigación básica, traslacional y clínica de nuestra disciplina y, para ello, se está elaborando un programa de calidad que incorpore los avances y conocimientos en Inmunología generados en el último año.

Os animamos a participar activamente mediante el envío de comunicaciones que den a conocer el trabajo que estáis realizando en vuestros grupos de investigación y en vuestros Servicios de Inmunología. Sin duda, tendremos la oportunidad de aprender los unos de los otros y disfrutar del reencuentro con los colegas y amigos/as, así como establecer nuevos contactos que puedan traducirse en nuevas colaboraciones.

La villa de Bilbao con su gastronomía, su cultura y su gente acogedora es el entorno perfecto para la celebración de nuestro Congreso y otra garantía para conseguir que el 44 Congreso de la SEI sea un éxito. Los y las bilbaínas os harán sentir como en casa. En breve, y a través de la página web, iréis disponiendo de toda la información referente al congreso.

Bilbon itxaroten zaituztegu

Os esperamos a todos/as en Bilbao.

Marcos Lopez-Hoyos
Presidente de la SEI



COMITÉS

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Yvelise Barrios del Pino
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Francisco Boix Giner

INFORMACIÓN GENERAL

SEDE DEL CONGRESO

Palacio de Euskalduna Jauregia
Abandoibarra Etorb., 4
48011 Bilbao, Vizcaya (España)

ORGANIZA

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Telf.: +34 976 435 857
sei2023@pacifico-meetings.com

HORARIO DE APERTURA DE LA SECRETARÍA TÉCNICA:

- **Miércoles 10 de mayo:** de 14:30h a 20:00h.
- **Jueves 11 de mayo:** de 08:30h a 20:30h.
- **Viernes 12 de mayo:** de 08:00h a 19:00h.
- **Sábado 13 de mayo:** de 08:30h a 14:00h.

ENTREGA DE DOCUMENTACIÓN

La entrega de documentación se efectuará mediante la presentación de la '*Entry card*' o presentación del bono de confirmación de su inscripción enviado vía e-mail por la secretaría técnica en horario de apertura de la secretaría durante el congreso. 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 16 de mayo** (una vez finalizado el congreso), a través del enlace que se publicará en la web: www.sei2023.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 una sala habilitada donde podrán entregar 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 Euskalduna Jauregia.

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.

SALIDA DE AUTOBUSES CENA DE GALA

Viernes 12 de mayo de 2023

20:30. Salida de autobuses desde la sede (Palacio Euskalduna).

21:00. Comienzo de la cena en la Terminal de Cruceros de Bilbao.

APP



Descargue la APP a través del código QR.

PLANO DE LA SEDE DEL CONGRESO

Planta 0

Salas principales del congreso

Sala 0B
Sala 0D
Sala 0C
Sala 0A

Exposición comercial

Zona póster

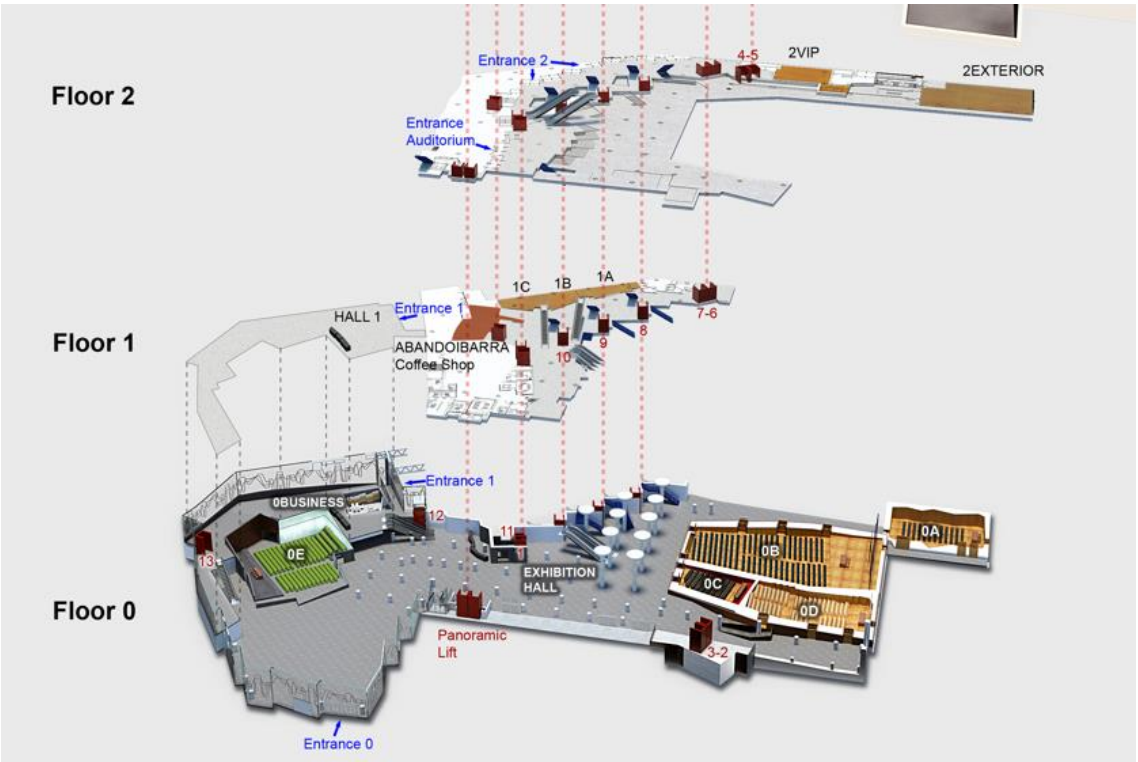
Planta 1

Secretaría técnica

Mostrador de recepción de ponencias

Planta 2

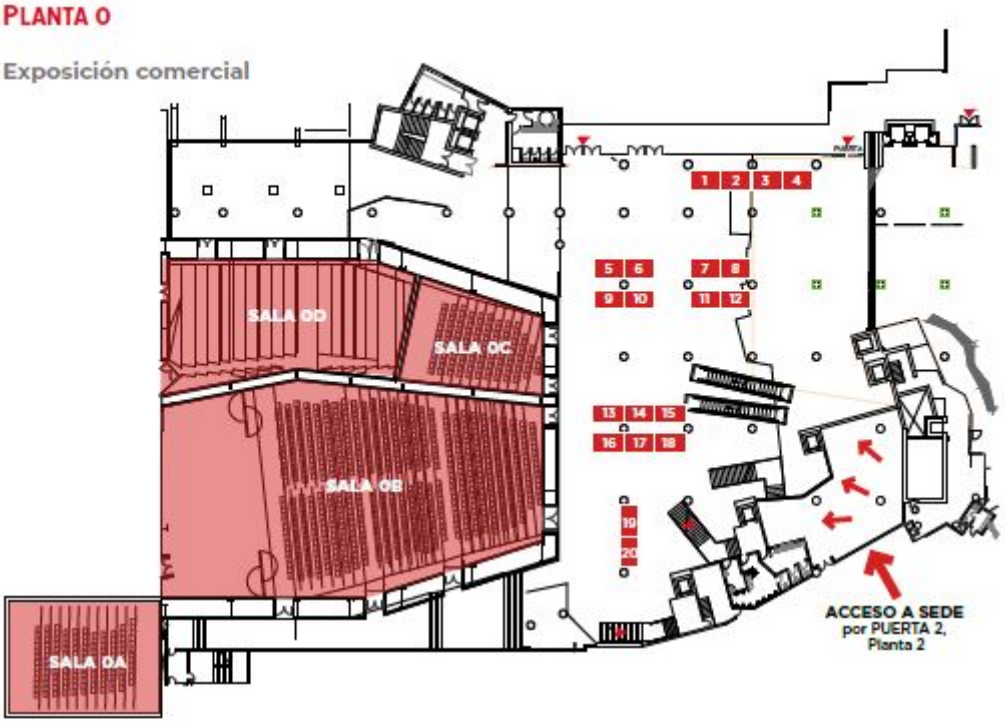
Entrada principal, acceso a la sede



EXPOSICIÓN COMERCIAL. 1-2-3-4 POSTER HALL

PLANTA 0

Exposición comercial



STAND	EMPRESA	STAND	EMPRESA
6	BONSAILAB	13	STEMCELL
7	Telstar	14	sysmex
8	werfen	15	EUROIMMUN
9&10	Palex	16	BD
		17	MABTECH
11	BECKMAN COULTER Life Sciences	18	LONGWOOD
12	moderna	19	Miltenyi Biotec

CRONOGRAMA GENERAL

Programa sujeto a modificaciones

CRONOGRAMA GENERAL

MIERCOLES 10 DE MAYO DE 2023				
14:30h	RECOGIDA DE CREDENCIALES Y DOCUMENTACIÓN			
	SALA 0B	SALA 0D	SALA 0C	SALA 0A
16:00 – 17:00h	Simposio satélite 1			
17:00 – 18:15h	LO MEJOR DEL AÑO (I)	RICORS TERAV Innovación española en Terapias Avanzadas.	REUNIÓN AEH	
18:15 – 18:45h	PAUSA CAFÉ			
18:45 – 19:00h	LO MEJOR DEL AÑO (II)	RICORS TERAV Innovación española en Terapias Avanzadas.	TALLER GIPSEI	
19:00 - 20:00h				

JUEVES 11 DE MAYO DE 2023				
	SALA 0B	SALA 0D	SALA 0C	SALA 0A
08:30 – 09:30h	PLENARIA 1: INMUNOTERAPIA			
09:30 – 10:30h	ACTO DE AUTORIDADES			
10:30 – 11:00h	PAUSA CAFÉ			
11:00 – 12:00h	SESIÓN 1: TRASPLANTE	SESIÓN 2: MICROBIOMA Y ENFERMEDAD	SESIÓN 3: COVID-19	
12:00 – 13:00h	PLENARIA 2: AUTOINMUNIDAD			
13:00 – 14:00h	Simposio satélite 4	Simposio satélite 5	Simposio satélite 6	Simposio satélite 7
14:00 – 15:00h	Simposio satélite 8	Simposio satélite 9	Simposio satélite 10	REUNIÓN GISEI
15:00 – 16:00h	VISITA A POSTERS, CAFÉ, COMIDA Y NETWORKING			REUNIÓN GEIE
16:00 – 17:30h	SOCIEDAD FRANCESA DE INMUNOLOGÍA			
17:30 – 17:45h	PAUSA			
17:45 – 19:15h	SESIÓN 4: AUTOINMUNIDAD E INFLAMACIÓN	CASOS CLÍNICOS ID	ASAMBLEA DE USUARIOS DEL PROGRAMA GECLID-SEI	
19:15 – 20:30h	COMUNICACIONES ORALES: INMUNOTERAPIA I	COMUNICACIONES ORALES: AUTOINMUNIDAD	COMUNICACIONES ORALES: MISCELÁNEA (INMUNIDAD ANTI-TUMORAL/ALERGIAS Y REACCIONES DE HIPERSENSIBILIADAD/VACUNAS)	

VIERNES 12 DE MAYO DE 2023				
	SALA 0B	SALA 0D	SALA 0C	SALA 0A
08:00 – 09:30h	SOCIEDAD PORTUGUESA DE INMUNOLOGÍA	COMUNICACIONES ORALES: INMUNIDAD FRENTE A INFECCIONES		
09:30 – 10:30h	PLENARIA 3. INMUNOLOGÍA TUMORAL			
10:30 – 11:30h	SESIÓN 5: CÉLULAS DEL SISTEMA INMUNITARIO	SESIÓN 6: INMUNODEFICIENCIAS PRIMARIAS	SESIÓN 7: INVESTIGADORES JUNIOR	
11:30 – 12:00h	PAUSA CAFÉ			
12:00 – 13:00h	PLENARIA 4. B CELLS			
13:00 – 14:00h	Simposio satélite 11	Simposio satélite 12	Simposio satélite 13	Simposio satélite 14
14:00 – 15:00h	Simposio satélite 15	Simposio satélite 16	Simposio satélite 17	
15:00 – 16:00h	VISITA A POSTERS, CAFÉ, COMIDA Y NETWORKING		REUNIÓN GEIT	REUNIÓN TALLER AUTOINMUNIDAD
16:00 – 17:15h	SESIÓN 8: INMUNOTERAPIA DEL CÁNCER	COMUNICACIONES ORALES: INFLAMACIÓN/ INMUNIDAD INNATA	COMUNICACIONES ORALES: TRASPLANTE	REUNIÓN GEAI
17:15 – 17:30h	PAUSA			
17:30 – 19:00h	SESIÓN 9: INMUNOLOGÍA DE LA INFECCIÓN	COMUNICACIONES ORALES: INMUNOTERAPIA II	REUNIÓN GETHI Y TALLER GETHIT	CASOS CLÍNICOS AI
19:00 – 20:00h	ASAMBLEA GENERAL SEI			

SÁBADO 13 DE MAYO DE 2023				
	SALA 0B	SALA 0D	SALA 0C	SALA 0A
08:30 – 09:30h	REUNIÓN GECIT	REUNIÓN GESEI	REUNIÓN GJSEI	
09:30 – 10:30h	PLENARIA 5: MYELOID CELLS IN HOMEOSTASIS AND DISEASE			
10:30 – 11:30h	COMUNICACIONES ORALES: INMUNODEFICIENCIAS: PRIMARIAS Y SECUNDARIAS	COMUNICACIONES ORALES: INMUNOLOGÍA BÁSICA	COMUNICACIONES ORALES: INMUNOGENÉTICA	
11:30 – 12:00h	PAUSA CAFÉ			
12:00 – 13:00h	SESIÓN 10: DEL PRESIDENTE DEL CONGRESO	SESIÓN 11: CHEMICAL IMMUNOLOGY	SESIÓN 12: DIVULGACIÓN	
13:00 – 14:00h	PLENARIA 6: GÉNERO Y DIVERSIDAD			
14:00 – 14:30h	ACTO FIN DE CONGRESO Y ENTREGA DE PREMIOS			

PROGRAMA

Programa sujeto a modificaciones

MIÉRCOLES 10 DE MAYO DE 2023

14:30h RECOGIDA DE CREDENCIALES Y DOCUMENTACIÓN

16:00 – 17:00h SIMPOSIOS SATÉLITE

Simposio satélite 1
SALA 0B

SIMPOSIUM: RETOS Y SOLUCIONES EN TERAPIAS CELULARES AVANZADAS.

Fabricación de terapias celulares avanzadas basadas en linfocitos de memoria específicos anti-virus enriquecidos con la plataforma CliniMACs Prodigy.
Dr. Óscar M. Pello.
Fundación Marqués de Valdecilla, Cantabria.

Inmunoterapia celular en cáncer ¿Hay algo que desde Miltenyi Biotec podamos hacer?
Dr Jose Luis Mateos Sanz.
Director de Área Clínica, Miltenyi Biotec España y Portugal.

17:00 – 19:00h RICORS TERAV
SALA 0D

INNOVACIÓN ESPAÑOLA EN TERAPIAS AVANZADAS

17:00 - 17:20h
Qué es la RED RICORS/TERAV: composición y objetivos.
Dr. José María Moraleda Jiménez.
Coordinador de TERAV.

17:20 - 17:40h
PROGRAMA 1. Terapia génica: Situación de partida y últimos avances.
Dr. Francisco Martin Molina.
Coordinador del programa.

17:40 - 18:00h
PROGRAMA 2. Inmunoterapia y terapias CAR: Situación de partida y últimos avances.
Dr. Manel Juan Otero.
Coordinador de programa.

18:00 - 18:20h
PROGRAMA 3. Terapia celular somática y medicina regenerativa. Situación de partida y últimos avances.
Dr. Fermín Sánchez-Guijo.
Coordinador de programa.

18:20 - 18:40h Pausa

18:40 - 19:00h
PROGRAMA 4. Desarrollos tecnológicos y terapéuticos: innovación, transferencia al sistema sanitario y educación. Situación de partida y últimos avances.

- Dra. Nuria Montserrat. *Coordinadora de programa.*
- Dra. Anna Sureda. *Coordinadora de programa.*

19:00 - 19:20h
Desarrollo de una terapia CAR-NK.
Dra. Cristina Eguizabal.

19:20 - 19:40h
Organoides y biomodelos en el desarrollo de terapias avanzadas.
Dra. Nuria Montserrat.

19:40 - 20:00h
Plataformas de producción celular y viral. ensayos clínicos de la red TERAV.
Dra. María Eugenia Fernández Santos.

20:00h Clausura

17:00 – 18:15h LO MEJOR DEL AÑO (I)
SALA 0B

Moderadores:

- Dra. África González Fernández. *Centro de Investigaciones biomédicas. Univerdidad de Vigo. Vigo, Pontevedra.*

- Dr. Rafael Sirera Pérez. *Universidad Politécnica de Valencia, Valencia.*

Inmunoterapia: cáncer y más allá.
Dr. Pedro Berraondo López.
Cima Universidad de Navarra, Pamplona.

Memoria: no B no T. Función de los progenitores hematopoyéticos en la inmunidad entrenada.
Dra. María Luisa Gil Herrero.
Universidad de Valencia, Valencia.

REUNIÓN AEH

SALA 0C

Ponentes:

- Dra. Sara Calleja Antolín. *Sanidad de Castilla y León (SACYL), Valladolid.*
- Dr. Roger Colobran Oriol. *Hospital Universitario Vall d'Hebron, Barcelona.*
- Alberto López Lera. *Hospital Universitario La Paz, Madrid.*
- Jaume Pons de Ves. *Hospital Universitario Son Espases, Mallorca.*

1. **Diagnóstico de laboratorio**
2. **Diagnóstico genético**
3. **Seguimiento clínico**

18:15 – 18:45h **PAUSA CAFÉ**

18:45 – 20:00h **LO MEJOR DEL AÑO (II)**

SALA 0B

Moderadores:

- Dra. África González Fernández. *Centro de Investigaciones biomédicas de la Universidad de Vigo, Vigo.*
- Dr. Rafael Sirera Pérez. *Universidad Politécnica de Valencia, Valencia.*

Respuesta inmunitaria y COVID.
Dr. Alfredo Corell Almuzara.
Catedrático de Inmunología. Universidad De Valladolid, Valladolid.

Inmunopatobiología fúngica: ¿Pueden los hongos favorecer la aparición o el desarrollo de cáncer?
Dr. Andoni Ramírez García.
Universidad del País Vasco / Euskal Herriko Unibertsitatea (UPV/EHU), Leoia.

TALLER GIPSEI

SALA 0C

Presentación del comité gestor.
Dr. Manuel Hernández González.
Hospital Universitario Vall d'Hebron, Barcelona.

La razón de ser del grupo.
Dra. Esther Moga.
Hospital de la Santa Creu i Sant Pau, Barcelona.

Objetivos 2023.
Dr. Juan Irure Ventura.
Hospital Universitario Marqués de Valdecilla, Santander.

Resultados de la encuesta a los miembros de la SEI sobre: "Uso de las citocinas en la clínica".
Dr. Joan Climent Martí.

Hospital Universitario Germans Trias i Pujol, Badalona.

Turno abierto de palabras.

JUEVES 11 DE MAYO DE 2023

08:30 – 09:30h PLENARIA 1: INMUNOTERAPIA

SALA 0B

Moderador: Dr. Francisco Borrego Rabaso. *Instituto de investigación sanitaria Biocruces, Barakaldo.*

Novel NK cell engineering strategies for enhanced immunotherapy.
Dr. Romee Rizwan.
Harvard Medical School, USA.

09:30 – 10:30h ACTO DE AUTORIDADES

SALA 0B

Presentación:
- Dr. Francisco Borrego Rabaso. *Presidente del Comité Organizador Local.*
- Dr. Marcos López Hoyos. *Presidente de la Sociedad Española de Inmunología (SEI).*

Keynote speaker:
La Inmunología en la Sociedad Actual.
Dra. África González Fernández.
Centro de Investigaciones biomédicas de la Universidad de Vigo. Vigo, Pontevedra.

10:30 – 11:00h PAUSA CAFÉ

11:00 – 12:00h SESIÓN 1: TRASPLANTE

SALA 0B

Moderador: Dr. Francisco Boix Giner. *Centro de Transfusión de Valencia, Valencia.*

Importancia del genotipaje HLA y KIR en la elección del mejor donante haploidéntico.
Dr. Rafael González Fernández.
Hospital Universitario Reina Sofía, Córdoba.

Contribución de células T cooperadoras foliculares a la respuesta a virus y aloantígenos en el paciente trasplantado.
Dra. Estela Paz Artal.
Hospital 12 de octubre, Madrid.

SESIÓN 2: MICROBIOMA Y ENFERMEDAD

SALA 0D

Moderadora: Dra. Leticia Abecia. *Universidad del País Vasco / Euskal Herriko Unibertsitatea (UPV/EHU), Leoia.*

Dra. Yolanda Sanz.
IATA-CSIC (Consejo Superior de Investigaciones Científicas), Valencia.

Microbiota in early life skin barrier function.
Dra. Mercedes Gómez de Agüero.
Universidad de Würzburg, Alemania.

SESIÓN 3: COVID-19
SALA 0C

Moderador: Dr. Ezequiel Ruiz-Mateos. *Instituto de Biomedicina de Sevilla, Sevilla.*

Specific myeloid cell subsets and pathogenic inflammatory innate responses associated with severe COVID-19.
Dr. Enrique Martín Gayo.
Hospital Universitario de la Princesa, Madrid.

Impairment of antiviral immune responses by SARS-COV-2 accessory proteins.
Dra. María Montoya González.
Centro de Investigaciones Biológicas (CIB - CSIC), Madrid.

12:00 – 13:00h PLENARIA 2: AUTOINMUNIDAD
SALA 0B

Moderadora: Dra. Eva Martínez-Cáceres. *Hospital Universitario Germans Trias i Pujol, Badalona.*

Impacto de las infecciones en la patología autoimmune.
Dra. Silvia Bolland.
NIH, USA.

13:00 – 14:00h SIMPOSIOS SATÉLITE

Simposio satélite 4
SALA 0B

Evolution of HLA antibody detection, from antigen to epitope Interpreting antibody data, balancing risk and opportunity.
Dr. Sean Carey.
Watershed Hydrology Group, Reino Unido.

Simposio satélite 5
SALA 0D

Explorando la inmunodeficiencia: aplicaciones y desafíos para la citometría de flujo.
Dra. Catarina Martins.
NOVA Medical School, Lisboa.

Simposio satélite 6
SALA 0C

El enigma de las Inmunodeficiencias Secundarias: nuevos biomarcadores.

Moderadora: Dra. Esmeralda Neves. *Centro Hospitalar Universitário Porto, Portugal.*

- Dra. Yvelise Barrios. *Hospital Universitario de Canarias, Santa Cruz de Tenerife.*
- Dra. Silvia Sánchez-Ramón. *Hospital Clínico San Carlos, Madrid.*

Simposio satélite 7
SALA 0A

Moderador: Dr. Alfredo Rodríguez-Antigüedad. *Hospital Universitario de Basurto, Bilbao.*

Neurofilamentos y neurodegeneración.
Dra. María Luisa Villar Guimerans.
Hospital Universitario Ramón y Cajal, Madrid.

Microbiota.
Dr. David Otaegui.
Instituto Biodonosti, San Sebastián.

Investigación propia en microvesículas.

- Dr. Alvaro Prada Iñurrategui. *Hospital Universitario de Donostia, San Sebastián.*
- Dra. Tamara Castillo. *Hospital Universitario Donostia, San Sebastián.*

14:00 – 15:00h SIMPOSIOS SATÉLITE

Simposio satélite 8
SALA 0B

DESENSIBILIZACIÓN EN TRASPLANTE RENAL EN PACIENTES HIPERINMUNIZADOS

Moderadores:
- Dra. Paca González Escribano. *Hospital Universitario Virgen del Rocío, Sevilla.*
- Dr. Rafael González Fernández. *Hospital Universitario Reina Sofía, Córdoba.*

Inmunoterapia celular en trasplante: uso de células T-CHAR para la eliminación específica de linfocitos B aloreactivos.
Dr. Eduard Palou.
Hospital Clinic, Barcelona.

Monitoring HLA antibodies Pre-Transplant Desensitization and Post-Transplant DSA Treatment.
Marcelo A. Fernández-Viña.
Stanford University, USA.

Simposio satélite 9
SALA 0D

Single Cell & Spatial Transciptomics para el estudio del sistema inmune.
Dra. Agnieszka Ciesielska.

PhD Science & Technology Advisor CEE & Southern Europe & Middle East & Africa, Distributors.

Simposio satélite 10
SALA 0C

Inmunometabolismo

- Dra. Miriam Contreras Mostazo. *Cell Analysis Product Specialist for Agilent XF, XB and XA product lines.*
- Dr. Alfredo Caro Maldonado. *Product Specialist of Agilent Seahorse (XF).*
- Dr. Igor Gak. *Field Application Scientist EMEA Region | Agilent BioTek (XB).*
- Dr. Gabriel Dammone. *Field Application Scientist Cell Analysis. Agilent xCELLigence and NovoCyte (XA).*

REUNIÓN GISEI
SALA 0A
1) Presentación del grupo GISEI y objetivos 2023.
2) Actualización del Registro Español de Inmunodeficiencias.
3) Propuestas líneas de trabajo.

14:00 – 16:00h **VISITA A POSTERS, CAFÉ, COMIDA Y NETWORKING**

SESIÓN POSTER DEFENSA 1

Pantalla 1. Autoinmunidad (I)
(P-001 a P-022)
Moderadores:
Dr. Álvaro Prada. *Hospital Universitario Donostia, San Sebastián.*
Dra. Arantza Pacho. *Hospital Universitario de las Cruces, Barakaldo.*

Pantalla 2. Autoinmunidad (II)
(P-023 a P-044)
Moderadores:
Dra. Esther Vergara Prieto. Complejo Hospitalario Universitario de Badajoz
Dr. Ricardo Rojo Amigo. Complejo Hospitalario Universitario A Coruña.

Pantalla 3. Inmunidad frente a las infecciones + Inmunodeficiencias (I)
(P-045 a P-068)
Moderadores:
Dr. Enrique Martín Gayo. *Hospital Universitario de la Princesa, Madrid.*
Dra. Carmen Cámara Hijón. *Hospital Universitario La Paz, Madrid.*

Pantalla 4. Inflamación + Inmunidad innata (I)
(P-069 a P-085)
Moderadores:
Dr. David Sancho. Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid.
Dr. Andoni Ramírez. Profesor Agregado, departamento de Inmunología, Microbiología y Parasitología. Universidad del País Vasco / Euskal Herriko Unibertsitatea (UPV/EHU), Leoia.

Pantalla 5. Miscelánea (Inmunidad anti-tumoral/Alergias y reacciones de hipersensibilidad/Vacunas) (I)
(P-086 a P-102)
Moderadores:
Dra. Alejandra Pera. Universidad de Córdoba, Córdoba.
Dr. Juan Irure. Hospital Universitario Marqués de Valdecilla, Santander.

Pantalla 6. Inmunoterapia
(P-103 a P-127)
Moderadores:
Dr. Asís Palazón. *Center for Cooperative Research in Bioscience (CIC bioGUNE), Derio.*
Dra. África González Fernández. *Centro de Investigaciones biomédicas. Universidad de Vigo. Vigo, Pontevedra*.

15:00 – 16:00h REUNIÓN GEIE
SALA 0A

GEIE. Visibilización de Enfermería en los Equipos Multidisciplinares. Liderando cuidados.
María Ángeles Escobar Palazón.
Hospital General Universitario Gregorio Marañón, Madrid.

Enfermera Pediátrica en Inmunodeficiencias. Más allá de la administración de Inmunoglobulinas.
Laura Ruiz López.
Hospital San Joan de Déu, Barcelona.

Humanización de los cuidados enfermeros en pacientes con IDCV.
- Beatriz Zazo Hernández. *Hospital Universitario de Getafe, Madrid.*
- Belén Carmona Sesmero. *Hospital Universitario de Getafe, Madrid.*

16:00 – 17:30h SOCIEDAD FRANCESA DE INMUNOLOGÍA
SALA 0B

Moderadores:
- Dr. Luis Fernández Pereira. *Hospital San Pedro de Alcántara, Cáceres.*
- Dr. Renato Moreira. *Institut national de la santé et de la recherche médicale, Francia.*

Retos del diagnóstico genético de las IDP. Nuevas etiologías genéticas en inmunodeficiencias combinadas.
Dra. Rebeca Pérez de Diego.
Instituto de Investigación Hospital Universitario La Paz, Madrid.

Deficiencia en IRF1 y predisposición a las infecciones por micobacterias en el hombre.
Dra. Jacinta Bustamante.
Université Paris Cité, Institut Imagine, Francia.

Development of inhibitors of CTP Synthase 1 for the treatment of lymphoproliferative disorders.
Dr. Andrew Parker.
Nifhtingale Hospital London, Reino Unido.

17:30 – 17:45h PAUSA

17:45 – 19:15h SESIÓN 4: AUTOINMUNIDAD E INFLAMACIÓN
SALA 0B

Moderadores:

- Dra. Mar Mendibe. *Universidad del País Vasco / Euskal Herriko Unibertsitatea (UPV/EHU), Leoia.*
- Dr. Alfredo Rodríguez-Antigüedad. *Hospital Universitario de Basurto, Bilbao.*

Terapia celular tolerogenica. Estrategia para el abordaje terapeutico de enfermedades autoinmunitarias.
Dra. Eva Martínez Cáceres.
Hospital Universitario Germans Trias i Pujol, Badalona.

Genetics of MS: multi-omic analysis of the region ANKRD55-IL6ST.
Dr. Koen Vandenbroeck.
Hospital Universitario de las cruces, Barakaldo.

70 años aprendiendo a usar los glucocorticoides en el lupus.
Dr. Guillermo Ruiz Irastorza.
Hospital Universitario de las cruces, Barakaldo.

CASOS CLÍNICOS ID
SALA 0D

Moderadores:
Dra. Carmen Cámara Hijón. *Hospital Universitario La Paz, Madrid.*
Dr. Roger Colobran Oriol. *Hospital Universitario Vall d'Hebron, Barcelona*

CCID-01

Defecto de ramificación de filamentos mediado POR ARP2/3 (deficiencia de ARPC1B): A propósito de un caso

Ferranti, Andrea Carolina; Vázquez Reyes, Paulina; Villegas Siles, Mildred Fabiola; Iguasnia Portilla, Daniel; Vergara Prieto, Esther; Fernández Pereira, Luis.

Complejo Hospitalario Universitario de Cáceres, Cáceres.

CCID-02

Identificación de una nueva mutación en el gen causante del síndrome IPEX (FOXP3) como causa de hidrops fetal y abortos de repetición

Gallardo García, Alberto; Marín Iglesias, Rosario; De La Varga Martínez, Raquel; Mora López, Francisco.

Hospital Universitario Puerta del Mar, Cádiz.

CCID-03

Síndrome de Griscelli tipo 2 en paciente adulto sin hipopigmentación

Lasa Lazaro, Maria[1]; Terol, María José[2]; Lopez Nevado, Marta[1]; Paz Artal, Estela[1]; Pleguezuelo, Daniel[1]; Allende, Luis M.[1].

[1]Hospital Universitario 12 de Octubre, Madrid; [2]Hospital Clínico de Valencia, Valencia.

CCID-04

Síndrome de Cogan y monocitosis en paciente con inmunodeficiencia común variable

López Cueto, María Fernanda; Pol Pol, Elisabet; Segura Guerrero, Marina; Julià Benique, María Rosa; Iglesias Alzueta, Julio F.; Pons De Ves, Jaime.

Servicio de Inmunología Hospital Universitario Son Espases, Palma de Mallorca.

CCID-05

¿Cuánto sabemos sobre las enfermedades asociadas a IKAROS? Un caso para pensar

Framil Seoane, Mario[1]; Bas Minguet, Jordi[1]; Colobran Oriol, Roger[2]; Esteve Solé, Ana[3]; Kuehn, Hye Sue[3]; Solanich Moreno, Xavier[1].

[1]Hospital Universitari de Bellvitge, L´Hospitalet de Llobregat; [2]Hospital Universitari Vall d´Hebrón, Barcelona; [3]National Institutes of Health, Bethesda.

CCID-06

Paciente con síndrome de deleción 22q11 y curso clínico atípico

González Torbay, Andrea; Cuesta Martín De La Cámara, Ricardo; Miguel Berenguel, Laura; Cámara Hijón, Carmen.

Hospital Universitario La Paz, Madrid.

ASAMBLEA DE USUARIOS DEL PROGRAMA GECLID-SEI

SALA 0C

Moderadora: Dra. Carmen Martín Alonso. *Centro de Hemoterapia y Hemodonación de Castilla y León, Valladolid.*

19:15 – 20:30h COMUNICACIONES ORALES: INMUNOTERAPIA I

SALA 0B

Moderadores:

- Dr. Asís Palazón. *Center for Cooperative Research in Bioscience (CIC bioGUNE), Derio.*
- Prof. Rafael Sirera Pérez. *Universidad Politécnica de Valencia, Valencia.*

CO-01

Characterization of the IGHA12/+ MOUSE: a quasi-monoclonal approach to study the atheroprotective antibody a12

Raposo Gutiérrez, Irene; Lorenzo Martín, Cristina; Rodríguez Ronchel, Ana; Rodríguez Ramiro, Almudena.

Centro Nacional de Investigaciones Cardiovasculares, Madrid.

CO-02

Treg cells are shaped by the tumor microenvironment to target specifically anti-tumor ΓΔ T cell subsets

Blanco-Domínguez, Rafael; Vaz Pinto Santos, André; Lopes, Noella; Condeço, Carolina; Mensurado, Sofia; Silva-Santos, Bruno.

Instituto de Medicina Molecular João Lobo Antunes, Lisboa.

CO-03

Intratumoral co-injections of NK cells and NKG2A neutralizing antibodies achieve efficacious anti-tumor responses in a manner dependent on CD8 T cell and CDC1.

Alvarez, Maite; Ochoa, Maria C; Molina, Carmen; Teijeira, Alvaro; Garasa, Saray; Sanchez-Gegrorio, Sandra; Berraondo, Pedro; Melero, Ignacio.

Centro de Investigación Medica Aplicada, Pamplona.

CO-04

Clinical status of chronic myelomonocytic leukemia patients with *KRAS* mutations present NLRP3 inflammasome activation and can be treated by IL-1 blocking therapy.

Hurtado Navarro, Laura[1]; Cuenca Zamora, Ernesto José[2]; Soler Espejo, Eva[2]; Such, Esperanza[3]; Bellosillo, Beatriz[4]; Zamora, Lurdes[5]; Hernández Rivas, Jesús María[6]; Marco Ayala, Javier[7]; Linares Latorre, Lola[8]; García Ávila, Sara[4]; Amat Martínez, Paula[9]; González, Teresa[6]; Arnan Sangerman, Montserrat[5]; Pomares Marín, Helena[5]; Hua Chen, Tzu[2]; Carreño, Gonzalo[10]; Jerez, Andrés[2]; Teruel Montoya, Raúl[2]; Ferrer Marín, Francisca[2]; Pelegrín, Pablo[1].

[1]Instituto Murciano de Investigación Biosanitaria (IMIB-Arrixaca), Murcia; [2]Hospital General Universitario Morales Meseguer, Centro Regional de Hemodonación, CIBERER, IMIB-Arrixaca, Murcia; [3]Hospital Universitario y

Politécnico La Fe, Valencia; [4]Hospital del Mar, IMIM, Barcelona; [5]Institut Català d'Oncologia (ICO), Badalona; [6]Complejo Asistencial Universitario de Salamanca, IBSAL, Salamanca; [7]Hospital Luis Alcanyis, Xátiva, Alicante; [8]Hospital General de Castellón, Castellón; [9]Hospital Clínico de Valencia, Valencia; [10]Hospital 12 de Octubre, Madrid.

CO-05

Memory features of CAR19-engineered natural killer cells as new immunomodulators of the safety and efficacy of CAR-NK based therapies.

Barriales San Miguel, Diego[1]; Herrera, Lara[1]; Etxebarria, Anne[1]; Anguita, Juan[2]; Juan, Manel[3]; Eguizabal, Cristina[1].

[1]CVTTH/IIS Biocruces Bizkaia, Galdakao; [2]CIC bioGUNE, Derio; [3]Hospital Clinic, Barcelona.

CO-06

New horizons through a multitarget approach: experience of a tertiary hospital with the use of dual cart 19/22 in B acute lymphoblastic leukemia (B-ALL)

Galán Gómez, Víctor; González Martínez, Berta; Mirones Aguilar, Isabel; Echecopar Parente, Carlos; Escudero López, Adela; Ferreras Puente, Cristina; Navarro Zapata, Alfonso; Mestre Durán, Carmen; Cobo, Marta; Pernas Sánchez, Alicia; Vallejo, Susana; De Paz Arias, Raquel; Minguillón Pedreño, Jordi; Martínez Romera, Isabel; Guerra García, Pilar; San Román Pacheco, Sonsoles; Pérez Martínez, Antonio.

La Paz University Hospital, Madrid.

CO-07

MRNAS encoding IL-12 and a decoy-resistant variant of IL-18 synergize to engineer T cells for efficacious intratumoral adoptive immunotherapy

Olivera, Irene[1]; Bolaños, Elixabet[1]; Gonzalez Gomariz, Jose[1]; Hervas Stubbs, Sandra[1]; Mariño, Karina V.[2]; Luri Rey, Carlos[1]; Etxeberria, Iñaki[1]; Cirella, Assunta[1]; Egea, Josune[1]; Glez Vaz, Javier[1]; Garasa, Saray[1]; Alvarez, Maite[1]; Eguren Santamaría, Iñaki[1]; Guedan, Sonia[3]; F. Sanmamed, Miguel[1]; Berraondo, Pedro[1]; Rabinovich, Gabriel A.[2]; Teijeira, Alvaro[1]; Melero, Ignacio[1].

[1]Centro de Investigación Médica Aplicada, Pamplona; [2]Instituto de Biología y Medicina Experimental (IBYME), Buenos Aires; [3]Institut d'Investigacions Biomèdiques August Pi iSunyer (IDIBAPS), Barcelona.

COMUNICACIONES ORALES: AUTOINMUNIDAD

SALA 0D

Moderadores:

- Dr. Álvaro Prada Iñurrategui. *Hospital Universitario Donostia, San Sebastián.*
- Dra. Arantza Pacho. *Hospital Universitario de las Cruces, Barakaldo.*

CO-08

The essential role of CCL17-producing inflammatory dendritic cells in lupus nephritis is uncovered by the suppressive effect of an infection with *plasmodium*

Amo, Laura[1]; Kole, Hemanta K[2]; Scott, Bethany[2]; Qi, Chen-Feng[2]; Wu, Monica[2]; Krymskaya, Ludmila[2]; Wang, Hongsheng[2]; Bolland, Silvia[2].

[1]Biocruces HRI, Barakaldo; [2]NIH, Maryland.

CO-09

RNA immunoprecipitation coupled with massive parallel sequencing for the identification of ribonucleoprotein autoantigens in systemic sclerosis

Perurena-Prieto, Janire[1]; Viñas-Giménez, Laura[1]; Sanz-Martínez, María Teresa[1]; Selva-O'callaghan, Albert[2]; Gordillo-González, Fernando[3]; Terrón-Camero, Laura C.[3]; Callejas-Moraga, Eduardo Luis[4]; Andrés-León, Eduardo[3]; Martín, Javier[3]; Colobran, Roger[1]; Guillén-Del-Castillo, Alfredo[2]; Simeón-Aznar, Carmen P.[2].

[1]Immunology Division, Vall d'Hebron University Hospital (HUVH), Vall d'Hebron Barcelona Hospital Campus, Barcelona; [2]Systemic Autoimmune Diseases Unit, Internal Medicine Department, Vall d'Hebron University Hospital (HUVH), Vall d'Hebron Barcelona Hospital Campus, Barcelona; [3]Institute of Parasitology and Biomedicine "López-Neyra", CSIC (IPBLN-CSIC), Granada; [4]Internal Medicine Department, Hospital Público de Monforte, Lugo.

CO-10

Crinosomes act as a major source of b-cell-derived proteins for peptide presentation by T1D-associated hla alleles

García Ayala, Manel[1]; Maggi, Jaxaira[2]; Auzmendi Aguirresarobe, Saioa[1]; Viñas Margalef, Xavier[1]; A. Arribas, Yago[3]; Carrascal, Montserrat[2]; Jaraquemada, Dolores[1]; Roura Mir, Carme[1].

[1]Institut de Biotecnologia i Biomedicina, Universitat Autònoma de Barcelona, Cerdanyola del Vallès; [2]CSIC/UAB Proteomics Laboratory, Facultat de Medicina, Universitat Autònoma de Barcelona, Cerdanyola del Vallès; [3]Inserm U932, Immunity and Cancer Unit, Curie Institute, París.

CO-11

frequency and referral patterns of neural antibody studies during the COVID-19 pandemic: experience from an autoimmune neurology reference center

Ruiz García, Raquel[1]; Ariño, Helena[1]; Rioseras, Beatriz[2]; Naranjo, Laura[1]; Martínez Hernandez, Eugenia[1]; Saiz, Albert[1]; Graus, Francesc[1]; Dalmau, Josep[1].

[1]Hospital Clínic De Barcelona, Barcelona; [2]Hospital Universitario Central de Asturias, Oviedo.

CO-12

Reduced expression of the transcriptional co-repressor CBFA2T3 in systemic lupus erythematosus t cells associates with nephritis and negatively correlates with disease activity and proteinuria

Pérez Lorenzo, María J; Suárez Fueyo, Abel; Galindo Izquierdo, María; García González, Javier; Criado Carrasco, Gabriel.

Hospital 12 de Octubre, Madrid.

CO-13

Discrepancias en la detección de anticuerpos anti-fibrilarina por diferentes técnicas comerciales y comparación con inmunoblot casero.

López Brull, Ángel[1]; Mariscal Rodríguez, Anaís[2]; Sánchez Pernaute, Olga[1]; Martínez-Becerra, María José[1]; Vegas-Sánchez, María Del Carmen[1].

[1]Fundación Jiménez Díaz, Madrid; [2]Hospital de la Santa Creu i Sant Pau, Barcelona.

CO-14

Fenotipo clínico de pacientes anti-KU positivos y su relación con otros autoanticuerpos asociados a polimiositis/dermatomiositis

Galván, Raquel; Olivas, Israel; González, Concepción; Romero, Pilar; León, Antonio.

Hospital Universitario Virgen Macarena, Sevilla.

COMUNICACIONES ORALES: MISCELÁNEA (INMUNIDAD ANTI-TUMORAL/ALERGIAS Y REACCIONES DE HIPERSENSIBILIADAD/VACUNAS)

SALA 0C

Moderadores:
- Dra. Alejandra Pera. *Universidad de Córdoba, Córdoba.*
- Dr. Juan Irure Ventura. *Hospital Universitario Marqués de Valdecilla, Santander.*

CO-15

Gold nanoparticles as platforms to prepare multivalent bacterial vaccines

Salcines Cuevas, David[1]; Zeoli, Andrea[2]; Teran Navarro, Hector[2]; Calvo, Jorge[2]; Perez-Del Molino, Concepcion[2]; Yañez Cuevas, Sonsoles[2]; Montoya, Noemi[3]; Ubeda, Maria[3]; Fresno, Manuel[4]; Marradi, Marco[5]; Alvarez Dominguez, Carmen[3].

[1]Universidad Internacional de La Rioja (UNIR), MEDONLINE group, Santande; [2]Instituto de Investigación Marqués de Valdecilla (IDIVAL), Santander; [3]Universidad Internacional de La Rioja (UNIR), MEDONLINE group, Logroño; [4]Centro de Biología Molecular "Severo Ochoa"-Universidad Autónoma de Madrid, Madrid; [5]Universidad degli Studi di Firenze (UNIFI), Florencia.

CO-16

Long-term adjuvant-induced transcriptional memory in hematopoietic stem and progenitor cells

Pasco, Samuel; Martín Ruíz, Itziar; Palacios, Ainhoa; Barriales, Diego; Seoane Alvarez, Iratxe; Castelo Careaga, Janire; Gutiez García, Naiara; Arajuo Aris, Sarai; Martin Rodriguez, Jose Ezequiel; Aransay, Ana M; Rodriguez, Hector; Anguita, Juan.

CIC bioGUNE, Derio.

CO-17

Humoral and cellular immunity to SARS-COV-2 after vaccination with MRNA vaccines in PWH with discordant immune response. Influence of the vaccine administered

López Cortés, Luis Fernando[1]; Saborido Alconchel, Abraham[1]; Trujillo Rodríguez, María[1]; Serna Gallego, Ana[1]; Llaves Flores, Silvia[1]; Muñoz Muela, Esperanza[1]; Pérez Santos, María J.[1]; Lozano, María Del Carmen[1]; Mejías Trueba, Marta[2]; Roca Oporto, Cristina[1]; Espinosa, Nuria[1]; Gutiérrez Valencia, Alicia[1].

[1]Division of Infectious Diseases, Microbiology and Parasitology. Biomedicine Institute of Seville/Virgen del Rocío University Hospital/CSIC/University of Seville, Sevilla; [2]Pharmacy Service. Virgen del Rocío University Hospital, Sevilla.

CO-18

Visualizing initial immune responses to liver metastasizing tumor cells

Pinci, Beatrice; Garasa, Saray; Manzanal, Almudena; Parrondo, Begoña; Luri, Carlos; Melero, Ignacio; Teijeira Sanchez, Alvaro.

CIMA, Universidad de Navarra, Pamplona.

CO-19

Estudio del papel de los neutrófilos en la inmunoestimulación frente ASARS-COV-2 con micropartículas de silicio mesoporoso (MSMPS)

López-Gómez, Ana[1]; Real Arévalo, Irene[2]; Juarez, Ignacio[1]; Vazquez Reyes, Sandra[3]; Garcia Culebras, Alicia[4]; Martín Palma, Raul[5]; Martínez Naves, Eduardo[1]; Gómez Del Moral, Manuel[4].

[1]Departamento de Inmunología, Oftalmología y ORL, Facultad de Medicina, Universidad Complutense de Madrid; [2]Inmunotek, Inmunotek; [3]Centro nacional de investigaciones Cardiovasculares, Centro nacional de investigaciones cardiovasculares; [4]Departamento de biología celular, Facultad de Medicina,

Universidad Complutense de Madrid; [5]Departamento de Física aplicada, Facultad de Ciencias, Universidad Autónoma de Madrid.

CO-20

Expansion of a decidual-like NK cell subset early after autologous hematopoietic stem cell transplantation in children with solid tumors

Astarloa-Pando, Gabirel[1]; Polanco-Alonso, Diego[1]; Orrantia, Ane[1]; Terrén, Iñigo[1]; Amarilla-Irusta, Ainhoa[1]; Pérez-Fernández, Silvia[1]; Pérez-Garay, Raquel[1]; Amo, Laura[1]; Arrese-Muñoz, Iria[2]; Sevilla, Arrate[1]; Sandá, Victor[1]; Lózpez-Pardo, Ainara[1]; Úriz, José J.[3]; Rey, Mercedes[4]; Astigarraga, Itziar[5]; González-Fernández, Rafael[6]; Zenarruzabeitia, Olatz[1]; Borrego, Francisco[1].

[1]IIS Biocruces Bizkaia, Barakaldo; [2]Immunology Division. Vall d'Hebron University Hospital,, Barcelona; [3]Biodonostia Health Research Institute, Service of Pediatrics, Donostia University Hospital, Donostia; [4]Biodonostia Health Research Institute, Service of Immunology, Donostia University Hospital,, Donostia; [5]Pediatric Oncology Group, Biocruces Bizkaia Health Research Institute, Service of Pediatrics, Cruces University Hospital, Barakaldo; [6]Clinical Management Unit of Immunology and Allergy, Reina Sofia Hospital, Córdoba.

CO-21

Desarrollo de un nuevo método para la predicción de epítopos lineales de linfocitos B basado en la similitud de secuencias

Ras Carmona, Álvaro[1]; Lehmann, Alexander A.[2]; Lehmann, Paul V.[2]; Reche, Pedro A.[1].

[1]Universidad Complutense de Madrid, Madrid; [2]Cellular Technology Limited (CTL), Ohio.

08:00 – 09:30h SOCIEDAD PORTUGUESA DE INMUNOLOGÍA

SALA 0B

Moderadores:

- Dr. Carlos Penha-Gonçalves. *Gulbenkian, Lisboa.*
- Dr. Balbino Alarcón. *Consejo Superior de Investigaciones Científicas (CSIC), Madrid.*

Tissue reprogramming of TLR4+ T cells in human inflammatory diseases.
Dra. Helena Soares.
Universidad de Nova, Lisboa.

Distinct role of antigen-specific T cell subsets in food allergy and tolerance induction.
Dr. Daniel Lozano Ojalvo.
Consejo Superior de Investigaciones Científicas (CSIC), Madrid.

Thymocyte self-renewal, autonomous thymopoiesis and leukemia.
Dra. Vera Martins.
Gulbenkian, Lisboa.

COMUNICACIONES ORALES: INMUNIDAD FRENTE A INFECCIONES

SALA 0D

Moderadores:

- Dr. Enrique Martín-Gayo. *Universidad Autónoma de Madrid, Madrid.*
- Dr. Manuel Hernández González. *Hospital Vall d'Hebron, Barcelona.*

CO-45

La presencia de candida albicans induce alteraciones metabólicas y moleculares en las células de melanoma B16-F10 relacionadas con un fenotipo tumoral más agresivo

Aparicio-Fernandez, Leire[1]; Antoran, Aitziber[1]; Iturbe, Santiago[1]; Areitio, Maialen[1]; Rodriguez-Ereñaga, Oier[1]; Martin-Souto, Leire[1]; Buldain, Idoia[1]; Benedicto, Aitor[1]; Márquez, Joana[1]; Arteta, Beatriz[1]; Pellon, Aize[2]; Moyes, David L.[2]; Aransay, Ana[3]; Macias-Cámara, Nuria[3]; Gonzalez, Monika[3]; Martin Rodriguez, Jose Ezequiel[3]; Anguita, Juan[3]; Rementeria, Aitor[1]; Ramirez-Garcia, Andoni[1].

[1]Universidad del País Vasco (UPV/EHU), Leioa; [2]King's College London, Londres; [3]CIC bioGUNE, Derio.

CO-46

Individually expressed SARS-COV-2 viroporin ORF3A alters inflammatory responses and cellular structure in lung epithelial cells

De Lucas Rius, Ana[1]; Zaldívar López, Sara[2]; Mendoza García, Laura[1]; Dies López-Ayllón, Blanca[1]; García García, Tránsito[2]; Redondo, Natalia[1]; Fernández Rodríguez, Raúl[2]; Suárez Cárdenas, José Manuel[2]; Jiménez Marín, Ángeles[2]; Oliva Blanco, María Ángela[1]; Garrido, Juan José[2]; Montoya González, María[1].

[1]Centro de Investigaciones Biológicas Margarita Salas, Madrid; [2]Universidad de Córdoba, Córdoba.

CO-47

Adoptive cell therapy with memory T lymphocytes to treat infectious diseases

Al-Akioui Sanz, Karima[1]; Ferreras, Cristina[1]; Martin Quirós, Alejandro[2]; Menéndez Ribes, Marta[1]; Navarro Zapata, Alfonso[1]; Mestre Durán, Carmen[1]; Clares Villa, Laura[1]; Minguillón, Jordi[1]; Echecopar Parente, Carlos[2]; Vicario, José Luis[3]; Balas, Antonio[3]; De Paz, Raquel[2]; Gasior, Mercedes[2]; López Granados, Eduardo[2]; Jiménez, Carlos[2]; Ramos, Esther[2]; Hernández Oliveros, Francisco[2]; Hernández Blanco, Clara[4]; Pérez Martínez, Antonio[2].

[1]IdiPaz, Madrid; [2]Hospital Universitario La Paz, Madrid; [3]Centro de transfusiones de la Comunidad de Madrid, Madrid; [4]Hospital Enfermera Isabel Zendal, Madrid.

CO-48

IL11 and fibrosis-related genes altered by the expression of SARS-COV-2 accessory proteins are present in whole virus-infected cells and COVID19 patient samples

Dies López-Ayllón, Blanca[1]; De Lucas Rius, Ana[1]; Mendoza García, Laura[1]; García García, Tránsito[2]; Fernández Rodríguez, Raúl[2]; Suárez Cárdenas, José Manuel[2]; Redondo, Natalia[1]; Pedrucci, Federica[1]; Zaldívar López, Sara[2]; Jiménez Marín, Ángeles[2]; Garrido, Juan José[2]; Montoya, María[1].

[1]CIB, Madrid; [2]UCO/IMIBIC, Córdoba.

CO-49

CD5-based adoptive cell transfer for systemic fungal infection

Velasco De Andrés, María[1]; Catalá, Cristina[1]; Carrillo Serradell, Laura[1]; Eitler, Jiri[2]; Krutz, Lydia[2]; Español, Marta[3]; Armiger Borrás, Noelia[1]; Pérez Amill, Lorena[4]; Suñe, Guillermo[4]; Puerta Alcalde, Pedro[5]; García Vidal, Carolina[5]; Urbano Ispizua, Álvaro[6]; Tonn, Torsten[7]; Martín Antonio, Beatriz[8]; Lozano Soto, Francisco[9].

[1]Fundació de Recerca Clínic Barcelona-Institut d'Investigacions Biomèdiques August Pi i Sunyer, Barcelona; [2]Experimental Transfusion Medicine, Faculty of Medicine Carl Gustav Carus, Dresden University of Technology, Germany, Dresden; [3]Servei d'Immunologia, Hospital Clínic de Barcelona, Barcelona; [4]Josep Carreras Leukaemia Research Institute,, Barcelona; [5]Infectious Disease Department, Hospital Clinic of Barcelona, Barcelona; [6]Servei de Hematologia, Hospital Clinic de Barcelona, Barcelona; [7]Experimental Transfusion Medicine,

Faculty of Medicine Carl Gustav Carus, Dresden University of Technology, Germany, Barcelona; [8]Department of Experimental Hematology, Instituto de Investigación Sanitaria-Fundación Jiménez Diaz (IIS-FJD), University Autonomous of Madrid (UAM), Madrid; [9]Fundació de Recerca Clínic Barcelona-Institut d'Investigacions Biomèdiques August Pi i Sunyer; Servei d'Immunologia, Hospital Clínic de Barcelona; Departament de Biomedicina, Facultat de Medicina, Universitat de Barcelona, Barcelona.

CO-50

Expansion of CD151+ NK cell subset correlates with COVID-19 severity

Amarilla-Irusta, Ainhoa[1]; Zenarruzabeitia, Olatz[1]; Sevilla, Arrate[2]; Terrén, Iñigo[1]; Astarloa-Pando, Gabirel[1]; Lopez-Pardo, Ainara[1]; Sandá, Víctor[1]; Arrese-Muñoz, Iria[3]; Pérez-Garay, Raquel[4]; Imaz-Ayo, Natale[5]; Arana-Arri, Eunate[5]; Amo, Laura[6]; Borrego, Francisco[6].

[1]Immunopathology Group, Biocruces Bizkaia Health Research Institute, Barakaldo; [2]Immunopathology Group, Biocruces Bizkaia Health Research Institute/Department of Genetics, Physical Anthropology and Animal Physiology, Faculty of Science and Technology, UPV/EHU, Barakaldo/Leioa; [3]Immunology Division, Vall d'Hebron University Hospital, Barcelona; [4]Immunopathology Group, Biocruces Bizkaia Health Research Institute/Clinical Analysis Service, University Hospital from Cruces, Barakaldo/Bizkaia (OSI Ezkerraldea-Enkarterri-Cruces); [5]Scientific Coordination Facility, Biocruces Bizkaia Health Research Institute, Barakaldo; [6]Immunopathology Group, Biocruces Bizkaia Health Research Institute/Ikerbasque, Basque Foundation for Science, Barakaldo/Bilbao.

CO-51

Combined dendritic cell stimulation and TIGIT blockade boosts adaptive NKG2C+ NK CELL agaisnt HIV-1

Sánchez-Cerrillo, Ildefonso[1]; Calvet-Mirabent, Marta[1]; Popova, Olga[1]; De Los Santos, Ignacio[1]; Sanz, Jesus[1]; García-Fraile, Lucio[1]; Fuentes, Patricia[2]; Alcain, Juan[2]; Tsukalov, Ilya[3]; Alfranca, Arantzazu[1]; Buzon, María Jose[4]; Toribio, María Luisa[2]; Muñoz-Fernandez, María Angeles[5]; Sánchez-Madrid, Francisco[1]; Martín-Gayo, Enrique[1].

[1]Hospital Universitario de La Princesa, Madrid; [2]Centro de Biología Molecular Severo Ochoa (CBM), Madrid; [3]Universidad Autónoma de Madrid (UAM), Madrid; [4]Hospital Universitari Vall d´Hebron, Barcelona; [5]Hospital Universitario Gregorio Marañon, Madrid.

CO-52

Association between high interferon-ɣ levels in response to avian tuberculin and candidate genes implicated in necroptosis and platelet activation

Alonso-Hearn, Marta[1]; Badia-Bringué, Gerard[1]; Vázquez, Patricia[1]; Garrido, Joseba M.[1]; Juste, Ramón A.[1]; Fernández, Almudena[2]; González-Recio, Oscar[2]; Canive, Maria[1].

[1]Department of Animal Health, NEIKER- Basque Institute for Agricultural Research and Development, Basque Research and Technology Alliance (BRTA), Derio, Bizkaia; [2]Departamento de Mejora Genética Animal, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), CSIC, Madrid.

CO-53

Dendritic cell-CD8+ T cell cooperation in lymphoid tissue is inversely associated to viral load and increases CD8+ T cell response in people living with HIV

Vitallé Andrade, Joana[1]; Bachiller, Sara[1]; Camacho-Sojo, María Inés[1]; Moysi, Eirini[2]; Dominguez-Molina, Beatriz[1]; Ferrando-Martínez, Sara[2]; López-Cortés, Luis Fernando[1]; Petrovas, Constantinos[3]; Koup, Richard[2]; Ruiz-Mateos, Ezequiel[1].

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09:30 – 10:30h PLENARIA 3. INMUNOLOGÍA TUMORAL

SALA 0B

Moderador: Dr. Asís Palazón. *Center for Cooperative Research in Bioscience (CIC bioGUNE), Derio.*

Immunological mechanisms of resistance to CDK4/6 inhibitors in HR+ breast cancer.
Dr. Lorenzo Galluzi.
Cornell Universit, USA.

10:30 – 11:30h SESIÓN 5: CÉLULAS DEL SISTEMA INMUNITARIO

SALA 0B

Moderador: Dr. Rafael Solana Lara. *Hospital Reina Sofía, Córdoba.*

How TCR knowledge derives in new immunomodulatory strategies/ Cómo el conocimiento sobre el TCR puede utilizarse en nuevas terapias inmunomoduladoras.
Dr. Balbino Alarcón Sánchez.
Consejo Superior de Investigaciones Científicas (CSIC), Madrid.

Harnessing NK cells for cancer immunotherapy.
Dra. Aura Muntasell.
Universitat Autònoma de Barcelona (UAB), Barcelona.

SESIÓN 6: INMUNODEFICIENCIAS PRIMARIAS

SALA 0D

Moderador: Dr. Óscar de la Calle Martín. *Hospital de la Santa Creu i Sant Pau, Barcelona.*

Single-cell genomics to study developmental immunology.
Dra. Cecilia Domínguez Conde.
Genomics Research Centre, Milán.

Single-cell analysis of common variable immunodeficiency shows epigenetic and transcriptional dysregulation during immune response.
Dr. Javier Rodríguez Ubreva.
Insituto de investigación Josep Carreras, Barcelona.

SESIÓN 7: INVESTIGADORES JUNIOR
SALA 0C

Moderadora: Dra. Ana Zubiaga Elordieta. *Universidad del País Vasco / Euskal Herriko Unibertsitatea (UPV/EHU), Leoia, Vizcaya.*

Of Innate immune cells in solid tumors: lessons learned from preclinical models to clinical settings.
Dra. María Casanova-Acebes.
Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid.

Understanding Tissue-Resident T cell ecology: from inflammation to cancer.
Dr. Miguel Muñoz Ruiz.
The Francis Crick Institute, Reino Unido.

11:30 – 12:00h **PAUSA CAFÉ**

12:00 – 13:00h **PLENARIA 4. B CELLS**
SALA 0B

Moderadora: Dra. Silvia Bolland. *NIH, USA.*

Antibodies meet the atheroma plaque: puzzles and perspectives.
Dra. Almudena Rodríguez Ramiro.
Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid.

13:00 – 14:00h **SIMPOSIOS SATÉLITE**

Simposio satélite 11
SALA 0B

COMPATIBILIDAD DONANTE-RECEPTOR BASADA EN EPÍTOPOS

Moderadores:
- Dr. Manuel Muro Amador. *Hospital Clínico Universitario Virgen de la Arrixaca, Murcia.*
- Dr. Jesús Ontañón Gómez. *Complejo Hospitalario universitario de Albacete, Albacete.*

Incompatibilidad molecular: ¿estamos preparados para la rutina clínica?

Dra. María Meneghini.
Hospital Universitario Vall D´Hebrón, Barcelona.

Novel Definition of HLA Antigens, their correlation with Eplets and evaluation of HLA mismatches.
Dr. Marcelo A. Fernández-Viña.
Stanford University, USA.

Simposio satélite 12
SALA 0D

PRIMARY CAUSES OF IMMUNE DYSREGULATION DISORDERS: THE ROLE OF THE GENETIC TESTING IN THE COMPLEX PROCESS OF SOLVING AUTOIMMUNE/AUTOINFLAMMATORY DISEASE

Moderadores:
Dr. José María García-Aznar. *Head of the Department-Immunogenetics division at Health in Code.*
Yvelise Barrios. MD, Immulogists. *Prof. of immunology. Universidad de La Laguna.*

The Role of Germline and Somatic Mutations in Monogenic Autoinflammatory Diseases.
Dra. Ivona Aksentijevich.
Head of Inflammatory Disease Section. Assoc Investigator, Medical Genetics Branch.

Hypomorphic defects in inborn errors of immunity: When infection meets autoimmunity/inflammation.
Dra. Elena Hsieh.
Department of Pediatrics, Section of Allergy and Immunology. Children's Hospital Colorado. Assoc Prof. School of Medicine, Department of Immunology and Microbiology. University of Colorado Denver.

Simposio satélite 13
SALA 0C

Aplicaciones del estudio del fenotipo T y B en la práctica diaria, defectos en anticuerpos u más allá.
Dra. Mónica Martínez Gallo.
Hospital Universitario Vall d'Hebron, Barcelona.

Utilidad de un Separador Celular en un Servicio de Inmunología Clínica.
Dr. Luis Allende.
Hospital 12 de octubre, Madrid.

Simposio satélite 14
SALA 0A

14:00 – 15:00h SIMPOSIOS SATÉLITE

Simposio satélite 15
SALA 0B

Mesa de Inmunidad Celular según perfiles de pacientes.

- Dra. Carmen Cámara Hijón. *Hospital Universitario La Paz, Madrid.*
- Dra. Estela Paz Artal. *Hospital 12 de octubre, Madrid.*
- Dr. Manuel Muro Amador. *Hospital Clínico Universitario Virgen de la Arrixaca, Murcia.*

Simposio satélite 16
SALA 0D

Retos en el diagnóstico y manejo de ADPS, una inmunodeficiencia primaria ultra rara.

- Visión del Inmunólogo: Dr. Eduardo García Granados. *Hospital Universitario La Paz, Madrid.*
- Visión del pediatra: Dr. Olaf Neth. *Hospital Virgen del Rocío, Sevilla.*

Simposio satélite 17
SALA 0C

Respuesta inmunitaria de las vacunas mRNA contra la Covid-19.

- Dr. José Gomez *Rial. Hospital Clínico Universitario de Santiago, Santiago de Compostela.*
- Dra. Gemma Moncunill Piñas. *Instituto de Salud Global, Barcelona.*

14:00 – 16:00h VISITA A POSTERS, CAFÉ, COMIDA Y NETWORKING

SESIÓN POSTER DEFENSA 2
Pantalla 1. Trasplante + Inmunogenética (I)
(P-128 a P-148)
Moderadores:
Dr. Marcos López-Hoyos. *Hospital Universitario Marqués de Valdecilla, Santander.*
Dra. Paula Arana Berganza. *Instituto de investigación sanitaria Biocruces, Barakaldo.*

Pantalla 2. Trasplante + Inmunogenética (II)
(P-149 a P-168)
Moderadores:
Dra. Nerea Olivares Beobide. *Hospital Universitario Cruces, Bilbao.*
Dr. Francisco Boix Giner. *Departamento de inmunología, Centro de Transfusión de Valencia, Valencia.*

Pantalla 3. Inmunidad frente a las infecciones + Inmunodeficiencias (II)
(P-169 a P-192)
Moderadores:

Dr. Manuel Hernández González. *Hospital Universitario Vall d'Hebron, Barcelona.*
Dra. Sara Calleja Antolín. *Sanidad de Castilla y León (SACYL), Valladolid.*

Pantalla 4. Inflamación + Inmunidad innata (II)
(P-193 a P-209)
Moderadores:
Dr. David Sancho. *Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid.*
Dr. Andoni Ramírez. *Profesor Agregado, departamento de Inmunología, Microbiología y Parasitología. Universidad del País Vasco / Euskal Herriko Unibertsitatea (UPV/EHU), Leoia*.

Pantalla 5. Miscelánea (Inmunidad anti-tumoral/Alergias y reacciones de hipersensibilidad/Vacunas) (II)
(P-210 a P-225)
Moderadores:
Dra. Alejandra Pera. *Universidad de Córdoba, Córdoba*.
Dr. Juan Irure. *Hospital Universitario Marqués de Valdecilla, Santander.*

Pantalla 6. Inmunología Básica
(P-226 a P-248)
Moderadores:
Dra. Raquel Tarazona Lafarga. *Universidad de Extremadura, Badajoz.*
Dr. Pedro Aparicio Alonso. *Universidad de Murcia, Murcia.*

15:00 – 16:00h REUNIÓN GEIT
SALA 0C

15:00 – 15:30h Reunión de trabajo: Desarrollo de nuevas iniciativas y actividades en el seno del GEIT.

15:30 - 16:00h Mesa redonda: Guía de monitorización de terapias CAR-T

REUNIÓN TALLER AUTOINMUNIDAD
SALA 0A

Organizado por el Servicio de Inmunología de Germans Trias i Pujol.

16:00 – 17:15h SESIÓN 8: INMUNOTERAPIA DEL CÁNCER
SALA 0B

Moderador: Dr. Manel Juan Otero. *Hospital Sant Joan de Déu, Barcelona.*

Influencia del microambiente tumoral en la inmunoterapia contra el cáncer: el rol de la hipoxia y ROS/ The influence of the tumor microenvironment in cancer immunotherapy: the role of hypoxia and ROS.
Dr. Asís Palazón.
Center for Cooperative Research in Bioscience (CIC bioGUNE), Derio.

Driving the development of next-generation CAR-T cel.

Dra. Sonia Guedan.
Hospital Clìnic, Barcelona.

COMUNICACIONES ORALES: INFLAMACIÓN/INMUNIDAD INNATA

SALA 0D

Moderadores:

- Dr. David Sancho Madrid. *Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid.*
- Dr. Andoni Ramírez García. *Profesor Agregado, departamento de Inmunología, Microbiología y Parasitología. Universidad del País Vasco / Euskal Herriko Unibertsitatea (UPV/EHU), Leoia.*

CO-38

The NLRP3 of cryopyrin-associated periodic syndromes forms a constitutively active inflammasome

Molina López, Cristina[1]; Hurtado Navarro, Laura[1]; Angosto Bazarra, Diego[1]; Tapia Abellán, Ana[2]; R. Marqués, Joana[3]; Bujan-Rivas, Segundo[3]; I. Arostegui, Juan[4]; Pelegrín, Pablo[1].

[1]Molecular Inflammation Group, Biomedical Research Institute of Murcia (IMIB), Murcia; [2]Present address: Interfaculty Institute for Cell Biology, Department of Immunology, University of Tubingen, Tubingen; [3]Department of Internal Medicine, Hospital Vall Hebron, Barcelona; [4]Department of Immunology, Hospital Clinic, Barcelona.

CO-39

Natural killer cell subsets in endometrial fluid: analysis of their association with the endometrial cycle and reproductive parameters

Herrera, Lara[1]; Martín-Inaraja, Myriam[1]; Bengoetxea, Ainara[2]; Vendrell, Alberto[3]; Pérez-Fernández, Silvia[3]; Eguizabal, Cristina[1]; Matorras, Roberto[2].

[1]CVTTH/IIS Biocruces Bizkaia, Galdakao; [2]Hospital Universitario de Cruces, Barakaldo; [3]IIS Biocruces Bizkaia, Barakaldo.

CO-40

The long-term immunomodulatory capacity of phloroglucinol, a microbiota derived metabolite, as treatment for acute colitis

Castelo, Janire[1]; Barriales, Diego[1]; Peña-Cearra, Ainize[2]; Seoane, Iratxe[2]; Palacios, Ainhoa[1]; Araujo, Sarai[1]; Atondo, Estibaliz[1]; Pasco, Sam T.[1]; Gutiez, Naiara[1]; Martín-Ruiz, Itziar[1]; Abecia, Leticia[2]; Anguita, Juan[1]; Rodríguez, Héctor[1].

[1]CiCbioGUNE, Derio; [2]EHU/UPV, Leioa.

CO-41

Calcineurin/NFAT pathway contributes to β-GLUCAN-induced trained immunity by epigenomic reprogramming of myeloid cells

Jarit Cabanillas, Aitor[1]; Dunphy, Gillian[1]; Galán, Miguel[1]; Robles, Iñaki[1]; Martínez, Sarai[1]; Gómez, Manuel J.[1]; Mañanes, Diego[1]; Quintas, Ana[1]; Mata, Pablo[2]; Bergón, Marta[2]; Negishi, Yutaka[3]; Mhlanga, Musa[3]; Redondo, Juan Miguel[1]; Del Fresno, Carlos[2]; Sancho, David[1].

[1]CNIC, Madrid; [2]IdiPaz, Madrid; [3]Radboud Institute for Molecular Life Sciences, Nijmegen.

CO-42

Single-cell analysis of the antibody immune response in PCSK9-AAV atherosclerotic MICE

Rodríguez-Ronchel, Ana; Raposo-Gutiérrez, Irene; Gómez-Escolar, Carmen; Del Monte-Monge, Alberto; Mur, Sonia M; Ramiro, Almudena R.

Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid.

CO-43

Inborn error of STAT2-dependent IFN-I immunity in a patient presented with hemophagocytic lymphohistiocytosis and multisystem inflammatory syndrome in children

López Nevado, Marta[1]; Sevilla, Julian[2]; Almendro Vázquez, Patricia[1]; Gil Etayo, Francisco Javier[1]; Garcinuño, Sara[1]; Serrano Hernández, Antonio[1]; Paz Artal, Estela[1]; González Granado, Luis Ignacio[1]; Allende, Luis Miguel[1].

[1]Hospital Universitario 12 de Octubre, Madrid; [2]Hospital Niño Jesús, Madrid.

CO-44

Two leukocytic cell lines deficient in membrane complement regulators as biosensors of abnormal complement deposition and their use for treatment efficacy

Megino, Rebeca F[1]; Del Prado, Claudia[1]; Marin, Ana V[1]; Cavero, Teresa[2]; Trujillo, Hernando[3]; Praga, Manuel[3]; Rodríguez De Córdoba, Santiago[4]; 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]Department of Nephrology, University Hospital 12 de Octubre, Madrid; [3]Department of Nephrology, University Hospital 12 de Octubre and Research Institute of University Hospital "12 de Octubre" (i+12), Madrid; [4]Center for Biological Research Margarita Salas and Center for Biomedical Network Research on Rare Diseases, Madrid.

COMUNICACIONES ORALES: TRASPLANTE

SALA 0C

Moderadores:

- Dr. Marcos López-Hoyos. *Hospital Universitario Marqués de Valdecilla, Santander.*
- Dra. Nerea Olivares Beobide. *Hospital Universitario Cruces, Bilbao.*

CO-31

Implications of hla mismatches at epitope level in clinical evolution of haploidentical stem cell transplantation with post-transplant cyclophosphamide

Gil Etayo, Francisco Javier[1]; Niño, Jairo Eduardo[1]; Terredillos Sánchez, Pilar[1]; Jiménez, Isabel[1]; Vicente, Ariadna[1]; Balanzategui, Ana[1]; Boix, Francisco[2]; Alcoceba, Miguel[1]; González De La Calle, Verónica[1]; Fonseca, Marta[1]; López Corral, Lucía[1]; Pérez, Estefanía[1]; Cabrero, Mónica[1]; Navarro, Almudena[1]; Sánchez-Guijo, Fermín[1]; González, Marcos[1]; García Sanz, Ramón[1]; Tejeda Velarde, Amalia[1].

[1]Hospital Universitario de Salamanca, Salamanca; [2]Centro de Transfusiones de la Comunidad Valenciana, Valencia.

CO-32

Expanding the applications of NGS-based HLA typing: HLA loss relapse detection after hematopoietic stem cell transplantation

Vera, Elsie[1]; Mantecon Ferrer, Silvia[1]; Mongay Berdet, Laura[1]; Caro Martínez, Josefa[1]; Benítez Carabante, María Isabel[2]; Peña, Marta[3]; Canelo Vilaseca, Marta[4]; Bertran, Silvia[5]; Nogués, Nuria[5]; Rudilla Salvador, Francesc[1]; Herrero Mata, María Jose[1]; Gómez Massa, Elena[1].

[1]Laboratori d'Histocompatibilitat i Immunogenètica, Banc de Sang i Teixits, Barcelona; [2]Servei de Pediatria oncològica i Hematologia Pediàtrica, Hospital Infantil Vall d'Hebron, Barcelona; [3]Servei d'Hematologia Clínica, Institut Català d'Oncologia-Hospitalet, L'Hospitalet de Llobregat; [4]Servei d'Hematologia, ICO-Badalona, Hospital Universitari Germans Trias i Pujol, Badalona; [5]Laboratori d'Immunohematologia, Banc de Sang i Teixits, Barcelona.

CO-33

Ruxolitinib no inhibe completamente las capacidades funcionales de células NK activas con ligandos de TLR4/9

Mestre Duran, Carmen[1]; Pertiñez, Lidia[1]; García Solís, Blanca[2]; Pernas, Alicia[3]; Galán, Victor[4]; Sisinni, Luisa[4]; Navarro Zapata, Alfonso[5]; Clares Villa, Laura[5]; Al-Akioui, Karima[1]; Escudero, Adela[6]; Ferreras, Cristina[1]; Pérez Martínez, Antonio[7].

[1]Grupo de Investigación Traslacional en Oncología Pediátrica, Trasplante Hematopoyético y Terapia Celular, Instituto de Investigación Sanitaria del Hospital La Paz (IdiPAZ), Hospital Universitario La Paz, Madrid; [2]Instituto de Investigación Hospital Universitario La Paz, Madrid; [3]Trasplante Hematopoyético y Terapia Celular, Instituto de Investigación Sanitaria del Hospital La Paz (IdiPAZ), Hospital Universitario La Paz, Madrid; [4]Servicio de Hemato-Oncología Pediátrica, Hospital Universitario La Paz, Madrid; [5]Grupo de Investigación Traslacional en Oncología Pediátrica, Trasplante Hematopoyético y Terapia Celular, Instituto de Investigación Sanitaria del Hospital La Paz (IdiPAZ), Hospital Universitario La Paz, Madrid; [6]Departamento de Genética, Instituto de

Genética Médica y Molecular (INGEMM), Hospital Universitario La Paz, Madrid; [7]Grupo de Investigación Traslacional en Oncología Pediátrica, Trasplante Hematopoyético y Terapia Celular, Instituto de Investigación Sanitaria del Hospital La Paz (IdiPAZ), Hospital Universitario La Paz, Madrid; Facultad de Medicina, Universidad Autónoma de Madrid, Madrid.

CO-34

Analysis of HLA-type imputation based on the most frequent allele in the calculation of the total eplet load

Renuncio García, Mónica[1]; González López, Elena[1]; Carreras, Enric[2]; Villa, Juliana[2]; Romón Alonso, Iñigo[3]; Roa Bautista, Adriel[1]; Gutiérrez Larrañaga, María[1]; Comins Boo, Alejandra[1]; Irure Ventura, Juan[1]; López Hoyos, Marcos[1]; San Segundo, David[1].

[1]Immunology Department. Marqués de Valdecilla University Hospital, Santander; [2]Josep Carreras Foundation, Barcelona; [3]Hematology Department. Marqués de Valdecilla University Hospital, Santander.

CO-35

Donor-derived cell-free DNA as a biomarker of kidney graft damage

González López, Elena[1]; Cañamero, Lucía[2]; Ocejo Vinyals, Javier Gonzalo[1]; Belmar, Lara[2]; Irure Ventura, Juan[1]; Valero, Rosalía[2]; García Berbel, Pilar[3]; Freire, Javier[3]; López Hoyos, Marcos[1]; Ruiz, Juan Carlos[2]; Rodrigo, Emilio[2].

[1]Servicio de Inmunología. Hospital Universitario Marqués de Valdecilla, Santander; [2]Servicio de Nefrología. Hospital Universitario Marqués de Valdecilla, Santander; [3]Servicio de Anatomía Patológica. Hospital Universitario Marqués de Valdecilla, Santander.

CO-36

Induction of tolerance in liver transplantation by manipulation of T cell metabolism: an *in vitro* and *in vivo* approach

Valverde Mateo, Sandra; Sánchez Lorencio, Maria Isabel; Vidal Correoso, Daniel; Baroja Mazo, Alberto.

IMIB, Murcia.

CO-37

POTENTIAL IMMUNOMODULATORY ROLE OF SPECIFIC ANTI-CYTOMEGALOVIRUS INtravenous immunoglobulin in solid organ recipients with difficult to control CMV disease

Alonso, Rocio[1]; Jarque, Isidro[2]; Sarmiento, Elizabeth[1]; Zatarain, Eduardo[1]; Salcedo, Magdalena[1]; Rodriguez-Ferrero, Marisa[1]; Garcia, Sandra[1]; Mejía González, Maria Alejandra[1]; Carbone, Javier[2].

[1]Hospital General Universitario Gregorio Marañón, Madrid; [2]Hospital Universitario Puerta de Hierro Majadahonda, Madrid.

REUNIÓN GEAI
SALA 0A

Presencia del grupo en la web.
Dr. Andrés Roncancio Clavijo.
Hospital Universitario Ramón y Cajal, Madrid.

Resumen del simposio de Autoinmunes.
- Dra. Laura Martínez Martínez. *Universitat Autònoma de Barcelona, Barcelona.*
- Dr. Juan Irure Ventura. *Hospital Universitario Marqués de Valdecilla, Santander.*
- Dra. Aurora Jurado Roger. *Hospital Universitario Reina Sofía, Córdoba.*

Presentación de los resultados de la encuesta de ANCA.
Dr. Juan Irure Ventura.
Hospital Universitario Marqués de Valdecilla, Santander.

Presentación de la propuesta del estudio multicéntrico sobre MSA postcovid.
Dra. Juliana Ochoa.
Hospital Ruber International, Madrid.

Presentación de la propuesta de anti-fibrilarina.
Hospital de la Santa Creu i Sant Pau y Fundación Jiménez Díaz.

Protocolos diagnósticos en Autoinmunidad.
Dr. Juan Francisco Delgado.
Hospital Universitario 12 de octubre Madrid.

17:15 – 17:30h **PAUSA**

17:30 – 19:00h **SESIÓN 9: INMUNOLOGÍA DE LA INFECCIÓN**
SALA 0B

Moderadoras:
- Dra. María Montoya González. *Centro de Investigaciones Biológicas (CIB - CSIC), Madrid*.
- Dra. Carmen Martín Alonso. *Centro de Hemoterapia y Hemodonación de Castilla y León, Valladolid.*

Impacto de la inmunidad entrenada en la patología cardiaca asociada a la enfermedad de Lyme.
Dr. Juan Anguita Castillo
CIC bioGUNE - Centro de Investigación Cooperativa en Biociencias, Derio.

Papel de las células NK en el control del reservorio del VIH.
Dra. María José Buzón Gómez.
Vall d'Hebron Institut de Investigación, Barcelona.

Factores virológicos e inmunológicos asociados al control de élite persistente del VIH.
Dr. Ezequiel Ruiz-Mateos.

Instituto de Biomedicina de Sevilla, Sevilla.

COMUNICACIONES ORALES: INMUNOTERAPIA II

SALA 0D

Moderadores:

- Dra. Yvelise Barrios del Pino. *Hospital Universitario de Canarias, Santa Cruz de Tenerife.*
- Dr. Manel Juan Otero. *Hospital Sant Joan de Déu, Barcelona.*

CO-22

Braintruck: turning NKG2D CAR-T therapy into an improved IIL18 truck as treatment for pediatric central nervous system tumors

Clares Villa, Laura[1]; Navarro Zapata, Alfonso[1]; Mestre Durán, Carmen[1]; Pertíñez, Lidia[1]; Martín Cortázar, Carla[1]; Ibáñez Navarro, Marta[2]; Al-Akioui Sanz, Karima[1]; Fernández, Lucía[2]; Morales, María Puerto[3]; Barber, Domingo Fernando[4]; Rodríguez, Antonio[5]; Pérez Martínez, Antonio[5]; Ferreras, Cristina[1].

[1]IdiPAZ - Instituto de Investigación del Hospital Universitario la Paz, Madrid; [2]Centro Nacional de Investigaciones Oncológicas, Madrid; [3]Instituto de Ciencias de Materiales de Madrid, Madrid; [4]Centro Nacional de Biotecnología, Madrid; [5]Universidad Autónoma de Madrid, Madrid.

CO-23

Unraveling immunosuppressive mechanisms to abrogate intravenous BCG efficacy in a lung metastatic mouse model

Guerrero, Claudia; Moreo, Eduardo; Uranga, Santiago; Gomez, Ana Belen; Martín, Carlos; Aguiló, Nacho.

Universidad de Zaragoza, Zaragoza.

CO-24

SWATH-MS proteomic analysis of response to immunotherapy in metastatic NSCLC patients

Mondelo Macía, Patricia[1]; García González, Jorge[2]; León Mateos, Luis[2]; Abalo, Alicia[1]; Bravo, Susana[1]; Chantada Vázquez, María Del Pilar[1]; Muinelo Romay, Laura[1]; López López, Rafael[2]; Dávila Ibáñez, Ana[1]; Díaz Peña, Roberto[1].

[1]Instituto de Investigación Sanitaria de Santiago, Santiago de Compostela; [2]Complexo Hospitalario Universitario de Santiago de Compostela, Santiago de Compostela.

CO-25

Addressing the effect of the inflammatory environment on the generation of monocyte-derived tolerogenic dendritic cell therapies in autoimmune diseases

Fondelli, Federico[1]; Godoy Tena, Gerard[2]; Mansilla, Maria José[1]; Ramo Tello, Cristina[3]; Ballestar, Esteban[2]; Martinez Caceres, Eva[3].

[1]*Institut German Trias i Pujol, Badalona;* [2]*Institut Josep Carreras, Badalona;* [3]*Hospital German Trias i Pujol, Badalona.*

CO-27

Fabricación de terapias avanzadas en los centros hospitalarios: el reto de las normas de correcta fabricación

García-Hoz, Carlota; Pariente, Roberto; Velázquez, Kyra; Villar, Luisa María.

Hospital Ramón y Cajal, Madrid.

CO-28

Immunocytokines based on IL-12 that target csf1r and PD-l1 retain anti-tumor effects while limiting systemic exposure

Di Trani, Claudia Augusta; Arrizabalaga, Leire; Cirella, Assunta; Álvarez, Maite; Bella, Ángela; Fernandez-Sendin, Myriam; Russo-Cabrera, Joan Salvador; Otano, Itziar; Gomar, Celia; Ardaiz, Nuria; Belsue, Virginia; Aranda, Fernando; Melero, Ignacio; Berraondo, Pedro.

Cima Universidad de Navarra, Pamplona.

CO-29

Novel monoclonal antibody developed through patient specific approach to treat sézary syndrome, an ultra-rare cancer of mature t lymphocytes

Herrero Alonso, Marta[1]; Marin, Ana V.[1]; Fernández Megino, Rebeca[1]; Chacón-Arguedas, Daniel[1]; Dominguez, Mercedes[2]; Moreno, Inmaculada[2]; Gaspar, Maria Luisa[2]; Viñuela Martín, Marcos[3]; Real Arévalo, Irene[3]; Subiza, José Luis[3]; 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]*Servicio de Inmunología Microbiana, Centro Nacional de Microbiología, Instituto de Investigación Carlos III, Majadahonda, Madrid;* [3]*Clinical Immunology-Experimental and Surgery Department, Hospital Clínico Universitario.*

CO-30

Unlocking the therapeutic potential of phosphatidylserine-liposomes in autoimmune type 1 diabetes

Almenara Fuentes, Lidia[1]; Rodriguez Fernandez, Silvia[1]; Perna Barrull, David[2]; Garcia Loza, Ivan[1]; Rosell Mases, Estela[3]; Salvado, Miriam[4]; Rodriguez Vidal, Silvia[4]; Aguilera, Eva[2]; Barneda Zahonero, Bruna[5]; Dalmases, Marti[5]; Verdaguer, Joan[3]; Vives Pi, Marta[2].

[1]*Ahead Therapeutics SL, Badalona;* [2]*Germans Trias i Pujol Research Institute, Badalona;* [3]*IRBLleida, Lleida;* [4]*Ahead Therapeutics SL, L'Arboç;* [5]*Ahead Therapeutics SL, Cerdanyola del Vallès.*

REUNIÓN GETHIT Y TALLER GETHIT

SALA 0C

17:30 – 18:00 Discusión del documento sobre las recomendaciones para la prueba cruzada por citometría de flujo.
Dr. Juan Manuel Torres Canizales.
Hospital Clínic, Barcelona.

18:00 -18:30h Papel de HLA y KIR en la tolerancia de la implantación placentaria.
Dra. Silvia Sánchez Ramón.
Hospital Ruber Internacional, Madrid.

18:30 – 19:00h

- Discusión de los temas tratados.
- Presentación guías de elección donante haploidéntico y de indicaciones de petición de B*27.
- Redacción de documento de consenso con las indicaciones de A*03 (farmacos anti ICI) y DQA1*05 (anti-TNF).
- Elección de los respectivos grupos de trabajo.

CASOS CLÍNICOS AI

SALA 0A

Moderadoras:
- Dra. Aurora Jurado Roger. *Hospital Universitario Reina Sofía, Córdoba*
- Dra. Laura Martínez Martinez. *Hospital de la Santa Creu i Sant Pau, Barcelona.*

CCAI-01

Trasplante autólogo de progenitores hematopoyéticos como tratamiento de la degeneración cerebelosa paraneoplásica asociada a anticuerpos ANTI-YO

Arnaldos Pérez, Cristina[1]; Naranjo Rondán, Laura[1]; Martínez Hernández, Eugenia[2]; Saiz, Albert[2]; Antón Monleón, María Del Carmen[1]; Ruiz García, Raquel[1].

[1]Servicio de Inmunología, Hospital Clínic de Barcelona, Barcelona; [2]Servicio de Neurología, Hospital Clínic de Barcelona, Barcelona.

CCAI-02

Alteración visual aislada en paciente con anticuerpos ANTI-GFAP

Martí-Morente, Daniel[1]; Sánchez-Sánchez, María Del Carmen[2]; Reche-Sainz, José Alberto[2]; Ruiz-García, Raquel[1]; Couso, Rocío Soledad[1]; Naranjo, Laura[1].

[1]Hospital Clínic de Barcelona, Barcelona; [2]Hospital Universitario 12 de Octubre, Madrid.

CCAI-03

Síndrome de Vexas: una nueva y grave entidad autoinflamatoria del adulto refractaria a múltiples terapias

Manterola Navarro, Elena; Ballester González, Rubén; Rodríguez Ramos, Rafael; Fernández-Velasco, José Ignacio; Martín Moro, Fernando; Rodríguez Martín, Eulalia.

Hospital Universitario Ramón y Cajal, Madrid.

CCAI-04

Determinación de anticuerpos ANTI-NF155 en el seguimiento y manejo de pacientes con nodopatía autoimmune
Lynton Pons, Elionor; Bravo Gómez, Andrea Carolina; Lleixà, M. Cinta; Pascual Goñi, Elba; Mariscal Rodríguez, Anaís; Martínez Martínez, Laura.

Hospital de la Santa Creu i Sant Pau, Barcelona.

CCAI-05

Anticuerpos anti-gad, implicaciones en paciente con DMT-1 y afectación neurológica
Peña, Virginia; Téllez, Raquel; Calabia, Olalla.

H.U, Fundación Jiménez Díaz, Madrid;

CCAI-06

Linfograma duodenal y enfermedad celiaca refractaria

García De La Torre, Iván; Stewart Delcid, Vivian; Rodríguez Ramos, Rafael; Ballester González, Ruben; Saiz González, Ana; Roy Ariño, Miren Garbiñe.

Hospital Universitario Ramón y Cajal, Madrid.

19:00 – 20:00h ASAMBLEA GENERAL SEI
SALA 0B

SÁBADO 13 DE MAYO DE 2023

08:30 – 09:30h REUNIÓN GECIT

SALA 0B

- **Resultados del proyecto GECIT 2023:** "Estandarización de un protocolo de fijación y envío para su posterior uso en citometría de flujo espectral".

- **Presentación de objetivos para 2023-2024.**

REUNIÓN GESEI

SALA 0D

8:30 - 8:40h Bienvenida y presentación del grupo de trabajo.
Isabel Cortegano.
Centro Nacional de Microbiología (CNM-ISCIII), Madrid.

8:40 - 8:50h Anécdotas inmunológicas: ¿en qué se parecen el esternón y el cerebro?
Iria Gómez-Touriño.
Universidad de Santiago, Santiago de Compostela.

8:50 - 9:00h Implantación de la e-consulta en Inmunología clínica.
Jimena Pérez.
Hospital Gregorio Marañón, Madrid.

9:00 - 9:10h El impacto de la apertura de una consulta de Inmunología clínica en un hospital complejo: Una experiencia.
Javier Carbone.
Hospital Universitario Puerta de Hierro, Madrid.

9:10 - 9:20h GESEI en la revista de la SEI: planificación y posibles contribuciones.
Pascual Martínez.
Universidad de Alicante, Alicante.

9:20 - 9:30h Debate, propuestas próximo año y cierre de la sesión.

REUNIÓN GJSEI

SALA 0C

Cuenta tu movida en 5 minutos

El GJSEI convoca la actividad "Cuenta tu movida en 5 minutos" con la finalidad de proponer a los miembros del GJSEI el reto de explicar su trabajo de investigación o de residencia en inmunología durante una intervención limitada en el tiempo, usando lenguaje sencillo e inteligible capaz de ser comprendido por un público con experiencia en inmunología, pero no en su especialidad. Su objetivo es acercar al resto de miembros jóvenes del GJSEI el trabajo que lleva a cabo en el laboratorio u hospital para fomentar el interés por dichos temas y favorecer el conocimiento entre áreas diversas.

09:30 – 10:30h PLENARIA 5: MYELOID CELLS IN HOMEOSTASIS AND DISEASE

SALA 0B

Moderador: Dr. Jordi Cano Ochando. *Instituto de Salud Carlos III (ISCIII), Madrid.*

Connecting myeloid cell metabolism and function.
Dr. David Sancho Madrid.
CNIC-Centro Nacional de Investigaciones Cardiovasculares, Madrid.

10:30 – 11:30h COMUNICACIONES ORALES: INMUNODEFICIENCIAS: PRIMARIAS Y SECUNDARIAS

SALA 0B

Moderadoras:
- Dra. Carmen Cámara Hijón. *Hospital Universitario La Paz, Madrid.*
- Dra. Sara Calleja Antolín. *Sanidad de Castilla y León (SACYL), Valladorlid.*

CO-54

From a candidate gene in CVID to a recurrent cause in the study of CVID with gastrointestinal symptoms: three novel mutations in IRF2BP2

García-Aznar, José María[1]; Ocejo, Javier Gonzalo[2]; Novoa, Erika[3]; Maneiro, Emilia[1]; García, Maite[1].

[1]Healthincode, A Coruña; [2]Hospital Universitario Marqués de Valdecilla, Santander; [3]Hospital Universitario Virgen de la Arrixaca, Murcia.

CO-55

Characterization of pathogenicity of a novel CTLA4 variant

Muñoz Sánchez, Guillermo[1]; Velasco De Andrés, Maria[2]; Esteve Sole, Ana[3]; Bolaños, Jennifer[4]; Bolos, Una[4]; Díaz Pavón, Mar[4]; Ortega González, Juan Ramón[4]; Gonzalez Navarro, Europa Azucena[4]; Serra Pagésº, Carles[4]; Lozano, Francisco[4]; Mensa Vilaro, Anna[4]; Yagüe, Jordi[4]; Alsina, Laia[4]; Aróstegui, Juan Ignacio[4]; Vlagea, Alexandru[4].

[1]Hospital Clinic de Barcelona, Barcelona; [2]IDIBAPS, Barcelona; [3]Hospital Sant Joan de Déu, Barcelona; [4]Hospital Clínic de Barcelona, Barcelona.

CO-56

Síndrome de HIPER-IGE por pérdida de función de STAT3: experiencia de un centro

Miguel Berenguel, Laura; Cuesta Martín De La Cámara, Ricardo; González Torbay, Andrea; Bravo García-Morato, María; Méndez Echevarría, Ana; Del Rosal Rabes, Teresa; Robles Marhuenda, Ángel; Gianelli, Carla; López Granados, Eduardo; Cámara Hijón, Carmen; Rodríguez Pena, Rebeca.

Hospital Universitario La Paz, Madrid.



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CO-01

CARACTERIZATION OF THE IGHA12/+ MOUSE: A QUASI-MONOCLONAL APPROACH TO STUDY THE ATHEROPROTECTIVE ANTIBODY A12

Raposo Gutiérrez, Irene; Lorenzo Martín, Cristina; Rodríguez Ronchel, Ana; Rodríguez Ramiro, Almudena.

Centro Nacional de Investigaciones Cardiovasculares, Madrid.

Atherosclerosis is the main determinant of **cardiovascular diseases**, the leading cause of death worldwide and an area where there is an urgent need for new **therapeutic and diagnostic tools in this area**. Atherosclerosis has a strong autoimmune component that involves innate and adaptive immunity. However, our knowledge on the antigenic triggers of the adaptive immune response is very limited. Recently, our lab has identified the self-protein ALDH4A1 as a novel atherosclerosis-associated antigen targeted by the atheroprotective antibody A12, revealing ALDH4A1 and A12 as potential therapeutic targets.

To study the molecular mechanisms underlying antibody A12 atheroprotection we have developed a quasi-monoclonal mouse model that will allow 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 and found a subset (3-7% of B cells) of hukappa positive cells, confirming the presence of the transgenic construct. Additionally, the proportion of peripheral B cells was reduced in IghA12/+ mice. 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. FACS analysis of the spleen showed a decrease in the proportion follicular B cells accompanied by an increase in the proportion of marginal zone B cells in IghA12/+ mice. In vitro functional analysis showed splenic B cells from IghA12/+ mice switched to IgG1 at wild type levels. Together IghA12/+ model provides additional evidence that A12 recognizes a self-antigen and provides a valuable tool to study A12 function in atherosclerosis.

CO-02

TREG CELLS ARE SHAPED BY THE TUMOR MICROENVIRONMENT TO TARGET SPECIFICALLY ANTI-TUMOR ΓΔ T CELL SUBSETS

Blanco-Domínguez, Rafael; Vaz Pinto Santos, André; Lopes, Noella; Condeço, Carolina; Mensurado, Sofia; Silva-Santos, Bruno.

Instituto de Medicina Molecular João Lobo Antunes, Lisboa.

γδ T cells are potent anti-tumor effectors, capable of orchestrating immune responses that control tumour progression. The antitumor properties of γδ T cells are mostly attributed to the fast and strong production of IFNγ. However, the cellular and molecular regulators of IFNγ-producing γδ T cells in the tumour microenvironment (TME) are largely unknown. Here, we used a murine orthotopic model of breast cancer (E0771) implanted in a FOXP3-DTR transgenic mouse strain, in which we were able to deplete regulatory T (Treg) cells upon diphtheria toxin injection, to study how Treg cells affect γδ T cell behavior in the TME. We showed that Treg cell depletion increased accumulation of anti-tumor IFNγ-producing γδ T cells and not IL-17-producing γδ T cell subsets in the TME, which was accompanied with decelerated tumor growth. We demonstrated that Treg cells specifically inhibit proliferation of IFNγ-producing γδ T cells in vitro and in vivo involving molecular mechanisms only sensed by this anti-tumor gd T cell subset. We observed that the TME induced the expression of regulatory molecules on Treg cells as well as the release of IL-13 and IL-10. In parallel, IFNγ-committed γδ T cells express high levels of IL-13 receptor and IL-10 receptor. Blockade of IL-10 and IL-13 impairs the inhibition of IFNγ-producing γδ T cells by Treg cells. This work identifies a new axis by which Treg cells specifically target anti-tumor γδ T cell subsets in the TME. Our findings open opportunities for the design of new therapeutic strategies to boost effective anti-tumour responses.

CO-03

INTRATUMORAL CO-INJECTIONS OF NK CELLS AND NKG2A NEUTRALIZING ANTIBODIES ACHIEVE EFFICACIOUS ANTI-TUMOR RESPONSES IN A MANNER DEPENDENT ON CD8 T CELL AND CDC1.

Alvarez, Maite; Ochoa, Maria C; Molina, Carmen; Teijeira, Alvaro; Garasa, Saray; Sanchez-Gegrorio, Sandra; Berraondo, Pedro; Melero, Ignacio.

Centro de Investigación Medica Aplicada, Pamplona.

Local intratumoral NK-cell activity is known to foster adaptive T cell-mediated immunity against cancer, that conceivably is inhibited by the interactions of the inhibitory receptor NKG2A with the MHC class I molecule HLA-E in humans or Qa-1b in mice. In this study, we found that intratumoral delivery of preactivated NK cells significantly attains therapeutic effects only if co-injected with blocking anti-NKG2A and anti-Qa1b monoclonal antibodies (mAb) in two mouse tumors models. Such therapeutic activity was contingent on endogenous CD8 T cells and on type-1 conventional dendritic cells (cDC1), which are known to mediate cross-priming of tumor antigens. Moreover, the anti-tumor effects can be enhanced upon combination with systemically delivered anti-PD-1 mAb. This combination achieves at least partial abscopal efficacy against distantly implanted tumor lesions that were not directly treated. In xenografted mice with HLA-E-expressing human cancer cells, intratumoral the co-injection of human NK cells and the clinical-grade anti-NKG2A mAb monalizumab synergistically achieved therapeutic effects and attained stronger antibody-dependent cellular cytotoxicity (ADCC) in conjunction with cetuximab. All together, these studies support the therapeutic potential of locally delivered NK cell-based immunotherapies that recruit endogenous T-cell responses.

CO-04

CLINICAL STATUS OF CHRONIC MYELOMONOCYTIC LEUKEMIA PATIENTS WITH *KRAS* MUTATIONS PRESENT NLRP3 INFLAMMASOME ACTIVATION AND CAN BE TREATED BY IL-1 BLOCKING THERAPY.

Hurtado Navarro, Laura[1]; Cuenca Zamora, Ernesto José[2]; Soler Espejo, Eva[2]; Such, Esperanza[3]; Bellosillo, Beatriz[4]; Zamora, Lurdes[5]; Hernández Rivas, Jesús María[6]; Marco Ayala, Javier[7]; Linares Latorre, Lola[8]; García Ávila, Sara[4]; Amat Martínez, Paula[9]; González, Teresa[6]; Arnan Sangerman, Montserrat[5]; Pomares Marín, Helena[5]; Hua Chen, Tzu[2]; Carreño, Gonzalo[10]; Jerez, Andrés[2]; Teruel Montoya, Raúl[2]; Ferrer Marín, Francisca[2]; Pelegrín, Pablo[1].

[1]Instituto Murciano de Investigación Biosanitaria (IMIB-Arrixaca), Murcia; [2]Hospital General Universitario Morales Meseguer, Centro Regional de Hemodonación, CIBERER, IMIB-Arrixaca, Murcia; [3]Hospital Universitario y Politécnico La Fe, Valencia; [4]Hospital del Mar, IMIM, Barcelona; [5]Institut Català d'Oncologia (ICO), Badalona; [6]Complejo Asistencial Universitario de Salamanca, IBSAL, Salamanca; [7]Hospital Luis Alcanyis, Xátiva, Alicante; [8]Hospital General de Castellón, Castellón; [9]Hospital Clínico de Valencia, Valencia; [10]Hospital 12 de Octubre, Madrid.

Mutations in the rat sarcoma gene (RAS) are frequently associated with the myeloproliferative variant of Chronic Myelomonocytic Leukemia (CMML). RAS mutations (NRAS, KRAS) acts triggering RAS-GTP proteins in the via RAS/MEK/ERK signalling.

Our aim was to study the mechanisms underlying NLRP3/IL-1β axis disruption and RAS mutations in patients with CMML and to analyze the clinical efficacy of IL-1β inhibitors in this setting. To do that, we collected a cohort of CMML patients with mutation in KRAS (n=19), and as controls we included CMML patients without KRAS mutations (n=8), healthy individuals (n=9) and intra-abdominal origin septic patients (n=5). Plasma levels of inflammatory cytokines associated with NFκB and NLRP3 inflammasome pathways were measured by Multiplex, and inflammasome activation was studied by flow cytometry and ELISA.

CMML patient with mutations in KRAS exhibit a constitutive activation of the NLRP3 inflammasome evidenced by an increased percentage of circulating monocytes with ASC oligomers under basal conditions and the increase of pyroptotic markers in plasma, such as HMBG1, ASC and the P2X7 receptor. Moreover, these patients also have an elevated

inflammatory cytokine signature evidenced by higher levels of cytokines associated with both the NLRP3 and NFκB pathways, which differ from control groups.

Ex vivo treatment of peripheral blood mononuclear cells from CMML patients harbouring KRAS mutations with MCC950, a specific NLRP3 inhibitor, slightly decreases the percentage of monocytes with ASC oligomers, while IL-1β release is significantly affected. Interestingly, one particular CMML patient with $KRAS^{G12D}$ mutation were treated with Anakinra, an IL-1R blocker, which reduced monocyte counts and improved the patient's clinical status. Interestingly, Anakinra also resulted in a reduced activation of the NLRP3 inflammasome in ex vivo assays from peripheral blood cultures.

Overall, these results may offer new therapeutics opportunities in patients with myeloid neoplasms and RAS mutations by using NLRP3 inflammasome blockers and IL-1 inhibitors.

CO-05

MEMORY FEATURES OF CAR19-ENGINEERED NATURAL KILLER CELLS AS NEW IMMUNOMODULATORS OF THE SAFETY AND EFFICACY OF CAR-NK BASED THERAPIES.

Barriales San Miguel, Diego[1]; Herrera, Lara[1]; Etxebarria, Anne[1]; Anguita, Juan[2]; Juan, Manel[3]; Eguizabal, Cristina[1].

[1]CVTTH/IIS Biocruces Bizkaia, Galdakao; [2]CIC bioGUNE, Derio; [3]Hospital Clinic, Barcelona.

Natural killer (NK) cells have arisen as a promising cell source to develop chimeric antigen receptor (CAR) based therapies since they exhibit antitumor responses in the absence of the side effects associated with CAR-T cells, such as cytokine release syndrome and immune effector cell-associated neurotoxicity syndrome. Besides, the lack of T-cell receptors in NK cells allows the generation of allogenic cell products without the risk to inducing graft-versus-host disease in infused patients. Despite encouraging results in the first clinical trial using anti-CD19 CAR-NK cells against CD19+ malignancies, the search for improved NK-based immunotherapeutic products is one of the main goals. In this regard, the discovery of memory-like responses in NK cells resembling the phenomenon of trained immunity in the myeloid compartment has redefined the functional complexity and heterogeneity of these cells. However, this phenomenon in the context of CAR-NK products as well as its impact in the clinical outcome in infused patients remains unexplored. In our laboratory, we seek to characterize this memory phenotype taking advantage of multidisciplinary approaches, including the regulatory network that governs this process at the transcriptomic and metabolic levels. We have established a reliable protocol to assess the functionality of memory responses in human primary NK cells repeatedly exposed to tumor cells, including non-transduced and CAR19-transduced NK cells. Our data show the modulation of several activation/inhibitory receptors along with a significant impact in the inflammatory output. Moreover, the same modulation was observed in frozen human NK cells after the first exposure to a tumor cell line, suggesting that these long-lasting secondary responses may rely on epigenetic marks preserved during the freeze/thaw cycle. Thus, the characterization of memory-like responses in CAR-NK products may open new venues to identify molecular traits amenable to be manipulated to improve the efficacy and safety of these cell products.

CO-06

NEW HORIZONS THROUGH A MULTITARGET APPROACH: EXPERIENCE OF A TERTIARY HOSPITAL WITH THE USE OF DUAL CART 19/22 IN B ACUTE LYMPHOBLASTIC LEUKEMIA (B-ALL)

Galán Gómez, Víctor; González Martínez, Berta; Mirones Aguilar, Isabel; Echecopar Parente, Carlos; Escudero López, Adela; Ferreras Puente, Cristina; Navarro Zapata, Alfonso; Mestre Durán, Carmen; Cobo, Marta; Pernas Sánchez, Alicia; Vallejo, Susana; De Paz Arias, Raquel; Minguillón Pedreño, Jordi; Martínez Romera, Isabel; Guerra García, Pilar; San Román Pacheco, Sonsoles; Pérez Martínez, Antonio.

La Paz University Hospital, Madrid.

BACKGROUND

About 30-50% of patients will suffer a relapse after CD19 CAR-T infusion, so new targets have been presented to be considered, such as dual CAR-T CD19/CD22.

METHODS

Five batches of the advanced therapy CD19/22 CAR-T cells have been manufactured under Good Manufacturing Practices, authorized under compassionate use program. The starting material was immobilized apheresis. After immunoselection of CD3+ T lymphocytes (CD4/CD8) and activation with CD3/CD28, they were transduced with a CD19/CD22 lentiviral vector provided by Miltenyi Biotec. Cell expansion was performed in the CliniMACS Prodigy® device with TexMACs culture medium supplemented with IL-7 and IL-15. During manufacturing, process and quality controls were carried out.

RESULTS

A total of 5 patients were treated. Three patients (60%) were diagnosed with a relapsed B-ALL after *tisagenlecleucel* infusion. Two patients did not present CD19 expression at relapse. One of them also expressed low CD22. One patient (20%) was diagnosed with a refractory B-ALL, and the last patient (20%) with a relapsed B-ALL. Both were no eligible for *tisagenlecleucel* treatment.

The age range of the patients was 6-17 years. The time range between the start of manufacturing and the availability of the CAR-T for infusion was 9-11 days. The pre-infusion minimal residual disease range was 0.01-87%. Bridging therapy was individualised in each patient, and lymphodepletion regimen consisted on fludarabine and cyclophosphamide. The infused dose range was 7.5x1e5-3x1e6 cells/kg.

Regarding toxicity, a total of 4 patients (80%) presented cytokine release syndrome: 2 patients

(50%) grade 3 (ASBMT guidelines). Two patients (20%) presented grade 3 immune effector cell-associated neurotoxicity syndrome.

On day +28, 4 (80%) of the patients were in complete remission.

CONCLUSIONS

The manufacturing of dual CD19/22 CAR-T therapy is feasible, reproducible and well tolerated without severe toxicities in the majority of patients. Its study under clinical trial should be recommended.

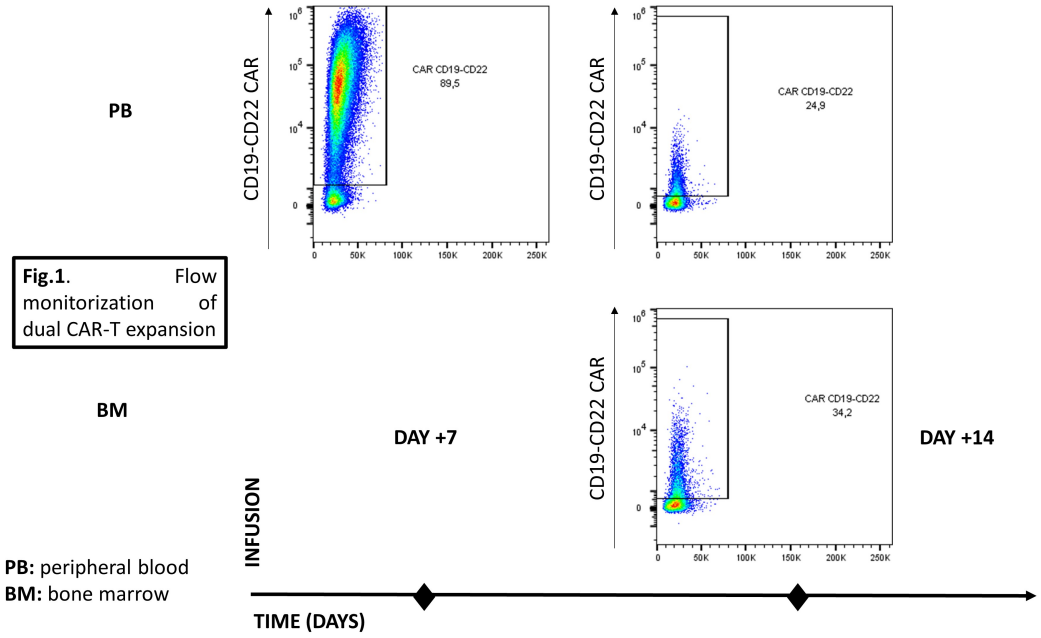


Fig.1. Flow monitorization of dual CAR-T expansion

CO-07

MRNAS ENCODING IL-12 AND A DECOY-RESISTANT VARIANT OF IL-18 SYNERGIZE TO ENGINEER T CELLS FOR EFFICACIOUS INTRATUMORAL ADOPTIVE IMMUNOTHERAPY

Olivera, Irene[1]; Bolaños, Elixabet[1]; Gonzalez Gomariz, Jose[1]; Hervas Stubbs, Sandra[1]; Mariño, Karina V.[2]; Luri Rey, Carlos[1]; Etxeberria, Iñaki[1]; Cirella, Assunta[1]; Egea, Josune[1]; Glez Vaz, Javier[1]; Garasa, Saray[1]; Alvarez, Maite[1]; Eguren Santamaría, Iñaki[1]; Guedan, Sonia[3]; F. Sanmamed, Miguel[1]; Berraondo, Pedro[1]; Rabinovich, Gabriel A.[2]; Teijeira, Alvaro[1]; Melero, Ignacio[1].

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Interleukin-12 (IL-12) is a potent immunotherapeutic cytokine which application in the clinical setting is hampered by IFNγ-dependent toxicity. On the other hand, IL-18 is a myeloid-derived cytokine that elicits IFN-γ expression on T and NK lymphocytes. IL-12 and IL-18 are known to synergize in terms of eliciting massive IFNγ production. For cancer immunotherapy, IL-18 has the caveat of being down-regulated in its function by a decoy receptor termed IL-18BP. Nevertheless, a mutant sequence of mouse IL-18 termed DRIL18 which preserves its bioactivity but lacks binding to IL-18BP has been recently reported.

Previous work of our laboratory has reported that intratumoral injection of transiently engineered T cells with IL-12 mRNA enhanced their systemic therapeutic efficacy. In this study, we sought to improve the therapeutic strategy of intratumoral delivery of T cells transiently engineered to express IL-12 with IL-18 mRNA electroporation.

Here, we mixed T cells electroporated with mRNAs to transiently express either single-chain IL-12 (scIL-12) or an IL-18 decoy-resistant variant (DRIL18). These cells were injected repeatedly into mouse tumors. Pmel-1 TCR-transgenic T cells electroporated with scIL-12 or DRIL18 mRNAs exerted powerful therapeutic effects in local and distant tumors. These effects were associated with T-cell metabolic fitness, enhanced miR-155 control of immunosuppressive target genes, enhanced expression of various cytokines and unique changes in the glycosylation profile of surface proteins, enabling enhanced adhesiveness to E-selectin. Efficacy of this intratumoral immunotherapeutic strategy was recapitulated using other clinically relevant adoptive T cell therapies as tumor-infiltrating lymphocytes (TILs) and CAR T cells upon IL-12 and DRIL18 mRNA electroporation

In conclusion, we report on a substantial improvement of adoptive T-cell therapies strategy based on mRNA transient gene-transfer and repeated intratumoral delivery. The synergistic immunobiology of IL-12 and IL-18, best represented in the form of DRIL18, holds promise for efficacious outcomes in the treatment of metastatic cancer patients.

Sesión Comunicaciones Orales 2 – Autoinmunidad

CO-08

THE ESSENTIAL ROLE OF CCL17-PRODUCING INFLAMMATORY DENDRITIC CELLS IN LUPUS NEPHRITIS IS UNCOVERED BY THE SUPPRESSIVE EFFECT OF AN INFECTION WITH *PLASMODIUM*

Amo, Laura[1]; Kole, Hemanta K[2]; Scott, Bethany[2]; Qi, Chen-Feng[2]; Wu, Monica[2]; Krymskaya, Ludmila[2]; Wang, Hongsheng[2]; Bolland, Silvia[2].

[1]Biocruces HRI, Barakaldo; [2]NIH, Maryland.

Systemic Lupus Erythematosus (SLE) is a disease of complex presentation, characterized by the presence of autoreactive lymphocytes, autoantibodies and systemic inflammation. Within the range of presentation, nephritis is considered a leading cause of mortality in severe cases of SLE. It is known that infections can alter the progression of autoimmune pathologies. For example, the host response against infection with *Plasmodium* commonly raises self-reactivity as a side effect, but it seems to spare damaging pathology caused by antibody deposition in kidney. Our studies of a mouse model of SLE and the effect of infection with the *Plasmodium* parasite have provided new insights into the mechanisms leading to destructive kidney tissue injury. Mice deficient in the inhibitory Fc receptor for IgG (FcγRIIB) are the animal model of SLE under study. We found that infection with *Plasmodium* protects SLE by modifying bone marrow cells and impairing leukocyte infiltration in the kidney, without altering systemic autoimmunity. Our investigations revealed a potential role of bone marrow-derived dendritic cells in promoting adaptive and destructive responses in the kidney. These cells were phenotypically and functionally characterized as type 2 inflammatory DCs (iDC2s) by the expression of CD11b, CD11c, MHC-IIhi, CD26 and SIRPα, together with the absence of CD88 and XCR1. During the end-stage nephritis, iDC2s infiltrate into the renal tissues and produce CCL17, an important chemokine associated with nephritis. After *Plasmodium* infection, renal iDC2s downregulated important genes for early maturation, immunomodulation and survival, resulting in a reduction of infiltration and progression to a severe nephritis. Overall, these results provide a possible route for lupus protection that specifically targets end-stage kidney disease, an essential regulated event in the transition from manageable glomerulonephritis to lethal tubular injury.

CO-09

RNA IMMUNOPRECIPITATION COUPLED WITH MASSIVE PARALLEL SEQUENCING FOR THE IDENTIFICATION OF RIBONUCLEOPROTEIN AUTOANTIGENS IN SYSTEMIC SCLEROSIS

Perurena-Prieto, Janire[1]; Viñas-Giménez, Laura[1]; Sanz-Martínez, María Teresa[1]; Selva-O'callaghan, Albert[2]; Gordillo-González, Fernando[3]; Terrón-Camero, Laura C.[3]; Callejas-Moraga, Eduardo Luis[4]; Andrés-León, Eduardo[3]; Martín, Javier[3]; Colobran, Roger[1]; Guillén-Del-Castillo, Alfredo[2]; Simeón-Aznar, Carmen P.[2]

[1]Immunology Division, Vall d'Hebron University Hospital (HUVH), Vall d'Hebron Barcelona Hospital Campus, Barcelona; [2]Systemic Autoimmune Diseases Unit, Internal Medicine Department, Vall d'Hebron University Hospital (HUVH), Vall d'Hebron Barcelona Hospital Campus, Barcelona; [3]Institute of Parasitology and Biomedicine "López-Neyra", CSIC (IPBLN-CSIC), Granada; [4]Internal Medicine Department, Hospital Público de Monforte, Lugo.

Background and objectives: systemic sclerosis (SSc)-specific autoantibodies allow the diagnosis and predict the prognosis of SSc patients with different clinical characteristics. The aim of this study was to develop a new assay for the identification of autoantibody targets in SSc using RNA immunoprecipitation coupled with massive parallel sequencing (RIP-Seq).
Methods: serum samples were collected from 307 SSc patients. Antinuclear autoantibodies were tested in all patients by indirect immunofluorescence (IIF) on HEp-2 cells. Specific autoantibodies (anti-Scl70, -centromere, -RNA-polymerase III, -U3-RNP, -NOR-90, -Th/To, -PM/Scl, -Ku, -U1-RNP) were evaluated with a commercial immunoblot and chemiluminescence immunoassay. Patients testing negative for all SSc-specific autoantibodies (n=68) were further tested by traditional RNA immunoprecipitation (IP). Fifty-seven patients from this last group and three healthy donors were also assayed by a new RIP-Seq protocol. In summary, RNA was obtained by traditional RNA IP, but instead of running a TBE-Urea-PAGE, RNA was subjected to massive parallel sequencing (RNA-Seq).
Results: overall, 30428 genes were found to be enriched in at least one of the 60 tested samples by RIP-Seq. Genes resulting with low expression were filtered out (median counts <100). Of the remaining genes, 400 were selected as they were considered to present at least one outlier. Among those genes, 7-2 and 8-2 RNA, Y1, Y3, Y4 and Y5 RNA, U3 RNA, and U11 RNA sequences were identified. These genes were highly enriched only in samples positive by RNA IP for anti-Th/To, -Ro60, –U3-RNP and –U11/12-RNP autoantibodies, respectively. U12 RNA enrichment was neither significant nor specific for U11/12-RNP positive samples.

Conclusion: we have developed and validated a new assay for the identification of ribonucleoprotein autoantigens in SSc patients using RIP-Seq. This assay could be more sensitive than RNA IP and may allow the detection of new autoantibodies in SSc.

CO-10

CRINOSOMES ACT AS A MAJOR SOURCE OF B-CELL-DERIVED PROTEINS FOR PEPTIDE PRESENTATION BY T1D-ASSOCIATED HLA ALLELES

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Autoreactive T-cell targeting of b-cells in type 1 diabetes (T1D) requires autoantigen processing and presentation by antigen presenting cells (APCs). An array of proteins has been described as targets of this autoimmune response. Proteolytic vesicles, which may reflect b-cell status, might be the major source of such proteins.

Fractions enriched in insulin secretory granules (ISGs) and crinosomes, the fusion of ISGs and lysosomes, were obtained from human b-cell lines. These were obtained via serial centrifugations and solubilised. Enrichment was assessed by LAMP1 (crinosomes) and phogrin (ISGs) content by western blot. Samples were further trypsin-digested for proteome study. Additionally, intact fractions and a complete b-cell lysate were used for pulsing moDCs expressing T1D HLA-DR risk alleles to characterise the derived immunopeptidome. Thus, MHC-II/peptide complexes were immunoprecipitated and peptides eluted in acidic conditions. All samples were analysed by mass spectrometry. On one hand, more than 800 proteins were identified in each granule fraction, including chromogranin A, CD81 and secretogranins. About 80% of the proteins were common to both crinosomes and ISGs, indicating their biological connection. On the other hand, analysis of the pulsed moDC's immunopeptidomes revealed that 47% of the peptide parental proteins were unique to the granule-pulsed samples, most being exclusive to the crinosome pulse, and a 36% were common between granule and lysate pulses. Interestingly, the peptides identified from these proteins were different between lysate and granule pulses. Around 23% of the peptides identified in the granule pulses belonged to new peptide families. These peptides derived from proteins such as EEF1A1 and lactotransferrin, both identified as targets of specific autoantibodies in T1D patients.

These results point at crinosomes as a source of b-cell proteins from compartments such as ISGs

or other cellular vesicles. Processing of these proteins by APCs increases the diversity of the peptides available for autoreactive T-cell recognition.

CO-11

FREQUENCY AND REFERRAL PATTERNS OF NEURAL ANTIBODY STUDIES DURING THE COVID-19 PANDEMIC: EXPERIENCE FROM AN AUTOIMMUNE NEUROLOGY REFERENCE CENTER

Ruiz García, Raquel[1]; Ariño, Helena[1]; Rioseras, Beatriz[2]; Naranjo, Laura[1]; Martínez Hernandez, Eugenia[1]; Saiz, Albert[1]; Graus, Francesc[1]; Dalmau, Josep[1].

[1]Hospital Clinic De Barcelona, Barcelona; [2]Hospital Universitario Central de Asturias, Oviedo.

Introduction: The activation of the immune system by SARS-CoV2 virus has emerged as a potential contributor to neurological complications in a few COVID-19 patients. In particular, a wide humoral immune activation seems to be frequent in patients with COVID-19 and some studies have suggested a high prevalence of neuronal or glial (neural) antibodies in the CSF of COVID-19 patients with neurological manifestations. We reasoned that any substantial association between COVID-19 and AE would be reflected by the pattern of case referrals to a center focused on the study of paraneoplastic neurological syndromes and AE.

Methods: We assessed the frequency and type of neural antibodies in a cohort of 15390 patients with suspected CNS autoimmunity over a period of 5 years covering the pre-pandemic and pandemic eras. We compared the frequency of antibody-positive patients identified in the pre-COVID-19 (2017-2019) and COVID-19 (2020-2021) periods for neural intracellular antigens (ICA), neuronal surface (NSA) or glial surface antigens (GSA).

Results: 801 positive results were identified from 794 different patients: 235 NSA, 216 ICA, 336 GSA, 6 NSA+ICA and 1 NSA+GSA. These findings demonstrate an overall positivity rate of 5.2% (95% CI 4.8% - 5.5%) for any type of neural antibody during the indicated 5-year period. Compared with the pre-COVID-19 era, the overall positivity rate changed from 4.9% to 5.4% ($p = 0.18$) for any type of neural antibody during COVID-19. The overall NSA and GSA positivity rate did not significantly change during the COVID-19 period ($p = 0.51$ and $p=0.11$ respectively). In contrast, the overall rate of ICA showed a 1.4 fold increase ($p = 0.01$), particularly for Hu and GFAP antibodies.

Conclusion: Our experience does not support that during the COVID-19 pandemic there was a clinically meaningful group of patients with CNS syndromes associated with novel antibodies against neuronal or glial antigens.

CO-12

REDUCED EXPRESSION OF THE TRANSCRIPTIONAL CO-REPRESSOR CBFA2T3 IN SYSTEMIC LUPUS ERYTHEMATOSUS T CELLS ASSOCIATES WITH NEPHRITIS AND NEGATIVELY CORRELATES WITH DISEASE ACTIVITY AND PROTEINURIA

Pérez Lorenzo, María J; Suárez Fueyo, Abel; Galindo Izquierdo, María; García González, Javier; Criado Carrasco, Gabriel.

Hospital 12 de Octubre, Madrid.

Objective

T cell activity is altered at different levels in systemic lupus erythematosus (SLE) patients. Since the transcriptional co-repressor CBFA2T3 (also known as MTG16 and ETO-2) is expressed by T cells and regulates several developmental processes and metabolism, we investigated its expression in SLE T cells.

Methods

T cells were isolated from peripheral blood of 32 SLE patients and 24 gender- and age-matched healthy controls by Ficoll gradient centrifugation and magnetic negative selection. After RNA purification and retrotrancription, expression of CBFA2T3, was assessed by quantitative PCR and analyzed in relation to clinical parameters.

Results

Expression of CBFA2T3 was significantly reduced in SLE T cells compared to HC samples (0.056 ± 0.006 RU in SLE vs 0.035 ± 0.003 RU in HC, P= 0.011).Analysis of CBFA2T3 expression in relation to clinical paramenters showed that reduced expression was associated with higher disease activity (0.022 ± 0.003 in SLEDAI > 6 vs 0.040 ± 0.004 in SLEDAI < 6) and the presence of lupus nephritis (0.019 ± 0.003 in LN^+ vs 0.042 ± 0.004 in LN^-). Furthermore, levels of CBFA2T3 negatively correlated with proteinuria in the group of lupus nephritis patients.

Conclusions

- Expression of CBFA2T3 in T cells from SLE patients is reduced compared to healthy individuals.
- Reduced CBFA2T3 levels associates with higher disease activity and the presence of lupus nephritis.

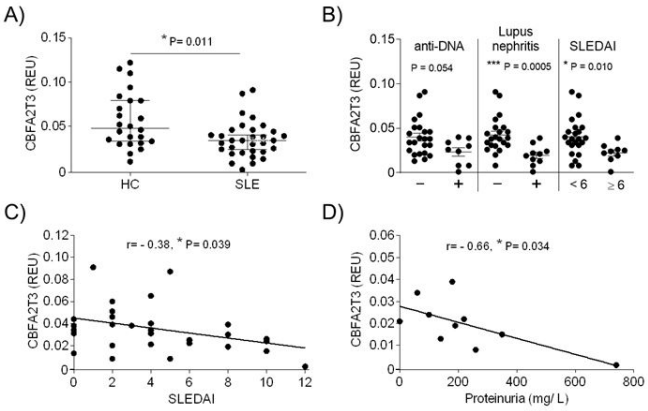


Figure 1. Reduced expression of CBFA2T3 in SLE T cells associates with disease activity and lupus nephritis. A) Levels of CBFA2T3 transcripts were quantified in T cells from 32 SLE patients and 24 healthy controls (HC). B) Expression of CBFA2T3 in relation to clinical parameters. Statistical differences were analyzed by Mann-Whitney U-test, *P < 0.05, **P< 0.01. C) CBFA2T3 expression in SLE T cells and SLEDAI correlation. D) CBFA2T3 expression and proteinuria correlation in SLE nephritic patients.

CO-13

DISCREPANCIAS EN LA DETECCIÓN DE ANTICUERPOS ANTI-FIBRILARINA POR DIFERENTES TÉCNICAS COMERCIALES Y COMPARACIÓN CON INMUNOBLOT CASERO.

López Brull, Ángel[1]; Mariscal Rodríguez, Anaís[2]; Sánchez Pernaute, Olga[1]; Martínez-Becerra, María José[1]; Vegas-Sánchez, María Del Carmen[1].

[1]Fundación Jiménez Díaz, Madrid; [2]Hospital de la Santa Creu i Sant Pau, Barcelona.

Los anticuerpos anti-fibrilarina (AAF) son marcadores de esclerosis sistémica (ES) difusa y afectación orgánica grave. Su correcta y temprana identificación es crucial, dado su valor pronóstico. Existen discrepancias entre las técnicas antígeno-específicas comercializadas para su detección. El objetivo de este estudio es analizar la concordancia entre distintas técnicas comerciales y comparar sus resultados con un inmunoblot (IB) casero con fibrilarina recombinante.

Se incluyeron 31 muestras de 28 pacientes de la FJD con patrón de IFI-HEp2-nucleolar (AC-8/9/10) y ELIA AAF positivo. Todas las muestras fueron analizadas también por dos IB comerciales (Dtek y Euroimmun) así como por IB casero.
Se analizó la concordancia entre los 4 métodos incluyendo 6 muestras con patrón IFI nucleolar y AAF ELiA negativos.

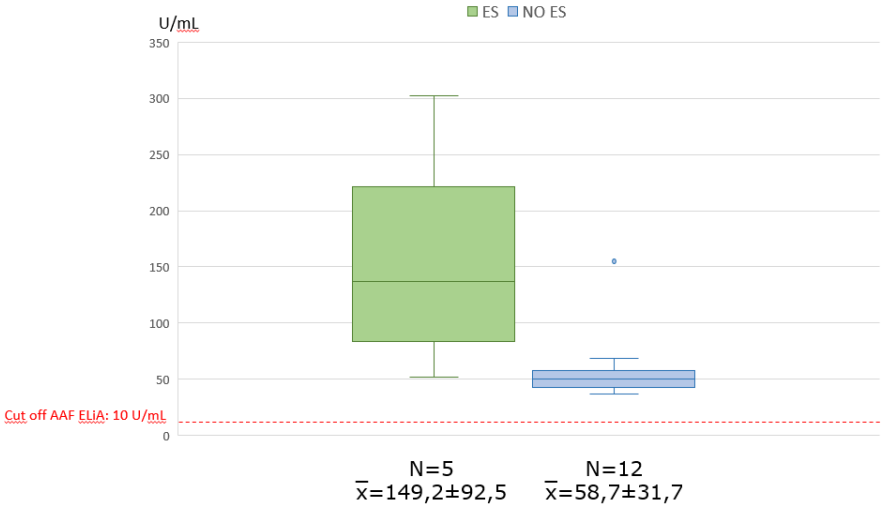
28/28 pacientes AAF positivos por ELiA fueron positivos por IB-Dtek (concordancia excelente, κ=1), 10/28 por IB-Euroimmun (concordancia pobre, κ=0.163) y 8/28 por IB casero (κ=0.125). El IB de Euroimmun y el casero mostraron una buena concordancia (κ=0.699). Disponíamos de información clínica de 17 pacientes.
Los pacientes con diagnóstico de ES (N=5) presentaban valores de ELiA más elevados (fig1) y un mayor porcentaje de positivos por todas las técnicas (80% vs 8% -Tabla 1-).

Existe un grupo mayoritario de pacientes con AAF que reconocen determinados epítopos representados de manera heterogénea en las diferentes técnicas empleadas y que actualmente no se asocian a ES. Futuros estudios permitirán conocer la implicación clínica de este hallazgo (estadíos preclínicos vs epítopos irrelevantes). El perfil AAF cuádruple positivo con niveles altos por ELIA es el más prevalente en pacientes con diagnóstico de ES.

	ID	AAF ELIA	DTEK	Euroimmun	IB Casero
Pacientes ES	1				
	2				
	3				
	4				
	5				
Pacientes no ES	6				
	7				
	8				
	9				
	10				
	11				
	12				
	13				
	14				
	15				
	16				
	17				

Positivo

Negativo


ES
NO ES
U/mL
350
300
250
200
150
100
50
0
Cut off AAF ELiA: 10 U/mL
N=5
x̄=149,2±92,5
N=12
x̄=58,7±31,7

CO-14

FENOTIPO CLÍNICO DE PACIENTES ANTI-KU POSITIVOS Y SU RELACIÓN CON OTROS AUTOANTICUERPOS ASOCIADOS A POLIMIOSITIS/DERMATOMIOSITIS

Galván, Raquel; Olivas, Israel; González, Concepción; Romero, Pilar; León, Antonio.

Hospital Universitario Virgen Macarena, Sevilla.

Ku es una proteína de unión al ADN implicada en reparación de las roturas de doble cadena. Los anticuerpos anti-Ku se describieron en 1981 en pacientes con síndrome de solapamiento esclerodermia-polimiositis. Aunque podrían considerarse anticuerpos asociados a miositis, muchos estudios han informado su positividad en pacientes con otras enfermedades autoinmunes sistémicas (EAS) y del tejido conectivo. Por tanto, nuestro objetivo fue estudiar el fenotipo clínico asociado a una población anti-Ku+ de nuestro hospital.
Estudio descriptivo y retrospectivo con 266 pacientes, entre 2020-2021, a quienes solicitaron la determinación de un panel de polimiositis/dermatomiositis (PM/DM) (Immunoblot, Euroimmun), incluyendo anticuerpos anti-Ku, bajo sospecha clínica o de laboratorio para dicha condición.
La mediana de edad de nuestra población (172 mujeres) fue 50 años y solo 36 pacientes tuvieron diagnóstico final de PM/DM. Dividimos nuestra población en 3 grupos: pacientes con EAS con positividad de anticuerpos antinucleares como criterio diagnóstico (n=66), pacientes con otras EAS o trastornos órgano-específicos (n=16) y pacientes sin diagnóstico de enfermedad autoinmune (n=184). En el primer grupo encontramos 6 pacientes con anticuerpos anti-Ku, 4 de ellos con diagnóstico de PM/DM (67%). No detectamos positividad anti-Ku en el segundo grupo, aunque encontramos 8 pacientes anti-Ku+ en el grupo sin trastornos autoinmunes (4%), con positividad débil la mayor parte de ellos. La detección de anti-Ku se asoció con otros anticuerpos del panel PM/DM en 4 pacientes: anti-OJ+anti-TIF1-γ (Neumonía intersticial con rasgos autoinmunes), anti-Ro/SSA+anti-TIF1-γ (DM juvenil y LES), Anti-Ro/SSA (esclerodermia) y anti-PMScl+anti-SRP (PM). Los síntomas clínicos descritos en pacientes con anticuerpos anti-Ku fueron principalmente musculares, articulares y fenómeno de Raynaud. El paciente con DM juvenil + LES presentó además un título elevado de anti-dsDNA.
Los autoanticuerpos anti-Ku demostraron una elevada sensibilidad en nuestros pacientes diagnosticados con PM/DM; no obstante, también pueden encontrarse esporádicamente asociados a otras enfermedades autoinmunes y no autoinmunes a concentraciones bajas.

Sesión Comunicaciones Orales 3 – Miscelánea (Inmunidad anti-tumoral/alergias y reacciones de hipersensibiliadad/Vacunas)

CO-15

GOLD NANOPARTICLES AS PLATFORMS TO PREPARE MULTIVALENT BACTERIAL VACCINES.

Salcines Cuevas, David[1]; Zeoli, Andrea[2]; Teran Navarro, Hector[2]; Calvo, Jorge[2]; Perez-Del Molino, Concepcion[2]; Yañez Cuevas, Sonsoles[2]; Montoya, Noemi[3]; Ubeda, Maria[3]; Fresno, Manuel[4]; Marradi, Marco[5]; Alvarez Dominguez, Carmen[3].

[1]Universidad Internacional de La Rioja (UNIR), MEDONLINE group, Santander; [2]Instituto de Investigación Marqués de Valdecilla (IDIVAL), Santander; [3]Universidad Internacional de La Rioja (UNIR), MEDONLINE group, Logroño; [4]Centro de Biología Molecular "Severo Ochoa"-Universidad Autónoma de Madrid, Madrid;[5]Universidad degli Studi di Firenze (UNIFI), Florencia.

The re-emergence of pathogens capable of causing human infections as well as the difficulty to develop effective vaccination strategies against several diseases, such as those caused by intracellular bacterial pathogens are an increasing problem for healthcare systems around the world and especially in developing countries. In search of new vaccines designs, nanomaterials offer a novel platform to deliver biomolecules with therapeutic and prophylactic applications. In this work, we explore the use of gold-based nanoparticles conjugated with polysaccharides and peptides as putative multivalent vaccines against non-tuberculous mycobacteria capable of causing cutaneous infection and listeriosis. Gold nanoparticles were conjugated with bacterial peptides of a common virulence factor of *Mycobacterium* and *Listeria* genera, the glyceraldehyde-3-phosphate dehydrogenase, GAPDH. The peptides used were GAPDH1-22 of *M. tuberculosis* (TB), and the peptide GAPDH1-15 of *Listeria monocytogenes* (LM). We justify the selection of these two peptides due to high homologous sequences among different species of mycobacteria and *L. monocytogenes.* We prepared NANO-GAPDH-TB (using GAPDH1-22 TB peptide) and NANO-GAPDH-LM (using GAPDH1-15 LM peptide) nanovaccines in formulation with two adjuvants, DIO-1 and Advax. Using mice models of cutaneous infections of *M. marinum* and *M. chelonae*, we assessed their safety, immunogenicity and protection they conferred. Here, we demonstrated that both, NANO-GAPDH-TB and NANO-GAPDH-LM formulated with Advax, besides lacking toxicity, showed good immunogenicity and protection against cutaneous mycobacteria and Listeria monocytogenes.

The protection was linked to the recruitment of innate immune cells, CD4 T cells and production of specific antibodies. We concluded that both nanovaccines, NANO-GAPDH-TB and NANO-GAPDH-LM formulated with Advax induced cross-proteciton immunity, confirmed the proof-of-concept that these nanovaccines served as multivalent vaccines.

CO-16

LONG-TERM ADJUVANT-INDUCED TRANSCRIPTIONAL MEMORY IN HEMATOPOIETIC STEM AND PROGENITOR CELLS

Pasco, Samuel; Martín Ruíz, Itziar; Palacios, Ainhoa; Barriales, Diego; Seoane Alvarez, Iratxe; Castelo Careaga, Janire; Gutiez García, Naiara; Arajuo Aris, Sarai; Martin Rodriguez, Jose Ezequiel; Aransay, Ana M; Rodriguez, Hector; Anguita, Juan.

CIC bioGUNE, Derio.

Traditional immunological dogma stipulates that memory is an antigen-specific, adaptive immune process, but clinical and epidemiological evidence shows that immunization with live, attenuated vaccines can confer broad nonspecific protection, mediated by enhanced innate immune mechanisms. Induction of this de facto innate immune memory, generally known as "trained immunity", causes a differential secondary response by innate cells following an initial training stimulus. Codependent metabolic and epigenetic rewiring mediate these effects, with long-term training imprinted in vivo in the hematopoietic stem and progenitor cell (HSPC) compartment. Understanding this phenomenon provides a unique opportunity to harness these effects for improved vaccine components, such as adjuvants. To this end, we examined the establishment of innate immune training by the commercially available Sigma Adjuvant System® (SAS), an oil-in-water formulation of monophosphoryl lipid A and trehalose dicorynomycolate. One month after treatment with SAS, murine bone marrow-derived macrophages produce lower pro-inflammatory cytokine levels upon ex vivo bacterial stimulation. To interrogate this phenomenon, we sorted bone marrow HSPCs and performed transcriptomic analysis, at bulk and single cell resolutions. Bulk sequencing showed a downregulation of epigenetic enzymes, alterations in specific metabolic pathways, and upregulated defense response genes. Single cell sequencing revealed changes in metabolic and interferon-mediated signaling genes in specific progenitor populations without affecting other hematopoietic lineages. Inducing this phenotype affects a subsequent immunization challenge, altering the adaptive responses. Together, these results reveal mechanisms behind the innate immune memory's persistence in the bone marrow.

CO-17

HUMORAL AND CELLULAR IMMUNITY TO SARS-COV-2 AFTER VACCINATION WITH MRNA VACCINES IN PWH WITH DISCORDANT IMMUNE RESPONSE. INFLUENCE OF THE VACCINE ADMINISTERED.

López Cortés, Luis Fernando[1]; Saborido Alconchel, Abraham[1]; Trujillo Rodríguez, María[1]; Serna Gallego, Ana[1]; Llaves Flores, Silvia[1]; Muñoz Muela, Esperanza[1]; Pérez Santos, María J.[1]; Lozano, María Del Carmen[1]; Mejías Trueba, Marta[2]; Roca Oporto, Cristina[1]; Espinosa, Nuria[1]; Gutiérrez Valencia, Alicia[1].

[1]Division of Infectious Diseases, Microbiology and Parasitology. Biomedicine Institute of Seville/Virgen del Rocío University Hospital/CSIC/University of Seville, Sevilla; [2]Pharmacy Service. Virgen del Rocío University Hospital, Sevilla.

Background. Data on SARS-CoV-2 mRNA vaccine immunogenicity in people with human immunodeficiency virus (PWH) with discordant immune response (DIR) are currently limited. Therefore, we compare immunogenicity of these vaccines in DIR and immunological responders (IR).

Materials and Methods. Prospective cohort that enrolled 89 participants. Finally, 22 IR and 24 DIR were analyzed before vaccination (T0), one (T1), and six months (T2) after receiving BNT162b2 or mRNA-1273. Additionally, 10 IR and 16 DIR were evaluated after a third dose (T3). Anti-S-RBD IgG, neutralizing antibodies (nAb), neutralization activity, and specific memory B cells were quantified. Furthermore, specific CD4+ and CD8+ responses were determined by intracellular cytokine staining and polyfunctionality indexes (Pindex).

Findings. At T1, all participants developed anti-S-RBD. 100% IR developed nAb compared to 83.3% DIR. Spike-specific B cells were detected in all IR and 21/24 DIR. Memory CD4+ T cells showed response in 5/9 IR and 7/9 DIR, mainly based on the expression of IFN- γ and TNF- α, with higher Pindex in DIR. Memory CD8+ T cells responded in only four participants in each group. At T2, anti-S-RBD and nAb titers were higher in DIR than IR. In both groups, there was an increase in specific B memory cells, higher in DIR. Six IR and five DIR maintained specific memory CD4+ response. Memory CD8+ response was preserved in IR but was lost in DIR. In a multivariate linear regression analysis, receiving mRNA-1273 instead of BNT162b2 played a prominent role in the results.

Conclusions. Our results suggest that PWH with DIR can mount an immune response similar to those with higher CD4+, provided they receive the mRNA-1273 vaccine instead of others less immunogenic.

CO-18

VISUALIZING INITIAL IMMUNE RESPONSES TO LIVER METASTASIZING TUMOR CELLS.

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INTRODUCTION

Tumor metastases are the main cause behind cancer related death. The liver is one of the most frequently targeted organs in cancer. The presence of liver metastases is known to promote systemic immunosuppression and poor response to immunotherapy. The nature of the liver organ specific immune response that is orchestrated upon tumor cell arrival has been poorly described and defines the immune response elicited against metastatis.

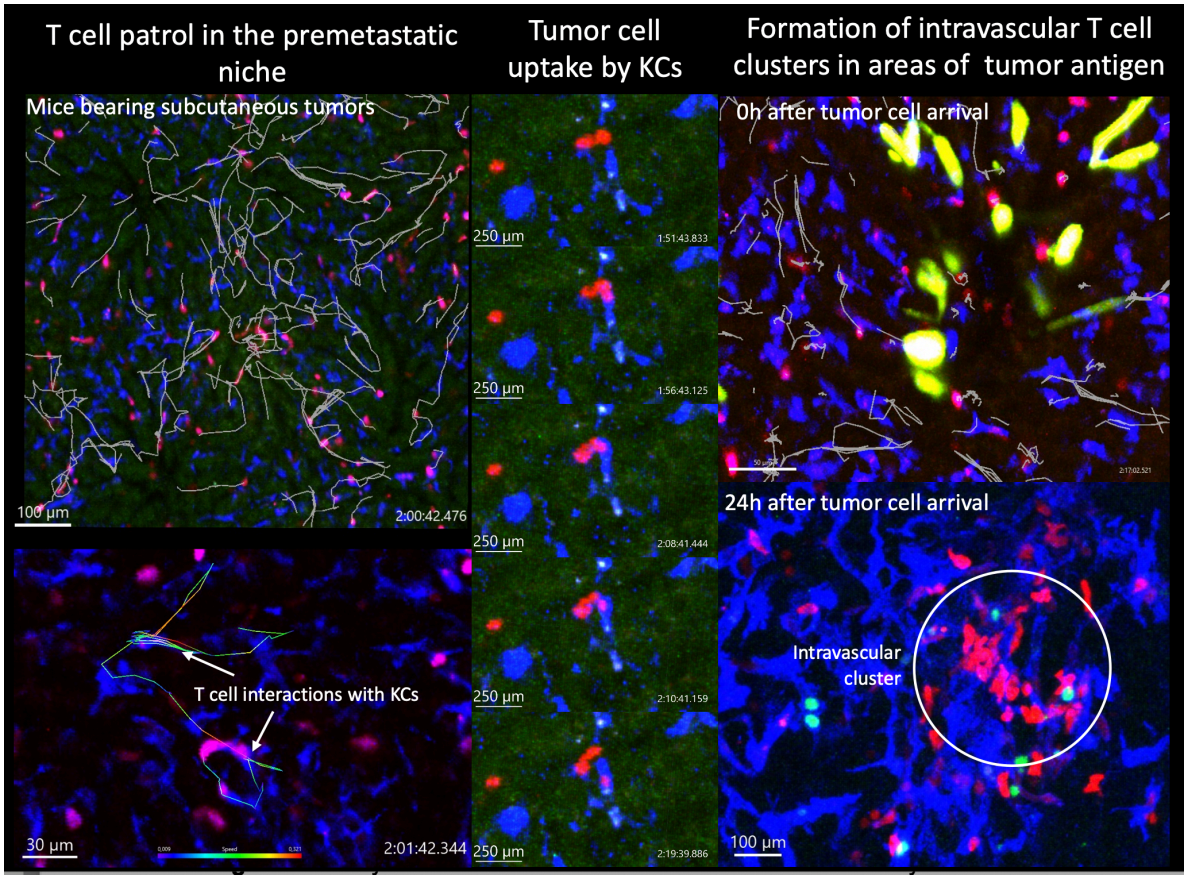
METHODS

Performing intravital microscopy in several immune-cell reporter mice we characterized the dynamics of immune responses against tumor cells from syngeneic colorectal cancer and melanoma cell lines after their intrasplenic injection. NK, T cell, dendritic cell, tumor and Kupffer cells behavior was tracked in time lapse microscopy videos.

RESULTS

We observed active surveillance of the premetastatic niche by T cells, a subset of circulating dendritic cells and NK cells. Specifically, T cells and, mainly $CD4^+$ T cells, patrol the liver sinusoids in the absence of metastatic cells and frequently perform short interactions with resident Kupffer cells. Tumor cells arriving to the liver rapidly break in vesicles that are usually uptaken by circulating or liver resident leukocytes. Depletion of immune effector leukocytes ($CD8^+$ T cells and NK cells) only minimally impacts the presence of whole tumor cells shortly upon cancer cell arrival. Kupffer cells were usually observed performing efferocytosis of large tumor cell pieces and were the main cells keeping fluorescent antigen in the liver. Intravascular clusters of T cells but not of NK cells are effectively formed in areas where tumor cell antigenic material is conserved 24 hours after tumor cell arrival and the appearance of such intravascular clusters is chemokine receptor dependent. We observe for the first time the dynamics of immune responses against tumor cells arriving to the liver and show tissue specific immune

responses dictating the outcome of metastatic cells.



CO-19

ESTUDIO DEL PAPEL DE LOS NEUTRÓFILOS EN LA INMUNOESTIMULACIÓN FRENTE ASARS-COV-2 CON MICROPARTÍCULAS DE SILICIO MESOPOROSO (MSMPS)

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1. Objetivos

Las micropartículas de silicio mesoporoso (MSMPs) tienen un gran potencial como adyuvantes, como ya demostramos en ensayos de inmunización frente al SARS-CoV-2. Los neutrófilos parecen jugar un papel importante en el efecto inmunoestimulador de las MSMPs, por ello estudiaremos tanto *in vitro,* como *in vivo*, la función de estas células.

2. Métodos

En neutrófilos humanos se estudió la capacidad de las MSMPs para inducir la formación de trampas extracelulares de neutrófilos (NETs) y la endocitosis mediante microscopia de fluorescencia. Además, se estudió la viabilidad de los neutrófilos tras ser estimulados con MSMPs mediante citometría de flujo.

En ratones se utilizó un modelo deficiente en neutrófilos, los ratones iDTRxMRP8Cre. En estos ratones se diseñó un protocolo para la eliminación de neutrófilos, basándose en la administración de la toxina diftérica. A estos ratones se les inmunizó frente al SARS-CoV-2 con el complejo vacunal MSMPs-S1 (Spike) y se estudió la respuesta humoral (IgM) mediante ELISA y la respuesta celular, medida como porcentaje de células CD4+ y CD8+ IFNγ+ en esplenocitos, mediante citometría de flujo.

3. Resultados

In vitro se observó como las MSMPs son endocitadas, y capaces de inducir la formación de NETs en neutrófilos humanos tras tres horas de estimulación. Los resultados de endocitosis observados se correlacionaron con la viabilidad de dichas células mediante por citometría de flujo.

In vivo, los experimentos de inmunización indican, que los ratones deficientes en neutrófilos no presentan ni respuesta humoral ni celular especifica frente al SARS-CoV-2. Los ratones no deficientes en neutrófilos, inmunizados con el complejo MSMPs-S1 presentan mayor respuesta humoral que los ratones inmunizados con S1, así como una mayor respuesta celular.

4. Conclusión

Los resultados muestran la importancia de los neutrófilos en la respuesta inmunoestimuladora mediada por las MSMPs, lo que podría ser clave para explicar el papel adyuvante de este material.

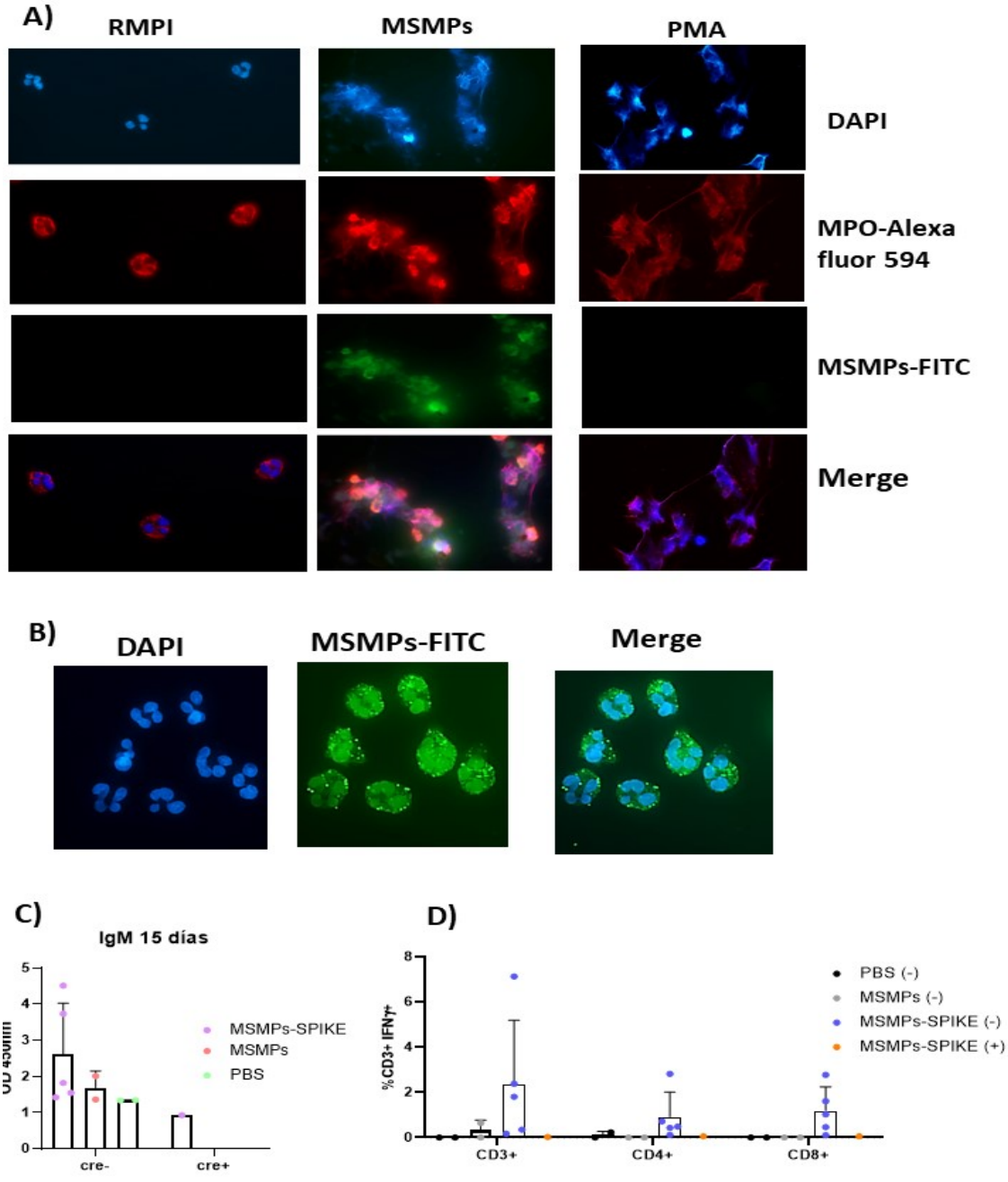


Figura 1: Representa el papel de los neutrófilos en la respuesta inmunitaria. **A)** Producción de NETs después de 3h de estimulación con MSMPs, en verde, y PMA como control positivo, medida como la liberación de la mieloperoxidasa, en rojo. En azul se observan los núcleos teñidos con DAPI. Aumento 60x **B)** Endocitosis de MSMPs por parte de neutrófilos humanos después de 1 hora de estimulación. En verde observamos las partículas y en azul los núcleos de los neutrófilos teñidos con DAPI. Aumento 100x **C)** Respuesta humoral, medida como producción de IgM, en ratones inmunizados con MSMPs-Spike deficientes en neutrófilos en comparación con ratones Wilde type (WT). **D)** Respuesta celular, medida como producción de IFNγ en células CD3+, CD4+ y CD8+, en ratones deficientes de neutrófilos inmunizados con MSMPs-Spike, en comparación con ratones WT.

CO-20

EXPANSION OF A DECIDUAL-LIKE NK CELL SUBSET EARLY AFTER AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN CHILDREN WITH SOLID TUMORS

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Rationale and objective:

Early immune reconstitution after autologous hematopoietic stem cell transplantation (autoHSCT) is associated with a better outcome in patients with cancer. Natural killer (NK) cells have a role in anti-tumoral responses and are the first lymphocyte subset recovering after autoHSCT. In order to understand the biology of NK cells after autoHSCT, we phenotypically characterized NK cells in pediatric patients with different solid tumors before and after autoHSCT.

Patients and methods:

Peripheral blood mononuclear cells and plasma from pediatric patients with different types of solid tumors were collected before and after autoHSCT (over one-year period). NK cells were studied by multiparametric flow cytometry and cytokine levels in plasma were measured.

Results:

It was found that NK cell subsets were altered early after autoHSCT. NK cells were more immature, activated, and their expression of several inhibitory receptors was increased, while the expression of activating receptors was decreased. There was a transitory acquisition of a decidual-like phenotype, characterized by a significant expansion of CD9+CD151+ cells. In addition, we observed that NK cell education and maturation are two uncoupled processes

following autoHSCT. Furthermore, plasma levels of cytokines with relevant roles in NK cell function were analyzed and it was observed a significant increase of IL-15, growth differentiation factor (GDF)-15 and placental growth factor (PlGF), together with a decrease of platelet derived growth factor (PDGF)-DD and PDGF-BB early after autoHSCT. *In vitro* experiments and correlation analysis showed that IL-15 and TGF-β, among other factors, could explain the acquisition of this characteristic decidual-like phenotype early after autoHSCT.

Conclusion:

NK cell phenotype significantly changed early after autoHSCT towards a phenotype that resembles NK cells from the decidua. IL-15 and TGF-β have a significant role in the acquisition of this phenotype.

CO-21

DESARROLLO DE UN NUEVO MÉTODO PARA LA PREDICCIÓN DE EPÍTOPOS LINEALES DE LINFOCITOS B BASADO EN LA SIMILITUD DE SECUENCIAS

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La predicción de epítopos de linfocitos B que puedan sustituir al antígeno para la producción y detección de anticuerpos es de gran interés tanto para la investigación como para la industria biotecnológica. En este trabajo desarrollamos un novedoso método basado en BLAST para la predicción de epítopos lineales de linfocitos B. Para ello, generamos una base de datos a partir de 62730 secuencias de epítopos lineales conocidas y consideramos como epítopo cualquier péptido con el que, utilizando esta base de datos, obtengamos al menos un hit sin gaps con una identidad ≥ 80 % y una longitud ≥ 8. Examinamos la capacidad predictiva de este método mediante la técnica de validación cruzada en la que consideramos como no epítopos de linfocitos B varios tipos de secuencias, entra las que incluimos 62730 péptidos productos de ensayos negativos verificados. Como resultado, obtuvimos valores de precisión, especificidad y sensibilidad del 72,54 ± 0,27 %, 81,59 ± 0,37 % y 63,49 ± 0,43 %, respectivamente. Con un conjunto de datos independiente que incorporaba 503 epítopos de linfocitos B, este método alcanzó una precisión, especificidad y sensibilidad del 74,85%, 99,20% y 50,50%, respectivamente, superando a los métodos más avanzados para predecir epítopos lineales de linfocitos B. Implementamos esta metodología basada en BLAST para la predicción de epítopos de linfocitos B en http://imath.med.ucm.es/bepib.

Sesión Comunicaciones Orales 4 – Inmunoterapia (II)

CO-22

BRAINTRUCK: TURNING NKG2D CAR-T THERAPY INTO AN IMPROVED IIL18 TRUCK AS TREATMENT FOR PEDIATRIC CENTRAL NERVOUS SYSTEM TUMORS

Clares Villa, Laura[1]; Navarro Zapata, Alfonso[1]; Mestre Durán, Carmen[1]; Pertíñez, Lidia[1]; Martín Cortázar, Carla[1]; Ibáñez Navarro, Marta[2]; Al-Akioui Sanz, Karima[1]; Fernández, Lucía[2]; Morales, María Puerto[3]; Barber, Domingo Fernando[4]; Rodríguez, Antonio[5]; Pérez Martínez, Antonio[5]; Ferreras, Cristina[1].

[1]IdiPAZ - Instituto de Investigación del Hospital Universitario la Paz, Madrid; [2]Centro Nacional de Investigaciones Oncológicas, Madrid; [3]Instituto de Ciencias de Materiales de Madrid, Madrid; [4]Centro Nacional de Biotecnología, Madrid; [5]Universidad Autónoma de Madrid, Madrid.

Central Nervous System (CNS) tumors are the most common solid pediatric tumors and the leading cause of cancer-related death in children. Although current therapies have improved their outcome, survivors present severe long-time sequelae from therapy.
Chimeric Antigen Receptor (CAR)-T cells is a therapeutic alternative for hematological malignancies but their application in solid tumors is still challenging. This is due to the scarce therapy infiltration, the lack of therapy persistence and the immunosuppressive tumor microenvironment (TME).
NKG2D CAR-T has shown substantial benefit in osteosarcoma studies in vivo, with some limitations. This project aims to improve CAR-T cell therapy on pediatric CNS tumors by generating different tools whose utility exceeds pediatric brain oncology itself.
To overcome TME limitations, we are manipulating NKG2D CAR-T cells to release engineered interleukin 18 (IL18) versions upon CAR T activation. It will transform the immunosuppressive TME, allowing immune cells to reach the tumor and exert their cytotoxic activity. IL18 is a proinflammatory cytokine that recruits immune cells, activating NK and T cells. Engineered IL18 is expected to increase the protein stability and release.
Furthermore, a magnetic nanoparticle (MNP) CAR-T delivery system is being developed. It will retain CAR-T cells acting on the tumor site in the presence of an external magnetic field, decreasing the effective dose, hence associated toxicities.

Four IL18 versions have been cloned under the control of NFAT and PGK promoters. We have compared IL18 release through ELISA, observing that one of the IL18 modifications causes an increase in the amount of cytokine secreted. Their functionality is currently being tested through migration and NK activation assays.

The more effective IL18 versions will be cloned in NKG2D CAR-T. Then, we will assess the therapy efficacy using 3D models. This interdisciplinary approach could improve current hurdles in CAR-T efficacy for the treatment of pediatric CNS tumors.

CO-23

UNRAVELING IMMUNOSUPPRESSIVE MECHANISMS TO ABROGATE INTRAVENOUS BCG EFFICACY IN A LUNG METASTATIC MOUSE MODEL.

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Universidad de Zaragoza, Zaragoza.

Bacillus Calmette-Guérin (BCG) is a live attenuated mycobacterium vaccine and it is widely used in clinic, mainly in two applications, as tuberculosis vaccine via intradermal route and as first-line therapy for non-muscle invasive bladder cancer by intravesical administration. In the past, a reduced number of studies in different animal models showed that no systemic severe toxicity has been associated with intravenous (i.v.) BCG. Recently, systemic delivery of BCG has been reported to provide a more efficient capacity of response against a subsequent lung tuberculosis infection in rhesus macaques. 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 KC8.1 lung tumor cells administration 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-derived signals might be influencing i.v. BCG response. This scenario might be helpful to unravel tumor mechanisms leading to i.v. BCG efficacy suppression. Here we show that NK cells are crucial in i.v. BCG antitumoral response against KC8.1 tumors. *In vitro* studies showed that KC8.1 cells resulted highly sensitive to IFNg-mediated induction of different cell surface molecules as MHC-I, or checkpoint ligands as PDL-1, suggesting a possible mechanism of immune suppression. Supporting this hypothesis, a KC8.1 subline lacking β2m (and therefore MHC-I surface expression) recovered sensitivity to i.v BCG therapeutic treatment.

In conclusion, KC8.1 tumor cells are immunoedited *in vivo* to subvert antitumoral response of i.v. BCG. Unraveling these mechanisms of immunosuppression could be crucial to avoid resistance to immunotherapy and to develop new cancer treatments.

CO-24

SWATH-MS PROTEOMIC ANALYSIS OF RESPONSE TO IMMUNOTHERAPY IN METASTATIC NSCLC PATIENTS

Mondelo Macía, Patricia[1]; García González, Jorge[2]; León Mateos, Luis[2]; Abalo, Alicia[1]; Bravo, Susana[1]; Chantada Vázquez, María Del Pilar[1]; Muinelo Romay, Laura[1]; López López, Rafael[2]; Dávila Ibáñez, Ana[1]; Díaz Peña, Roberto[1].

[1]Instituto de Investigación Sanitaria de Santiago, Santiago de Compostela; [2]Complexo Hospitalario Universitario de Santiago de Compostela, Santiago de Compostela.

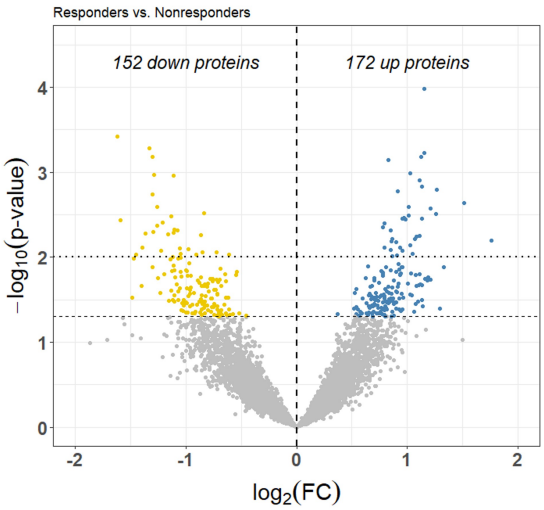
Objective: In the present study, we hypothesized that differential proteomic quantitative analysis based on Sequential Window Acquisition of all Theoretical Mass Spectra (SWATH–MS) technology, can allow us to identify a blood-based proteomic signature and to select and predict the response to first line immunotherapy treatments in new diagnosed metastatic NSCLC patients.

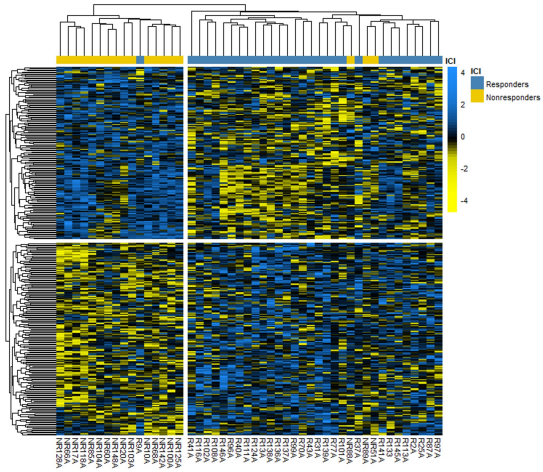
Methods: We included 48 advanced NSCLC patients treated with pembrolizumab as first line therapy at the Medical Oncology Service of Complexo Hospitalario Universitario de Santiago de Compostela. Blood samples were collected from each patient prior to the treatment onset, obtaining the corresponding plasma sample. Plasma samples were depletioned and digested, and peptides samples were analyzed using a micro-LC-MS/MS. Relative quantification was obtained using the data-independent acquisition (DIA) SWATH–MS method. Differential proteomic quantitative analysis between responders (n=30) and non-responders (n=18) groups were performed.

Results: A total of 6,364 proteins with 1% false discovery rate (FDR) on the peptide and protein level were quantified after samples filtering. Three hundred and twenty-four differentially expressed proteins (DEPs) (Student's t tests, p-value<0.05) between responder and non-responder patients were identified. Of them, 172 proteins appeared upregulated, and 152 proteins were downregulated (Figure 1). A proteomic signature with 7 proteins was developed using a stepwise forward variable selection. The combination of 7 proteins allowed to separate both groups with high sensitivity and specificity.

Conclusions: Proteomic analyses from circulating proteins allowed us to discover and quantified a high number of potential biomarkers in plasma samples, showing their likely value as a screening tool to find new prognostic and predictive biomarkers in the immunotherapy field. Moreover, we reported a proteomic signature of 7 proteins that allowed us to predict the

response to immunotherapy in advanced NSCLC patients.





CO-25

ADDRESSING THE EFFECT OF THE INFLAMMATORY ENVIRONMENT ON THE GENERATION OF MONOCYTE-DERIVED TOLEROGENIC DENDRITIC CELL THERAPIES IN AUTOIMMUNE DISEASES.

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[1]Institut German Trias i Pujol, Badalona; [2]Institut Josep Carreras, Badalona; [3]Hospital German Trias i Pujol, Badalona.

Introduction: Antigen-specific immunotherapies represent an approach to re-educate immunity toward homeostasis without general immunosuppression and has the potential to stop the progression of autoimmune diseases. Our group developed an autologous tolerogenic dendritic cell product (VitD3-TolDCs) differentiated *in vitro* from peripheral blood monocytes, which showed safety in a Phase I clinical trial in patients with active Relapsing-Remitting Multiple Sclerosis (RRMS). However, given MS-intrinsic systemic inflammation, we aimed to compare, at a multiomic and functional level, monocytes and VitD3-TolDCs from MS patients and HD, to identify putative dysfunctional pathways that could be modulated to produce stronger VitD3-TolDCs.

Methods: 18vs18 DNA monocyte and 7vs7 VitD3-TolDCs samples from naïve active MS patients and HD have been profiled via flow cytometry and EPIC methylation Arrays. Next, targeted gene expression was evaluated through qPCR and bulk RNAseq. Finally, we evaluated the capability of VitD3-TolDCs to induce allogeneic PBMCs proliferation in Mixed Lymphocyte Reactions in comparison to HD.

Results: MS patients' monocytes shift from classical to the intermediate subset and show increased expression of inflammation markers. Moreover, in MS monocytes we identified methylation changes and enrichment of specific transcription factors binding motifs in regions of genes involved in immune activation and response. Methylation data was validated through qPCR, confirming implication of the AHR pathway. Moreover, RNAseq identified differences among monocytes and tolDCs from patients and controls. Finally, VitD3-tolDCs from MS patients, despite presenting less methylation changes than monocytes, resulted less able to suppress allogenic proliferation than those from HD.

Conclusions: MS monocytes present a more inflammatory phenotype in comparison to HD and when used to produce VitD3-tolDCs generate less powerful tolerogenic cells. Given the involvement of specific TFs in the gene signature of MS patients' vitD3-tolDCs, we are currently exploring if these proteins could be targeted to increase the potency of our cellular product.

CO-27

FABRICACIÓN DE TERAPIAS AVANZADAS EN LOS CENTROS HOSPITALARIOS: EL RETO DE LAS NORMAS DE CORRECTA FABRICACIÓN

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Hospital Ramón y Cajal, Madrid.

INTRODUCCIÓN: En los últimos años la práctica clínica está siendo protagonista de una revolución con el éxito de nuevos tratamientos conocidos como Terapias Avanzadas (ATMP, Advance Therapy Medicinal Products).
Algunas ATMP han demostrado su eficacia contra enfermedades que hasta el momento carecían de tratamientos eficaces. Este es el caso de las terapias CAR-T, actualmente autorizadas para el tratamiento de cánceres hematológicos de estirpe B y mieloma múltiple.
Pero las terapias CAR-T al ser productos personalizados presentan dos retos importantes: su elevado coste y un tiempo de fabricación demasiado largo, factor crítico para el éxito del tratamiento dada la gravedad de algunos pacientes.
Con el objetivo de reducir costes y tiempo de fabricación, facilitando el acceso a esta innovadora terapia a los pacientes, algunos hospitales como el Hospital Clinic de Barcelona (HCB) o el Hospital La Paz en Madrid, han puesto en marcha con éxito sus propias unidades de producción de CAR-T.
OBJETIVO: Poner en marcha una unidad para la producción de CAR-T en el Hospital Ramón y Cajal que cumpla los requisitos para obtener el certificado de Normas de Correcta Fabricación (NCF) por parte de la Agencia Española de Medicamentos y Productos Sanitarios (AEMPS).
RESULTADOS: Actualmente disponemos una unidad de producción con instalaciones y equipamiento para la producción de terapia CAR-T bajo cumplimiento de las NCF. Se han llevado a cabo con éxito las validaciones requeridas por esta normativa y hemos implantado un sistema de calidad farmacéutico que asegura que nuestros productos no suponen ningún riesgo para los pacientes por una inadecuada seguridad, calidad o eficacia.
CONCLUSIÓN: Supone un gran reto para los hospitales adaptarse a las directrices y normas que regulan la producción de ATMP. Nos gustaría compartir nuestra experiencia esperando poder orientar a otros servicios de inmunología que se estén planteando iniciar un proyecto de este tipo.

CO-28

IMMUNOCYTOKINES BASED ON IL-12 THAT TARGET CSF1R AND PD-L1 RETAIN ANTI-TUMOR EFFECTS WHILE LIMITING SYSTEMIC EXPOSURE

Di Trani, Claudia Augusta; Arrizabalaga, Leire; Cirella, Assunta; Álvarez, Maite; Bella, Ángela; Fernandez-Sendin, Myriam; Russo-Cabrera, Joan Salvador; Otano, Itziar; Gomar, Celia; Ardaiz, Nuria; Belsue, Virginia; Aranda, Fernando; Melero, Ignacio; Berraondo, Pedro.

Cima Universidad de Navarra, Pamplona.

IL-12 is a highly effective cytokine for cancer immunotherapy, but its systemic delivery as a recombinant protein has caused toxic side effects in early clinical trials. To overcome this, current clinical trials are exploring the use of IL-12-coding mRNA delivered directly into the tumor. This study aimed to improve this method by improving IL-12's anchoring to the tumor. We created mRNA encoding single-chain IL-12 fused with bispecific diabodies targeting CSF1R and PD-L1, which are molecules abundantly expressed by macrophages in the tumor microenvironment. We demonstrated the binding ability and effectiveness of these constructs *in vitro*. Even small doses of the IL-12-coding mRNA resulted in a potent anti-tumor effect in two types of subcutaneous-implanted tumor models, associated with elevated circulating IL-12p70 and IFN-γ levels. Fusion of IL-12 to diabodies targeting PD-L1, CSF1R, or both resulted in similar anti-tumor effects, with IL-12 and IFN-γ levels barely detectable in circulation. The anti-tumor efficacy was linked to changes in the phenotype of $CD8^+$ and $CD4^+$ T lymphocytes, NK cells, and myeloid cells in the tumor niche. In conclusion, the intra-tumor delivery of mRNA-encoding immunocytokines represents a promising approach to improve IL-12-based cancer immunotherapy.

CO-29

NOVEL MONOCLONAL ANTIBODY DEVELOPED THROUGH PATIENT SPECIFIC APPROACH TO TREAT SÉZARY SYNDROME, AN ULTRA-RARE CANCER OF MATURE T LYMPHOCYTES.

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[1]Department of Immunology, Ophthalmology and ENT, Complutense University School of Medicine and 12 de Octubre Health Research Institute (i+12), Madrid; [2]Servicio de Inmunología Microbiana, Centro Nacional de Microbiología, Instituto de Investigación Carlos III, Majadahonda, Madrid, Spain., Madrid; [3]Clinical Immunology-Experimental and Surgery Department, Hospital Clínico Universitario San Carlos, Madrid.

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 their TCR, against which we aim to generate specific mouse monoclonal antibodies (mAbs).

Using CD4 and PD-1, primary SS PBL were sorted as SS (tumoral, PD-1+) and non-SS (healthy, PD-1-)) T cells were expanded *in vitro* using different immortalization procedures, such as exposure to HTLV-I. We showed that *in vitro* expanded SS cell lines recapitulate the clonal malignant TCR by PCR. The SS CDR3β was obtained by sequencing ex vivo SS PBL cells.

Mice were immunized with a SS-specific CDR3β synthetic peptide and mAb-producing hybridomas were generated by fusion of splenocytes with mouse myeloma cells (SP2). MAb reactivity screening was tested against the CDR3β peptide by ELISA, and against non-SS and SS cells with a FITC-Rabbit Anti-Mouse polyclonal Ab by flow cytometry. Three selected subclones (28.2.6, 28.3.10 and 28.4.21) consistently recognized immortalized SS cells much better than non-SS T cells, using an anti-CD3 hybridoma (Leu-4) as a positive control. We are currently purifying the hybridomas and characterizing their VH and VK specificity by sequencing. In conclusion, we have generated three mAbs specific for SS cells that could become patient-specific immunotherapy to treat SS.

CO-30

UNLOCKING THE THERAPEUTIC POTENTIAL OF PHOSPHATIDYLSERINE-LIPOSOMES IN AUTOIMMUNE TYPE 1 DIABETES

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Recent advances in the field of immunotherapy are moving towards the development of antigen-specific treatments designed to inhibit autoimmune aggression. However, new therapeutic approaches are still needed to restore self-tolerance in a safe and effective manner. We have previously demonstrated that phosphatidylserine (PS) rich liposomes encapsulating Type 1 Diabetes (T1D) autoantigens have the potential to prevent experimental T1D through the induction of tolerogenic dendritic cells and regulatory T cells. Our aim was to better understand the biodistribution of the PS-liposomes and the cells involved in their tolerogenic mechanism of action in the context of T1D. To this end, fluorescence was monitored in organs of mice treated intravenously (i.v.) with fluorescently labelled PS-liposomes, and the *in vivo* interaction with immune cell subsets in different organs was explored. In addition, to study the antigen-specific tolerogenic effect, immune cells of NODBDC2.5 transgenic mice were evaluated after treatment with 2.5mi loaded PS-liposomes. Rapid biodistribution was observed after the i.v. route, with peak values reaching 1 hour after injection and little bioaccumulation 24 hours after. Considerable amounts of fluorescence were detected in the spleen and liver, and phagocytic cells in these organs confirmed the *in vivo* engulfment. Furthermore, we found that antigen-specific PS-liposomes induced an increase in TR1 cells, conventional regulatory T cells, and B cell regulatory cells, while promoting anergy and exhaustion of T cells in NODBDC2.5 mice. To further explore translationality to humans, PS-liposomes were tested *in vitro* with APCs from T1D patients, revealing efficient phagocytosis and increased secretion of tolerogenic cytokines in those cells that were interacting with PS-liposomes. These findings advanced the understanding of the tolerogenic mechanism of action of PS liposome-based immunotherapy, while expanding the possibilities for future treatments against human autoimmune diseases.

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Sesión Comunicaciones Orales 5 – Trasplante

CO-31

IMPLICATIONS OF HLA MISMATCHES AT EPITOPE LEVEL IN CLINICAL EVOLUTION OF HAPLOIDENTICAL STEM CELL TRANSPLANTATION WITH POST-TRANSPLANT CYCLOPHOSPHAMIDE

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Background: The activity of hematopoietic stem cell transplantation from haploidentical donors is steadily increasing in recent years. However, haploidentical transplantation presents an increased risk for complications and requires elevated doses of immunosuppression, since the number of HLA mismatches between donor and patient is higher.
The aim of this study was to determine which HLA mismatches could affect clinical evolution including the risk of graft vs host disease in patients transplanted with haploidentical donors and post-transplant cyclophosphamide as graft-versus-host disease prophylaxis (PTCy).
Methods: A retrospective cohort of 127-haploidentical stem cell transplanted patients at Hospital Universitario de Salamanca was analysed. HLA-A, -B, -C and DRB1 loci were typed in each pair of donor-recipient by Sequence Specific Oligonucleotide or Next Generation Sequencing. HLA compatibility was evaluated calculating the presentable epitopes by PIRCHE algorithm, according to serological mismatches.
Results: The median age of transplanted cohort was 58 (IQR: 41-64). The analysis of the presentable epitopes of HLA Class-I illustrates the potential role of HLA-C mismatches in graft-versus-host disease: acute (OR: 1.93, 95% CI: 1.08-3.35), chronic (OR: 2.15, 95% CI: 1.19-3.83) and total graft vs host disease rates (OR: 2.26, 95% CI: 1.27-3.96). The analysis of epitopes showed that higher discrepancies in HLA-DRB1 were significantly associated with poorer engraftment (OR: 7.34, 95% CI: 3.93-13.3), relapse rate (OR: 2.33, 95% CI: 1.28-4.21) and death (OR: 2.12, 95% CI: 1.19-3.74). Finally, epitopes evaluation demonstrates that HLA-C PIRCHE score >3 behaved as an independent risk factor for unfavourable evolution (OR: 3.54,

95% CI: 1.12-11.16, AUC: 0.689).

Conclusions: Our results suggest that bioinformatics scores, such as PIRCHE, could be used as predictive tool to improve HLA compatibility between donor and patient, contributing to minimize the development of clinical complications in haploidentical stem cell transplantation with PTCy.

CO-32

EXPANDING THE APPLICATIONS OF NGS-BASED HLA TYPING: HLA LOSS RELAPSE DETECTION AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION

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HLA loss relapse (HLA-LR) consists on genomic loss of mismatched HLA molecules in re-emerging leukemic cells after hematopoietic stem cell transplantation (HSCT). Donor lymphocyte infusion is no effective as a rescue therapy for HLA-LR so relapse classification is crucial for guiding therapeutic decisions. Commercial kits specifically designed to detect HLA-LR are based on qPCR so they provide high sensitivity but allele and gene HLA coverage limitation. Assessing HLA-LR by Next-generation sequencing (NGS)-based HLA typing not only could overcome this limitation but also involves high-throughput and flexibility.
The aim of this study is to optimize and validate a NGS-based HLA typing strategy for HLA-LR detection.
As NGS-based HLA typing is not commonly used to analyse chimeric samples, we firstly performed artificial chimeric curves (N=7) in order to assess the sensitivity in detecting the low-represented allele for each HLA gene (allele 3) (Figure 1A). Allele 3 was only detected in all HLA genes until point 90%-10% so optimization was required to improve its detection in samples with more extreme chimerism (Figure 1B). By increasing deep coverage and/or performing single-gene PCR, allele 3 was detected until point 97%-3% (Figure 1C).
For the validation phase, 23 post-HSCT patient samples with known chimerism were analysed according to the optimized procedure and allele 3 was successfully detected in 20/23 samples (Figure 1D). In addition, 13 samples from 4 relapsed patients were also analyzed and allowed the classification of relapse in classical or HLA loss (Figure 1E).
The classification of relapse though NGS-based HLA typing has been implemented as a new test in our lab with minimal workflow modification of the technique and in a range from

0%Donor-100%Patient to 95%Donor-5%Patient.

In conclusion, classification of post-HSCT relapse by NGS-based HLA typing has been implemented in our lab as a new test with complete HLA gene and allele coverage.

CO-33

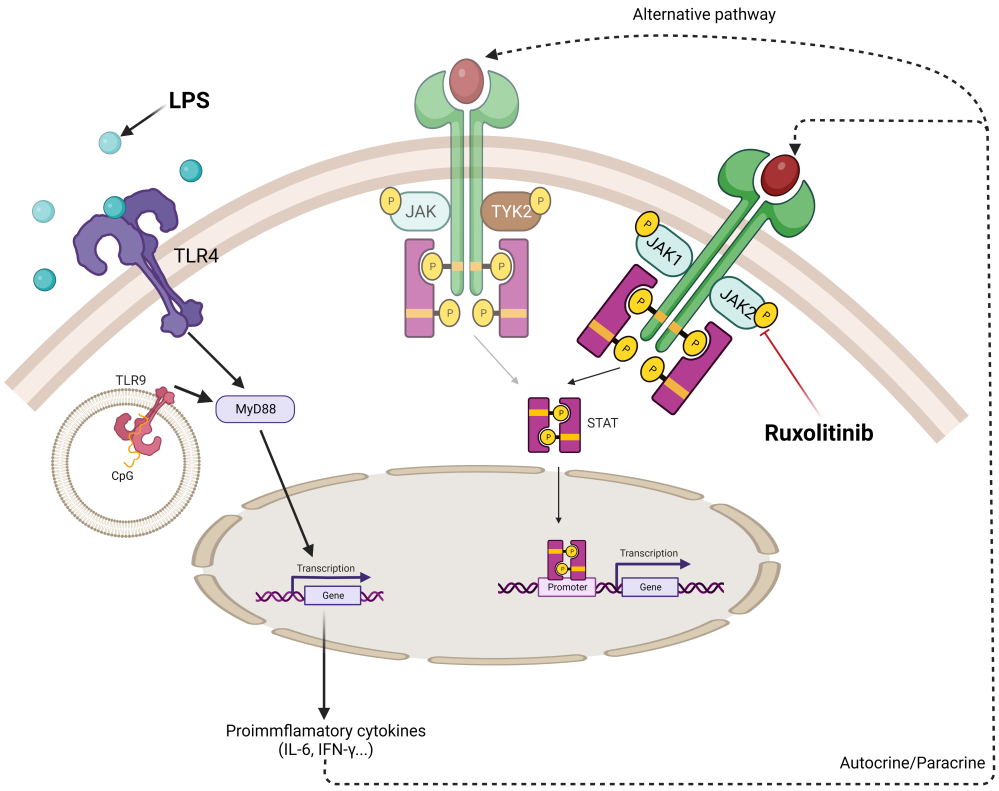
RUXOLITINIB NO INHIBE COMPLETAMENTE LAS CAPACIDADES FUNCIONALES DE CÉLULAS NK ACTIVAS CON LIGANDOS DE TLR4/9

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Las células *natural killer* (NK) son linfocitos que forman parte de la primera defensa contra las células infectadas y transformadas. La capacidad citotóxica de las células NK está controlada por un equilibrio entre los receptores inhibidores y activadores expresados en su superficie, que reconocen a sus ligandos en las células estresadas. Las celulas NK son activadas por citoquinas a traves de la vía de señalización JAK-STAT, pero también se activan mediante receptores tipo Toll (TLR) expresados en su superficie. Ruxolitinib (Novartis) es un inhibidor específico de JAK1/2 que ha sido aprobado para el tratamiento de la mielofibrosis y la enfermedad aguda y crónica de injerto contra receptor refractaria a esteroides (SR-EICR). En este estudio, se obtuvieron células NK purificadas a partir de donantes sanos que fueron incubadas durante 16h con ligandos de los receptores tipo Toll TLR4 y TLR9, en presencia o ausencia de dosis crecientes de ruxolitinib (0.1, 1 y 10 µM). Observamos que ruxolitinib no inhibe completamente la fosforilación de STAT3. Las dosis más altas de ruxolitinib (10µM) disminuyeron la producción de citoquinas proinflamatorias en células NK activas con ligandos de TLR4/9. La

capacidad citotóxica y degranulativa de las células NK estimuladas frente a las líneas celulares K562, SEM y MV-4-11 se redujo en presencia de dosis crecientes de ruxolitinib, pero no se abolió por completo. Se observaron cambios fenotípicos en las células NK activadas en presencia de ruxolitinib. En una pequeña cohorte de pacientes pediátricos tratados con ruxolitinib para la EICR, no observamos ninguna disminución en el número de células NK, aunque serían necesarios más estudios prospectivos con una cohorte mayor. En resumen, nuestros resultados muestran que las capacidades funcionales y el fenotipo de las células NK activadas a través de agonistas TLR4/9 no fueron completamente abolidos mediante la inhibición de la vía JAK-STAT por ruxolitinib.



CO-34

ANALYSIS OF HLA-TYPE IMPUTATION BASED ON THE MOST FREQUENT ALLELE IN THE CALCULATION OF THE TOTAL EPLET LOAD

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Introduction

In solid organ transplantation, HLA class-I and -II eplets disparity is associated with *de novo* development of donor specific anti-HLA antibodies, rejection, and graft failure. To accurately define eplet load, high-resolution typing is required, so HLA imputation is needed in studies with low-resolution typing. Recently, it has been published that a high percentage of the imputed types do not correspond to the real type, affecting eplets load. We ought to investigate the potential bias in total eplest load measurement after imputation of most frequent alleles.

Materials and methods

The allelic frequency of the different HLA loci was calculated using a database of high-resolution typing of voluntary bone marrow donors (23,406 for class-I HLA and 23,628 for class-II HLA), obtained from the Spanish Registry of Bone Marrow Donors (REDMO), managed by the Carreras Foundation.

The eplets count in the different alleles was calculated using the HLA-Matchmaker v3.1 algorithm.

Results

The probability of assigning the correct eplet load with imputation typing in HLA class-I was 93.8%, 91.1 and 83.9 for loci A, B and C, respectively. For HLA-DRB1 and DQB1 loci, it was 84.8% and 69.1%, respectively (Figure 1).

When HLA-A, HLA-B and HLA-DRB1 imputation is incorrect, the most frequent bias is the underestimation of one eplet (in 3.7%, 3.4% and 11.5%, respectively). Unlike locus C, where the most frequent bias corresponds to increase one eplet in the 12.5% of the cases. On the other hand, in 12% of cases, two eplets are lost at locus DQB1 (Figure 1).

Conclusion

The disparity of eplets is not significantly overestimated or underestimated, despite the error in

the imputation of HLA class-I type. However, in class-II the risk of bias for eplets load increases after imputation. In retrospective studies for total eplets mismatch load, high-resolution for class-II loci is highly recommended when possible.

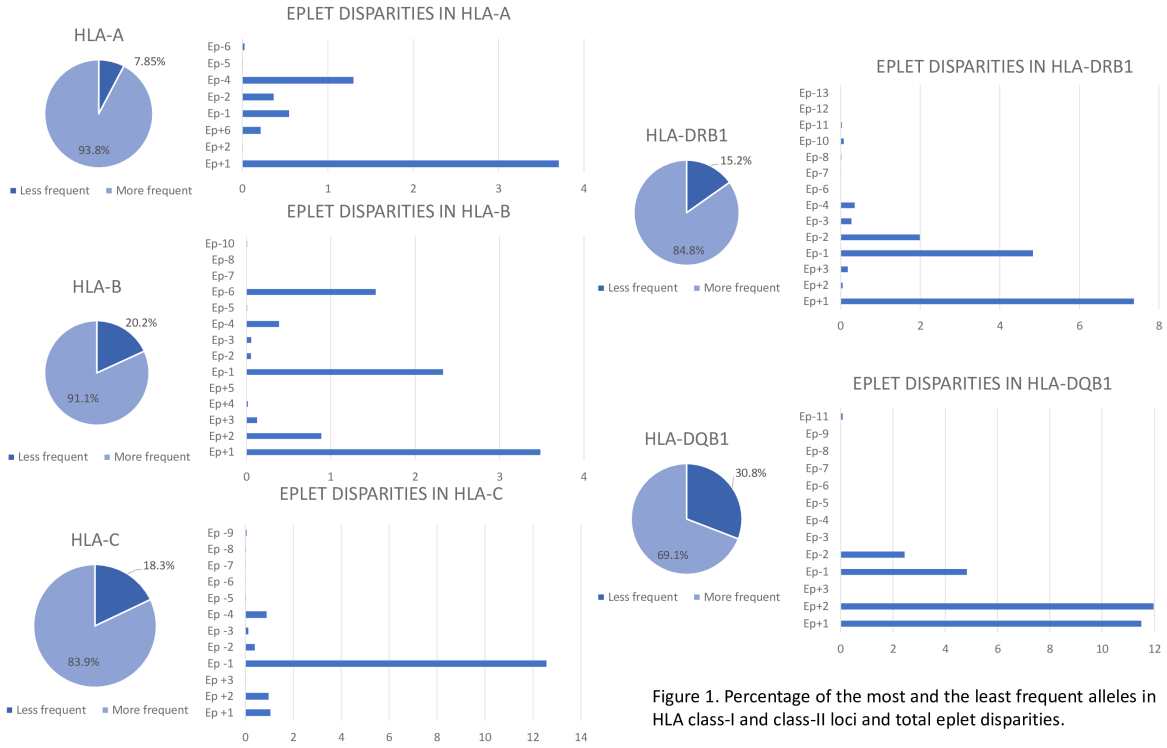


Figure 1. Percentage of the most and the least frequent alleles in HLA class-I and class-II loci and total eplet disparities.

CO-35

DONOR-DERIVED CELL-FREE DNA AS A BIOMARKER OF KIDNEY GRAFT DAMAGE

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Background: Despite the improvement in renal transplant results, the alloimmune response remains the leading cause of renal graft loss. Currently, the only way to detect the alloimmune graft damage is to perform a surveillance or by indication renal biopsy. Recent studies have shown that donor-derived cell-free DNA (dd-cfDNA) is a non-invasive biomarker of allograft injury related to rejection before clinical allograft dysfunction. Our objective was to analyze the role of this biomarker in detecting kidney graft damage and acute rejection.

Methods: A prospective study was carried out including 36 renal transplants performed in our unit throughout 2021. All acute rejections were biopsy proven (BPAR). "Troubled" graft was defined if the graft was suffering acute rejection or any other problem such as obstruction or graft infection. Blood samples were obtained on streck tubes at 1-month post-transplant. Cell free DNA was extracted from plasma using QIAamp Circulating Nucleic Acid Kit (Qiagen), amplified by using multiplex PCR with CareDX AlloSeq cfDNA kit and sequenced on MiSeq sequencing instrument (Illumina, Inc.) to calculate the percentage of circulating dd-cfDNA.
Results: Six (17%) patients suffered acute rejection before the second month. dd-cfDNA was significantly higher in patients with BPAR and antibody-mediated rejection (AbMR) compared to patients without rejection (1.54%, IQR 1.56% vs 0.49%, IQR 0.47% p = 0.002 and 1.83%, IQR 1.33% vs 0.50%, IQR 0.45% p = 0.001, respectively) .dd-cfDNA discriminated BPAR and AbMR patients with an AUC of 0.889 and 0.923, respectively. Among 5 patients with dd-cfDNA above 1% without BPAR, 2 suffered hydronephrosis and one of them developed a graft pyelonephritis. dd-cfDNA identified "troubled" graft (OR 37.96, 95%CI 3.13-460.23, p = 0.004) independently of renal function.

Conclusions: Using Alloseq to determine dd-cfDNA enables minimally invasive identification

of kidney transplant damage, especially rejection, independently of kidney function.

ISCII funding: PI20/01710 and RD21/0005/0010

CO-36

INDUCTION OF TOLERANCE IN LIVER TRANSPLANTATION BY MANIPULATION OF T CELL METABOLISM: AN *IN VITRO* AND *IN VIVO* APPROACH

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The paradigm for successful organ transplantation relies on a balance between regulatory T cells (Treg) and alloantigen-reactive effector T cells (Teff). CD4+ FOXP3+ Treg present regulatory properties, inhibiting Teff cells activity. While Teff cells employ aerobic glycolysis and amino acid uptake and metabolism, Treg rely more on fatty acid oxidation to generate energy.

The principal aim was to determine if manipulation of metabolism (*in vitro* and *in vivo*) induces differential immunomodulatory responses between Treg and Teff cells, promoting a scenario of immunological tolerance to liver transplantation.
Proliferation degree, metabolic-related gene expression, pro-inflammatory cytokines release or FOXP3 demethylation ratio of CD4+ T cells obtained from mouse spleen was analyzed by *in vitro* culture, using chemical or natural compounds as well as formulated media trying to replicate different metabolic scenarios. An adoptive OT-II splenocyte transfer assay was used to determine the effect of the manipulation of the metabolism (ketogenic diet) over the proliferation and function of antigenic-specific CD4+ Thelper cells compared to Tregs *in vivo*.
A decrease of cell proliferation was observed, mainly affecting Teff. Proliferation of Treg was favored when glutaminolysis inhibitors, as well as media poor in glucose and glutamine were added to the cell culture. Likewise, we observed a decrease in the expression of genes related to glycolysis, glutaminolysis and fatty acid synthesis, as well as an increase in the expression of genes related to fatty acid oxidation. Also, a decrease in the secretion of proinflammatory cytokines, as well as a lower percentage of methylation of the TSDR region of the FOXP3 gene of Treg was found. Moreover, transferred OT-II CD4+ cells presented a lesser proliferation rate against ovalbumin immunization when mice were under a ketogenic diet.
These results indicate that manipulation of cellular metabolism may create an environment where Treg prevail over Teff cells.

CO-37

POTENTIAL IMMUNOMODULATORY ROLE OF SPECIFIC ANTI-CYTOMEGALOVIRUS INTRAVENOUS IMMUNOGLOBULIN IN SOLID ORGAN RECIPIENTS WITH DIFFICULT TO CONTROL CMV DISEASE

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Immune response to cytomegalovirus (CMV) infection is complex, including cellular, humoral, acquired and innate immune responses. Specific anti-cytomegalovirus intravenous immunoglobulin (CMV-IVIG) is used to prevent CMV infection mainly in high risk heart and lung recipients [Donor(+)/Receptor(-)]. Recent studies are reporting that CMV-IVIG is also used in patients with difficult to control CMV disease after solid organ transplantation (SOT). CMV-IVIG has the potential to influence the immune response exerting immunomodulatory activity, but its complex mode of action has not been well assessed.

We aimed to evaluate CD4+, CD8+ and CD19+ immunophenotypic subsets before and after CMV-IVIG (Cytotect CP 5% or Megalotect 5%) use in SOT recipients with difficult to control CMV disease (lack of control despite antiviral therapy). Patients were evaluated by flow cytometry at the time of diagnosis of difficult to control CMV disease and at the end of therapy or similar time in controls (treated with conventional antimicrobial therapy alone). 9 SOT treated with conventional antimicrobial therapy (Heart 7, Liver 2) and 5 with conventional antimicrobial therapy and CMV-IVIG (Kidney 2, Liver 1, Heart 2) were evaluated.

Baseline and post-therapy CD8+ producing IFN-gamma levels after incubation with IE1-CMV antigen were 0,27% and 0,44% (+0,17%) in CMV-IVIG group and 0.15% and 0,19% (+0,04%) in conventional therapy alone group. Kinetics of CD8+DR+ cells was 60,89% and 37% (-23,88%) in CMV-IVIG group and 54,73% and 53,29% (-2,87%) in non CMV-IVIG group. Levels of naïve B-cells were 49,21% vs 61,43% (+14,35%) and 41,34 vs 40,05 (-0,34%) in CMV-IVIG and non CMV-IVIG groups respectively.

The kinetics of T-cell activation and of naïve B-cells is different in CMV-IVIG treated patients with difficult to control CMV disease suggesting a possible immunomodulatory effect of this specific anti-CMV therapy in difficult to control CMV disease in SOT. Clinical trials in this indication are warranted.

Sesión Comunicaciones Orales 6 – Inflamación / Inmunidad innata

CO-38

THE NLRP3 OF CRYOPYRIN-ASSOCIATED PERIODIC SYNDROMES FORMS A CONSTITUTIVELY ACTIVE INFLAMMASOME

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Cryopyrin-associated periodic syndrome (CAPS) is a hereditary autoinflammatory disease due to a gain-of-function mutation in the NLRP3 gene. Patients with this syndrome suffer from inflammatory episodes without any type of infection, resulting in activation of the NLRP3 inflammasome and overproduction of pro-inflammatory cytokines such as IL-1β or IL-18. CAPS-associated NLRP3 inflammasome is often presented as a hypersensitive inflammasome but the exact activation mechanism of the NLRP3 CAPS inflammasome is not well understood. In this study, we used blood from patients with different NLRP3 CAPS variants (D303N, R260W, T348M, and A349T), and immortalized mouse macrophages that express different CAPS variants (D303N, R260W, and T348M) under a doxycycline-inducible system. Inflammasome activation was assessed by ELISA, Western blot, flow cytometry, and immunofluorescence.

Contrasting with the wild-type NLRP3, the NLRP3 with CAPS-associated variants presented a constitutive oligomerization of ASC, activation of caspase-1, cleavage of gasdermin D, the release of IL-18, and pyroptotic cell death. All these were reversed using MCC950 (a specific NLRP3 inhibitor). Furthermore, this basal inflammasome activation was further modulated by NF-κB activators such as lipopolysaccharide or Pam3-CSK and endogenous molecules such as IL-6, S100A9, and palmitate, which also expanded the secretome of CAPS-associated inflammasomes. We also found that post-translational deubiquitination was also involved in the activation of CAPS-associated inflammasomes. Additionally, we observed that the NLRP3 CAPS variants conditioned the metabolism of macrophages, reducing both glycolysis and mitochondrial respiration, which could be reversed by NLRP3 inhibition.

This study shed new light on the origins of non-infectious inflammatory flares in CAPS patients and elucidated new pharmacological targets associated with the activation of the inflammasome.

CO-39

NATURAL KILLER CELL SUBSETS IN ENDOMETRIAL FLUID: ANALYSIS OF THEIR ASSOCIATION WITH THE ENDOMETRIAL CYCLE AND REPRODUCTIVE PARAMETERS

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Endometrial fluid is a protein-rich liquid that contains secretions from the endometrial glands and cleavage products of both the secreted proteins and the glycocalyx. Depending on the methodology employed, more than 4,600 proteins, more than 270 lipids and more than 920 micro-RNAs have been detected. Moreover, different proteic, lipidomic and micro-RNA patterns have been described as prognostic factors of implantation in the very same cycle in which endometrial fluid was obtained. Natural Killer (NK) cells are a type of lymphocyte consisting of large granular lymphocytes that belong to the innate immune system. They are derived from hematopoietic progenitor cells (HPCs) in the bone marrow and express the surface marker CD56, and they are defined as CD3−CD56+ lymphocytes. There are two main types of NK cells: i) peripheral blood NK cells (pbNK), with low CD56 expression (CD56dim) and high levels of CD16, whose activity is predominantly cytotoxic; and ii) uterine NK cells (uNK), which are mostly CD56bright CD16− cells and which correlate with a more immune-regulatory role. NK cells have been thought to be associated with implantation failures, recurrent miscarriage (RM) or infertility (IF), but results are controversial. The aim of our study is to investigate the presence of NK cells in endometrial fluid and their immunophenotype, to ascertain their changes, if any, during the endometrial cycle and to determine their association with reproductive parameters. We analyzed EF from 44 patients and found that the pattern of the NK cells presence in this fluid were neither pbNK nor uNK. Nonetheless, the surface markers present in the NK cells were more similar to pbNK, while the pattern of the population was different. Also, we found that CD16 marker in EFNK cells increases during the endometrial cycle, suggesting it plays a role in implantation. This could be an interesting point for future studies.

CO-40

THE LONG-TERM IMMUNOMODULATORY CAPACITY OF PHLOROGLUCINOL, A MICROBIOTA DERIVED METABOLITE, AS TREATMENT FOR ACUTE COLITIS

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Inflammation is an essential defensive process in response to physical, chemical, and biological insults. However, prolonged inflammation can become a risk factor in the development of diseases by itself or leading to chronic inflammatory diseases, such as inflammatory bowel disease or cancer. High dietary intake of phenolic compounds via fruit, vegetables and cereals has been related to a lower incidence of these diseases. Among phenolic compounds, gallotannins and their microbial metabolites have barely been studied in this context. Depending on the composition of the individual microbiota, gut bacteria can differentially transform these compounds, resulting in different final metabolites with the ability to modify the composition of the microbiota and/or modulate the immune response. Phloroglucinol (PG), one of the key metabolites resulting from this gut microbial metabolism of polyphenols, has been widely used as a treatment for Irritable Bowel Syndrome, although its mode of action is still unclear. In this work, we have studied the capacity of PG to modulate the immune response. Our research, using in vitro studies and mouse models of inflammation, explores its properties as a modulator of the innate immune response and its ability to modify the microbiota. Our work describes how this compound is able to strongly attenuate inflammatory bowel disease in the mouse modulating the response of macrophages to pro-inflammatory pathogens in acute experimental settings and more importantly, in the long term, through the induction of long lasting innate immune training in the hematopoietic lineages.

CO-41

CALCINEURIN/NFAT PATHWAY CONTRIBUTES TO B-GLUCAN-INDUCED TRAINED IMMUNITY BY EPIGENOMIC REPROGRAMMING OF MYELOID CELLS

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β-glucan stimulation of macrophages induces changes in their epigenetic landscape, which result in a boosted inflammatory response against a secondary heterologous challenge, a process termed trained immunity (TI). In addition to this epigenetic rewiring, trained macrophages experience a significant metabolic shift that helps to maintain the epigenetic modifications required for the long-lasting trained phenotype. Nevertheless, the initiating events leading to this reprogramming in TI have not been fully elucidated. Here, we show that early signalling processes induced by β-glucan through its receptor Dectin-1, specifically the calcineurin/NFAT signalling pathway, are crucial for the establishment of TI. We found that both pharmacological inhibition and genetic deficiency of calcineurin in macrophages (LysMΔ*Cnb1* mice) dampen the β-glucan-induced trained response, *in vitro* and *in vivo*. Of note, calcineurin inhibition or deficiency did not affect the mTOR/HIF-1α-mediated glycolytic switch induced by β-glucan. Instead, activation of the calcineurin/NFAT signalling pathway promotes chromatin accessibility at genomic regions related to immune signalling and function, priming the response to a secondary stimulation. *In vivo*, calcineurin inhibition decreases the β-glucan-enhanced heterologous response to LPS or Staphylococcus aureus infections. Moreover, calcineurin deficiency in myeloid cells decreases TI induced by Western diet and reduces the development of atherosclerotic lesions in mice fed a high cholesterol diet. Additionally, monocytes from kidney transplant patients receiving chronic treatment with calcineurin inhibitors failed to mount β-glucan-induced TI. These findings highlight the crucial role of the calcineurin/NFAT pathway in epigenetic priming and suggest it may be a target for modulating trained innate immune responses.

CO-42

SINGLE-CELL ANALYSIS OF THE ANTIBODY IMMUNE RESPONSE IN PCSK9-AAV ATHEROSCLEROTIC MICE

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Atherosclerosis is a chronic inflammatory disease of the arteries that can lead to thrombosis, infarction and stroke, being one of the primary causes of mortality worldwide. The adaptive immune response, orchestrated by T- and B-lymphocytes, is critical during atherosclerosis. Recently, our lab performed a study of the atherosclerosis-associated antibody repertoire and identified an antibody that delays atherosclerosis progression, highlighting the importance of B cells in the disease.

Here we aim at developing a map of the B-cell response during atherosclerosis at single cell resolution. We made use of the pro-atherosclerotic PCSK9-AAV mouse model, combined with the *Aicda*-Cre$^{+/ki}$ *R26tdTom*$^{+/ki}$ mouse strain, which allows tracing activated B cells. *Aicda*-Cre$^{+/ki}$ *R26tdTom*$^{+/ki}$ mice were single-injected with PCSK9-AAV and fed with either high fat diet (HFD) or chow diet. A group of mice injected with control AAV and fed with chow diet were included as an additional control. Flow cytometry analysis of peripheral blood at different timepoints showed a progressive increase in Tom+, activated B cells, in the HFD-fed PCSK9-AAV mice group compared to control mice. After 16 weeks of HFD, we detected a bona fide antibody immune response that includes a germinal center response as well as plasma cell and memory B cell generation. To address clonal expansion and selection, we have performed single-cell V(D)J and transcriptome analysis of activated B cells. We have found that atherosclerotic mice showed shifts in the proportions of activated B cell subpopulations and have specific expanded clones. Our results reinforce the idea that atherosclerosis associated with a specific humoral immune response.

CO-43

INBORN ERROR OF STAT2-DEPENDENT IFN-I IMMUNITY IN A PATIENT PRESENTED WITH HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS AND MULTISYSTEM INFLAMMATORY SYNDROME IN CHILDREN

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Human inborn errors of innate immunity affecting the type I interferon (IFN-I) pathway have been associated with susceptibility to severe viral illness. Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening systemic hyperinflammatory syndrome that has been increasingly associated with inborn errors of IFN-I-mediated innate immunity. Here is reported a novel case of complete deficiency of STAT2 in a 3 years-old child that presented with typical features of HLH after mumps, measles and rubella vaccination at the age of 12 months. Due to the life-threatening risk of viral infection, she received SARS-CoV-2 mRNA vaccination. Unfortunately, she developed multisystem inflammatory syndrome in children (MIS-C) after SARS-CoV-2 infection, four months after the last dose. NGS revealed a novel homozygous loss of function variant (c.633+2T>C) in the *STAT2* gene altering the correct splicing. Expression of IFN-I-stimulated genes such as *IRF7, ISG15, USP18 and IFIT1* were severely decreased in the patient. Functional studies showed an impaired IFN-I-induced response and a defective IFNα expression at later stages of STAT2 pathway induction. Furthermore, a possible transient defect of NK-cell degranulation was observed during HLH presentation. These results suggest a possible more complex mechanism for hyperinflammatory reactions in this type of patients involving a possible defect in the IFN-I production. Understanding the cellular and molecular links between IFN-I-induced signaling and hyperinflammatory syndromes can be critical for the diagnosis and tailored management of these patients with predisposition to severe viral infection.

CO-44

TWO LEUKOCYTIC CELL LINES DEFICIENT IN MEMBRANE COMPLEMENT REGULATORS AS BIOSENSORS OF ABNORMAL COMPLEMENT DEPOSITION AND THEIR USE FOR TREATMENT EFFICACY

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Tight regulation of the complement system is critical to avoid uncontrolled activation and self-tissue damage. Plasma complement dysregulation has been involved in the pathology of many human diseases, particularly renal disorders, that usually entail costly treatments that are difficult to monitor. Currently there are no available standardized tests to demonstrate dysregulation with diagnostic, prognostic, and therapeutic implications. Thus, there is a need to develop tools to monitor the involvement of complement in diseases and to explore the complex interplay between complement activation and regulation on human cells *ex vivo*.
The analysis of complement deposition on human cells after incubation with dysregulated human plasma could be used as a reference and inexpensive assay for this purpose, as it closely resembles the physiological situation. We have developed the immortalized human cell lines K562 and EBV devoid of certain membrane complement regulators (to enhance their susceptibility to complement deposition) as biosensors of abnormal plasma complement activation and have shown that they can be used to detect by flow cytometry early (C3b) or late (C5b9) complement fragment deposition on their cell membranes by plasma obtained from patients with renal disorders such as atypical hemolytic uremic syndrome or C3 glomerulopathies. Our next aim was to test the efficacy of the biosensors to monitor the treatment of these patients.
We observed an increased surface C3b and/or C5b9 deposition in patients with different renal disorders carrying C3 or unknown mutations. Moreover, a reduction of C3b or C5b9 deposition was observed when we tested samples from the patients after they were treated with anti-factor B or anti-C5, respectively.

Together, the results indicated that the combination of the two biosensors could be useful to characterize complement deposition-related renal disorders and suggest the potential utility to monitor the treatment efficacy in these patients.

Sesión Comunicaciones Orales 7 – Inmunidad frente a las infecciones

CO-45

LA PRESENCIA DE CANDIDA ALBICANS INDUCE ALTERACIONES METABÓLICAS Y MOLECULARES EN LAS CÉLULAS DE MELANOMA B16-F10 RELACIONADAS CON UN FENOTIPO TUMORAL MÁS AGRESIVO.

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Candida albicans es un hongo patógeno oportunista que forma parte de la microbiota humana en localizaciones como la cavidad oral, el intestino, o la piel. Además, en los últimos años su presencia también se ha asociado con la aparición de tumores primarios y metástasis, principalmente en la cavidad oral y el colon. Sin embargo, hasta el momento existen pocos estudios sobre el efecto que este hongo ejerce sobre las células tumorales y, concretamente, ninguno en melanoma. Por lo tanto, el objetivo de este trabajo fue estudiar el efecto del hongo *C. albicans* sobre las células tumorales de melanoma *in vitro* e *in vivo*.

Para ello, se realizaron ensayos *in vitro* de migración, capacidad adhesiva a células endoteliales, y proliferación celular, utilizando células de melanoma tras su exposición a *C. albicans*. Los tres procesos mostraron un aumento significativo tras el contacto con el hongo. Asimismo, se realizaron análisis de expresión génica por RNA-seq en estos cultivos detectándose que la presencia de *C. albicans* induce una sobreexpresión de genes relacionados con el efecto Warburg (*Hk2*, *Eno2*) y con la aparición y progresión del cáncer (*Vegfa*, *Jun*, *Fos*, …) lo que fue confirmado mediante ensayos ECAR, RT-qPCR y el uso de inhibidores específicos de distintas rutas metabólicas.

Los datos anteriores parecen indicar que el contacto con el hongo provoca el desarrollo de un un fenotipo tumoral más agresivo. Para confirmarlo, se realizaron ensayos *in vivo* en ratones C57BL/6. Las células de melanoma se estimularon con *C. albicans* y se inocularon intraesplénicamente en los ratones. Tras dos semanas, los hígados extraídos de ratones inoculados con células previamente estimuladas con el hongo mostraron un mayor número de

focos y de área metastática.

En conclusión, *C. albicans* promueve cambios metabólicos y moleculares en las células tumorales de melanoma que conllevan una mayor capacidad pro-tumoral y metastática.

CO-46

INDIVIDUALLY EXPRESSED SARS-COV-2 VIROPORIN ORF3A ALTERS INFLAMMATORY RESPONSES AND CELLULAR STRUCTURE IN LUNG EPITHELIAL CELLS.

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The SARS-CoV-2 genome encodes for eleven accessory proteins. Their functions are still unclear, but increasing evidence suggests they play important roles in viral pathogenesis, and even several variants of concern have mutations among them. ORF3a, the biggest accessory protein, is a unique viroporin that impairs immune responses. It has been described to activate the NF-kB pathway and NLRP3 inflammasome, upregulate cytokine expression, induce oxidative stress and cell apoptosis, inhibit interferon-activated JAK/STAT pathway and reduce MHC-I levels among other functions. In this study, we have analysed transcriptional and structural changes induced by the expression of SARS-CoV-2 ORF3a in transduced A549 lung epithelial cells (Wuhan-Hu-1 isolate). Whole transcriptome sequencing has shown that A549-ORF3a cells differentially express 1559 genes compared with control A549 cells, which are significantly associated with inflammatory pathways according to GO and KEGG enrichment analyses. Particularly, we have found that ORF3a downregulates CCL2 and mediates NF-kB signaling, activating IL-1, TNF and interferon pathways. In parallel, we have studied the cellular landscape of A549 cells transduced with a GFP N-terminal tagged version of SARS-CoV-2 ORF3a. Cryo nano-tomography in the water window performed at full-field transmission X-ray microscopy beamline MISTRAL (ALBA synchrotron) has revealed that the number of vesicles is significantly higher than in control A549-GFP cells. ORF3a localizes in these vesicles and induces the formation of tubular structures. In addition, altered mitochondria are often next to those ORF3a-containing vesicles and the cytoplasm displays an unusual increased amount of lipid drops. Together, our results show that the individual expression of viroporin ORF3a is enough to alter inflammatory responses and cellular structure, suggesting that this protein could have an important role in viral pathogenesis. Further research about SARS-CoV-2 accessory proteins could result in new therapeutic targets for COVID-19.

CO-47

ADOPTIVE CELL THERAPY WITH MEMORY T LYMPHOCYTES TO TREAT INFECTIOUS DISEASES

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Background. Immunocompromised patients are susceptible to high-risk opportunistic infections and malignant diseases. If available, most antiviral and antifungal drugs are quite toxic, relatively ineffective, and induce resistance in the long term.

Methods and results. We have previously demonstrated the safety and efectiveness of adoptive cell therapy for COVID-19 patients with CD45RA$^-$ memory T cells containing SARS-CoV-2-specific T cells from a donor chosen based on HLA compatibility and cellular response to SARS-CoV-2 peptide pools (RELEASE CLINICAL TRIAL, NCT04578210).

To use adoptive cell therapy to treat COVID-19 it would be necessary to develop a biobank of living drugs. For that, we examined the immune evolution performing a longitudinal analysis from previously SARS-CoV-2 infected and infection-naïve individuals covering 21 months from infection and 10 months after vaccination. Cellular responses were maintained over time while humoral responses increased after vaccination but were gradually lost. Therefore, the best donors would be recovered individuals two months after vaccination. We also evaluated the effect of dexamethasone and Interleukin-15 on CD45RA$^-$ cells (standard of care treatment for COVID-19 and other infectious diseases, and a cytokine involved in T-cell maintenance and survival respectively). Dexamethasone did not alter cell functionality, proliferation or phenotype at a clinical-practice concentration, while interleukin-15 increased the memory T-cell and T-regulatory cell activation state, and interferon gamma release.

We applied the adoptive passive transfer of CD45RA$^-$ cells containing pathogen-specific memory T-cells to other infectious diseases characterized by sustained lymphopenia. We

infused six immunocompromised patients with *Cytomegalovirus*, BK virus, *Aspergillus*, and Epstein-Barr virus lymphoproliferative disease. Patients experienced pathogen clearance, resolution of symptoms and lymphocyte increase.

Conclusion. The use of $CD45RA^{-}$ cells containing specific memory T cells of a third-party donor for treating severe pathogenic diseases in immunocompromised patients is feasible, safe, and effective, and has lower costs and a less complex regulatory environment than other cell therapies.

CO-48

IL11 AND FIBROSIS-RELATED GENES ALTERED BY THE EXPRESSION OF SARS-COV-2 ACCESSORY PROTEINS ARE PRESENT IN WHOLE VIRUS-INFECTED CELLS AND COVID19 PATIENT SAMPLES

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SARS-CoV-2, the cause of the COVID19 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. In this study, interaction with cellular responses of A549 lentivirus-transduced expressing individual viral accessory proteins ORF6, ORF8, ORF9b or ORF9c from SARS-CoV-2 (Wuhan-Hu-1 isolate) were analyzed. Transcriptomic analysis revealed that Interleukin-11 (IL11), a member of IL6 cytokine family, was up-regulated in all transduced cells, and several fibrosis-related genes were differentially expressed. Previous studies revealed that the expression of IL11 correlates with the extent of fibrosis and IPA software analysis showed gene data sets of each ORF6, ORF8, ORF9b or ORF9c transduced cell lines implicated in pulmonary fibrosis idiopathic disease. Subsequently, bioinformatics analysis revealed that several of these genes were differentially expressed in other whole virus-infected cell lines, such as A549-ACE2 or Calu3, where IL11 appeared among them. Extended bioinformatics analysis performed with data from COVID19 patient samples and ORF6, ORF8, ORF9b or ORF9c transduced cells showed 28 genes differentially expressed in both samples and transduced cell lines. From this list, 4 were commonly down-regulated and 10 were commonly up-regulated. IL11 was again up-regulated in these COVID19 patient samples. A further enrichment study revealed that these genes were mostly present in the extracellular space and associated with the ECM organization and collagen biosynthesis. Taken together, our results suggest that ORF6, ORF8, ORF9b and ORF9c could be involved in profibrotic responses in SARS-CoV-2 infection. Thus, these accessory proteins could be targeted by new therapies for COVID19 disease.

CO-49

CD5-BASED ADOPTIVE CELL TRANSFER FOR SYSTEMIC FUNGAL INFECTION

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Invasive fungal infections are emerging as a worldwide problem mainly due to the widespread adoption of aggressive immunosuppressive and surgical interventions, as well as the emergence of antifungal resistant strains. The paucity of the antifungal pipeline and the important side effects reported for the available ones place the need to develop alternative strategies. Immunotherapeutic approaches involving the adoptive transfer of engineered immune cells harboring Chimeric Antigen Receptors (CARs) are gaining interest. The pre-clinical efficacy of human T cells redirected against fungi (*Aspergillus fumigatus*, *Cryptococcus neoformans*) and virus (Human Immunodeficiency Virus, Hepatitis B Virus, Hepatitis C virus, Human Cytomegalovirus, Epstein-barr Virus) by the expression of specific CARs has been already reported. Previous work by our group reported that CD5, a scavenger-like lymphocyte surface receptor, shows a high binding affinity for β-glucans from fungal cell walls, marking it as a good candidate for anti-fungal CAR designs. Thus, a second generation CD5-based CAR was expressed on primary human T and NK cells and NK cell lines by lentiviral transduction. The functionality of CD5CAR-T/NK cells was assessed *in vitro* by flow cytometry, ELISA and killing assays. When co-cultured with fungal conidia, CD5CAR-T/NK cells showed higher

surface CD107a and CD69 expression, and higher IFN-γ production together with lower CFU counts as compared to un-transduced cells. Moreover, infusion of CD5CAR-T/NK cells to immunocompromised NSG mice undergoing systemic fungal infection showed higher survival rates than untreated controls. In conclusion, the antifungal properties of immune cells expressing CD5-based CARs pave the way to novel off-the-shelf adoptive cellular immunotherapies against invasive fungal infections, alone or in combination with conventional antimycotics.

CO-50

EXPANSION OF CD151+ NK CELL SUBSET CORRELATES WITH COVID-19 SEVERITY

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COVID-19 is a highly transmittable and pathogenic viral infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The clinical presentation varies among patients ranging from asymptomatic, pneumonia to an acute respiratory distress syndrome and death. The innate and adaptive immune responses are essential to control infection, but an uncontrolled inflammatory response contributes to severe clinical manifestations due to an elevated serum level of pro-inflammatory cytokines. Natural killer (NK) cells play a central role in the defense against viral infections, thanks to their ability to recognize and eliminate infected cells without prior sensitization, as well as regulating adaptive responses. Several studies have revealed an important role of NK cells during SARS-CoV-2 infection, but their exact contribution is not well elucidated. In COVID-19 patients, the number of circulating NK cells is reduced and they display a phenotype characterized by higher levels of inhibitory receptors, altered cytotoxic activity and immunomodulatory properties. Studies identified an expansion of adaptive-like NK cells that correlates with moderate and severe disease. Other studies showed NK cell activation and proliferation. The present work aims to identify novel NK cell-based biomarkers that associate with the degree of disease severity. For that, we have studied a cohort of COVID-19 patients with mild, moderate and severe disease. We have observed an increase of relevant cytokines in NK cell biology like IL-15, Growth Differentiation Factor-15 (GDF-15) and Placental Growth Factor (PlGF) in moderate and severe patients, while TGF-β remains

unchanged. Flow cytometry studies identified a significant expansion of a CD151+ NK cell subset in patients with severe COVID-19. This subset has an increased cytotoxic potential, defined by higher granzyme B content. Furthermore, CD151+ NK cells are characterized by a higher expression of activation markers. This phenotype resembles that of decidual NK cells and may have a role in COVID-19 severity.

CO-51

COMBINED DENDRITIC CELL STIMULATION AND TIGIT BLOCKADE BOOSTS ADAPTIVE NKG2C+ NK CELL AGAISNT HIV-1

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BACKGROUND

Adaptive NKG2C+ Natural Killer (NK) cells have been associated with control of viral infections and could be useful as immunotherapies against HIV-1. However, in People living with HIV (PLWH), the expression of checkpoint receptors may limit their functionality. Here, we studied TIGIT as a checkpoint receptor limiting restoration of NKG2C+ NK and the impact of its blockade *in vivo*.

METHODS

Dendritic cells (DCs) from PLWH (n=29) activated with nanoparticle-loaded poly I:C were cultured with autologous NK cells *in vitro* and abilities to eliminate autologous HIV-1 infected CD4+ T cells and the association with NKG2C and TIGIT expression were analyzed by FACS. n=42 immunodeficient NSG mice were xenotransplanted with CD4+ T cells from PLWH individually (n=14) or in combination with autologous DC-NK cells in the presence of isotypic (n=14) or anti-TIGIT (n=14) mAbs. Presence of HIV-1 CD4+ p24+ T cells in the blood was evaluated by FACS and histological distribution of p24+ in the spleen was analyzed by confocal microscopy after 7 days.

RESULTS

Proportions of NKG2C+ CD57- were enriched in NK cells from PLWH capable of eliminating autologous p24+ infected CD4+ T cells after DC-stimulation. This phenotype was negatively correlated with higher expression of TIGIT. Importantly, i.p. administration of anti-TIGIT into NSG mice xenotransplanted with CD4+ T cells from PLWH and autologous NK cell and Nano-PIC-DC immunotherapy led to a significant reduction of p24+ cells (mean reduction 57%, p= 0.0019) compared to mice receiving only CD4+ T cells, in contrast to mice receiving isotypic control mAb. In addition, significantly lower areas of p24+ foci (p=0.0078) and a significant

reduction of CD57 expression (p=0.0011) on adaptive NKG2C+ cells were detected in the spleen of aTIGIT compared to isotype-control mice group.

CONCLUSION

Combination of DC immunotherapy and TIGIT blockade is a promising strategy to enhance functional NKG2C+ NK cells against HIV-1.

CO-52

ASSOCIATION BETWEEN HIGH INTERFERON-ɣ LEVELS IN RESPONSE TO AVIAN TUBERCULIN AND CANDIDATE GENES IMPLICATED IN NECROPTOSIS AND PLATELET ACTIVATION

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Although several studies have characterized the host genetics behind the susceptibility to *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection, the genetic loci influencing individual resistance to MAP infection and the mechanisms underlying host resistance are still largely unknown. During the course of MAP infection, Th1 lymphocytes produce interferon-gamma (IFNɣ) which is involved in the activation and recruitment of new macrophages to the site of infection. In the current study, we hypothesize that cows with an ability to produce higher levels of IFNɣ might control MAP infection more successfully. To test this hypothesis, IFNɣ production was measured in response to the avian purified protein derivative (aPPD) in blood samples collected from 152 Holstein cattle using a specific IFNɣ ELISA kit. DNA isolated from peripheral blood samples of the same animals was genotyped with the EuroG MD bead Chip and the genotypes were imputed to the whole-genome sequence. A genome-wide association analysis (GWAS) revealed that high levels of IFNɣ in response to the aPPD were associated with a specific genetic profile (heritability = 0.69) and allowed the identification of 71 SNPs (P-value $<5\times 10^{7}$), 40 quantitive trait loci (QTL), and 101 candidate genes associated with high production of IFNɣ. Some of the identified QTLs overlapped with QTLs previously associated with bovine diseases and traits including paratuberculosis susceptibility and tolerance, infectious keratoconjunctivitis susceptibility, spongiform encephalopathy, respiratory disease susceptibility, gastrointestinal nematode burden, tick resistance, somatic cell count and score, clinical mastitis, IgG levels, length of productive life, heat tolerance, and reproductive traits. Functional analysis using the 101 candidate genes revealed a significant enrichment of the necroptosis (bta04217) and platelets activation (bta04611). Taken together, our results define a heritable and distinct immunogenetic profile designed to produce high IFNɣ levels, lyse the infected macrophage by necroptosis, and induce a strong pro-inflammatory response.

CO-53

DENDRITIC CELL-CD8+ T CELL COOPERATION IN LYMPHOID TISSUE IS INVERSELY ASSOCIATED TO VIRAL LOAD AND INCREASES CD8+ T CELL RESPONSE IN PEOPLE LIVING WITH HIV

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Introduction

Plasmacytoid dendritic cells (pDCs) are main producers of interferon-I, playing a key role in HIV control. CD141+ myeloid dendritic cells (mDCs) function consist principally in CD8+ T cell co-stimulation, but their role during HIV infection is not well known. Our objective was to investigate the cooperation of pDCs and CD141+ mDCs with CD8+ T cells in human lymph nodes (LN) and peripheral blood, determining the effect of this interaction in HIV viremia and CD8+ T cell response.

Methods

Inguinal LN biopsies from untreated people living with HIV (PLWH) were obtained for immunostaining and confocal microscopy including histocytometry analysis. CD8-DC co-culture experiments were performed with isolated cells from blood and tonsils of HIV negative subjects and blood from PLWH. Here, pDCs and CD141+ mDCs were pre-stimulated with toll-like receptor agonists for 18h, then they were co-cultured with CD8+ T cells for 6h in the presence of SEB or Gag peptides and T cell response was measured by flow cytometry.

Results

Confocal microscopy and histocytometry analysis showed a differential pDC and CD141+ mDC distribution in LN from PLWH, being pDCs closer to the follicles than CD141+ mDCs. An interaction of pDCs and CD141+ mDCs with CD8+ T cells was observed in LN. However, the triple interaction was only observed in the patient showing the highest viral load. Importantly, the distance of CD8-pDC interaction to the follicle was inversely associated to viral load. Lastly, we found a higher cytokine production and cytotoxic capacity by CD8+ T cells through the cooperation of pDCs and CD141+ mDCs by co-culture experiments, which partially

depended on interferon-I.

Conclusions

There is an interaction between pDCs, CD141+ mDCs and CD8+ T cells in LN from untreated PLWH, associated to viral load. The interaction with activated pDCs and CD141+ mDCs increases antigen-specific CD8+ T cell response.

Sesión Comunicaciones Orales 8 – Inmunodeficiencias: primarias y secundarias

CO-54

FROM A CANDIDATE GENE IN CVID TO A RECURRENT CAUSE IN THE STUDY OF CVID WITH GASTROINTESTINAL SYMPTOMS: THREE NOVEL MUTATIONS IN IRF2BP2

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Abstract

The first mutation in the *IRF2BP2* gene reported to cause common variable immunodeficiency (CVID) was a missense variant (p.Ser551Asn) described in 2016. Very recently, a more accurate phenotype associated with loss of function of *IRF2BP2* has been defined. Only three truncating variants in *IRF2BP2* have been reported in association with CVID: p.Ala209Glnfs*31, p.Gln536delins* and p.Gln540*.

In this work, we published three novel heterozygous truncating variants located in the first exon of *IRF2BP2* that were found to be responsible for a specific CVID presentation with inflammatory manifestations. The patients were studied using a high-throughput sequencing (HTS) protocol. Candidate variants in *IRF2BP2* were confirmed by Sanger sequencing in the three patients and their family members.

The first index patient harbored a heterozygous variant (c.217_244del; p.Pro73Serfs*72). This 27-nucleotide deletion with 92.6% GC content was inherited from the father, who was also affected by a similar condition. The second index patient carried a heterozygous GC-rich 11-nucleotide insertion (c.324_325insAGGCGGCCCCG; p. Ala110Argfs*48). This change was absent from the healthy parents, revealing a *de novo* origin in the proband. The third patient harbored a heterozygous 10-nucleotide deletion located in the same region as the previous case (c.314_324del; p. Glu105Alafs*24). This variant also occurred *de novo*.

Monoallelic variants in *IRF2BP2* causing frame shifts lead to a lack of protein expression, considering that other truncating mutations previously described in this gene and located after

exon 1 are pathogenic. The findings of this study support the pathogenicity of truncating mutations in *IRF2BP2*, which are highly penetrant for autosomal dominant CVID and variable manifestations of gastrointestinal symptoms. Before 2023, only four families with CVID caused by defects in *IRF2BP2* had been published. This study reports three additional families with three novel truncating variants, which suggests that this gene could be undertested in the context of primary immunodeficiency and inflammatory disorders.

CO-55

CHARACTERIZATION OF PATHOGENICITY OF A NOVEL CTLA4 VARIANT

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Introduction

CTLA-4 is a transmembrane receptor constitutively expressed in regulatory T cells (Tregs). To down regulate the adaptive immune response, CTLA-4 removes the CD80/86 molecule from the surface of antigen presenting cells through a mechanism known as transendocytosis. Monoallelic *loss-of-function CTLA4* variants cause an immune disorder named CTLA-4 haploinsufficency.

Objective

Herein we aimed to characterize the pathogenicity of a novel *CTLA4* variant identified in a family with multiple individuals affected by an immune dysregulatory disorder.

Methods

Genetic analyses were performed by both Sanger and Next-Generation Sequencing. To evaluate transendocytosis, Chinese Hamster Ovarian (CHO) cells were transfected with a vector containing CD80 linked to GFP reporter. Patient's PBMCs were activated with CD3/CD28 MoAb in the presence of CHO cells expressing CD80-GFP, and stained for FACS analysis. T cells were gated on $CD4^{+}FOXP3^{+}CD25^{+}$ and analyzed for their ability to uptake the CD80 from CHO cells and for CTLA-4 expression.

Results

We analyzed a non-consanguineous Spanish family with four affected individuals in consecutive generations. The propositus was a 12-year-old male (onset at 5 years-old) who presented recurrent episodes with fever, diarrhea, abdominal pain, atopic dermatitis and joint pain (wrists and knees). Genetic analyses detected the novel, heterozygous p.(Arg51Glufs*21) variant in the *CTLA4* gene predicted to generate a truncated protein. Analyses of affected (n:3)

and unaffected (n:3) relatives detected the variant *CTLA4* genotype exclusively in affected individuals. CTLA-4 expression in patient's PBMCs was lower than in healthy controls, and transendocytosis assay revealed a deficient uptake of CD80-GFP by patient's $CD4^{+}FOXP3^{+}CD25^{+}$ cells.

Conclusions

*We ide*ntified a family with multiple affected individuals carrying the novel, heterozygous p.Arg51Glufs*21 CTLA4 variant, with additional studies revealing an impaired functional outcome. These evidences support the diagnosis of CTLA-4 haploinsufficency in all affected individuals, and demonstrate the utility of transendocytosis assay to evaluate the functional consequences of CTLA4 variants.

CO-56

SÍNDROME DE HIPER-IGE POR PÉRDIDA DE FUNCIÓN DE STAT3: EXPERIENCIA DE UN CENTRO

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El Síndrome de Hiper-IgE por pérdida de función de STAT3 (STAT3-HIES) es una enfermedad monogénica autosómica dominante con amplio espectro clínico que, además de condicionar un estado de inmunodeficiencia, abarca otras manifestaciones extrainmunológicas, siendo características las anomalías del tejido conectivo.

Se realiza un análisis retrospectivo de 9 pacientes estudiados en el Hospital Universitario La Paz entre los años 1998 y 2022. Se revisan las características epidemiológicas, presentación clínica y tratamientos, así como los hallazgos inmunológicos y genéticos.

Se trata de 6 mujeres y 3 hombres con edades comprendidas entre los 8 y 64 años, de los cuales 7 tienen variantes patogénicas germinales en el gen *STAT3* y otros dos en mosaico. A nivel clínico, las manifestaciones más frecuentes fueron las infecciones cutáneas y abscesos por *S. aureus* (6/9), infecciones pulmonares recurrentes (5/9), fracturas óseas (5/9), dermatitis atópica (4/9) y candidiasis mucocutánea (3/9). Como profilaxis infecciosa, 5/9 pacientes emplean septrin, 3/9 itraconazol y 2/9 gammaglobulina intravenosa. Respecto a la patología cutánea, 1/9 se trata con tacrólimus y 1/9 con dupilumab y corticoides. Lo más característico del estudio inmunológico fue disminución de linfocitos Th17, elevación de IgE y disminución de linfocitos B de memoria y ausencia de producción de anticuerpos específicos, en ese orden.

El STAT3-HIES es una entidad clínica a considerar ante un paciente con abscesos cutáneos, infecciones respiratorias recurrentes, candidiasis mucocutánea y anomalías del tejido conectivo, teniendo en cuenta que pueden existir familiares portadores de la variante asintomáticos y casos de mosaicismo. En nuestra serie se confirma que la elevación de la IgE no está presente en todos los casos, siendo más característica la disminución de linfocitos Th17. El tratamiento actual se centra en la prevención de las complicaciones infecciosas mediante profilaxis antimicrobiana,

siendo el septrin la primera opción terapéutica, además de azoles en caso de infecciones recurrentes por hongos.

CO-57

THREE MUTATIONS IN BCL11B WITH IMMUNE DYSREGULATION PHENOTYPES

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Heterozygous *BCL11B* mutations have been related to neurodevelopmental delay (NDD), facial dysmorphia, recurrent infections, absence of ILC2, and T-cell compartment alterations. Here, we report three mutations in three unrelated patients from our center.

First, a 14-year-old Caucasian girl presented with facial dysmorphia, NDD, and early autoimmune neutropenia and thrombocytopenia. She also suffered recurrent infections, mainly by herpesvirus. Genetic studies showed a described *de novo* frameshift mutation in heterozygosis in *BCL11B*: c.1887_1893del, p.Gly630Thrfs*91. Immunophenotyping showed slight T cell baseline activation, $CD21^{low}$ B cell expansion, and ILC2 absence.

Second, a 6-year-old Caucasian girl with facial dysmorphia, mild NDD, autoimmune anemia and thrombocytopenia, allergies and asthma but no recurrent infections showed a *de novo* heterozygous *BCL11B* mutation: c.1944_1965del, p.G649Afs*67. Immunological studies are ongoing.

Third, an 11-year-old Asian girl without NDD presented with an ALPS-like phenotype with early severe autoimmune pancytopenia, recurrent infections, adenopathies, and splenomegaly refractory to sirolimus or rituximab. Immunophenotyping showed a proinflammatory T-cell phenotype lacking Treg. An undescribed heterozygous missense mutation in *BCL11B* was detected: c.398G>A, p. G133D. Interestingly, her brother and father carried the same mutation but did not suffer any symptomatology and their immunophenotype was normal. Since they were HLA-identical, the patient received an HSCT from her brother. However, pancytopenia seems to be persisting. BCL11b is a transcription factor essential for T-cell development. Thus, to further assess the pathogenicity of the c.398G>A mutation, CD34+ progenitors isolated from the patient's PBMCs were developmentally interrogated in an artificial thymic organoid (ATO)

assay. ATO cultures showed the generation of double-negative T-lineage progenitors with impaired proliferation and a differentiation blockage at the beta-selection check-point, suggesting that defective generation of double-positive cells is probably affecting Treg development.

These cases highlight the complexity of the relationship between *BCL11B* mutations and immunological defects, relating them to immune dysregulation symptoms and beyond the well-described NDD.

CO-58

MOLECULAR CHALLENGES IN DIAGNOSTICS OF X-LINKED CHRONIC GRANULOMATOUS DISEASE: CNVS, INTRONIC VARIANTS, SKEWED X-CHROMOSOME INACTIVATION AND GONOSOMAL MOSAICISM.

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Chronic granulomatous disease (CGD) is a prototypical inborn error of immunity (IEI) affecting phagocytes and characterized by the inability of these cells to produce reactive oxygen species. CGD is caused by defects in genes encoding the subunits of the NADPH oxidase enzyme complex (*CYBB, CYBA, CYBC1, NCF1, NCF2, NCF4*), making patients highly susceptible to recurrent severe bacterial and fungal infections and dysregulated inflammatory responses. X-linked CGD (XL-CGD), caused by mutations in *CYBB* gene, is the most frequent and severe form of CGD. To date, more than 1,000 different mutations affecting the *CYBB* have been reported, including large deletions affecting also other neighbouring genes and causing additional diseases. In this study we present 3 families that illustrate different molecular challenges in the genetic diagnosis of XL-CGD. In family 1, we report a female patient with a heterozygous deletion of *CYBB* exon 13 and a skewed X-chromosome inactivation (XCI). In family 2, we report a male patient with a hemizygous deletion of *CYBB* exon 7 also defining its consequences at mRNA level. In family 3, we report two male patients carrying the same novel intronic variant in *CYBB* (c.1151+6T>A). This variant affected the splicing process, although a minor fraction of wild type mRNA was also produced. Their mother was heterozygous carrier while maternal grandmother was a carrier in form of gonosomal mosaicism.
In summary, using a variety of techniques, including NGS-based targeted gene panel and deep amplicon sequencing, copy-number variation calling strategies, microarray-based comparative

genomic hybridization, and cDNA analysis to define splicing defects and XCI, we here show how to face and solve some uncommon genetic mechanisms in the diagnosis of XL-CGD.

CO-59

INBORN ERRORS OF IMMUNITY IN ECUADOR: HOW TO CREATE A STRUCTURED DIAGNOSIS SYSTEM?

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Ecuador has historically lacked an Inborn Errors of Immunity (IEI) identification strategy. Although diagnostic difficulties are seen in all developing countries, Ecuador has lagged far behind others in Latin America.

We have structured a diagnosis system for IEI for the first time in the Ecuadorian population. We have trained medical personnel in the country about early PIDD suspicion. The patients were referred to our Immunology department and were clinically and immunologically evaluated, then selected for genetic analysis by NGS using an extensive panel for IEI. Until now, 57 patients met the inclusion criteria.

We have identified 21 patients with gene mutations related to PIDD. We found patients in the following groups: 1. Immunodeficiencies affecting cellular and humoral immunity: CD3D: c.274+5G>A (Intronic). 2. Combined immunodeficiencies with syndromic features: WAS: c.176del (p.Pro59Leufs*17); 3. Predominantly antibody deficiencies: BTK: c.176del (p.Pro59Leufs*17); c.216_220delins15 (p.Pro73Leufs*15); c.1931T>C (p.Phe644Ser). 4. Diseases of immune dysregulation: PRF1: c.1528T>C (p.Cys510Arg); BACH2: c.452G>A p.Arg151His); CARMIL2: c.668C>T (p.Ser223Phe). 5. Congenital defects of phagocytes: ITGB2: c.562C>T (p.Arg188*), G6PD: c.202G>A (p.Val68Met); c.[202G>A;376A>G] (p.[Val68Met;Asn126Asp]), CYBB:c.252G>A (p.Ala84=). 6. Defects in intrinsic and innate immunity: IFNAR1: c.789-2A>G (Splice acceptor), STAT1: c.511G>A (p.Asp171Asn); c.1154C>T (p.Thr385Met); c.1162A>G (p.Lys388Glu). 7. Autoinflammatory diseases: NLRP3: c.1223T>C (p.Met408Thr); two siblings with MEFV: c.2082G>A (p.Met694Ile); two familial members with CARD14: c.349G>A (p.Gly117Ser). 8. Complement deficiencies: SERPING1: c.685+2T>G (Splice donor). Five were novel mutations, including WAS, BTK, BACH2, CARMIL2, and IFNAR1. CD3D was identified as a recurring founder mutation in Ecuadorian SCID patients.

Follow-up of patients: CYBB was successfully HSC-Transplanted in our Pediatric Department. CD3D and WAS patients are waiting for HSCT. 2 BTK patients are in immunoglobulin

replacement therapy. G6PD, STAT 1, NLRP3, MEFV, CARD14, and SERPING1 patients under the surveillance of different medical specialties. Unfortunately, PRF1, BACH2, ITGB2, IFNAR1, and one BTK patients died before suitable treatment was provided.

Sesión Comunicaciones Orales 9 – Inmunología Básica

CO-60

ROLE OF THE PHOSPHATASE SLINGSHOT-1 (SSH-1) IN THE IMMUNOLOGICAL SYNAPSE STRUCTURE AND T-CELL METABOLISM

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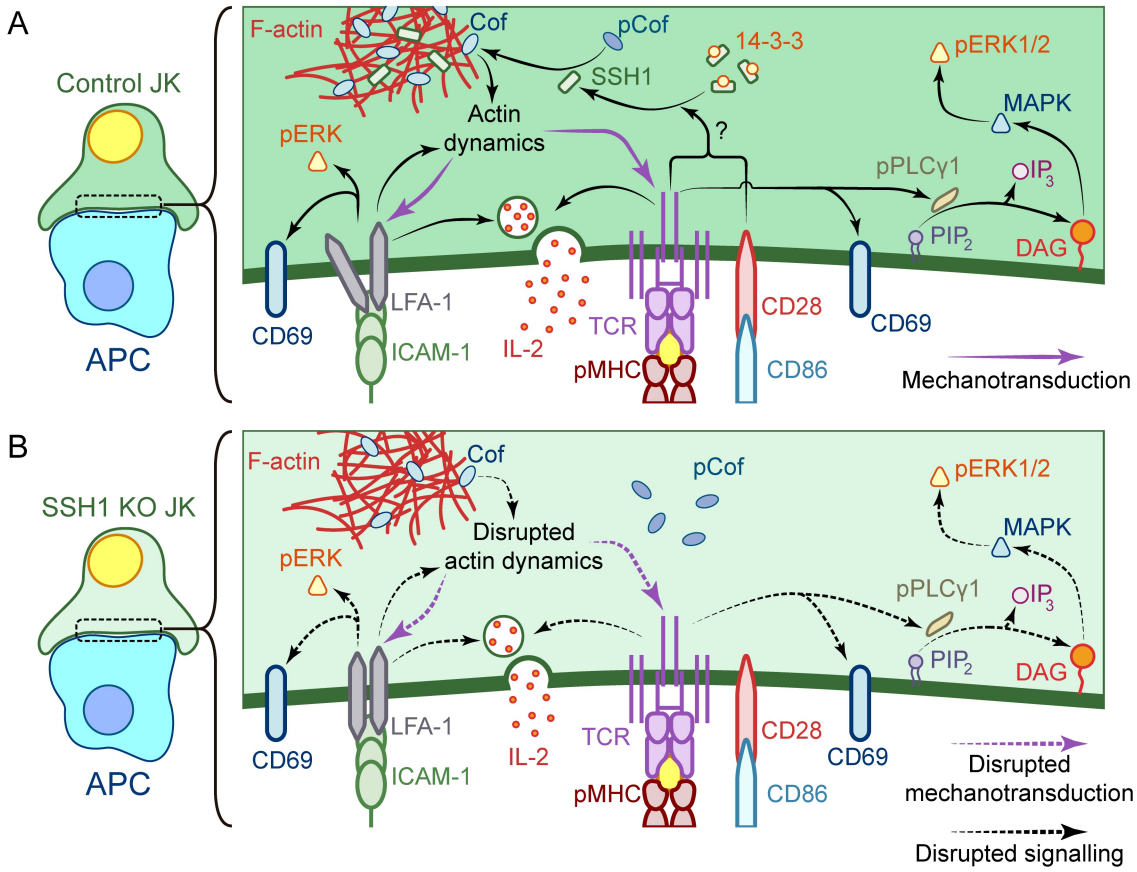
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The Immunological Synapse (IS) is a specialized adhesion where extracellular signals are integrated to properly regulate lymphocyte activation and effector functions. At the T cell side, the IS is organized as a central area, called central supra molecular activation cluster (cSMAC), which contains the T cell receptor (TCR) and signalling molecules, and a peripheral (p)SMAC, which contains the integrin LFA-1 and cytoskeleton components. The actin and tubulin cytoskeletons and their dynamics are essential for efficient TCR signalling and proper immunological synapse (IS) organization, facilitating sustained T cell activation and effector function. Slingshot-1 phosphatase (SSH-1) is an important regulator of the actin cytoskeleton by dephosphorylating and activating cofilin, an actin depolymerising and severing factor. However, the regulatory role of SSH-1 during T-cell activation and effector function, as well as in the metabolic response occurring in activated T lymphocytes is largely unknown.

The aim of this work is to determine the role of SSH-1 in the structure of the immunological synapse and metabolism of T lymphocytes. For this purpose, Jurkat SSH-1 KO cells were used to perform metabolic assays to evaluate the role of SSH-1 in glycolysis and mitochondrial respiration, and confocal microscopy assays were performed to study the function of SSH-1 in the structure of the IS.

The results obtained in this work demonstrate that SSH-1 is relevant for the control of T cell

activation and immune synapse topology and also for mitochondrial activity due to the connection of SSH-1 with the tubulin cytoskeleton, allowing the correct polarization of mitochondria, LFA-1 and VLA-4 integrins at the IS.



CO-61

NOTCH SIGNALING IN THYMUS EPITHELIAL CELLS HOMEOSTASIS AND THYMUS INVOLUTION

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Notch signaling is crucial for fate-specification and maturation of thymus-seeding progenitors along the T-cell lineage. Recent studies have extended the role of Notch signaling to thymic epithelial cells (TECs), showing that Notch regulates TEC progenitor maintenance and differentiation into medullary TECs (mTECs) during fetal development. Here we show that *in vivo* Notch activation is not constrained to embryonic thymus, but it is also induced in postnatal TECs, and increases significantly with age in both humans and mice, suggesting a conserved role for Notch signaling in TEC homeostasis during thymus aging. To investigate the functional impact of Notch activation in postnatal TEC biology, we used a mouse model in which RPBJκ, the transcriptional effector of canonical Notch signaling, is deleted in epithelial cells under the promoter of Foxn1 transcription factor. Immunohistochemistry and flow cytometry revealed no significant differences in TEC composition in mutant (RPBJκ-KO) and wild-type littermate mice at early postnatal ages. However, a significant reduction of the medullary region was observed in mutant compared to WT aged thymi, which was accompanied by an accelerated decrease of postnatal mTEC numbers. Also, we found that thymic medulla organization and integrity critically depends on activation of the canonical Notch signaling pathway, as its abrogation in TECs led to the medullary thymic microenvironment disruption and to an accelerated thymus atrophy. These features paralleled a significant increase in the proportion of intrathymic non-T lineage cells, and a decrease of DP thymocyte numbers compatible with a compromised thymic function in mutant mice. Therefore, impaired Notch signaling induced in embryonic development impacts postnatal TECs and leads to an accelerated mTEC degeneration and a premature thymus involution. Collectively, our data have uncovered a new role for Notch1 signaling in the control of adult mTEC homeostasis, and point toward Notch signaling manipulation as a novel strategy for thymus regeneration.

CO-62

DEFECTIVE MITOCHONDRIA REMODELLING IN B CELLS LEADS TO AN AGED IMMUNE RESPONSE

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B cells are central players of the humoral immune response, conferring protection against infections through the production of high affinity antibodies and the establishment of a memory compartment. Upon antigen encounter B cells activate and start proliferating establishing a characteristic structure called Germinal centre (GC), where B cells additionally undergo somatic hypermutation and class switch.

For their correct activation, and the establishment of an effective GC, B cells need to interact with a special subset of CD4 T cells called T follicular helper cells (TFH). This interaction shapes the activation of a fined tuned transcriptional program in both types of cells; Bcl6 is one of the indispensable transcriptional factors. How the GC is initiated and consequently, what drives Bcl6 activation is the great interest nowadays.

Immunometabolism, and more precisely mitochondria activity, has recently emerged as a driving force of B cell differentiation. Our group, among others, has described that B cell activation at the GC couples with a mitochondria remodelling to fulfil B cell bioenergetic requirements supplying B cell with ATP and anabolic blocks. However, the precise role of mitochondria in B cells is still uncertain.

In this study we worked with mice that lack Tfam (an essential transcription factor for replication and transcription of mitochondrial DNA) in early B cell development. After antigen encounter TfamKO B cells are unable to perform mitochondrial remodelling. Surprisingly, KO B cells are able to maintain ATP levels. In consequence, this model has allowed us to unmask mitochondrial functions, beyond energetic homeostasis.

We proved mitochondria remodelling is crucial controlling lysosome function shaping B cell antigen presentation capacity at the GC. Importantly, these defect lead to an aberrant GC reaction, and triggers an accelerated aging of the immune response. We believe these defects could correlate with pathogenic implication such as increase autoimmunity and lymphoma development.

CO-63

THE IMPACT OF BATF3 ON THE GENERATION AND MAINTENANCE OF ΓΔ T17 CELLS.

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The development of the γδ T cell lineage begins during fetal life and is characterized by waves of progenitors defined by TCR Vg usage. The first wave is defined by Vγ5 usage and constitute the subset termed dendritic epidermal T cells. This wave is followed successively by waves defined by Vγ6 and Vγ4. Innate γδ T cells are also committed to produce IL-17 (Vγ6 and Vγ4) or IFN-γ (Vγ5) in fetal thymus. This preprogramming of γδ T cells is a poorly understood and complex process that relies on activating ligands not strictly restricted by classical MHC molecules. We hypothesized that the transcription factor Batf3, provides instructions to generate functional innate γδ T cells and it has an impact on the generation and maintenance of γδ T cells in the peripheral tissues.

Our results showed that Batf3-deficiency impacts on γδT17 commitment in the neonatal thymus. Consequently, adult Batf3-deficient mice had also a substantial decrease in the amount of γδT17 cells in the adipose tissue, skin and lungs. Therefore, the absence of Batf3, influences the differentiation of innate γδ type 17 lymphocytes. Then, using mice deficient in cDC1 (XCR1-DTR/XCR1creDTA), we observed that this DC subtype, which depends on the Batf3 transcription factor for their development, is not required for development of γδT17 cells.
We performed a retrospective analysis of expression profiling by array (GSE34723, PMID: 25284794), and observed that, besides DCs, BATF3 is expressed by Medullary thymic epithelial cells (mTECs). mTEC are divided into $mTEC^{lo}$ ($MHCII^{lo}$ $CD80^{lo}$) and $mTEC^{hi}$ ($MHCII^{hi}$ $CD80^{hi}$). The expression of both Batf3 and Autoimmune regulator (AIRE), is upregulated during $mTEC^{hi}$ maturation, impacting on the generation of γδT17 cells.

Thus, our data suggest that the upregulation of Batf3 in $mTEC^{hi}$ is necessary for a proper differentiation of innate IL-17-producing γδ thymocytes and their further maintenance in the peripheral tissues.

CO-64

PHOSPHATASES OF REGENERATING LIVER (PRLS) AS REGULATORS OF IMMUNE SYNAPSE ARCHITECTURE THROUGH THE INTERACTION WITH THE WDR1 ACTIN CYTOSKELETON REGULATOR IN T LYMPHOCYTES.

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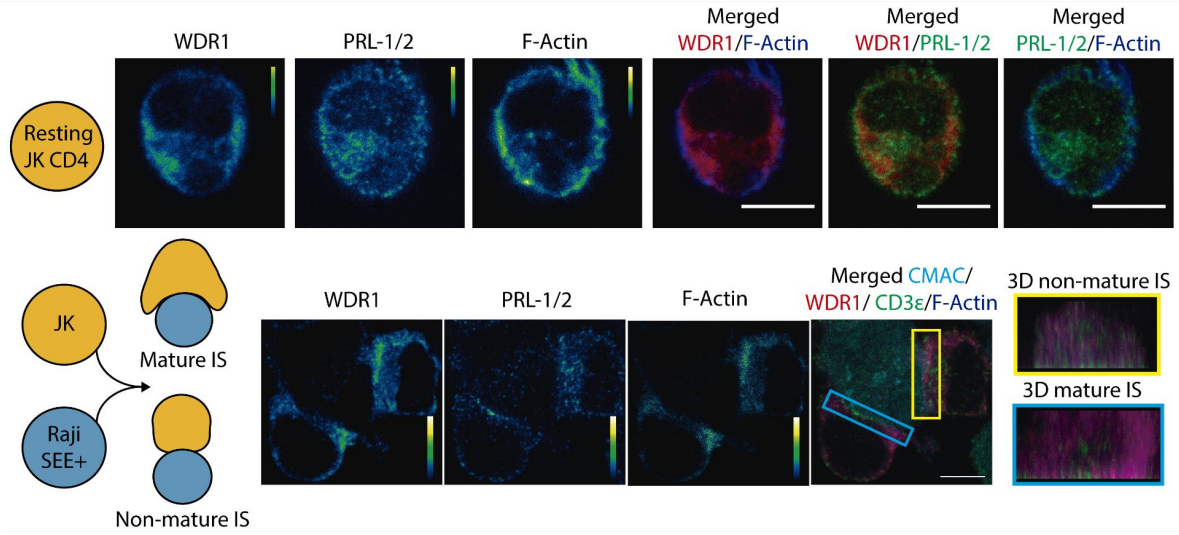
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Phosphatases of regenerating liver (PRLs: PRL-1, PRL-2 and PRL-3) are three highly-homolog proteins that belong to dual specificity phosphatases family. Their regulatory role in lymphocyte biology is not completely understood. However, we have previously reported that PRLs are relevant in the effector function and activation of T lymphocytes. By identifying substrates of PRLs we would be able to better understand the mechanisms mediating these functions. We then developed proteomics of GFP-PRL-1 immunoprecipitates in activated Jurkat cells and found peptides of WDR1, cofilin and coronin, three coordinated regulators of F-actin turnover, which is crucial to achieve a correct T lymphocyte activation.

In Hek293 cells, the interaction of PRL-1 with WDR1 was dependent on the farnesylation state of PRLs and the actin cytoskeleton integrity. Moreover, immunoprecipitation assays with β-actin showed that PRL-1/2 and WDR1 are together complexed with actin. When the immunological synapse was studied by confocal microscopy, a colocalisation of the PRLs, with actin and WDR1 was found at the non-mature IS, while in the mature IS, actin and WDR1 were distributed to distal areas of the IS and PRLs were located in inner regions (figure).

Knockouts of PRL-1, PRL-2 and WDR1 in Jurkat cells were used to study immunological synapse architecture when these proteins were missing. Several differences were found, such as WDR1 altered distribution in PRL-1 and PRL-2 knockouts and impaired filamentous actin dynamics in PRL-2 knockout cells.

All together, we propose that PRLs regulate immunological synapse architecture by regulating actin filament dynamics through the interaction with WDR1.


WDR1
PRL-1/2
F-Actin
Merged
WDR1/F-Actin
Merged
WDR1/PRL-1/2
Merged
PRL-1/2/F-Actin
Resting
JK CD4
JK
Raji
SEE+
Mature IS
Non-mature IS
WDR1
PRL-1/2
F-Actin
Merged CMAC/
WDR1/ CD3ε/F-Actin
3D non-mature IS
3D mature IS

CO-65

RETINOID X RECEPTORS CONTROLS MATURATION, LIPID HANDLING AND SURVIVAL OF ALVEOLAR MACROPHAGES.

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Tissue resident macrophages (TRMs) are immune cells that act as the first line of defense against external microorganisms. In addition, TRMs exert a wide range of specific functions in the tissue in which they reside. Alveolar macrophages (AMs) are embryo-derived TRMs with self-renewal capacity that populate the lumen of the alveoli with a minimal contribution from circulating monocytes. AMs phagocyte the pathogens that enter through the respiratory airways and maintain adequate levels of surfactants, preventing alveolar collapse and ensuring lung homeostasis. Retinoid X Receptors (RXRs) are ligand-dependent transcription factors activated by retinoic acid metabolites and endogenous and dietary specific lipids. Although they are necessary for other nuclear receptors to exert their function, RXRs work as active transcriptional units since they can form permissive heterodimers or even work as homodimers. RXRs are expressed in TRMs and have been described to control the transcriptional regulation of diverse processes including cell proliferation, development, differentiation, metabolism, and cell death. Using specific knock-out mouse models, we induced the deletion of *Rxrs* in murine AMs and observed a reduction in the number of mature AMs, and an impaired surfactant clearance that led to pulmonary alveolar proteinosis. Additionally, we observed increased values of phospholipids in the bronchoalveolar lavages of RXR-deficient mice. Lipid stainings and FACS assays revealed that RXR-depleted AMs presented excessive lipid accumulation and reduced survival likely due to lipotoxicity. The study of *Rxr* expression in AM embryonic and postnatal development, as well as in adult differentiating circulating monocytes into mature AMs, suggests that RXRs play an essential role in AM maturation.

In sum, our results indicate that RXRs are required for correct surfactant clearance and lipid handling, and highlight their importance for AM survival and maturation. Our studies represent an open front for the discovery of promising therapies for lung malignancies modulating RXR in AMs.

Sesión Comunicaciones Orales 10 – Inmunogenética

CO-66

ALTERNATIVE SPLICING OF PRE-MRNA MODULATES THE IMMUNE RESPONSE IN PERIPHERAL BLOOD AND GUT TISSUES OF HOLSTEIN CATTLE NATURALLY INFECTED WITH *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS*

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Alternative splicing (AS) is an important mechanism of gene expression regulation that can influence pre-mRNA stability, structure, function, and localization of corresponding proteins with important physiological consequences. The role of AS in the regulation of the host response to *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection is still unclear. In the current study, differential AS was analyzed using RNA-Sequencing data from peripheral blood (PB) and ileocecal valve (ICV) samples from Holstein cattle without lesions (N=4) and with focal (N=5) or diffuse (N=5) paratuberculosis (PTB)-associated lesions in gut tissues. Using the RNA-Seq mapped reads, a multivariate analysis of AS events was performed with rMATS 3.2. rMATS uses a likelihood-ratio test to confirm that the difference in the mean exon inclusion levels between two sample groups exceeds a given threshold with $p<0.05$. In the PB samples of the infected animals, several genes of the neutrophils' degranulation pathway and clathrin-mediated endocytosis showed significant AS perturbations upon MAP infection. In the ICV samples of the infected cows, proteins with coiled-coil domains showed differential AS events when compared with control cows. Changes in the AS of several genes correlated with changes in gene expression. In the ICV of animals with diffuse lesions versus controls, for instance, AS impacted the expression of several genes (*BOLA, MHCI-A,* and *BOLA-NCI*) associated with the antigen presentation pathway, endocytosis, and with several human autoimmune and viral diseases. In addition to these genes, AS impacted the expression of genes of the complement and coagulation cascades (*C2* and *C4A*) in the comparison of animals with

diffuse versus focal lesions. Taken together, our results demonstrate the important role that AS plays in the regulation of MAP-induced immune response and opened a new way for the development of therapeutical interventions to control bovine PTB.

CO-67

EVOLUTION OF THE MHC-I GENES IN *SERINUS CANARIA* FROM THE CANARY ISLANDS IS DIFFERENT FROM THAT OF ASIAN AND AFRICAN CONTINENTAL *SERINUS* SPECIES

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The radiation of canaries (genus Serinus) occurred in Africa and Eurasia during the Miocene Epoch (9 million years ago) according to maximum parsimony(MP), neighbor-joining (NJ), maximum likelihood (ML)and Bayesian methodologies. Serinus canaria (wild canary) and S. serinus (European serin) together form one ofthe several polytomies within the genus Serinus phylogenetic trees. In a relatively late period, a wild ancestor of S. canaria invaded the Canary Islands, and these birds are the origin of all existing cage canaries, including the first genetically engineered animal: the red canary. The present analysis of the major histocompatibility complex (MHC) molecules in the Canary Islands' species S. canaria showsthat the evolution of the MHC in this species is overall different and faster than that of continental species –namely, S. thibetanus (Asia) and S. mozambicus (Africa) – but particularly so in the peptide binding region. These data support the hypothesis that oceanic islands may be evolutionary reservoirs and not evolutionary dead ends. Also, the lack of two canonic residues in canaries and all songbirds (5500 species) MHC-I molecules among vertebrates is discussed in the frame of songbirds been a different dinosaurian evolutionary lineage.

CO-68

DIVERSITY OF *NKG2C* GENOTYPES IN A EUROPEAN POPULATION

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NK cells detect altered molecular patterns in infected or tumor cells, including abnormal HLA class I expression levels. Among the receptors that sense HLA-I levels, Killer-cell Immunoglobulin-like Receptors (KIR) and their ligands HLA-A, -B and -C present a wide genetic variety in contrast to CD94/NKG2 heterodimers (Killer-cell Lectin-like Receptors, KLR) that recognize HLA-E, which appear to be well conserved. However, both CD94/NKG2 receptors and HLA-E display some degree of diversity. The CD94/NKG2 family, like KIRs, comprises different activating (CD94/NKG2C) and inhibitory (CD94/NKG2A) members generated by duplication and diversification. In addition, there are isoforms derived from alternative splicing, and a complete deletion of the *NKGC* gene affects one third of individuals in homo or heterozygosis. CD94/NKG2C marks an NK cell subset with a distinctive phenotype that expands in response to HCMV and participates in anti-viral immunity.

We have studied the genotypic diversity of the *NKG2C* (or *KLRC2*) gene by analyzing the distribution of both CNV and SNPs in its coding and non-coding sequence by PCR and SBT methods in 240 individuals. We have assessed two SNPs in its coding region that define two major alleles – *NKG2C*01* (Ser2-Ser102) and *NKG2C*02* (Asn2-Phe102) – and the hybrid allele *NKG2C*03* (Ser2-Phe102). We extended the analysis to non-coding regions, finding one SNP in the promotor and five in the 3'UTR. All SNPs are tightly linked and define three major haplotypes (frequencies: 0.456, 0.221 and 0.117) that, in combination with complete gene deletion (0.175) and additional rare haplotypes (< 0.015), generate a wide diversity of genotypes. The most common of these is seen in only 22% of individuals, revealing a noticeable level of population diversity.

Further investigation is needed to ascertain whether and how genetic diversity modifies NKG2C function and, possibly, immune responses and health conditions conditioned by the receptor.

CO-69

DEVELOPMENT AND VALIDATION OF A NEXT-GENERATION SEQUENCING-BASED STRATEGY TO CHARACTERIZE THE B-CHAIN OF THE T-CELL RECEPTOR

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The T cell receptor (TCR) is responsible for antigen recognition and T-cell activation. It consists of the α and β main chains, which are encoded by variable (TRBV), diversity (TRBD) and joining (TRBJ) gene rearrangements. Next-generation sequencing (NGS) of TCR is useful for determining the diversity of a T repertoire and for detecting and monitoring expanded T-cell clones.

The aim of this study was to develop and validate a NGS-based strategy to characterize the TCR β-chain. For this purpose, we designed TRB specific primers and tested different amplification, indexing and purification strategies. Sequencing was performed with MiSeq system and MIXCR was used for data analysis. Multiplex PCR bias was optimized using an equimolar pool of synthetic DNA fragments consisting of different TRBV and TRBJ combinations, and by increasing or decreasing primer concentrations. Sensitivity was tested by mixing well-characterized T-cell lines in a T-cell diverse repertoire in known proportions (from 1:1 to $1:10^4$).

A total of 51 forward and 14 reverse primers located in the TRBV framework region 1 and the TRBJ 3'UTR were required to amplify all functional genes. The final optimized strategy consisted of a fusion PCR for DNA amplification and library preparation, and a purification step by gel extraction. The specificity of the sequenced product for TRB genes was >85%, regardless of the type of sample tested (peripheral whole blood, peripheral blood mononuclear cells or pure T lymphocytes) and the concentration of the initial DNA (5-150 ng/ml). We obtained a considerable reduction in PCR bias after testing several primer mixes (**Fig.1**). Furthermore, high reproducibility (R^2=0.97) and sensitivity up to 10^{-4} were demonstrated using the optimized primer mix.

In summary, a NGS-based strategy for TCR β-chain characterization has been developed, validated and already implemented in our laboratory, with high specificity, sensitivity, reproducibility and minimal PCR bias.

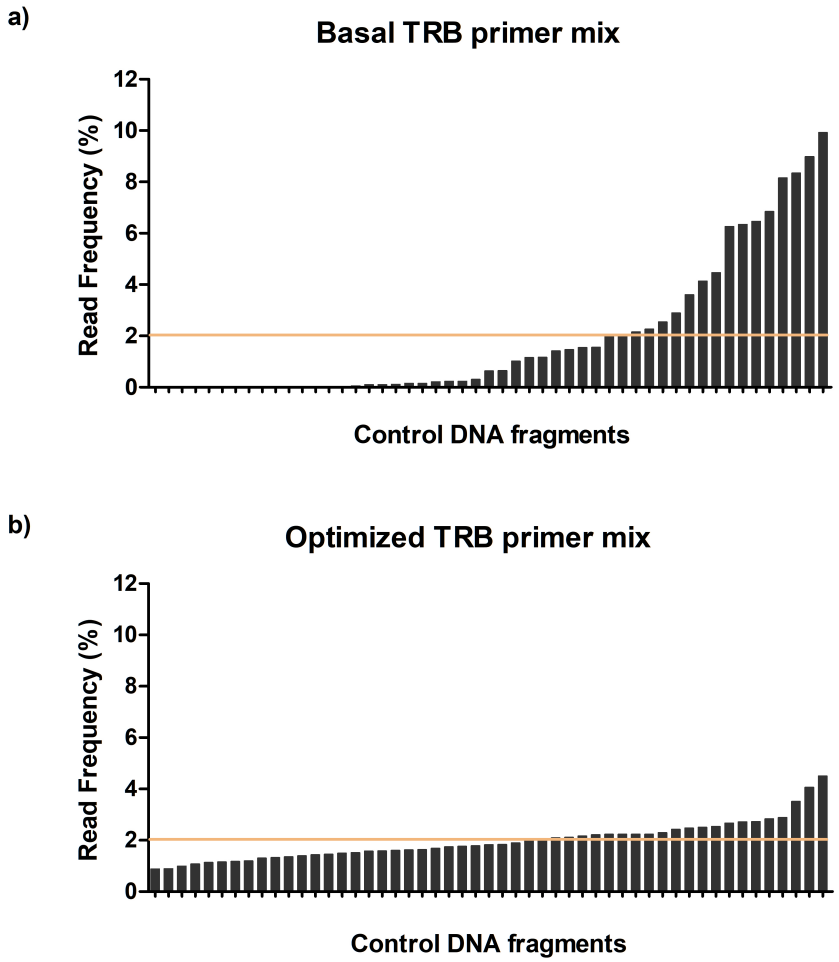


Figure 1. Reduction of PCR bias by optimizing of primer efficieny. Read frequency of 51 synthetic DNA fragments mixed at equimolar concentrations and amplified with the basal TRB primer mix **(a)** and the optimized TRB primer mix **(b)**. Orange line represents the expected read frequency of each DNA fragment.

CO-70

CONTRIBUTION OF HLA CLASS I AND II ALLELES TO SUSCEPTIBILITY AND SEVERITY OF COVID-19 IN THE SPANISH POPULATION

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Objective: To analyze the association between variation at the human leukocyte antigen (HLA) system and susceptibility to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, and the severity of the disease in COVID-19 positive cases recruited within the Spanish Coalition to Unlock Research on Host Genetics on COVID-19 (SCOURGE) consortium.

Methods: A total of 15,047 individuals, 9,373 COVID-19 positive cases and 5,674 population controls were considered for the analysis of the present study. The classic HLA alleles at *HLA–A*, *B*, *C*, *DPB1*, *DQA1*, *DQB1*, and *DRB1* loci were imputed to two-digit typing resolution, using the R package HLA Genotype Imputation with Attribute Bagging (HIBAG) and its corresponding European Ancestry reference panel.

Results: We found association of the allele HLA-B*14:02 with COVID-19 infection (4.65% in controls vs. 3.96% in cases, *p-value* after Bonferroni correction <0.05). Interestingly, four additional alleles had suggestive association with susceptibility to COVID-19 infection (*p-value*<0.01). Distribution of HLA-C*04:01, B*50:01 and B*35:01 alleles were higher in COVID-19 patients compared with controls (13.4% vs. 12.2%, 2.4% vs. 2% and 4.9% vs. 4.2%, respectively), whereas HLA-C*08:02 allele frequency was higher in controls than in COVID-19 patients (6.2% vs. 5.4%). None of the HLA alleles showed significant association with asymptomatic infection, hospitalization, or severity. In addition, we observed interesting trends concerning HLA evolutionary divergence (HED) scores for HLA-A and HLA-C (*p-value* =0.08 and *p-value* =0.02; respectively), as well as the value of the mean HED, increased among the COVID-19 cases (*p-value* =0.07).

Conclusions: These findings suggest that the common genetic variation at the HLA is not a major factor determining COVID-19 susceptibility and/or severity. However, the results allow us to hypothesize about a potential role of HLA class I alleles in the development of a specific immune response to COVID-19.

CO-71

INFLUENZA VIRUS INDUCES BMDM AND S. PNEUMONIAE TRANSCRIPTIONAL SHUT-OFF IN CO- AND SUPERINFECTION *IN VITRO* MODELS.

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Influenza virus infections are one of the main causes of morbidity and mortality worldwide. Most infections by Influenza A virus (IAV) generally cause mild or moderate symptoms in healthy adults, but bacterial infections frequently worsen their prognosis. Among them, *Streptococcus pneumoniae* (Spn) is the most common pathogen in human respiratory coinfections. Despite the many described mechanisms used by Influenza viruses to neutralize the immune response of the host, specific antagonistic activities in macrophages require a more detailed description. Transcription shut-off is a common antagonistic function shared by all Influenza viruses. To better understand the cooperation between Influenza virus and Spn, we performed transcriptomic analysis (RNA-Seq) of bone marrow-derived macrophages (BMDM) differentiated for 7 days with GM-CSF and infected at an MOI of 1 with Influenza A/PR/8/34 and coinfected or superinfected for 8h with Streptococcus pneumoniae (ATCC6301). Coinfection presented a synergistic induction in the expression of specific cytokines, chemokines and IFNs. We identified 445 genes with significant increased expression in coinfection, including inflammatory chemokines CCL3 and CCL4. Similarly, we identified 495 genes with decreased responses, including the Target of rapamycin complex subunit LST8 (MLST8) or NOD-Like receptor P1 (NLRP1). The association network between the synergistic genes showed that coinfection increases inflammasome signaling via NF-κB and Tyk2. Similarly, despite macrophage transcription shut-off caused by Influenza virus, superinfection with Spn showed that 227 genes were synergistically upregulated, especially inflammasome, and 333 synergistically downregulated. Surprisingly, analysis of bacterial transcripts showed that Influenza virus also induces bacterial transcriptional shut-off during superinfection. Regarding bacterial gene expression, we found no differences during coinfection, but superinfection showed a decrease in the expression of most virulent genes. Our results link in vitro infection of Influenza virus and Spn to transcriptomic changes, validated with RT-qPCR

experiments, and highlight genes that are putatively responsible for the severity of complications in coinfections.

POSTERS



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Posters Defensa

Sesión Poster Defensa 1. Pantalla 1. Autoinmunidad (I)

P-001

ANALYSIS OF THE INTERSTITIAL LUNG DISEASE PATHOGENESIS IN PSGL-1 KO AND P-SELECTIN KO MICE

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Background

Systemic sclerosis (SSc) and systemic lupus erythematosus (SLE) are connective tissue-related autoimmune diseases. The most severe complication is the development of interstitial lung disease (ILD), one of the main causes of death in these diseases.

Our previous work described that PSGL-1 KO mice develop a SSc-like syndrome and P-Selectin KO mice induce a SLE-like syndrome. In addition, both KO mice develop ILD with aging.

There is no curative treatment for ILD and the treatment used is the same for both of SSc-ILD and SLE-ILD. In order to find more specific and effective treatments it is crucial a better understanding of the cellular processes implicated in the pathogenesis of SSc-ILD and SLE-ILD. For that reason, the objective of this work is to study the implication of myofibroblasts and apoptosis in the development of ILD in both diseases.

Methods

Bronchioalveolar lavage of Wild Type (WT), PSGL-1 KO and P-Selectin KO mice was obtained. The presence of myofibroblasts and apoptotic cells was detected by flow cytometry.

Results

Our data indicate that PSGL-1 KO mice have statistically increased number of myofibroblasts compared with WT and P-Selectin KO mice. Although most of the myofibroblasts were CD45+CD44+ α-SMA+ cells they were also increased in the CD45- populations including fibroblasts, epithelial and endothelial cells.

We observed an increased number of CD45- apoptotic cells in PSGL1 KO mice compared with

WT and P-Selectin KO mice. Regarding CD45+ population, both KO mice showed increased number of apoptotic cells, but only in PSGL-1 KO mice the increase was statistically significant.

Conclusion

Our results show that ILD pathogenesis is different in both autoimmune syndromes in mice. In order to find personalized treatments for each disease, a more in-depth analysis of ILD is essential.

P-002

INCREASE OF CIRCULATING MONOCYTE-PLATELET CONJUGATES IN RHEUMATOID ARTHRITIS RESPONDERS TO IL-6 BLOCKAGE

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Platelets (PLT) bind to a significant percentage of circulating monocytes and this immunomodulatory interaction is increased in several inflammatory and autoimmune conditions. The therapeutic blockage of IL-6 with Tocilizumab (TCZ) alters PLT and the phenotype and function of monocytes in rheumatoid arthritis (RA). However, the relationship between monocyte-PLT conjugates (CD14+PLT+) and clinical and immunological variables and the regulation of this interaction by IL-6 blockage are still unknown. Here, we compared the presence of monocyte-PLT conjugates (CD14+PLT+) and membrane CD162 expression using flow cytometry, and, by ELISA, the markers of PLT activation (sCD62P and sCD40L) in healthy donors (HD) and patients with long-standing RA before TCZ (baseline). We found higher percentages and absolute counts of CD14+PLT+, and higher plasmatic levels of sCD62P and sCD40L but lower CD162 expression on monocytes from RA patients than those from HD. Additionally, the levels of CD14+PLT+ inversely correlated with inflammatory parameters. Interestingly, 95% of patients with lower percentages of CD14+PLT+ and only 63% of patients with higher percentages of CD14+PLT+ achieved a EULAR-defined response at four weeks ($p = 0.036$). After TCZ, the percentage of CD14+PLT+ increased in 92% of RA patients who achieved 12 w-remission ($p < 0.001$). Our results suggest that the binding of PLTs has a modulatory effect, accentuated by the increased binding of PLTs to monocytes in response to the therapeutic blockage of IL-6.

P-003

PREDICTING PARTIAL REMISSION IN TYPE 1 DIABETES USING IMMUNOLOGICAL PARAMETERS

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Introduction

Type 1 diabetes (T1D) is a chronic autoimmune disease characterized by the destruction of pancreatic insulin-producing β cells. Partial remission (PR), defined as a temporary reduction in insulin requirement that is associated with β-cell regeneration and downmodulated autoimmunity, is a poorly explored phenomenon in T1D and a potent therapeutic window. Critical unmet needs in clinical trials and disease management are the early stratification and selection of patients with a better disease prognosis.

Objective

To develop a predictive model that utilizes metabolic and immunological data to identify those patients with T1D who are likely to experience PR.

Methods

We analyzed up to fifty-five different peripheral immune cell subpopulations using multiparametric flow cytometry at T1D diagnosis of a pediatric cohort (n=59). To test the PR predictive ability of all the immunological and metabolic parameters, machine learning logistic regressions were used.

Results

Patients at T1D diagnosis and who experienced PR were older, had a higher body mass index, required lower insulin doses, and had higher stimulated C-peptide concentrations compared to non-remitters. Immune cell analysis showed decreased Tregs but more activated, and an increase in DN γδ T cells and $CD27^+$ EM $CD8^+$ T cells in future PR patients at their diagnosis. All ROC curves for these parameters showed a good discriminatory capacity between future PR and non-PR, and logistic regressions confirmed their predictive role. Based on these variables, we created a potent predictive model of PR, which will be tested on an independent cohort in the future.

Conclusion

The proposed PR predictive model based on immunological, clinical, and metabolic data could improve patient care and clinical trial selection by their early stratification and allow for the implementation of strategies to maintain or induce the PR phase.

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P-004

MONITORIZACIÓN DE LA FUNCIÓN HEPÁTICA EN PACIENTES CON ANTICUERPOS ANTI-SP100 Y ANTI-GP210.

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Introducción: La colangitis biliar primaria (CBP) es una enfermedad inflamatoria crónica que compromete la funcionalidad hepática, debido a la degeneración de los canalículos biliares. El diagnóstico de CBP se basa en parámetros bioquímicos, pruebas de imagen y marcadores inmunológicos (anticuerpos antimitocondriales [AMA] y anticuerpos frente a antígenos nucleares [ANA] anti-sp100 y anti-gp210). El objetivo de este trabajo es evaluar la evolución y posible desarrollo de CBP en pacientes en los que se detectaron precozmente anticuerpos anti sp100 y gp210.
Materiales y métodos: La detección de ANAs y AMAs se realizó mediante inmunofluorescencia indirecta (IFI). La cuantificación de anticuerpos anti sp100 y gp210 mediante ELISA.
Resultados: Un total de 60 mujeres y 5 varones con ANA+, con positividad para anti-sp100 y gp210, hallados de manera incidental, fueron monitorizados desde 2015 a la actualidad. En el momento de la detección, 30 pacientes presentaban además de los anti gp210 o sp100, patrón compatible con AMA, confirmado en 26 casos (grupo AMA+). Los restantes 35 pacientes presentaban exclusivamente anticuerpos anti-sp100 y/o gp210 (grupo AMA-). 22 de los 30 pacientes AMA+ presentaban en el momento de detección de los ant1-gp210 o sp100, alteración de las enzimas de función hepática. Tras el periodo de seguimiento, el 90% presentaban alteradas las enzimas hepáticas, y el 54% habían sido diagnosticados de CBP. 11 de los 35 pacientes AMA- (31,4%) presentaban alteración bioquímica en el momento inicial. Tras el seguimiento, el 65,7% presentaban alteración de la función hepática en algún momento de la evolución, habiendo sido diagnosticados 3 (8%) de CBP.
Discusión/conclusión: Se considera que los marcadores inmunológicos de CBP no clásicos (anti Sp100 y anti Gp210) tienen una alta especificidad diagnóstica. Su aparición precoz aún en ausencia de otros signos y síntomas de la enfermedad, obliga a una monitorización estrecha de estos pacientes, para poder aplicar tratamiento oportuno.

P-005

ESTUDIO RETROSPECTIVO SOBRE LA FRECUENCIA DE ANTICUERPOS ONCONEURONALES Y SU POSIBLE ASOCIACIÓN CON SÍNDROMES PARANEOPLÁSICOS

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Los síndromes neurológicos paraneoplásicos (SNP) incluyen un grupo de trastornos en los que una respuesta inmunitaria contra un tumor sistémico subyacente se dirige erróneamente frente al sistema nervioso. La mayoría de estos SNP están ligados a la presencia de anticuerpos dirigidos contra antígenos neuronales intracelulares. Estos anticuerpos no tienen un papel patogénico conocido, pero son útiles como biomarcadores diagnósticos al asociarse comúnmente a la existencia de tumores específicos, por lo que se conocen como anticuerpos onconeuronales. El objetivo principal de este estudio fue determinar la frecuencia de SNP en pacientes de nuestro hospital con positividad para anticuerpos onconeuronales.

Se revisaron los resultados de 504 pacientes a los que se les solicitó un análisis rutinario para detectar la presencia de anticuerpos onconeuronales (contra amfifisina, CV2, Ma2, Ri, Yo, Hu, Recoverina, Sox1 y titina) en los años 2021-2022, mediante dos técnicas complementarias: Inmunofluorescencia indirecta sobre cortes de tejido e Inmunoensayo en línea (ambos Euroimmun). Se recogieron, además, los datos clínicos de aquellos pacientes con resultados positivos.

La población de estudio presentaba 275 hombres y 229 mujeres, con una mediana de edad de 60 años. En 55 pacientes se encontró positividad para al menos uno de los anticuerpos estudiados, resultando anti-Yo el más prevalente (31%). De ellos, 18 pacientes (33%) tenían diagnóstico tumoral y 9 de éstos, además, un SNP (50%), asociados sobre todo a anti-Yo (56%) y anti-SOX1 (22%). Los tumores más frecuentemente hallados fueron el cáncer de pulmón de células pequeñas (22%) asociado a anti-Hu, anti-Yo y anti-Sox1, cáncer de mama (22%) asociado a anti-recoverina y neoplasias digestivas (17%) asociadas a anti-Yo y anti-Hu.

La detección de anticuerpos onconeuronales permite adelantar un posible diagnóstico de SNP y dirigir la búsqueda hacia tumores potencialmente implicados. En nuestro hospital, la presencia de estos anticuerpos detectó un porcentaje moderado de pacientes con SNP.

P-006

ANÁLISIS DE LA CORRELACIÓN ENTRE ANTICUERPOS ANTI-MÚSCULO LISO, F-ACTINA Y CÉLULAS TRANSFECTADAS VSM47 EN PACIENTES CON PATOLOGÍA HEPÁTICA AUTOINMUNE

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Introducción: La hepatitis autoinmune (HAI) es una patología inflamatoria crónica inmuno-mediada. Entre los criterios inmunológicos para su diagnóstico se encuentra la presencia de anticuerpos anti-músculo liso (SMA), los cuales van dirigidos frente a proteínas del citoesqueleto como la F-actina.
El estudio mediante inmunofluorescencia indirecta (IFI) sobre triple tejido de rata (TTR) permite el reconocimiento de los SMA, mientras que la caracterización específica de los anticuerpos anti-F-actina se puede realizar mediante ensayos en fase sólida (ELISA) o IFI sobre células transfectadas (CBA). **El objetivo** del presente trabajo es analizar la concordancia entre estas metodologías, la asociación con la enfermedad y su utilidad en el diagnóstico.

Material y Métodos: Se incluyeron un total de 47 pacientes remitidos para su estudio al servicio de Inmunología, que tras el diagnóstico definitivo se clasificaron en los siguientes grupos: pacientes con HAI (13), pacientes con diagnóstico o sospecha de otras hepatopatías (20) o pacientes con otras patologías autoinmunes (LES, Graves, artritis…) o sin diagnóstico definitivo (14).
Para realizar el estudio se emplearon tres aproximaciones diferentes basadas en técnicas de IFI sobre TTR (Biosystems) y CBA-VSM47 (Euroimmun), así como mediante ELISA IgG-Actina (Werfen, positivo >= 20 UR/mL).

Resultados:
Se analizaron los resultados obtenidos por las tres metodologías para cada uno de los grupos de pacientes:

	SMA ≥1/320*	CBA- VSM47 +	ELISA IgG-Actina +
HAI	54%	77%	100%
Otras hepatopatías	42,1%	36,4%	78,9%
Otras patologías	53,3%	20%	80%

*En el estudio por IFI sobre TTR el 100% de los casos analizados fueron positivos, por lo que destacamos aquellos con título superior o igual a 1/320.

Conclusión:

Tanto la IFI sobre TTR como el ELISA para F-actina son técnicas muy sensibles pero poco específicas, ya que presentan resultados positivos en pacientes con otras patologías. El ensayo CBA-VSM47 presenta una alta sensibilidad y especificidad para el diagnóstico de HAI.

P-007

NON-CLASSICAL ANCA SPECIFICITIES: HOW FREQUENT ARE THEY?

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Introduction

Non-classical antineutrophil cytoplasm antibodies (ANCA) are defined here as ANCA detected by IIF and not directed against the predominant ANCA antigens, proteinase 3 (PR3) and myeloperoxidase (MPO). These ANCA are found in a variety of clinical conditions. They are directed against a variety of neutrophil antigens such as cathepsin G, elastase, lactoferrin and bactericidal permeability-increasing protein (BPI).

Objective

To establish the positivity rate and of Non-classical ANCA specificities in our cohort analysed between January 2020 and December 2022.

Materials & Methods

The results of patients tested for ANCA between January 2020 and December 2022 were retrospectively reviewed. IFA were performed on a composite slide that included: human neutrophils fixed with ethanol, human neutrophils fixed with formalin and HEP2 cells with neutrophils fixed with ethanol. Antibodies against MPO and PR3 were tested by a commercial Chemiluminescent Immuno-Assay. Antibodies against cathepsin G, elastase, lactoferrin and BPI were tested by a commercial Enzyme Immuno-Assay.

Results

A total of 9131 patients were analysed. ANCA were positive in 645 (7.06%) patients. Out of these 645 ANCA positives, 405 were negative and 179 (27.75%) were positive for MPO and/or PR3. Of the 405 ANCA-positive MPO/PR3-negative patients, non-classical ANCA were performed in 95 patients obtaining positive results in 27 (28.42%) of them. Anti-elastase antibodies were the most frequents (n=15, 15.79%), 13 with perinuclear pattern (P-ANCA) and 2 Atypical pattern (X-ANCA), followed by anti-BPI antibodies (n=8, 8.42%), 4 P-ANCA, 3 cytoplasmatic (C-ANCA) and 1 X-ANCA, anti-lactoferrin (n=3, 3.16%) and anti-cathepsin G (n=1, 1.05%).

Conclusion

The positivity rate for classical and Non-classical ANCA was similar in our cohort although the clinical significance of the latter is not yet well established. Anti-elastase and anti-BPI

antibodies were the most common antibodies detected. There is an incomplete correlation between indirect immunofluorescence patterns and the specificity of antibodies.

P-008

DIAGNOSTIC ACCURACY OF A COMMERCIAL LINE BLOT ASSAY FOR SOX1 ANTIBODIES

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Introduction: SOX1 antibodies (SOX1-abs) are associated with the presence of small-cell lung cancer (SCLC) and paraneoplastic syndromes (PNS), mainly Lambert-Eaton myasthenic syndrome. Many centers use commercial line blots for testing SOX1-abs, however the diagnostic yield is low. The aim of this study was to assess if the intensity of the reactive band of the line blot along with the findings in the tissue-based assay (TBA) were able to improve the predictive value of the line blot.

Material and methods: Samples from 34 consecutive patients with a positive line blot, tested between 2018 and 2022, were further examined using indirect immunohistochemistry on a TBA of rat cerebellum and indirect immunofluorescence on a cell-based assay (CBA) of HEK293 cells expressing SOX1, SOX2, and SOX3. Clinical data was also obtained.

Results: SOX1-abs detected by line blot were confirmed by CBA in 50% (17/34) of cases, all of them with lung cancer (SCLC in 16/17, 94%) and 15/17 (88%) with PNS. None of negative CBA cases had cancer or PNS. All patients (17/17) without SOX1-abs by CBA were also negative by SOX2 and SOX3 CBAs, whereas 16/17 (94%) patients with SOX1-abs by CBA were also SOX2 and/or SOX3 positive. TBA was evaluable in 30 of 34 patients. Among the 17 cases positive by CBA, 15 (88%) showed a TBA pattern compatible with SOX1-abs, and among the 13 cases negative by CBA, all were TBA negative. The frequency of SOX1-abs by CBA in TBA negative patients was 10% (1/10) in low-positive intensity bands and 20% (1/5) in samples with stronger band intensity.

Conclusions: Confirmation by CBA of positive samples by line blot should be mandatory in cases not evaluable or negative in the TBA but could be spare in those who are positive on the TBA.

P-009

COMPARACIÓN DE DOS PUNTOS DE CORTE UTILIZADOS EN PRÁCTICA CLÍNICA PARA EL LINFOGRAMA INTRAEPITELIAL CELIACO EN ADULTOS

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Introducción: La enfermedad celíaca (EC) es un trastorno sistémico inmunomediado desencadenado por la ingesta de gluten en individuos genéticamente susceptibles. El linfograma intraepitelial celíaco, definido como un aumento de linfocitos intraepiteliales (LIEs) TCRγδ+ concomitante a una disminución de LIEs CD3-, es una herramienta útil para el diagnóstico de EC.

Objetivos: Comparar dos puntos de corte del linfograma intraepitelial celiaco utilizados en práctica clínica y calcular el umbral con la mayor sensibilidad (S) y especificidad (E) conjunta para el diagnóstico de EC.

Metodología: Se realizó un estudio retrospectivo de pacientes adultos con sospecha de EC. El estudio del linfograma intraepitelial se desarrolló mediante citometría de flujo en muestras de biopsia duodenal. Los puntos de corte fueron: **1.**TCRγδ+≥10% y CD3-≤20; **2.**TCRγδ+≥15% y CD3-≤6%, utilizados en nuestro laboratorio en 2021 y 2022 respectivamente. Para el análisis estadístico se utilizó el software XLSTAT.

Resultados: 120 pacientes reclutados y clasificados en celiacos (n=43) y no celiacos (n=77). Los criterios diagnósticos de EC utilizados fueron: Marsh 1 a Marsh 3; anticuerpos anti-Ttg o anti-endomisio positivos; genética compatible HLA-DQ2 y/o DQ8 y remisión clínica y serológica tras dieta sin gluten. El estudio mostró que utilizando el punto de corte **1** del linfograma intraepitelial celiaco se obtuvo una S (95.34%) mayor que con el punto de corte **2** (65.11%). Sin embargo, la E fue mayor utilizando el punto de corte **2** (94.80%) respecto al **1** (71.42%). Seguidamente, se representó la curva ROC para calcular el umbral con la S (74.41%) y E (92.20%) conjuntas más alto: ≥14% para TCRγδ+ y ≤7% para CD3-.

Conclusión: En nuestra cohorte de pacientes con sospecha de EC, el umbral con mayor S y E conjunta para el linfograma intraepitelial celiaco fue: TCRγδ+≥14% y CD3-≤7%. Por tanto, el punto de corte 2 tiene mayor capacidad para discriminar entre pacientes celiacos y no celiacos.

P-010

IMPORTANCE OF THE CUT-OFF POINT FOR THE DETECTION OF ANTI-TISSUE TRANSGLUTAMINASE IGA ANTIBODIES IN CELIAC DISEASE PATIENTS USING TWO AUTOMATED PLATFORMS

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Introduction: Celiac disease (CD) is an autoimmune condition where the detection of anti-tissue transglutaminase (tTG) IgA antibodies is of great importance for the diagnosis and monitoring of gluten free diet (GFD). The prevalence of CD has increased in the last years mostly due to the improved sensitivity of serological methods to detect these autoantibodies.

Aim: To assess the sensitivity and the concordance of two automated methods for the detection of anti-tTG IgA antibodies in CD patients at diagnosis and after GFD, respectively.

Methods: Sera from 68 patients at diagnosis, and 136 after starting a GFD from our serum collection were analyzed for the presence of anti-tTG IgA using BioCLIA (Palex) based on chemiluminescence and Aptiva Celiac IgA (Werfen) based on Particle-Based Multi-Analyte Technology (PMAT).

Results: Using the cut-off values established by the manufacturers (BioCLIA: 20 RU/mL; Aptiva Celiac disease: 5 FLU), the sensitivity of BioCLIA and Aptiva Celiac was 79%, and 94%, respectively. In addition, the concordance of both methods to detect anti-tTG IgA antibodies in GFD patients was 73%, being Aptiva Celiac the method able to detect a higher number of patients with a positive result (34 out of 37; 91.9%) between those with discordant results. As it is shown in Figure 1, modifying the cut-off points of Aptiva and BioCLIA, the sensitivity of both methods showed limited variations for the detection of anti-tTG IgA antibodies in CD patients at diagnosis.

Conclusion: BioCLIA (Palex) and Aptiva Celiac disease IgA (Werfen) are two available methods for the detection of anti-tTG IgA antibodies and the management of CD. The establishment of own cut-off points in each laboratory is very important for a correct

performance.

	Aptiva 5 FLU	Aptiva 10 FLU	Aptiva 15 FLU	Aptiva 20 FLU
BioCLIA 20 RU/mL	Aptiva: 94% BioCLIA: 79%	Aptiva: 93% BioCLIA: 79%	Aptiva: 87% BioCLIA: 79%	Aptiva: 84% BioCLIA: 79%
BioCLIA 15 RU/mL	Aptiva: 94% BioCLIA: 84%	Aptiva: 93% BioCLIA: 84%	Aptiva: 87% BioCLIA: 84%	Aptiva: 84% BioCLIA: 84%
BioCLIA 10 RU/mL	Aptiva: 94% BioCLIA: 85%	Aptiva: 93% BioCLIA: 85%	Aptiva: 87% BioCLIA: 85%	Aptiva: 84% BioCLIA: 85%

P-011

CLINICAL SIGNIFICANCE OF ANTI-SM ANTIBODIES IN THE ABSENCE OF ANTI-U1RNP

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Introduction

Anti-Sm and anti-U1RNP antibodies are biomarkers of lupus, being anti-Sm the most specific. The analysis of U1RNP by BIO-FLASH chemiluminescent analyser (Werfen) uses an antigen that includes also Sm antigen, therefore patients with anti-Sm antibodies should be positive for both Sm and U1RNP. However, in routine clinical practice with this assay, some discordant Sm+U1RNP- results are obtained.

Objective

To establish the frequency of Sm+U1RNP- results obtained by BIO-FLASH and study its clinical significance in the context of lupus diagnosis.

Methods

Retrospective study of 106 serum samples anti-Sm+ was performed by BIO-FLASH (2018–2022).

Immunological and clinical data were collected based on 2019 EULAR/ACR lupus classification criteria and compared between Sm+U1RNP+ and Sm+U1RNP- patients.

Results

40 patients (38%) were Sm+U1RNP-. Median values of anti-Sm in Sm+U1RNP- patients were lower than Sm+U1RNP+ (31 CU (IQR 25-60) *vs* 84 CU (IQR 34-179);p<0.001).

Clinical data showed a higher percentage of patients with lupus diagnosis in Sm+U1-RNP+ group compared to Sm+U1-RNP- (69% *vs* 30%, p=0.003)(OR: 3.4, IC95%:1.46-7.72).

Sm+U1RNP+ group present more arthritis cases than Sm+U1RNP- (65% *vs* 21%;p<0.001) as well as more lupus mucocutaneous symptoms (62% *vs* 25%;p<0.001)(Table 1).

Regarding immunological criteria, in Sm+U1RNP+ group the percentage of either ANA+ patients (98% vs 73%;p=0.001) or with low C3 (30% vs 8%;p=0.046) was significantly higher

than in Sm+U1RNP-(Fig. 1).

Conclusions

The frequency of Sm+U1RNP- results obtained with BIO-FLASH was higher than expected. With this assay, the association of Sm antibodies with lupus is lower if is not accompanied by a positive anti-U1RNP result.

Variable	Sm+U1RNP+(n=66)	Sm+U1RNP-(n=40)	*p*-value
Demographics			
Age, yr;median(IQC)	44(31-57)	50(41-62)	**0,038**
Sex (female);n(%)	52(79%)	28(70%)	n.s.
Lupus clinical criteria;n(%)			
Arthritis	41(65%)	8(21%)	**<0,001**
Serositis	10(16%)	5(13%)	n.s.
Renal	16(25%)	6(15%)	n.s.
Neuropsychiatric	2(3%)	1(3%)	n.s.
Mucocutaneous	39(62%)	10(25%)	**<0,001**
Leukopenia/Lymphopenia	22(38%)	11(30%)	n.s.
Thrombocytopenia	7(12%)	1(3%)	n.s.

Table 1. Demographic and clinical variables.

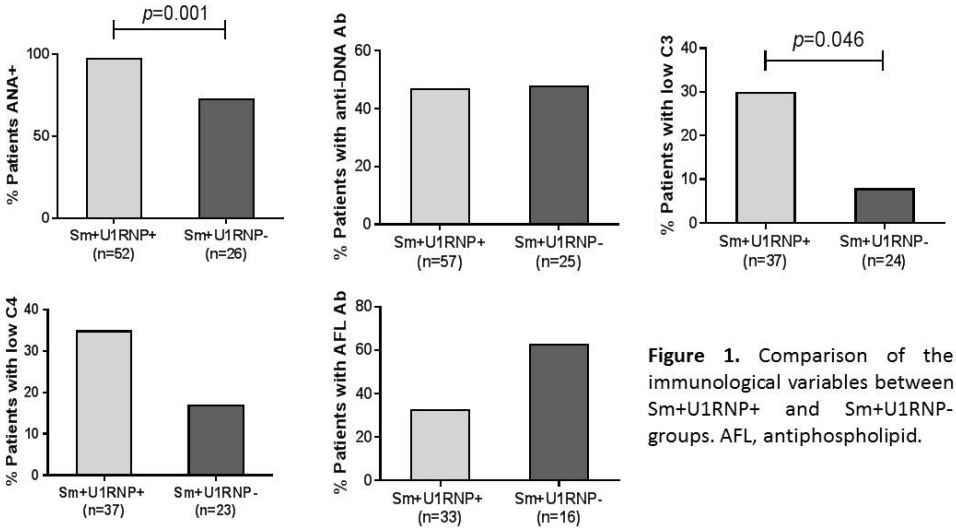


Figure 1. Comparison of the immunological variables between Sm+U1RNP+ and Sm+U1RNP- groups. AFL, antiphospholipid.

P-012

PSGL-1/P-SELECTIN INTERACTION REDUCES THE AMOUNT OF DNA RELEASED IN MONOCYTES EXTRACELULAR TRAPS: LACK OF REGULATION IN SLE PATIENTS

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Background:

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the presence of autoantibodies, that can be generated by the impaired clearance of extracellular traps. Interestingly, monocyte homeostasis in SLE is altered. In addition, P-selectin KO mice develop an autoimmune syndrome similar to human lupus. Moreover, patients with cutaneous lupus have low expression of P-selectin in skin vessels. Althought, it has been described that human monocytes generate METs, it is unknown the role of P-selectin in this process.

Objectives:

1. To study the basal levels of METs in monocytes from healthy donors and SLE patients
2. To study the contribution of P-selectin to MET generation in monocytes from healthy donors and SLE patients

Materials and methods:

Monocytes were obtained from SLE patients and healthy donors (HD). Cells were left to adhere on BSA for 1 h at 37ºC (basal conditions) or incubated with BSA or P-selectin+BSA in rolling-like conditions (shaking at 60 rpm) for 15 min and allowed to adhere at 37°C for 1 hour. METs were quantified using DAPI and Sytox Green. All data were obtained and analyzed by flow cytometry and confocal microscopy.

Results:

In basal conditions, we have observed an increased susceptibility to MET generation in SLE patients. In addition, our results indicate that in healthy donors monocytes, PSGL-1/P-selectin interaction reduces the amount of extruded DNA without afecting the percentage of cells generating METs. This regulation is lost in SLE patients.

Conclusions:

Dysregulation of PSGL-1/P-selectin axis might contribute to SLE pathogenesis.

P-013

P-SELECTIN/PSGL-1 AXIS REGULATION OF AUTOPHAGY IN MONOCYTES FROM HEALTHY DONORS AND LUPUS PATIENTS

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BACKGROUND

The interaction between PSGL-1, a transmembrane protein expressed in leukocytes, and its selectin ligands, induces Syk activation. Also, it has been described that AKT is activated in T cells by PSGL-1 ligation. Autophagy can be modulated by PI3K-AKT-mTOR signaling pathway as well as by chaperones. Previous work demonstrated the importance of P-selectin/PSGL-1 interaction in regulating immune homeostasis and SLE development, where autophagy and proteostasis play a key role. In fact, autophagy alterations have been associated to SLE pathogenesis.

OBJECTIVES

1. Study basal levels of LC3B-II, pmTOR, HSP40 and HSP70 in monocytes from HD and lupus patients.
2. Determine levels of LC3B-II, pmTOR, HSP70 and HSP40 upon P-selectin/PSGL-1 binding in monocytes from HD and lupus patients.

MATERIALS AND METHODS

Monocytes from healthy donors (HD), active (aSLE) and inactive (iSLE) lupus patients were incubated on BSA or BSA+P-selectin under rolling-like conditions (shaking at 60rpm for 15 minutes), followed by a 1-hour adhesion time at 37ºC, or incubated on BSA without rolling. Autophagy (LC3B-II), pmTOR, HSP40 and HSP70 levels were analyzed by flow cytometry.

RESULTS

Monocytes from aSLE patients present higher basal expression levels of autophagy and HSP40, and a tendency towards increased HSP70 levels compared to HD. In HD monocytes, P-selectin/PSGL-1 interaction reduces autophagy, HSP40 and HSP70 expression levels, while in SLE patients these remain unchanged. Regarding pmTOR, no significant changes are seen

between HD and patients, either in basal conditions or upon P-selectin binding.

CONCLUSIONS

P-selectin/PSGL-1 interaction modulates autophagy in HD monocytes, reducing LC3B-II levels. Because pmTOR levels remain unchanged upon P-selectin binding, mTOR might not be involved in autophagy regulation by P-selectin. Instead, HSP70 and HSP40 may be modulating this process as their levels are also decreased following P-selectin/PSGL-1 interaction. However, monocytes from SLE patients show an impaired autophagy and chaperone regulation by interaction with P-selectin.

P-014

DIAGNOSTIC PREDICTIVE VALUE OF SOLUBLE PSGL-1 AND ITS LIGANDS IN PATIENTS WITH SYSTEMIC SCLEROSIS (SSC)

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Systemic sclerosis (SSc) is a systemic autoimmune disease characterized by skin fibrosis, vascular damage and presence of autoantibodies. Based on the extent of skin involvement, SSc can be classified as limited cutaneous SSc (lcSSc) and diffuse cutaneous SSc (dcSSc). Interstitial lung disease (ILD) is the main cause of death. There is not curative treatment, hence finding predictive biomarkers for early SSc diagnosis and prognosis is mandatory. PSGL-1 and its ligands –P-, L-, E-selectins– are crucial for leukocytes extravasation to inflammation sites, thus play an important role in autoimmune diseases. There are controversial data about serum levels of selectins in SSc patients. In this work, we have analysed the serum pattern of PSGL-1, P-, E- and L-selectins and ADAM8 in SSc patients. Serum samples from 52 SSc patients (38 lcSSc and 14 dcSSc) and 55 healthy donors (HD) sex and age matched per group were collected, and proteins levels were measured by ELISA.

SSc patients showed higher E-Selectin and lower L-Selectin serum levels regarding HD. Levels of PSGL-1, E-Selectin and ADAM8 were higher in dcSSc compared to lcSSc patients. In addition, low levels of sPSGL-1 were associated with the presence of anti-centromere autoantibodies whereas high sPSGL-1 levels were associated with the presence of anti-Scl70 autoantibodies and ILD. Furthermore, high sE-Selectin levels were associated with the presence of ILD and pulmonary arterial hypertension (PAH). In a multivariable binary logistic regression model, a combination of two consecutive ratios —sL-Selectin/sE-Selectin (cut off<39,80; S=44%, Sp=91%) and sE-Selectin/sPSGL-1 (cut off>0,27; S=52%, Sp=85%)— classified correctly 38 out to 52 patients. Therefore, these molecules can be used as predictive markers of SSc. Our results show that PSGL-1, E-Selectin, L-Selectin and ADAM8 could contribute to the pathogenesis of SSc and could be a potentially valuable tool for disease diagnosis.

P-015

ANCA ASOCIATED VASCULITIS: CORRELATION BETWEEN ANTIBODY TITER AND SEVERITY OR PROGNOSIS

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Introduction

ANCA-associated vasculitis (AAV) is a group of pathologies characterized by necrotizing inflammation that mainly affects small and medium-sized vessels. Serum anti-neutrophil cytoplasmic antibodies (ANCA), mainly anti-myeloperoxidase (anti-MPO) and anti-proteinase 3 (anti-PR3), levels may correlate to severity, prognosis or recurrence of the disease.

Methods

A retrospective analysis of 97 patients with ANCA-positive vasculitis was performed, using laboratory established cut-off value, measured by chemiluminescence. 60 patients were anti-MPO positive while 37 had anti-PR3 antibodies. Out of the 97 patients that were included in the study, 43 were diagnosed granulomatosis and polyangiitis, 35 with microscopic polyangiitis, 9 with eosinophilic granulomatosis and polyangiitis, and 10 with an unclassified AAV. Furthermore, data of renal disease (hematuria and/or proteinuria) and pulmonary involvement (hemoptysis, asthma and/or respiratory insufficiency) were collected at vasculitis diagnosis, as well as whether the illness progressed to the stage of requiring dialysis, transplantation, or mortality. The usefulness of the initial antibody titer to estimate a higher probability of disease recurrence was also examined.

Results

There were significant differences in ANCA levels between patients with renal or pulmonary dysfunction (n=76) versus those without them (n=21) (p=0.0026), and a cut-off threshold of 60.5 IU/ml was established (AUC=0.7118), as is shown in Figure 1.

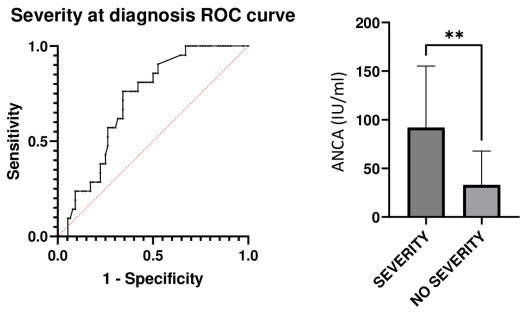
After evaluating the illness's prognosis, an AUC= 0.5209 was found, being no significant differences between those patients who had a worse disease progression (n=23) and those who did not (n=74) (p=0.7664). When comparing individuals who experienced a recurrence of

vasculitis (n=27) to those who did not (n=70), similar findings were obtained (AUC=0.5476; p=0.472).

Conclusion

Anti-MPO and anti-PR3 levels at the moment of vasculitis diagnosis are related with disease severity but not with disease outcome or vasculitis recurrence. For improved patient care, establishing a threshold at which the disease is more severe may be helpful.

Figure 1. Receiver Operating Characteristic (ROC) plot for AAV and disease severity at diagnosis (AUC=0.7118) and column barr graph (p=0.0026).



P-016

ANTICUERPOS ANTI TIF1Γ: LIMITACIONES TÉCNICAS Y LA IMPORTANCIA DEL CONTEXTO CLÍNICO.

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Introducción

Los anticuerpos anti-TIF1γ son de gran trascendencia para el diagnóstico de patologías como la dermatomiositis paraneoplásica. La inespecificidad de su patrón en inmunofluorescencia indirecta (IFI) hace especialmente relevante la confirmación por otras técnicas como el inmunoblot.

Objetivos

Evaluar, en nuestra población, la correspondencia entre clínica y patrón de IFI con las positividades de anti-TIF1γ mediante inmunoblot.

Material y métodos

Estudio observacional retrospectivo de 28 pacientes (2017-2022) con anti-TIF1γ positivo moderado o fuerte mediante inmunoblot (Euroline, Euroimmun). Se recopilaron los datos de autoinmunidad, neoplasias y patrones de IFI (células Hep2000) de las historias clínicas. El estudio estadístico se realizó mediante SPSS.

Resultados

De los 28 pacientes estudiados la dermatomiositis como síndrome paraneoplásico se confirmó en 3 casos, aunque 2 más tenían historia de neoplasia (pulmón, cérvix, mama y ovario). Otros dos pacientes presentaron dermatomiosistis o síndrome antisintetasa sin neoplasia asociada. Además, 3 pacientes más presentaron miopatías no filiadas y otros 3 afectaciones pulmonares compatibles sin clara asociación.

Respecto a la IFI, fue negativa en 11 pacientes, 12 presentaban patrón moteado fino y 5 patrón citoplásmico. Del grupo con patrón moteado, 5/12 presentaban anticuerpos con patrón compartido (anti-Ro y anti-ku) y sólo uno se diagnosticó de dermatomiositis paraneoplásica. En el grupo con patrón citoplásmico 1/5 presentó sd. Antisintetasa aunque en probable relación anti-Jo-1+ y sin neoplasia. Del grupo con inmunofluorescencia negativa, 3/11 tenían dermatomiositis, 3/11 presentaban alteraciones miopáticas y 2/11 alteraciones pulmonares (EPID y fibrosis). Ningún paciente con IFI negativa y manifestaciones compatibles tenía otros anticuerpos asociados a miopatías por inmunoblot.

Conclusión

En nuestro medio y tiempo de estudio la detección de TIF1γ por inmunoblot no parece tener la correlación clínica esperada y esta no se incrementa si se acompaña de patrón moteado en IFI. Los datos parecen evidenciar la importancia del contexto clínico para no incurrir en el error diagnóstico.

P-017

USEFULNESS OF SERUM CALPROTECTIN AS BIOMARKER IN PEDIATRIC RHEUMATIC DISEASES

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Introduction

Pediatric rheumatic diseases are a diverse group of illnesses characterized by inflammation and autoimmunity. Due to the complexity and progressive course of these disorders, specific biomarkers are desirable for patient follow up and therapeutic decisions. In the past few years, serum calprotectin has emerged as a potential biomarker.

Objectives

The main objectives of the present study were:

a. To assess the correlation between serum calprotectin levels and clinical inflammation in pediatric rheumatic patients.
b. To compare serum calprotectin quantification by two different methods: Enzyme Immunoassay (EIA) and Chemiluminescence Enzyme Assay (CLIA).
c. To evaluate the concordance of each method with several clinical variables.

Material and Methods

First, we studied 72 samples from 37 patients (3 to 23 years old) with rheumatic diseases, mainly Juvenile Idiopathic Arthritis (JIA). Serum calprotectin concentration measured by EIA (Bülmann®) was compared with clinical inflammation in terms of JADAS27 index, and with other general inflammation markers (C-reactive protein (CRP) and Erythrocyte Sedimentation Rate (ESR)).

Second, we studied 144 samples from 41 patients with similar characteristics. Serum calprotectin measured by EIA and by CLIA (QUANTA Flash®) were compared and correlated with sex, age, clinical remission, number of inflamed joints, and JADAS27 index.

Results

Our study showed that serum calprotectin is a biomarker with high specificity (78.2%) and

sensitivity (80%) (Cut-off: 3,36 µg/ml) in our cohort. We found a good correlation between EIA and CLIA results (r=0.87). However, we observed significant differences in values >4 µg/ml ($p<0.01$). Both methodologies were associated with clinical remission and JADAS27 index.

Conclusions

Serum calprotectin is a usefulness biomarker to evaluate clinical inflammation in pediatric patients with rheumatic diseases. Ongoing studies will tell us if serum calprotectin is also helpful in therapeutic decisions.

P-018

ANTICUERPOS ANTI-GSTT1, ¿DETECCIÓN MEDIANTE INMUNOFLUORESCENCIA INDIRECTA O LUMINEX?

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Introducción

La tecnología Luminex ha revolucionado la detección de anticuerpos en pacientes trasplantados y podría sustituir a técnicas convencionales como la Inmunofluorescencia indirecta (IFI), empleada en la determinación de anticuerpos anti-GSTT1.

Objetivos

Comparar ambas técnicas (Luminex e IFI) en la detección de anticuerpos anti-GSTT1 en trasplantados renales con alelo GSTT1-nulo y que reciben un riñón de donante GSTT1-positivo.

Materiales y métodos

Se estudian anticuerpos anti-GSTT1 en sueros, pre-trasplante y post-trasplante, de 42 receptores "Donantes *GSTT1-positivos*/Receptor *GSTT1-nulos",* mediante IFI sobre portas de triple tejido (Biocientífica, Menarini Diagnostics) y tecnología Luminex (anti-no HLA LIFECODES Non-HLA Antibody Kit, Immucor). Se verifica la especificidad del Luminex con sueros de 20 receptores *GSTT1*-positivo. Programa estadístico utilizado: SPSS 26,0.

Resultados

Mediante IFI, se detectó la presencia de anticuerpos anti-GSTT1 en 12 receptores (título 1/40-1/320; mediana 1/160). En 4 de ellos, se detectaron en el momento del trasplante y en el resto a los 20.8 meses de mediana de seguimiento. Mediante Luminex, los anticuerpos se detectaron en 16 receptores (Intensidad media de fluorescencia (MFI): media 11700, DS 6360). De ellos, 12 son positivos pre-trasplante y el resto a los 34.5 meses de mediana del seguimiento. No se detectaron anti-GSTT1 en los pacientes GSTT1-positivo. El estudio comparativo de ambas técnicas revela menor sensibilidad de la IFI respecto al Luminex (85.7%), con un Valor Predictivo positivo de 100%. Existen diferencias estadísticamente significativas en la MFI obtenida por Luminex entre los receptores con anticuerpos detectados por IFI (mediana MFI 14.126) y los que no son detectados por IFI (mediana MFI 2.627); p= 0.022, U de Mann-Whitney.

Conclusiones

La determinación de anticuerpos anti-GSTT1 mediante Luminex presenta mayor sensibilidad

con respecto a la IFI, manteniendo una alta especificidad, lo que implica una elevada eficacia diagnóstica en la detección de anticuerpos anti-GSTT1 en pacientes trasplantados de órganos sólidos con alelo GSTT1-nulo.

P-019

SENSITIVITY AND SPECIFICITY OF TWO CHEMILUMINESCENCE METHODS FOR THE DETECTION OF ANTI-CCP ANTIBODIES IN RHEUMATOID ARTHRITIS AND NON-RHEUMATOID ARTHRITIS PATIENTS

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Introduction: Rheumatoid arthritis (RA) is one of the most prevalent autoimmune diseases, being its diagnosis a complex and highly personalized process, in which clinical manifestations and serological results, including autoantibodies and acute-phase biomarkers, are considered. The presence of autoantibodies is characteristic of this disease, being rheumatoid factor (RF) and anti-cyclic citrullinated peptides (CCP) antibodies the main ones.

Aim: To compare the sensitivity and specificity of two chemiluminescence methods to detect the presence of anti-CCP antibodies in RA and non-RA patients.

Methods: Sera from 161 RA patients and 49 from non-RA patients, including psoriatic arthritis, polyarthralgia or osteoarthritis, from our serum collection were analyzed for the presence of anti-CCP using BioFlash (Werfen) and BioCLIA (Palex).

Results: Using manufacturers´cut-offs (BioFlash CCP: 20 CU; BioCLIA CCP: 20 RU/mL), BioFlash showed a sensitivity and specificity of 89% and 92%, respectively, whereas the sensitivity and specificity of BioCLIA was 86% in both cases. After reviewing the clinical records of those patients cataloged as non-RA patients, 3 of them finally developed RA, and 1 developed rhupus. Therefore, considering this final diagnosis, the sensitivity of BioFlash and BioCLIA was 88% and 86%, respectively, and the specificity was 98% and 91%, respectively (Table 1).

Table 1.

	Initial diagnosis		Final diagnosis	
	BioFlash CCP	BioCLIA CCP	BioFlash CCP	BioCLIA CCP
Sensitivity	89%	86%	88%	86%
Specificity	92%	86%	98%	91%
LR+	10.88	6.04	39.32	9.68
LR-	0.12	0.16	0.12	0.15
PPV	0.97	0.95	0.99	0.97
NPV	0.71	0.66	0.70	0.64

Conclusion: BioFlash (Werfen) and BioCLIA (Palex) are two comparable methods for the detection of anti-CCP antibodies, which are very useful for the diagnosis of RA.

P-020

COMPARATIVE RESULTS OF AUTOANTIBODIES BY APTIVA TECHNIQUE BETWEEN AN EXTERNAL QUALITY SERVICE AND HOSPITAL UNIVERSITARIO REINA SOFIA

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Background. Autoantibody laboratory techniques are always a diverse and a constant changing subject, which let us analyze biological samples in a better and faster way. Hence, an annually quality control between immunology labs and regulatory agencies is desirable to review possible disparities and keep improving the diagnostic methods.

Material and Methods. A total of 32 serum samples received between 2017 and 2022 were tested for a specific panel of autoantibodies (ANA, dsDNA, centromere, ENA, Ro, La, RNP, Sm, Scl-70 and Jo-1). The detection was made by commercial technique APTIVA, which is particle-based multi-analyte technology. We compared these results with the data obtained from an external quality service and we calculated the percentage of mismatch results and attached the specific autoantibodies that were on these results.

Results. We observed a total of 6 mismatches (1,5%) of all the samples analyzed between the external quality service data and ours. In these mismatches, we found that anti-RNP was the most common antibody of the study (n=3) followed by Ro (n=2) and Sm (n=1).

Conclusion. We conclude that our results are satisfactory because of the low differences found with regard to an international quality reference and encourage us to keep renewing laboratory techniques for the benefit of patients.

P-021

VALIDATION OF A MULTIPARAMETRIC ASSAY FOR DETECTING SLE-ASSOCIATED ANTIBODIES AND CORRELATION WITH CLINICAL MANIFESTATIONS

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

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

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

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

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

Anti-dsDNA autoantibodies by PMAT showed a positive strong correlation with cSLEDAI-2K ($p<0.001$) and a moderate negative correlation with C3 and C4 levels ($p<0.001$). Anti-Sm and

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

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

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

P-022

ANTICUERPOS AISLADOS FRENTE A RO60 Y/O RO52 SIN CLÍNICA ASOCIADA. UN PROBLEMA EN LA CONSULTA

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Introducción:
El aumento de solicitudes de análisis de anticuerpos antinucleares está incrementando la aparición de especificidades positivas no esperadas, como anticuerpos anti-Ro. Esto implica, en muchos casos, derivaciones a consultas especializadas para ampliación de estudio.

Objetivos:
Analizar, en nuestra población, la relación de la presencia entre anticuerpos anti-Ro60 y/o Ro52 y el diagnóstico de conectivopatías.

Material y métodos:
Se realizó un estudio observacional retrospectivo descriptivo de 112 pacientes con anticuerpos anti-Ro60 y/o Ro52 (determinados por FEIA, Thermofisher, y confirmación con Line-Blot Euroimmun), sin otra especificidad. Fueron clasificados en 3 grupos: anti-Ro52+ (29), anti-Ro60+ (33), y anti-Ro60+/anti-Ro52+ (50), y de la historia clínica se obtuvo la información diagnóstica relativa a la presencia de conectivopatía. El análisis estadístico se realizó con SPSS Statistics 18.

Resultados:
En el grupo anti-Ro52+, un 62,07 % no se asocia a conectivopatías, y del 37,93% si asociado, el 81,8% era Sd. de Sjögren. En el grupo anti-Ro60+, un 42,42 % no se asoció a conectivopatías, del 57,58% si asociado, el 68,4% era Sd. de Sjögren. En el grupo anti-Ro60+/anti-Ro52+ tenían conectivopatías el 64%, dentro ellos, el 75% eran Sd. de Sjögren.

Conclusiones:
En la población estudiada, la aparición aislada de anticuerpos anti-Ro60 y/o Ro52 no siempre se asocia al diagnóstico de conectivopatías, sobre todo cuando detectamos anti-Ro52 como único anticuerpo positivo. Esto supone un incremento de derivaciones innecesarias a consultas de

especialistas que se podrían evitar si las solicitudes de anticuerpos antinucleares se ajustaran más a la clínica del paciente.

Sesión Poster Defensa 1. Pantalla 2. Autoinmunidad (II)

P-023

OXYGEN SENSING BY THYMIC DCS CONTROLS NEGATIVE SELECTION IN THE THYMUS.

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Thymic dendritic cells (DCs) present self-antigens to developing thymocytes to determine which self-reactive T cells must be deleted. The thymus is an organ that contains hypoxic foci along both the cortex and medulla. However, DCs in the thymus show high *Vhl* levels, along with low expression of HIF (Hipoxia Inducible Factor) target genes. How sensing hypoxia by DCs could regulate thymic selection remains unexplored. To delete the oxygen sensing capacity in DCs we generated a CD11cΔ*Vhl* mouse line, where *Vhl* (the negative regulator of HIF) was deleted in DCs under the CD11c promoter. We found that thymi from 8-week old CD11cΔ*Vhl* mice were smaller compared with control littermates, reducing the frequencies of $CD8^+$ single positive (CD8SP) and CD4SP thymocytes. Thymocytes undergoing negative selection (TCR-$\beta^{hi}CD5^+$) showed reduced frequency along with increased Nur77 and Caspase-3 staining, indicating enhanced TCR stimulation and increased clonal deletion in CD11cΔ*Vhl* mice compared with wildtype controls. Supporting this finding, *Vhl* deficient thymic dendritic cells loaded with OVA protein showed increased antigen presentation to OTI OVA-specific $CD8^+$ T cells. Furthermore, in a model of colitis, the transfer of $CD4^+CD25^-$ T cells from CD11cΔ*Vhl* mice to Rag1 -/- mice induced reduced weight loss compared to the transfer of $CD4^+CD25^-$ T cells from control mice, indicating reduced autoreactivity. These results may imply that DCs with stabilized HIF boost negative selection.

P-024

DIFFERENTIAL EPIGENETIC AND TRANSCRIPTOMIC PROFILES OF MONOCYTES SUBSETS IN SYSTEMIC LUPUS ERYTHEMATOSUS.

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Monocytes are an innate immune cell type responsible for pathogen phagocytosis and cytokine production, among other functions. Although they have been historically studied as a single population, monocytes are actually composed by multiple subpopulations. The most common classification is based on the cell surface expression of the proteins CD14 and CD16. In this way, we can differentiate between classical (CM), intermediate (IM) and non-classical monocytes (NCM) which constitute 85%, 5% and 10% of the total monocyte population. These subpopulations correspond to differentiation stages of monocyte physiological development. Nevertheless, they perform different roles in the innate immune response and, notably, their representation changes in many diseases involving the immune system. In our study, we set to understand the differential participation of each subpopulation in the autoimmune disease of Systemic Lupus Erythematosus (SLE). To this end, we have studied the DNA methylation and expression profile of 20 SLE patients and 10 healthy donors. On the one hand, DNA methylation changes appearing in CM are maintained in the other subsets while NCM present unique and differential changes. This would agree with the notion that CM give rise the other subsets and, thus, semi-permanent epigenetic changes are maintained throughout this differentiation. The changes in CM link this population's alterations with Fos/Jun pathway while NCM's alterations associate it to the response to interferon pathway. On the other hand, transcriptomic changes do not seem to follow this preserved profile and the different subsets present several hundreds of uniquely dysregulated genes. In this case, CM dysregulated genes are associated to metabolism and myeloid differentiation while NCM's have a clear relation with Th17 mediated immune response. In all, our preliminary results show that each monocyte subpopulation probably has a differential role in the development of SLE which could help understand the variability observed in this complex disease.

P-025

RELEVANCIA CLÍNICA DEL LINFOGRAMA DUODENAL COMO HERRAMIENTA DIAGNÓSTICA DE ENFERMEDAD CELIACA EN ADULTOS

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INTRODUCCIÓN: La enfermedad celiaca (EC) es una patología autoinmune caracterizada por lesiones en la mucosa duodenal desencadenadas por el gluten. El linfograma duodenal analiza las subpoblaciones de linfocitos intraepiteliales totales ($LIEs_T$), LIEs TCR-γδ+ y LIEs CD3-, y se ha propuesto como prueba complementaria para el diagnóstico diferencial de EC en adultos.
OBJETIVO: Comparar el linfograma duodenal de pacientes celiacos en dieta con gluten (DCG), celiacos en dieta sin gluten (DSG) y no celiacos (patología digestiva diferente a EC).
METODOLOGÍA: Se reclutaron 120 pacientes y se dividieron en: I) celiacos en DCG (n=16), II) celiacos en DSG (n=27) y III) no celiacos (n=77). El estudio del linfograma duodenal se realizó en muestras de biopsia duodenal mediante citometría de flujo. Los puntos de corte fueron: $LIEs_T \geq 15\%$; TCR-γδ+≥15%; y CD3-≤6%. El análisis estadístico se realizó mediante las pruebas no paramétricas de Kruskal-Wallis. Los valores fueron expresados como media±desviación típica ($p \leq 0,05$).
RESULTADOS: Al comparar las subpoblaciones del linfograma duodenal entre los tres grupos, los pacientes celiacos en DCG presentaron valores de $LIEs_T$ significativamente más altos que los observados en pacientes celiacos en DSG (18.18±6.35 versus 13.48±6.31, p=0.05), y que los pacientes no celiacos (11.81±9.28, p=0.001). Al comparar los TCR-γδ+, los valores medios obtenidos en celiacos en DCG (31.93±12.72) y en celiacos en DSG (24.59±9.08) fueron significativamente superiores que en los no celiacos (9.55±7.51) ($p<0.0001$). Por el contrario, los CD3- fueron significativamente menores en pacientes celiacos en DCG (2.93±4.43) y celiacos en DSG (7.70±9.71) comparados con los obtenidos en no celiacos (17.10±13.49) ($p<0.0001$).
CONCLUSIÓN: Los resultados obtenidos enfatizan la utilidad del linfograma duodenal en el diagnóstico diferencial de la EC con otras patologías digestivas. La dieta es un parámetro fundamental a tener en cuenta, ya que algunas subpoblaciones linfocitarias no se ven alteradas por la misma (TCR-γδ+ y CD3-), mientras que otras sí ($LIEs_T$).

P-026

ASSOCIATION BETWEEN CHANGES IN THE PATTERNS OF ANTINUCLEAR AUTOANTIBODIES AND EFFICACY AND TOXICITY OF IMMUNE CHECKPOINT INHIBITION THERAPY IN SOLID TUMORS.

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Immune-related Adverse Events (irAEs) are unpredictable autoimmune-like toxicities induced by immune checkpoint inhibitors (ICI) as a consequence of a breakdown in self-tolerance. ICIs can induce autoantibody formation, and the presence of antinuclear autoantibodies (ANAs) has been reported in patients who developed irAEs. Moreover, irAEs have been associated to improved outcome in treatment with ICIs. Our goal was to compare ANA patterns by indirect immunofluorescence at different timepoints before (baseline) and after the initiation of ICI treatment and to analyze the role of ANA pattern changes as predictors of irAEs development and treatment responses. This study includes 152 consecutive patients with solid tumors treated with anti-PD-(L)1 blockade agents in Hospital de la Santa Creu i Sant Pau, Barcelona. We grouped patients into three groups: ANA de novo (patients who showed new ANA patterns at any time after ICI initiation), ANA (ANA positive at baseline without changes in the ANA patterns after initiation of treatment) and non-ANA (ANA negative at baseline and after ICI initiation). We did not find any association between the appearance of ANAs and irAE rates or the number and types of irAEs. However, patients in the ANA de novo group showed higher severe irAE rates (grade ≥ 3), superior OS and significant superior PFS than the other groups. Additionally, in most of the patients with severe irAEs (83.3%), changes in ANA patterns preceded irAE onset. In conclusion, we found ANA induction during ICI therapies in a subgroup of patients and our results suggest that the appearance of ANAs may predict the severity of the irAE.

P-027

INFLUENCIA DE ANTICUERPOS ANTI-RO52 EN PACIENTES CON SÍNDROME ANTISINTETASA ANTI-JO1 POSITIVO.

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Introducción

El anticuerpo ant-Jo1 se detecta hasta en un 70% de los pacientes con Síndrome anti sintetasa (SAS). Se ha descrito mayor afectación cuando se detectan autoanticuerpos anti-Ro52

Objetivo:

Analizar en nuestro medio las manifestaciones y evolución de los pacientes anti-Jo1 positivos.

Material y métodos:

Búsqueda retrospectiva de pacientes con anticuerpos anti-Jo1 determinados mediante Linedot específico de miositis (Euroimmun ©) y confirmados con FEIA (Termofisher©) entre los años 2008-2022. Se evaluó la presencia conjunta de anticuerpos anti-SSA (Ro60 y/o Ro52), determinados por Linedot (Euroimmun©). Se obtuvieron los datos clínicos tras revisión de sus historias clínicas.

Resultados:

Se detectaron 37 pacientes anti-Jo1+ (edad media: 57 años); 73% mujeres y 27% hombres; 22 (59%) fueron positivos para Ro52; 14 (38%) negativos para otros autoanticuerpos (Ro52-) y un paciente presentó Ro60 sin Ro52.

Se confirmó SAS en 28 pacientes, (tiempo medio de seguimiento: 8.53 años). De éstos, 17 (61%) son Ro52+, 10 (36%) Ro52- y un paciente Ro60 aislado.

Distribución de síntomas entre pacientes Ro52+: miositis (n=17, 100%), EPID (n=13, 77%), artritis (n=8, 47%), Raynaud (n=3, 18%), “manos de mecánico” (n=1, 6%), y entre pacientes Ro52-: miositis (n=9, 90%), EPID (n=7, 70%), artritis (n=6, 60%), Raynaud (n=3, 30%), "manos de mecánico" (n=3, 30%)

Evolución de los pacientes: entre los Ro52 +**,** 14 (82%) presentaron brotes/refractariedad a tratamiento, 2 (12%) fallecieron por insuficiencia respiratoria aguda en el contexto de EPID, 2 (12%) por procesos oncológicos y 2 (12%) por infecciones. De los pacientes Ro52 negativos, 5 (50%) presentaron refractariedad y no se registró ningún exitus ni proceso neoplásico.

Conclusiones:

En nuestra población, la presencia conjunta de anti-Ro52 y anti-Jo1 en pacientes con SAS no

parece relacionarse con un fenotipo diferente en cuanto a distribución de manifestaciones clínicas. Sí se asocia, aparentemente, con peor evolución (número de brotes/refractariedad a tratamiento).

P-028

NON CRITERIA ANTI-PHOSPHOLIPID ANTIBODIES IN APS-PATIENTS: BEYOND THE LUPUS ANTICOAGULANT.

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Hospital Universitario 12 de Octubre, Madrid.

Recently, non criteria Anti-phospholipid antibodies (aPL) have gained great interest in the Antiphospholipid Syndrome (APS) for their possible role in patients with clinical manifestations but negative for the Sydney consensus aPL.

Here we study the presence of the non-criteria aPL among patients fulfilling APS clinical manifestations. We also analyze the differential clinical characteristics of positive patients for non-criteria aPL.

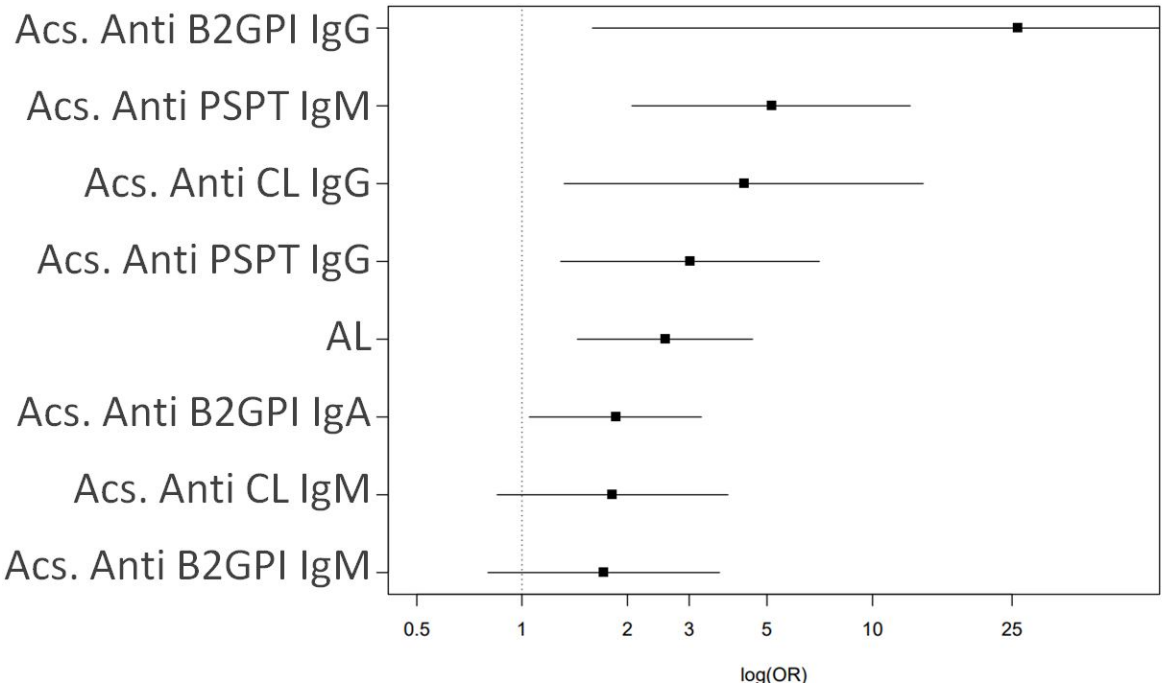
A cohort of 838 patients with clinical APS classification criteria obtained from 4487 patients with suspected-APS was evaluated for Anti-phosphatidylserine/prothrombin (aPS/PT) (IgG/M), IgA anti-Beta 2 glycoprotein I (aB2GPI) and criteria aPL. A control group of 691 healthy donors was also recruited.

317 patients were positive for at least one aPL. 187 (22%) met the Sidney laboratory-criteria, 51 were anti-PS/PT isolated positive and 72 for the aB2GPI IgA. Patients isolated positive for LA were 54. Agreement between aPS/PT (IgG/M) and LA was moderate: Kappa index 0.51 (CI:0.44-0.57). The inclusion of non-criteria aPL identify 41% of the patients positives for any aPL, and 34,3% considering only the patients with obstetric morbidity.

All aPL showed a significant association with clinical manifestations, with better odds-ratios for the IgG isotype and for the aPS/PT.

In the multivariable analysis, LA showed a slightly lower association (2.04, 95% CI 1.17–3.56) than the aPL included in the classification criteria (2.36, 95% CI 1.21-4.6). Both variables showed lower associations than extra-criteria aPL. aB2GPI IgA (2.46, 95% CI 1.4–4.32) and aPS/PT (2.36, 95% CI 1.34–4.18) were independently associated with thrombotic events. aB2GPI showed a higher association among all the antibodies evaluated, including the aPL of the classification criteria.

The inclusion of non-criteria aPL may help in APS diagnosis. LA and aPS/PT identify different types of patients. Although 58% of LA positives were also PS/PT positive, there were isolated positive for both determinations with clearly different clinical characteristics.


Acs. Anti B2GPI IgG
Acs. Anti PSPT IgM
Acs. Anti CL IgG
Acs. Anti PSPT IgG
AL
Acs. Anti B2GPI IgA
Acs. Anti CL IgM
Acs. Anti B2GPI IgM
0.5
1
2
3
5
10
25
log(OR)

P-029

ANTICUERPOS ANTI-RIBOSOMAL P AISLADOS. ¿TIENEN UTILIDAD CLÍNICA?

Just Balerdi, María Begoña; Casado Navarro, Natalia; Dávila Rosales, Marian Alexandra; Sancho Cadenas, Elena; Martínez Becerra, María José; Vegas Sánchez, María Del Carmen; Guzmán López, Karina.

Hospital Universitario Fundación Jiménez Díaz, Madrid.

Los anticuerpos anti-ribosomal P(aRibP) son marcadores descritos en Lupus eritematoso sistémico (LES), generalmente asociados a la presencia de nefritis lúpica, manifestaciones psiquiátricas o hepatitis. Sin embargo, su papel como marcador aislado no está definido.

Se realizó un análisis retrospectivo de las peticiones de aRibP procesadas en el laboratorio de autoinmunidad del Hospital Universitario Fundación Jiménez Díaz, durante el período del 28/12/2018 al 28/12/2022. Se seleccionaron a todos aquellos que presentaron aRibP positivo. Se definió como aRibP aquellos sueros con patrón compatible por inmunofluorescencia indirecta en células HEp2(AC-19), resultado positivo por, al menos, una técnica de inmunoensayo de fase sólida (Biorad-Bioplex®/ELIA-Thermofisher®) y confirmación por inmunoblot (Euroimmun® /DTEK®).
Se excluyeron todos aquellos que presentaron anticuerpos específicos de LES de manera concomitante.

De las 296.606 determinaciones de aRibP, se obtuvieron 137 resultados positivos, de los cuales 110 asociados a otros anticuerpos específicos de LES. aRibP de manera aislada estuvo presente en 27 determinaciones. Se tuvo acceso a historias clínicas de 13 pacientes (23 determinaciones). De estos, 3/13 cumplían criterios de LES, 4/13 en proceso inflamatorio activo, 2/13 en proceso oncológico, y el resto sin diagnóstico definitivo. Cuatro de los 13 pacientes presentaron seguimiento analítico por una o dos técnicas de inmunoensayo sin recidivas en ese periodo.

Según nuestros resultados la presencia de aRibP de forma aislada fue menor de lo esperado. Aparece mayoritariamente asociado a otros anticuerpos, fundamentalmente asociados a LES. En los pacientes con aRibP aislado de nuestra serie no se observan casos de neuroLES ni hepatitis, describiéndose mayoritariamente en otros escenarios clínicos diversos. Debido a la baja prevalencia de los aRibP de forma aislada y a su limitada asociación con LES, la n no permite extraer conclusiones sobre la utilidad de monitorizar niveles en esta serie. Más estudios son necesarios para ver su comportamiento a largo plazo, incluyendo episodios de recaída.

P-030

ESTUDIO PARA LA DETERMINACIÓN DEL CUT-OFF DEL ÍNDICE KAPPA EN EL DIAGNÓSTICO DIFERENCIAL DE ESCLEROSIS MÚLTIPLE

Peña Blanque, Virginia; Calabia González, Olalla; García Cabello, Ana; Téllez Pérez, Raquel.

H.U. Fundación Jiménez Díaz, Madrid.

Introducción

Las bandas oligoclonales IgG (BOC) forman parte de los criterios diagnósticos de McDonald 2017 para esclerosis múltiple (EM). Existen biomarcadores cuantitativos que apoyan la síntesis intratecal de inmunoglobulinas como las cadenas ligeras kappa en LCR (KFLC) y el índice kappa (índice-K) que aumentan en pacientes con CIS y EM respecto a grupos control.

Objetivos

Establecer el cut-off del índice-K en una cohorte de pacientes, comparando los resultados con la técnica gold-estándar (BOC) en el diagnóstico diferencial de EM.

Métodos

Estudio retrospectivo sobre muestras de suero y LCR para el estudio de BOC recibidas desde marzo a diciembre de 2022. Se realizó: BOC (Inmuno-isoelectroenfoque, HydrasysFocusing, Sebia), cuantificación de albúmina (COBAS8000®, Roche/Hitachi) y KFLC (Turbidimetría, Optilite, Bindingsite). Se utilizó la fórmula Índice-K = (KFLC-LCR x sAlb)/(sKFLC x Alb-LCR). Para las curvas ROC se incluyeron pacientes con diagnóstico de EM/CIS, siendo el grupo control pacientes con enfermedades neurodegenerativas, neuromusculares, inflamatorias e infecciosas. En el análisis se consideró positivo BOC ≥ 2 bandas aisladas en LCR y el "patrón mayor que".

Resultados

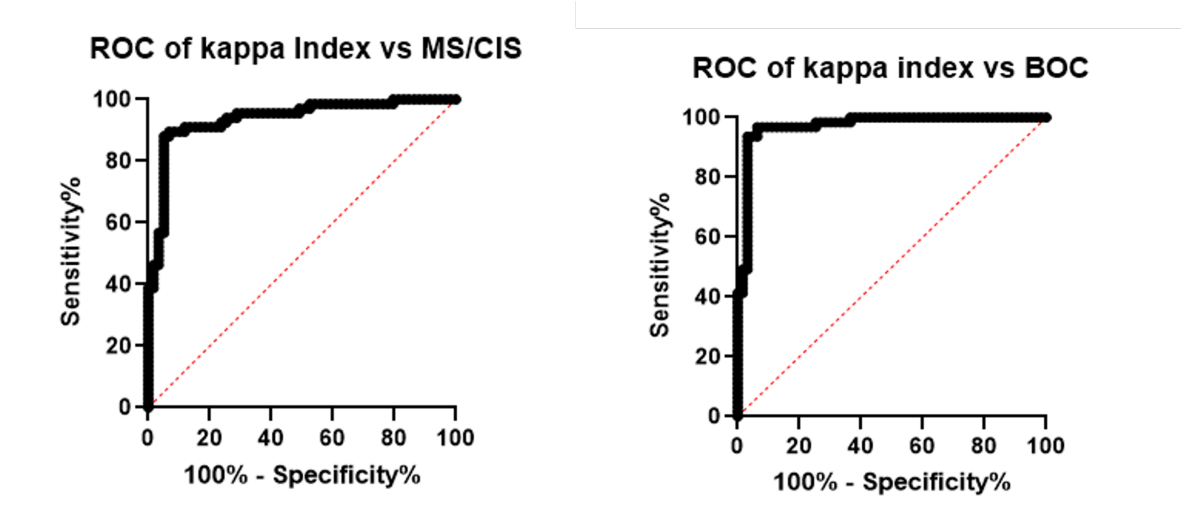
126 pacientes, 59 EM/CIS [80% mujeres, edad media 39 (15-66)] y 67 controles [50% mujeres, edad media 48 (19-83)]. Mediana Indice-K EM/CIS significativamente más elevada respecto controles (62.12 vs 3.96, p<0.0001).

El 50% los pacientes presentaban BOC positivas y niveles superiores de índice-K (62.11 vs 3.93, p< 0.0001). La curva ROC mostró buen rendimiento del índice-K para diagnóstico EM/CIS (AUC=0,9378) y discriminación de pacientes BOC positivos vs negativos (AUC=0,9728) (**Figura 1**).

El cut-off óptimo para EM/CIS=8.7, sensibilidad 89.55% (IC95% 79.97-94.85) y especificidad 93.22% (IC95% 83.82-97.33). Para BOC=9.85, sensibilidad 96% (IC95% 89.14-99.44) y especificidad 93.22% (IC95% 82.73-96.56).

Conclusión

El cut-off obtenido en nuestra cohorte está en línea con lo publicado (rango 3.045-10.62). La buena sensibilidad y especificidad del índice-K aporta un valor añadido a tener en cuenta para el diagnóstico de EM.



P-032

TRANSCRIPTOMIC APPROACH TO IDENTIFY BIOMARKERS FOR PRIMARY PROGRESSIVE MULTIPLE SCLEROSIS IN POPULATIONS OF DENDRITIC CELLS

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Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) characterized by inflammation and demyelination as well as axonal and neuronal degeneration. In the majority of patients, the disease begins with a relapsing remitting course (RRMS). However, a minority of patients (10%) show progressive disease from onset (PPMS). These patients have a shorter life expectancy and twice the risk of mortality than RRMS. To date, there is no cure for this disease, although several treatments have been developed to improve the quality of life of RRMS patients. Almost all of the drugs developed to treat MS are for RRMS, PPMS is considered less responsive to these therapies and patients are generally excluded from trials intended to demonstrate the efficacy of the drugs. Although the exact causes of this disease are unknown, MS is considered to be predominantly a T-lymphocyte-mediated disease, with professional antigen-presenting cells, i.e., dendritic cells (DCs), playing an important role in the onset and course of the disease. Extensive evidence suggests that DCs are implicated in immune dysregulation observed in MS; as well, they display altered functionality and may express different levels of specific genes in PPMS compared to RRMS. For this reason, we propose that DCs may be a source of biomarkers distinguishing the two main forms of the disease, which may uncover novel therapeutic targets for treating PPMS. We have performed whole-genome gene expression profiling by means of RNAseq in dendritic cells, purified from peripheral blood mononuclear cells (PBMCs) of PPMS and RRMS patients, and healthy controls. We were able to identify several genes that were differentially expressed between PPMS and RRMS patients. Functional enrichment analysis also showed that the pathways identified as most significant were different between the two mayor forms of the disease.

P-033

A MICROGLIAL ANKRD55 NUCLEAR INTERACTOME REVEALS A ROLE IN TRANSCRIPTION-CENTERED PROCESSES

Tulloch Navarro, Raquel[1]; Mena, Jorge[1]; Fiat, Ainhoa[1]; Aldekoa, Ane[1]; Azkargorta, Mikel[2]; Lindskog, Cecilia[3]; Alloza, Iraide[1]; Alortza, Felix[2]; Vandenbroeck, Koen[1].

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Genomic variants in the ANKRD55 gene have been associated with risk for autoimmune disease. Risk alleles of these variants modulate expression levels of ANKRD55 in $CD4^+$ T cells and monocyte-derived dendritic cells. Nevertheless, in the murine mouse model of experimental autoimmune encephalomyelitis (EAE), ANKRD55 was overexpressed in neurons and microglia, and its subcellular location predominates in the nucleus. The biological function of ANKRD55 and its role in autoimmune diseases is unclear. Ankyrin repeat domains, of which nine are found in the main ANKRD55 isoform, are one of the most common platforms engaging in protein-protein interactions. The aim of this study was to characterise the nuclear interactome of ANKRD55 extracted from the nuclei of the immortalized human microglia-SV40 cell line (IMhu-M), which is derived from primary microglia and is known to express typical microglial markers. For this purpose, cells were transfected with ANKRD55 mRNA, nuclei were purified by sucrose gradient, protein complexes with ANKRD55 were isolated by immunoprecipitation anti-FLAG tag and identified by mass spectrometry (LC MS/MS). 150 proteins were identified as possible partner proteins of ANKRD55. Proteins with high Normalized Spectral Abundance Factor (SFAT) values were Nucleolin (NCL), Insulin like growth factor 2 mRNA binding protein 1 (IGF2BP1), RNA binding motif protein 39 (RBM39), Nuclear RNA export factor 1 (NXF1), Cleavage and polyadenylation specific factor 1 (CPSF1), Structural maintenance of chromosomes 3 (SMC3). The functional enrichment analysis done with Ingenuity Pathways Analysis (IPA) suggested that ANKRD55-protein partners are related to RNA binding, nucleotide binding, structural constituent of ribosome, processes associated with mRNA processing, RNA splicing, via splicosome, mRNA export from nucleus, mitotic cell cycle, tRNA export from nucleus. The interaction of ANKRD55 and RBM39 in microglia SV40 and monocyte-derived microglia (translational model) was validated by microscopy. Our study suggests that ANKRD55 exerts functions in transcription processes.

P-034

SERUM IGM DETECTION HAS SIMILAR SENSITIVITY TO OLIGOCLONAL BANDS FOR THE DIAGNOSIS AND PROGNOSIS OF MULTIPLE SCLEROSIS.

Muñoz Morón, Úrsula[1]; Escudero Lirola, Esther[1]; Sánchez-Vera Gómez-Trelles, Isabel[1]; Sebal Neira, Cristina[1]; García Sánchez, Maria Isabel[2]; Piédrola Pedraza, Ignacio[1]; Álvarez Lafuente, Roberto[3]; Sádaba Argaiz, María Cruz[1].

[1]Universidad San Pablo Ceu, Madrid; [2]Biobanco del SSPA. Hospital Universitario Virgen Macarena, Sevilla; [3]Instituto de Investigación Sanitaria del Hospital Clínico San Carlos, Madrid.

Background. Multiple sclerosis (MS) is an autoimmune demyelinating disease of the central nervous system. The detection of oligoclonal IgG (BOCIgG) and IgM (BOCIgM) bands is a diagnosis and prognosis biomarker respectively, but the assay is cumbersome. We developed the highest sensitive technique to detect antibodies to lipids, demonstrating that almost 90% of MS patients showed IgM to phosphatidylcholine (IgMPC) in serum, similar sensitivity to those showed by BOCIgG. We aimed to validate the role of serum IgMPC as the most sensitivity for the diagnostic of MS, and to analyze the role of these antibodies as prognosis biomarker.
Materials. Cerebrospinal fluid and serum samples from 66 MS patients, classified as clinically isolated syndrome (CIS; n=26), secondary progressive (SP; n=26) and primary progressive (PP; n=21). Detection of BOCIgG and BOCIgM using electro focusing and immunodetection. High sensitive ELISA assay was developed to detect serum IgMPC
Results. The 92,4% of the MS patients showed BOCIgG and 90,9% of them had serum IgMPC. 90.2% of MS patients with BOCIgG also showed serum IgMPC. All MS patients without BOCIgG (n=5) showed serum IgMPC. CIS patients with BOCIgM showed higher levels of serum IgMPC (0,335 ± 0,158) than those without them (0,154 ± 0,105).
Conclusions. Similar results were observed when the presence of serum IgMPC and BOCIgG were analyzing. Serum IgMPC showed the same sensitivity as that for the presence of BOCIgG for the diagnosis of MS. In addition, those patients with BOCIgM showed the highest levels of serum IgMPC, demonstrating that the detection of IgMPC using our ELISA assay is also a prognosis biomarker.
The ELISA assay has some advantages compared with the detection of oligoclonal bands, the technique can be developed in all the clinical laboratories and does not use cerebrospinal fluid, that can provoke important side effects.

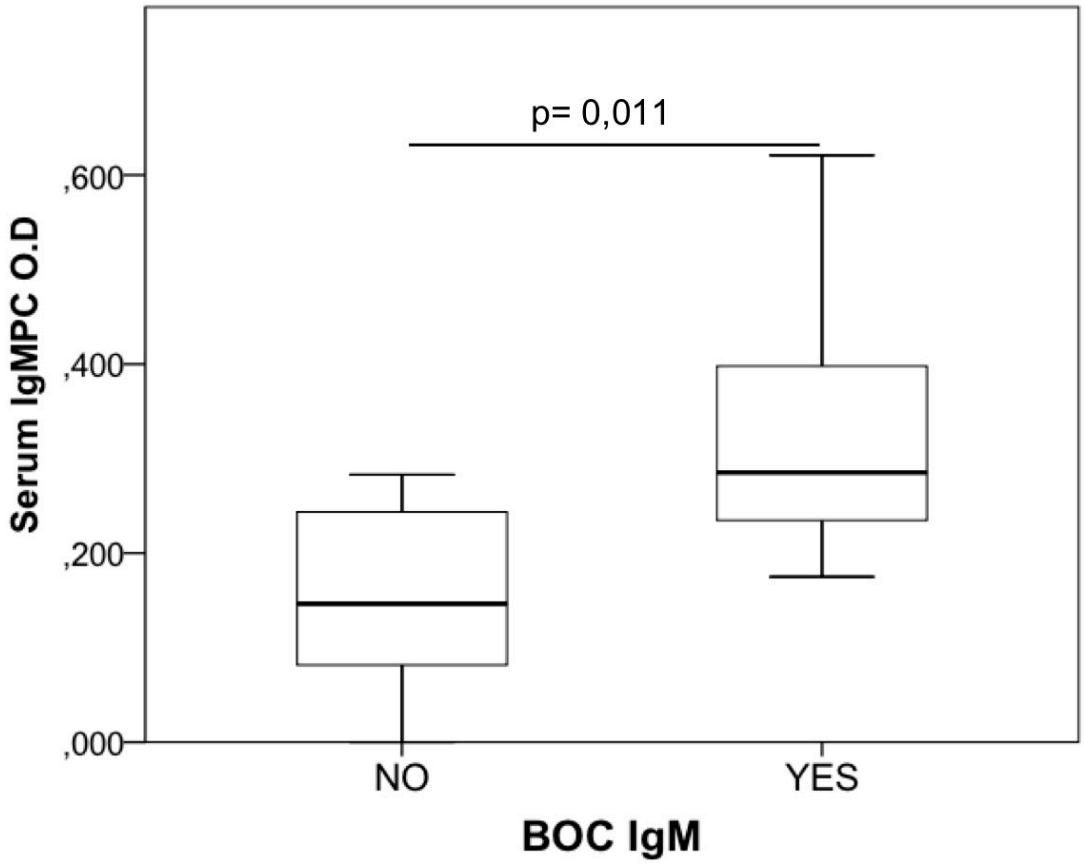


Fig 1. Levels of serum IgMPC measured as optic density (O.D) in patients with (YES) or without (NO) oligoclonal IgM bands (BOCIgM).

Table 1. Relation between the detection of serum IgMPC and oligoclonal IgG bands.

	BOC IgG-	BOCIgG+
Serum IgMPC +	n=0 (0,0%)	n=6 (9,8%)
Serum IgMPC -	n=5 (100%)	n=55 (90,2%)

P-035

AUTOANTICUERPOS FRENTE A RECEPTORES ACOPLADOS A PROTEÍNA G EN DISAUTONOMÍAS

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Hospital Universitario Virgen del Rocío, Sevilla.

Introducción: La disautonomía es una alteración del sistema nervioso autónomo caracterizada por taquicardia, mareo, debilidad o cansancio extremo que engloba varias entidades como el síndrome de taquicardia postural ortostática (POTS), la encefalomielitis miálgica/síndrome de fatiga crónica (EM/SFC) o disautonomia tras infección, como long-COVID. Su etiología puede ser primaria (causa desconocida), o secundaria (autoinmune), detectándose la aparición de autoanticuerpos frente a receptores acoplados a proteína G (GPCR-fAAB).
Objetivos: Analizar la presencia de GPCR-fAAB tres grupos de pacientes (POTS, EM/SFC y long-COVID).
Pacientes y Métodos: determinación de niveles de 7 autoanticuerpos mediante ELISA (CellTrend GmbH): anti- receptores alfa (Rα1) y beta (Rβ1 y Rβ2) adrenéricos, anti- receptores muscarínicos colinérgicos (M2,3,4 AChR) y receptor de la angiotensina II tipo 1 (AT1R) en 83 pacientes derivados desde una consulta especializada de medicina interna.
Resultados: en el grupo de pacientes con **POTS** el 100% de los mismos fueron positivos para Rα1, un 70% para RM2, y un 60% y un 20% para Rβ1 y Rβ2 respectivamente. Tanto para receptores M3 como para anti-ATG se encontró una frecuencia del 10%. Respecto del grupo de pacientes con **SFC** un 67,2% fueron positivos para Rα1 y un 46.5% para RM2. Así mismo en torno al 30% de pacientes fueron positivos para Rβ1, Rβ2 y anti-ATG, siendo del 20% en el caso de RM3. Finalmente, en el grupo **Long-COVID,** un 53% fue positivo para Rα1 y un 20% para M2. Respecto de anti-ATG se detectó en un 13% de los pacientes, siendo del 6% para Rβ1, Rβ2.
Conclusiones: Los anticuerpos frente a receptores α1-adrenérgicos son los más frecuentes en los tres grupos analizados. Los pacientes con POTS presentan autoanticuerpos frente a Rα1, Rβ1 y RM2 mayoritariamente. Destacar que un 30% de pacientes con SFC presentaron anti-ATG, mientras que en el grupo de POTS fue del 10%.

P-036

ANTI-MI2 Y ANTI-CENPB, ¿UNA INESPERADA PAREJA?

Jiménez De Las Pozas, Yesenia; Vegas Sanchez, María Del Carmen; Casado Navarro, Natalia; Davila Rosales, Mariam Alexandra; Martínez Becerra, María José.

H.U. Fundación Jiménez Díaz, Madrid.

En el estudio previo de una cohorte de 46 pacientes con anti-Mi2 por ELiA® (>7 U/mL-Thermo), encontramos un 22% de pacientes que asociaban anti-CENPB (>0,8 IA - Bioplex2200®-BioRad). Los niveles medios en esta cohorte para anti-Mi2y anti-CENPB fueron 47±46 U/mL y 3,1±1,8 IA respectivamente.
Ante este hallazgo, buscamos activamente (Junio/2022-Enero/2023) la presencia de anti-Mi2 por EliA® en pacientes con valores de anti-CENPB entre 0,5 y 7,9 IA. En los pacientes positivos para anti-Mi2, este se estudió por dos inmunoblot (Euroimmun® y Dtek®). Se registró el patrón de inmunofluorescencia en Hep2, otros anticuerpos asociados a EAIs y se revisaron las historias clínicas.
En total, encontramos anti-Mi2 y anti-CENPB concomitantemente en 18 pacientes (17 mujeres). La edad media fue de 30 años [5-74] y el 44% eran menores de 16 años. En 5 pacientes se confirmó este hallazgo en otras muestras, con un tiempo medio de seguimiento de 68 meses [23-164]. En 13 pacientes se detectó, además, algún otro anticuerpo (2/13 anti-Ro52). Disponemos de información clínica de 15 pacientes, 2 tienen diagnóstico de conectivopatía, 5 de enfermedad celiaca.
Los niveles medios de anti-Mi2 EliA® fueron 55±40U/mL y en 14/18 pacientes se confirmó la positividad por al menos un inmunoblot. Para anti-CENPB los valores medios fueron 2,7±1,6 IA. Anti-CENPB se confirmó por otra técnica en 16 pacientes [2/15 por inmunoblot (Euroimmun/Dtek), 9/15 por ELiA->7U/mL-y 5/15 por ambas]. Respecto a la inmunofluorescencia indirecta, 6/18 pacientes presentaron un patrón mixto AC-3+AC-4, 2/18 AC-3+Otros AC, 8/18 AC-4±OtrosAC y2/18 no AC-3/AC-4.
Describimos una cohorte con una asociación atípica de anticuerpos que se relaciona con una baja prevalencia de diagnóstico de conectivopatía. Es destacable el alto porcentaje de población pediátrica, de pacientes con enfermedad celiaca y de asociación con otros anticuerpos en esta serie. Son necesarios más estudios para determinar la implicación de este hallazgo.

P-037

DETERMINATION OF "NON-CRITERIA" ANTIPHOSPHOLIPID ANTIBODIES IN PATIENTS WITH SUSPECTED ANTIPHOSPHOLIPID SYNDROME.

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Background: Antiphospholipid syndrome (APS) is a systemic autoimmune disorder characterized by thrombosis and/or pregnancy morbidity with persistent antiphospholipid antibodies (aPLs). According to Sydney Classification criteria, the IgG/IgM anticardiolipin antibodies (aCL), anti-β2-glycoprotein I antibodies (aβ2GPI), and lupus anticoagulant (LA) were defined as criteria aPLs (CaPLs). Several other "non-criteria" aPL are believed to be associated with APS; however, the lack of evidence confirming their applicability prevents their inclusion into criteria. We aim to determine the prevalence and clinical association of "non-criteria" aPL antibodies in patients with pregnancy complications or suspected APS.

Methods: Monocentric study including 29 women with suspected APS with negative results for CaPLs. We evaluated by ELISA the presence of IgG and IgM against a mix of phospholipids (Screening from Orgentec®), anti-annexin V (Orgentec®), and anti-aPS/PT (Inova®), and anti-prothrombin (Orgentec®). The clinical characteristics of the patients were analyzed according to their serologic status.

Results: The Screening Orgentec® and the anti-annexin V did not add new positive determinations. With the anti-prothrombin and anti-aPS/PT determinations, 12 of the 29 previously negative patients are now positive (8 anti-PT, 3 anti-PS/PT). The fetal death percent was higher in the positive group (5.9% vs 25%), with a relation between this and the presence of IgM aPS/PT. There were more miscarriages <12 weeks in the positive group (58,3% vs 35,3%) with association with IgG aPT. There is a relation between systemic autoimmune diseases and positive IgM aPS/PT (p 0.028).

Conclusions: The determination of aPS/PT and aPT provide 12 new diagnoses in 29 patients negative for the CaPLs. Non-criterion aPL determination is recommended, especially in patients with history of fetal death and systemic autoimmune disease. The limitation is the small sample size, so this methodology should be implemented in multicenter studies to confirm our findings.

P-038

COMPARISON OF TWO AUTOMATED TECHNIQUES IN PATIENTS WITH GESTATIONAL AND THROMBOTIC ANTIPHOSPHOLIPID SYNDROME.

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Antiphospholipid syndrome (APS) is an autoimmune disease characterized by thrombosis and reproductive failure. The classification criteria require the concomitant presence of at least one clinical event, and positivity either for lupus anticoagulant, or for at least one antiphospholipid antibody (aPL) IgG and/or IgM: anticardiolipin antibody, or anti-β2 Glycoprotein-I antibody. The detection of aPL has commonly been carried out with enzyme-linked immunosorbent assays (ELISA), however, alternative solid-phase automated techniques have been developed but with considerable variability between assays.

Considering that there is no gold standard, and the diagnosis of APS depends on antibody positivity, is important to validate new assays. In this study, we aim to analyze the diagnostic accuracy and agreement of two different methodologies. Between January 2019 and November 2020, 1020 biosamples were collected sequentially. In order to establish a cutoff, we also collected samples from 230 blood donors. Patients and donors were tested for aPL with two automated methods: a fluorescence enzyme-immunoassay (T1) and a luminescence bead based assay (T2). To evaluate the diagnostic accuracy of both tests we calculated the area under the curve (AUC).

In our population, 18.13% of the patients were identified as positive with any of the assays. T1 detected a higher number of positives than T2 (135 vs 105 patients). The weighted kappa for both techniques was 0.39 (0.30-0.47). This value increased to 0.56 (0.38-0.73) when only patients with an autoimmune disorder were included. Regarding patients with clinical manifestation, patients with gestational APS presented a higher weighted kappa (0.46) than patients with thrombotic APS (0.33). Moreover, regarding gestational APS, only T1 presented a significant AUC.

Our findings confirm the lack of harmonization between both methods. The higher correlation between the techniques found in autoimmune disease patients might be due to a potential overrepresentation of this population in the standardization of techniques.

P-039

PROFILING THE ANTIBODY COMPOSITION OF PHAGE DISPLAY LIBRARIES DERIVED FROM PERIPHERAL BLOOD OF CELIAC PATIENTS

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Monoclonal antibodies have become one of the most powerful tools for therapy and diagnosis. In 2021, the 100th therapeutic antibody was approved. Since their discovery, many technologies have been refined to develop new monoclonal antibodies, highlighting the phage display technology. This methodology encompasses three main steps: 1) Generation of a diverse antibody library, from natural sources (e.g from lymphocytic RNA) or by artificial means (mutagenic PCRs). 2) Selection of leading clones that bind an antigen (thanks to the attachment of antibodies to phage protein III). 3) Amplification of leading clones within the phage population (due to the direct coupling of the phenotype and genotype, by the expression of antibodies in the auto-replicative bacterium-phage system). Although this methodology has proved very useful, some issues have been raised regarding how the selection works and how it can be improved.

Monitoring of the selection process along the rounds of panning (successive rounds of selection and amplification of phage-antibodies) has been performed by colony picking and Sanger sequencing since the development of phage display technology in the 1990s. Although the method can be automated and thousands of colonies can be picked, it only covers a small amount of the clones implied in the process. This problem can be solved by using massive sequencing techniques.

In this work, two different libraries derived from celiac patients, one fully immune and, another library that merges semisynthetic heavy chains with immune light chains. Both libraries were used in panning processes to select high-affinity Fabs against gliadin. The libraries themselves have been analyzed, and the sub-libraries obtained after each round of panning were characterized by sequencing with Oxford Nanopore Technologies.

The comparison of how the composition of antibody chains changed through different panning rounds gives a global picture of how the selection works for the different antibody libraries studied.

P-040

EVALUATION OF TWO AUTOMATED PLATFORMS FOR THE DETECTION OF ANTI-TISSUE TRANSGLUTAMINASE IGA ANTIBODIES IN CELIAC DISEASE PATIENTS AT DIAGNOSIS AND AFTER STARTING GLUTEN-FREE DIET

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Introduction: Celiac disease (CD) is an autoimmune disease and anti-tissue transglutaminase (tTG) IgA antibodies are of great importance for the diagnosis and monitoring of gluten free diet (GFD). The prevalence of CD is increasing in the last years mostly due to the increased sensitivity of new serologic methods to detect antibodies.

Aim: To compare the sensitivity and the concordance of two methods to detect the presence of anti-tTG IgA antibodies in CD patients at diagnosis and after GFD, respectively.

Methods: Sera from 60 patients at diagnosis, 90 after starting a GFD and 50 from HLA-DQ2/DQ8 negative subjects from our serum collection were analyzed for the presence of anti-tTG IgA using EliA Celikey IgA (Thermofisher) and Aptiva Celiac IgA (Werfen).

Results: Using manufacturers´cut-offs (EliA Celikey: 10 UI/mL; Aptiva Celiac: 5 FLU), the sensitivity of EliA Celikey and Aptiva was 83%, and 90%, respectively. In addition, the specificity of both methods was 100%, since none antibodies were detected in sera from HLA-DQ2/DQ8 negative subjects. Furthermore, the concordance of both methods to detect anti-tTG IgA antibodies in GFD patients was 71%, being Aptiva the method able to detect a higher number of patients with a positive result (20 out of 26; 76.9%) between those with discordant results. As it is shown in Table 1, keeping the Elia Celikey cut-off point fixed and increasing the Aptiva one to 10, 15 or 20 FLU, the sensitivity of both methods was similar.

Table 1.

	EliA Celikey 10UI/mL	Aptiva 5FLU	Aptiva 10FLU	Aptiva 15FLU	Aptiva 20FLU
Sensitivity	83%	90%	88%	80%	80%

Conclusion: EliA Celikey (Thermofisher) and Aptiva Celiac disease (Werfen) are two comparable methods for the detection of anti-tTG IgA antibodies, which show similar behavior for the management of CD. Setting of cut-off values must be set up at each laboratory for a correct performance.

P-041

RELEVANCIA DE LA BANDA ÚNICA EN PACIENTES CON SOSPECHA DE ESCLEROSIS MÚLTIPLE

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Introducción

La esclerosis múltiple (EM) es la enfermedad desmielinizante crónica del sistema nervioso central (SNC) más frecuente y la mayor causa de discapacidad en adultos jóvenes. La detección de la síntesis intratecal de IgG es una herramienta importante para el diagnóstico de EM. Raramente se observa una banda única (BU) de IgG en líquido cefalorraquídeo (LCR), aunque podría estar asociada a diferentes enfermedades como la EM con una frecuencia variable (0 – 44%) y otras enfermedades inflamatorias, desmielinizantes, autoinmunes, infecciones del SNC, linfomas, etc. Sin embargo, la interpretación y aplicación de la BU es controvertida.

Objetivos

Análisis de la frecuencia de presencia de BU en pacientes con sospecha de EM.

Materiales y métodos

Análisis emparejado de muestras de suero y LCR mediante isoelectroenfoque. Estudio de la frecuencia de aparición de BU IgG en las 2033 muestras de pacientes analizadas desde 2009, así como su asociación a los diferentes diagnósticos tras una media de 36 meses de seguimiento.

Resultados

1. Veinte pacientes presentan BU (0.98%).

Diagnóstico por paciente	IgM
EMRR	+
Demencia senil	
Demencia cuerpos de Lewy-EP	
Lesiones sustancia blanca	
Mielitis	
EMRR	+
Mielitis aguda	+
Mielitis transversa	+
Mielitis	
EMRR	+
Enfermedad Creutzfeldt-Jakob	
Ataxia cerebelosa	
Ictus isquémico	
Alteración memoria	
Mielitis	
EMRR	+
Listeria	
Neuropatía óptica bilateral progresiva	
Parkinson	
Cefalea y parálisis facial	

2. Cuatro tienen diagnóstico de EMRR (20%), todos ellos con bandas oligoclonales (BOC) IgM +.

3. Cinco presentan enfermedades inflamatorias del SNC (25%).

Conclusión

1. La frecuencia de BU en nuestra serie es baja (0.98%).
2. En nuestra serie, el 20% de los pacientes presentan diagnóstico de EMRR (BOC IgM+).
3. Informar de la detección de BU podría ser útil en el diagnóstico de pacientes con EM.

P-042

HEPATITIS AUTOINMUNE INDUCIDA POR FÁRMACOS TRAS LA INGESTA DE CÚRCUMA.

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La hepatitis autoinmune (AIH) es una enfermedad crónica del hígado de etiología desconocida. Puede aparecer tras la exposición a tóxicos y en ocasiones es difícil realizar un diagnóstico diferencial con el daño hepático inducido por fármacos inmunomediado (DILI) y con la hepatitis autoinmune inducida por fármacos (DI-AIH). La principal diferencia entre AIH y DI-AIH es la necesidad de tratamiento inmunosupresor a largo plazo en la AIH.
Presentamos el caso de un varón de 28 años admitido en nuestro Hospital por hepatitis aguda grave. Resultados de laboratorio: elevación de transaminasas, bilirrubina y otras enzimas hepáticas. ANA a título 1/160. IgG 823 mg/dL. Presencia del alelo HLA-DRB1*13 de predisposición a AIH tipo I en caucasoides.
El paciente negó consumo habitual de tóxicos, aunque consumía diariamente TURMERIC+ (suplemento a base de cúrcuma, jengibre y piperina). Cinco meses antes, coincidiendo con el inicio de la toma de este suplemento, ya se había detectado elevación de transaminasas.
Se interrumpió su consumo, dada la sospecha inicial de DILI, aunque los parámetros de función hepática empeoraron. La biopsia hepática mostró hallazgos compatibles con AIH. La administración de corticosteroides y posteriormente azatioprina, mejoró los datos bioquímicos, que se estabilizaron a pesar de la interrupción voluntaria del tratamiento inmunosupresor.
La cúrcuma se ha implicado en el origen del daño hepático severo, que se resuelve tras la interrupción de la ingesta. Así mismo, se ha descrito una asociación con el alelo HLA-B*35:01.
En nuestro caso, diferentes criterios permitían llegar a un diagnóstico de AIH probable o de DILI probable. Los hallazgos histológicos y la presencia del alelo HLA-DRB*13 respaldaban el diagnóstico de AIH. Sin embargo, la normalización de la bioquímica hepática a pesar de la suspensión del tratamiento inmunosupresor y el hallazgo del alelo HLA-B*35:01, apoyan el diagnóstico de DI-AIH.

P-043

NEW VASCULITIS ASSOCIATED IMMUNOFLUORESCENCE CYTOPLASMIC PATTERN ON HEP-2

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Background

Indirect immunofluorescence assay (IIFA) on Hep-2 cells is the gold standard technique for autoantibodies detection in the diagnosis of many autoimmune diseases. Here we describe an atypical cytoplasmic Hep-2 IIFA pattern found in two patients with suspected autoimmune disease.

Methods

We performed Hep-2 IIFA using commercial slides. Sera from two patients and positive control with known pattern were diluted 1/160. Monoclonal antibodies against LC-3 (rabbit), SQT-1/p62 (rabbit) and ubiquitine (mouse) were diluted 1/50 and incubated individually or in combination with patients' sera after blocking. Secondary antibodies were diluted 1/500 (anti-human IgG FITC; anti-mouse IgG AF546; anti-rabbit IgG AF555). DAPI was also added. Imaging was done with a confocal fluorescence microscope (SPE; Leica Microsystems).

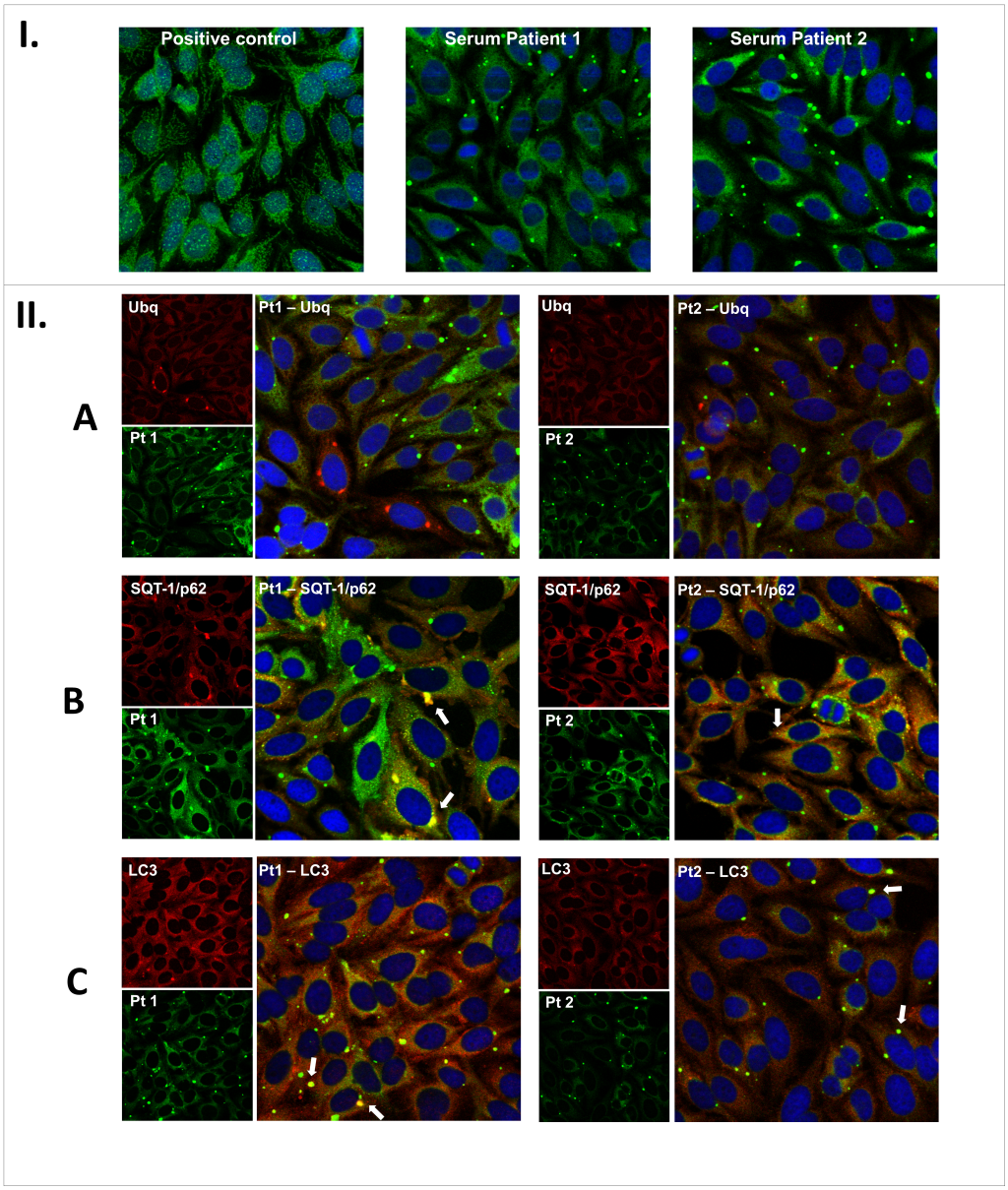
Results

Both patients shared a coarse speckled pattern with big dots that varies in number and size between cells, overlapped with a fine speckled pattern (Figure 1.I.). Fluorescence seemed to be vesicle-related, but different to lysosomes, Golgi and GW-bodies patterns. As autophagy is involved in immune processes and autoimmune disease pathogenesis, we studied if autoantibodies in these patients may be recognizing antigens from the autophagosomes or autophagy pathway (LC-3, SQT-1/p62 and ubiquitine), by co-incubation with patients' sera (Figure 1.II, A-C respectively). Bigger dots and fine splecked pattern observed in serum overlapped partially with SQT-1/p62 and LC-3 (white arrows), but not with ubiquitine. Patient 1 had vasculitis symptoms, but follow-up could not be completed. Patient 2 had small vessel vasculitis with negative ANCA, diagnosed two years after this pattern was found.

Conclusions

Our results show an atypical cytoplasmic pattern that could be associated with autoantibodies against autophagy proteins in the context of small vessel vasculitis, although we could not

conclude the antigen specificity of these autoantibodies. More studies must be done to clarify the role of autophagy in autoimmune diseases.



P-044

COEXISTENCE OF ANTI GABA B RECEPTOR AND ANTI AMPHIPHYSIN ANTIBODIES WITH PARANEOPLASTIC LIMBIC ENCEPHALITIS IN SMALL CELL LUNG CANCER: A CASE REPORT.

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Introduction

Paraneoplastic neurological syndromes are a group of clinical syndromes associated with cancer involving the nervous system, including paraneoplastic limbic encephalitis (PLE).
PLE is an inflammatory process that involves structures at the limbic system, including the hippocampus, hypothalamus and amygdala. This syndrome is an immune-mediated response associated with anti-neuronal antibodies. The mechanism is the cross-reactivity of antibodies to ectopic antigens expressed by tumor cells with antigens of the nervous system. Approximately 50% of patients with anti-GABAB receptor antibodies develop tumors, frequently small cell lung cancer.

Objectives

To report a case of a patient with coexistence of anti AMPHIPHYSIN and anti GABAB receptor antibodies with paraneoplastic limbic encephalitis in small cell lung cancer.

Material and Methods

Anti AMPHIPHYSIN antibodies were determinated using EUROLINE ASSAY and Neurology Mosaics for indirect Immunofluorescence test. Anti GABAB receptor antibodies were determined using a cell-based assay: BIOCHIP KIT of EUROIMMUN AG (Lübeck, Germany) with employs human HEK293 cell expressing recombinant glutamate receptors type NMDA and type AMPA, CASPR2, LGI1, GABA B receptors and DPPX. Cerebrospinal fluid (CF) 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 76-year-old male patient with dysarthria, loss of consciousness, confusion and tonic movements. Laboratory results showed positivity for autoantibodies against

AMPHIPHYSIN at serum dilution of 1/800 (inmunoblot positive +++) and antibodies anti GABAB receptor at serum dilution of 1/400. CF analysis was also positive for both antibodies. The patient was treated with pulses of methylprednisolone and intravenous immunoglobulin. The final diagnosis was small cell lung carcinoma with clinical onset PLE.

Conclusions

The determination antibodies again neuronal antigens provides the clinician with additional information for the diagnosis and treatment of PLE and helps the early diagnosis of lung cancer and other tumors.

Sesión Poster Defensa 1. Pantalla 3. Inmunidad frente a las infecciones + Inmunodeficiencias (I)

P-045

CANDIDA ALBICANS ALTERA EL METABOLISMO Y PROMUEVE UNA RESPUESTA INFLAMATORIA EN CÉLULAS ENDOTELIALES DEL SINUSOIDE HEPÁTICO

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Candida albicans es un hongo patógeno oportunista que puede penetrar en el torrente circulatorio y causar infecciones diseminadas. Para su eliminación, el principal órgano filtrador del ser humano es el hígado. Una vez allí, el hongo es reconocido por las células hepáticas, siendo las células endoteliales del sinusoide hepático (LSECs) unas de las primeras células en interactuar. Puesto que la respuesta de las LSEC frente a este hongo no ha sido aún descrita, el objetivo de este trabajo fue estudiar los mecanismos moleculares implicados en la respuesta de las LSEC tras el contacto con *C. albicans*.

Para ello, las LSECs fueron expuestas a *C. albicans* durante 6 horas y se realizaron análisis de expresión génica por RNA-seq, ensayos ECAR (*Extracellular Acidification Rate*) y medida de la producción de interleucina 6 (IL-6). Se demostró tras el contacto con el hongo un aumento de la expresión de Receptores de Reconocimiento de Patrones (PRRs; Mincle, Dectina-2, TLR-2…), citocinas (IL-6, TNF, CCL-3…) y moléculas de adhesión (VCAM-1, CADM-1), lo que conllevaría la activación de un estado proinflamatorio que implicaría el inicio de una respuesta inmune. Los ensayos también mostraron un aumento en la actividad metabólica a través de un incremento de la glicólisis aerobia (*Eno2*, *Slc1a1*…), así como de la producción de IL-6.

Los resultados obtenidos en el RNA-seq fueron confirmados por análisis de RT-qPCR y mediante el uso de inhibidores específicos de las rutas implicadas en el proceso inflamatorio y en el aumento de metabolismo producido por la presencia de *C. albicans*, estudiando su efecto sobre la producción de IL-6.

En conclusión, estos resultados indican que las LSECs responden frente a la presencia de *C. albicans* aumentando su actividad metabólica y promoviendo un ambiente proinflamatorio a través de la producción de citocinas y moléculas de adhesión.

P-046

DISEASE-CORRECTED HEMATOPOIETIC PROGENITORS FROM APDS2 SYNDROME DERIVED IPS CELLS

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APDS2 syndrome is a primary immunodeficiency disease caused by mutations in PIK3R1 gene. Such genetic alterations affect to immune cells, resulting in an immunologic dysfunction. To date, the only available treatment against APDS2 disease consists in hematopoietic stem cells transplantation, bringing poor efficacy and many adverse effects. For this reason, our aim is to develop novel gene and cell strategies to uncover effective specific-therapies directed to APDS2 patients. For this purpose, we use CRISPR/Cas9 molecular tool for genetic edition to correct the PIK3R1 mutation in the genome of an APDS2-derived iPS cell line generated in our laboratory. Our results shows that our nucleofected CRISPR/Cas9 complex present an efficiency of a 60% without off-target effects or chromosome aberrations. The corrected clones maintain their identity as pluripotent stem cells and are able to differentiate towards the three germ layers more efficiently compared to the APDS2-mutated cells. Furthermore, our clones are able to be driven towards CD34+ hematopoietic progenitors, obtaining functional immune cell precursors from the APDS2 patient. Finally, our corrected clones show a better differentiation potential towards the hematopoietic lineage respect to the mutated cells. Indeed, our hematopoietic progenitors are able to differentiate towards the different types of blood cells.
To this extent, our therapeutic approximation perfectly fits with personalized medicine being

able to obtain a personal source of healthy transfusable components from the APDS2 patient. Taking into account such results, our work represents an innovative therapy that could 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.

P-047

VALIDATION OF CONVENTIONAL AND NOVEL LABORATORY CRITERIA FOR LATE-ONSET COMBINED IMMUNODEFICIENCY

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Background and aims: Preliminary data indicate that a subset of patients with common variable immunodeficiency (CVID) actually suffer from more severe disease, term late-onset combined immunodeficiency (LOCID). Different TCD4+ naïve cell count-based criteria have been proposed in the past to identify patients with LOCID (the DEFI and the Freiburg criteria). However, these criteria have been arbitrary established without considering age-matched reference values for naïve TCD4-cell counts.

Methods: In this multicentric study, 157 CVID patients were studied in parallel to 270 healthy donors using flow cytometry based on the Euroflow standard operation procedures (www.EuroFlow.org).

Results: More LOCID cases were identified within CVID patients when age-matched reference values were used (n=56) as compared to Freiburg (n=35) and DEFI criteria (n=21). The new age-matched criteria was highly efficient for the identification of LOCID among the younger CVID patients (vs. the Freiburg and DEFI criteria): 18-39y (36% *vs.* 23% and 9%; $p<0.01$) and 40-59y (46% *vs.* 28% and 19%; $p<0.001$) CVID age-groups. Frequency and the extension of infections were associated with IgG and IgA serum levels at diagnosis, but presence of non-infectious complications were exclusively found among individuals with low TCD4+ naïve cells counts, independently of the LOCID criteria. However, the new age-matched criteria showed a higher sensitivity for identifying of patients suffering of these complications, including: autoimmune cytopenia (66% *vs.* 41% and 24%), enteropathy (60% *vs.* 31% and 31%) splenomegaly (60% *vs.* 40% and 24%), hepatomegaly (69% *vs.* 42% and 35%), lymphadenopathy (71% *vs.* 49% and 27%), granulomas (70% *vs.* 44% and 25%) and interstitial lung disease (59% *vs.* 47% and 24%) than the Freiburg and DEFI criteria, respectively.

Conclusions: Age-matched reference values for the identification of CVID patients with decreased TCD4+ naïve cell counts translate into a more accurate diagnosis of LOCID, associated with more severe clinical phenotypes.

P-048

CD64 EXPRESSION ON NEUTROPHILS AS A POTENTIAL BIOMARKER FOR BACTERIAL INFECTION IN ASCITIC FLUID

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Introduction

The assessment of CD64 expression on neutrophils surface (CD64Neu) by flow cytometry has been validated as a rapid biomarker for bacterial infections in both peripheral blood and other biological fluids. The immune system produces type II interferon during bacterial infections, which induces CD64Neu. Ascites is a common complication in cirrhotic patients that a variety of factors, including bacterial infections, can cause. Manual counting of polymorphonuclear (PMN) cells in ascitic fluid and microbiologic culture are essential for its diagnosis. Rapidly identifying a possible bacterial infection in this patient would be helpful for therapeutic intervention. We aimed to validate the determination of CD64Neu by flow cytometry in ascitic fluid and assess its potential usefulness in the rapid identification of bacterial infections.

Materials and methods

The expression of CD64 on neutrophils of 77 ascitic fluid samples corresponding to the first episode of hospitalization, recruited from November 2021 to December 2022 in the Immunology Laboratory at Marqués de Valdecilla University Hospital, was analyzed by flow cytometry.

Results

A total of 17 out of 77 (22.1%) samples were diagnosed with bacterial infection by a positive microbiologic culture or PMN count. The median of CD64Neu MFI was significantly increased in the bacterial infection group (3,690.49 MFI [1,635.23-6,521.18] vs. 1,105.90 MFI [737.31-2,048.21], $p<0.001$). The CD64 MFI ratio of granulocytes to lymphocytes was calculated and was elevated in the bacterial infection group (13.06 [6.38-24.58] vs. 5.01 [3.38-7.36], $p<0.001$). A CD64N ratio higher than 9.9 identified those patients with bacterial infection with 70.6 and 86.7% sensitivity and specificity, with an area under the curve (AUC) of 79.4%.

Conclusion

The CD64Neu determined by flow cytometry on ascitic fluid could help quickly identify bacterial infections in ascites patients, allowing early antibiotic treatment.

P-049

REDUCED LEVELS AND FUNCTIONAL ABILITY OF NAÏVE T-LYMPHOCYTES IN ELDERLY PEOPLE MIGHT INFLUENCE THE DEVELOPMENT OF IMMUNOLOGICAL MEMORY AGAINST NEOANTIGENS

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We wanted to study age's influence in the development of immunological memory against neoantigens using the SARS-CoV-2 vaccination as model of a first encounter with an antigen. For this, we analysed 35 elderly donors (ED; age:75.5, IR:6) and 35 young donors (YD; age:40, IR:10). We performed immunophenotypes by flow cytometry, analysed activation (CD25, CD69, HLA-DR) and proliferation (Ki-67) ability of naïve T-lymphocytes in response to anti-CD3 and studied the development of immunological memory against a neoantigen a month after receiving SARS-CoV-2 vaccine. Specific memory T-lymphocytes to SARS-CoV-2 were quantified using IFN-g-Enzyme-Linked ImmunoSpot Assay (ELISpot) after their stimulation with viral peptide pools.

As expected, ED presented higher percentages of highly-differentiated subpopulations effector memory (EM) and EMRA and lower naïve than YD in both, $CD4^+$ and $CD8^+$ T-lymphocytes ($p<0.05$ and $p<0.001$). CD31 expression in naïve $CD4^+$ T-lymphocytes was lower in ED ($p<0.001$). We also found lower absolute numbers of naïve B-lymphocytes in ED ($p<0.05$). Activation and proliferation ability of naïve T-lymphocytes was studied. Naïve $CD4^+$ T-lymphocytes expressed lower CD25 and CD69 ($p<0.05$) and naïve $CD8^+$ T-lymphocytes expressed lower CD25 ($p<0.05$) in ED. Reduced percentages of $Ki\text{-}67^+$ cells were found in both, naïve $CD4^+$ and $CD8^+$ T-lymphocytes, in ED ($p<0.001$). We observed that naïve $CD8^+$/EMRA $CD8^+$ ratio, but not naïve $CD4^+$/EMRA $CD4^+$ ratio, was positively correlated with the $Ki\text{-}67^+$ cells percentage in $CD4^+$, naïve $CD4^+$, $CD8^+$ and naïve $CD8^+$ T-lymphocytes ($p<0.01$, $p<0.05$, $p<0.001$ and $p<0.001$, respectively).

ED presented more SARS-CoV-2 non-responders than YD (29/35 vs 34/35) and lower number of specific IFN-g-producing T-lymphocytes/10^6PBMC ($p<0.05$). No relationship was found between lymphocyte subpopulations and response to vaccination.

In summary, ED had a highly-differentiated immune system and a defective functionality of naïve T-lymphocytes, with lower level of activation and proliferation compared to YD, and this might negatively influence in the development of immunological memory against neoantigens.

P-050

CCR6 AND CCR9 EXPRESSION DEFINES A POPULATION OF SARS COV-2 SPECIFIC REGULATORY T LYMPHOCYTES WITH PATHOGENIC POTENTIAL

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Chemokine receptors regulate immune cell migration in many situations, including autoimmune**,** inflammatory and infectious diseases. GWAS studies have identified genetic loci, including those for CCR9 and CCR6, associated with worse prognosis in COVID-19 infection. CCR9 associates with worse outcome especially when expressed in TReg. There are few studies about SARS CoV-2-specific Treg, so it is very relevant to analyze in depth phenotypic and functional characteristics of this immune subset.

We found an increased percentage of CD25+CD127low TReg population within SARS-CoV-2-specific CD4+ lymphocytes. Furthermore, these cells expressed higher levels of CCR9 and CCR6 than non-specific ones. TReg functional profile was assessed through the analysis of TNFa, IFNg, IL-10, IL-17 and IL-4 by flow cytometry, in both SARS-CoV-2-specific and non-specific cells. Overall, cytokine profile was similar in the two populations analyzed, with the exception of IL-17, whose levels were selectively higher in SARS-CoV-2 specific TReg cells. The analysis of Foxp3 expression within CD127lowCD25+ lymphocytes showed a remarkable variability in SARS-CoV-2-specific CD127lowCD25+ cells, which was not observed in their non-specific counterparts. CCR9 expression directly correlated with that of FoxP3 and IL-10, while CCR6 was associated with increased IL-17 and decreased FoxP3 levels. Accordingly, higher Foxp3 and CCR9 expression and lower CCR6 levels correlated with increased regulatory potential in SARS-CoV-2-specific TReg population. Of note, CCR6/CCR9 ratio was significantly higher in critical COVID-19 patients, thus suggesting a pathogenic role of CCR6-expressing specific TReg cells in the disease.

In summary, SARS-CoV-2-specific CD4+ T lymphocytes are enriched in TReg cells with increased levels of CCR9 and CCR6 chemokine receptors. CCR9 and CCR6 expression defines TReg subpopulations with differential functional characteristics that may play a pathogenic role in COVID-19.

P-051

ROLE OF TAP AND THE PROTEASOME IN CYTOMEGALOVIRUS ANTIGEN PRESENTATION

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$CD8^+$ T lymphocytes selectively detect and kill infected cells by the recognition of viral epitopes presented in the cell surface. Viral peptides in the surface of infected cells are mainly presented by the classical antigen presentation pathway, although presence of alternative antigen presentation pathways has been recently proved in some viral infections. Understanding these alternative mechanisms is relevant in future vaccine design since certain viruses can affect and alter the function of key elements of antigen presentation pathway to avoid immune system control. Thus, the goal of this work was to study alternative antigen presentation pathways during an infection by murine cytomegalovirus (MCMV), specifically in the absence of the transporter associated with antigen processing (TAP) and the proteasome. First, we observed just a partial role of TAP in the control of an MCMV infection, since despite a small delay TAP-deficient mice efficiently controlled the virus. Moreover, we found a limited ability of MCMV-infected TAP-deficient cells to present viral antigens *in vitro*, although we identified an MCMV epitope that was presented *in vitro* in the absence of TAP and we determined the relevance of the presentation of this epitope *in vivo*. Finally, we found a partial resistance of MCMV antigen presentation to the inhibition of the proteasome and importantly we demonstrated that proteasome activity was necessary to the presentation of the TAP-independent epitope. Therefore, our data showed that although relevant, TAP and the proteasome are not required in MCMV antigen presentation and proved the presence of alternative antigen presentation pathways during an MCMV infection.

P-052

HIV INFECTION INDUCES THE DEVELOPMENT OF NEUTRALIZATION-INTERFERING ANTIBODIES THAT HAMPER THE FUNCTION OF NEUTRALIZING ANTIBODIES.

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Background: The human immunodeficiency virus (HIV) induces a complex humoral response marked by the coexistence of neutralizing (NAbs) and non-neutralizing antibodies (non-NAbs). Although NAbs have been well characterized, little is known about the role of non-NAbs during infection. Non-NAbs can mediate anti-viral Fc-effector functions, such as antibody-dependent cellular cytotoxicity (ADCC) or antibody-dependent cellular phagocytosis (ADCP). However, they can also interfere with the action of NAbs by competing with them for binding to the Envelope glycoprotein (Env). Therefore, we aimed to determine whether HIV infection promotes the generation of neutralization-interfering antibodies (NiAbs).
Methods: Poor neutralizing plasma samples from chronically viremic HIV-infected (CHI) individuals were selected. The presence of NiAbs in these samples was determined by ELISA, flow cytometry (FACS) and neutralization competition assays. Recombinant Env-specific antibodies were generated by RT-PCR from single memory B cell microcultures or from single Env-specific memory B cells isolated by FACS.
Results: Plasma IgGs from CHI individuals were able to block the binding of NAbs to HIV Env, reducing its neutralizing capacity. Fifteen Env-specific recombinant monoclonal antibodies were isolated from a CHI individual. These antibodies were clonally unrelated, and showed limited or non-neutralizing capacity (3/15 and 12/15, respectively). Among non-NAbs, five recognized gp41 and seven targeted gp120. Three anti-gp120 non-NAbs were identified as NiAbs as they competed with NAbs for binding to Env, reducing their neutralizing activity.
Conclusions: HIV infection elicits NiAbs, which may be used by the virus as an immune escape mechanism to hamper the development and function of NAbs. The identification of Env regions inducing NiAbs might be crucial for the design of a successful HIV vaccine.

P-053

DESCRIPTION OF THREE DIFFERENT *DE NOVO* AD GOF MUTATIONS IN STAT1 RESPONSIBLE FOR CMC IN ECUADORIANS

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Several inborn errors of immunity (IEI) stem from different signal transducer and activator of transcription 1 (STAT1) alterations. STAT1 is a cytoplasmic transcription factor that participates in interferon responses through the JAK-STAT1 pathway. Hence, we focused on AD GOF mutations in STAT1 that impair the development of IL-17-producing T cells (T_H17), leading to Chronic Mucocutaneous Candidiasis (CMC). CMC is characterized by recurrent or permanent infections of the skin, nails, and oral or genital mucosa by *Candida* species, predominantly *Candida albicans*.

We describe three non-related Ecuadorian girls, from non-consanguineous families, P1, P2, and P3, with different heterozygous *de novo* STAT1 AD GOF mutations (STAT1 c.511G>A [p.Asp171Asn], STAT1 c.1154C>T [p.Thr385Met], and STAT1 c.1162A>G [p.Lys388Glu], respectively). They all displayed several *Candida* infections during infancy affecting mainly the oral cavity. Nonetheless, the infections occasionally spread towards the gingiva (P1), amygdala (P2), oropharynx, or eyes (P3). Currently, infections are controlled with Fluconazole. Also, the three patients experienced non-infectious dermatological manifestations. All of them suffered repeated viral and bacterial respiratory infections, which eventually required hospitalization. Remarkably, at six years old, P1 was infected with *Mycobacterium tuberculosis*. Regarding laboratory tests, P1 showed lymphopenia, thrombocytosis, high ESR, and increased IgG3 and IgA levels. Neither abnormal lymphocyte populations nor anomalous Ig levels were observed for P2. P3 showed neutropenia, thrombocytosis, and, in contrast with P1, lymphocytosis with normal subpopulations.

The three mutations were identified by an extensive clinical exome panel analysis for IEI and

were validated by Sanger sequencing. Asp171Asn is present in STAT1 exon 7 and affects the coiled-coil domain (CCD). Alternatively, Thr385Met and Lys388Glu are displayed in exon 14, thus, both struck the DNA-binding domain (DBD). These mutations clearly correlated with previously described symptomatology and clinical phenotype for CMC. However, the case of CCD mutation presented with lymphopenia, which is probably linked with a more severe presentation.

P-054

SECUENCIACIÓN MASIVA DE NUEVA GENERACIÓN EN EL DIAGNÓSTICO DE ERRORES CONGÉNITOS DE LA INMUNIDAD EN EL PACIENTE ADULTO.

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Introducción: Los errores congénitos de la inmunidad (ECI), abarcan un conjunto de patologías caracterizadas por el aberrante funcionamiento de uno o varios componentes del sistema inmune. Debido a que la mayoría de estas enfermedades son producidas por una alteración genética, su aparición y diagnóstico suele darse en las primeras etapas de la vida. A pesar de ello, algunos pacientes pueden manifestar discordancia genotipo-fenotipo, lo que puede provocar que la sospecha diagnóstica se realice en la edad adulta. Una de las razones del aumento de casos diagnosticados se debe a la incorporación de la secuenciación masiva de nueva generación (NGS) en los algoritmos diagnósticos.
En este estudio exponemos la experiencia recopilada en el uso de la NGS cómo herramienta diagnóstica de ECI en pacientes adultos.
Metodología: Se ha realizado un análisis retrospectivo de pacientes secuenciados mediante NGS con sospecha de ECI entre 2015 y 2023 (Panel de 192 genes entre 2015-2021 y panel de 434 genes entre 2022-2023). Del total de pacientes analizados (n=302) se descartaron los menores de 18 años y aquellos sin una alteración monogénica definida.
Resultados: De los 302 pacientes estudiados a través de NGS, se hallaron, en 69 de ellos, variantes patogénicas (22.8%). Finalmente, 15 adultos fueron diagnosticados de ECI (5%). Los genes en los que se determinó la presencia de variantes patogénicas productoras de ECI en pacientes mayores de 18 años fueron *ADA2*, *C8B*, *CD8A*, *CTLA4*, *MAGT1*, *NFKB1*, *PGM3*, *PIK3R1*, *RAB27A*, *TAP1* y *TET2*.
Conclusión: La incorporación de la NGS como herramienta diagnóstica en enfermedades tan heterogéneas como los ECI es esencial a nivel asistencial en un servicio de inmunología. Los datos que se presentan también demuestran la utilidad de la NGS en el paciente adulto.

P-055

LONGITUDINAL STUDY OF ADAPTIVE NK CELLS IN CMV-SEROCONVERTED HEALTHY DONORS

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Cytomegalovirus (CMV) infection is one of the most prevalent viral infections in the population with a global incidence of 56 to 94%. It is one of the most common infections in immunocompromised individuals and can be very serious, while in healthy individuals it is asymptomatic.

The involvement of innate immunity and, particularly, of natural killer (NK) and γ/δ T cells in the primary line of defense against primary CMV infection, has been broadly reported. Specifically, a stable expansion of adaptive CD94/NKG2C+ (hereafter NKG2C+) NK cells with memory-like properties is observed after the infection and/or reactivation, for example, in immunosuppressed patients undergoing kidney transplantation.

The aim of this project is to study the circulating NK cell pool before and after CMV infection in healthy people in order to describe the kinetics of the adaptive NK cell expansion.

We have enrolled a cohort of healthy donors (n=9) before and after seroconversion. These individuals are not apparently immunocompromised, therefore our results do not have this bias, which is the limitation we usually encounter in studies aimed to analyze the kinetics of adaptive NK cells expansion in response to CMV. Eight samples from each donor were obtained at different times (3 months between samples), four of which were pre-seroconversion and four were post-seroconversion. A group of non-seroconverted donors (n=15) was also studied to compare the variability of the frequency of adaptive NK cells over time. Phenotypic characterization of NK cell subsets analyzing the expression of surface receptors (CD59, CD3, CD14, CD19, CD123, HLA-DR, CD2, NKp80, NKG2C, CD7, CD57, CD161, KIR3DL1, KIR2DL2/L3/S2, KIR2DS4, NKG2A, KIR2DL1) and intracellular molecules (FcεRγ, syk,

PLZF), was performed by flow cytometry.

In accordance with the literature, preliminary analysis revealed an expansion of adaptive NKG2C+ NK cells in donors following seroconversion. Supervised and unsupervised analysis are ongoing.

P-056

HIV-SPECIFIC CD4$^+$ AND CD8$^+$ T CELL RESPONSES AND MARKERS OF CELL EXHAUSTION IN PEOPLE WITH HIV AFTER SWITCHING TO DUAL THERAPY VS. MAINTAINING TRIPLE THERAPY

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Background

Triple therapy (TT) has been considered the gold standard for antiretroviral treatment (ART) for years. However, dual therapy (DT) has shown to have comparable virological efficacy to TT in virologically suppressed and naïve-treatment people living with HIV (PLWH). Moreover, similar effect on immune recovery, immune activation and inflammation, and HIV reservoir has been observed. Even so, the effect of ART simplification on HIV-specific T-cell response remains unknown.

Our aim was to evaluate whether HIV-specific T-cell response and markers of cell exhaustion are affected by simplifying DT versus maintaining TT after two years of follow-up.

Methods

T-cell specific response was measured as the frequency of cells expressing intracellular cytokines after stimulation with HIV (Gag)-specific peptide pool, normalized with the unstimulated condition. To classify an individual as responder, this expression must be higher than 0.001 at least in one cytokine. Polyfunctionality was defined as the capacity of the cells to express intracellular cytokines (IL-2, TNF-α, and IFN-γ), the degranulation markers CD107a and perforin (PRF) simultaneously. Total CA-DNA and usRNA were quantified by droplet digital PCR (ddPCR).

Results

Forty participants matched by sex and age were selected (20 per group, TT and DT). HIV-specific memory CD4+ and CD8+ T cells showed a higher level of CD107a expression in TT than DT. However, we did not find differences in the quality of those responses measured as the polyfunctionality index, and the cytokines and degranulation markers combinations. Regarding the exhausted phenotype, we observed a decrease in PD1 and TIM3, as well as in several combinations, in memory CD4+ and CD8+ T cells during the follow-up, without finding

significant differences between treatments.

Conclusions

Our data show that ART simplification to DT seems not to have an impact on the HIV-specific T-cell response nor the cell exhaustion after two years of follow-up.

P-057

PATIENTS WITH END STAGE RENAL DISEASE SHOW REDUCED ABILITY TO RESPOND AGAINST BOTH NEW AND RECALL ANTIGENS

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Patients with End Stage Renal Disease (ESRD) show immunosenescence secondary to the renal dysfunction. To analyse the relationship between this process and the ability to respond to new or recall antigens we studied the cellular memory generated against both the SARS-CoV-2 new vaccine, and the seasonal Influenza vaccine (InV).

We analysed 48 ESRD patients on dialysis (ESRD; age: 67,3 ± 14,9) and 51 healthy donors (HD; age: 68,7 ± 13,8). Donors were studied a month after receiving the first complete scheme of RNA-based SARS-CoV-2 vaccine, and previously (eleven months after InV season 19-20: InV-t0) and one month after receiving InV season 20-21 (InV-t1). Differentiated degree of $CD4^+$ and $CD8^+$ T lymphocyte subpopulations were evaluated by flow cytometry in peripheral blood (CD45RA/CCR7). Specific memory T-lymphocytes to SARS-CoV-2 and Influenza vaccines were quantifying using IFN-γ-Enzyme-Linked ImmunoSpot Assay (ELISpot).

ESRD patients presented higher percentages of highly differentiated subpopulations effector memory (EM) and EMRA and lower naïve than HD in both, $CD4^+$ and $CD8^+$ T lymphocytes ($p<0.001$). We found significant differences between ESRD and HD in the cellular immune responses against both SARS-CoV-2 vaccine ($p<0.001$) and InV-t1 ($p<0.001$), nevertheless there were not significant differences between of ESRD and HD in InV-t0. The immune response in ESRD patients against the SARS-CoV-2 vaccine correlated positively with naïve and negatively with EM $CD4^+$ T lymphocytes, while we found a negative correlation between InV-t0 and EMRA $CD4^+$ T lymphocytes ($p<0.05$). We did not find any association between memory to InV-t1 and T lymphocyte subpopulations.

Our results support that immunosenescence in patients with ESRD may influence the ability to generate immune responses against both new and recall antigens. These two kinds of responses are related to differentiation degree of T lymphocyte subpopulations.

P-058

HIV IMMUNE CLUSTERS AS A POTENTIAL TOOL FOR STRATIFICATION TO TAILOR INTERVENTIONS BASED ON THEIR CLINICAL NEEDS.

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Hospital Universitario Germans Trias i Pujol, Badalona.

Background

The body of evidence in HIV research has focused on CD4 lymphocytes and CD4/CD8 ratio, but other actors of the immune system have gained relevance due to their role in the chronic immune activation and inflammation. The identification of different profiles of HIV patients according to lymphocyte subpopulations and clinical correlations will allow tailoring interventions and care based on their needs.

Objective: To identify, describe and quantify different clusters of HIV patients based on immune subpopulations.

Methodology: Transversal study of HIV patients attended at HUGTP between 2020 and 2022. Inclusion criteria: at least three determinations of CD4 and CD8 subpopulations by flow cytometry, and a stable ratio CD4/CD8 (CV <25%). A total of 2364 HIV patients were enrolled. Cluster analysis was performed using R statistical packages.

Results:

Five clusters have been identified based on their immunological pattern.

- Cluster 1, 2 and 3 represent 60% of patients. All of them showed CD3+CD4+ cells counts >500 cells/mm3. Cluster 1 (21.1%) had CD4/CD8 ratio<1, having not achieved a complete recovery of their TCD4. Cluster 2 (27.1%) and 3 (12.4%), proved ratio >1, but Cluster 3 showed higher lymphocyte cells counts (3,11x109cells/L) than Cluster 2 (2,25x109cells/L). In both groups, CD4+ cells counts were >700 cells/mm3.

- Cluster 4 represents 33,7% of patients. It showed CD4 cells counts below 500 cells/mm3, CD4/CD8 ratio ≈ 1 and high counts of CD3-CD8+ cells.

- Cluster 5 represents 5,7% of patients, with CD8 cells counts >1500 cells/ mm3, CD4 counts >700 cells/ mm3 and CD4/CD8 ratio <0,5.

Conclusion:

According to T cell subpopulations, five different HIV profiles have been identified. Further analysis of clinical correlations from each cluster will be performed to define their relevance as a stratification tool.

P-059

DIAGNOSIS OF SEVERE COMBINED INMUNODEFICIENCY (SCID) CAUSED BY A NOVO VARIANT IN IL2GR GENE.

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Introduction

SCID encompasses a group of heterogeneous genetic disorders characterized by weakened cellular and humoral immunity.

Objetive

A 3-month-old infant with no family history is monitorized at the pediatric unit for severe growth retardation since the first month of life. Being hospitalized several times for mild bronchiolitis by Rinovirus and Adenovirus, pneumonia by Pneumocystis Jirovecii and gastrointestinal tract infection by Rotavirus and Astrovirus. The radiological study showed thymic hypoplasia.

Results

The inmunological study revealed lymphopenia, accompanied by low T and NK cells as well as elevated γδ and activated T lymphocytes with absence of surface CD25. Recent thymic emigrants (RTEs) were below the normal range for his age and switched memory B cells and T-cell receptor excision circles (TRECS) displayed a value of 0, along with a defect in T cell proliferation in response to mitogens.

Lymphocyte subpopulations		
Total lymphocyte	240 (cells/µL)	(3000 - 13.500)
CD3+ cells	92% 221 (cells/µL)	(51,80 - 74,20) (2.284 - 4.776)
CD4+ cells	32% 77 (cells/µL)	(34,90 - 53,10) (2.284 - 4.776)
CD8+ cells	39% 94 (cells/µL)	(12,80 - 27,10) (524 - 1.583)
NK cells	0,07% 17 (cells/µL)	(4,0 - 15,10) (230 - 801)
B cells	7,93% 19 (cells/µL)	(17,0 - 37,20) (776 – 2.238)
γδ cells	21%	> 15%
(CD4+ CD45RA+ CD31+) cells	2,85%	(55,7 – 76,7)
(CD3+ DR+) cells	99%	

As these data suggested severe combined immunodeficiency, a genetic study was requested. The genetic results showed that the patient presents a probably pathogenic variant NM_000206.2:c 2T> C p.(Met1?) in IL2RG gene in hemizygosis. Both parents are not carriers of this variant, pointing to the presence of "de *novo* variant".

Conclusions

Based on the clinical and immunological data and the genetic results, the patient was diagnosed with X-linked severe combined immunodeficiency (X-SCID) and underwent hematopoietic stem cell transplantation (HSCT) with umbilical cord blood.

P-060

INMUNODEFICIENCIA COMBINADA GRAVE EN LA REGIÓN DE MURCIA ENTRE 2010 Y 2020

González López, Rosana; López Martínez, María Concepción; Las Heras Ferre, Beatriz; Jiménez Coll, Víctor; Soto Ramírez, María Fernanda; Sánchez Rodríguez, Carlos; Fernández González, Marina; Moya Quiles, María Rosa; Salgado Cecilia, Gema; Montes Ares, Olga; Muro Amador, Manuel.

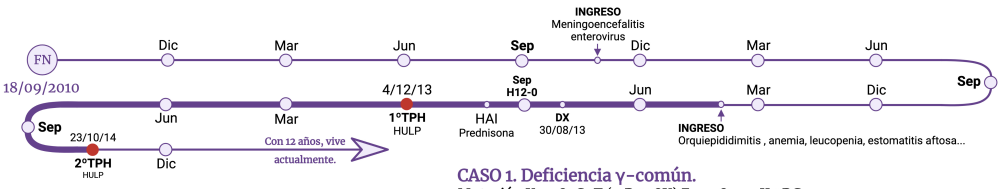
Hospital Clínico Universitario Virgen de la Arrixaca, Murcia.

La inmunodeficiencia combinada grave (IDCG) representa la forma más grave de las inmunodeficiencias primarias. La incidencia mundial es de 1:50.000-100.000 nacidos vivos, con diferencias regionales. En la Comunidad Catalana, pionera en España en el acceso al cribado neonatal mediante la cuantificación de TREC, se ha comunicado una incidencia de 1:74.285 entre 2017 y 2020. En el Hospital Universitario Virgen de la Arrixaca, centro de referencia para el diagnóstico de inmunodeficiencias en la Región de Murcia, la incidencia entre 2010 y 2020 fue de 1:34.756. En este periodo, se diagnosticaron cinco pacientes: dos casos de deficiencia de cadena común gamma, dos de deficiencia de HLA clase II (RFXAP y CIITA), y un caso de deficiencia de RAG1. El inicio de la clínica se produjo entre los 2 y 6 meses de vida, con un retraso diagnóstico de entre 3 meses a 3 años. Tres casos debutaron con infección grave con ingreso en UCI. Cuatro pacientes se sometieron a TPH (trasplante de progenitores hematopoyéticos) tras un periodo de 3 a 11 meses tras el diagnóstico. Hasta la fecha actual, sólo uno de los cinco pacientes ha sobrevivido tras un segundo TPH. Los pacientes restantes fallecieron a una edad comprendida entre 7 meses y 2 años. Actualmente en esta región al no haberse implantado aún el cribado neonatal, la clínica principalmente infecciosa es la única "vía" para el diagnóstico y posterior tratamiento, a pesar de la suficientemente demostrada superioridad en efectividad de un tratamiento precoz en el periodo de latencia previo a debut clínico.

El último informe de evaluación de tecnologías sanitarias (2020) estableció que el desconocimiento de la incidencia nacional es una de las limitaciones para la inclusión del cribado neonatal de IDCG. Es necesario establecer una incidencia real y una re-evaluación de recursos actuales en cada una de las comunidades autónomas.

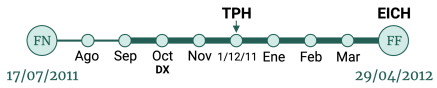
Evolución temporal de los casos de IDCG nacidos en la Región de Murcia entre 2010 y 2020

Hospital Clínico Universitario Virgen de la Arrixaca- Murcia



CASO 1. Deficiencia γ-común.

Mutación X c.982C>T (p.R.328X) Exon 8 gen IL2RG



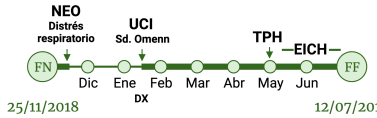
CASO 2. Deficiencia γ-común.

Mutación X 2T>C (p.MIT) Exon 1 gen IL2RG CD132-



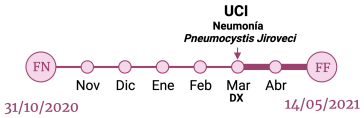
CASO 3. Deficiencia de HLA de clase II.

Homocigota con una variante que afecta al splicing del gen RFXAP.
NM_000538: exon 2: c.601-2A>T



CASO 4. Síndrome de Omenn.

Homocigoto con dos variantes RAG1 frameshift:
-NM_000448:exon2:c.519delT
-NM_000448:exon2:c.1184C>T



CASO 5. Deficiencia de HLA de clase II.

Homocigota con una variante tipo frameshift en el gen CIITA:
NM_001286402.1:C715_716insC;NM_001273331.1:p.His239Profs*8
Ambos progenitores portadores en heterocigosis.

P-061

COMPARATIVA DE LA RESPUESTA INMUNE ESPECÍFICA TRAS PRIMO-INFECCIÓN POR SARS-COV-2 EN NIÑOS Y ADULTOS JÓVENES NO VACUNADOS CON Y SIN ERRORES CONGÉNITOS DE LA INMUNIDAD

García García, Ana[1]; Fortuny, Claudia[1]; Fumadó, Victoria[1]; Jordan, Iolanda[1]; Ruiz, Laura[1]; González Navarro, Europa Azucena[2]; Egri, Natalia[2]; Esteve, Ana[1]; Luo, Yiyi[1]; Vlagea, Alexandru[2]; Monsonís, Manel[1]; Launes, Cristian[1]; Soler, Aleix[1]; Codina, Anna[1]; Juan, Manel[2]; Pascal, Mariona[2]; Deyà, Àngela[1]; Alsina, Laia[1].

[1]Hospital Sant Joan de Déu, Esplugues de Llobregat, Barcelona; [2]Hospital Clínic de Barcelona, Barcelona.

Objetivos: Comparar la respuesta inmune específica humoral y celular aguda y a largo plazo de pacientes pediátricos y adultos jóvenes tras la infección por SARS-CoV-2 con errores congénitos de la inmunidad(ECI) vs. controles sanos(CS) pareados.

Métodos: Pacientes con ECI y CS primo-infectados por SARS-CoV-2, no vacunados, hasta 22 años de edad: se analizan respuesta humoral (inmunoglobulinas-Ig- G,M,A frente a la región del receptor binding-domain de la proteína espícula) y respuesta celular T (ELISpot S y N) específicas a SARS-CoV-2 en fase aguda (hasta 6 semanas post-infección) a los 3, 6, 9, 12 meses.

Resultados: 25 ECI (mediana edad 14,3 años, mín.-máx. 4,5-22,8; 15/25 varones, 4/25 neumonía COVID-19; 48% inmunodeficiencias combinadas asociadas a síndromes, 16% defectos de anticuerpos), vs. 17 CS (sin diferencias en sexo, edad ni gravedad de la infección). Se muestran únicamente resultados con diferencias significativas. Fase aguda: Ig específicas positivas en todos los CS vs. ECI: IgG^+ 58,8%(10/17); IgM^+ 41,2%(7/17); IgA^+ 52,9%(9/17); respuesta cuantitativa humoral (índice medio) menor en ECI: IgG ECI(3,1±4,4) vs. CS(3,5±1,5), IgM ECI(1,9±2,4) vs. CS(3,9±2,4), IgA ECI(3,3±4,7) vs. CS(4,6±2,5); ELISpot positivos, cualitativamente (S-ELISpot ECI: 3/11, 27,3% vs. CS: 10/11, 90,9%; N-ELISpot ECI: 3/9, 33,3% vs. CS: 11/11, 100%) y cuantitativamente (índice medio) menor en ECI (S-ELISpot ECI: 3,2±5,0 vs. CS 21,2±17,0; N-ELISpot ECI: 9,3±16,6 vs. CS: 39,1±23,7). Respuesta a largo plazo: SARS-CoV-2-IgM^+ a los 6 meses cualitativamente (3/8, 37,5% vs. 9/10 CS: 90,0%), y cualitativamente inferior en ECI en IgG, IgM, e IgA (ECI n=9, 1,1±0,9 vs. CS n=10, 2,1±0,9; ECI n=9, 1,3±1,5 vs. CS n=10, 2,9±2,8; ECI n=9, 0,6±0,5 vs. CS n=10, 1,7±0,8, respectivamente).

Conclusiones: Esta cohorte pediátrica de pacientes con ECI, comparada con CS, mostró menor

respuesta humoral y celular, cualitativa y cuantitativamente, tras la fase aguda de la infección; a los 6 meses post-infección, la respuesta humoral fue menor.

P-062

NEUMONÍA GRAVE POR COVID-19 EN SÍNDROME DE GOOD: EXPERIENCIA DE UN CASO

Eguía Núñez, Jorge[1]; Cos Esquis, María Lourdes[2]; López Montesinos, Inmaculada[2]; Gimeno Martínez, Ramón[2]; Caballero Rabasco, M. Araceli[2]; Sánchez González, Blanca[2]; López García, Alicia[2]; Mellibovsky, Leonardo[2].

[1]Laboratori de Referència de Catalunya (dibi), El Prat de Llobregat; [2]Hospital del Mar, Barcelona.

El síndrome de Good es una enfermedad rara consistente en la asociación de timoma e hipogammaglobulinemia con cociente CD4/CD8 invertido. La fisiopatología es desconocida y pueden estar implicados factores que afectan a la maduración de linfocitos T y B. Las manifestaciones pueden estar ausentes o producir una inmunodeficiencia con o sin proceso autoinmune.

Se informa un caso de una mujer de 79 años con diagnóstico de síndrome de Good, en tratamiento con inmunoglobulinas subcutáneas, y que presentó neumonía grave por COVID-19 con evolución favorable.

La paciente fue ingresada en el Hospital del Mar para biopsia ganglionar durante la pandemia COVID-19, detectándose una PCR de rutina positiva. A los 5 días del ingreso, inició sintomatología compatible con infección por SARS-CoV-2. Inicialmente, la radiografía de tórax no mostró neumonía, pero progresó a síndrome de dificultad respiratoria aguda confirmándose una neumonía bilateral en la radiografía de tórax a los 12 días. La citometría de flujo mostró un porcentaje de linfocitos B dentro de la normalidad, con diferenciación interrumpida y poblaciones de memoria residual, incremento de linfocitos T gammadelta+, una inversión del cociente CD4/CD8 y un predominio de las células de memoria. La paciente recibió tratamiento con corticoides y Tocilizumab. A los 16 días la PCR fue negativa y los anticuerpos anti-spike fueron positivos. La inmunidad celular fue negativa en el día 7 y positiva en el día 30. La paciente fue dada de alta a los 22 días.

Se trata del primer caso reportado de un paciente son síndrome de Good con COVID-19 grave que sobrevivió. Existe controversia sobre cómo la infección por SARS-CoV-2 afecta a los pacientes con deficiencia de anticuerpos. Nuestra hipótesis, es que mantuvo las células implicadas en la inmunidad humoral y celular durante la infección. En conclusión, el conocimiento previo de los linfocitos B y T podría ser útil para anticipar el curso de la enfermedad infecciosa.

P-063

HIPOGAMMAGLOBULINEMIA, TROMBOCITOPENIA Y LINFOCITOPENIA GRAVES GESTACIONALES EN PACIENTE CON INMUNODEFICIENCIA VARIABLE COMÚN. UN AGUJERO NEGRO DE LA INMUNOLOGÍA A EXPLORAR.

Mansilla Ruiz, María Dolores; Villegas Mendiola, Ángela; Palacios Ortega, María; De La Fuente Muñoz, Eduardo; Mohamed Mohamed, Kauzar; Guerra Galán, Teresa; Pereiro Rodríguez, Alejandro; García Bravo, Laura; López Uceda, Bárbara; Fernández Arquero, Miguel; Gúzman Fulgencio, María; Sánchez Ramón, Silvia.

Hospital Clínico San Carlos, Madrid.

La hipogammaglobulinemia y el déficit de producción de anticuerpos son dos de los criterios inmunológicos característicos de la inmunodeficiencia variable común (IDVC), cuyas manifestaciones clínicas son heterogéneas con predominio de infecciones recurrentes y citopenias periféricas. Se sabe que la gestación en la IDVC puede complicar las citopenias periféricas, sin existir marcadores de riesgo. Se describe el caso de una paciente de 35 años, con diagnóstico de IDVC en seguimiento por nuestro Servicio y en terapia con gammaglobulina intravenosa, presentando buena evolución clínica y analítica, por lo que inicia terapia subcutánea. Primera gestación en abril de 2022, presentando un descenso progresivo de IgG, que se agrava durante el tercer trimestre (IgG de 165 mg/d), pese a cambio a terapia intravenosa quincenal a dosis altas, asociado a trombocitopenia y linfocitopenia progresivas y refractarias a tratamiento con corticoides y GGIV a dosis altas (2g/kg/mes). Una vez descartadas causas secundarias de hipogammaglobulinemia, se plantea la hipótesis de un posible “secuestro” de IgG a nivel placentario, en paciente con buena respuesta a la terapia tanto en perfusión intravenosa como subcutánea previo a la gestación. Se realizaron determinaciones seriadas de IgG en suero, linfocitos y plaquetas durante el embarazo y tras el parto, al neonato y en la muestra de placenta. Los valores valle de la paciente, previos a la gestación eran alrededor de 1.000 mg/dl, durante la gestación, disminuyen hasta valores alrededor de 200 mg/dl; 48 horas previas al parto, se administra dosis de gammaglobulina, con medición de valores inmediatamente posteriores, de 1.950 mg/dl; 48 horas postparto, mantiene valor de 1.400 mg/dl y una semana después del parto, mantiene valor de 1.200 mg/dl. Este caso clínico pone de relevancia el papel inmunoactivo de la placenta y las complicaciones derivadas de su patología de base como posible factor predisponente a las mismas.

P-064

MICRORNA SEQUENCING ANALYSIS OF ILEOCECAL VALVE AND PERIPHERAL BLOOD FROM HOLSTEIN CATTLE WITH DISTINCT PARATUBERCULOSIS LESIONS IN GUT TISSUES

Badia Bringué, Gerard[1]; Canive Ruiz, María[1]; Torremocha, Rosana[2]; Blanco Vázquez, Cristina[3]; Ramos, Ricardo[2]; Casais, Rosa[3]; Alonso-Hearn, Marta[1].

[1]Department of Animal Health, NEIKER- Basque Institute for Agricultural Research and Development, Basque Research and Technology Alliance (BRTA), Derio; [2]Genomic Unit, Scientific Park of Madrid, Madrid; [3]Center of Animal Biotechnology, SERIDA-Regional Service of Agri-Food Research and Development, Deva.

MicroRNAs regulate the post-transcriptional expression of genes implicated in the immune responses against pathogens by binding to their target mRNAs. In this study, whole RNA-Sequencing (RNA-Seq) was used to compare microRNAs expression in ileocecal valve (ICV) and peripheral blood (PB) samples from Holstein cows (N=14) with focal or diffuse paratuberculosis (PTB)-associated lesions in gut tissues versus control cows without any evidence of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection. Our results showed PB and ICV-specific microRNAs expression. As expected, the number of differentially expressed microRNAs was larger in the ICV that in the PB samples. In the ICV samples, we identified 4, 5, and 18 microRNAs differentially expressed in cows with focal lesions versus vs. controls, with diffuse lesions vs. controls, and with diffuse vs. focal lesions, respectively. Among the 8 microRNAs differentially expressed in the PB samples from cows with focal lesions vs controls, 3 were also down-regulated in cows with diffuse lesions (miR-19a, miR-144, miR32). miR-2425-3p and miR-139 were significantly upregulated in PB samples from cows with focal lesions when compared with control cows, which suggests that they could be used as biomarkers of early MAP infection. The changes of expression in 5 of the microRNAs differentially expressed in PB samples from infected cows vs. controls (miR-19a, miR-144, miR-2425, miR-139, miR-101) were validated using RT-qPCR. Gene targets were predicted for each differentially expressed microRNA using TargetScan, PiTa, and miRanda algorithms, and RNA-seq of mRNA of the same animals. A functional analysis using the predicted gene targets revealed enrichment in RNA polymerase and mitogen-activated protein kinase (MAPK) signaling pathways in the ICV from the cows with focal and diffuse lesions, respectively. The detection of some of the microRNAs identified in the current study could be used for the development of novel microRNA-based diagnostic and therapeutical tools for PTB control.

P-065

USO DE LA TERAPIA DE REEMPLAZO CON INMUNOGLOBULINAS: EXPERIENCIA REAL EN UNA UNIDAD DE INMUNODEFICIENCIAS PRIMARIAS.

Moral Moral, Pedro; Núñez Beltrán, María; Puig Navarro, Carlos; Cabañero Navalón, Dafne; Ciscar Fernandez, Pascual; García Bustos, Victor.

Hospital Universitari i Politècnic La Fe, Valencia.

OBJETIVO

Describir de las características clínicas y el tratamiento sustitutivo con inmunoglobulinas (Ig) que utilizan los pacientes en una Unidad de Inmunodeficiencias Primarias y comparar sus características clínicas, analíticas, terapéuticas y los efectos sobre su calidad de vida según la vía de administración.

MATERIAL Y MÉTODOS

Estudio observacional retrospectivo con 105 pacientes diagnosticados de inmunodeficiencia primaria o secundaria durante 14 meses. Los pacientes se dividieron según la vía de administración. Se recopilaron características clínicas, posología del tratamiento (tratamiento previo, tolerancia, visitas formativas, consultas médicas relacionadas con su patología o tratamiento) y resultados clínicos (niveles de Ig, número de sesiones de trabajo y/o días perdidos). El análisis estadístico descriptivo e inferencial se realizó con software R (versión 4.0.0).

RESULTADOS

El diagnóstico más frecuente fue inmunodeficiencia común variable (44,76%). El 69% de los pacientes utilizaron vía de administración subcutánea (SC). Los pacientes con terapia de reemplazo de Ig SC eran en promedio significativamente más jóvenes que aquellos con reemplazo de Ig intravenoso (IV) (46,76 Vs 55,31 años, $p < 0,001$). No se observaron diferencias en los niveles sanguíneos entre ambos grupos. Hubo más consultas en el grupo IV ($p<0,001$), pero no se observó diferencia significativa en el número de consultas médicas urgentes. La tasa de efectos adversos fue baja y no difirió entre los grupos SC o IV. Los pacientes con terapia de Ig SC continuaron con dicha vía después del período de seguimiento, pero aquellos tratados con Ig IV tuvieron más probabilidades de cambiar a SC ($p<0,001$).

CONCLUSIONES

Según nuestros datos, la terapia de Ig SC conduce a una disminución del número de días de trabajo perdidos, sin documentarse un aumento significativo del número de infecciones, ni una disminución significativa de niveles de Ig. La ruta SC se prefirió entre los pacientes más jóvenes.

P-066

MULTIPARAMETRIC FLOW CYTOMETRY APPROACH IN HUMORAL PRIMARY IMMUNODEFICIENCIES INCLUDING IGG AND IGA SUBCLASSES

Castro Hernández, Carolina; González López, Elena; Roa Bautista, Adriel; Renuncio García, Mónica; Mikhalkovich, Dzmitry; Mota Pérez, Nerea; Ocejo Viñals, Javier Gonzalo; Irure Ventura, Juan; Comins Boo, Alejandra; López Hoyos, Marcos; San Segundo Arribas, David.

Hospital Universitario Marqués de Valdecilla, Santander.

Introduction

The study of serum immunoglobulins is a key tool for the diagnosis of humoral primary immunodeficiencies (HPID). Recently, a profile of monoclonal antibodies has recently been described for the study of memory B subpopulations producing different IgG and IgA subclasses based on membrane surface markers by multiparametric flow cytometry.
The aim of this study was to validate the panel proposed by Euroflow for the study of the subpopulation of switched memory B lymphocytes (smLB) with membrane surface markers and study in HPID.

Material and Methods

Serum immunoglobulin levels were quantified by turbidimetry in 35 patients with HPID. Moreover, the PBMCs were obtained and stained with monoclonal antibodies for the analysis of the expression of Ig subclasses in smLB by multiparametric flow cytometry.

Results

Nine patients with confirmed IgA deficiency and 24 patients with other HPID were identified. The smLB were identified as CD19+CD5-CD27+/-IgD-IgM-. In patients with IgA deficiency, a lower % of IgA1+ [0.37-3.75; 4.35-13.57] and IgA2+ [<0.01-1.03; 2.66-14.37] smLB compared with other HPID group was observed, respectively. Moreover, a positive correlation between serum IgA levels and % of IgA1+ ($p = 0.01$) and % of IgA2+ ($p < 0.01$) smLB subpopulations in IgA-deficiency patients was observed.

Discussion

The detection of different smLB subpopulations based on membrane subclasses by flow cytometry is a feasible approach in the study of HPID suspicion. Here, we confirm the reduction of smLB compartment in IgA1+ and IgA2+ in IgA deficient patients. However, longitudinal studies should be performed to confirm the potential role of multiparametric flow cytometry approach in HIPD diagnosis.

P-067

TRATAMIENTO CON INMUNOGLOBULINA SUBCUTÁNEA FACILITADA EN PACIENTE CON HIPERCATABOLISMO SEVERO DE IGG. PAPEL DE LA ENFERMERIA ESPECIALIZADA

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HGU Gregorio Marañón, Madrid.

INTRODUCCION

Tener una buena calidad de vida (CV) es indispensable en pacientes con inmunodeficiencia primaria (IDP), porque la falta de ella, podría afectar negativamente la vida del paciente. La terapia de reemplazo de IgG en estos pacientes mediante el uso de inmunoglobulina subcutánea (SCIG) tiene buenos resultados en mantener niveles óptimos de inmunoglobulina G (IgG).

OBJETIVO

En un paciente con hipercatabolismo IgG grave, se administró terapia con inmunoglobulina subcutánea facilitada (FSCIG) y se evaluó la CV relacionada con la salud.

MATERIAL Y MÉTODOS

Paciente de 38 años con inmunodeficiencia común variable (IDCV). Antecedentes de infecciones recurrentes tratadas con inmunoglobulina intravenosa (IGIV). Debido al hipercatabolismo proteico no lograba mantener niveles óptimos de IgG por lo que se cambió a terapia combinada (IVIG 40 g/21 días + SCIG 8g/semana; 2g/kg/mes). El paciente vive a 50 km del hospital, con un entorno familiar complicado que requiere apoyo psicológico. Debido al importante deterioro de la CV, se cambió el tratamiento a FSCIG, con el objetivo de mantener la IgG por encima de 400 mg/dL, en ausencia de infecciones recurrentes, con una dosis menor de IgG. Se inició FSCIG a una dosis de 25g/15 días (1g/kg/mes). La calidad de vida se evaluó con SF-36 v.2.0.

RESULTADOS

Durante un seguimiento de 3 años de la autoadministración de IGSCF en domicilio, los niveles medios de IgG fueron de 960 mg/dL. Según el SF-36, mejoró la CV en 30 de 36 elementos. No hubo nuevas complicaciones infecciosas. La IGSCF fue bien tolerada.

CONCLUSION

El uso de FSCIG es una alternativa en pacientes hipercatabólicos severos de IgG, mejorando la calidad de vida, manteniendo niveles aceptables de IgG a través de dosis más bajas. La enfermera especializada en el manejo y entrenamientos de este tipo de inmunoglobulinas, es esencial en la salud del paciente.

Sesión Poster Defensa 1. Pantalla 4. Inflamación + Inmunidad innata (I)

P-068

ESTUDIO DE COMPARACION DE LA DETERMINACION DE LAS SUBCLASES DE IgG POR DOS METODOLOGÍAS, NEFELOMETRIA VS TURBIDIMETRIA, EN LA EVALUACIÓN DE PATOLOGIA INMUNOLOGICA

Perez Garay, Raquel[1]; Martinez De La Puente Molina, Eduardo[2]; Jimenez Ros, Leyre Cristina[2]; El Moutawakil El Alami, Alae El Moutawakil El Alami[2]; Conde Rubio, Paula[2]; Salazar Ibañez, Lorena[2]; Santos Zorrozua, Borja[3].

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INTRODUCCIÓN

La medición de las subclases de IgG es parte de la evaluación inmunológica ante la sospecha de una inmunodeficiencia. Binding Site® desarrolló un ensayo turbidimétrico, preciso y fiable, minimizando el efecto prozona y calibrado a estándares internacionales más recientes (ERM-DA470 (CRM 470), frente al clásico Sanquin-Siemens WHO67/97 (M1590).

OBJETIVOS

Comparar las subclases IgG por nefelometría BNII® (Siemens®) vs turbidimetría Optilite® (BS®) y Atellica (Siemens®) vs Optilite® (BS®) para la IgG total.

MATERIAL Y MÉTODOS

Se analizaron 79 sueros: alteraciones de la inmunidad (13%), síndrome de hiperIgG4 (6%), pancreatitis (6%) y el resto otras patologías.

La comparativa estadística se realizó por el método de Passing-Bablok, regresión lineal y análisis Bland-Altman, y la concordancia se evaluó por cálculo de coeficientes ICC y de correlación Lin, mediante software R (V.4.0.1).

RESULTADOS

	Media (mg/dL) BS vs Siemens	**Passing-Bablok**	**Pearson's R**
IgG1	474,6-622	y=54,07 CI95%(25,78-96,97) + 1,173 CI95%(1,105-1,246)x	0,984
IgG2	286,78-260	y=12,39 CI 95%(-2,478-23,88) + 0,8962 CI95%(0,8463-0,9503)x	0,89
IgG3	53,15-31,8	Y=1,235 CI95%(-0,429-3,198) + 0,5702 CI95%(0,5208-0,6131)x	0,864
IgG4	29,0-31,1	y=-2,587 CI95%(-4,263--0,8245) + 1,584 CI95%(1,507-1,658)x	0,989
Total IgG	918,5-981,0	y=14,2 CI95%(-8,88-50,95) + 1,002 CI95%(0,971-1,034)x	0,986
IgG (BS) vs suma	918,5-896,92	y=17,16 CI95%(-6,076-46,07) + 0,9493 CI95%(0,912-0,979)x	0,975
IgG (SIEMENS) vs suma	981,0-1054,1	y=-23,78 CI95%(-82,7-23,53) + 0,9672 CI95%(0,919-1,019)x	0,936
		Coeficiente ICC	**Coeficiente de correlación de Lin**
		0,9023 CI95%(0,852-0,937)	0,906 CI95%(0,869-0,932)
		0,887 CI95%(0,829-0,926)	0,886 CI95%(0,827-0,925)
		0,542 CI95%(0,367-0,681)	0,586 CI95%(0,488-0,669)
		0,794 CI95%(0,690-0,866)	0,797 CI95%(0,753-0,834)

CONCLUSIONES

Los resultados no son intercambiables entre sí, observándose un error constante y proporcional IgG1 e IgG4 y proporcional IgG2 e IgG3. El modelo de regresión ofrece un buen ajuste, para valores altos la dispersión es mayor. Los coeficientes ICC y Lin son ligeramente superiores para BS vs Siemens, probablemente debido a que no usan el mismo material de referencia de la calibración (CRM vs WHO).

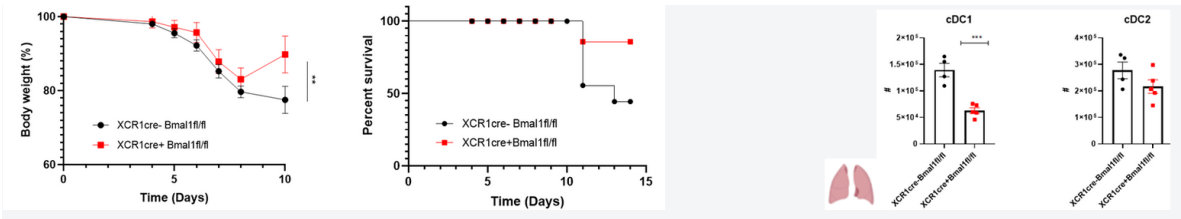
P-069

DISRUPTING THE MOLECULAR CLOCK OF CDC1 INCREASES SURVIVAL AGAINST INFLUENZA A VIRUS

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Circadian rhythms are endogenous oscillations in physiological processes that repeat with a ~24h periodicity imposed by the rising and setting of the sun, and are controlled by oscillating core-clock transcription factors. Bmal1 is one of the main clock genes and its abrogation leads to circadian rhythm disruption. Influenza virus infection is a leading cause of respiratory mortality and morbidity. Time of infection determines survival against Influenza A virus (IAV). In mice, susceptibility increases when infection occurs at the beginning of the resting phase (7 pm, ZT13) or after abrogating the circadian clock by inducing constitutive Bmal1 deficiency ($Bmal1^{fl/fl}ERcre^{+}$). Dendritic cells (DC) provide a first line of defence following IAV infection. Here we studied the response to IAV infection in mice bearing a specific depletion of the molecular clock in a DC population called cDC1 (XCR1DBmal1 mice). We have observed that, upon IAV infection at ZT13, XCR1DBmal1 mice lose less weight and survive more than wild-type mice. The increased survival of XCR1DBmal1 mice correlated with lower cDC1 numbers in the lung and lung-draining lymph nodes. In this line, cDC1-deficient mice (Batf3 KO and $XCR1^{cre}$ DTA mice) are more resistant to flu infection than wild-type mice. We are studying the cellular mechanisms involved in this potential detrimental role of cDC1 in the early immune response against IAV infection, and whether the cDC1 molecular clock is key in this immune response.



P-070

THE COMPLEMENT SYSTEM: A NEGATIVE REGULATOR OF TRAINED IMMUNITY

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Trained immunity, an innate immune memory program characterized by enhanced pro-inflammatory cytokine production in monocyte-derived cells, protects against fungal and bacterial infections. The complement system is also a major component of innate immunity, regulating inflammation and host defense against fungal and bacterial pathogens. While these innate immunity systems are necessary to successfully control active infections, the relationship between trained immunity and the complement system has not yet been explored. To characterize the importance of the complement pathway in the regulation of training we developed we developed an innovative in vitro trained immunity protocol using murine bone marrow-derived macrophages (BMDM) induced with β-glucan. Using wild-type and complement C3-deficient mice, we analyzed the secretion of pro-inflammatory cytokines by enzyme-linked immunosorbent assay (ELISA); the regulation of genes by RNA-seq, and the epigenetic rewiring of trained macrophages using ATAC-seq and CUT&RUN. Our results indicate that β-glucan significantly upregulates the expression of C3 mRNA in BMDM upon LPS stimulation. Unexpectedly, C3-deficient trained macrophages significantly increased the secretion of TNF, IL6, and CXCL10 cytokines. Our data reveal for the first time that the complement system acts as a negative regulator of trained immunity. Identification of natural inhibitors of trained immunity represents a novel approach to direct innovative therapies to regulate the immune response.

P-071

ACTIVATION OF THE NLRP3 AND PYRIN INFLAMMASOMES DURING SEPSIS.

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Sepsis is the leading cause of death in critical care units, and it's characterized by a systemic inflammatory response which is followed with an immunosuppression of the host. To study the role of the NLRP3 and Pyrin inflammasomes in sepsis: individuals with intraabdominal origin sepsis (n=32), abdominal surgery individuals that had not developed sepsis (n=32), and a healthy control group (n=32) were recruited.

The systemic inflammatory markers CRP and PCT were elevated in septic patients' plasma 24h after sepsis initiation when compared to the control groups, indicating an inflammatory state of septic patients. Moreover, elevated levels of IL1-RA, sIL-1RI, sIL-1RII, IL-38, IL-18BP, TNFα, IL-6, IL-15, IL-8, M-CSF and GM-CSF were identified in plasma from sepsis patients on admission compared to control subjects.

The release of IL-1β in whole blood samples and the formation of ASC specks in monocytes after NLRP3 stimulation resulted in a differential response among septic patients, identifying a group of individuals with an impaired NLRP3 inflammasome activation. In deep, these patients also had biochemical and clinical scores of disease severity above the average. On the other hand, all septic patients presented a non-impaired activation of the Pyrin inflammasome.

To analyse the role of the immunometabolism in this differential response, expression of the genes LDHA, PDK1, PKM2, and SLC2A1 and the concentration of basal ATP, lactate, NAD^+/NADH ratio and phosphoenolpyruvate were measured among patients.

Moreover, NLRP3-immunocompromised septic patients who survived and recovered from sepsis had an improvement on NLRP3 activation at hospital discharge, suggesting that NLRP3

inflammasome impairment is a transitory state.

Our study confirms that during the initial inflammatory response in sepsis there is a differential response of the NLRP3 inflammasome, which could be considered a marker of immunosuppression, while the Pyrin inflammasome presents different regulation mechanisms inflammasome during sepsis.

P-072

JNK DEFICIENCY IN INTESTINAL EPITHELIAL CELLS LEADS TO SPONTANEOUS DUODENAL TUMORS ASSOCIATED WITH COMPLEMENT SYSTEM UPREGULATION

Rivas Pardo, Benigno; Martín Adrados, Beatriz; González García, Raquel; Solsona García, Adela; Chen, Chaobo; Gómez Del Moral, Manuel; Cubero, Francisco Javier; Martínez Naves, Eduardo.

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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, and this increased activation frequently results in pathology via the regulation of the transcription of numerous inflammation-related genes. The general objective of this work was to identify specific mechanisms of the JNK signaling pathway involved in intestinal 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 analyzed samples of small and large intestine using histological techniques (H&E and immunohistochemistry) and RNAseq. Moreover, we induced colorectal carcinoma using AOM/DSS method.

Results

We analyzed samples of 10, 52 and 72 weeks old $JNK^{\Delta IEC}$ mice. Young $JNK^{\Delta IEC}$ mice developed spontaneous mild inflammation in the duodenum and colon, which progress to an aberrant architecture and epithelial-dysplasia together with cellular infiltrates in the duodenum. These lead to an end-stage spontaneous duodenal tumors development in the oldest mice. We observed several significant changes in the intestinal transcriptome. Interestingly, the complement pathway was one of the most enriched pathways in duodenal samples, while in the colon we found a dramatic downregulation of defensin genes. C3 protein expression was restricted to intestinal crypts in healthy tissues, while it was expressed in the whole tumoral tissues. Moreover, we found that $JNK^{\Delta IEC}$ mice were more susceptible to AOM/DSS induced colorectal carcinoma than control mice.

Conclusions

Jnk1 and *Jnk2* deletion in IECs is associated with the development of spontaneous duodenal tumors and an increased susceptibility to induced colorectal carcinoma. The complement system, highly upregulated and expressed in the tumoral tissues, could be a key factor in this process.

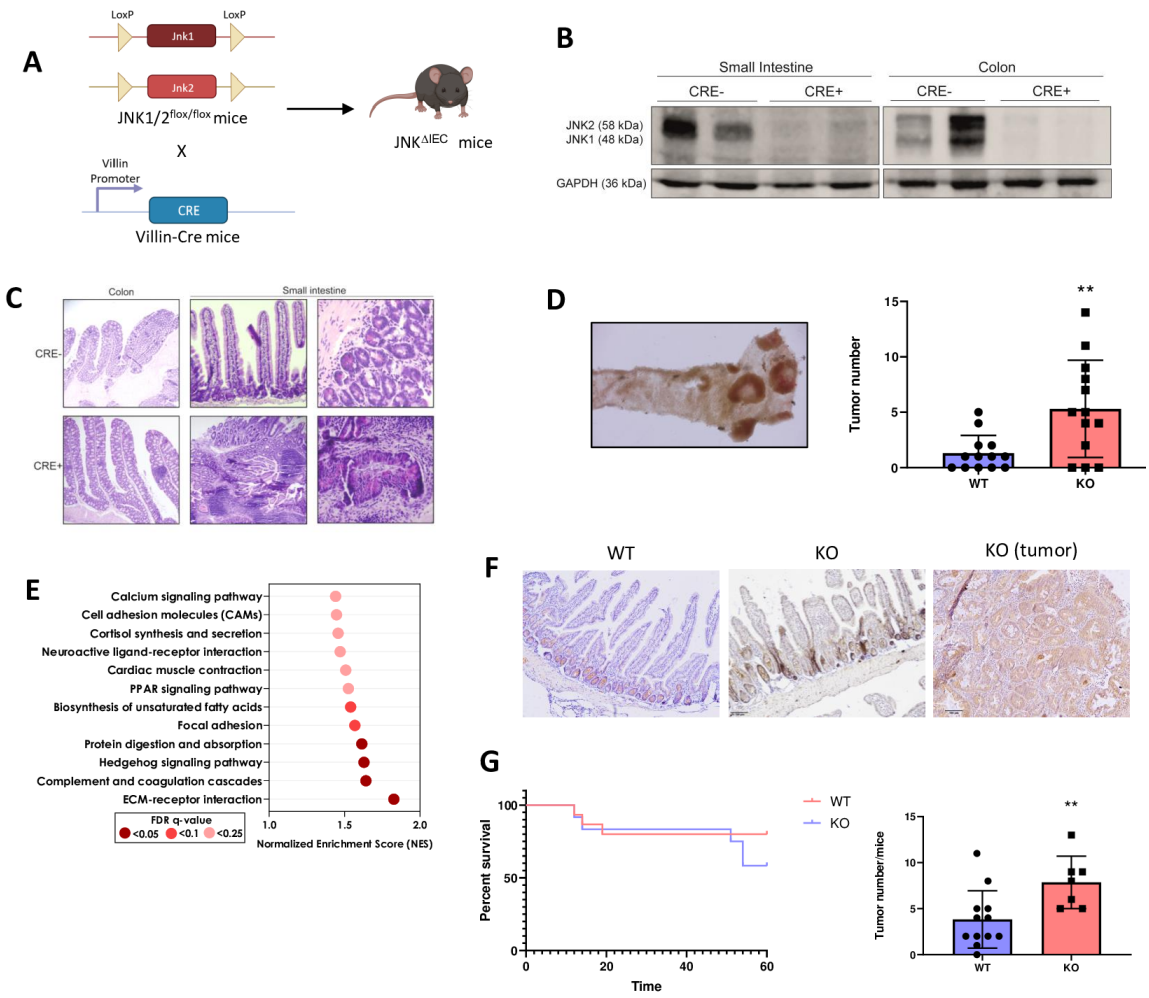


Figure. A) Generation of mice with conditional deletion of *Jnk1* and *Jnk2* in intestinal epithelial cells ($JNK^{\Delta IEC}$) using VillinCre-Loxp system. **B)** Western Blot analysis to demostrate the deletion of JNK1 and JNK2 in small intestine and colon of CRE+ mice. **C)** Hematoxylin and eosin staining of colon and duodenum of 52 weeks old $JNK^{flox/flox}$ and $JNK^{\Delta IEC}$ mice. D) . **E)** Representative image of the duodenum of 72 weeks old $JNK^{\Delta IEC}$ mice and number of tumors. **E)** Gene Set Enrichment Analysis of duodenal of $JNK^{\Delta IEC}$ mice compared with $JNK^{flox/flox}$ mice. **F)** C3 immunohistochemistry of the duodenum of WT and KO mice**. G)** Survival curve and tumor number of WT and KO mice after AOM/DSS colorectal cancer induction

P-073

EFFECT OF NANOPLASTICS ON THE INFLAMMATORY RESPONSE

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Most of the plastic-based products are single use, which leads to their environmental accumulation and degradation thus generating micro- and nano-plastic (MNPLs) residues. Little is known about human exposure to MNPLs and their potential health risks. In this regard, the effect of MNPLs on the immune system and specifically on the inflammatory response is not well characterized. In this study, we have checked the effect of two nanoplastics; polystyrene (PS) and polyethylene terephthalate (PET), on the inflammatory response in two different situations: i) we have evaluated the inflammatory response after a previous exposure to nanoplastics. ii) we have assessed whether these nanoplastics are able to generate inflammasome activation after a previous inflammatory state.

In the first approach, NLRP3 and Pyrin inflammasome activation levels were assessed in mouse bone marrow derived macrophages (BMDMs) and human peripheral blood mononuclear cells (PBMCs) after a previous exposure to different concentrations of PS and PET particles. In the second approach, BMDMs and PBMCs were primed with PAMPs and then treated with different concentrations of PS and PET particles to check inflammasome activation.

Our results show that prior exposure to nanoplastics reduces the expression of different inflammatory and inflammasome-related genes and the subsequent activation of NLRP3 and pyrin inflammasomes in a dose-dependent manner. On the other hand, nanoplastics are able to produce NLRP3 inflammasome activation in PAMP-primed BMDMs and PBMCs, thus inducing the oligomerization of ASC, the activation of caspase-1 and gasdermin D processing, with the release of inflammatory cytokines such as IL-1β and IL-18. Our data also show that decrease of intracellular K^+, cathepsin B and macroautophagy may have a role in the NLRP3 inflammasome activation.

In summary, our study shows that nanoplastics can be internalized by immune cells and have an

important role in the production and modulation of the inflammatory response.

P-074

THE BET PROTEINS INHIBITOR, JQ1, REDUCES THE RELEASE OF IL-1B INDUCED BY THE NLRP3 INFLAMMASOME ACTIVATION UNDER ISCHEMIC RENAL DAMAGE.

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The NLRP3 inflammasome is a multi-protein complex that plays a critical role in the innate immune system by mediating the caspase-1 activation and secretion of the IL-1β and IL-18 pro-inflammatory cytokines in response to microbial infections and cellular damage. Their exacerbated activation has been linked to several inflammatory diseases, and thus, to the search for new therapeutic strategies to block it.

Here, we have analysed the role of the epigenetic inhibitors of BET (bromodomain and extra-terminal domain) proteins in the NLRP3 inflammasome activation. For that, the inflammasome components and the IL-1β production were assayed in primary monocytes and macrophages activated with LPS and ATP/Nigericin. The effect of the JQ1 inhibitor was assayed in a murine model of renal ischemic/reperfusion injury (IRI) where inflammasome activation contributes to the development of the disease.

Results show that treatment of monocyte/macrophages with the JQ1 and I-BET-762 inhibitors, significantly decreases the IL-1B and CASP-1 transcriptional and protein levels, and it is associated with a decreased of the IL-1β release. Using ChIP assay, we determine that BRD4, a member of the BET protein subfamily, is recruited to the promoter region of the *CASP1* and *IL1B* genes inducing their expression. Moreover, treatment with SGC/CBP30, a specific inhibitor of the CBP/P300 HATs, significantly reduces the expression of CASP-1 and IL-1B, suggesting that histone acetylation is required for the further recruitment of BRD4 and P-TEFb complex, aimed to initiate the transcriptional elongation. Administration of JQ1 in a mice model of renal damage inhibits the induced CASP-1 and IL-1B expression, avoiding the NLRP3 inflammasome activation and IL-1β release, and contributing to a better renal function.

Therefore, the treatment with BET protein inhibitors, such as JQ1, could ameliorates the NLRP3 inflammasome activation, through downregulation of CASP-1 and IL-1B expression, and consequently, the inflammation triggered by innate cells under ischemic renal damage.

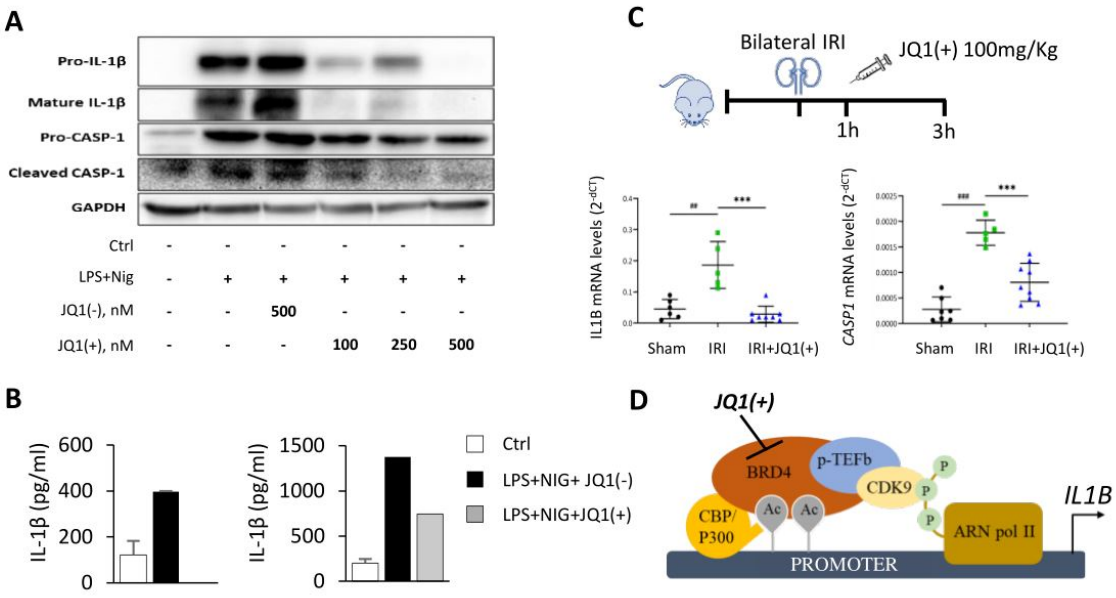


Figure 1.- The BET proteins inhibitor, JQ1, downregulates the IL-1B and CASP-1 expresion impairing the IL-1β release. A. Western blot análisis of proforms and active forms of IL-1B and CASP-1 in monocytes unactivated or activated with LPS (100ng/mL, 16h) and Nigericin (4μM, 1h) and treated with JQ1(+) at different doses or its enantiomer JQ1(-). **B.** IL-1β production levels in monocytes (left) and macrophages (right) activated with LPS/Nig and treated with JQ1(-) or JQ1(+) at 500nM. **C.** Scheme of the renal damage model induced by IRI and IL-1B and CASP-1 mRNA expresión levels in groups; sham, IRI and IRI+JQ1(+). **D.** Model propossed for the regulation of IL-1B expresión by BRD4 and inhibition by JQ1.

P-075

MAPPING THE DIVERSITY OF TRAINED IMMUNITY AGAINST INTESTINAL PATHOGENS AND SYMBIONTS

Palacios, Ainhoa[1]; Barriales, Diego[1]; Castelo, Janire[1]; T. Pasco, Samuel[1]; Araujo, Sarai[1]; Gutiez, Naiara[1]; Seoane, Iratxe[2]; Abecia, Leticia[2]; Rodríguez, Héctor[1]; Anguita, Juan[1].

[1]CICbioGUNE, Derio; [2]UPV/EHU, Leioa.

In the past decade, the existence of long-term consequences of the stimulation of innate immune cells has been recognized, resulting in divergent immune responses to secondary challenges. Trained immunity has redefined our understanding of the innate immune system modulation. Using certain single molecular patterns or vaccine-like formulations, it has been proposed as a promising strategy against inflammatory/infectious insults due to its capacity to generate heterologous cross-protection. However, the phenotype and regulatory mechanisms of innate immune memory against live bacteria has been largely unaddressed. These interactions are particularly relevant in the context of persistent infections and the maintenance of homeostasis by gut commensals, where the repeated exposure of innate cells to microorganisms might shape the evolving response.

To address this issue, we compared the acute and trained responses of mouse bone-marrow derived macrophages exposed to a phylogenetically diverse panel of gut-related commensal and potential pathogenic bacteria. Critical aspects of phagocyte responses were measured in trained macrophages, including their ability to internalize and kill microorganisms and the ensuing release of proinflammatory cytokines. Moreover, the regulatory mechanisms of memory generation were explored by analyzing the underlying transcriptional and metabolic rewiring of these cells. Our results show that macrophages trained with live bacteria present substantially different functional phenotypes from those in acute conditions (first stimulation), diverging from the responses observed with isolated microbial products. Common memory features include the dampening of proinflammatory molecule release and an enhanced internalization capacity of bacteria. Bactericidal activity of trained macrophages, however, differs depending on the bacterial species employed. Interestingly, we identified transcriptional traits in BMMs linked to distinctive memory regulation after the secondary exposure to pathogens and commensals. This information might pave the way to develop tailored therapies targeting the modulation of ongoing innate memory responses, specifically those that are associated with the persistence of pathogens.

P-076

GENERATION OF HUMAN MICROGLIA CELLS FROM PLURIPOTENT STEM CELLS LINES (HIMG) FROM AGED AND AD PATIENTS.

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Age-related neurodegenerative disease is a leading medical and societal challenge. Existing treatments for neurodegenerative diseases, particularly Late Onset Alzheimer Disease (LOAD), are very limited, and only treat the symptoms, rather than addressing the cause. GWAS analyses have shown the importance of the **brain innate cells** in the increasing risk for developing LOAD.

One of the main caveats to our knowledge of these processes is the research material used. The use of rodent microglia cells, mouse cellular lines (BV2) or human cells lines (HMC3/CHME3) has proved that do not recapitulate many of the human microglia features, and therefore halt the progress toward the finding of relevant molecular targets. An effort to establish better and more relevant screening platforms is required, and here we propose to study human origin microglia from AD patients by using already established hiPSCs cell lines from official cellular repositories.

Our aim is to establish a robust protocol to obtain the main cell type involved in the innate immunity in the brain: microglia cells from human source. We are working in different protocols aiming to obtain human microglia from human inducible pluripotent stem cells (hiPSC) and human brain organoids. Once these protocols are attained, our final goal is to study signalling pathways associated to microglia associated to degeneration (DAM) phenotypes. This particular microglia cells are characterized by an increase of specific genetic signature, with APOE and TREM2 genes among them. Furthermore, recent data has shown that APOE binds to TREM2, a microglial surface receptor, and imply that the different association to LOAD risk between APOE alleles might be due to the differential binding to the TREM2 receptor. **The use of human microglia will help us to understand the molecular cues and contribute to the knowledge and design of new tools to understand and modulate neuroinflammation in a human.**

P-077

CYTOMEGALOVIRUS: AN INFECTIOUS COMPONENT IN AORTIC STENOSIS

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[1]Maimonides Biomedical Research Institute of Cordoba (IMIBIC, Córdoba; [2]Cardiovascular Surgery Unit. Reina Sofía university Hospital of Cordoba, Córdoba; [3]Department of Cell Biology, Physiology and Immunology, University of Cordoba, Maimonides Biomedical Research Institute of Cordoba (IMIBIC), Córdoba.

Immune cells suffer alterations with age leading to immunosenescence. Additionally, chronic stimulation of the immune system by persistent antigens can lead to 'early immunosenescence'. In this regard, we have demonstrated that proatherogenic $CD28^{null}$ T cells expansion is driven by cytomegalovirus (CMV) chronic infection and not by age. Furthermore, CMV infection has been associated with several autoimmune conditions, and CMV-seropositivity has been associated with an increased risk of cardiovascular death by up to 20%. Recent studies suggest that aortic stenosis (AS) is an active inflammatory atherosclerotic-like process. As in atherosclerosis, AS has been associated with $CD28^{null}$ T cells expansion, suggesting that CMV infection could have a role in the degeneration of the aortic valve. We developed a novel cytometry-based protocol for the study of AS that has allowed us to perform, for the first time, an in-depth characterization of the inflammatory infiltrating cell subsets present in the degenerated (calcified) aortic valve. We performed a detailed phenotypical and functional characterization of both peripheral and valvular T cells from AS patients (49-80 years), and sex/age matched healthy volunteers (HD). The results showed an increase of peripheral and valvular $CD28^{null}$CD57+ T cells in AS patients compared with HD. The presence of CMV-specific T cells in the diseased valves was further corroborated by functional assays (TNF and IFN-g production) using CMV peptides, and by CMV-specific tetramer staining. Our results show for the first time an association of AS with CMV chronic infection and supports a potential role of CMV in the degeneration (calcification) of the aortic valve, probably mediated, at least in part, by CMV-specific T cells and by the CMV-driven expansion of highly cytotoxic and proinflammatory $CD28^{null}$ T cells.

Funding: This research was funded by the Spanish Instituto de Salud Carlos III Ministry of Health, PI19/00075 (to Pera A.) and FI20/00194 (to Alvarez-Heredia P.).

P-078

THERAPEUTIC TARGETING OF IRF5 FOR *BORRELIA BURGDORFERI* INFECTION

Araujo-Aris, Sarai; Barriales, Diego; Martín-Ruíz, Itziar; Jiménez-Osés, Gonzalo; Rodríguez, Héctor; Anguita, Juan.

CIC bioGUNE, Derio.

Transmission of *Borrelia burgdorferi*, the causative agent of Lyme disease and the most common tick-borne infection, occurs when infected ticks feed on vertebrates. Days after the bite, the bacteria spread to various organs, including the heart where they can persist for months to years. This long-term cohabitation of bacteria and infiltrating immune cells triggers continuous inflammation, resulting in a modified inflammatory response via the MyD88-Interferon regulatory factor (IRF) signalling pathway. MyD88 protein serves as a toll-like receptor (TLR) adaptor, with IRF4 and IRF5 competing for MyD88 interaction at the same binding site while acting as mutual inhibitors. In a basal state, both have a similar expression but, upon an inflammatory stimulus, IRF5 expression increases and facilitates the production of pro-inflammatory cytokines, while IRF4 conversely dampens this response.

We hypothesize that molecules that interfere with the interaction IRF5/MyD88 can modulate *B. burgdorferi*-induced inflammation. *In silico*, we studied the IRF4/IRF5 interaction site in MyD88 and discerned two regions putatively responsible for their binding. Next, we developed MyD88-based membrane-permeable peptides designed to compete with MyD88 for IRF5 binding. We tested this library of peptides in bone marrow derived macrophages and observed that some candidates reduced the inflammatory output upon a *B. burgdorferi* challenge, demonstrating potential therapeutic value. The *in vivo* activity of these successful peptides was assessed during *B. burgdorferi* cardiac infection and will be presented.

P-079

PHENOTYPIC AND FUNCTIONAL STUDY OF NK CELLS STIMULATED IN VITRO WITH IL-12/15/18: INDUCTION OF MEMORY-LIKE NK CELLS.

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NK cells are a key component of the innate immune system, and their function is regulated by a rigorous balance of signals mediated by activating and inhibitory receptors. These cells are essential not only in host defence against virus-infected cells, but also in tumour immune surveillance. A memory-like phenotype can be induced in NK cells by IL-12/15/18 under *in vitro* culture conditions. Cytokine-activated memory-like (CIML) NK cells have been considered an excellent candidate for future immunotherapies due to their longer half-life and their highly cytotoxic nature. In our study, we aimed to analyse CIML NK cells in terms of phenotype and function. NK cells were isolated from peripheral blood obtained from healthy donors, then stimulated with IL-12/15/18 (CIML) or IL-15 alone (control) and analysed at day 7 by multiparametric flow cytometry to determine the phenotypic and functional profile of both control and CIML NK cells.

Our phenotypic analyses revealed that CIML NK cells had significantly higher levels of CD25, NKG2D, NKp30, NKp46 and the inhibitory receptors NKG2A, TACTILE and TIM-3. On the contrary, these cells express significantly lower levels of CD57, KIR2D, CD16 and perforin. Our functional studies demonstrated that CIML NK cells exhibit degranulation capacity against K562, however, our results demonstrated inter-donor variability.

In conclusion, based on the heterogeneous phenotype and function of *in vitro* expanded CIML NK cells, a more exhaustive phenotypical characterization will help to increase the therapeutic potential and improvement of expansion protocols.

(*Los primeros tres autores han contribuído igualmente al trabajo).

P-080

CARACTERIZACIÓN DE LA MICROBIOTA INTESTINAL DE PACIENTES CON ENFERMEDAD INFLAMATORIA INTESTINAL DE RECIENTE DIAGNÓSTICO.

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Introducción: La disbiosis juega un papel importante en la fisiopatología de la enfermedad inflamatoria intestinal (EII). Los estudios previos incluyen pacientes con tratamiento, pudiendo esto modificar la microbiota. Objetivos: Caracterizar la microbiota de pacientes con EII de reciente diagnóstico en comparación con controles sanos (CS), y las diferencias entre pacientes con enfermedad de Crohn (EC) y colitis ulcerosa (CU).

Métodos: Se realizó un abordaje metagenómico shotgun en heces de pacientes con EII de reciente diagnóstico (<1 mes) antes del inicio de cualquier tratamiento para la EII y de CS, analizando los resultados con Cutadapt, Kraken2 y Bracken. La actividad de la EII se clasificó mediante el subíndice endoscópico del índice de Mayo en la CU y mediante el SES-CD en la EC.

Resultados: Se incluyeron 104 pacientes con EC, 145 con CU y 49 CS. Los pacientes con EC

presentaron menos α-diversidad comparado con aquellos con CU y los CS, observándose una relación inversa entre este índice y la gravedad de la actividad. La α-diversidad fue similar en los pacientes con CU leve, moderada y grave, comparados con los CS. Atendiendo a la abundancia taxonómica a nivel de género microbiano, las mayores diferencias globalmente en los pacientes con EC y en aquellos con actividad grave, se encontraron en Adlercreutzia y Wujia, comparado con los CS. Sin embargo, los pacientes con EC leve presentaron las mayores diferencias en Desulfovibrio y aquellos con EC moderada en Ruminococcus. Los pacientes con CU globalmente, y particularmente con actividad leve, moderada y grave, presentaron las mayores diferencias en Toxoplasma y Gemella, comparado con los CS.
Conclusiones: Los pacientes con EC de reciente diagnóstico tienen una diversidad microbiana en heces menor que los CS, a diferencia de aquellos con CU. Además, los pacientes con EC presentan diferencias en la abundancia taxonómica en función de la gravedad

P-081

A NOVEL MECHANISM OF CROSS-TALK BETWEEN INTERFERON SIGNALLING, INFLAMMATION AND CELL DEATH

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Interferon Regulatory Factor 9 (IRF9) is a transcription factor that participate in interferon type-I signalling by associating with STAT-1 and -2 proteins, to form the ISGF3 heterotrimer. After IFN-I stimulation this complex translocates to the nucleus and drives the transcription of hundreds of Interferon Stimulated Genes. We have previously described two Primary Immunodeficient patients who were unable to control viral infection due to IRF9 deficiency. However, another striking feature of those patients was hyper-inflammatory reactions and we have gone on to explore whether IRF9 could modulate inflammation as well as the Interferon response.

Bioinformatic analyses showed that expression of the HOIP ubiquitinase directly correlated with IRF9 expression. Experiments using Luciferase reporter constructs revealed that HOIP can be regulated by ISGF3. Moreover, we also observed that, HOIP protein expression is upregulated after Interferon stimulation. Thus, HOIP is a novel ISG, whose expression is reduced in the absence of IRF9.

HOIP deficient patients' present with immunodeficiency and autoinflammatory manifestations, and this phenotype depends on alterations in CD14+ monocytes. Strikingly, silencing of either IRF9 or HOIP led to higher secretion of proinflammatory cytokines by differentiated monocytic cell lines after IL-1β or LPS stimulation. HOIP deficiency is also known to sensitize cells for death ligand induced cell death, and again, very similar patterns of necroptosis were observed after silencing of either HOIP or IRF9 in THP1 cells. This evident correlation between the actions of IRF9 and HOIP being further studied to define the molecular mechanisms underlying this phenotype.

All in all, these data provide novel insights into the cross-talk between the interferon response, inflammation and cell death, giving an increase knowledge of this complex interplay. This new knowledge also provides a better comprehension of the pathogenesis of disease in these patients and opens new possibilities for diagnosis and therapy.

P-082

PAPEL DE LA LAMINA A/C COMO REGULADOR DE LA FUNCIÓN DE LAS CÉLULAS PRESENTADORAS DE ANTÍGENO EN EL CONTEXTO DE LA ENFERMEDAD INFLAMATORIA INTESTINAL

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La enfermedad inflamatoria intestinal (EII) es una inflamación intestinal crónica relacionada con factores genéticos y ambientales, disbiosis de la microbiota, exacerbación de la inmunidad innata y adaptativa y disfunción de la barrera intestinal epitelial. Clásicamente, su patogénesis se asocia a un desequilibrio entre la acumulación exacerbada de células Th1 y Th17 proinflamatorias y una supresión Treg insuficiente. Las células presentadoras de antígeno (APCs) activan a las células T naïves, promoviendo la respuesta inmune adaptativa.
El filamento intermedio Lamina A/C se localiza en la envoltura nuclear favoreciendo la activación y diferenciación hacia Th1 de las células T CD4+ naïves e inhibiendo la generación de Tregs. No obstante, su papel en las APCs es casi desconocido.
Nuestro objetivo es analizar el papel de la lamina A/C en la capacidad de las APCs para promover respuestas inmunitarias adaptativas en la EII. Para ello, analizamos un ratón que carece de lamina A/C en parte del compartimento mieloide (Lmna-/-) y su control WT.
Los ratones Lmna-/- mostraban una pérdida de peso menor que los WT tras el tratamiento con uno o dos ciclos de DSS para la inducción de colitis. Los ratones Lmna-/- presentaban una menor sintomatología, un menor acortamiento de la longitud del colon y un menor ratio de células Th1/Treg en los ganglios mesentéricos. Menores signos de colitis fueron observados en cortes histológicos de colon de los ratones Lmna-/-.
Además, la transferencia adoptiva de células dendríticas derivadas de médula ósea de ratones lmna-/- previa al tratamiento con DSS de ratones WT, resultó en una menor pérdida de peso, mayor longitud del colon y menor inflamación en cortes histológicos de colon en comparación con ratones WT transferidos con células WT.

Como conclusión se observa que la reducción de los niveles lamina A/C en las APCs podría tener un papel protector frente a la EII.

P-083

REGULATION OF IMMUNE AND STROMAL CELLS LAMIN A/C IN INFLAMMATORY BOWEL DISEASE AS A THERAPEUTIC TARGET

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Inflammatory bowel disease (IBD) is a heterogeneous state of chronic intestinal inflammation of unknown cause. IBD is an interplay between genetic and environmental factors, microbiota dysbiosis, exacerbated innate and adaptive immunity and epithelial intestinal barrier dysfunction. However, the mechanism controlling all these steps is not completely known. Here, we explore the effect of regulating the levels of lamin A/C, a structural, epigenetic, and transcriptional modulator, in some immune cells and in stroma cells in a mouse model of IBD with dextran sulfate sodium salt (DSS).

IBD evolution was studied 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). Two approaches were followed to preferably stimulate innate or adaptive immunity, with one or two cycles of DSS, respectively.

The results point to a beneficial role of lamin A/C deficiency in our model of colitis: in comparison to wild-type mice ($Lmna^{+/+}$), the absence of lamin A/C in total immune cells, T cells, myeloid cells, or stroma cells upon one dose of DSS protects from colitis, while lamin A/C overexpression in T cells provokes increased colitis disease. These differences are more accentuated when mice were exposed to two doses of DSS, especially in mice with changes in lamin A/C levels in T cells.

In summary, lamin A/C levels in immune and stromal cells modulates innate and adaptive immune responses, and can be involved in the development of IBD. Lamin A/C modulation in specific cell types can be a potential target for IBD therapy.

P-084

CINÉTICA DE LA EXPRESIÓN DE SUPERFICIE DE TL2 Y TLR 4 EN DISTINTAS SUBPOBLACIONES CELULARES EN LA ETAPA TEMPRANA POST TRASPLANTE CARDIACO

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Las infecciones, el fallo primario y el rechazo agudo del injerto son barreras para la supervivencia los primeros meses post trasplante cardiaco (TC). Hay pocos estudios de componentes de la inmunidad innata en búsqueda de biomarcadores no invasivos en sangre periférica en TC.

OBJETIVO: Mediante un estudio prospectivo de seguimiento se evaluó la cinética de expresión de superficie de receptores tipo toll (TLR2 y TLR4) en distintas subpoblaciones linfocitarias (CD8, B, NK), monocitos, células dendríticas plasmocitoides y mieloides en una cohorte de TC.

METODOS: Se incluyeron 41 pacientes con diagnóstico de cardiopatía terminal que ingresaron para evaluación de TC. Se estudió la expresión porcentual de TLR2 y TLR4 en las diferentes subpoblaciones mediante análisis de citometría de flujo en inclusión en lista de espera (basal), y a los 7, 30 y 90 días post-TC. Inmunosupresión convencional con anti-receptor de IL2 como inducción, y triple inmunosupresión con prednisona, micofenolato y un inhibidor de calcineurina.

RESULTADOS:

Observamos que la expresión de TLR-2 disminuyó el día 7 en todas las poblaciones estudiadas en relación con el basal y este nivel se observó nuevamente a los 30 días post-trasplante. La expresión de TLR2 por células dendríticas plasmocitoides fue mayor antes del trasplante que post-TC. La expresión de TLR-4 sigue cinética diferente, Experimentó un aumento entre los días 7 y 30 después del trasplante en todas las subpoblaciones, excepto en células NK CD56+/CD16+. Post-TC, el nivel basal de la expresión de TLR-4 de CD8, B, NK y monocitos se observó a los 90 días.

CONCLUSIONES:

Tras el TC se observan cambios en la expresión de TLR2 y TLR4 en distintas subpoblaciones. Estos cambios pueden estar relacionados por la exposición a antígenos HLA, el proceso inflamatorio postquirúrgico, la inmunosupresión, infecciones tempranas, rechazo alogénico o mecanismos de tolerancia, lo cual debe explorarse en futuros estudios.

P-085

CHARACTERIZATION OF EXTRACELLULAR VESICLES IN AUTISM SPECTRUM DISORDER BY FLOW CYTOMETRY

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Background

Autism spectrum disorder (ASD) is a complex and frequent neurodevelopmental disorder with a prevalence of around 1 in 60 children. Extracellular vesicles (EVs) are nanovesicles containing proteins, mRNA, and microRNA. They are secreted by many immune cells, including macrophages, dendritic cells, and T and B lymphocytes, among others, and this mechanism allows cell-to-cell communication. Genetic biomarkers and proteins encompassed in EVs have recently been identified that have helped to understand the biological mechanisms and the diagnosis of different neurodegenerative diseases.

Aim

Assess the expression of exosomal surface epitopes in a cohort of patients with ASD versus controls.

Methods

Plasma samples of 50 patients with ASD and 50 healthy volunteers were recruited. The MACSPlex Exosome Kit (MiltenyiBiotec) was used to simultaneously detect 37 exosomal surface epitopes plus two isotype controls by flow cytometry (CytoFLEX, Beckman Coulter). The data was calculated as the ratio of each surface epitope's median fluorescence intensity to the corresponding isotype control.

Results

The antigens differentially expressed on EVs between groups are summarized inTable1.

Table1. Expression of the statistically significant antigens in EVs of healthy controls and ASD patients

Antigens	**Controls**	**ASD**	**P value**
Increased in ASD			
HLA-DRDPDQ	3.15(1.84-5.60)	5.40(3.00-6.74)	0.016
CD56	1.02±0.48	1.28±0.55	0.015
CD105	1.49(0.70-2.02)	1.83(1.33-2.44)	0.018
CD49e	0.90(0.61-1.14)	1.16(0.83-1.51)	0.008
SSEA-4	1.26(0.69-1.80)	1.76(1.40-2.35)	<0.001
CD326	1.51±1.07	2.45±3.15	0.050
Decreased in ASD			
CD8	1.68±0.98	1.24±0.73	0.012
CD9	1.57(1.26-3.66)	1.17(0.91-2.06)	0.003
CD40	0.94(0.77-2.15)	0.83(0.71-0.98)	0.010
CD62P	9.93(2.44-19.60)	1.95(1.28-3.56)	<0.001
CD31	1.40(1.12-3.40)	1.17(1.02-1.50)	0.009

Conclusions

The analysis of antigen expression on EVs in ASD showed a profile of expression of antigens involved in activation and molecular adhesion. However the role in the pathogenesis of ASD is still unclear and further studies should be addressed in order to establish a link between inflammation and ASD.

Sesión Poster Defensa 1. Pantalla 5. Miscelánea (Inmunidad anti-tumoral / Alergias y reacciones de hipersensibilidad / Vacunas) (I)

P-086

EVALUATION OF PREDICTIVE CAPACITY OF BASOPHIL ACTIVATION TEST IN ANAPHYLAXIS TO PENICILLIN-DERIVATES: OUR EXPERIENCIE

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Background: Basophil activation test (BAT) is a useful non-invasive assay used in the diagnosis of drug allergy, as it allows the recreation of the natural immune reaction in an "ex-vivo" manner. However, its low sensitivity implies the use of other invasive and life-threatening tests when negative results. The aim of this analysis was to evaluate the sensibility (S), specificity (E), positive predictive value (PPV) and negative predictive value (NPV) of BAT to penicillin-derivates in our centre.

Material and methods: A cohort of 50 patients with a history of anaphylaxis to penicillin- derivates drugs (according to EAACI classification) from 2018 to 2021 were subjected to BAT using penicillin G (PEN), amoxicillin (AMX), ampicillin (AMP), amoxicillin-clavulanic acid (AMX-C) and/or clavulanic-acid (CLA). Skin test (ST) was accomplished using penicilloylpolylysine (PPL), minor determinant mix (MDM), AMX, AMP and/or CLA. Some ST, specific IgE (sIgE) and oral provocation tests (OPT) were not performed because they were not available or because of a positive BAT result.

Results: Fifty patients (64% female; average age: 45±20,97 years) were studied for anaphylaxis to penicillins (32 patients had anaphylaxis grade 2 EAACI, 18 grade 3 EAACI; <12months in 74%). Drugs involved were AMX in 26 (52%) patients, AMX-C in 23 (46%) patients and PEN in 1 (2%) patient. Percentages of positivity to BAT were 34% to AMX, 34% to PEN, 64% to AMX-C and 68% to CLA. OPT was performed in 31 (62%) patients, in 14 (28%) with

the involved drug, while in 19 (34%) with an alternative drug. Test sensibility and specificity were
75% and 100% respectively. PPV value was 100%, whereas VPN was 50%.

Conclusions. Our results are in line with those previously published. The poor specificity and NPV compel the performance of other diagnostic tests. A standardization of BAT is needed to improve its predictive capacity.

P-087

INCREASED FREQUENCY OF CTLA-4 AND PD-1 EXPRESSING REGULATORY T CELLS AND BASOPHILS WITH ACTIVATING PROFILE IN INFANTS WITH MODERATE-TO-SEVERE ATOPIC DERMATITIS HYPERSENSITIZED TO FOOD ALLERGENS

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Infants with severe Atopic Dermatitis (AD) may be sensitized to foods that have not been introduced into their diet, posing a risk for developing an immediate hypersensitivity reaction on the first exposure to the food that they are sensitized to. The aim of this work was to perform an analysis of the sensitization profile in infants with moderate-to-severe AD and to identify cellular and molecular markers for food allergy (FA).

Using ImmunoCAP FEIA system and ISAC technology, we measured specific IgE to several allergens in blood samples from healthy donors and children with moderate-to-severe AD. Furthermore, using flow cytometry-based studies, basophils and regulatory T (Treg) cells were phenotypically characterized.

We observed that 90% of children with AD were sensitized to food antigens before introducing them into the diet, and that 100% developed FA. Phenotypic analysis showed a significantly higher percentage of CTLA-4 and PD-1 expressing Treg cells in AD patients than in healthy controls. In addition, basophils from patients exhibited a marked reduction in the expression of CD300a, higher expression of FcγRI and CXCR4, and to some extent higher expression of CD63 and CD300c.

Altogether, our results demonstrate that infants with moderate-to-severe AD are at high risk of being sensitized to food allergens. Therefore, to avoid allergic reactions, broad-spectrum sensitization studies would be necessary before introducing complementary diet. Increased expression of CTLA-4 and PD-1 suggests a greater suppressive potential of Treg cells in infants with AD than healthy controls. Furthermore, our results also suggest a role for CD300 molecules on circulating basophils as biomarkers for FA susceptibility.

P-088

LAS CÉLULAS NK INDUCEN MUERTE POR NECROPTOSIS A TRAVÉS DEL TNF-A EN CÉLULAS TUMORALES SUSCEPTIBLES

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Las células NK pertenecen al sistema inmunitario innato y se caracterizan por tener una elevada capacidad citotóxica gracias, principalmente, al sistema perforina-granzimas y los miembros de la familia del TNF. Tras el reconocimiento de una célula tumoral o infectada, la célula NK provoca la muerte de la célula diana, mediante diferentes vías de muerte. No obstante, no está claro el papel del TNF-α y de la muerte celular por necroptosis en la acción antitumoral de las células NK. En este estudio se ha demostrado como, tanto con la utilización de células NK activadas de donantes sanos, como con la línea celular NK92 que las células NK son capaces de inducir necroptosis a través de la producción de TNF-α en células tumorales sensibles a este tipo de muerte, relacionada con la expresión de la quinasa RIP3. Células que sobreexpresan RIP3, son sensibles a la citotoxicidad inducida tanto por el TNF-α, como por las células NK, especialmente, en condiciones donde la apoptosis se encuentra disminuida mediante el inhibidor de caspasas Z-VAD-FMK y en presencia de un inhibidor de cIAP (miméticos de Smac). En estas condiciones, la muerte inducida tanto por células NK como por los sobrenadantes de estas, se encuentra drásticamente reducida cuando se elimina el receptor de TNF-α de la célula o la proteína RIP1. Este fenómeno se observa también en células tumorales que de forma natural expresan RIP3.

Por lo tanto, los resultados obtenidos sugieren que el TNF-α secretado por las células NK tras el reconocimiento de una célula tumoral, puede inducir necroptosis especialmente en presencia de moléculas que sensibilizan a esta vía de muerte. Este hecho podría ayudar a desarrollar nuevas inmunoterapias, potenciando la citotoxicidad de las células NK sobre células tumorales resistentes a la apoptosis y favoreciendo la activación hacía vías de muerte más inmunogénicas.

P-089

ORAL SQUAMOUS CARCINOMA INDUCES IMMUNOSUPRESSIVE MACROPHAGES

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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 cultured primary monocytes from healthy donors' peripheral blood for 5 days in the presence of conditioned media derived from the H413 and TR146 OSCC lines. Also, we have co-cultured conditioned-monocytes with allogeneic 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 imprint an immunosuppressive phenotype on monocytes related to M2 macrophages, judged by the lower expression of HLA-DR, CD86, CD11c and increase of CD163, CD206, 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. In addition, the phagocytic ability of these monocytes-conditioned was decrease. In contrast, the medium derived from these conditioned-monocytes had the ability to enhance the proliferation of the OSCCs, presumably due to the secretion of VEGF.

Conclusion

Our data indicate that OSCCs promote the differentiation of immunosuppressive monocytes into an M2-like phenotype, which may facilitate tumor progression.

P-090

DIAGNÓSTICO INTEGRADO DE LEUCEMIAS MIELOIDES AGUDAS (LMA) EN PACIENTES PEDIÁTRICOS-ADOLESCENTES: A PROPÓSITO DE UN CASO CLÍNICO.

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La leucemia mieloide aguda (LMA) es una neoplasia hematológica muy agresiva caracterizada por la proliferación clonal de células mieloides inmaduras en diferentes estadios madurativos. Afecta predominantemente a adultos, siendo poco frecuentes los casos en que dicha patología se presenta en pacientes pediátricos o en la adolescencia.

El presente caso supone un ejemplo de abordaje multidisciplinar frente a una sospecha de neoplasia hematológica en una paciente de 14 años. El examen de médula ósea y el estudio inmunofenotípico mostraron resultados compatibles con una LMA M5 según la clasificación Francesa-Americana-Británica (FAB), de predominio promonocítico (70%) con expresión aberrante de diversos marcadores (CD14-, CD15+, CD13-, CD11b-).

El cariotipo hematológico puso de manifiesto un cariotipo hiperploide, observándose trisomías de prácticamente todos los cromosomas. En los estudios de FISH (Hibridación *In Situ* Fluorescente), no se observaron ninguna de las alteraciones cromosómicas habituales en casos de LMA. La paciente tampoco presentaba mutaciones en *FLT3* ni *NPM1*.

Los resultados obtenidos mediante técnicas de secuenciación masiva confirmaron el complejo perfil molecular de la paciente: el estudio de variaciones en el número de copias (CNV) fue concordante con los resultados aportados por el cariotipo hematológico; el estudio de mutaciones, por su parte, fue positivo, portando variantes patogénicas en *EZH2, CBL*, *PHF6* y *KRAS*. Dicho perfil mutacional es poco frecuente en casos de LMA, y solo EZH2 se encuentra recogido como marcador de riesgo adverso en clasificaciones de riesgo genético en LMA de adultos (European LeukemiaNet (ELN) 2022).

El trabajo multidisciplinar de los servicios de Hematología e Inmunología permitió el diagnóstico diferencial de la paciente con LMA M5, la cual fue calificada como riesgo adverso y estableciéndose un régimen de inducción con citarabidina y antraciclina (inducción 7+3). Actualmente, la paciente se encuentra en progresiva mejoría y en búsqueda de un donante compatible para trasplante de progenitores hematopoyéticos.

P-091

CHARACTERIZATION OF CYTOKINE INDUCED KILLER CELLS: ANALYSIS OF THE EXPRESSION OF ACTIVATING AND INHIBITORY RECEPTORS

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Cytokine-induced killer cells (CIK) cells are a heterogeneous group of immune effector cells exhibiting a mixed T- and natural killer (NK) cell-like phenotype, obtained by in vitro incubation of human peripheral blood mononuclear cells (PBMC) with interferon (IFN)-γ, interleukin (IL)-2, and anti-CD3 monoclonal antibody. Their cytotoxic capacity is mainly attributable to $CD3^+$ $CD56^+$ cells and it is not restricted by the major histocompatibility complex (MHC), allowing them to recognize virus-infected or malignant cells in the absence of MHC. The regulation of CIK cell function by inhibitory and activating receptors is a crucial aspect of immune regulation and phenotype characterization is essential to understand their functional properties and optimize their use in cancer therapy.

In this study, CIK cells were cultured and expanded from PMBC of healthy donors with the addition of IFN-γ, IL-2, and anti-CD3 antibody for 21 days. The phenotype of CIK cells has been characterized, before and after the expansion, to determine the expression of activating and inhibitory receptors.

The results show that CIK cells mainly express the TCR-alpha/beta and CD8 cells at the end of expansion. There was a significant increase in the activating receptors of DNAM-1 and NKG2D, while the most expressed inhibitory receptors were TIM-3 and LAG-3. Furthermore, we found a significant increase in CD6, Fas, and Perforin expression. On the contrary, a significant decrease was observed in the expression of KIR2D and NKp80. Analysis of CIK function showed that CD3 and to a lesser extent NKG2D play a role in redirected degranulation and cytokine production. Inhibitory receptors also regulate the functional capacity of these cells.

In summary, the results support that advances in the phenotype and functional analysis of CIK cells are critical for the development of effective immunotherapies for cancer treatment.

P-092

HLA-G Y SU RECEPTOR ILT2 EN TLS DE PACIENTES CON CÁNCER GÁSTRICO

Vaquero Yuste, Christian[1]; Molina Alejandre, Marta[1]; Juárez Martín-Delgado, Ignacio[1]; Suárez Trujillo, Fabio[1]; Gutiérrez Calvo, Alberto[2]; López García, Adela[2]; Lasa Unzue, Inmaculada[2]; Gómez Sanz, Remedios[2]; Fernández-Cruz Pérez, Eduardo[3]; Rodríguez Sáinz, Carmen[3]; Arnaiz Villena, Antonio[4]; Martín Villa, José Manuel[4].

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Introducción: Las estructuras linfoides terciarias (TLS), son agregados inmunitarios ectópicos frecuentemente asociados a una exposición prolongada a señales inflamatorias (infección, autoinmunidad y cáncer). Las TLS llevan a cabo funciones de activación, proliferación y diferenciación de células T y B a nivel local.

La presencia de TLS en cáncer gástrico se ha relacionado con un mejor pronóstico de los pacientes, aunque se desconocen muchos de los mecanismos de origen o función de estas estructuras en el contexto de la respuesta anti-tumoral.

HLA-G es una molécula HLA no clásica capaz de inducir tolerancia a través de su receptor ILT2 en linfocitos T y otras células inmunocompetentes. Se desconoce la implicación de la vía HLA-G/ILT2 en las TLS, pero su presencia podría suponer la inhibición de la respuesta anti-tumoral. Bloquear la vía HLA-G/ILT2 podría suponer una nueva terapia ICI.

Objetivos: Describir la expresión de HLA-G y sus receptores en TLS de pacientes con cáncer gástrico.

MyM: Se fenotiparon PBMCs de 14 pacientes de cáncer gástrico y 15 controles mediante citometría de flujo, y se realizaron tinciones de IHQ en cortes de tejido tumoral y distal de los pacientes.

Resultados: Encontramos un aumento de la expresión de ILT2 en linfocitos CD8+ de PBMCs de pacientes (40,6%) con respecto a los controles (9,9%, $p<0,001$) (Figura 1). Además, encontramos la expresión de HLA-G e ILT2 en las TLS (Figura 2). Por último, observamos una correlación negativa entre la presencia de HLA-G y la cantidad de células CD8+ en las TLS ($R^2=0,3201$; $p=0,0439$), indicando una posible inhibición mediada por HLA-G (Figura 3).

Conclusión: La presencia de HLA-G y su receptor ILT2 en las TLS de los pacientes con cáncer gástrico sugiere una implicación de esta vía de señalización en la respuesta anti-tumoral, y

serviría como marcador pronóstico y como nueva diana terapéutica para tratar a estos pacientes.

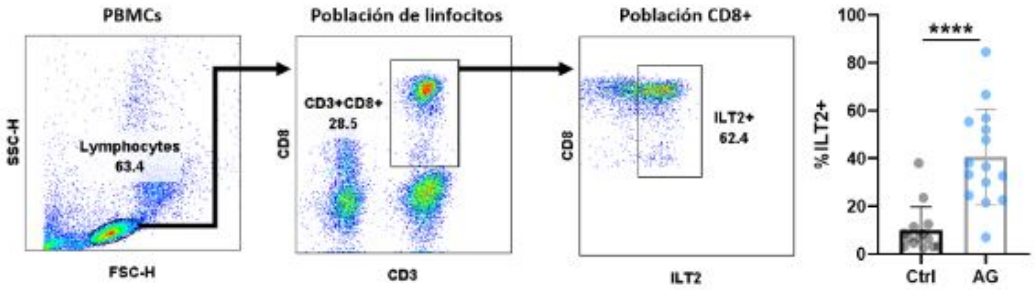


Figura 1: *Aumento de la población ILT2+ en células T citotóxicas de sangre periférica en pacientes con adenocarcinoma gástrico (AG).* Estrategia de fenotipado y gating para seleccionar la población citotóxica y determinar la frecuencia de células ILT2+. Se observa una mayor frecuencia de células ILT2+ en pacientes con AG (40,6%) en comparación con la población Ctrl (9,9%, p<0,001). Estos datos apuntan a que la molécula ILT2, y por tanto la vía HLA-G/ILT2, puede ser una diana relevante en el tratamiento de estos tumores. **** = p<0,001.

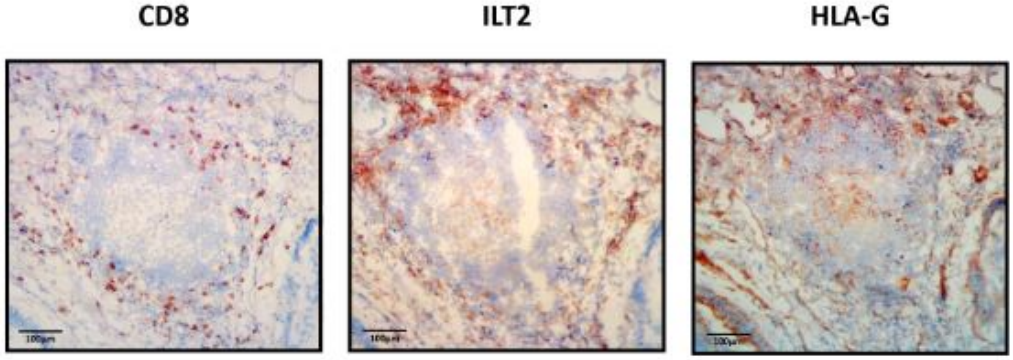


Figura 2: *Expresión de HLA-G, ILT2 y CD8 en tejido de pacientes.* Observamos la presencia de células CD8+ principalmente en la periferia de las TLSs, lo que coincide con lo descrito en la bibliografía. También se puede apreciar la expresión de ILT2, repartido por toda la TLS. Parte de estas células serán CD8+ILT2+, como reflejo de lo observado en la sangre de los pacientes. Por último, destaca la presencia de HLA-G en estas TLSs, lo que puede inducir un microambiente tumoral inmunosupresor. Esta observación podría ser de gran relevancia en el contexto del desarrollo de una inmunoterapia dirigida a bloquear la vía HLA-G/ILT2.

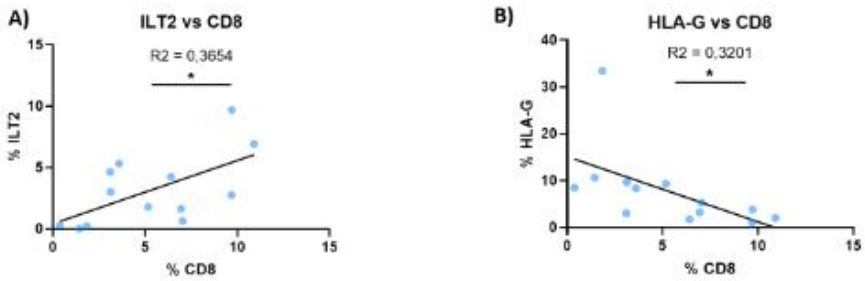


Figura 3: *Correlación de los marcadores estudiados en TLSs de pacientes con cáncer gástrico.* Se observa una correlación positiva entre la cantidad de células ILT2+ (A) y negativa (HLA-G) (B) con la frecuencia de células CD8+ (p= 0,0286 y p=0,0439, respectivamente) en las TLS de los tumores gástricos. Estos datos sugieren que parte de las células CD8+ en el interior de las TLS expresan ILT2, y la presencia de HLA-G reduce la frecuencia de las células CD8 en el interior de estas estructuras. * = p<0,05.

La presente investigación fue financiada por una subvención del ISCIII número PI18/00626.

P-093

OPTIMIZATION OF DRUG SPECIFIC MEMORY T-LYMPHOCYTES DETECTION IN A CASE OF SEVERE DRESS SYNDROME

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Drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome is a severe multiorgan hypersensitivity reaction caused by some drugs and mediated by T-lymphocytes. When a DRESS is diagnosed, it is necessary to confirm that the suspicious drug is the cause of the reaction in order either to avoid a new exposition to this drug or to prevent from an unnecessary avoidance of a drug. However, in vitro techniques currently used have quite limitations. The aim of this work is to study the case of a severe DRESS syndrome probably caused by minocycline that triggered in a fulminant hepatitis and the requirement of liver transplantation in a fifteen years old patient . We also tried to improve the available techniques to detect these specific antigen responses. Three different techniques to test the activation and expansion of the drug-specific memory T-lymphocytes were performed: Cell Trace Violet Proliferation test, Ki67 staining, both analyzed through flow cytometry, and interferon-gamma IFN-γ-based Enzyme-Linked Immunospot (ELISpot) assay. Different variables as time of co-incubation of PBMCs and the drug, use of different cytokines and different concentrations of the drug were tested in order to improve the sensibility of these techniques. The results obtained showed that both Cell Trace Violet Proliferation and the Ki67 detection did not show enough sensitivity for drug specific T-lymphocytes detection in this patient. ELISPOT assays allowed the detection of this response and a great improvement in sensitivity was achieved when PBMCs were cultured in presence of 0.1-2 µg/ml of minocycline for 6 days prior to ELISPOT. The costimulation with IL2 or IL7+IL15 in these cultures potentiated the response although it also generated a nonspecific response in negative controls. All together, our results suggest that an optimized ELISPOT technique could be the best option for the detection of specific drug memory T-lymphocytes in DRESS syndrome.

P-094

SAPONIN-BASED ADJUVANTS: NEW INSIGHTS INTO THE MOLECULAR MECHANISMS BY DECIPHERING A POTENTIAL RECEPTOR.

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Cic Biogune, Derio.

Adjuvants are key substances in vaccine formulations that have been studied for almost a century. They enhance vaccines' immunogenic capacity and, therefore, provoke a stronger immune activation. Nonetheless, toxicity problems, among others, restrict the use of many of them in humans. Aluminum-based adjuvants have been traditionally used in the clinic but more recently other molecules have been developed, being the saponin-based family one type of adjuvants that have gained relevance. However, little is known about their molecular mechanism and scant mechanistic studies have been performed to date. Therefore, our work seeks to get more insights on the mechanisms of saponin immunopotentiation. More concretely, we seek to identify the molecular receptor responsible for their activity using a chemical proteomics approach. Synthetic saponin variants were chemically developed deriving from QS-21's structure, in order to obtain a simplified and less toxic molecule, but with the same effector capacity. Moreover, a photoaffinity probe consisting of a light-activatable group and a biotin tag was added to the structure of our saponin, enabling subsequent photo-crosslinking and detection with streptavidin. Saponin was first incubated with RAW264.7 cells at different times, followed by staining and confocal microscopy observation, to set an optimized time of incubation. Then, western blotting assays and proteomics were performed, so that the receptor to which saponin does bind could be detected. Preliminary western blot results show a potential receptor, which will be compared to the results obtained by the proteomics. Furthermore, the incubation and immunofluorescence assays will be repeated in RAW264.7 cells (wildtype and with the corresponding potential receptor silenced), aiming to confirm that protein as the saponin receptor.

P-095

FINDING OF A SUBCLINICAL PNH CLONE IN A 5-YEAR-OLD GIRL WITH BONE MARROW APLASIA

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Introduction

A 5-year-old girl came to the Hospital Emergency Department with her parents due to the appearance of multiple petechiae on the trunk and face of 3-4 days' evolution, as well as haematoma on the leg. She had not had any accompanying symptoms. The initial study revealed pancytopenia whose origin was determined by bone marrow biopsy as bone marrow aplasia, ruling out myelodysplastic syndrome and acute leukaemia.

Objective

Study and affiliation of pancytopenia to exclude paroxysmal nocturnal haemoglobinuria (PNH) by flow cytometry analysis (FCM).

Material and method

Patient's peripheral blood was assessed by FCM to analyse CD157 and FLAER expression on mature monocyte and neutrophil population and glycophorin (CD235a), CD55 and CD59 expression on erythrocytes.

Results

In the context of pancytopenia, the FCM study revealed a peripheral blood population of monocytes and neutrophils (0.7 and 0.5%, respectively) with negative expression for CD157 and FLAER markers, and a population with simultaneous negative expression for CD59 and CD55 as well as CD235a representing 0.06% of erythrocytes, and could be classified as subclinical PNH.

Conclusions

PNH is a rare disease, especially in children, caused by a mutation in the PIG-A gene that prevents proper protein binding to GPI, resulting in the presence of a haematopoietic clone. The form of presentation of this disease is very varied and its association is relatively frequent in patients with bone marrow aplasia. Therefore, screening for the presence of a possible PNH clone is necessary in patients diagnosed with bone marrow aplasia. This association has also been described as having a better prognosis.

We present a rare case of subclinical PNH in a 5-year-old girl diagnosed with bone marrow

aplasia. The determination of this clone supported the diagnosis of acquired haematological bone marrow aplasia, making congenital aetiologies such as Fanconi anaemia or dyskeratosis congenita less likely.

P-096

CLINICAL RELEVANCE OF MOLECULAR DIAGNOSIS IN PATIENTS SENSITIZED TO THE HOUSE DUST MITE *DERMATOPHAGOIDES PTERONYSSINUS*. RETROSPECTIVE STUDY OF CASES IN THE PROVINCE OF CADIZ

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Hospital Universitario Puerta del Mar, Cádiz.

INTRODUCTION

Dust mite allergy is the principal cause of respiratory allergy and the main trigger of asthma exacerbations. *Dermatophagoides pteronyssinus* (d1) is the main house dust mite and contains two main allergens: der p 1 and der p 2. Der p 23 is another major d1 allergen, which has been demonstrated to be a predictive marker of seriousness for asthma and is underrepresented in allergen-specific immunotherapy (AIT).

OBJECTIVES

To describe a cohort of patients sensitized to the allergenic components of d1 and to determine those who are monosensitized to der p 23. As well as to analyse whether the molecular study of major allergens is an advantage in deciding and/or modifying the AIT decision.

PATIENTS AND METHODS

The cohort consists of 319 patients from the province of Cadiz selected from the Der p 23 study from January to December 2022. Clinical data were collected for each patient and the levels of specific IgE (UIa/mL) against the full extract (fe) of d1 and the specific components (sc) der p 1, der p 2 and der p 23. The ratio 2 ($_s$IgE-sc/$_s$IgE-fe) of these components was determined.

RESULTS

Thirty patients (9.4%) were monosensitized to der p 23 and 18 (5.6%) were not sensitized to any sc. Thirty-one percent of the patients monosensitized to der p 23 had a medical record of asthma. Thirteen patients who were not monosensitized had der p 23 as the major allergen according to ratio 2.

CONCLUSIONS

Forty-three patients of the total cohort could have a less effective AIT as they are

monosensitized to der p 23 or have a majority sensitivity to it. The determination of der p 23 allows us to identify these patients and to have a better criterion on the potential success of AIT.

P-097

HLA-CLASS II ALLELES AS RISK FACTOR OF FOLLICULAR LYMPHOMA

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Department of Haematology, University Hospital of Salamanca (HUS-IBSAL), CIBERONC, and Cancer Research Institute of Salamanca-IBMCC (CSIC-USAL University), Salamanca, Spain, Salamanca.

Introduction: Follicular lymphoma (FL) is an indolent cell lymphoproliferative disorder that arises from germinal centre B-cells of the lymphoid follicle. It accounts for ~20-30% of the total non-Hodgkin lymphoma (NHL). Gene variants at 6p21.3 region have been related to NHL susceptibility, including FL. The key role of the HLA system in tumour antigen presentation could be involved in the disease susceptibility and control.

Objective: We aimed to analyse the role of HLA polymorphisms in a cohort of patients with FL at our centre and determine their impact in FL development.

Methods: HLA-A, -B, -C, -DRB1 and -DQB1 typing was performed by PCR sequence specific oligonucleotide (SSO) technique (Lifecodes, Immucor) in 213 FL patients. Data from 1950 healthy individuals from the same geographical area previously published by our group were used as control group. Comparisons between populations were performed by Fisher's exact test and corrected *P*-values (*Pc*) were obtained using the Bonferroni method to mitigate the error of multiple comparisons.

Results: We found that patients carrying *HLA-DRB1*01* allele [43.7% *vs.* 19.5%, OR=3.2 (95%CI 2.4-4.3), *P*c<0.001] or *HLA-DQB1*05* allele [50.7% *vs.* 30.9, OR=2.3 (95%CI 1.7-3.1), *P*c<0.001], alleles that are in linkage disequilibrium, have higher incidence of FL when compared with healthy controls.

Conclusions: Our results suggest a role for HLA class II alleles in the risk of FL development.

These findings are in line with previous studies, which have identified an association between *HLA-DRB1*01* allele and higher risk of FL (Wang *et al*, Blood 2010; Baecklund *et al*, Am J Epidemiology 2017), and other germinal centre lymphomas such as diffuse large B-cell lymphoma (Alcoceba *et al*, Blood 2013). Further analysis regarding haplotype and impact on survival are ongoing.

P-098

COMPUTER-ASSISTED IDENTIFICATION OF TREG CELL EPITOPES

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Universidad Complutense Madrid, Facultad de Medicina, Departamento Inmunologia, Madrid.

Regulatory CD4 T cells (Tregs) play an important role in the maintenance of self-tolerance, avoiding immune responses against self-antigens. The most important and numerous group of Tregs expresses CD25 and FoxP3, develops in thymus and recognizes epitopes derived from self-antigens with high affinity. Tregs can be stimulated to expand and respond with epitopes known as Tregitopes. Clearly, the identification of Tregitopes is relevant to the development of new immunosuppressive therapies. Here we predicted ~200 Tregitopes in thymus-specific antigens by anticipating peptides that can bind with high affinity to class II human leukocyte antigen molecules (HLA II). Within the computationally predicted epitopes we synthesized 11 predicted Tregitopes and by using FACS analysis we have been able to find 4 peptides in human and 2 peptides in wild type mice that can expand IL-10 producing CD4+CD25+FoxP3+ Tregs both in human PBMCs and in mice splenocytes. We are confident that the results derived from this work will be instrumental to understand/study T regulatory cell immunobiology and will be useful for developing pan-specific tolerogenic treatments.

P-099

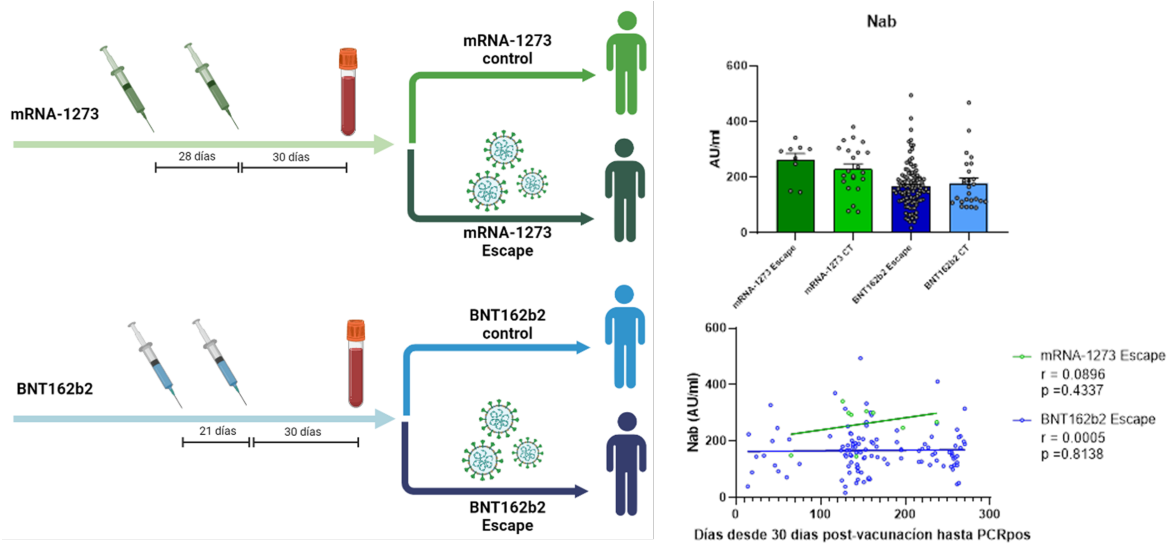
NEUTRALIZING ANTIBODY LEVELS CONFERRED BY MRNA-BASED VACCINES ARE NOT PREDICTIVE FOR BREAKTHROUGH INFECTIONS OF SARS-COV-2

Gil Manso, Sergio[1]; Alonso, Roberto[2]; Catalán, Pilar[2]; Sánchez-Arcilla, Ignacio[3]; Marzola, Marco[3]; Correa, Rafael[4]; Pion, Marjorie[1]; Muñoz, Patricia[2].

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The development of COVID-19 vaccines against the SARS-CoV-2 was an unprecedented achievement during an urgent medical need. During the first trimester of 2021, healthcare workers were placed first in line to receive the mRNA-derived COVID-19 vaccines (Hospital General Universitario Gregorio Marañón) and received the two doses of the mRNA vaccines BNT162b2 and mRNA-1273, developed by Pfizer/BioNTech and Moderna, respectively. After vaccination, a seroprevalence study was performed 30 days' post-vaccination on all those who volunteered, determining the levels of IgG Anti-RBD and neutralizing antibodies (Nab) against SARS-CoV-2. These determinations were pivotal to verify that the vaccination induces immunoglobulins (Ig) that protect from virus infection and spreading. Between March and November of 2021, breakthrough infections were reported in vaccinated healthcare workers. A breakthrough is defined as an infection in an individual without COVID-19 history and who is vaccinated with two doses. 116 breakthroughs were reported in individuals vaccinated with BNT162b2 and 12 in those vaccinated with mRNA-1273. We compared the anti-SARS-CoV-2 Nab levels in samples obtained 30 days' post-vaccination in those individuals and in a control cohort to determine if levels of anti-SARS-CoV-2 Ig could be a predictive factor of breakthroughs. First, no significant differences were observed between the control cohort and the breakthrough cohort. Further, we correlated Nab AU/ml levels with the elapsed time between 30 days' post-vaccination and the infection. We wanted to determine if individuals with higher levels of antibodies just after the vaccination took longer to be infected. No correlation was observed for both vaccines, and we concluded that anti-SARS-CoV-2 immunoglobulines levels cannot be used as predictive factors of potential future infection.

Likely other factors (individuals, specific cellular response, repetitive virus exposition, viral load, variants, etc) could be determinant to signal the loss of protection.



P-100

SECOND BOOSTER (FOURTH DOSE) OF MRNA-1237 PARTIALLY RESCUES THE RESPONSE TO VACCINATION IN KIDNEY TRANSPLANT RECIPIENTS.

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

[1]Dpto. de Inmunología, Oftalmología Y ORL. Facultad de Medicina. Universidad Complutense de Madrid, Madrid; [2]Nephrology Department, Institute San Carlos for Medical Research (Instituto de Investigación Sanitaria del Hospital Clínico San Carlos (IdISSC), San Carlos Clinical University Hospital, Madrid; [3]Dpto. de Biología Celular. Facultad de Medicina. Universidad Complutense de Madrid, Madrid.

Introduction

Immunocompromised patients have an increased risk of developing severe COVID disease and show a limited response even to booster vaccination. Patients undergoing kidney transplantation have pharmacological immunosuppression, which hinders their potential response to vaccination.

Objectives

To study the cellular and humoral response to SARS-CoV-2 after second booster (fourth dose) mRNA-1273 (Moderna-BioNTech) vaccination in a cohort of kidney transplant recipients (KTR)

Material and methods.

146 KTR from the Hospital Clínico San Carlos received four doses of mRNA-1217. Blood was obtained prior to vaccination and at 3 time-points after the administration third and fourth doses with an interval of six months between shots. Anti-RBD IgG and the neutralization capacity of the sera were analyzed by ELISA. Cellular response (CD4 and CD8 T-cells) against Spike-protein peptides was analyzed by flow cytometry.

Results

Follow-up data were obtained after 2 months of the 4th dose and 55 patients were found to have passed the infection. After the fourth dose, naive patients (91/146) had the following response

rates: 75.6% humoral and cellular response, 12.2% only cellular response, 9.8% only humoral and 2.4% no response (vs 54.2%, 18%, 22.2% and 5.6% and 3rd-dose, respectively).

Regarding the immunosuppressive treatments, only mTOR inhibitor showed association with SARS-CoV-2 IgG >50 AU/ml (technique positivity range) [OR 7.31 95% CI (0.92-58.99), p=0.056,] or neutralizing activity >30% (50th percentile of the sample) [OR 3.23 95% CI (1.17-8.90), p=0.030]. No association with cellular response was found.

Exposure to SARS-CoV-2 after receiving the second booster increased anti-Spike IgG titer and neutralizing activity in patients (Table 1).

Conclusions

Administration of a second booster to KTRs increased their humoral and cellular responses. Treatment with mTOR inhibitors was associated with enhanced humoral and neutralizing antibody responses. SARS-CoV-2 infection was a marker of increased both humoral and cellular response.

Table 1.

	SARS-CoV-2 *naïve*	SARS-CoV-2 covalescent	p-value
IgG anti-S (AU/mL)	3640 (279-15940)	23867 (12025-59346)	**<0.001**
Neutralizing Activity (%)	30.0 (7.9-62.9)	74.4 (51.3-82.4)	**<0.001**
IFNγ-CD4 (%)	0.28 (0.02-0.56)	0.31 (0.09-0.55)	0.546
IFNγ-CD8 (%)	0.15 (0.01-0.48)	0.30 (0.15-0.83)	**0.005**

P-101

ELEVATED FREQUENCY OF NON-CLASSICAL (CD16+) MONOCYTES IN COLORECTAL CANCER

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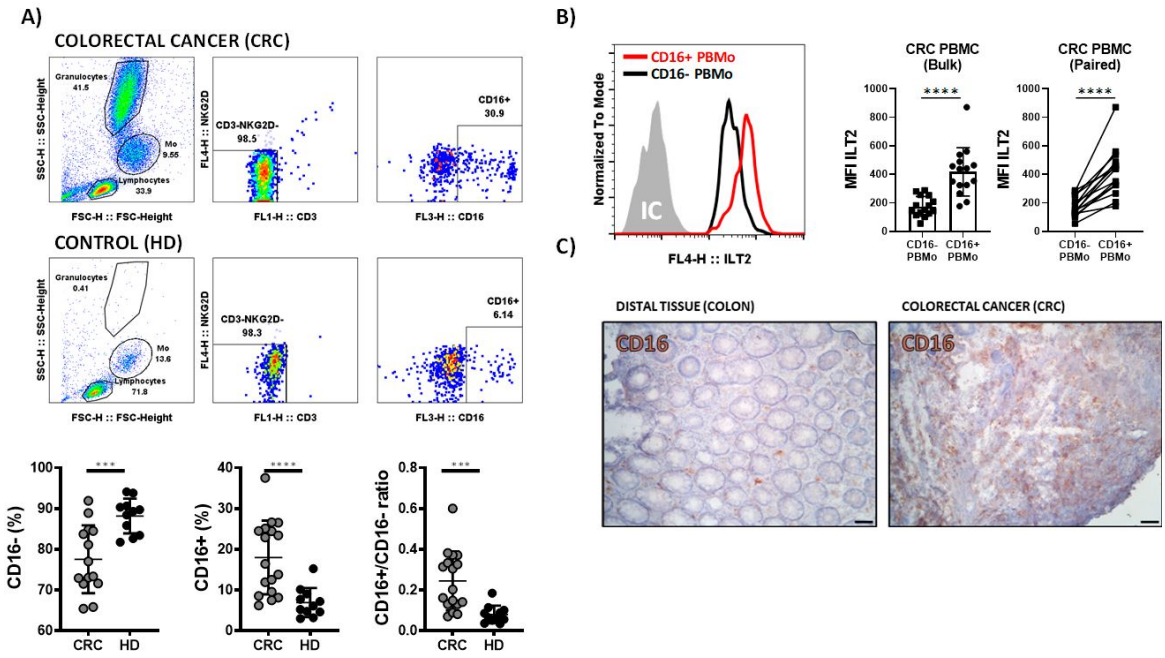
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The CD14- CD16+ monocytes population is known as non- classical monocytes and represent a type of mononuclear cells with both pro- (angiogenesis, immunosuppression) and anti-tumorgenic (metastasis prevention, antibody- mediated cytotoxicity, NKs recruitment) functions (Olingy et al. 2019). Because of their pro- and anti-tumorigenic capacity, the study of non-classical monocytes in the context of cancer is of clinical relevance.

In this work, the frequency of CD16+ peripheral blood monocytes (PBMo) and the expression of different markers associated with their function by flow cytometry in 17 patients with colorectal cancer (CRC, Hospital Clínico San Carlos) and 11 healthy donors (HD) was evaluated.
Increase in the CD16+ PBMo population (18.6%±9.1) compared to the control population (6.0% ± 3.9; $p<0.0001$ t- test) was found, in detriment of the CD16- population (77.4%±8.3 vs 88.9%±4.7, $p=0.0002$ t- test), affecting the CD16+/CD16- ratio (0.25±0.15 in CRC vs 0.07±0.04 in HD, $p=0.0002$ t- test) (Figure 1A). The CD16+ PBMo population exhibits higher ILT2 expression (MFI=398.3±175.8) compared to the CD16- PBMo (MFI=164.7 ± 72.6, $p<0.0001$, paired and unpaired t- test) (Figure 1B). The presence of CD16+ cells in CRC tumors was also confirmed by IHC (Figure 1C).

The results shown reveal an increase in CD16+ monocytes in peripheral blood of CRC patients, probably being the non- classical (CD14- CD16+) or intermediate (CD14+ CD16+) monocyte population, which may have a dual role in tumor development and progression. Remarkably, we found that the CD16+ population presents higher levels of the ILT2 receptor, with a suppressor activity, suggesting a regulation of CD16+ PBMo activity through ILT2 ligands (class- I HLA molecules and a non- classical MHC molecule, HLA- G).

Therefore, we consider of potential interest the study of non- classical (CD16+) PBMo in the context of the immune response in colorectal cancer.



P-102

PREVALENCE OF THE L265P MUTATION IN MYD88 IN PATIENTS WITH HAEMATOLOGICAL MALIGNANCIES AT THE HOSPITAL PUERTA DEL MAR (CADIZ)

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Introduction

The L265P mutation in MYD88 has only been described in neoplastic B cells, and it has been described to promote lymphomagenesis. This mutation is found in 90% of Waldenström's macroglobulinemia, a type of lymphoplasmacytic lymphoma (LPL). However, it has also been identified in other haematological malignancies with variable prevalence.

Objective

To analyse the prevalence of the L265P mutation in MYD88 in haematological malignancies over a one-year period.

Study and comparison of the analytical characteristics of patients diagnosed with chronic lymphocytic leukaemia (B-CLL) with positive determination for this mutation.

Materials and Method

MYD88 L265P determination was performed by fragment analysis with FAM-labelled primers. Descriptive analysis of cases classified according to diagnosis was performed. Mann-Whitney U test was used for the statistical study of patients diagnosed with B-CLL.

Results

Eighty-seven L265P mutation determinations were performed. The most prevalent group was patients diagnosed with LPL. 38 B-CLL patients were studied due to the investigation performed, 7.9% were positive. These patients had a monoclonal IgM peak in the proteinogram. One of the three cases presented an atypical B-CLL immunophenotype with bright expression of CD20 and light chains and negative expression for CD43. These patients had no significant differences in age (p=0.085), lymphocyte count (p=0.223) and percentage (p=0.645) or β2-microglobulin quantification (p=0.261).

Conclusions

The study detected the presence of the MYD88 L265P mutation in all diagnostic groups with the exception of DLBCL as it was determined in only one case.

The highest prevalence was found in LPL, as expected.

The diagnostic, prognostic and therapeutic implications of this mutation make its determination necessary in other pathologies in addition to LPL.

Diagnosis	MYD88 L265P	Total	% Positive
Non diagnostic	0	2	0,0
B-CLL	3	38	7,9
LPL	15	23	65,2
MGUS	3	13	23,1
MZL	1	10	10,0
DLBCL	0	1	0,0
Total	22	87	25,3

Sesión Poster Defensa 1. Pantalla 6. Inmunoterapia

P-103

GUIDING PERSONALIZED CANCER IMMUNOTHERAPY DESIGN WITH EPITOPE PREDICTION MODELS: A VALIDATION CASE ON UROTHELIAL AND HEAD AND NECK CANCERS.

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Identifying immunogenic epitopes -peptidic fragments from cellular proteins that are presented at the cell surface bound to HLA molecules and have the ability to trigger a cellular immune response- is fundamental to rationally design immune-based therapies. A computational pipeline that could predict such immunogenic epitopes would have vast potential applications in biomedicine, ranging from vaccine design against viruses or bacteria to therapeutic vaccination of cancer patients.

Here, we have built a pipeline upon in-house developed and publicly available tools that aims to predict neoepitopes, immunogenic epitopes derived from tumor mutations. The classical approach typically combines identification of mutations through variant calling and a prediction of the HLA binding affinity of the candidate neoepitopes. As a novelty, we have included in our pipeline an ML-based predictor of T cell epitope immunogenicity, PredIG. This tool enables the prioritization of epitopes based on an immunogenicity score. To validate our pipeline in a cohort of human cancer patients, we present a use-case on nine urothelial and head and neck cancer specimens, where we prioritized the initial number of candidate neoepitopes (up to 170,000) to a list of top 15 peptides per patient. Such bioinformatic prioritization is crucial to downsize the candidates to a feasible number for experimental validation and further use in immunotherapies. Moreover, we show functional validation experiments of T cell recognition, including ELISpot assays, using autologous T cells from cancer patients, which determine the in vivo immunogenicity of the selected neoepitopes.

Overall, our pipeline simulates the antigen processing, antigen presentation and TCR recognition processes that an epitope needs to undergo to trigger an adaptive immune response. Hence, we can offer a more comprehensive prediction of actionable targets for a rational cancer immunotherapy design.

P-104

MEMORY T CELLS AS ADOPTIVE THERAPY IN SARSCOV2 PNEUMONIA AND/OR LYMPHOPENIA (RELEASE NCT04578210)

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Background

There are no efficient anti-viral treatments for hospitalized COVID-19 patients. Unspecific treatments like dexamethasone have become the standard of care (SoC). Lymphopenia is a biomarker of disease severity usually presented in hospitalized patients. We performed a phase 2 randomized multicenter trial to evaluate the safety and efficacy of infusion of memory T cells containing SARS-CoV-2 specific T cells versus the SoC treatment.

Methods

Patients were enrolled from January 2021 to January 2022 at 3 hospitals in Spain. 86 patients were randomized into the SoC arm and SoC plus infusion of $1x10^6$/kg memory T cells. Donors were chosen based on the expression of SARS-CoV-2 specific response within the $CD45RA^-$ memory T cells and the most frequent HLA typing. Primary outcome was defined as proportion of participants with normalization of fever and oxygen saturation and lymphopenia recovery through day 14.

Results

Primary outcome for recovery was met in the experimental arm, 74.1% versus 47.5% (p=0.03). Also in the experimental arm, a higher number of patients reached normal lymphocyte values on days 3 and 14 (p= 0.04) with a higher median of normalizing lymphocyte levels on days 3 and

14 (p=0.004, p=0.048). On day 7 the same group showed an increase in NK cells (p=0.04) and higher median of the percentage $CD8^{+}RA^{+}$ T cells (p<0.0001). Donor microchimerism was observed for 3-4 weeks after infusion. Two patients died in the SoC group and none in the experimental one on day 28 (p= 0.19). Total mortality accounted for 4 deaths in the SoC and 1 in the experimental arm (p=0.17). There were no treatment-related adverse events.

Conclusions

Adoptive cell therapy with off-the-shelf $CD45RA^{-}$ memory T cells containing SAR-CoV-2 specific T cells is effective and accelerates lymphocyte recovery of patients with COVID-19 pneumonia and/or lymphopenia. This is a promising targeted therapy for future viral pandemics.

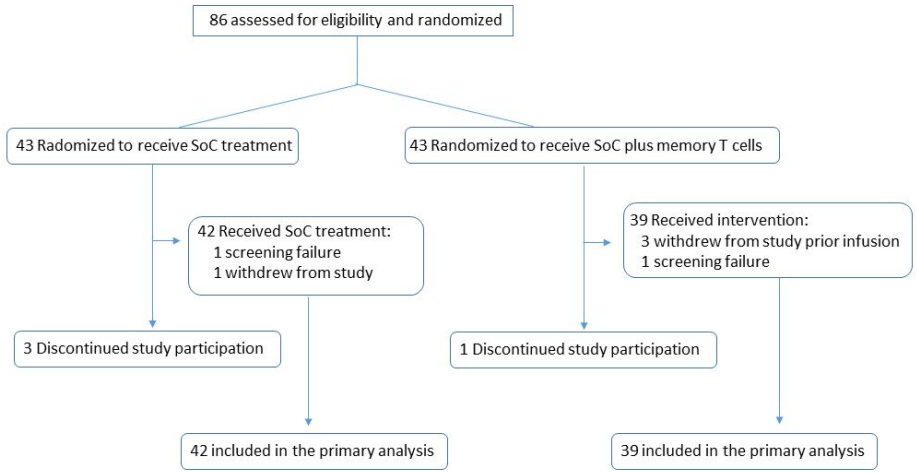


Figure 1. Flow chart of the study. Patients screening, enrollment and treatment assignment

P-105

COMPARATIVA DE LA PRODUCCIÓN DE TERAPIA CAR-T A PARTIR DE AFÉRESIS FRESCA VERSUS CONGELADA

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Hospital Clínic de Barcelona, Barcelona.

Introducción

La terapia de linfocitos T modificados mediante receptores de antígeno quimérico (CAR-T) es producida a partir de la leucoaféresis del paciente. A partir de esta, se aíslan linfocitos T para después modificarlos genéticamente. Posteriormente, son cultivados hasta conseguir la dosis necesaria y el producto se criopreserva hasta la infusión (sólo en dos pacientes, el producto se infundió en fresco).

Materiales y métodos

En el Hospital Clínic de Barcelona se han realizado >240 producciones CAR-T con el mismo biorreactor siguiendo el mismo procedimiento. Una pequeña cohorte (12%, n=29) de estos CAR-T se han producido a partir de aféresis congeladas por diversos motivos: (a) Tiempos de espera para iniciar la producción >48 horas, por alta ocupación de los biorreactores. (b) Fallos en la producción y recuperación de parte de la aféresis congelada. En este trabajo se pretende analizar en detalle parámetros de calidad y de producción CAR-T en ambas situaciones.

Resultados

Así se han comparado diferentes parámetros para producir la terapia CAR-T en ambas situaciones:

Variable	Media		Desviación estándar		P(T£t) dos colas
	Fresca	Congelada	Fresca	Congelada	
Coeficiente de expansión	25,77	14,61	11,15	17,03	0,051
Population doubling level	4,53	3,35	0,74	1,15	0,004
Doubling time (horas)	121,88	434,44	11,03	20,84	0,002
Ratio crecimiento	0,392	0,264	0,061	0,068	$2x10^{-5}$
Eficiencia transducción (%CAR)	36,90	29,47	1,42	1,28	0,103
Tiempo de respuesta (días)	46,95	48,25	51,36	34,66	0,948

Conclusiones

1. Las células CAR-T producidas con ambas estrategias son aceptables pues permiten producir la dosis final necesaria.
2. Las células CAR-T producidas a partir de aféresis congeladas crecen más lento y tardan más en iniciar los ciclos de duplicación.
3. Las células CAR-T producidas con ambas estrategias poseen niveles de transducción y tiempos de respuesta similares.
4. Ambas estrategias de producción son válidas para la producción CAR-T y la criopreservación de la aféresis permite organizar mejor las producciones.

P-107

IMMUNOGENICITY OF CD19 CHIMERIC ANTIGEN RECEPTOR (ARI0001) IN RELAPSED/REFRACTORY CD19 POSITIVE B-CELL MALIGNANCIES

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[1]Plataforma Inmunoterapia, HSJD-Hospital Clínic Barcelona, Servicio de Inmunología del Hospital Clínic de Barcelona, Instituto de Investigaciones Biomédicas August Pi i Sunyer, Barcelona; [2]Plataforma Inmunoterapia, HSJD-Hospital Clínic Barcelona, Servicio de Inmunología del Hospital Clínic de Barcelona, Barcelona; [3]Benaroya Research Institute, Seattle; [4]Plataforma Inmunoterapia, HSJD-Hospital Clínic Barcelona, Servicio de Inmunología del Hospital Clínic de Barcelona, Instituto de Investigaciones Biomédicas August Pi i Sunyerr, Barcelona; [5]Servicio de Hematología, ICMHO, Hospital Clínic Barcelona, Barcelona; [6]Servicio de Hematología, ICMHO, Hospital Clínic Barcelona, Instituto de Investigaciones Biomédicas August Pi i Sunyer, Universidad de Barcelona, Barcelona; [7]Servicio de Inmunología del Hospital Clínic de Barcelona, Instituto de Investigaciones Biomédicas August Pi i Sunyer, Barcelona; [8]Servicio de Hematología, Hospital Sant Joan de Déu de Barcelona, Barcelona; [9]Plataforma Inmunoterapia, HSJD-Hospital Clínic Barcelona, Servicio de Inmunología del Hospital Clínic de Barcelona, Instituto de Investigaciones Biomédicas August Pi i Sunyer, Universidad de Barcelona, Barcelona.

Background: ARI-0001 cells were approved by AEMPS indicated to treat adult patients with relapsed/refractory B-cell acute lymphoblastic leukemia. These ARI-0001 cells express an anti-CD19 single-chain variable fragment (scFv), originated from a murine monoclonal antibody called A3B1, conjugated with the transmembrane and co-stimulatory domains. It has been examined the humoral and cellular immune responses in patients included in the clinical trial CART-BE-01.

Methods: Regarding humoral immune response, human anti-murine antibodies (HAMA) in patients' sera were determined through flow cytometry. Afterwards, cytotoxicity assays were performed to study the effect of HAMA on the CART-cell function. Regarding cellular immune response, predicted CAR19 peptides were used to stimulate PBMCs from treated patients. CD4+ T cells were obtained through activation induced markers (CD154 and CD69). After 30-

35 days of expansion, CD4+ T-cell lines were screened with peptide pools and then it was assessed which HLA present peptides which triggers the T-cell activation.

Results: Twenty out of 47 patients (42.5%) tested positive for the presence of anti-CAR antibodies after ARI-0001 cells infusion. Even though 42.5% had anti-CAR antibodies, only 20% of these HAMA positive patients diminish the cytotoxic effect on ARI-0001 cells *in vitro*. Regarding cellular immune response, a set of 35 prioritized immunogenic HLA-II peptides were chosen for assessing the T-cell activation. In total 43 cell lines were obtained from 5 different patients who developed human anti-murine antibodies. Eight out of 43 cell lines were reactive against CAR19 peptides after 30-35 days of expansion. In relation to CAR19 peptides, immunogenic peptides for these eight cell lines were located at scFv (VL, linker and VH) and were presented by HLA-DR.

Conclusions: These data suggest that our CAR19 induces humoral and cellular immune responses. Thus, immunogenicity induced by ARI-0001 cells could be clinically important, specially before considering a second infusion.

P-108

GENERATION OF CD19 CAR VARIANTS WITH NK CELL SPECIFIC MOTIFS TO DETERMINE THE BEST CHOICE FOR TREATING RELAPSED OR REFRACTORY LEUKEMIAS AND LYMPHOMAS

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B-cell hematologic cancers such as leukemia and lymphoma are common forms of pediatric and adult cancers worldwide. Acute Lymphoblastic Leukemia (ALL) is the most common cancer among children with a prevalence of 20-25% of all cases. The survival rate for these patients at 5 years is 79.2%, but it is still an incurable disease in many patients. Chimeric Antigen Receptor (CAR) T cells therapy has arisen as a new alternative to conventional therapies in order to treat advanced refractory cancers. However, this therapy has some undesirable side effects such as cytokine release syndrome (CRS) and neurotoxicity. Also, despite the good results in clinic, some patients are refractory or relapsed after CART cell treatment. Moreover, another potential problem with this treatment could be the need of using allogenic T cells when is not possible to perform an apheresis to the patient, as allogenic T cells carry a risk of graft versus-host disease (GVHD). NK cells could be a good alternative for CAR based therapy, as they do not cause this kind of side effects, and exhibit a potent graft versus leukemia (GVL) effect without causing GVHD. In this study, we adapted CD19 CAR to NK cells signaling motifs, obtaining different variants of the CAR ARI-0001. These variants were tested in adult peripheral blood and cord blood NK cells in order to determine the best choice to perform a clinical trial phase I to evaluate the safety and viability of allogenic CD19 CARNK cells for treating patients with previous autologous CART treatment.

P-109

INTRATUMORAL NEOADJUVANT IMMUNOTHERAPY BASED ON BO-112 AS A VIRAL MIMETIC

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Many preclinical experiments and clinical studies are demonstrating the beneficial effects of the intratumoral delivery of immunotherapeutic agents with the idea of turning one of the metastatic tumor lesions into an in-situ vaccine capable of unleashing a systemically efficacious immune response. Similarly, several studies have shown the advantages of the pre-surgical neoadjuvant therapy for cancer, including chemotherapy, radiotherapy or hormone therapy. Indeed, presurgical immunotherapy with checkpoint inhibitors has gained evidence to become standard-of-care in resectable cases of melanoma, non-small cell lung cancer and MSI colon cancer. However, local injection of immunotherapy agents has not been explored in the neoadjuvant setting for cancer patients as of yet, with the only exception of the vaccinia virus vector T-Vec. Since the intratumoral injection of the viral RNA analogue BO-112 has shown to be efficacious against established transplanted tumors in mice and in humans when combined with anti-PD-1 mAbs, we sought to evaluate the anti-tumor efficacy of BO-112 applied in neoadjuvant settings in mouse models. We have observed that repeated intratumoral injections of BO-112 prior to surgical excision of the primary tumor significantly reduced tumor metastasis in orthotopic (BALB/c-derived 4T1) and subcutaneous (C57BL/6-derived MC38) tumor mouse models. Such effect was enhanced when combined with anti-PD-1 mAb systemic injections. The anti-tumor efficacy of this neoadjuvant immunotherapeutic approach depended on the presence of antigen-specific effector CD8 T cells and cDC1 antigen-presenting cells. Such a conclusion was drawn from the fact that intratumoral treatment with BO-112 on CD8 T cell-depleted or cDC1-deficient ($BATF3^{-/-}$) mice suffered similar metastatic rates as compared with untreated control mice. Given the fact that intratumoral BO-112 has been successful in phase-two clinical trials for metastatic melanoma, these results provide a strong rationale for translating this neoadjuvant intratumoral immunotherapy to clinical presurgical settings, especially in combination with established checkpoint inhibitors.

P-110

ASSESSMENT OF IMMUNE EFFECTOR CELL-ASSOCIATED NEUROTOXICITY SYNDROME (ICANS) FOLLOWING TREATMENT WITH CD19 CAR-T CELLS: A CASE REPORT.

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Hospital Universitario Ramón y Cajal, Madrid.

Introduction

The use of CAR-T cells against haematological malignancies can result in significant toxicities such as immune effector cell-associated neurotoxicity syndrome (ICANS). Although the clinical features are readily recognizable, its pathophysiology remains poorly understood.

Case description

55-year-old man receiving *axicabtagene-ciloleucel* (anti-CD19 CAR therapy) for diffuse large B cell lymphoma arising from follicular lymphoma, refractory to several lines of treatment. On day +12 post-infusion, he started with symptoms compatible with grade-I ICANS, which evolved to grade-IV (cerebral edema) on day +14.

Materials and methods

We performed flow cytometry analysis on day +14 in paired samples, cerebrospinal fluid (CSF) and peripheral blood (PB), to detect and immunophenotype CAR-T cells. As CAR detection reagent, we used a human recombinant CD19-FITC protein (Acrobiosystems). Cytokine levels were quantified in serum and CSF using Human Inflammatory Cytokine Cytometric Bead Array (Becton Dickinson).

Results

Firstly, we detect infiltrating CAR-T cells in CSF during grade-IV ICANS, representing 5.5% of total CD3+ lymphocytes, higher than PB detection (4.1%). Interestingly, in the CAR+ compartment, the proportion of CD4+ CAR-T cells in CSF was higher than PB (81.4% vs 40.5% respectively) and the proportion of CD8+ CAR-T cells was lower (9.3% vs 46.1% respectively). In contrast, CD4+/CD8+ ratio in non-transduced (NT) counterpart showed a large number of CD8+ T lymphocytes in CSF or PB (0.4 vs 0.03 respectively). Moreover, the subpopulation of CD56+ CAR-T cells was more represented in CSF (14.3%) than PB (11.2%), while its NT counterpart was decreased in CSF compared to PB (57.2% vs 80.5% respectively). Regarding to cytokine levels, IL-6 and IL-8 were increased both in serum or CSF.

Conclusion

We have observed a predisposition of CAR-T cells to migrate to the CNS in a grade-IV ICANS, being mostly CD4+ CAR-T cells. In addition, IL-6 and IL-8 were increased in CSF during acute phase of ICANS.

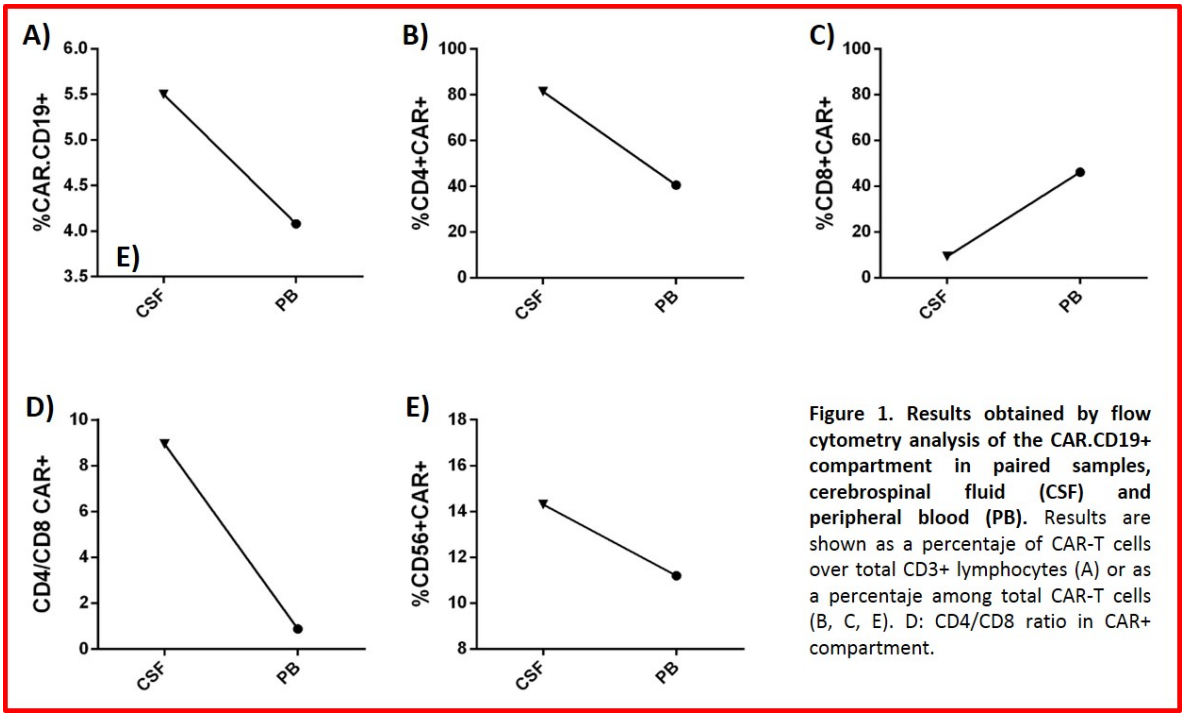


Figure 1. Results obtained by flow cytometry analysis of the CAR.CD19+ compartment in paired samples, cerebrospinal fluid (CSF) and peripheral blood (PB). Results are shown as a percentaje of CAR-T cells over total CD3+ lymphocytes (A) or as a percentaje among total CAR-T cells (B, C, E). D: CD4/CD8 ratio in CAR+ compartment.

P-112

SECOND CART19 CELL INFUSIONS IN ADULT RELAPSED / REFRACTORY B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS AFTER RITUXIMAB + CFM/FLU BASED LYMPHODEPLETION

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Hospital Clínic de Barcelona, Barcelona.

Background

Prognosis of relapsed/refractory (R/R) B-cell acute lymphoid leukemia (ALL) is poor, particularly after allogeneic hematopoietic cell transplantation (alloHCT). Similar prognosis is seen in relapse after CD19- CAR-T therapy (CART19), which have been associated with absence of CAR-T expansion/persistence and/or B-cell aplasia (BCA), as well as the development of HAMAs (human anti-mouse antibodies).

Methods

We report safety and efficacy of adult patients with R/R ALL after ARI-0001 CART19 cells that received a 2nd ARI-0001 infusion in our center (from the CART19-BE-01 trial and the compassionate use program). We also analyzed CART expansion, BCA and presence of HAMAs on both groups.

Results

A total of 12 adult patients with R/R ALL that relapsed after ARI-0001 received 2nd ARI-0001 infusions. At 1st infusion 92% (11/12) patients had alloHCT. All patients received LD with fludarabine and cyclophosphamide, but all 2nd infusions (except 1) also included rituximab on day -6. Patients received a median of 1 (0.5-1) and 3 (0.5-3)x10^{6} ARI-0001 cells/kg for 1st and 2nd infusions, respectively. There was no severe CRS nor any grade ICANS for both groups. Complete response-rates after ARI-0001 were seen in 100% (12/12) and 67% (8/12) for 1st and 2nd infusions, respectively. Among responders after 2nd infusions (8/12), half of them remain in remission (4/8) at month +27, +25, +13 and +8, two of which 2nd remission is lasting longer than their 1st one without further treatment. Data of PFS, BCA, CART persistence and HAMAs for both groups are shown on Table 1:

Median	First infusion	Second Infusion
PFS (months)	11.6	5.2
Duration BCA (months)	2.3	3
CART persistence (days)	35	16
Presence of HAMAs+ (%)	42	30

Conclusions

Second ARI-0001 CART19 infusions were safe and able to achieve long term remissions in a subset of patients, with an increase on BCA duration despite less persistence of CART.

P-113

MONITORIZACIÓN DE PACIENTES TRASFUNDIDOS CON CAR-T MEDIANTE PCR DIGITAL

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Introducción: La terapia de células T con receptores quiméricos de antígenos (CAR-T) ha demostrado resultados prometedores en leucemia linfoblástica (LL) y linfoma de células B. Los factores determinantes de la respuesta a CAR-T son la expansión y persistencia de este subconjunto de células por lo que su monitorización es crucial. Si bien la citometría de flujo (CF) es una herramienta muy útil, se están buscando métodos de cuantificación más sencillos. En este trabajo se ha puesto a punto un método de detección y cuantificación de CAR-T mediante PCR digital (dPCR), técnica que tiene la misma sensibilidad que la qPCR pero una mayor reproductibilidad.

Objetivo: validación de técnica de dPCR para cuantificación de CAR-T

Materiales y métodos: se usó ADN genómico de células mononucleares de sangre periférica de 46 pacientes con LL o linfoma B en recaída/refractarios tratados con CD19 CAR-T, en diferentes puntos tras la infusión: día 5, 7, 11, 14, 20 y 28. Para dPCR empleamos QuantStudio™ Absolute Q™ usando cebadores y sondas TaqMan diseñadas para amplificar fragmentos en la región FMC63 de la construcción CAR-T. Como referencia del número de copias se amplificó de forma simultánea el gen RNasaP, asumiendo que hay dos copias de este por célula. Para normalizar los resultados se expresaron en número de copias de CAR-T/célula.

Resultados: el 82 % de los pacientes muestra una expansión del producto infundido entre los días 7-11 seguida de una rápida pérdida hasta el día 20, tras el cual es indetectable, el 4% tras el día 11 y el 4% no muestra expansión.

Conclusiones: la cinética de expansión de CAR-T es muy similar a los resultados publicados con CF. Debido a su sencillez y reproductibilidad, la dPCR podría sustituir a la CF en la cuantificación de CAR-T en el seguimiento y monitorización.

P-114

A 2-step algorithm spares corticosteroids in two-thirds of patients with severe immune-mediated hepatitis due to checkpoint inhibitors

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Abstract

Background & aims: Corticosteroids are the treatment of choice for immune-mediated hepatitis (IMH) due to checkpoint inhibitors (ICI). Our aim was to develop a 2-step algorithm for management of severe IMH, based on ICI temporary discontinuation (first step) and, if lack of improvement, indication of corticosteroids based on the degree of necroinflammation at biopsy (second step).

Approach & Results: Prospective study that included all subjects with grade-3/4 IMH. Peripheral extended immunophenotyping was performed in all cases. Indication for corticosteroids was severe necroinflammation at biopsy, or mild or moderate necroinflammation but progressive worsening at analytical values in the following days.

From January/2020 to November/2022, 94 patients were assessed, and 59 (62.7%) were excluded, with cancer progression as the most common cause. Finally, 35 individuals with grade-3/4 IMH were included: 68.5% anti-PD1/anti-PD-L1, 8.6% anti-CTLA-4, 22.8% both; CTCAE severity: 27 grade-3, 8 grade-4.

Overall, 24/35 (68.6%) patients benefited from avoidance of corticosteroids: 18 (51.4%) according to the first step (ICI discontinuation), and 6 (17.1%) to the second (necroinflammation degree). No patient with mild necroinflammation required corticosteroids, though 3/5 with moderate required corticosteroids during follow-up due to lack of improvement. Biopsy mainly impacted on grade-3 IMH, sparing corticosteroids in 6/11 non-improved patients with just ICI discontinuation. Patients requiring corticosteroids had an increase in HLA-DR expression on CD8+ lymphocytes (p=0.002), in central memory CD4+ (p=0.065), and a lower effector-memory CD4+ (p=0.014).

Conclusions: A two-step algorithm including discontinuation of immunotherapy, immunophenotyping and liver biopsy allows avoidance of corticosteroids in almost 70% of patients with severe immune-mediated hepatitis.

P-115

A COMPARATIVE STUDY OF NON-VIRAL SYSTEMS TO IMPROVE HUMAN NK CELLS TRANSFECTION

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In the last decades, natural killer (NK) cells are been considered promising specialized immune cells that can be genetically engineered for improving the cancer treatment for patients who are resistant to conventional therapies. These immune effector cells present an efficient recognition and cytolytic activity against malignant cells. However, the success of NK-based therapies is limited by the availability of safe and effective vectors that ensure entry and proper intracellular disposition of the nucleic acid in the target cell. In this sense, non-viral vectors are easier to produce in large scales and a safer alternative to virus based vectors, but present low transfection efficacy. Lipid nanoparticles (LNPs) are a very promising non-viral vectors that have a great capacity to condense nucleic acids and protect them from the nucleases-mediated degradation. LNPs are made of biocompatible and biodegradable materials that facilitate their internalization into target cells. Moreover, they can be functionalized and redirected selectively to specific tissues in order to increase their efficiency, and have an excellent safety profile supported by their use as mRNA delivery systems in vaccines against the SARS-CoV-2. In the present study, we have designed different non-viral LNP-based systems for transfection of NK cells using a plasmid DNA and evaluated both transfection efficiency and in cell viability.

P-116

PROINFLAMMATORY PRIMING OF MENSTRUAL BLOOD-DERIVED MESENCHYMAL STROMAL CELLS ALTERS THE PROTEIN CARGO OF THEIR EXTRACELLULAR VESICLES ENHANCING THEIR IMMUNOSUPPRESSIVE PROPERTIES.

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The immunomodulatory properties of mesenchymal stromal cells (MSCs) have been demonstrated in preclinical and clinical studies. Increasing evidence indicated that MSCs develop their immunosuppressive properties by paracrine regulation of the phenotypes and activities of innate and adaptive immune cells. The mechanism of MSC-mediated immunosuppression is slightly changed depending on the MSC source. Menstrual blood-derived stromal cells (MenSCs) are a new source that is notable for being isolated from an abundant and continuous font, by a non-invasive procedure without any ethical concerns. In this work, we focus on defining the mechanisms of immunosuppression of MenSCs by studying the protein cargo of their released extracellular vesicles (EVs) and whether proinflammatory preconditioning of the cells can improve these immunosuppressive properties. Therefore, MenSCs from five healthy premenopausal women were cultured under inflammatory stimuli (IFNγ and TNFα) or basal conditions. After 48h, the conditioned medium was collected and concentrated by ultrafiltration using 3 kDa cut-off devices, to obtain the concentrated secretome. EVs were isolated from the secretome by differential ultracentrifugation and proteomic analysis of their cargo was performed. Interestingly, our results revealed the presence of ICAM-1, an adhesion molecule, in addition to PD-L1, PD-L2, NECT2, NECT3, PVR, and LEG9, ligands of the checkpoint molecules, associated with EVs. All of them are widely involved in

immunosuppressive responses of different immune cells, such as monocytes, macrophages, T cells, and NK cells. Inflammatory priming significantly increased the expression of these molecules (ICAM1 log2FC= 3.098 FDR<0.005, CD274 log2FC= 1.703 FDR<0.005, and LEG9 log2FC= 2.038 FDR<0.005), indicating an enhanced immunosuppressive capacity of these cells. Our study has underlined potential molecular mechanisms involved in the immunosuppressive capacity of MenSCs and advocates the use of inflammatory priming to enhance this property.

P-117

EFFECT OF EPIGENETIC DRUGS IN NKG2D CAR T TREATMENT FOR NEUROBLASTOMA

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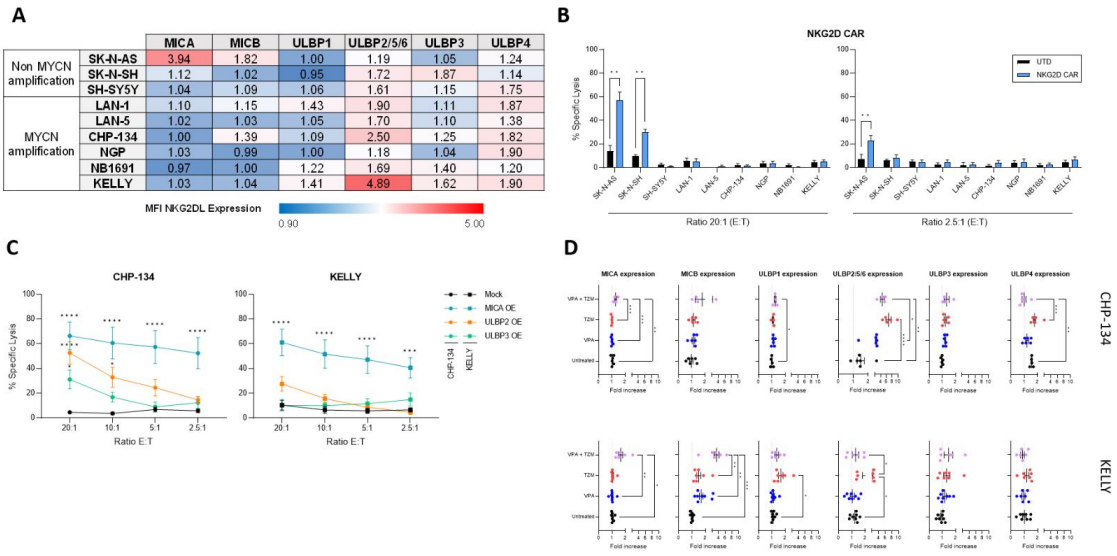
Background: Neuroblastoma (NB) is the most common extracranial solid tumor in childhood and is responsible for 11% of pediatric cancer deaths. Interactions between Natural Killer Group 2D (NKG2D) receptor, expressed in cytotoxic immune cells, and its ligands (NKG2DLs), that are upregulated in many types of tumor cells as neuroblastoma, are important for anti-tumor immune surveillance. However, neuroblastoma cells may develop mechanisms of resistance to NKG2D mediated cell-lysis such as NKG2DL release, downregulation of NKG2DL surface expression by epigenetic repression. Epigenetics drugs could facilitate NKG2DL expression for use of engineered T lymphocytes with a NKG2D CAR and to become in an effective therapeutic strategy for neuroblastoma patients.

Methodology: CD45RA- memory T cells were isolated from PBMCs after depletion of CD45RA+ cells of healthy donors. NKG2D-CAR T cells were generated by lentiviral (NKG2D-41BB-CD3z) transduction with MOI=2.5. Overexpression of NKG2DL was performed by lentiviral transduction in resistant neuroblastoma cell lines. NKG2DL expression was analyzed by flow cytometry (FCM) after epigenetic drugs exposition. Cytotoxicity of NKG2D-CAR T cells against neuroblastoma cells was evaluated in vitro by Europium-TDA assays or FCM.

Results: Almost neuroblastoma cell lines analyzed showed heterogeneous NKG2DL expression. NKG2D-CAR T cells were cytotoxic against 2/9 neuroblastoma cell lines, with a specific lysis over 30% at higher effector: target ratio, while the other cell lines appeared to be resistant. However, NB resistant cell lines became susceptible to lysis by NKG2D-CAR T cells after NKG2DL overexpression. Using epidrugs, NKG2DL expression was upregulated and showed synergetic effect between drugs but did not allowed NKG2D-CAR T cell functionality.

Conclusions: Analyzed neuroblastoma cell lines expressed NKG2DLs, but only 2/9 were

susceptible to NKG2D-CAR T cells lysis. NB cell lines became in susceptible to NKG2D CAR-T lysis after different NKG2DL overexpression. Epigenetic target regulation directly affects NKG2DL upregulation in NB but it was not sufficient to overcome resistance



		MICA	MICB	ULBP1	ULBP2/5/6	ULBP3	ULBP4
Non MYCN amplification	SK-N-AS	3.94	1.82	1.00	1.19	1.05	1.24
	SK-N-SH	1.12	1.02	0.95	1.72	1.87	1.14
	SH-SY5Y	1.04	1.09	1.06	1.61	1.15	1.75
MYCN amplification	LAN-1	1.10	1.15	1.43	1.90	1.11	1.87
	LAN-5	1.02	1.03	1.05	1.70	1.10	1.38
	CHP-134	1.00	1.39	1.09	2.50	1.25	1.82
	NGP	1.03	0.99	1.00	1.18	1.04	1.90
	NB1691	0.97	1.00	1.22	1.69	1.40	1.20
	KELLY	1.03	1.04	1.41	4.89	1.62	1.90

MFI NKG2DL Expression: 0.90 – 5.00

P-118

ADMINISTRATION OF MODIFIED VACCINIA VIRUS ANKARA VIA THE PERITONEAL ROUTE CONFERS EXPRESSION OF SINGLE-CHAIN INTERLEUKIN-12 IN THE OMENTUM, RESULTING IN IMMUNE-MEDIATED EFFICACY AGAINST PERITONEAL CARCINOMATOSIS

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Peritoneal carcinomatosis is an advanced stage of cancer where the disease has spread to the peritoneal cavity. To restore the antitumor immunity subverted by the tumor cells in this location, we evaluated the effects of intraperitoneal administration of vaccinia virus Ankara (MVA) engineered to express single-chain interleukin 12 (scIL-12). We found that only intraperitoneal administration resulted in a potent immune response, characterized by increased levels of tumor-specific $CD8^+$ T lymphocytes capable of producing both IFN-γ and TNF-α. The antitumor immune response was detected not only in the peritoneal cavity but also systemically. Intraperitoneal treatment with a single administration of MVA.scIL-12 completely eradicated MC38 tumors implanted in the peritoneal cavity and also protected cured mice from subsequent subcutaneous rechallenges. Bioluminescence imaging showed that intraperitoneal administration targets the transgene to the omentum. Furthermore, the safety profile of intraperitoneal administration was better than that of intravenous administration, as no weight loss or hematological toxicity was observed when the vector was locally delivered into the peritoneal cavity. In conclusion, intraperitoneal administration of MVA vectors encoding scIL-12 targets the omentum and results in a potent tumor-specific immune response.

P-119

CAR-VSTS THERAPY: OPTIMIZATION OF THE ACTIVATION AND EXPANSION OF EPSTEIN-BARR VIRUS-SPECIFIC T LYMPHOCYTES FOR TRANSDUCTION WITH ARI-0001 (CAR-T.CD19) FOR B-CELL NEOPLASMS

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Chimeric Antigen Receptor T cells against CD19 (CAR-T.CD19) has proven to be one of the most promising treatments for B-cell neoplasms. However, CAR-T therapy has some limitations, such as the lack of cell proliferation and persistence *in vivo*. CAR-T action could be prolonged and enhanced using virus-specific T cells. Epstein-Barr virus (EBV) is highly prevalent in human population, and it is involved in the pathophysiology of some tumors.
The aim of this work was to optimize the production of EBV-specific T cells. PMBCs from healthy EBV-seropositive donors were activated with overlapping peptides of EBV-specific antigens and different culture conditions were tested in G-Rex® 24-well plate with expansion medium and cytokines. Final product was characterized by flow cytometry. Three products were TCRβ sequenced to analyze the diversity and clonality. The final selected culture condition was tested in 4 expansions and showed a fold expansion of $15.3\pm10_{SD}$, a cell product based mainly on $CD3^+$T lymphocytes ($93.2\%\pm12.1_{SD}$), a mean of $75.9\%\pm33.4_{SD}$ of $CD8^+$ on T cells, and >80% of $CD8^+$T cells expressed IFN-γ in the presence of EBV antigens. Study of the phenotype of T cells by flow cytometry showed that T lymphocytes mainly exhibited an effector memory phenotype. Production of virus-specific T cells has been scaled up under Good Manufacturing Practices (n=1), and it has been seen that the method is replicable. TCRβ sequencing demonstrated increased clonality in the expanded products and detected specific clonotypes of EBV antigens presented by donor-expressed HLA molecules. Next step will be to optimize the CAR-T.CD19 transduction protocol, since the starting material will be cryopreserved EBV-specific T cells.
In conclusion, we obtained a T cell product highly specific against EBV antigens.

P-120

OVERCOMING CURRENT CHALLENGES IN CAR-T CELL IMMUNOTHERAPY FOR T-ALL WITH ANTI-PRE-TCR TRUC-T CELLS

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T-cell acute lymphoblasyic leukemia (T-ALL) is an aggressive haematological malignancy with high rates of relapse and ineffective options for refractory disease associated with dismal outcomes. Therefore, there is an urgent need for developing novel effective therapies for T-ALL patients. Adoptive therapy with T cells armed with chimeric antigen receptors (CAR-T) has emerged as a powerful tool against refractory haematopoietic malignancies. However, CAR-T therapy still has serious limitations including poor anti-tumour activity, T-cell toxicities, accelerated T-cell exhaustion and reduced *in vivo* persistence, resulting from antigen recognition and tonic signalling through transduced non-physiological receptors like CARs.

To overcome these challenges, we aimed at developing TCR fusion constructs (TRuCs) composed of a T-ALL-specific antigen-recognition domain fused to the CD3ε subunit of the αβ TCR, to be expressed on effector T cells. Unlike conventional CAR-T, TRuC-T cells use the entire signalling machinery of the αβTCR, generating a more powerful T-cell response, with longer persistence and potentially fewer adverse effects. Thus, we took advantage of our previous results showing that the pre-TCR (pTa-TCRb) is a T-ALL biomarker involved in T-ALL pathogenesis and progression, for developing an innovative TRuC recognition structure targeting the human pre-TCR, as the basis of a novel, efficacious and safe T-ALL-specific immunotherapy.

Using a proprietary anti-pTα mAb, we generated a pTa-specific single chain variable fragment that was fused to the N-terminus of full-length human CD3ε through a (Gly4Ser)3 linker and cloned into a lentiviral vector. Jurkat T-cells were then transduced with lentiviral particles and *in vitro* assays validated both the surface expression of TRuC-pTα and its potential for inducing the activation of TRuC-pTα Jurkat T cells upon recognition of pre-TCR+ T-ALL cell lines. Current work focusses on the generation and validation in preclinical *in vivo* assays of a T-ALL immunotherapy based on primary TRuC-pTα T cells.

P-121

GENERATING NK CELLS *IN VITRO* FROM CORD BLOOD UNITS IN A FEEDER FREE GMP SYSTEM

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Recently, hematologic cancers are being treated with Chimeric Antigen Receptor (CAR) T cells therapy as an alternative to conventional treatments such as chemotherapy or radiology. However, patients treated with CAR-T therapies have shown some non-desired side effects such as cytokine release syndrome (CRS) and neurotoxicity. Besides, it will be more convenient to be able to use allogenic T cells, but it is not possible as there is a risk of developing graft versus-host disease (GvHD) on infused patients. Natural Killer (NK) cells are a promising alternative in these CAR-based cell therapies as they do not cause these side effects while they allow the generation of allogenic off-the-shelf products since they lack T-cell receptors (TCR). To develop this product, NK cells are usually isolated from adult peripheral blood and cord blood units. Nevertheless, these cells have shown low fold expansion *in vitro*, which may complicate reaching the number of cells required for the doses usually employed in these CAR-based therapies. In this study, we aim to obtain the best NK cell source with a higher fold expansion to improve the efficacy of these immunotherapeutic products. In this regard, we have obtained a mature and functional NK cell population derived from *in vitro* cord blood-derived CD34+ hematopoietic stem cells. Moreover, we overcame the challenge to implement the differentiation protocol from a feeder dependant culture to a GMP system, so generated NK cells could be used in CAR-NK therapies. Our results prove that cord blood-derived CD34+ cells are a promising allogenic mature and functional NK cell source with a high fold expansion in a feeder free GMP system.

P-122

GENERATING NKG2D-CAR-TRANSDUCED NATURAL KILLER (NK) CELLS FOR TREATING OSTEOSARCOMA

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Osteosarcoma (OS) is the most common primary malignant bone tumor, with an incidence worldwide of approximately one to three cases annually per million. It typically affects children and young adults with a second smaller peak of incidence in those individuals over the age of 50. The current main treatment is based on the combination of surgery and neoadjuvant chemotherapy leading to a long-term survival rate of 70%. However, these strategies are inefficient for the metastatic or relapsed osteosarcoma patients, in who the 5-year survival rate is less than 20%. As a result, in the last decades, alternative novel therapeutical approaches are being developed, including **chimeric antigen receptors (CARs)-based immunotherapy**, in which immune effector cells such as T cells are genetically engineered to express CARs in their surface membrane for improving the recognition and killing of tumor cells. **CAR-transduced T (CAR-T) cells** have revolutionized the cancer therapy, however toxicities associated to this autologous therapy such as cytokine release syndrome and immune effector cell-associated neurologic syndrome limit its widespread. Therefore, efforts have been made to find a more appropriate cell type for being genetically engineered with CARs. Among other immune cell types, **natural killer (NK) cells** have been proposed as a potential allogenic alternative due to their cytolytic capacity and their safety profile**.** Since **NKG2D receptor (NKG2DR)** is one of the most important activating receptor expressed in NK and osteosarcoma cells express ligands for NKG2D receptor, the aim of this study is the production of NKG2D-CAR-NK cells in order to target and kill more effectively osteosarcoma cells. The increased expression of NKG2DR on the surface of effector cells is correlated with an increased cytotoxic capacity. We hope that these results will stimulate the development of experimental studies, testing the potential effectiveness of the new concepts of CAR-NK therapies against aggressive solid tumors.

P-123

DEVELOPMENT AND CHARACTERIZATION OF OPTIMIZED VIRUS-SPECIFIC T CELLS MANUFACTURING

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Adoptive cell therapy based on the infusion of virus-specific T lymphocytes is used when pharmacological treatment fails to reduce the viral load. Infections caused by adenovirus (AdV) and BK virus generate a low intensity T cell memory response. Usually, products obtained with the CliniMACS® cytokine capture system (IFN-γ) reach specificities below 50% for these viral antigens as well as limited total number of specific T cells, therefore, in most cases a single therapeutic dose is available. In this context, manufacturing optimization is crucial to obtain substantial amount of highly specific T lymphocytes for these antigens.

The aim of this project was to obtain highly specific T cells by expanding previously selected T lymphocytes with the IFN-γ capture method. In order to set up the protocol and to get preliminary data, we used remaining samples of IFN-γ capture method done under compassionate uses, to treat viral infections after HSCT (5 cytomegalovirus (CMV) and 2 AdV). Our results showed fold expansion up to 14000, and with a mean percentage of cell viability of 81% in the T lymphocytes of the final cell product, of which > 94% were specific against the viral antigen of interest. Therefore, the specificity against pp65-CMV antigens increased 22.69% compared to the pre-expanded cells obtained by the IFN-γ capture method. T-cell receptor sequencing of the final product demonstrated that the increased specificity correlated with higher clonality and lower diversity of the expanded products. $CD8^+$ T lymphocytes expressed CD107a, meaning cytotoxic functionality. Two expansions were performed obtaining highly specific T cells against AdV-5 hexon (93.8% $CD3^+IFN\text{-}\gamma^+$, SD 0.283), increasing 85.11% the specificity compared to AdV product obtained by IFN-γ Cytokine Capture System.

In summary, this strategy increases the specificity, clonality and cellularity of the T cell product obtained by IFN-γ Cytokine Capture System.

P-124

CD137 (4-1BB) REQUIRES PHYSICALLY ASSOCIATED CIAPS FOR SIGNAL TRANSDUCTION AND ANTITUMOR EFFECTS

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CD137 (4-1BB) is a member of the TNFR family, whose expression is induced on antigen-primed T cells and that mediates potent costimulatory signals upon ligation by CD137L or agonist monoclonal antibodies. CD137 agonists attain immunotherapeutic antitumor effects in cancer mouse models and multiple agents of this kind are undergoing clinical trials. We show that cIAP1 and cIAP2 are physically associated with the CD137 signaling polyprotein complex. Moreover, cIAPs are required for CD137 signaling towards the NF-κB and MAPK pathways and for costimulation of human and mouse T lymphocytes. Functional evidence was substantiated with SMAC-mimetics that trigger proteasomal degradation of cIAPs and transfecting dominant-negative variants of cIAPs. Importantly, antitumor effects of agonist anti-CD137 mAbs are critically dependent on the integrity of cIAPs in cancer mouse models.

P-125

LA TASA DE NEUTRÓFILOS DEL LAVADO BRONCOALVEOLAR COMO GUÍA PARA PERSONALIZAR LA TERAPIA INMUNOSUPRESORA EN PACIENTES CON ENFERMEDAD PULMONAR INTERSTICIAL

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Las enfermedades pulmonares intersticiales difusas (EPIDs) son un grupo de enfermedades caracterizadas por presentar diferente grado de inflamación y fibrosis en el pulmón. En la mayoría de las EPIDs, diferentes a la Fibrosis Pulmonar Idiopática, el tratamiento es inmunosupresión con corticoides u otros inmunosupresores sistémicos (Micofenolato, Rituximab, Metotrexato, Abatacept). El estudio del contenido leucocitario del lavado broncoalveolar (LBA) aporta gran valor diagnóstico y ayuda a diferenciar patologías con predominio de linfocitos, eosinófilos, células de Languerhans o macrófagos, y a diferenciarlas de procesos agudos o infecciosos con predominio de neutrófilos. Se ha descrito, además, que la tasa de neutrófilos del LBA tiene valor pronóstico.

Este trabajo investiga el impacto del tratamiento inmunosupresor en la supervivencia global (SG) de 581 pacientes con diversas EPIDs, en función del porcentaje de neutrófilos del LBA estimado mediante citometría de flujo.

El seguimiento medio de los pacientes fue de 69,3±37,2 meses. El porcentaje de neutrófilos del LBA mostró valor gran pronóstico sobre la SG: porcentajes >10% de neutrófilos se asocian a tasas inferiores de SG a 15 años (53,8% vs. 71,9%, p<0,000). En pacientes con porcentaje de neutrófilos menores al 10% el tratamiento con otros inmunosupresores mostró tasas de SG a 15 años superiores (79,4%, p<0,05) a la de los tratados con corticoides (66,4%) y similar a la de pacientes sin tratamiento inmunosupresor (77,1%), que eran los pacientes con patología más leve. Sin embargo, en pacientes con porcentajes de neutrófilos superiores al 10% la SG a 15 años de ambos tratamientos fue comparable (52,7% y 54,3%) y muy inferior a la de los pacientes sin tratamiento inmunosupresor (72,7%, p>0,05).

Evaluar el porcentaje de neutrófilos en el LBA de pacientes EPID puede ayudar a personalizar las terapias, ya que los pacientes con porcentajes de neutrófilos inferiores al 10% pueden beneficiarse de tratamientos inmunosupresores diferentes a los corticoides.

P-126

EVALUATION OF THE CD4/CD8 RATIO DYNAMICS DURING THE MANUFACTURING AND TREATMENT WITH THE ACADEMIC ANTI-CD19 ARI-0001 AND ANTI-BCMA ARI-0002H CAR T-CELL THERAPIES

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Background

The composition of CD4+ and CD8+ T-cells in the starting material (SM) and final product (FP) varies during anti-CD19 ARI-0001 and anti-BCMA ARI-0002h manufacturing processes, and among patients treated with them. Changes in CD4/CD8 ratio of SM and FP, and during follow-up of patients were studied.

Methods

The %CD4+ and %CD8+ in SM and FP were measured by flow cytometry in ARI-0001 (n=152) and ARI-0002h (n=32) products, as well as the %CAR+ T-cells in FP. CD4/CD8 ratio was analyzed in peripheral blood at 1, 3, 7 and 12 months (M) after FP infusion.

Results

Regarding ARI-0001, differences in CD4/CD8 ratio between SM (median=0.81) and FP (median=1.69) were observed ($p<0.0001$). In those SM with ratio<1, it significantly increased (median SM=0.51, median FP=1.5; $p<0.0001$). If initial ratio>1, the difference was not significant. A weak positive correlation was shown between FP ratio and FP CAR expression ($r=0.3185$, $p<0.0001$).

Regarding ARI-0002h, differences between SM and PF ratio were not observed. However, like for ARI-0001h, the ratio increased (median SM=0.57, median FP=0.68; $p=0.0191$) for those products with SM ratio<1. In those with initial ratio>1, no differences were observed.

No differences were observed when comparing either ARI-0001 or ARI-0002h SM. However, the ARI-0001 ratio was higher in the FP (median FP ARI-0001= 1.69, median FP ARI-0002h= 0.73; $p<0.0001$).

Regarding patients´ follow-up after ARI-0001 infusion, the ratio at 1M markedly decreased compared to FP ratio, and then it gradually increased (median 1M=0.5, 3M=0.47, 7M=0.56,

12M=0.66). The same was seen for ARI-0002h (median 1M=0.29, 3M=0.3, 7M=0.35, 12M=0.42).

Conclusions

The CD4/CD8 ratio increases in ARI-0001 FP in comparison to SM.

When CD4/CD8 ratio<1 in the SM, the CD4+ population seems to expand more than CD8+ subset for both ARI-0001 and ARI-0002h.

Comparing to FP, CD8+ T-cells expand more than CD4+ T-cells immediately after ARI-0001 or ARI-0002h infusion.

P-127

IMMUNE PROFILE OF CANCER PATIENTS IN THE SPANISH IMMUNOTHERAPY REGISTRY OF CARDIOVASCULAR TOXICITY (SIR-CVT) DURING THE COURSE OF TREATMENT.

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The advancement of immunotherapies has shifted the standard of care in cancer treatment. Immune checkpoint inhibitors (ICIs), such as monoclonal antibodies targeting PD-1, PD-L1 and CTLA-4, stimulate the patient´s immune system, through inhibition of regulatory T cells, to attack cancer cells that evade the immune response. However, the mechanism of action of ICIs can also lead to undesirable events (irAEs). During the mechanism of action of ICIs, autoimmune T cell clones are activated. As a consequence, many systemic autoimmune diseases may arise, including myocarditis, which is one of the rarest but most fatal irAEs with a mortality rate of up to 50%. This sub-study, part of the Spanish Immunotherapy Registry of Cardiovascular Toxicity (SIR-CVT), aims to investigate the immune profile of cancer patients' peripheral blood before and during the course of treatment with immunotherapies. The registry focuses on the potential cardiovascular toxicities associated with these therapies, and aims to provide insight into the underlying mechanisms of these toxicities.

Patients were recruited from various hospitals and blood samples were collected before and after 2-4 weeks, 10-12 weeks, 6 months, and 1 year of starting immunotherapy. Flow cytometry analysis was performed on the blood samples from 92 patients receiving immunotherapy. The data was acquired in a FACSymphony (BD) instrument and analyzed using FlowJo10.1 software.

We present a new understanding of the dynamics in peripheral blood lymphocytes during cancer immunotherapy. Results suggest a decrease in activated regulatory T cells, which play a key role in maintaining immune homeostasis, and an increase in effector T cells, as autoimmune Th17 subset. These shifts can cause disruption in immune homeostasis and may result in autoimmunity phenomena, including myocarditis. By combining these findings with clinical data, we have the potential to identify biomarkers that could predict or prevent adverse events and ultimately enhance patient outcomes.

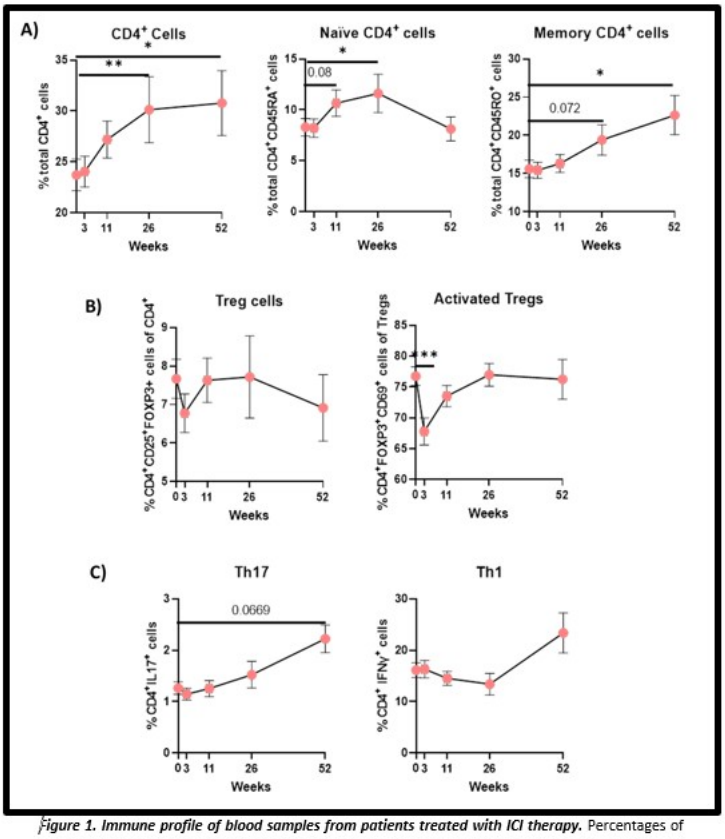


Figure 1. Immune profile of blood samples from patients treated with ICI therapy. Percentages of peripheral blood lymphocytes analyzed by flow cytometry in longitudinal assays from patient's blood samples with cancer before ICI treatment (n=92), after 2-4 weeks (n=75), 10-12 weeks (n=64), 6 months (n=29), and 1 year (n=11) since the first therapy cycle. ***A)*** Percentages of total $CD4^+$, $CD4^+CD45RA^+$ and $CD4^+CD45RO^+$ cells of PBLs. ***B)*** Percentages of $CD4^+CD25^+FoxP3^+$ cells $CD4^+$-gated and $CD4^+CD25^+FoxP3^+CD69^+$ cells gated in $CD4^+FoxP3^+$ cells. ***C)*** Percentages of $CD4^+IL17^+$ cells and $CD4^+IFN\gamma^+$ cells gated in $CD4^+$ cells. Data is represented as mean ± SEM. Statistical differences between means was measured using mixed-effects analysis of variance followed by Dunnett's post-hoc test. * $P < 0.05$, ** $P < 0.01$, *** $p < 0.001$.

Sesión Poster Defensa 2. Pantalla 1. Inmunoterapia

P-128

IMPROVEMENT OF LYMPHOCYTE PURITY IN FLOW CYTOMETRIC CROSSMATCH ASSAYS: A COMPARISON OF CELL ISOLATION METHODS

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Introduction: The flow cytometric crossmatch (FCXM) assay is used to determine whether donor specific HLA antibodies (HLA-DSA) are present in recipient sera. Isolated cell purity is a widely recognized critical factor in the test outcome. The aim was to compare the performance of two cell isolation methods in FCXM assays carried out with peripheral blood from living donors for kidney transplant.

Materials and methods: Hematological cell fractions from living donor whole blood samples were evaluated before and after being processed by erythrocyte aggregation and total lymphocyte enrichment (EA&TLE) through negative magnetic selection. Results from FCXM assays carried out with EA&TLE and a conventional cell isolation technique by gradient density centrifugation using Ficoll were compared and correlated with class I and class II HLA-DSA detection by Luminex single-antigen bead assay (LSA).

Results: The average of lymphocytes in the samples processed by EA&TLE was 94% (95% confidence interval [95%CI] 89%-97%), with a recovery of 24 to 50% of the lymphocytes present before processing and a lymphocyte count after enrichment of 3 to 10 million cells/mL. Analysis of 65 FCXM assays performed with EA&TLE and 66 with Ficoll showed that EA&TLE has a higher sensitivity for the detection of both class I and class II HLA-DSA (67% EA&TLE vs 43% Ficoll) and higher kappa correlation coefficient (0.61, 95%CI=0.318-0.90 EA&TLE vs 0.40, 95%CI=0.01-0.82 Ficoll) when compared to HLA-DSA detection by LSA. Furthermore, FCXM assays performed with EA&TLE showed a lower detection threshold for class II HLA-DSA in comparison to FCXM assays performed with Ficoll (lowest anti-IgG MFI 5193 for EA&TLE vs MFI 10289 for Ficoll).

Conclusions: Implementation of EA&TLE for whole blood cell isolation increased lymphocyte purity, improved FCXM assay sensitivity and resulted in a greater correlation with HLA-DSA identification by LSA. Thus, EA&TLE can increase cell isolation efficiency and cost-effectiveness in FCXM assays.

P-129

DESIGN, OPTIMIZATION AND VALIDATION OF A PROTOCOL FOR HLA TYPING USING OXFORD NANOPORE TECHNOLOGIES' MINION PLATFORM

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The accurate, high-resolution typing of HLA genes is essential for assessing donor-recipient compatibility in hematopoietic stem cell transplantation. The Next Generation Sequencing (NGS) is currently the gold-standard technique for this purpose. However, their main disadvantage is the relative short reads, which lead to ambiguities regarding the phase establishment. One possibility to overcome this inconvenience is the third-generation sequencing (TGS) platforms, such as nanopore sequencing, which represents a cost-effective and scalable new technology for HLA genotyping, even for challenging samples.
Our aim was to design and optimize a protocol for HLA typing using the MinION sequencer (Oxford Nanopore Technologies - ONT); find the optimal software and settings to obtain the accurate genotype and perform a validation with 12 samples showing phase establishment difficulties.
Amplification of HLA class I (*HLA-A, -B, -C*), HLA class II (*HLA-DRB1, HLA-DQB1, DPB1*), and non-classic HLA (*HLA-E and HLA-G*) was performed with an in-house designed multiplex long-range PCR. Barcoded libraries were generated using the native barcoding (EXP-NBD104) and ligation kits (SQK-LSK109) from ONT. Sequencing was performed on a MinION R9.4.1 flow cell for 4h. NGSengine software was used for the analysis.
To obtain optimal results, the recommended enzymatic incubation times were increased and homopolymer regions of each gene were excluded from the analysis. The 79.5% of reads were correctly mapped against the HLA region, with an average read depth of 724 nucleotides. The majority of the reads measured between 3.71 Kb and 8.83 Kb, consistent with the amplicon profile. Moreover, the percentage of concordance was more than 90% between platforms and the 80% of inconsistencies belonged to nucleotide changes in short homopolymer regions of HLA class II genes.
In conclusion, we have designed a strategy for sequencing the entire HLA genes with TGS that allows phasing. However, HLA class II analysis needs to be optimized.

P-130

FCYRIIIB RECEPTOR (CD16B) DEFICIENCY IN PATIENTS WITH HAEMATOLOGICAL AND INMUNOLOGICAL DISORDERS

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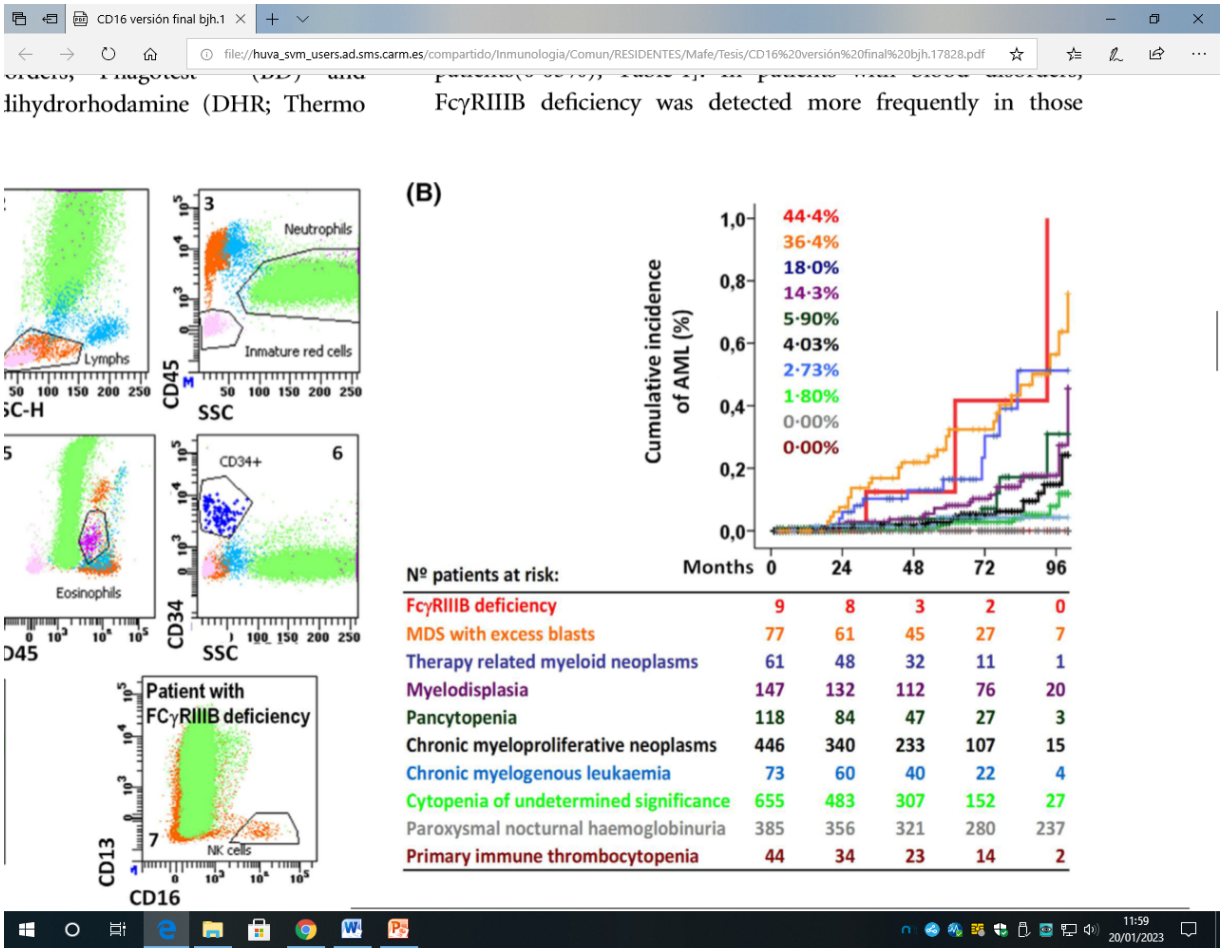
[1]Hospital Clínico Universitario Virgen de la Arrixaca, Murcia; [2]Hospital Rafael Mendez de Lorca, Murcia; [3]Hospital General Universitario Santa Lucía, Murcia.

Background: Low-affinity immunoglobulin gamma Fc region III-B (FcyRIIIB) receptor is present mainly in neutrophils and its core function is to eliminate immune complexes. It is encoded by the FCGR3B gene, and presents copy number variation (CNV). The genotype without any copy (FCGR3B null) derives in lack of FcyRIIIB receptor, and is present in approximately 0.05% worldwide. The FCGR3B null genotype is not associated with any clinical alteration. The possible reason that could explain this is that the immunoglobulin gamma Fc region receptor III-A (FcyRIIIA) receptor is also present in neutrophils and develop the same cellular activation role, making neutrophils fully functional.
Aims: To evaluate the incidence of CD16B deficiency in haematological and immunological patients and its clinic impact.

Methods: we used peripheral blood and bone marrow samples from patients with hematologic and immunologic alterations from 2013 until 2021, the FcyRIIIB receptor was marked with CD16B. We processed them with the FACSCanto-II and FACSLyric cytometers and analysed with the DIVA program. To evaluate the neutrophil function, we used Phagotest and the oxidation-phagocytosis test. The data obtained was studied using the Statistical Package for Social Sciences (SPSS). The acute myeloid leukemia (AML) progression was determined using the Kaplan-Meier method.

Results & Conclusion: FcyRIIIB receptor deficiency was 8 times more frequent in patients with haematological disorders than immunological ones. We found a total of 9 out of 2147 haematological patients with this deficiency (*0.42%*) and with diverse diagnosis, compared with 1 out of 2147 immunological patients (0.05%, similar to global prevalence), who had only

bronchiectasis. It was observed that 4 of these 9 haematological patients had or later developed LMA, which results in an AML cumulative incidence of 44.4%. This might suggest an association, although it is recommended to validate these findings in larger studies and have a stricter follow-up of these patients.



P-131

IMPACTO DE LA INCOMPATIBLIDAD EN HLA-C EN LA FERTILIZACIÓN IN VITRO CON DONACIÓN DE OVOCITOS.

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La Fertilización In vitro con donación de ovocitos (FIV-O), fue descrita inicialmente en 1984. Actualmente representa el 8% de los tratamientos de reproducción asistida realizados en Europa.

A diferencia de lo que ocurre en el embarazo natural y en la FIV convencional, en la FIV-O, el embrión puede expresar un repertorio de moléculas de HLA completamente distinto al de la receptora. Se ha planteado que esta incompatibilidad, puede ser causa de que un número significativo transferencias no conduzcan a la obtención de un recién nacido vivo, a pesar de que en la FIV-O se empleen embriones de óptima calidad.

El adecuado desarrollo de la placenta depende de la interacción entre el trofoblasto extravellositario (TEV) y diferentes células del sistema inmunitario presentes en la decidua. Debido a que las únicas moléculas de HLA polimórficas que expresa TEV, son las moléculas de HLA-C, el estudio del HLA-C embrionario y de los donantes de gametos reviste particular interés.

Con el objetivo de determinar la influencia de la incompatibilidad HLA-C en la FIV-O, se realizó un estudio prospectivo, observacional, en 100 parejas que acudieron de forma consecutiva al Instituto Europeo de Fertilidad entre el 2016-22. Se excluyeron parejas con fallo reproductivo recurrente, enfermedades autoinmunes sistémicas. Se incluyeron 282 individuos: 100 parejas y 82 donantes de ovocitos que generaron 104 tripletes. Se registraron un total de 188 transferencias embrionarias. Se realizo seguimiento en cada trimestre del embarazo y en el post-parto.

El resultado de la FIV-O se analizó en función del número de incompatibilidades en HLA-C presentes en el triplete. Los tripletes con ≥ 3 incompatibilidades en HLA-C tuvieron una probabilidad significativamente menor de lograr un embarazo clínico o un RN vivo en comparación con los tripletes con ≤ 2 incompatibilidades, 40% vs 63% %, ($p < 0.01$) y 29% vs 54% ($p < 0.01$).

P-132

DETECTION OF IGG ANTIBODIES AGAINST SELF HLA CLASS II MOLECULES AFTER RED BLOOD CELL TRANSFUSION

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Background:

A 13-year-old male with end-stage renal disease, currently waiting for kidney transplantation and anti-HLA antibodies undetectable in previous studies, developed a humoral response against self-HLA class-II molecules two weeks after several transfusions.

Aim:

To clarify the reaction pattern of anti-HLA antibodies in order to identify the unacceptable HLA antigens in the setting of transplant

Methods:

For screening and specific identification of IgG anti-HLA antibodies we used Luminex-Based Assays (Immucor). For high-resolution HLA-genotyping we used next-generation-sequencing (NGSgo®MX11, GENDX).

Results and discussion:

Sera obtained after two weeks and one and two months from the transfusion events had similar anti-HLA antibody patterns, consistent with an ongoing immune response but not with the presence of contaminating antibodies in the transfusion product. Screening test was negative for HLA-Class-I and positive for HLA-class-II (MFI=6000, in bead group enriched with DR52 molecules). LSA-II test showed the following reactivity pattern: DRB3*01,02,03 (maximum MFI=10,000 for DRB3*02), DRB1*08 (MFI=6,000), DRB1*04, 07, 09 and DRB4*01 (MFI values around 2,500) and DRB1*01 (MFI<1000). Eplet analysis revealed a specific reaction pattern against 104AK epitope (a highly exposed epitope according to the Epitope Registry) that is shared by several HLA-DR molecules and explains most of the reactivity of the serum. The similarity of the antibody pattern obtained by testing with another Luminex-Based-Assay supplier (One Lambda) in a different laboratory makes the recognition of cryptic or denatured epitopes unlikely. Self-crossmatch by complement-dependent-cytotoxicity-assay using B-cells was negative suggesting insufficient strength or the presence of complement non-activating antibodies.

NGS-genotyping discarded null/new alleles in the relevant loci that would explain the observed reactivity (DRB1*03:01:01, 07:01:01; DRB3*02:02P; DRB4*01:01P).

A sustained humoral immune response against a highly exposed epitope shared by several HLA-DR antigens, including self-HLA-DR molecules, was generated following transfusion. Although the relevance of this finding is unclear it seems reasonable to exclude self-HLA antigens from unacceptable antigens.

P-133

ASSOCIATION BETWEEN HLA-DP MISMATCH AND OUTCOME OF IDENTICAL SIBLING HSCT: SINGLE CENTER EXPERIENCE

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Background and objective: the biological significance of HLA-DP in hematopoietic stem cell transplantation (HSCT) has been best studied in unrelated pairs, however, the impact in sibling donor transplants is not well-established, despite the potential frequency of single mismatch is ~5%. In this study, we compared donor-recipient mismatch in HLA-DP loci and its effect on the clinical evolution of patients with hematological malignancies who underwent HSCT using 10/10 identical sibling donors.

Methods: HLA compatibility was established according to low resolution of HLA-A, -B, -C, -DRB1 and -DQB1 loci and HLA haplotypes identity. The DP locus was first evaluated using Lifecodes PCR-SSOP, followed by high-resolution testing by Next Generation Sequencing (NGSgo-MX11-3, GenDx) that were carried out on those pairs showing discrepancies. HLA-DPB1 mismatch status was classified as permissive or non-permissive using DPB1 T-Cell Epitope Algorithm v2.0 (IPD-IMGT/HLA), based on the T-cell-epitope (TCE) alloreactivity groups.

Results: in 411 transplants, we found 10 pairs (2,43%) with DPB1 disparity; five of these pairs included an additional DPA1 mismatch. In the mismatched group, eight pairs were permissive and two were non-permissive. In non-permissive group, one case showed predicted immunogenicity in the graft-versus-host direction and one in the host-versus-graft direction. No significant association was observed between overall survival, disease relapse, death, graft versus host disease and HLA-DPB1 mismatch.

Conclusions: our results indicate that the incidence of crossing-over events in DPB1 of related pairs is low and does not seem to be associated with allo-HSCT outcome. However, it would be interesting to promote a multicenter study and to apply an algorithm that includes the epitopes of the whole HLA-DP molecule, beyond the peptide binding site, since IPD-IMGT/HLA algorithm only uses the hypervariable regions defined in exon 2.

P-134

HLA-DRB1*04:02~DRB4*01:03:01:02N ASSOCIATION IS IDENTIFIED IN SPANISH POPULATION

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The beta chain of the HLA-DR molecule is encoded by multiple genes, and depending on the DRB1 allele, a second DRB gene can be encoded on the haplotype. DRB1*04, *07 or *09 alleles have been associated with DRB4, which counts 230 reported alleles in IMGT/HLA Database and 26 of them are considered nulls. DRB4*01:03:01:02N is one of these un-expressed alleles which is frequently accompanied by DRB1*07:01~DQB1*03:03, but it has been also unusually reported with DRB1*04. Recently, the first case of association between DRB4*01:03:01:02N and DRB1*04 in a Spanish solid organ donor was published; however, it was unable to determine the protein level of DRB1*04. Here, we investigate the association between HLA-DRB4*01:03:01:02N allele and DRB1~DQB1 haplotype in Spanish population.

310 volunteer bone marrow donors positive for DRB4 were full HLA typed using commercial next-generation sequencing and the MiniSeq. 35 donors (11.3%) carried DRB4*01:03:01:02N allele, and 25 of them (71.4%) showed the well-established association with DRB1*07:01~DQB1*03:03 haplotype. In six (17.2%) donors we were unable to determine the HLA class II haplotype, due to the presence of a second DRB4 associated allele. Interestingly, in 4 (11.4%) donors DRB4*01:03:01:02N allele was observed as part of the DRB1*04:02~DQB1*03:02 haplotype. No cases of DRB4*01:03:01:02N~DRB1*09 haplotype was detected.

Our results confirm the presence of the unusual DRB1*04~DRB4*01:03:01:02N association in Spanish population and indicate that this null allele is not only encoded on the haplotype DRB1*07~DQB1*03. Moreover, our data suggest that DRB4*01:03:01:02N could be included in DRB4*01:03:01:02N~ DRB1*04:02~DQB1*03:02 haplotype in Spanish population.

P-135

VIGILANCIA A 6 MESES POST-TRASPLANTE CON MEDICIÓN DE DNA LIBRE PROCEDENTE DEL DONANTE (DD-CFDNA) EN PACIENTES TRASPLANTADOS RENALES CON ANTICUERPOS ANTI-HLA DONANTE ESPECÍFICOS (DSA) PREFORMADOS.

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Actualmente la monitorización de los pacientes trasplantados con DSA preformados se realiza mediante seguimiento de DSA y marcadores subrogados de la función renal. La hipótesis es que la medición de dd-cfDNA es útil para su monitorización e intervención temprana.
Se ha medido el dd-cfDNA (AlloSeq cfDNA, CareDx) en 9 pacientes con DSA preformados a los 15 días, 1, 3 y 6 meses post-trasplante.
Tres pacientes presentaron valores de dd-cfDNA mayores al 1% (1,6 a 7,3%) a los 15 días post-trasplante. Uno, con DSA de alta MFI (>10000) frente a DP2 y DP3, presentó elevación de creatinina y biopsia compatible con rechazo. Tras el tratamiento, mejoró clínicamente y los valores de dd-cfDNA disminuyeron hasta normalización (0,6% y 0,3% a 1 y 6 meses post-trasplante). Los otros dos pacientes no presentaron signos de rechazo y sus valores de dd-cfDNA se normalizaron.
Seis pacientes presentaron valores de dd-cfDNA menores al 1% a los 15 días post-trasplante. Uno de ellos presentó empeoramiento progresivo de los niveles de dd-cfDNA (0,6% a 2,6%), anticipando a la disfunción renal en más de un mes. Dos pacientes tuvieron niveles mayores al 1% a los 6 meses post-trasplante, uno con valores altos de DSA, ambos sin síntomas clínicos de deterioro. Los otros tres pacientes tuvieron valores de dd-cfDNA menores al 1%, buena evolución clínica y DSA indetectables post-trasplante.
Se observó una diferencia significativa en los valores de dd-cfDNA entre las muestras con DSA+ y DSA- ($p<0.01$; n=15, 0.7% [CI95% 7.3–0.2] vs n=12, 0.4% [CI95% 2.5–0.1]). Por el contrario, no se observaron diferencias significativas en los valores de creatinina ($p=0.1$).
En pacientes trasplantados con alto riesgo inmunológico, la evaluación del dd-cfDNA puede ayudar a valorar la situación del injerto, riesgo de rechazo agudo y anticipar el empeoramiento clínico. Asimismo, evita la realización de biopsias en pacientes con injertos estables.

P-136

PRE-TREATMENT OF KIDNEY TRANSPLANTATION SERA SIGNIFICANTLY REDUCES FALSE POSITIVE RESULTS

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Background: anti-HLA antibodies is one of the major causes of graft rejection in kidney transplantation. Single Antigen bead(SAB) assays have improved the sensibility of anti-HLA antibodies detection. Nevertheless, some treatments or autoimmune diseases could interfere with this analysis, increasing background (negative control bead, NCB, with high MFI: Median Fluorescence Intensity) and false positive results. This could extend the time in the waiting list for these patients, especially in hypersensitized with elevated cPRA.

The aim of this study is to evaluate a protocol of sera pretreatment to eliminate background in sera with SAB with values of MFI >250 in NCB.

Methods: A prospective study of 5 sera, with a NCB >250 MFI in SAB (1/2/EDTA-pretreated), from patients included in deceased waiting list for kidney transplantation. These sera and negative and positive pools were retested by SAB assay using Luminex after EDTA-0.6%/BSA-5%/PBS pre-treatment at the following dilutions: 1/2, 1/4 and 1/8.

Results: The results of negative and positive pools maintained stable after pretreatment at the three dilutions. The MFI value of positive bead control of the five pretreated sera remained in correct range for the three dilutions. The MFI value of NCB decreased to correct range for the five pretreated sera, but the dilution which reach the correct MFI range vary for each patient. The dilution taken to the analysis of anti-HLA antibodies was the lowest with a NCB below 250 MFI. Before pretreatment, the five patients included antibodies against their own HLA molecules, and they clarified after treatment. For the five patients, the antibodies with highest MFI values remained stable after treatment, but antibodies with MFI values below 4000 became negative, causing a significant reduction of cPRA.

Conclusions: Sera pretreatment with EDTA-0.6%/BSA-5%/PBS allows to reduce background and false positive results, increasing transplantation opportunities for those patients with interferences in SAB.

P-137

KIR3DL1/BW4 ALLOREACTIVITY IS INVOLVED IN THE OUTCOME OF HLA-HAPLOIDENTICAL STEM-CELL TRANSPLANTATION WITH POST-TRANSPLANT CYCLOPHOSPHAMIDE.

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Introduction and objective: During the haploidentical stem cell transplantation (haplo-SCT), natural killer cell (NK) alloreactivity may contribute to graft-versus-tumor effect. NK cell lineage, amongst others, appears in peripheral blood within the first month post-SCT so may aid clearing of residual leukemic cells. Donor's NK showcase alloreactivity if there are not inhibitory signals coming from the patient's HLA class I ligands. In this study, we retrospectively evaluated the impact of *KIR* genes and KIR/ligand interactions on post-transplant cyclophosphamide haplo-SCT outcomes in 184 patients with hematological malignancies at our center.

Methods: Patient's HLA class I and donor's KIR genotypes were determined by sequence specific oligonucleotide (SSO) genotyping kits (Lifecodes, Immucor, CT, USA). *HLA-C* alleles were assigned to groups C1 or C2, *HLA-B* alleles were assigned to groups Bw4 or Bw6, while *HLA-A* locus was classified according either to the presence of *A*03* and *A*11* alleles or presence of Bw4 epitope.

Results: 30.8% of patients received myeloablative conditioning regimen whereas reduced intensity was provided to the remaining. The recipient's median age was 56 years (IQR: 40-63). KIR3DL1/Bw4 interaction was associated with an excess of mortality [OR=2.4 (95%CI 1.2-4.8), p=0.01] and the Kaplan-Meier curve shows a lower survival in patients positive for KIR3DL1/Bw4 [26.7% vs 45.8%, Log Rank= 0.02]. No significant association was observed between other KIR receptors nor KIR/ligand interactions with disease relapse, death, graft versus host disease or engraftment.

Conclusion: KIR3DL1/Bw4 may influence the survival of patients undergoing haplo-SCT, hence, on the basis of our results, the analysis of KIR/ligand remains of importance for donor selection to improve transplant outcome.

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LA PRESENCIA DE MOLÉCULAS KIR2DL2/S2,KIR2DL5 Y KIR3DL1 EN RECEPTORES DE TRASPLANTE HEPÁTICO CON CIRROSIS ALCOHÓLICA PODRÍA ESTAR IMPLICADA EN LA MUERTE POR FRACASO DEL INJERTO

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Alcoholic liver disease is the second most common diagnosis among patients undergoing liver transplantation-LT. The innate immune system and the inflammatory cascade are essential to the multifactorial pathophysiology of alcoholic liver disease. Recent studies on KIR receptors have suggested that these play a role in sepsis, liver rejection, and virus relapse. We aimed to study theinfluence of preclinical complications such as ascites and encephalopathy and KIR genetic characteristics on death due to sepsis, multiorgan-MF, and graft failure-GF in AC patients undergoing LT.164 consecutive and deceased Caucasian AC patients who underwent LT were retrospectively reviewed. Pre-transplant complications, cause of death, and patient survival were analyzed. Genomic DNA was extracted from PBLs, and KIR genotyping was performed by PCR-SSO. A statistically significant increase in the presence of KIR2DL2+ in patients with MF compared to GF. An increase was also observed in KIR2DS2+ in sepsis compared to GF. Patients who died of MF, a decrease in KIR2DL5+ was observed in AC patients with and without encephalopathy. The presence of KIR3DL1+ in the AC patients significantly increased the mortality from sepsis, and this fact was confirmed by multivariate logistic regression. The presence of KIR3DL1+ in the AC patients significantly increased the mortality from sepsis and was confirmed by multivariate logistic regression. KIR2DS1+ and KIR2DS4+ showed an increase in mortality due to GF compared to patients without these genes. However, this was confirmed only for KIR2DS1+ by multivariate logistic Cox regression. Our results show that the presence of KIR2DL2/S2, KIR2DL5, and KIR3DL1 genes in the patient influences MF and GF after LT, according to our findings. Our findings highlight the AC patient's vulnerability to LT during hospitalization following the transplant and outside of it and the need to adopt critical preventive actions to build a healthcare routine screening to improve and modify treatments to increase survival.

P-139

IDENTIFICATION AND CHARACTERIZATION OF THE NOVEL *HLA-DPA1*02:66*:02N ALLELE, WITH A PREMATURE STOP CODON IN EXON 2

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Introduction: the failure to identify HLA null alleles in bone marrow transplantation could be life-threatening because this could result in an HLA mismatch with the ability to trigger graft-vs-host disease and to reduce patient's survival. Here we describe the identification of the novel *HLA-DPA1*02:66:02N* allele during routine HLA-typing using next-generation sequencing (NGS).

Material and methods: *HLA-DPA1*02:66:02N* was first identified in two unrelated bone marrow donors by NGS. We also typed them by sequence specific oligonucleotide (SSO) and sequence specific primer (SSP).

Results: HLA sequencing of bone marrow donor #1 gave a *DPA1*01:03:01:04* and *DPA1*02var* assignment after complete genomic HLA-DPA1 analysis in a single phase. HLA sequencing of bone marrow donor #2 gave a *DPA1*01:03:01:12* and also *DPA1*02var*. The *DPA1*02var* showed a single nucleotide difference respect to *DPA1*02:01:01:03* in exon 2, genomic position 3825 (C>T), codon 50 (CGA>TGA) producing the replacement of the amino acid arginine (Arg, A) by a premature stop codon.

Given that the substitution was located in exon 2, we also typed both donors by SSO, which showed two possible allele combinations for both: *DPA1*01:03:03* and *DPA1*01:06:01* or *DPA1*01:03:03* and *DPA1*02:45*. Beads which target *DPA1*02* were negative, suggesting that they were negative for *DPA1*02:01:01:01*. Finally, we typed both donors by SSP who were positive for *DPA1*01* and *DPA1*02*.

Since both unrelated individuals share the same common Caucasoid HLA haplotype we would suggest that the novel allele could be encoded on the haplotype: *A*30:01:01:01~C*06:02:01:01~B*13:02:01:01~DRB4*01:01P~DRB1*07:01:01~DQA1*02:01:01~DQB1*02:02:01:01~DPA1*02:66:02N~DPB1*17:01:01*.

Conclusions: we identified the novel *DPA1*02:66:02N* allele by NGS and confirmed the existence of a possible new allele by SSO, but not by SSP. Therefore, this illustrates how NGS

has benefit histocompatibility labs since it increases HLA genes coverage, reduces ambiguities, permits to identify new alleles, allows to easily analyze HLA loci usually omitted in clinical practice and permits to improve transplantation outcome.

P-140

SEGUIMIENTO DE LOS VALORES DE DD-CFDNA EN PACIENTES TRASPLANTADOS RENALES COMO BIOMARCADOR PREDICTIVO DE RECHAZO

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En el campo del trasplante renal, la biopsia es el Gold Standard para la monitorización del órgano trasplantado. Sin embargo, dista mucho de ser un screening ideal, por su carácter invasivo y las incomodidades que genera. Surge por lo tanto la necesidad de encontrar un nuevo biomarcador no invasivo que permita la detección del rechazo subclínico. Actualmente la creatinina sérica y los indicadores urinarios cumplen ese rol, por ser económicos, fácilmente interpretables y relativamente fiables. Sin embargo, su sensibilidad y especificidad para detectar daño al aloinjerto son escasas. Por ello, la reciente aprobación de tests para detectar el dd-cfDNA ha supuesto una gran esperanza en el campo del reconocimiento no invasivo del daño alogénico, asociandose valores de dd-cfDNA superiores al 1% con la presencia de daño al injerto.

Se presentan los resultados obtenidos en una cohorte de pacientes trasplantados renales (18 en el momento del envío de la comunicación) cuyos valores de dd-cfDNA han sido determinados a 15 días, 3, 6 y 9 meses pos-trasplante y contrastados con su evolución clínica. De los 18 pacientes analizados, 9 mostraron valores de cfDNA inferiores al 1% en las determinaciones realizadas, mientras que los 9 restantes exhibieron un incremento significativo en algún momento. Contrastando dichos datos con su evolución clínica; ninguno de los 9 pacientes con cfDNA inferior al 1% y 3 de los pacientes con incremento del mismo mostraron algún tipo de evento adverso relevante, mientras que los 6 pacientes restantes con incremento significativo de dichos niveles mostraron o bien DSAs a alta MFI o historial de rechazo cuya aparición podría haberse predicho hasta varios meses antes estudiando los valores de este biomarcador. En conclusión, pendientes de ampliar la serie, el dd-cfDNA se postula como una herramienta diagnostica de alto valor predictivo para la detección y anticipación del rechazo subclínico.

P-141

INHIBITION OF TRAINED IMMUNITY BY IMMUNOSUPPRESSANTS USED IN TRANSPLANT MAINTENANCE THERAPIES

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Solid organ transplantation is made possible by the use of immunosuppressive drugs, although due to their detrimental effects, there is a pressing need to reduce toxicity and improve long-term allograft survival. Generally, immunotherapies aimed at delaying transplant rejection target the lymphoid compartment. Although these drugs have decreased acute rejection in the short and medium term, long-term graft survival rates remain suboptimal. A recent publication demonstrates for the first time that trained immunity is an obstacle to transplant acceptance and tolerance in an animal model and shows that it can be manipulated by nanoimmunotherapy.

To study which immnosuppressive drugs used in patients' maintenance therapies can inhibit the induction of trained immunity we developed a novel in vitro trained immunity protocol using bone marrow-derived monocytes from mice and human monocytes from healthy donors. Monocytes were trained with β-glucan and subsequently stimulated with LPS. Different concentrations of rapamycin, tacrolimus, cyclosporine, mycophenolic acid, and prednisolone were added before the first stimulus. To evaluate the inhibitory capacity of the drugs, IL-6 and TNF-α levels upon LPS stimulation were measured by enzyme-linked immunosorbent assay (ELISA). Furthermore, the gene expression of pro-inflammatory genes and the epigenetic rewiring of macrophages were measured by qPCR and CUT&RUN, respectively.

Our results indicate that tacrolimus, cyclosporine, and prednisolone inhibited the increased cytokine production characteristic of trained immunity. Therefore, these immunosuppressants could be potential candidates for use in nanotherapies to reduce their toxicity and improve long-term allograft survival.

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PROTOCOLO PARA TRASPLANTE CARDÍACO EN PACIENTES HIPERINMUNIZADOS CON ANTICUERPOS ANTI-HLA I TRATADOS CON RITUXIMAB: A PROPÓSITO DE UN CASO.

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H.U. 12 de Octubre, Madrid.

Paciente AB+ con miocardiopatía restrictiva entra en lista de espera para **trasplante** cardiaco en 2009. A finales de ese año el estudio de anticuerpos citotóxicos anti-HLA, resulta negativo. Continúa el seguimiento hasta 2022, cuando positivizan los anticuerpos anti-HLA I siendo su PRAv=78%. Un estudio de anticuerpos fijadores de complemento detecta anticuerpos C1q+ de las especificidades **A2, B57 y B58**. Dada la dificultad para encontrar un donante, se toma la decisión (diciembre 2022) de **desensibilizar** al paciente mediante ciclos de **inmunoglobulina intravenosa y Rituximab**. En enero de 2023 los niveles de anticuerpos disminuyeron hasta detectarse sólo las especificidades A2 y B57 con MFI <2000, más posterior negativización. A finales de enero se recibe una oferta de donante cadáver haploidéntico con DSA (A24 histórico, pero C1q negativo). Se procede al trasplante y se realiza la prueba cruzada a la vez. Uno de los problemas que existen en pacientes tratados con Rituximab es que las **pruebas cruzadas suelen dar resultado falso positivo** debido a la unión del fármaco a los linfocitos B. En nuestro laboratorio, hemos diseñado un **protocolo** para estos pacientes que consiste en extraer células mononucleares de ganglio y bazo para posteriormente aislar por **selección negativa linfocitos T**. Luego realizamos una prueba cruzada por **CDC** con linfocitos T y por **citometría** de flujo con células mononucleares. En el paciente en cuestión ambas pruebas resultaron negativas y no precisó ningún tratamiento desensibilizador adicional.

Conclusiones: la detección de anticuerpos fijadores de complemento y la realización de pruebas cruzadas por CDC con linfocitos T aislados y citometría de flujo en pacientes hiperinmunizados y en desensibilización con IgG-IV-Rituximab permite ampliar la ventana de antígenos permitidos aumentando por tanto el acceso al trasplante de pacientes con posibilidades muy limitadas.

P-143

ENFERMEDAD DE WALDMANN, A PROPÓSITO DE UN CASO Y REVISIÓN DE LA LITERATURA.

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La enfermedad de Waldmann se caracteriza por presentar linfangiectasia intestinal primaria, con enteropatía pierde-proteínas, linfopenia e hipogammaglobulinemia secundaria a hipoproteinemia. Se presenta el caso clínico de una paciente mujer de 36 años, con historia desde la infancia de infecciones respiratorias recurrentes tanto víricas como bacterianas, alteraciones dentarias con horizontalización de los mismos, astenia y diarrea crónicas, siendo diagnosticada en 2004 de linfangiectasias duodenales, cursando con una enteropatía pierde proteínas. En el estudio inmunológico presenta hipogamaglobulinemia IgG e IgM moderadas (416 y 18 mg/dL, respectivamente), déficit de producción de anticuerpos frente a antígeno polisacárido, linfocitopenia T CD4+ (197 cell/uL), e hipocomplementemia C4 (9.6 mg/dL). La paciente no presenta clínica ni signos de derrame pleuropericárdico ni de edemas en el momento actual. El estudio de exoma clínico objetivó una variante heterocigota en el sitio de splicing del gen RASGRP1 (c.327-1C>T) (NM_005739.3) clasificada como probablemente patogénica y asociada a defecto en la activación de los linfocitos T y B mediada por Ras. El producto codificado por este gen actúa como intercambiador de nucleótidos de guanina activando la proteína Ras, lo que lleva a su vez, a la activación de la ruta Ras-MAPK, de gran importancia en la supervivencia, desarrollo y proliferación de células T. Alteraciones en este gen se relacionan con un tipo de inmunodeficiencia de herencia recesiva (OMIM:618534) descrita en pacientes que asocian características clínicas y analíticas compartidas por la paciente. Además, se hallaron otras variantes de significado incierto en el análisis cuya implicación clínica no ha sido del todo esclarecida. Actualmente, gracias a las técnicas de secuenciación, se está obteniendo información de gran relevancia clínica, si bien, vemos necesario una validación funcional de los hallazgos, para poder enmarcar las variantes dentro de los mecanismos de enfermedad y poder realizar un seguimiento clínico personalizado.

P-144

HIGH RESOLUTION HLA TYPING INCREASES THE ACCESS TO KIDNEY TRANSPLANT IN HIGHLY SENSITIZED RECIPIENTS

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Background: Renal transplantation is the best curative therapy for chronic kidney disease. One of the most relevant parameters for successful kidney transplantation is HLA compatibility. The development of new high resolution (HR) HLA typing technologies, such as Next Generation Sequencing (NGS), has allowed to easily typify HLA loci to protein level, permitting to optimize HLA compatibility between donor and recipient.
Case presentation: A pre-sensitized (two deliveries) 65 years-old woman, diagnosed with chronic kidney disease secondary to p-ANCA+ vasculitis, was selected for kidney transplant from a deceased donor. HR HLA typing of the recipient was: A*11:01, 32:01; B*15:01, 51:01; C*03:03, 15:02; DRB1*04:01, 04:04; DRB4*01:01P; DQA1*03:01, -; DQB1*03:02, -; DPA1*01:03, -; DPB1*04:01, -. Her pretransplant panel reactive antibody (PRA) was 100%, calculated by Single Antigen bead assay. The HLA typing of the deceased donor was: A*03, 23; B*15(63), 50; C*04, 07; DRB1*04:03, 04:05; DRB4*01:01P; DQA1*03, -; DQB1*03(8), 04; DPA1*01:03P, -. DPB1*02:01, -. The evaluation of HLA compatibility at low resolution indicated that donor and recipient shared DRB1 locus, but the recipient had IgG anti-DRB1*04 antibodies (MFI: 5773) and should be discarded. However, it was decided to re-evaluate the case according to HR typing and protein level for Single Antigen. We realized that she only had antibodies against DRB1*04:02, which was not present at donor typing and she did not have antibodies targeting other DRB1*04 molecules. The cytotoxic dependent complement cross-match was negative and she received the kidney graft without showing any early post-transplant complications. After three months of follow-up, the patient presents a stable clinical and hemodynamic profile without *de novo* anti-HLA antibodies synthesis.
Conclusions: This case highlights the importance of including HR HLA typing in kidney transplantation in order to optimize HLA compatibility, especially for highly pre-sensitized patients.

P-145

COLLATERAL ACUTE ANTIBODY-MEDIATED KIDNEY REJECTION BY REACTIVATING HLA SPECIFIC MEMORY CELLS AFTER INFECTION.

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Background and aim

Circulating donor-specific anti-HLA antibodies (DSA) before or arising after transplantation constitute a critical threat to allograft function. Hence, HLA- allosensitization, either classically through pregnancy, blood transfusion or previous transplant, or through non-classically infections, involves a major barrier to transplant success. Our aim was to understand the cause of an early allograft humoral rejection in the absence of preformed DSA.

Material and methods

Anti-HLA antibodies were analyzed by Single Antigen Beads (One Lambda). High-resolution HLA typing was performed by PCR-SSO (Immunocore).

Results

A 64-year-old woman with chronic kidney disease secondary to Alport syndrome and pregnancy as HLA-sensitization event, received a blood-isogroup asystole-donor kidney allograft. Virtual and flow-cytometry crossmatches were negative at the moment of transplantation. The patient received thymoglobulin and triple immunosupressive therapy with early start of tacrolimus. On the 13th day after transplantation, kidney allograft started to fail, overlapping with diarrhoea and acute phase reactants increase. Microbiology uro-analyses isolated the presence of E. coli and E. faecalis. Biopsy showed features compatible with a mixed cellular IA rejection with capillaritis and strong Cd4 staining. Anti-HLA antibody analyses revealed the presence of a de-novo profile, including the emergence of a prior negative DSA-A2 (pre-transplantation MFI <200; current MFI>;8000). The rejection was treated with steroid bolus, plasmapheresis and intravenous immunoglobulin with good response. Interestingly, although DSA-A2 explained humoral findings, the remaining de-novo antibodies seemed not to be secondary to an allograft response. Thus, after deciding to genotype the patient's husband-HLA, the de-novo profile could be completely understood.

Conclusions

An infection in the context of allograft transplantation could awake quiescent B-lymphocyte clones in HLA-sensitized patients with the subsequent emergence of DSA,invisible in the pre-transplantation analyses, leading to collateral allograft rejection. Beyond the mechanism triggering the immune activation, those patients pre-exposed to alo-HLA, even with negative preformed DSA, should be thoroughly monitored.

P-146

STUDY OF FAMILIAL HCL IN THE HOSPITAL UNIVERSITARIO PUERTA DEL MAR

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Introduction

We present the study of 2 siblings diagnosed with BRAF V600E mutation-positive hairy cell leukaemia (HCL). The sister was diagnosed with HCL 9 years ago. the brother was being studied for pancytopenia. We studied a bone marrow sample to rule out a possible myelodysplastic syndrome by flow cytometry (FCM); however, an infiltration of 15% of the total cellularity was observed, corresponding to mature clonal lambda B lymphocytes.

OBJECTIVE

Study and affiliation of proliferative syndrome in a patient with pancytopenia and with a sister previously diagnosed with HCL, by means of FCM, genetic study and HLA typing.

Material and methods

The patient's peripheral blood was evaluated by FCM, the LST tube and Euroflow B-CLPD tube 2 and 3 were used. BRAF V600E mutation was studied by qPCR. HLA typing was performed using LinkSēq™ HLA Typing Kits.

Results

Flow cytometry study showed a larger mature lambda clonal B population with CD19+h CD20+h CD103+h CD25+ phenotype compatible with tricholeucocytes accounting for 10% of the lymphocytes. Genetic testing for BRAF V600E mutation was positive.

Both siblings were HLA-identical. Both had B58 and DR11 alleles.

Conclusions

HCL is an acquired chronic B lymphoproliferative syndrome characterised by the presence of tricholeucocytes and accounts for 2% of all leukaemias.

We present the rare case of two siblings diagnosed with HCL and tested positive for the BRAF V600E mutation. Familial HCL is a very rare type of HCL for which only about 20 families have been described. However, no genetic variations have been found to be determinant for germline predisposition to the disease. To our knowledge, the most extensive study of familial HCL and HLA association included the study of 38 patients. It found a higher frequency of B17 (B57 and B58 split antigen serotypes) and DR11 alleles, as in our patients.

P-147

RECHAZO RENAL MEDIADO POR ANTICUERPOS ANTI-MICA (SOBRE UN CASO CLÍNICO)

Fernández-Perea, Beatriz; Ballesta-Alcaraz, Lucía; Barrera Aguilera, Maria Del Carmen; Vilchez Gutierrez, Jose Ramón; López-Nevot, Miguel Ángel.

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El rechazo es una de las complicaciones más temidas en el trasplante. A pesar de que la mayoría de rechazos mediados por anticuerpos se atribuyen a anticuerpos anti-HLA, algunos estudios muestran que la presencia unicamente de anticuerpos anti-MICA con MFI (Intensidad Media de Fluorescencia) elevada es suficiente para producir un rechazo hiperagudo y agudo.

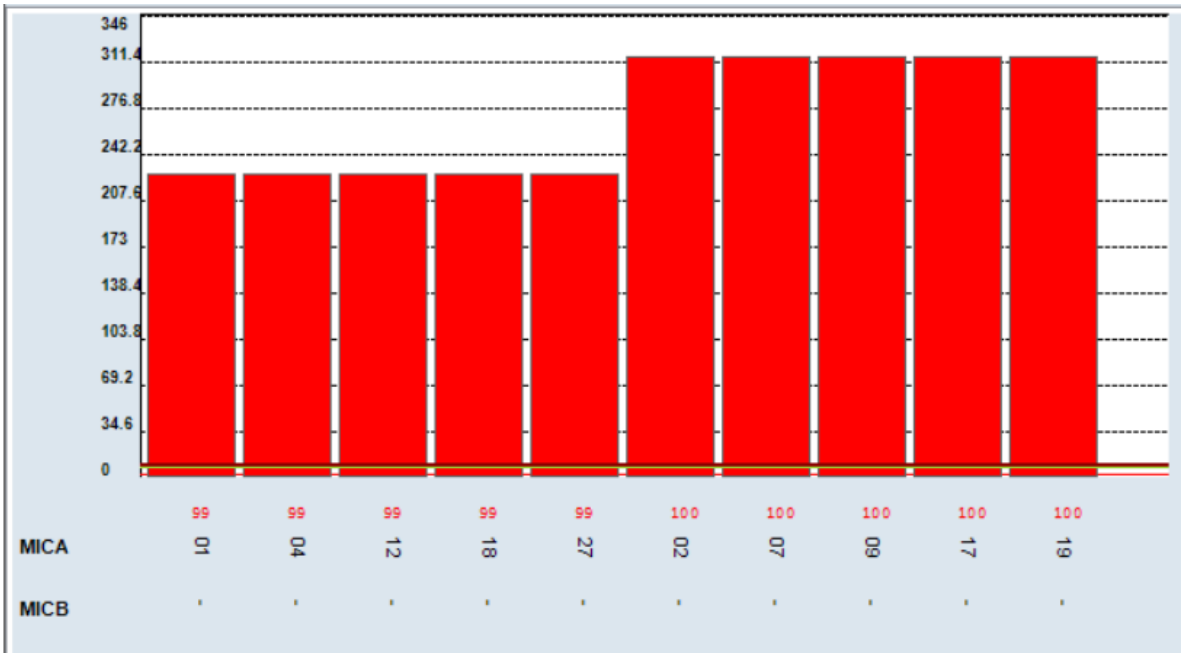
Se presenta el caso de un paciente que recibe trasplante renal de donante cadáver en junio de 2016. El acto quirurgico ocurre sin complicaciones En marzo de 2022, ingresa por deterioro de la función renal y sospecha de rechazo agudo por infrainmunosupresión. El paciente reconoce irregularidad en la toma de tacrolimus .Se observa un aumento de creatinina (3,5-4 mg/dl) y en la exploración ecográfica no se aprecian daños.

La biopsia revela un rechazo celular agudo y humoral.Se observa un rechazo agudo mediado por celulas T IA con componente de celulas plasmáticas. Presencia de capilaritis (ptc 2) asociada a positividad para C4d en capilares peritubulares, lo que indica un rechazo humoral activo acompañante (criterios de BANFF 2019). El estudio de anticuerpos por Luminex (LABScreen, One Lambda) revela la ausencia de anticuerpos anti- HLA y la presencia de anti-MICA “de novo” con MFI elevada (15.700-18.900).

Se trata con metilprepdnisolona y sesiones de plasmaféresis durante 2 semanas. El deterioro renal persiste con cifras de creatinina superiores a 2,5 mg/dl y los Ac-anti MICA post-trasplante permanecen positivos a alta intensidad tras el tratamiento (14600-17900 MFI).

En revisiones posteriores en mayo y noviembre del mismo año la tendencia se mantiene: creatinina elevada (3 y 4 mg/dl) ,anticuerpos anti- HLA de clase I y II negativos y anticuepos anti-MICA permanece con valores de MFI muy elevada. Este hecho nos lleva a plantearnos un posible rechazo mediado por MICA que podría desencadenar una lesión endotelial y deterioro

crónico del injerto.



P-148

Potenciales Donantes Con Patología Oncológica En La Región De Murcia

González López, Rosana; Encarnación Navarro, Juan Antonio; Lozano Roig, Miguel Ángel; Jiménez Coll, Víctor; Sánchez Rodríguez, Carlos; Soto Ramírez, María Fernanda; Fernández González, Marina; Botella Martínez, Carmen; Galián Megías, José Antonio; Manso Murcia, Clara; Alonso Romero, José Luis; Moya Sánchez, José; Royo-Villanova Reparaz, Mario; Muro Amador, Manuel.

Hospital Clínico Universitario Virgen de la Arrixaca, Murcia.

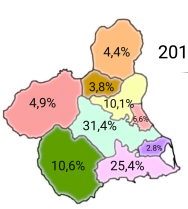
A lo largo de los años la tasa de donantes ha bajado debido a las mejoras sociosanitarias y preventivas. Por esta razón, los donantes tienden a ser población añosa con diversidad patológica acumulada y las listas de espera engrosan la mortalidad y el gasto sanitario.

La tasa de mortalidad oncológica sigue en la cima de causas de mortalidad en menores de 50 años en España. Entre estos casos, aquellos tumores primarios con bajo riesgo de metástasis podrían ser candidatos. En concreto, se ha estimado retrospectivamente la potencialidad de donantes con tumores del SNC en los últimos 10 años en la Región de Murcia (1/1/2011 al 30/6/2021), obteniéndose 667 pacientes que cumplían los criterios de inclusión para el estudio propuesto. Con respecto a la distribución por edad, hubo 151 pacientes fallecidos menores de 50 años, lo que representa el 22 % del total de la muestra. Se estima que en la Región de Murcia fallecen alrededor de 15 pacientes al año con tumor cerebral primario menores de 50 años a los que se podría ofrecer de manera sistemática la posibilidad de donar.

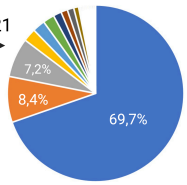
Mediante una revisión bibliográfica se estima que la tasa de transmisión de los donantes con tumor cerebral primario maligno sea <1%, por tanto, estos donantes podrían ser considerados de bajo riesgo. Mientras que en el trasplante cardíaco se ha mantenido tasa 0 de transmisión según la evidencia, para el resto de los órganos habría que estudiar individualmente el balance riesgo-beneficio según la situación del receptor. Debido a estos resultados, se está realizando un estudio con donantes diagnosticados de un tumor maligno primario cerebral desde 2021 en la Región de Murcia para analizar del riesgo de transmisión individualizado por cada tipo de órgano trasplantado, identificar los órganos y receptores adecuados para este tipo de donantes y establecer pautas de actuación.

Potenciales donantes con patología oncológica en la Región de Murcia

Hospital Clínico Universitario Virgen de la Arrixaca- Murcia



2011-2021



Tumores SNC
- Glioblastoma
- Astrocitoma anaplásico
- Astrocitoma grado II
- Ependimoma
- Meningiomas
- Oligodendroglioma anaplásico

Sin diseminación externa al SNC

667:2505 casos cumplen criterios para donación

MÍN 18años ← **Media de edad 65,2 años** → **MÁX 80años**	
Estadios tumorales	Nº de pacientes
I	22 (3,3%)
II	**88 (13,2%)**
II	**80 (12%)**
IV	**477 (71,5%)**
Tratamientos	
Cirugía+RT+QT	**58%**
Cirugía+RT	7,2%
Biopsia+RT+QT	2,4%
Sólo biopsia/Cirugía	**27,9%**
Cirugía+QT	1,8%
Alteración hepática	
SÍ	21%
NO	**79%**
Aclaramiento renal >90ml/min	
SÍ	**78%**
NO	22%

Estimación de tasa de transmisión tumoral **<1%**

Trasplante cardíaco seguro. Lista de espera nacional 2021: 141 pacientes.

Trasplante hepático. Lista de espera nacional 2021: 376 pacientes.
El beneficio del trasplante en situación de urgencia vital o hepatocarcinoma con alto riesgo de diseminación extrahepática supera al riesgo de transmisibilidad.

Trasplante renal a individualizar. Lista de espera nacional 2021: 3.945 pacientes.
Aquellos pacientes con tumores primarios del SNC que deseen donar podrán someterse a estudio pre-mortem de manera sistemática.

1.



Cuidados paliativos → **Consentimiento informado** → **Estudio de funcionalidad, transmisibilidad y compatibilidad no invasivo**

Pre-mortem: Elección individualizada de potenciales receptores según situación clínica y/u oncológica (Servicio de Nefrología) y compatibilidad (Servicio de Inmunología).

- Selección de receptores con consentimiento informado de conformidad con las características del donante.

Trasplante de órganos: Primera muestra de ADN circulante 1º día postrasplante +TC TAP al alta postrasplante.

- Inicio del seguimiento analítico-clínico al 3º mes postrasplante.
- Se mantendrá un seguimiento cada 6 meses durante los 3 primeros años con TC TAP + Analítica ADN tumoral + Tasa de anticuerpos DSA + ADN de donante circulante.
- Seguimiento anual a partir del 3º año hasta el 5º año.

2.

Peri-mortem: TC TAP → **Mortem**
- Muerte encefálica
- Donación con asistolia controlada Maastrich tipo III

- Extracción de órganos con análisis intraoperatoria de viabilidad por el Servicio de Anatomía patológica. Se analizarán biopsias ante posible diseminación vista en TC para su descarte.
- Toma de biopsia de los órganos trasplantados y no trasplantados para búsqueda a posteriori de células gliales

Sesión Poster Defensa 2. Pantalla 2. Trasplante + Inmunogenética (II)

P-149

POSTTRANSPLANT CYCLOPHOSPHAMIDE SEEMS TO PREVENT GRAFT-VERSUS-HOST DISEASE MORE EFFECTIVELY COMPARED TO STANDARD THYMOGLOBULIN UNDER HLA-MISMATCHED SETTINGS IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

Diaz-Fernandez, Paula[1]; Marcos-Jimenez, Ana[1]; Gomez Garcia De Soria, Valle[2]; Feijoo Monroy, Samantha[2]; Portero-Sainz, Itxaso[1]; Iturrate Basaran, Isabel[2]; Montes Casado, Nuria[3]; Figuera Alvarez, Angela[2]; De La Camara, Rafael[2]; Muñoz-Calleja, Cecilia[1].

[1]Immunology Department, Hospital Universitario de la Princesa, Madrid; [2]Hematology Department, Hospital Universitario de la Princesa, Madrid; [3]Instituto de Investigación Sanitaria Princesa, Madrid.

The quality of immune reconstitution largely determines the outcome of allogeneic hematopoietic stem cell transplantation (alloHSCT). In order to study its effect on the incidence and severity of graft-versus-host disease (GvHD), relapse or survival, we have prospectively studied the kinetics of immune recovery for 275 alloHSCT patients, up to one year after the transplant.

As a first approach we have compared the effect of GvHD prophylaxis schemes incorporating thymoglobulin (THY) (64% of mismatched unrelated donors (mMUD) in our cohort) or posttransplant cyclophosphamide (PTCy) (haploidentical donors and 36% of mMUD), on the development of acute GvHD (aGvHD) and chronic GvHD (cGvHD).

THY is an immunosuppressant depleting whole T-cells, which is administered before the infusion of the progenitors. On the other hand, PTCy is administered at days +3 and +5 and specifically eliminates proliferating alloreactive lymphocytes.

The incidence of aGvHD, either as a whole or when divided into degrees, did not show any significant differences between the group of patients receiving THY or PTCy. Conversely, moderate/severe cGvHD occurred in 27% of mMUD treated with THY, while no patients treated with PTCy did (p=0.0011).

Additionally, considering cGvHD degrees, PTCy seemed to protect against the severity of cGvHD (OR=0.33105; p=0.058) compared to THY. Conversely, neither overall survival nor GvHD-free, relapse-free survival at five years differed among the different types of transplants (p=0.677-and p=0.699, respectively). Furthermore, any differences in the cause of death was observed between these groups (p=0.399).

Our analysis shows that the targeted depletion of alloreactive lymphocytes derived from PTCy-based prophylaxis results in a decrease in the development of moderate-severe cGvHD compared to T-cell depletion resulting from the treatment with THY. We hypothesized that this may result from the broader effect of THY, that could be depleting regulatory T cells that may have otherwise contributed to protect against the development of GvHD.

P-150

SURVEY ON METHODOLOGICAL ASPECTS OF FLOW CYTOMETRY CROSSMATCHING: TOWARDS VALIDATION AND STANDARDISATION ACCORDING TO CURRENT REGULATION.

Torres Canizales, Juan[1]; San Segundo, David[2]; Gomez Rial, José[3]; Begoña Vazquez, María[4]; Nieto, Israel[5]; Bernardo González, Iván[6]; Caballero, Abelardo[7]; Molina, Juan[8]; González, Rafael[8]; Castro, María José[9]; Ontañón, Jesus[10]; Viola Solano, Luz Marina[11]; Marco, Francisco[12]; Cunill Monjo, Vanesa[13]; Olivares, Nerea[14]; Merino Roncal, Juana[15]; Vílchez Gutiérrez, José Ramón[16]; Cid Fernández, José Javier[17]; Caro Oleas, Jose Luis[1].

[1]Hospital Clinic de Barcelona, Barcelona; [2]Hospital Marqués de Valdecilla, Santander; [3]Hospital Clinico Universitario Santiago, Santiago; [4]Hospital Universitario de Badajoz, Badajoz; [5]Hospital Ramón y Cajal, Madrid; [6]Hospital San Pedro, Logroño; [7]Hospital Regional Universitario de Málaga, Málaga; [8]Hospital Universitario Reina Sofía, Córdoba; [9]Hospital 12 de Octubre, Madrid; [10]Hospital General Universitario de Albacete, Albacete; [11]Fundación Valle del Lili, Cali; [12]Hospital General de Alicante Dr. Balmis, Alicante; [13]Hospital Son Espases, Mallorca; [14]Hospital Universitario de Cruces, Bilbao; [15]Clínica Universidad de Navarra, Pamplona; [16]Hospital Universitario Virgen de las Nieves, Granada; [17]Complejo Hospitalario Universitario A Coruña, A Coruña.

Introduction: The flow cytometric crossmatch (FCXM) assay allows the detection of donor-specific antibodies (DSA). However, the validation method is hard to establish due to the nature of this technology. In addition, this procedure must comply with legal regulation and requirements for Laboratory Developed Tests (LDT). We aim to identify the status of the main technical and methodological FCXM aspects.

Materials and methods: A Redcap survey was distributed to members of the Spanish Working Group on Histocompatibility and Transplantation Immunology (GETHIT). Pre-analytical, analytical, and post-analytical phases of the technique were addressed, including type of reagents used, their characteristics and classifying each of the reagents according to the current regulations.

Results: A total of 17 centres participated (5 with > 10 years of experience, 8 centres with 5-10 years, 4 with 1-5 years). The median of FCXM performed was 30 tests/year (IQR= 5-90) for living donor (LD) simples and 96 tests/year (IQR= 29-137) for deceased donors (DD). Cell source mainly included peripheral blood, both from LD and DD, and spleen samples and lymph node from DD. The majority reagents used were categorized as RUO, although CE-IVD

reagents exist but not validated in FCXM. Up to 6 different anti-IgG are used (three are F(ab')2 IgG fragment and three are Fc specific antibodies). Fluorochrome-labelled antibodies included anti-IgG, CD3, CD19 or CD20, CD45 or viability dyes. 9/17 centers use commercial reagents, while 8/17 centers use “in house” preparations of phosphate buffered saline and fetal calf serum. Methodological variations, besides reagents, were identified in cell processing, serum thawing, cell/serum ratio, incubation times and temperature.

Conclusions: Collaborative efforts to identify and classify methodological aspects in alignment with the associated regulatory environments will allow in the context of complex techniques such as FCXM, the standardisation of the procedure allowing steps forward in a LDT validation process.

P-151

SUMMARY-DATA BASED MENDELIAN RANDOMIZATION IDENTIFIES GENE EXPRESSION REGULATORY POLYMORPHISMS ASSOCIATED WITH BOVINE PARATUBERCULOSIS BY MODULATION OF THE NUCLEAR FACTOR KAPPA β (NF-κß)-MEDIATED INFLAMMATORY RESPONSE

Badia Bringué, Gerard[1]; Canive Ruiz, María[1]; Fernandez-Jimenez, Nora[2]; Lavín, Jose Luís[3]; Casais, Rosa[4]; Blanco Vázquez, Cristina[4]; Vázquez, Patricia[1]; Fernández, Almudena[5]; Bilbao, Jose R.[2]; Garrido, Joseba M.[1]; Juste, Ramón A.[1]; González-Recio, Óscar[5]; Alonso-Hearn, Marta[1].

[1]Department of Animal Health, NEIKER- Basque Institute for Agricultural Research and Development, Basque Research and Technology Alliance (BRTA), Derio; [2]Department of Genetics, University of the Basque Country (UPV/EHU), Leioa; [3]Department of Applied Mathematics, NEIKER- Basque Institute for Agricultural Research and Development, Basque Research and Technology Alliance (BRTA), Derio; [4]Center of Animal Biotechnology, SERIDA-Regional Service of Agri-Food Research and Development, Deva; [5]Department of Animal Breeding, Instituto Nacional de Investigacion y Tecnologia Agraria y Alimentaria (INIA-CSIC), Madrid.

Genome-wide association studies (GWAS) have identified genetic variants associated with paratuberculosis (PTB) susceptibility. Most of the GWAS-identified single-nucleotide polymorphisms (SNPs) are in non-coding regions. Connecting these non-coding variants and downstream affected genes is a challenge and, up to date, only a few functional mutations or expression quantitative loci (cis-eQTLs) associated with PTB susceptibility have been identified. In the current study, the associations between imputed whole-genome sequence genotypes and whole RNA-sequencing data from peripheral blood (PB) and ileocecal valve (ICV) samples of Spanish Holstein cows (N=16) were analyzed with TensorQTL. This approach allowed the identification of 88 and 37 cis-eQTLs regulating the expression levels of 90 and 37 genes in PB and ICV samples, respectively (false discovery rate, FDR ≤ 0.05). Next, we applied summary-based data Mendelian randomization (SMR) to integrate the cis-eQTL dataset with GWAS data obtained from a cohort of 813 culled cattle that were classified according to the presence or absence of PTB-associated histopathological lesions in gut tissues. After multiple testing corrections (FDR ≤ 0.05), we identified two novel cis-eQTLs affecting the expression of the early growth response factor 4 (EGR4) and the bovine neuroblastoma breakpoint family member 6-like protein isoform 2 (MGC134040) that showed associations

with the presence of multifocal and diffuse lesions in gut tissues, respectively. While EGR4 acts as a brake on T-cell proliferation and cytokine production by limiting the strength and duration of the nuclear factor Kappa β (NF-κß) activation, MGC134040 is a target gene of NF-κß. Our findings provide a better understanding of the genetic factors influencing PTB outcomes, confirm that the multifocal lesions have different underlying host genetics than the diffuse lesions, and highlight regulatory SNPs and regulated-gene targets to design future functional studies.

P-152

DQA1 EPLET MISMATCH LOAD AS AN INDEPENDENT RISK FACTOR OF CLAD AFTER LUNG TRANSPLANTATION

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[1]Servicio de Inmunología. Hospital Universitario Marqués de Valdecilla, Santander; [2]Servicio de Neumología. Hospital Universitario Marqués de Valdecilla, Santander.

Background

Lung transplantation remains the finest choice for end-stage lung diseases, and the recipient selection is currently based on clinical urgency, ABO compatibility, and donor size. The risk of allosensitization is classically based on HLA mismatch but eplet mismatch load is gaining importance in long term outcomes in solid organ transplantation. Chronic lung allograft dysfunction (CLAD) is relatively common and relevant, affecting almost 50% of patients five years after transplantation and being the first cause of death from the first year after transplantation. The overall class-II eplet mismatch has been associated with CLAD development.

Methods

Based on clinical data, 240 lung transplant recipients were eligible for CLAD and HLA and eplet mismatch was analyzed using the HLAMatchmaker 3.1 software.

Results

A total of 92 (38.3%) lung transplant recipients developed CLAD. The time free-of- CLAD was significantly decreased in patients with presence of DQA1 eplet mismatches (p=0.015). Furthermore, when other previously described CLAD risk factors were studied in a multivariate analysis, the presence of DQA1 eplet mismatches was found to be independently associated with the early onset of CLAD.

Conclusion

The concept of epitope load has arisen as a new tool to better define donor-recipient immunological compatibility. The presence of DQA1 eplet mismatches potentially would decrease the time free of CLAD.

P-153

THE EVACADE STUDY: DD-CFDNA FRACTION FOR THE DIAGNOSIS OF ACUTE REJECTION IN KIDNEY TRANSPLANTATION ON BEHALF OF THE SPANISH WORKING GROUP ON TRANSPLANT IMMUNOLOGY

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[1]Immunology Department. University Hospital Marqués de Valdecilla, Santander; [2]Nephrology Department. University Hospital of Salamanca (HUS). Institute for Biomedical Research of Salamanca (IBSAL), Salamanca; [3]Nephrology Department. Renal Transplant Unit. Bellvitge University Hospital, Barcelona; [4]Haematology Department. University Hospital of Salamanca (HUS). Institute for Biomedical Research of Salamanca (IBSAL). CIBERONC-CB16/12/00233 and Centre for Cancer Research-IBMCC (USAL-CSIC), Salamanca; [5]Immunology Department. University Hospital Reina Sofía, Córdoba; [6]Immunology Department. Clinic Hospital of Barcelona, Barcelona; [7]Nephrology Department. University Hospital Marqués de Valdecilla, Santander; [8]Nephrology Department. Clinic Hospital of Barcelona, Barcelona; [9]Nephrology Department. University Hospital Doctor Peset, Valencia; [10]Nephrology Department. Renal Transplant Unit. Clinic University Hospital La Fe, Valencia; [11]Immunology Department. University Hospital Son Espases, Palma de Mallorca; [12]Immunology Department. Hospital del Mar, Barcelona; [13]Immunology Department. University Hospital Cruces, Barakaldo; [14]Nephrology Department. University Clinical Hospital of Valencia, Valencia; [15]Nephrology Department. Hospital del Mar Medical Research Institute (IMIM). Hospital del Mar, Barcelona; [16]Immunology Department. Transfusion Centre of the Valencian Community, Valencia.

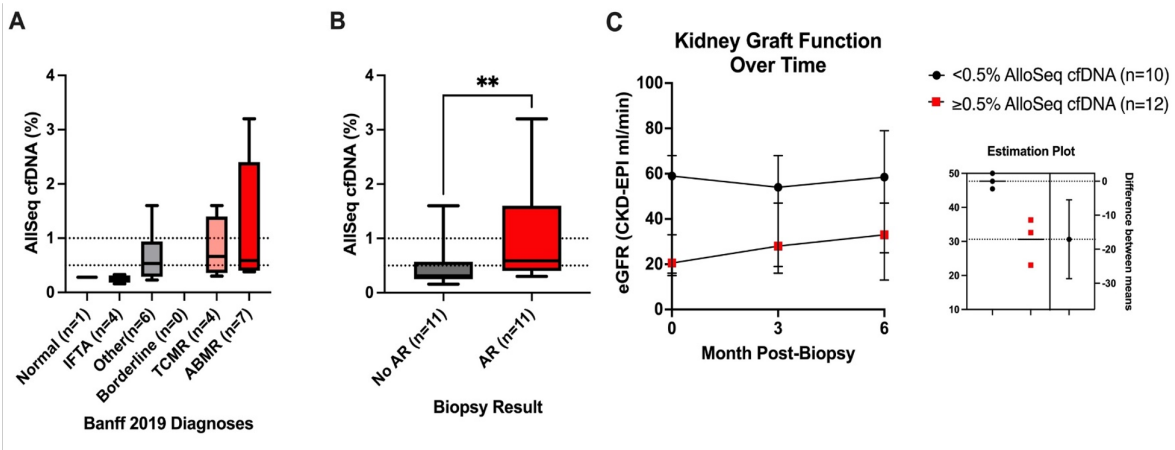
Background: Non-invasive surveillance with routine monitoring of donor-derived cell-free DNA (dd-cfDNA) after kidney transplant may allow to identify risk of allograft injury. Availability of standardized high-throughput methodologies across centres might open the opportunity to conduct dd-cfDNA testing as part of clinical routine with short turn-around times. We aimed to validate the clinical performance of dd-cfDNA as a surrogate marker of active acute rejection (aAR) for the identification of allograft injury, based on histology Banff 2019 criteria diagnoses, in kidney transplant recipients (KTr) with a follow-up of 6 months to

assess clinical outcomes.

Methods: The EvAcADE is a multicentric cross-sectional study enrolling KTr at clinical suspicion of allograft injury undergoing indication biopsy. Since January 2022, 42 patients were included from twelve different Spanish Renal Transplant Units. So far, dd-cfDNA data was available from 22 patients for trend analysis. Overall, blood was drawn paired with biopsy at each site, whereas dd-cfDNA fraction from plasma samples was quantified in a centralised laboratory using the AlloSeq® cfDNA assay (CareDx®). The nonparametric U-Mann Whitney and Spearman's rho tests were used with $p<0.05$ as statistically significant.

Results: 50% (n=11) of the indication biopsies had histology classified as rejection. Antibody-mediated (ABMR) and T-cell mediated (TCMR) rejection had higher levels of dd-cfDNA compared to other Banff categories as shown in Fig1A. dd-cfDNA at the time of biopsy was significantly elevated in patients with aAR compared to those without aAR (0.57 % vs 0.31%, $p=0.008$) (Fig1B). Elevation of dd-cfDNA (≥0.5%) was significantly correlated with reduced estimated glomerular filtration rate (eGFR) ($p=0.01$) (Fig1C).

Conclusions: Testing for dd-cfDNA in plasma efficiently discriminates aAR at the time of clinical presentation. Both ABMR and TCMR elevated dd-cfDNA levels and values ≥0.5% were associated with declined renal allograft function. Testing for dd-cfDNA could be an interesting tool to non-invasively predict renal acute allograft insults.



P-154

ONE SHOT TO DECIPHER THE COMPLEX GENETIC MAP OF ACUTE LYMPHOBLASTIC LEUKEMIA BY USING A NEW APPROACH OF NGS

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Multiple techniques are required to be simultaneously used in the genetic diagnosis of acute lymphoblastic leukemia (ALL). For this instance, karyotype, FISH, Arrays, flow cytometry, PCR and NGS are necessary to identify relevant rearrangements, clone repertoire and germline/somatic variants. The limitations of these techniques and increased expenses associated with the implementation in the laboratory, makes the diagnostic process complex, occasionally redundant, and inefficient. To overcome these limitations, we designed a new customized NGS approach that enabled us to analyze in a single test, gene mutations, rearrangements and CNVs, as well as to identify the Ig/TCR repertoire.

By using this procedure, we evaluated 26 samples from 12 patients (6 B-ALL and 6 T-ALL). In some of them, the test was applied in different moments of the disease, so we could compare the genetic map at diagnosis, remission, and relapse status. In addition, peripheral and bone marrow samples were analyzed simultaneously in some cases.

We found potential candidate germline variants in *BCL11B, STAT5B, TERT* and *ABL1* genes. All of them were classified as VUS, so further functional studies would be required to establish their pathogenicity. Mutations with prognostic o therapeutic significance in *TP53* and *FLT3* genes were found in ALL-B patients, whereas variants in *JAK3* and *STAT5* were found in a patient with early T precursor ALL. Loss of large fragments of *IKZF1*, *CDKN2A/B* genes and *BCR/ABL1* and *TAL1/STIL* rearrangements were also common findings. Finally, we were able to detect the decrease of BCR/TCR repertoire diversity in 66% of B-ALL and 83% of T-ALL patients, as well as specific clonal expansion.
In conclusion, we successfully applied an NGS-based method to analyze in one unique sample the genetic landscape of patients with ALL. This approach may offer advantages over classical techniques to identify genetic alterations with prognostic and therapeutic significance.

P-155

HLA-DQA1*05 E INMUNOGENICIDAD A INFLIXIMAB EN UNA COHORTE DE PACIENTES CON ENFERMEDAD INFLAMATORIA INTESTINAL (EII).

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Hospital Universitario Donostia, San Sebastián.

HLA-DQA1* 05 se postula como marcador genético de susceptibilidad a la producción de anticuerpos frente a fármacos anti-TNFα y útil en la orientación del tratamiento bien en monoterapia o en combinación con Azatioprina, para minimizar su inmunogenicidad en Enfermedad Inflamatoria Intestinal (EII). Diferentes estudios muestran resultados discordantes.
Objetivo: Evaluar la asociación del alelo HLA- DQA1* 05 y el desarrollo de anticuerpos frente a fármacos anti-TNFα y la relación del tipo de tratamiento con la producción de anticuerpos en el grupo de DQA1*05 positivos.
Pacientes y métodos: Se analizó la presencia del HLA-DQA1*05 en un total de 168 pacientes con EII tratados con anti-TNFα. De estos pacientes, en el 25,6% (43/168) se había estudiado la presencia de anticuerpos anti-Infliximab o anti-Adalimumab por indicación médica.
Resultados: El 44,19% (19/43) son DQA1*05 positivos. Entre los pacientes DQA1*05 positivos, el 47% (9/19) tienen anticuerpos, mientras que de los DQA1*05 negativos, el 45% (11/24) presentan anticuerpos (anti-Infliximab en todos los casos), sin embargo, la asociación del DQA1*05 con la presencia/ausencia de anticuerpos no es significativa (p>0,05). La mediana del tiempo de aparición de los anticuerpos es de 10 meses (6,5-17,5) en el grupo de pacientes con HLA-DQA1*05 positivo y de 7 meses (3-26) en el grupo con HLA-DQA1*05 negativo (p>0,05).
La pauta farmacológica en estos pacientes es heterogénea. Entre los pacientes DQA1*05 positivos (n=19) se observaron anticuerpos en 2/9 pacientes con tratamiento combinado y en 7/10 pacientes con Infliximab en monoterapia.
Conclusión: No podemos confirmar la asociación de DQA1*05 y aparición de anticuerpos anti-Infliximab ni con un menor tiempo de aparición de los mismos. Nuestra cohorte, con baja incidencia de anticuerpos positivos (11.9%), ha de ser ampliada para realizar un análisis más robusto de la relación de DQA1*05 con la inmunogenicidad a anti-TNFα en monoterapia o combinada con Azatioprina.

P-156

SEQUENCING OF TWO NEW HLA ALLELES BY NGS, *HLA-B*50:22* AND -*DRB1*03:200*, IDENTIFIED IN TWO SPANISH BONE MARROW DONORS.

Planelles, Dolores[1]; Boix, Francisco[1]; Rodriguez-Cebriá, Manuel[1]; Luis-Hidalgo, Mar[1]; Castro, Emma[1]; Callao, Virginia[2]; Larrea, Luis[1]; Ortiz De Salazar, Mabel[3]; Roig, Roberto Jesus[1]; Arbona, Cristina[1].

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Introduction

The search for an HLA-matched unrelated donor for patients in need for hematopoietic stem cell transplantation maybe difficult, especially for patients with rare HLA alleles. It is therefore important to increase both donors and HLA typing resolution in the registries using high throughput techniques such as Next Generation Sequencing (NGS).

Objective

Molecular characterization of two novel HLA alleles, *B*50:22* and *DRB1*03:200*, in two Spanish bone marrow donors.

Material And Methods

The NGS procedure was performed with the AllType™ FASTplex™ NGS kit (Thermo Fisher Scientific Inc.) following manufacturer's instructions. Our high-resolution typing strategy includes full-gene coverage for A, B, C, DQA1 and DPA1; and full nucleotide coding sequence coverage for DRB1/3/4/5, DQB1 and DPB1. Ion Torrent™ based-NGS was performed in an Ion GeneStudio™ S5 Plus instrument (Thermo Fisher Scientific Inc.). Data were analysed using the TypeStream Visual® Software (One Lambda) and the 3.46-3.47 versions of the IPD-IMGT/HLA Database.

Results

The new *HLA-B*50:22* was detected after completing full-length genomic HLA-B analysis (genomic sequence 4000bp) showing one single nucleotide (SNP) difference from its closest allele *B*50:01:01:01* in exon 5, cDNA position 916 (A916G, ATC>GTC), resulting in an aminoacid change in codon 282 (I282V). The new allele has been included in the designation groups B*50:01P and B*50:01:01G.

The new *HLA-DRB1*03:200* (coding sequence 663bp) differs from its most closely related *DRB1*03:01:01:02* allele by one SNP at cDNA position 175 (T175C, TAC>CAC) within exon

2, resulting in an aminoacid substitution at codon 30 (Y30H). Both HLA alleles were officially named as *B*50:22* (HWS10062210) and *DRB1*03:200* (HWS10061746) by the WHO Nomenclature Committee for Factors of the HLA System in 31 August 2022 and 30 June 2022, respectively.

Conclusions

The application of NGS to HLA typing is enlarging HLA diversity in registries, thus increasing the likelihood of finding an HLA-compatible stem cell donor more accurately.

P-157

PAPEL DE LOS ANTICUERPOS ANTI-DPB1 EN EL RECHAZO RENAL HIPERAGUDO (SOBRE UN CASO CLÍNICO)

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La presencia de pacientes en lista de espera de trasplante renal con anticuerpos frente a multitud de especificidades precisa de la existencia de programas que amplíen el número de posibles donantes al que tiene acceso este perfil de paciente.

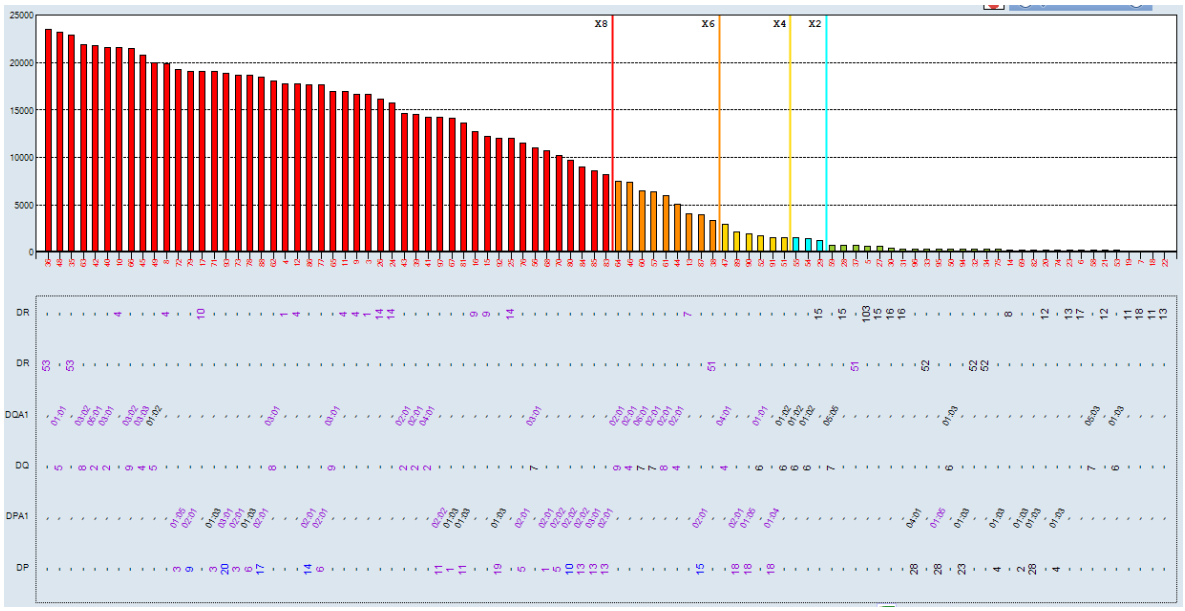
Se presenta el caso de un paciente incluido en SICATA (Sistema de Información de la Coordinación Autonómica de Trasplantes de Andalucía). Se comunica al servicio la presencia de un potencial donante de riñón compatible con dicho paciente para el que se ofrecen el tipaje HLA del donante que incluye los alelos A, B, C, DRB1 y DQB1, pero no se conoce las especificidades de DPB1,ya que el centro de procedencia del órgano no tipa dicho locus en alarmas de trasplante.

El posible receptor había recibido dos trasplantes previos, en los que había generado anticuerpos frente a clase II, entre los que se encuentran anticuerpos anti-DPB1 frente a algunas especificidades con elevada intensidad de fluorescencia. Se realiza el cross-match por CDC (Citotoxicidad Dependiente de Complemento) separando subpoblaciones linfocitarias T y B y éste resulta negativo. Ante la falta de consenso frente a la relevancia de los anticuerpos anti-DPB1 en el trasplante renal y escasas posibilidades de trasplante de dicho paciente (PRA>95%) se decide efectuar el trasplante.

Posteriormente, se realiza el tipaje completo del donante mediante tipificación por PCR a tiempo real (LinkSēq™, One Lambda) en el que se obtienen las especificidades DPB1*04, *17, confirmándose así la presencia de anticuerpos frente al donante ya que en análisis previos por Luminex (LABScreen, One Lambda) se mostraron anticuerpos frente a DPB1*17 con elevada intensidad de fluorescencia (MFI>18.000).

Actualmente el paciente se encuentra con tratamiento inmunosupresor y cifras de creatinina bien controladas. Por tanto, podemos afirmar que el paciente no realizó un rechazo hiperagudo a

pesar de la presencia de anticuerpos anti-DPB1 con elevada intensidad.



P-158

HLA-DMB IN AMERINDIANS: SPECIFIC LINKAGE OF DMB*01:03 AND DRB1 ALLELES

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Universidad Complutense de Madrid, Madrid.

HLA-DMB proteins are important for intracellular microbial metabolism in order other major histocompatibility complex (MHC) molecules present peptides to lymphocytes. In addition, HLA-DMB alleles have been found linked to diseases in some ethnic groups and HLA-DMB molecules may be important to explain HLA disease association. In present work, we aimed to detect HLA-DMB alleles profile in Amerindians for the first time and compare them to other populations. This will establish the bases to study HLA-DMB linkage to disease in Amerindians. A group of 168 Amerindians volunteered to donate blood after signed consent and have been typed for HLA-DMB alleles. They have been characterized both, by genetic and genealogical bases. Cloning and automated HLA-DMB-DNA (exons 2, 3 and 4) sequencing have been performed for allele assignation. HLA-DMB*01:01:01 and HLA-DMB*01:03:01 show the highest frequencies. These have been compared to other World wide populations. HLA-DMB*01:03:01 is tightly associated to certain specific HLA-DRB1 alleles in Amerindians. The specific Amerindian HLA-DMB allele frequencies and their linkage disequilibrium with other MHC alleles may be crucial to determine HLA-DMB World wide variation, evolution and specific linkage to disease in Amerindians and other populations.

P-159

CHARACTERIZATION OF A NOVEL HLA-A ALLELE, *A*31:208*, BY FULL-LENGTH PCR-NGS-BASED SEQUENCING IN A PATIENT WITH B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA.

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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 characterization of a novel *HLA-A* allele by NGS.

MATERIAL AND METHODS

The new allele *HLA-A*31:208* was identified in a Paraguayan patient diagnosed of B-cell acute lymphoblastic leukemia. The NGS procedure was performed using commercially available reagents for multiplexed HLA amplification of 6 loci (NGSgo®-MX6-1, GenDX) and a MiniSeq platform (Illumina™). In order to confirm the new allele, verification was performed with a second patient's sample obtained from epithelial buccal cells. Confirmatory typing was performed by polymerase chain reaction-sequence specific oligonucleotide (PCR-SSO) method (Lifecodes HLA-A SSO test, Immucor) using Luminex® technology (Luminex Corporation) and MATCH IT!® software (Immucor) and HLA-A locus singleplex NGS (NGSgo®-AmpX, GenDX). NGS data were analysed using the NGSengine® Software (GenDX) and the 3.44.1 version of the IPD-IMGT/HLA Database.

RESULTS

The new *HLA-A*31:208* was detected after completing full-length genomic *HLA-A* analysis (genomic sequence 3153 bp) and differs from its most closely related *A*31:01:02:01* allele by one nucleotide substitution at genomic position 1794 A>G, cDNA 844, codon 258 ACC>GCC, within exon 4 (Figure 1). The nucleotide change results in a substitution at amino acid 258Thr>Ala. The new allele has been included in the designation groups *A*31:01P* and *A*31:01:02G*.

Family haplotype analysis demonstrated that this allele is included in the haplotype: *A*31:208~C*04:01:01~B*35:20:01~DRB1*08:02:01~DQB1*04:02:01*.

The new *HLA-A* allele was officially named as *A*31:208* by the WHO Nomenclature Committee for Factors of the HLA System in 31 October 2021.

CONCLUSIONS

NGS technology is revealing greater polymorphism in the HLA system and facilitates the detection of new alleles.

```
cDNA                    620        630        640        650        660        670        680        690        700        710
A*31:01:02:01             A CCCCCCCAAG ACGCATATGA CTCACCACGC TGTCTCTGAC CATGAGGCCA CCCTGAGGTG CTGGGCCCTG AGCTTCTACC CTGCGGAGAT
A*31:208                  - ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

cDNA                    720        730        740        750        760        770        780        790        800        810
A*31:01:02:01       CACACTGACC TGGCAGCGGG ATGGGGAGGA CCAGACCCAG GACACGGAGC TCGTGGAGAC CAGGCCTGCA GGGGATGGAA CCTTCCAGAA GTGGGCGTCT
A*31:208            ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

cDNA                    820        830        840        850        860        870        880        890
A*31:01:02:01       GTGGTGGTGC CTTCTGGACA GGAGCAGAGA TACACCTGCC ATGTGCAGCA TGAGGGTCTC CCCAAGCCCC TCACCCTGAG ATGGG
A*31:208            ---------- ---------- ---------- ---G------ ---------- ---------- ---------- ---------- -----
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Figure 1. Exon 4 nucleotide sequence alignment of A*31:01:02:01 and A*31:208. Mismatch position E4-844 is shown in red.

P-160

LA MUTACIÓN C282Y EN HOMOCIGOSIS CONDICIONA LOS VALORES DEL ÍNDICE DE SATURACIÓN DE TRANSFERRINA (IST) EN PACIENTES CON HEMOCROMATOSIS GENÉTICA

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La hemocromatosis genética (HCGT) es una enfermedad relacionada con el metabolismo del hierro, en la cual se produce acumulación del mismo en diversos órganos debido a un exceso de su absorción. Desde el punto de vista genético, la causa principal de la enfermedad es la mutación C282Y en el gen HFE (6p21.3), que provoca un plegamiento anómalo de la proteína y, en consecuencia, un inadecuado funcionamiento de la misma. Existen dos mutaciones adicionales (H63D y S65C) en este gen que, en heterocigosis junto con C282Y, producen formas más leves de HCGT. Según la Asociación Europea para el Estudio del Hígado (EASL), los valores de ferritina y, especialmente, el índice de saturación de transferrina (IST), son los parámetros de referencia a la hora de solicitar un estudio genético, que en nuestro centro se realiza por RT-PCR. El objetivo de este estudio fue analizar los valores de ferritina y del IST al diagnóstico en pacientes homocigotos (C282Y/C282Y) y heterocigotos (C282Y/H63D), comparando dichos parámetros entre ambos grupos. Se analizaron un total de 145 pacientes, 51 homocigotos y 94 heterocigotos, pertenecientes al Área IV del Servicio de Salud del Principado de Asturias (SESPA). Se consideraron como patológicos valores de IST > 50% y de ferritina > 300 ng/ml en hombres, y valores de IST > 45% y ferritina > 200 ng/ml en mujeres. En el momento del diagnóstico, la ferritina media fue de 1042,65 ng/ml en pacientes homocigotos y 738,3 ng/ml en heterocigotos, y el IST medio fue de 88,92% en homocigotos y 67,83% en heterocigotos. Se encontraron diferencias significativas (p=0,02) entre los valores del IST de homocigotos y heterocigotos, pero no así en los valores de ferritina entre ambos grupos.

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DISTRIBUCIÓN DE GENOTIPOS *KIR* EN 113 PACIENTES CON ENFERMEDAD HEMATOLÓGICA MALIGNA Y SUS DONANTES.

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[1]Centro de Transfusión de la Comunidad Valenciana, Valencia; [2]Hospital Clínico Universitario de Valencia, Valencia; [3]Instituto de investigación del Hospital Clínico Universitario de Valencia (INCLIVA), Valencia; [4]Hospital Universitario y Politécnico La Fe, Valencia; [5]Hospital Regional Universitario de Málaga, Málaga; [6]Hospital Universitario de Salamanca, Salamanca; [7]Hospital Universitario Son Espases, Palma.

INTRODUCCIÓN: En el trasplante haploidéntico de progenitores hematopoyéticos (haplo-TPH), a diferencia del sistema HLA, se dispone de menor información referente al genotipo *KIR*.

OBJETIVO: Conocer las frecuencias de los genotipos *KIR* en pacientes hematológicos sometidos a haplo-TPH y sus donantes.

MATERIAL Y MÉTODO: Se realizó un estudio multicéntrico prospectivo en 113 pacientes oncohematológicos, con diversas hemopatías, sometidos a haplo-TPH y sus donantes. Las variables cualitativas se expresaron como frecuencia y porcentaje. Las cuantitativas como mediana y rango. El genotipo *KIR* se obtuvo mediante *PCR-SSO* y *PCR-SSP*. Se compararon los resultados con los descritos en *Allele Frequency Net Database.*

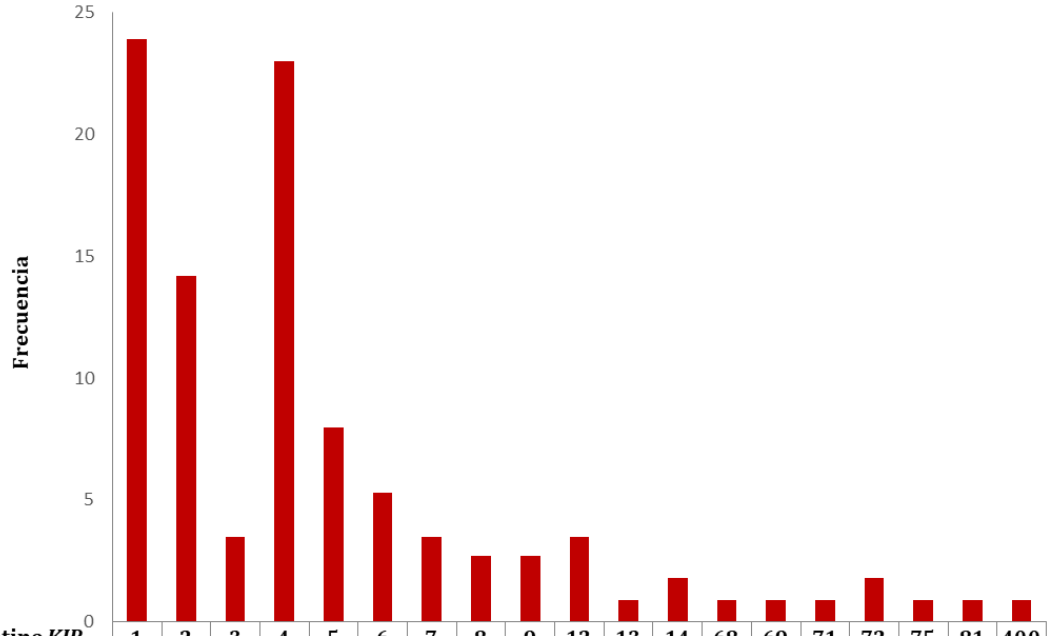
RESULTADOS: Respecto a la caracterización *KIR* de la población de pacientes los genotipos más prevalentes fueron el 1 (23,9%), seguido del 4 (23%) y posteriormente el 2 (14,2%) (Figura 1). En cambio, en los donantes el genotipo *KIR* más frecuente fue el 1 (28,3%), seguido del 2 (12,4%) y después el 4 (10,6%).

DISCUSIÓN: Estos datos concuerdan con los publicados en población española, cuyos genotipos *KIR* 1, 2 y 4 son los más prevalentes: frecuencias de 26% (16,9%-31,3%), 12,7% (7,1%-12,9%) y 15% (8,3%-17,3%) respectivamente. Sin embargo, el genotipo *KIR* 4, obtuvo

una frecuencia superior a la esperada en el grupo de pacientes. Esto podría explicarse por el limitado tamaño muestral, o que el genotipo 4 pudiera ser de riesgo frente a enfermedades hematológicas (hipótesis que requiere mayor investigación). No obstante, a nuestro conocimiento, es de los primeros estudios que analiza la distribución de frecuencias de los genotipos *KIR* en pacientes oncohematológicos.

CONCLUSIÓN: La distribución de genotipos *KIR* en la cohorte de pacientes si bien se aproxima a la referenciada en población española sana en sus genotipos más prevalentes 1, 2 y 4 (frecuencias superiores al 10%), se desvía en el caso del genotipo 4, que aparece con frecuencia mayor a la esperada.

Figura 1. Frecuencia del genotipo *KIR* en los pacientes



Genotipo *KIR*	1	2	3	4	5	6	7	8	9	12	13	14	68	69	71	72	75	81	400
Porcentaje (%)	23,9	14,2	3,5	23	8	5,3	3,5	2,7	2,7	3,5	0,9	1,8	0,9	0,9	0,9	1,8	0,9	0,9	0,9

P-162

EVOLUCIÓN DE LOS SUBTIPOS DE LA POBLACIÓN LINFOCITARIA CD4 CD25HIGH DURANTE LOS DOS PRIMEROS AÑOS DEL TRASPLANTE RENAL CON INMUNOSUPRESIÓN ESTÁNDAR Y FUNCIÓN ESTABLE

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El marcador CD127 se corresponde con la cadena alfa del receptor de la IL7 (IL7Rα) y su expresión permite dividir a la población CD4 CD25high en dos subtipos: CD4 CD25high CD127high con función efectora y CD4 CD25high CD127low con función reguladora. En este trabajo hemos medido la población linfocitaria CD4 CD25high y sus fenotipos CD127low y CD127high durante los dos primeros años del trasplante renal en pacientes con función estable e inmunosupresión estándar, con el objetivo de conocer si existen variaciones en las proporciones de estas poblaciones linfocitarias con respecto a los valores pretrasplante.

El seguimiento se llevó a cabo en 11 pacientes, mediante la extracción de sangre periférica pre-trasplante y a los 3, 12 y 24 meses postrasplante los datos obtenidos fueron los siguientes: CD4 CD25high (6,3±2,9; 12,9±10,7; 4,2±4,1 y 6,1±4,6; p=0,006), CD4 CD25high CD127high (2,4±1,6; 5,8±4,9; 2,1±2,6 y 3,3±3,6; p=0,004), CD4 CD25high CD127low (3,2±1,5; 6,5±6,1; 1,7±1,3 y 2,6±1,2; p=0,008). En todas ellas, se observa un incremento desde el pre-TR hasta el 3 mes y tras ello, un descenso a los 12 y 24 meses hasta niveles pre-TR.

El incremento de la población CD4 CD25high hasta el tercer mes postransplante indicaría una posible alorespuesta, no obstante, este incremento se ve acompañado tanto de la subpoblación efectora como de la reguladora, el aumento de la subpoblación reguladora podría estar relacionado con la estabilidad clínica.

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MONITORIZACIÓN EN TIEMPO REAL DE LOS ANTICUERPOS ANTI HLA DONANTE ESPECÍFICOS EN EL SEGUIMIENTO POST-TRASPLANTE DE PACIENTES BAJO PROTOCOLO DE DESENSIBILIZACIÓN

Walo Delgado, Paulette E.; Vionnet Salvo, Ana Paula; Jarque Canalías, Isidro; Laporta, Rosalía; Aguilar Pérez, Myriam; Bueno, José Luis; Carbone Campoverde, Javier.

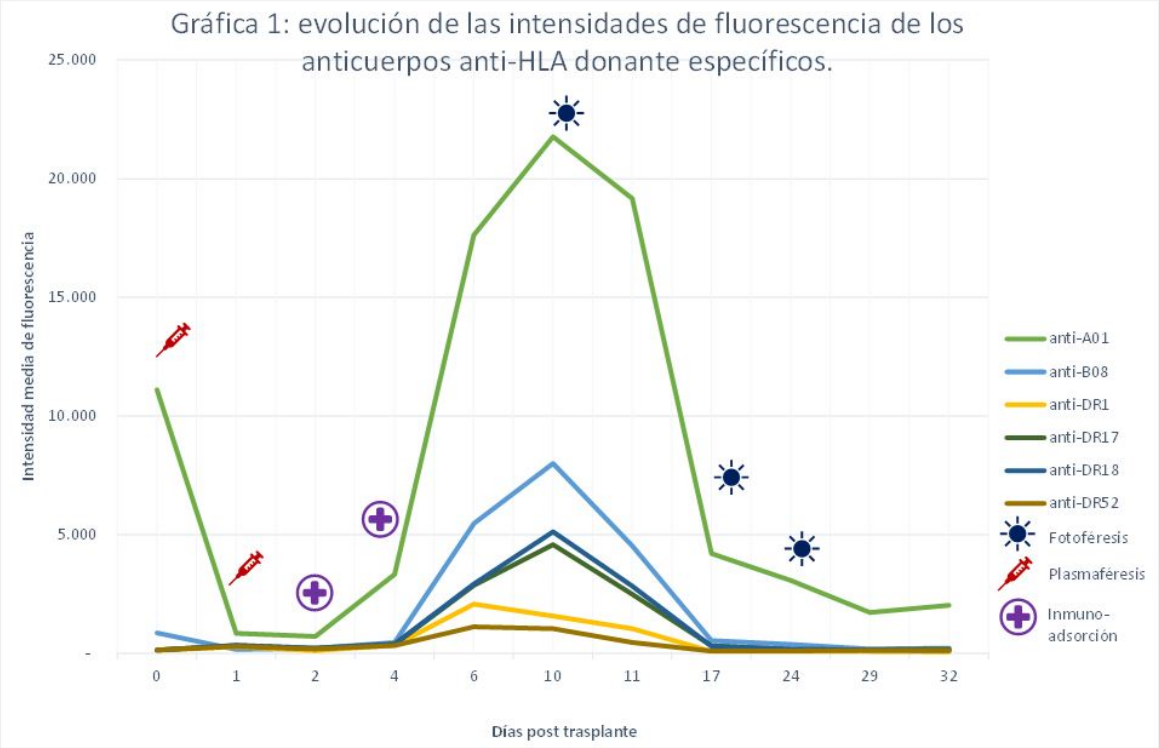
Hospital Universitario Puerta de Hierro, Madrid.

Los pacientes sensibilizados con anticuerpos anti-HLA ven reducidas las posibilidades de encontrar un donante adecuado. En algunos casos, su situación clínica no permite esperar al donante ideal, sino que se debe hacer un balance entre el riesgo y el beneficio, y optar por proceder al trasplante. Ahí entran en juego los protocolos de desensibilización para tratar de prevenir posibles rechazos mediante inmunomodulación.

Presentamos el caso de una mujer de 53 años, a quien se le realizó un trasplante bipulmonar por EPOC enfisematoso, que presentaba pre-trasplante anticuerpos anti-HLA donante específicos (ADEs) frente a uno de los alelos HLA-A con intensidad media de fluorescencia (IMF) >10.000 IMF. La prueba por citotoxicidad celular dependiente de complemento con células T del donante pre-trasplante fue negativa. Se inició protocolo de desensibilización con plasmaféresis, inmunoglobulinas intravenosas, tacrolimus y micofenolato, y se realizaron extracciones de sangre seriadas para estudio de anticuerpos anti-HLA. A las 48 horas del trasplante, los ADEs habían negativizado; sin embargo, la paciente empeoró clínicamente, lo que obligó a suspender la plasmaféresis. Adicionalmente, comenzó a presentar datos de disfunción del injerto, y la biopsia reportó infiltrado celular con eosinófilos y depósitos de C4d, sugestivos de rechazo de componente mixto, humoral y celular. Se decidió iniciar inmunoadsorción, la cual también debió ser suspendida por mala tolerancia, presentando un incremento en las IMF de los ADEs pre-trasplante (IMF >20.000) y desarrollo de ADEs frente a nuevas especificidades (Gráfica 1).

En el décimo día postrasplante se inició fotoféresis y se mantuvo terapia periódica con inmunoglobulinas, con buena tolerancia y evolución clínica favorable. Los ADEs presentaron un descenso significativo de las IMF, alcanzándose valores similares a los pre-trasplante a los 30 días de seguimiento.

La monitorización de los anticuerpos anti-HLA a tiempo real contribuye a la toma de decisiones terapéuticas en pacientes con ADEs bajo protocolo de desensibilización.



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ANÁLISIS DE POLIMORFISMOS EN LA SECUENCIA CODIFICANTE DEL GEN *NKG2C* EN TRASPLANTE RENAL

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El citomegalovirus humano (HCMV) provoca una infección persistente y muy prevalente que, si bien suele cursar de manera leve o asintomática en individuos inmunocompetentes, supone un riesgo grave en inmunodeficiencias o tratamientos de inmunosupresión. Como respuesta a la infección por HCMV se ha descrito la expansión de una población de células NK que parece implicada en el control del virus. Dicha población se caracteriza principalmente por la alta expresión en superficie del receptor activador CD94/NKG2C, el cual reconoce HLA-E. Aunque los receptores de tipo lectina para moléculas HLA son considerados poco polimórficos en comparación con la alta diversidad de las moléculas HLA-I clásicas o los KIR, aproximadamente un tercio de los individuos porta una deleción completa del gen *NKG2C* en homo o heterocigosis. Además, se han identificado varios polimorfismos tanto en la región codificante como en regiones no traducidas del gen. Hasta la fecha, esta diversidad genética apenas ha sido estudiada en patología.

Hemos analizado los polimorfismos de la secuencia codificante del gen *NKG2C* (o *KLRC2*) en pacientes sometidos a trasplante renal (n=202) y comparado los resultados con una cohorte de donantes sanos (n=240). Los polimorfismos estudiados codifican para los alelos comunes NKG2C*01 (Ser2-Ser102) y NKG2C*02 (Asn2-Phe102), junto con el alelo híbrido NKG2C*03 (Ser2-Phe102). Ambas poblaciones presentan frecuencias de portador similares para los tres alelos, así como para la deleción en homocigosis, salvo por una representación ligeramente inferior del alelo mayoritario NKG2C*01 en la cohorte de pacientes trasplantados, cuyo significado aún está por determinar.

Este estudio posibilita futuros análisis sobre el posible papel de la diversidad genética de *NKG2C* en el control del HCMV en pacientes inmunodeprimidos y, particularmente, en trasplantes de órgano sólido y hematopoyético.

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CHARACTERIZATION OF TWO NEW HLA ALLELES, *HLA-C*03:582* AND -*DRB1*07:133*, IDENTIFIED IN TWO SPANISH BONE MARROW DONORS BY NEXT GENERATION SEQUENCING.

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Introduction

In order to optimize the Spanish registry of voluntary 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). The application of NGS to routine HLA typing of donors is allowing the detection of a large number of new HLA alleles

Objective

Molecular characterization of two novel HLA alleles: *C*03:582* and *DRB1*07:133*.

Material And Methods

The new alleles were identified in two Spanish 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 3.44.1 version of the IPD-IMGT/HLA Database.

Results

The new *HLA-C*03:582* was detected after completing full-length genomic *HLA-C* analysis (genomic sequence 3070 bp) and shows one single nucleotide difference from its closest allele *C*03:03:01:01* in exon 5, genomic position 2034 (T>G), cDNA 950, codon 293 (GTC>GGC). This change results in an aminoacid substitution 293Val>Gly. The new allele has been included in the designation groups *C*03:03P* and *C*03:03:01G*. Sample showed the following typing: *A*02:01:01,-; B*15:01:01,*44:02:01; C*03:582,*05:01:01; DRB1*01:01:01,*16:01:01; DQB1*05:01:01,*05:02:01; DPB1*04:01:01,*04:02:01.*

The new *HLA-DRB1*07:133* (coding sequence 552 bp) differs from its most closely related *DRB1*07:01:01:01* allele by one nucleotide substitution at genomic position 5402 T>A, cDNA 130, codon 15 TGT>AGT, within exon 2. This nucleotide change results in a substitution at

amino acid 15 Cys>Ser. Complete HLA typing was: *A*02:01:01,*23:01:01; B*07:02:01,*44:03:01; C*07:02:01,*16:01:01; DRB1*07:133,*15:01:01; DQB1*02:02:01,*06:02:01; DPB1*04:01P,*05:01P*.

The new HLA alleles were officially named as *C*03:582* (HWS10063665) and *DRB1*07:133* (HWS10063669) by the WHO Nomenclature Committee for Factors of the HLA System in 31 October 2021.

Conclusions

Due to the extreme polymorphism of the HLA system, NGS typing is the suitable procedure for a complete and reliable HLA typing.

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HLA ALLELES AND HAPLOTYPES IN SUDANESE ETHNICITIES: THE SAHARA DESERT AS THE CRADLE OF MEDITERRANEANS

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The contribution of migrated people from once green Sahara (about 6000 years BC) towards Mediterranean area had probably a double effect: both genetic and cultural connections have been described with Western Europe and North Africa. Sudanese populations from different ethnicities have been studied for HLA-A, -B, -DRB1 and -DQB1 antigens by a standard microlymphotoxicity method. Results found show that Nubians are genetically related with black Sub-Saharan populations and distant from other Sudanese tribes, who are closer to Mediterranean populations than to Sub-Saharan ones. This is concordant with other authors and metanalysis data. This is, to our knowledge, the first and only HLA work that studies Sudanese people according to different Sudan ethnic groups; samples were collected before Sudan partition between North and South. A prehistoric genetic and peoples exchange between Africa and the Mediterranean basin may be observed and is supported with the results obtained in this Sudanese HLA study. However, demic diffusion model of agriculture and other anthropological traits from Middle East to the West do not exist.

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CAMBIO DE VISIÓN EN EL DIAGNOSTICO KIR Y HLA-C EN PACIENTES CON PERDIDAS RECURRENTES DE EMBARAZO.

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Aunque el síndrome antifosfolípido sea la primera causa de aborto recurrente, existe un porcentaje muy grande de casos que no se sabe la procedencia de la pérdida de embarazo.
Se está apostando por el papel del sistema inmune, ya que se ha visto que el contacto en los primeros meses entre estas células inmunes y las del feto es crucial.
Las células NK podrían ser las responsables de este contacto directo, presentándose en un 70% los primeros meses. Estas células, tienen unos receptores llamados KIR, formados por un conjunto de receptores activadores e inhibidores. Nuestros resultados muestran que la posible hipótesis de pérdida de ligando inhibitorio iKIR utilizada en trasplante, podría estar presente en el embarazo como factor beneficioso.
En primer lugar, decir que los resultados más vistosos, fueron un aumento de C1 en las parejas control cuando las mujeres tenían KIR AA (22.86% frente a un 10%) y además un aumento de C2 en pacientes con KIR AA (18,18% frente a un 0% en controles).
Además, según nuestros resultados, un 5,71% de parejas control presentaban ligando específico (C2) para KIR2DL1, frente a un 29,63% en las parejas con abortos recurrentes, dato significativo con una p=0.008. Y, además, se presentó un 54,2% de ligando específico (Bw4) para KIR3DL1 en controles frente a un 92,6% en pacientes, dato también significativo con una p<0,001.
Es decir, las pacientes control, con por lo menos dos embarazos sin complicaciones, podría tener sentido la hipótesis de pérdida de ligando específico para receptores activadores ya que es presentada de manera significativa en comparación con las mujeres con pérdida recurrente de embarazo.
Por tanto, este hallazgo podría ser un cambio revolucionario en el diagnóstico de estas pacientes y posterior tratamiento.

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EVALUACION DE TRASPLANTE RENAL DE DONANTE VIVO EN PACIENTES CON PRUEBA CRUZADA VIRTUAL POSITIVA

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Introduccion

La presencia de anticuerpos donante específicos (ADEs) ha sido habitualmente una contraindicación para el trasplante renal independientemente del resultados de las pruebas cruzadas por CDC y/o citometría de flujo. En los últimos tiempos, gracias a la evolución de los protocolos de desensibilización, se puede superar esta barrera al trasplante.

Objetivo

Evaluar el pronóstico del trasplante renal de donante vivo en pacientes con ADEs.

Materiales Y Metodos

Se presenta el caso de un varón de 29 años candidato a trasplante renal de donante vivo emparentado (madre) por ERC de etiología vascular. Previo al trasplante se realiza estudio de anticuerpos anti-HLA mediante tecnología Luminex por bead arrays (Labscreen Single Antigen (SA) Clase I y II, One Lambda, INC). Se objetivan ADEs a título de > 40.000 SFI (A11), recibiendo protocolo de desensibilización: administración de Rituximab y 3 sesiones de plasmaféresis.

Se realizaron 3 pruebas cruzadas (CDC y citrometría de flujo) previas al trasplante que resultaron negativas. Se realiza determinación de antiHLA tras protocolo de desensibilización con desaparición de todos los antiHLA clase I (incluyendo A11) y negativización de clase II.

Resultados

Tras realizarse el trasplante, recibe inmunosupresión de inducción con timoglobulina e inmunosupresión de mantenimiento con tacrolimus, micofenolato y prednisona con buena evolución inicial.

A las 2 semanas postraplante, se realiza determinación de anticuerpos anti-HLA por sospecha de rechazo humoral vs celular. Se observa reaparición de ADEs >40.000 SFI (A11). Se plantea biopsia renal, que el paciente rechaza. Se realizan 5 sesiones de plasmaférisis + IGIV y se administran 3 dosis de Rituximab. Buena evolución posterior.

Conclusion

La mejora de los protocolos de desensibilización y las terapias inmunosupresoras está permitiendo la realización de trasplante renal con prueba cruzada virtual positiva en pacientes no hipersensibilizados y con SFI<100.000, observándose buena evolución del injerto.

Sesión Poster Defensa 2. Pantalla 3. Inmunidad frente a las infecciones + Inmunodeficiencias (II)

P-169

IDENTIFICATION OF GENOMIC COVID-19 SEVERITY VARIANTS IN A BASQUE RESIDENT HOSPITAL COHORT

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Over the past three years, the COVID-19 pandemic caused by the SARS-CoV-2 aetiological agent has resulted in approximately 500,000,000 confirmed cases and more than 6,000,000 deaths worldwide. This highly contagious virus causes severe acute respiratory symptoms and has become the most consequential global crisis since the time of the 1918 influenza pandemic. This disease remains under study due to its ability to relapse and the heterogeneity of clinical outcomes among patients. In this study, we set out to identify host marker variants associated with severity of the disease at the cohort level, by analysing over 1.000 patients entered into the Galdakao and Cruces Universitary Hospitals associated with IIS Biocruces. Using the Axiom™ Human Genotyping SARS-CoV-2 Research Array, a whole-genome genotyping tool, more than 820.000 markers were scrutinized. The design of this array involves a dense coverage of genes implicated in underlying conditions that may affect the severity, susceptibility and outcome of the SARS-CoV-2 infection, including cytokine genes and genes associated with viral entry points and relevant pharmacogenetics, blood groups including ABO and population markers. This data resource allows deep mining for interactions and stratification of variants specific to this Basque resident population. Using both a genome-wide association (GWAS) approach and a candidate gene approach, several potential and viable severity markers were established. The GWAS approach revealed multiple SNPs with genome wide significance (*P* values < 10^{-8}) located in various chromosomes, including X where we identified association signals for instance in ACE2, a key gene enabling SARS-CoV2 infection, as well as various other genes.

We will present in depth analysis of the complete results of the Axiom™ analysis. Overall, our work underscores the value of direct and fast genotyping of medium-sized hospital-based cohorts in order to identify genomic variants associated with severity at genome-wide significance levels.

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ESTUDIO DE LA DEFICIENCIA DEL FACTOR DEL COMPLEMENTO C4 EN EL SURESTE ESPAÑOL

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El factor del complemento C4 está codificado en humanos por dos genes muy similares, C4A y C4B, los cuales codifican dos isoformas con apenas diferencias aminoacídicas, pero con ciertas diferencias a nivel funcional. Los genes C4 aparecen repetidos en número variable de copias, siendo lo más usual dos copias de C4A y otras dos de C4B, aunque en la población normal varía desde 0 copias hasta 4 o más copias de alguna de las isoformas por genoma diploide. Está ampliamente descrita una alta asociación entre un bajo número de copias de los genes C4A y el riesgo de padecer enfermedades autoinmunes por inmunocomplejos y, en menor medida, de C4B e infecciones de repetición por bacterias encapsuladas. Las frecuencias de deficiencia genética de C4 en población sana en distintos trabajos ronda el 1% para C4A y algo mayor para el C4B. Mediante un método de PCR-Real Time desarrollado en nuestro laboratorio con sondas Taqman que hibridan en los nucleótidos isotípicos, hemos sido capaces de detectar la deficiencia de ambas isoformas no solo en ADN, sino también en ARN (ADNc). El objetivo del trabajo fue comprobar si la deficiencia funcional (ARN) de C4 era mayor que la genética (ADN) en una población de estudio sin sospecha de inmunodeficiencia. Se utilizaron muestras archivadas en nuestra unidad de oncohematología, la mayoría de pacientes con sospecha de síndrome mieloproliferativo crónico. Se realizó el test a 266 ARNs, siendo negativos para C4A un 1.13%, para C4B un 10,15%, y doble negativos un 0%. Se tomó posteriormente los ADNs de las muestra negativas para comprobar si las deficiencias parciales eran funcionales o genéticas. El resultado fue un 0,38% para C4A, un 3,01% para C4B. Nuestros resultados sugieren que la deficiencia parcial de C4 puede ser has tres veces más prevalente de lo que se cree.

P-171

FIRST CASE OF C5 DEFICIENCY DESCRIBED IN BASQUE COUNTRY.

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Introduction

The complement system plays a major role in the innate immune system´s defense against encapsulated microorganisms through opsonization and membrane attack complex (MAC). Complement activation is carried out through three pathways (classical, lectin and alternative) that give rise to the formation of C5-convertasa necessary for the formation of MAC. Primary immunodeficiency of complement C5 (C5D) is a rare autosomal recessive disease associated to infections, particularly meningitis and gonorrhea by Neisseria species. Two mutations of complement C5 has been described in north-African families (Morocco).

Objectives

To report the first case in our laboratory of a family with the complement C5 mutation **c.960_962del/p.Asn320del**. The index case was a boy, who at the age of 12 years enters our hospital for meningococcal infection by **serotype Y**.

Material and Methods

Serological study: CH50 was carried out with the DiaSorin CAE kit. The study of complement is extended to the rest components C3, C4, C1 Inhibitor, C2, C1q, C5, C6, C7, C8, Factor B, Factor H, Factor I and properdin.

Genetic study: search for the mutation that causes deletion of the amino acid Asparagine in position 320 of the protein associated with C5 deficiency described in the Moroccan population, by amplifying C5´s exon 9 with part of adjacent intronic sequences and Sanger sequencing.

Results

We present a pediatric patient with symptoms of fever (39°C), vomiting, headache and prostration. Positive for Meningococcus (serotype Y). Result of CH50 4.35 (normal >70) and C5 <2.9 mg/dL (normal 7.00-23.00).

The genetic study shows that is homozygous for the mutation **c.960_962del/pAsn320del** (deletion of three consecutive nucleotides in exon 9) causing deletion of Asparagine associated

with C5 deficiency. Father and mother (consanguineous) heterozygous, sister negative.

Conclusions

We describe the first case in Basque Country of complement C5 deficit with mutation found in several unrelated families, all of Moroccan origin.

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RECURRENT MYCOBACTERIAL INFECTION IN A NOVEL CARMIL2 MUTATION FOUND IN ECUADOR

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We present a case of a female patient born in Loja- Ecuador, who at 13 years old, presented pleural and epicardial effusion. She was diagnosed with a miliary mycobacterial infection; she received 6-month triple therapy with good response. Afterwards, at 14 years old, she debuted with intense headache, fever, and left hemiparesis. A cerebral CT scan revealed an irregular heterogeneous lesion with marked ring enhancement. It was identified as a space-occupating brain lesion that required decompressive craniotomy and brain abscess drainage. Samples obtained from drainage were sent microbiological study. *Mycobacterium tuberculosis* infection, non-resistant to conventional treatment, was found. The definitive diagnosis was tuberculous abscess in the central nervous system.

The patient was referred to our immunology department with a suspicion of Inborn Errors of Immunity (IEI). We found a novel homozygous CARMIL2 missense mutation (exon 9, c.668C>T, (p.Ser223Phe), by NGS panel for Primary Immunodeficiencies. This variant is not present in population databases, nor has been described in the literature in individuals affected with CARMIL2-related conditions. The parents are from the same city Loja- Ecuador with a possibility of consanguinity.

CARMIL2 is a critical protein signaling from CD28 to CARD11 and PKC-Θ in human T cells. In the last IUIS Classification, CARMIL2 deficiency is classified within Diseases of immune dysregulation. It has been classified as an autosomal recessive disorder and it is clinically characterized by recurrent viral, bacterial, fungal, and mycobacterial infections. Some patients also develop EBV-lymphoproliferative disorders, and other malignancies. In 2022, Levy et al. reported 2 out of 89 cases with CARMIL2 gene variants that presented with susceptibility to mycobacterial infection.

Now the patient is 17 years old, in surveillance of the infectious diseases department, and waiting for a suitable treatment to be provided. She has not present new infections so far.

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PFIZER-BIONTECH COVID-19 (BNT162B2) VACCINATION MAY PROTECT AGAINST T CELL IMMUNOSENESCENCE DRIVEN BY SARS-COV-2 IN CMV-SEROPOSITIVE INDIVIDUALS

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Cytomegalovirus (CMV) infection drives accelerated T cell immunosenescence characterized by the loss of CD28 receptor. These cells co-express CD57 and CX3CR1 and are associated with inflammation and vascular damage. Expansions of these proatherogenic cells have been associated with a higher risk of cardiovascular disease. We have previously shown an expansion of $CD28^{null}$CD57+CX3CR1+ T cells in mild/asymptomatic SARS-CoV-2 infected (mCOVID-19) individuals similar to those in aortic stenosis patients. Notably, this expansion only occurred in CMV-seropositive individuals. Here we aimed to evaluate whether Pfizer-BioNTech COVID-19 (BNT162b2) vaccine has a protective effect against the expansion of these proatherogenic T cells in mCOVID-19 individuals. Consequently, we analysed by multiparametric flow cytometry, the frequency of peripheral $CD28^{null}$CD57+CX3CR1+ (CD4, CD8 and TcRgδ) T cells in CMV-seropositive mCOVID-19 volunteers stratified by vaccination status (vaccinated, n=8 and unvaccinated, n=16), and CMV-seropositive healthy donors (HD) (n=15). As expected, an increase of $CD28^{null}$CD57+CX3CR1+ T cells (CD4 $p<0.01$, CD8 $p<0.001$, TcRgδ $p<0.001$) percentage was observed in unvaccinated-mCOVID-19 individuals compared to HD. In vaccinated-mCOVID-19 individuals $CD28^{null}$CD57+CX3CR1+ (CD8 and TcRgδ) T cells were increased compared to HD, but to a lesser extent ($p<0.05$). Notably, CD4+ $CD28^{null}$CD57+CX3CR1+ T cells percentage was similar among vaccinated-mCOVID-19 individuals and HD. These results suggest that Pfizer COVID-19 vaccine may offer a protective effect against the expansion of proinflammatory and proatherogenic T cells in CMV-seropositive mCOVID-19 individuals, thus potentially reducing the SARS-CoV-2 associated cardiovascular risk. Funding: This research was funded by grant PI1900075, Instituto de Salud Carlos III, Proyectos de I+D+I de Investigación en Salud 2019 (to Pera, A.), Grant PE-COVID-

0053-2020, Consejería de salud y familias Junta de Andalucía, Subvenciones para la financiación de la I+D+i biomédica y en ciencias de la salud en Andalucía 2021, co-funded by European Union (FEDER Andalucía 2014-2020) (to Pera, A. and Juan Molina). Grant FI20/00194 (to Álvarez-Heredia, P.).

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INMUNODEFICIENCIA COMBINADA SEVERA POR DEFICIENCIA DE ADA: EVOLUCIÓN SEGÚN TRATAMIENTO RECIBIDO. EXPERIENCIA DE UN CENTRO.

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Introducción y Objetivos

La adenosin deaminasa (ADA) es un enzima clave en el metabolismo de las purinas y su deficiencia puede dar lugar a una inmunodeficiencia combinada severa T-B-NK- (IDCS). Actualmente exsisten tres tipos de tratamiento para esta inmunodeficiencia: trasplante de progenitores hematopoyéticos (TPH), terapia génica (TG) y terapia de sustitución enzimática con ADA-PEG. Los dos primeros tienen intención curativa mientras que la terapia sustitutiva solo corrige parcialmente la deficiencia. El objetivo de este trabajo es describir la experiencia en nuestro centro con estos pacientes.

Métodos

Se realizó un análisis retrospectivo de los 6 pacientes con deficiencia de ADA seguidos en el Hospital Universitario La Paz desde 1998.

Resultados

Todos los pacientes presentaron síntomas clásicos de IDCS en los primeros 6 meses de vida. Se encontró la variante p.Leu107Pro en cinco de ellos, dos de los cuales eran homocigotos. Tres pacientes, diagnosticados en 2002, 2016 y 2017, respectivamente, se sometieron a TPH, mientras que el resto no tenía disponible un donante de médula ósea compatible. Dos pacientes continúan con terapia sustitutiva (desde 2003 y 2005, respectivamente), y una fue incluida en un ensayo de terapia génica en el Great Ormond Street Hospital for Children, Londres. Todos han conseguido evitar la susceptibilidad a infecciones aunque solo dos trasplantados y la paciente de terapia génica han alcanzado una reconstitución inmunológica completa. El principal problema de cuatro pacientes es la sintomatología neurológica, independientemente del tratamiento

recibido.

Conclusiones

La deficiencia de ADA es la forma más frecuente de IDCS autosómica recesiva. Afortunadamente, existen tratamientos curativos y de sustitución enzimática que han permitido una supervivencia del 100% en nuestra serie. Nuestros principales retos son el manejo de pacientes con terapia sustitutiva a largo plazo y el abordaje de las complicaciones neurológicas que presentan la mayoría de nuestros pacientes.

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ESTIMATING ADMISSIBLE TOTAL ERROR IN LYMPHOCYTE POPULATIONS' ANALYSIS BY FLOW CYTOMETRY: 10 YEARS' RESULTS OF SPANISH SOCIETY FOR IMMUNOLOGY EQAS GECLID-SEI

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Introduction

Flow cytometry analyses of lymphocyte subpopulations (T, B, NK) are crucial for an increasing number of clinical algorithms and research workflows such as those for leukaemia, lymphoproliferative disorders, immunodeficiencies, or CAR-T therapies' monitoring. Growing quality and safety standards for in vitro diagnostic medical devices (IVD) are to be implemented by every clinical laboratory to fulfil the European directive IVD-Regulation EU 2017/746, and fully applicable in the EU since May 26th, 2022.

Aim

To estimate admissible Total Error (aET) values of percentage and absolute number of lymphocyte subpopulations based on the current state of the art (SOTA) using real data from a proficiency testing scheme of the Spanish Society for Immunology (GECLID program) and to compare them with previously published BV specifications.

Material and Methods

44,998 results from 75 laboratories were analysed from 2012 to 2021. Data come from IC-1 scheme (Lymphocytes) of GECLID Program. This quantitative scheme includes both percentage and absolute numbers of CD3+, CD4+, CD8+, CD19+, and NK cells. %TE was calculated as: [(reported value – robust mean)/robust mean] *100/laboratory/parameter. The cut off for aTE was set at 80% best results of the laboratories.

Results

The aTE was calculated for 2 different sets of data, the first with all data from 2012-2021 (green bars), and a second data from 2017-2021 (blue bars) in Figure 1. The previously TE specifications calculated in 2019 based in BV were widely achieved by the laboratories included

in the study (orange bars). The aTE for each parameter from 2017-2021 was lower than that calculated from the whole dataset.

Discussion

The decrease of % aTE observed with 2017-2021 data could be related to technical advances in flow cytometry. The aTE specifications for lymphocyte subsets´ determination based on proficiency testing results are a useful tool for analytical improvement.

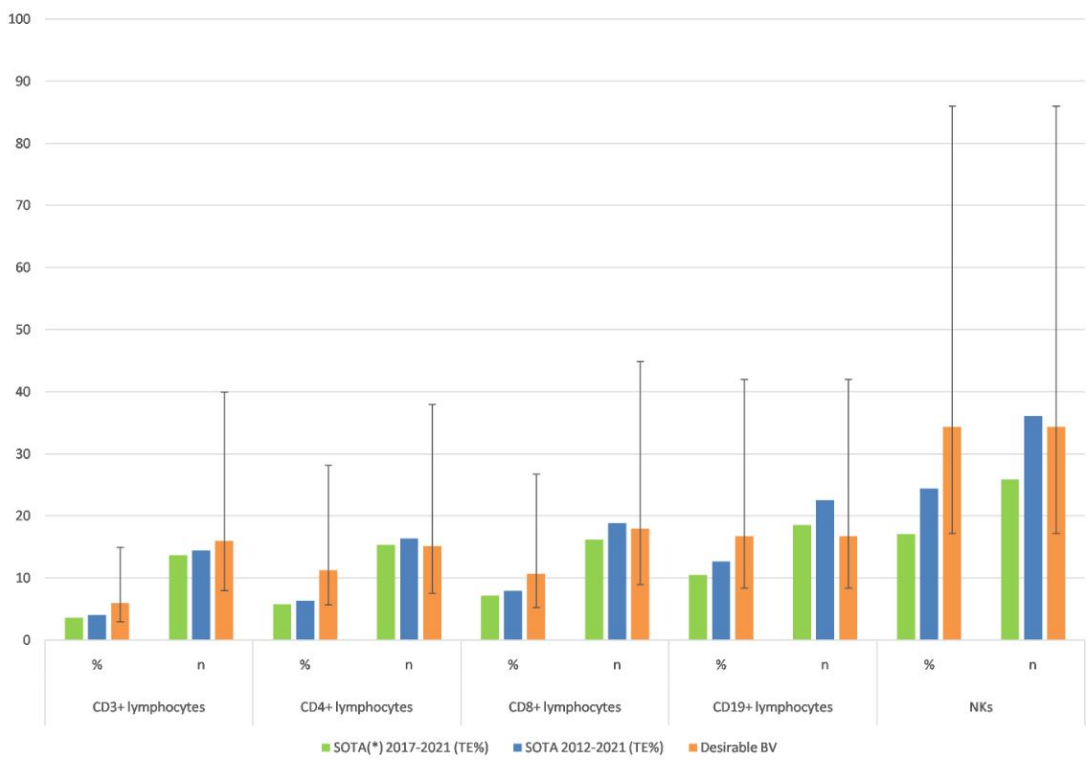


Figure 1. Calculation of admissible Total Error based on state of the art and comparison with biological variation specifications for lymphocyte subpopulation parameters. Data from a proficiency testing scheme of the Spanish Society for Immunology (GECLID program) for lymphocyte subsets were analised for admissible Total Error calculation (from 2012-2021, blue bars; from 2017-2021, green bars) and compared with aET measured by desirable biological variation (BV) up to 2019 (orange bars, whiskers show the optimun and minimun BV)

P-176

ASSESMENT OF LONG – TERM IMMUNITY IN THYMECTOMIZED PATIENTS WITH DEXTRO-TRANSPOSITION OF THE GREAT VESSELS AFTER CARDIAC SURGERY.

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Introduction

Dextro - transposition of the great vessels (D-T.G.V.) is a birth heart defect that occurs in 1 out of 2,000 to 4,000 newborns. During D-T.G.V. surgery in the neonatal period, it becomes necessary to perform partial or total thymectomy.

Objetive

The objective of this study is to evaluate the long-term effect of thymectomy on the immune response in these patients.

Methods

Lymphocyte subpopulations of 30 patients with D-T.G.V. undergoing surgery during the first month of life and with a ranged age from 6 to 17 were compared with 16 non-thymectomized healthy subjects with a ranged age from 11 to 17. Comparison of the means of both groups for several values was conducted using t-student test, and a p-value < 0.05 was considered statistically significant.

Results

Neonatal thymectomized have low total lymphocytes and lymphocyte subpopulations, specially naive and regulatory T lymphocytes and recent thymic emigrants (RTEs) and elevated memory T cells.

Lymphocytes (cells /µL)	Thymectomized group	Control group	P-valor
Total	1719,00	2242,50	0,033
CD3+	931,03	1528,20	0,001
CD4+	557,97	828,40	0,017
CD8+	307,30	585,47	0,001
CD19+	346,45	274,69	0,283
NK	390,67	322,67	0,460

Lymphocytes (%)	Thymectomized group	Control group	P-valor
Subpopulations CD4+			
RTEs	17,96	45,43	0,000
Naive	33,87	56,53	0,004
Central memory	49,76	28,70	0,001
Effector memory	14,82	8,01	0,027
Effectors	1,55	0,80	0,204
Regulatory T	4,09	6,37	0,004
Subpopulations CD8+			
Naive	37,86	57,04	0,007
Central memory	37,31	22,52	0,004
Effector memory	14,40	8,98	0,068
Effectors	10,12	11,46	0,644

Conclusions

These results show that early thymectomy could have an impact on inmunity later in life, increasing the risk of developing autoimmune diseases and infections in adulthood and highlight the necessity of carrying out a periodic clinical – analytical follow up of these patients over time.

P-177

SPANISH EXPERT CONSENSUS RECOMMENDATIONS ON THE CURRENT MANAGEMENT OF SECONDARY IMMUNODEFICIENCY IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

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Adequate management of secondary immunodeficiency (SID) patients in daily practice is challenging due to the lack of standardized protocols, disparities related to appropriate vaccination coverage, immunization schedules, use of antibiotic prophylaxis, and uncertainties regarding when to initiate or discontinue immunoglobulin replacement therapy (IgRT). A cross-sectional Delphi-based survey was created to gauge the perspectives of clinical hematology and clinical immunology experts from Spain (n= 17 and n= 18 respectively) on SID related to the treatment of oncohematological patients. A scientific committee formed by four hematologists and one immunologist was established to develop the study questionnaire. In accordance with the recommendations that were raised, there was unanimous acknowlegdment in the need to have available guidelines for the management of immunodeficiencies in hematological patients, to perform a baseline immunological evaluation in patients with chronic lymphocytic leukemia (CLL), multiple myeloma (MM) and hematopoietic stem cell transplantation (HSCT) recipients, and to quantify IgG, IgA and IgM levels when SID is suspected. In addition, more than 90% of participants agreed that patients with CLL, MM, and lymphoma should get active vaccination against seasonal influenza, H1N1, pneumococcus, and Haemophilus influenzae. When IVIG treatment is administered, there was general agreement on IgG levels should be checked every three months (83%) and IVIG should be stopped when IgG levels had recovered after a 12-month follow-up (77%). There was unwavering consensus that a clinical protocol for the use of IVIG in the treatment of SID should be readily available (94%), that trough IgG levels should be monitored to establish the appropriate IVIG dose (86%), and that IVIG therapy should be

stopped after IgG levels had recovered (77%). In conclusion, results of this study may help hematologists and immunologists make better management decisions for SID in clinical practice and encourage tighter collaboration between them for the benefit of this particular patient.

P-178

EXPRESIÓN Y FUNCIONALIDAD DE TLR2 Y TLR4 EN LA POBLACIÓN LINFOIDE PRESENTE EN PULMÓN Y ÓRGANOS LINFOIDES DURANTE LA VIDA NEONATAL

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Introducción: La activación de los receptores de tipo Toll (TLRs) por parte de las células innatas provoca una cascada de eventos, que a su vez activan la respuesta inmunitaria adaptativa. En el compartimiento linfoide existen linfocitos pseudoinnatos, que actúan de forma rápida y poco específica en la primera defensa frente a patógenos. Entre ellos están los linfocitos B1Rel, descritos por nuestro grupo por su semejanza con los linfocitos B1. Actualmente se sabe poco sobre la expresión y funcionalidad de los TLRs en células linfoides pseudoinnatas neonatales.

Objetivos: Determinar la implicación de TLR2 y TLR4 en el establecimiento de la población B1Rel en etapas tempranas de la vida en el pulmón como órgano barrera y en otros órganos linfoides.

Material y Métodos: Este estudio se ha realizado en modelo de ratón (WT, TLR4-/- y MyD88-/-). La metodología utilizada incluyó microdisección, detección de expresión génica por RTqPCR, expresión de proteínas por inmunofluorescencia y citometría de flujo. Tratamientos *in vivo* con antibióticos y estimulación con agonistas de TLRs *in vivo* e *in vitro*.

Resultados. Los linfocitos B expresan TLR2+ en momentos perinatales en pulmón, hígado y bazo, siendo mayor la expresión de TLR2 en B1Rel. La microbiota modula esta expresión de TLRs en linfocitos B. La estimulación *in vitro* con ligandos de TLR2 y TLR4 mostró que las células B neonatales responden aumentando su viabilidad, proliferación y diferenciación. La inmunización con la bacteria *Streptococcus pneumoniae* inactivada (agonista de TLR2) produjo un aumento de linfocitos B1Rel, modulando su expresión de TLR2.

Conclusión: Desde momentos tempranos de la vida existen linfocitos B1Rel TLR2+. Estos linfocitos responden a través de TLR2, y en menor medida de TLR4, a estímulos microbianos presentes tanto en homeostasis como en procesos infecciosos.

P-179

DISTINCT PROVIRAL RESERVOIR LANDSCAPE IN TRANSIENT ELITE CONTROLLERS BEFORE LOSING THE VIREMIA CONTROL COMPARED TO PERSISTENT CONTROLLERS

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Background: Elite controllers (EC) represent less than 1% of people living with HIV-1 who can maintain undetectable viral load in absence of antiretroviral treatment. EC represent a dynamic and heterogeneous group regarding immunological, virological, proteomic, metabolomic and miRNA profiles. EC can be classified in two groups: “transient elite controllers” (TC), who lose viremia control (VC), and “persistent controllers” (PC), who maintain VC indefinitely overtime. It is important to characterize the quality of HIV reservoir in TC, before losing the VC, to determine the causes of the loss of spontaneous VC.
Methods: Genomic DNA was extracted from peripheral blood mononuclear cells of 18 PC (>23 years maintaining the VC), 10 TC (detectable viral load during >1 year of follow-up) before losing the VC (0.3-2 years) and 41 antiretroviral-treated individuals (ART) for a median of 9 years (2-19 years). Next-generation sequencing techniques were used, such as full-length individual proviral sequencing and matched integration site and proviral sequencing, to characterize the HIV-1 reservoir.
Results: ART presented significantly higher total, intact and defective proviruses compared to PC and TC. Interestingly, TC presented more intact provirus ($p=0.005$) and a tendency to have higher proportion of defective provirus ($p=0.072$) than PC. Furthermore, intact proviruses were not found in 70% of the PC. No differences were found in total provirus in TC and PC and clonally expanded intact proviruses were not found in TC. Intact provirus in PC were located preferentially in centromeric regions and zinc-finger genes, associated with heterochromatin

features, in comparison to TC, whose intact provirus, were located in permissive genic euchromatic positions.

Conclusions: Distinct proviral reservoir was found in PC, TC (before losing the VC) and ART. This study goes one step further to identify PC as the right model of functional HIV cure and to determine the causes of the loss of VC.

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IMPACT OF HYBRID IMMUNITY, BOOSTER VACCINATION AND OMICRON BREAKTHROUGH INFECTION ON CROSS-NEUTRALIZATION AGAINST DELTA, BA.1, BA.2 AND BA.4/5 SARS-COV-2 VARIANTS

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Background: The elicitation of cross-variant neutralizing antibodies against SARS-CoV-2 represents a major goal, for current COVID-19 vaccine strategies, which can be reached using vaccines based on ancestral and variants of concern (VoCs) sequences and is modulated by natural infection. The goal of this study was to assess the contribution of vaccines and natural infection to the elicitation of cross-neutralizing antibodies

Methods: We cross-sectionally analyzed six groups of individuals, organized according to the number of vaccine doses they received and their SARS-CoV-2 infection status prior to and after vaccination. Plasma neutralization titers were determined against ancestral, Delta, BA.1, BA.2, and BA.4/5 spike-bearing pseudoviruses. In addition, to better evaluate the intra-individual cross-neutralization breadth we calculated ratios between ancestral and variant individual titers.

Results: Two doses of vaccine had a limited capacity to generate sufficient cross-neutralizing antibodies against omicron VoCs in uninfected individuals, but efficiently synergized with previous natural immunization in convalescent individuals. Booster dose, on the other hand, had a critical impact on the broadening of the cross-neutralization response in uninfected individuals, to levels similar to hybrid immunity, while still improving cross-neutralizing responses in convalescent individuals. Omicron breakthrough infection specifically improved omicron cross-neutralization in fully vaccinated individuals but the level of protection was only incrementally increased compared to pre-vaccination infection.

Conclusions: Our results show similar benefits of hybrid immunity and booster vaccines improving titers of cross-neutralization against matched and unmatched variants. Hybrid immunity associated with omicron breakthrough infection further improved cross-neutralizing titers against omicron subvariants but only marginally compared to previous infection-

associated hybrid immunity. Therefore, ancestral Spike-based immunization, via infection or vaccination, contributes to broadening SARS-CoV-2 humoral immunity.

P-181

ESTUDIO DE METILACIÓN DEL ADN DE CÉLULAS T EN PACIENTES CON INMUNODEFICIENCIA VARIABLE COMÚN Y DESREGULACIÓN INMUNOLÓGICA

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[1]Hospital 12 de Octubre, Madrid; [2]Hospital Universitario Central de Asturias, Asturias; [3]Hospital Universitario Virgen del Rocío, Sevilla; [4]Hospital Universitario Vall d'Hebron, Barcelona; [5]Hospital Universitario de Gran Canaria Doctor Negrín, Las Palmas de Gran Canaria; [6]Hospital General Universitario de Alicante, Alicante; [7]Hospital Clínico San Carlos, Madrid; [8]Hospital Fundación Jiménez Díaz, Madrid.

La inmunodeficiencia variable común (IDVC) es la inmunodeficiencia primaria sintomática más frecuente en adultos. Se caracteriza por presentar niveles bajos de anticuerpos y una mayor susceptibilidad a infecciones bacterianas. Aproximadamente, el 30% de los pacientes con IDVC desarrolla complicaciones con desregulación inmunológica que incluyen autoinmunidad y linfoproliferación (del tipo síndrome linfoproliferativo autoinmune-like, SLPA-like), así como trastornos inflamatorios crónicos y/o tumores, que comprometen alteraciones inmunológicas no solo en el compartimento B sino también en el T. A pesar de los avances en las herramientas de diagnóstico molecular, solo el 20% de los casos presenta defectos monogénicos, lo que sugiere la contribución de otros factores que expliquen la fisiopatología de esta enfermedad, como la poligenia, mutaciones somáticas o modificaciones epigenéticas. Recientemente, se han reportado alteraciones en la metilación del ADN asociado a IDVC en gemelos monocigóticos discordantes para la enfermedad. También se han descrito por primera vez defectos en la metilación debido a mutaciones germinales en *TET2* en pacientes con fenotipo SLPA-like. La metilación del ADN está sometida a una regulación dinámica, en la que se reprime la expresión génica al prevenir la unión a activadores transcripcionales o al aumentar la asociación a represores. Con el objetivo de demostrar si existen cambios en el patrón de metilación del ADN, que pueda contribuir a la fisiopatología de esta enfermedad, se realiza en este trabajo un análisis de metilación de ADN en células T CD4 y CD8, mediante la detección de niveles de 5-metilcitosina utilizando una cohorte de pacientes IDVC/SLPA-like con desregulación

inmunológica (en la mayoría de los casos con citopenias autoinmunes) en comparación con individuos sanos como grupo control. El resultado de este análisis pretende profundizar en el posible papel de las alteraciones epigenéticas, en las manifestaciones clínicas y alteraciones inmunofenotípicas, en este tipo de pacientes con fenotipo IDVC/SLPA-like.

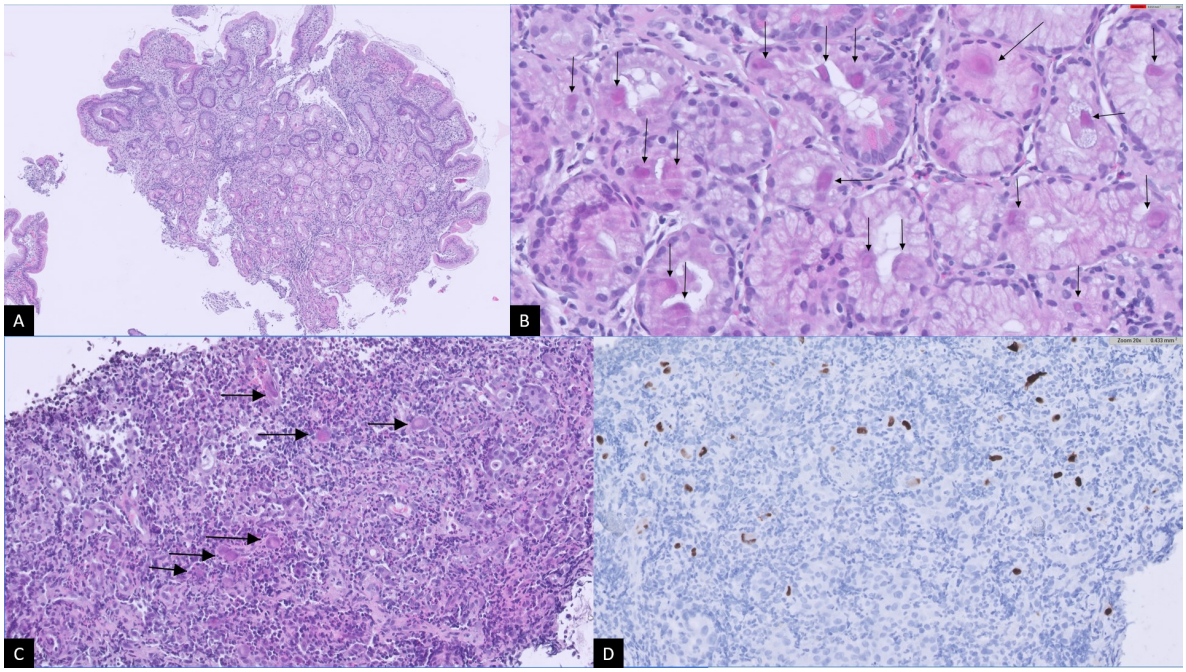
P-182

INFECCIÓN POR CITOMEGALOVIRUS Y ADENOCARCINOMA GÁSTRICO EN PACIENTE CON INMUNODEFICIENCIA COMÚN VARIABLE

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Varón 46 años, esplectomizado a los 34 años y diagnosticado de probable inmunodeficiencia común variable tras ingreso por neumonía y bacteriemia por *Streptococcus pneumoniae*, a partir de lo cual se detectó hipogammaglobulinemia IgG, IgA e IgM. Desde entonces inició tratamiento con inmunoglobulinas intravenosas. En 2021 es derivado a la consulta de Inmunología con cuadro grave gastrointestinal y anorexia.
Datos inmunológicos: niveles indetectables de IgA e IgM, IgG 285 mg/dL [540-1822]. L*infocitos B 398/µL [122-632], células NK 14/µL [127-509] y notable linfocitosis T CD3+ 9654/µL [960-2600], con linfocitos TCD8+ 8244/µL [270-930] y linfocitos TCD4+ 1036/µL [540-1660], ratio CD4+/CD8 0.13 [0.9-4.5]. Se estudia clonalidad de linfocitos T mediante citometría de flujo y por PCR multiplex, con resultado de policlonalidad. Además, la mayoría de linfocitos TCD4+y CD8+ expresan CD57+. En las subpoblaciones B se advierte* ausencia de linfocitos B de memoria con cambio de isotipo 0% [4-22] e incremento de linfocitos B CD21low 28.3% [0.4-4.5]. Se analiza panel virtual de 554 genes asociados a alteraciones del sistema inmunitario no detectando ninguna variante.
Estudios microbiológicos revelan la presencia de *Campylobacter jejuni* resistente a macrólidos, siendo ingresado con imipenem intravenoso durante 10 días, siendo dado de alta con coprocultivo negativo. A las dos semanas, vuelve a ser ingresado por recaída, detectando también el antígeno de *Helicobacter pylori* en heces. La endoscopia digestiva alta reveló inflamación y ulceración a nivel gástrico como intestino delgado, junto a pseudoinclusiones y megalias nucleares en ambas regiones, que sugerían efecto citopático por proceso infeccioso viral, con posterior inmunohistoquímica positiva para citomegalovirus. La cuantificación de la carga viral fue 17.500 copias/mL, adicionando el antirretroviral ganciclovir. El estudio histológico y laparoscopia posterior determinan la presencia de adenocarcinoma gástrico. Tras reunión multidisciplinar, se decide realizar gastrectomía. Dos meses después, la carga viral del CMV era indetectable y los linfocitos volvieron a la normalidad.


A
B
C
D

P-183

EL ANTÍGENO DE MADURACIÓN DE CÉLULAS B SOLUBLE (SBCMA) COMO POSIBLE BIOMARCADOR PARA LA DIFERENCIACIÓN DE FORMAS MÁS SEVERAS DE INMUNODEFICIENCIA HUMORAL

Guerra Galán, Teresa; Palacios Ortega, María; Rodríguez De La Peña, Antonia; Pereiro Rodríguez, Alejandro; Mohamed Mohamed, Kauzar; Mayol Hornero, Elsa; García Bravo, Laura; Villegas Mendiola, Ángela; Mansilla Ruíz, María Dolores; Fernández Arquero, Miguel; Sánchez Ramón, Silvia.

Hospital Clínico San Carlos, Madrid.

Introducción

El antígeno de maduración de células B (BCMA) es un miembro de la superfamilia de receptores del factor de necrosis tumoral (TNFR) restringido a plasmablastos y células plasmáticas. La unión a sus ligandos BAFF y APRIL desencadena señales vía NF-κB fundamentales para la proliferación y mantenimiento de las células plasmáticas. Además de su forma anclada a la membrana existe una forma soluble (sBCMA) fruto del clivaje por una γ-secretasa. La medición de los niveles de sBCMA en sangre se ha propuesto como biomarcador pronóstico en ciertas enfermedades autoinmunes y en las gammapatías monoclonales, así como para discernir inmunodeficiencias humorales como el déficit selectivo de IgA (DSIgA) de otros defectos con un curso más grave, como la inmunodeficiencia variable común (IDVC).

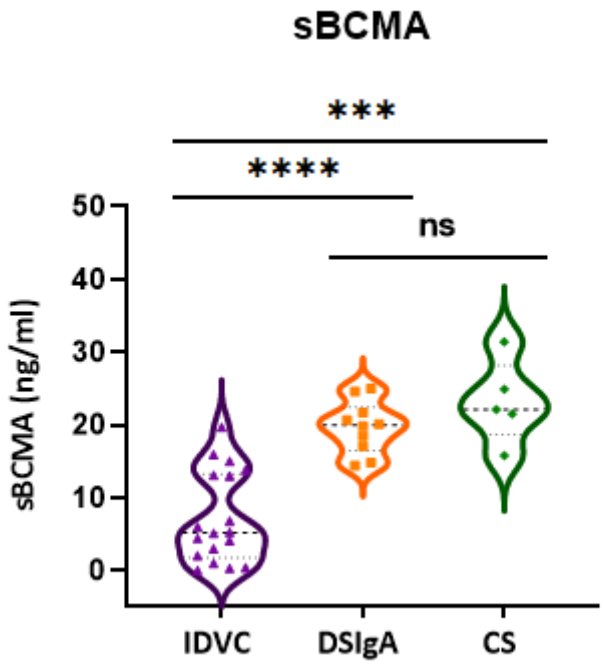
Materiales y Métodos

Para valorar la posible utilidad diagnóstica se llevó a cabo un estudio piloto de cohortes en 28 pacientes del Servicio de Inmunología del Hospital Clínico San Carlos diagnosticados con IDVC (n=18) y DSIgA (n=10) y 5 controles sanos. La medición de sBCMA fue llevada a cabo mediante ensayo ELISA utilizando anticuerpos policlonales anti-BCMA (R&D Systems) y los resultados obtenidos fueron analizados utilizando un equipo *Triturus* (Grifols) y mediante el test estadístico U de Mann-Whitney.

Resultados y Conclusión

El 88,9% (n=16) de los pacientes con IDVC presentó niveles de sBCMA ≤15ng/ml (mediana=5,19) frente al 80% de los pacientes con DSIgA con niveles >15ng/ml (mediana=19,94). La diferencia entre ambos grupos fue estadísticamente significativa (***p*** <0,0001) y consistente con los resultados de otros autores (*Cunningham-Rundles C. et al.*), no observándose diferencias significativas entre los controles sanos analizados y los DSIgA. Estos resultados preliminares son prometedores de cara a la posible utilización del sBCMA como un

nuevo biomarcador al diagnóstico de IDVC. No obstante, un mayor número de estudios retrospectivos y prospectivos son necesarios.



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LACK OF FUNCTIONAL CONSEQUENCES OF PATHOGENIC VARIANT C.205T>C (P.W69R) IN IL10RA (IL-10R ALPHA CHAIN) IN VERY EARLY ONSET INFLAMMATORY BOWEL DISEASE (VEO-IBD)

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Very -early-onset inflammatory bowel disease (VEO-IBD) is a clinical entity characterized by IBD starting before the sixth year of life (often before 3 months), associated to genetic defects in the IL-10 signaling pathway (IL-10R Alpha chain (IL10RA), Beta chain or IL-10 itself), of autosomal recessive inheritance, with homozygous or compound heterozygous patients. We present a case of a consanguineous patient of VEO-IBD, homozygous for variant of IL10RA (c.205T>C-p.W69R), predicted as probably damaging (score 1 at PolyPhen-2), showing normal activity in IL-10 signaling assays.

Immunologic tests: phospho-STAT3-pY705 (BD) assay in response to rh-IL-10 (BD) 20 ng/ml and inhibition of TNF-alpha induction in response to LPS by rh-IL-10. IL-10 test specificity was determined by incubation with a mAb to human IL-10 (and control mAb), that specifically abrogated STAT3 phosphorylation in response to rh-IL-10.

The patient showed an initial very severe clinical presentation at 3 months of age, with proctitis, perianal abscesses and fistulae, diarrhea with failure to thrive, and also a familial history concordant with VEO-IBD (one sister bearing the same variant died at 5 months of complications of IBD). Treatment with sulfasalazine and parenteral nutrition achieved disease control. After recovery the patient is currently 1 year, off treatment and doing well on modular diet, which is not a typical course of monogenic VEO-IBD (HSCT indication). On segregation study of IL10RA variant the father was a heterozygotic carrier and surprisingly the mother was homozygous in spite of being asymptomatic.

Although to date 4 series have been published in which IL10RA p.W69R variant was linked to VEO-IBD, functional consequences of this mutation have not been assessed. Our results highlight the importance of immunological testing in the workout of inborn errors of immunity (in contrast to isolated genetic testing) and might reflect a higher level of VEO-IBD biological complexity than previously supposed.

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T-SPOT COVID: ¿ES SUFICIENTE LA RESPUESTA HUMORAL PARA PREDECIR LA PROTECCIÓN FRENTE AL SARS-COV-2?

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Para estudiar la respuesta celular al SARS-CoV-2 en pacientes con diferentes antecedentes (vacunados, sin vacunar, con patología inmunologíca, sin patologías, con infección por SARS-CoV-2, sin infección previa) se ha utilizado la técnica T-SPOT COVID. Este ensayo nos permite detectar la respuesta de células T específicas frente a antígenos del SARS-CoV-2 (espícula y nucleocápside) con alta sensibilidad y sin que exista reactividad cruzada con otros coronavirus. La metodología consiste en detectar la producción de la citocina interferón gamma (IFN-γ) por las células T CD4 y CD8 efectoras en respuesta a la estimulación con estos antígenos específicos. La respuesta celular es nuestro principal sistema de defensa contra los virus que nos infectan, siendo complementada por la respuesta humoral (de anticuerpos). Para evaluar la respuesta inmunológica completa hemos realizando también pruebas serológicas para cuantificar la presencia de anticuerpos IgG frente al SARS-CoV-2. Nuestro estudio demuestra que existen pacientes vacunados y/o infectados que no generan anticuerpos (respuesta humoral) pero sí generan respuesta de células T (respuesta celular). También hemos detectado pacientes vacunados y/o infectados por SARS-CoV-2 que tienen respuesta humoral pero no presentan respuesta celular. En varios de nuestros pacientes se ha detectado una respuesta celular potente (frente a espícula y nucleocápside) y títulos muy bajos de anticuerpos. Las vacunas basadas en mRNA han frenado eficazmente la propagación de la enfermedad, pero el epítopo antigénico producido debe ser presentado en el HLA de los individuos. Esto limita la efectividad de la producción de anticuerpos por los linfocitos B, pero estos pacientes podrían presentar una respuesta celular efectiva. Por lo tanto, sería conveniente evaluar la respuesta humoral y celular para conseguir un status inmunológico de los pacientes que nos permita predecir con certeza el nivel de protección que presentan frente al SARS-CoV-2.

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LATE ONSET HYPOGAMMAGLOBULINEMIA, *CAMPYLOBACTER JEJUNI* REINFECTION AND LYMPHOPROLIFERATIVE T SYNDROME: THE THREE FACES OF PID?

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The correlation between primary immunodeficiencies (PID) and the development of secondary lymphoproliferative disorders is well recognized. Indeed, diagnosing PID in a patient with late-onset hypogammaglobulinemia requires excluding an ongoing malignant process. However, what if both approaches were different phenotypes of a common underlying pathology? Here we present and discuss this PID/SID intersection in an illustrative case of a Caucasian woman with a previous selective IgA deficiency, who was diagnosed with late-onset combined immunodeficiency (LOCID) at the age of 72 after the finding of marked panhypogammaglobulinemia (serum IgG: 163; IgA <2; IgM <10 mg/dL) and a CD3+ T cell defect (CD4/CD8 inverted ratio of 0.2) in the setting of a severe infectious condition (*Campylobacter jejuni* diarrhea, CMV colitis and viremia, muguet, *Candida sp*. esophagitis, and SARS-CoV2 asymptomatic viremia). CVID-LOCID-like diagnosis was made after excluding a potential hidden malignant lymphoproliferative disorder or neoplasia in July 2022. After several recurrent *C. jejuni* bacteremia, progressive thrombocytopenia and a decay of her overall health, despite adequate immunoglobulin replacement therapy, a lymphoproliferative T disorder was detected in January 2023. Clinical exome analysis disclosed a heterozygous mutation in WIPF1 gene, a gene that can be related to Wiskott-Aldrich-like syndrome. Variants of this gene could also manifest in other syndromic immunodeficiencies. Pursuing a definitive diagnosis, we aim to study the levels of expression of intracellular WASp and WIP proteins.
We discuss this case report and argue on how PID-related gene variants could play a role in her overall condition and on the crossroads between germinal PID-genetic lesions relating to PID, recurrent or latent infections, and malignant lymphoproliferative disorders.

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ANÁLISIS PRELIMINAR DE LA RESPUESTA T-ESPÉCIFICA FRENTE AL VIRUS EPSTEIN-BARR POR CITOMETRÍA DE FLUJO EN PACIENTES PEDÍATRICOS CON TRASPLANTE HEPÁTICO

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La infección/reactivación del virus Epstein-Barr (VEB) es una complicación post-trasplante frecuente. El análisis de la respuesta T-específica frente a VEB permitiría adecuar la inmunosupresión en pacientes trasplantados infectados. El objetivo del trabajo es definir la respuesta T-específica frente al VEB en receptores pediátricos de un injerto hepático.
Nuestra cohorte incluye 15 receptores hepáticos pediátricos (11 seropositivos, 4 seronegativos) (7/8 hombres/mujeres, mediana 5 años) y 12 controles seropositivos (7/5 hombres/mujeres, mediana 51 años) del Hospital La Paz. Se incubaron células mononucleares de sangre periférica con péptidos de VEB (PepTivator® EBV) durante 6 horas para marcaje de citoquinas intracelulares (ICS) y 48 horas para marcadores inducidos por activación (AIMS). Mediante citometría (Beckman Coulter) se analizó la respuesta monofuncional/polifuncional ICS en linfocitos TCD3+ (CD4+/CD8+) (CD107a-APC, IL-2-FITC, IFNγ-PB, TNFα-PE) y AIMS en TCD4+ (CD25-PC7, CD134-PE). Los resultados se expresaron como fold increase respecto al sin estímulo (FI>1.00 ICS, FI>3.00 AIMS).
Todos los controles y pacientes mostraron respuesta positiva por ICS. El estudio de AIMS fue positivo en el 83% de controles y negativo en el 100% de pacientes. La respuesta TCD3+ mediante ICS fue significativamente mayor en pacientes (FI 100.50 vs. 22.17, p=0.018), incluso analizando TCD4+ (FI 27.57 vs. 11.91, p=0.014) y TCD8+ (FI 139.10 vs. 41.29, p=0.030) individualmente. Los perfiles polifuncionales CD4+CD107a+IFNg-IL2-TNFa+ (FI 1.40 vs. 0.72, p=0.0457) y CD8+CD107a+IFNg-IL2-TNFa+ (FI 12.04 vs. 1.46, p=0.007) estaban significativamente aumentados en pacientes. Respecto a los AIMS, la respuesta TCD4+ fue significativamente menor en pacientes (FI 0.87 vs. 4.28, p<0.0001).
Los receptores hepáticos pediátricos presentan una respuesta T-específica frente a VEB mediante ICS significativamente mayor que los controles, al contrario que mediante AIMS. Esto puede deberse a diferencias cinéticas de expresión de biomarcadores en subpoblaciones

TCD4+. Este estudio sigue aumentando el tamaño muestral para analizar los pacientes en función de su seropositividad y/o infección activa por VEB.

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LOS MICROSPORIDIOS Y SU ASOCIACIÓN CON CÁNCER Y ENVEJECIMIENTO

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El cáncer y el envejecimiento son dos males que en la actualidad según datos de la Organización Mundial de la Salud, causan graves problemas a nivel social y clínico.
Nuestras últimas investigaciones se han centrado en aprovechar la capacidad que tienen los parásitos Microsporidios de evadir la respuesta del Sistema Inmunitario para desarrollar conocimiento todavía muy básico pero probablemente aplicable a la inducción de la expresión de PD-L1 en los tumores que no lo expresan, optimizando así el tratamiento con determinados anticuerpos antitumorales, actualmente utilizados en terapia oncológica y también para desarrollar conocimiento aplicable en un futuro en el desarrollo de fármacos senolíticos, ya que la infección por éstos podría estar implicada en la regulación de rutas de inducción de senescencia, como es la vía de señalización ISS.
Estos parásitos son protozoos intracelulares obligados, que se han definido como parásitos oportunistas humanos. Poseen el genoma eucariota más compactado que se conoce y son capaces de infectar a todos los *Phyla* animales. Poniendo de manifiesto su éxito y extremada capacidad para regular el ciclo celular y evadir el sistema inmunitario.
En investigaciones realizadas hemos observado que el microsporidio humano *Enecephalitozoon cuniculi,* establece diferentes mecanismos de Evasión del Sistema Inmunitario, relacionados con la presentación antigénica, el ciclo celular, apoptosis y la respuesta inmunitaria adaptativa ya que induce en modelo de cultivos infectados un aumento de la expresión de la proteína inmunosupresora PD-L1, por otro lado la infección por esté parásito también está implicado en el aumento de la expresión de PTEN y disminución de la proteína AKT componentes intermedios de la vía ISS.
Por ello nuestras investigaciones se centran en el conocimiento de estas vías de señalización celular. Ya que en un futuro pudiesen dar lugar a nuevas alternativas terapéuticas, procedentes de productos derivados de estos microorganismos patógenos.

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SERUM CALPROTECTIN IN COVID-19 PATIENTS.

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Aim

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

Material and methods

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

Results

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

Discussion

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

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THE ROLE OF NAD+ IN MITOCHONDRIAL DYSFUNCTION IN PATIENTS WITH SECONDARY IMMUNODEFICIENCY

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Hematological malignancies constitute a heterogeneous group of diseases including chronic lymphocytic leukemia (CLL) or multiple myeloma, an advanced stage of monoclonal gammopathy of undetermined significance (MGUS). CLL and MGUS are two diseases that are diagnosed predominantly in the elderly and usually progress over time. These patients may develop secondary immunodeficiency (SID), suffering from numerous infections, some of them severe, and second neoplasia, which affect their quality of life and prognosis.
Mitochondria play a key role in immune cell activation, proliferation, differentiation and effector function. Nicotinamide adenine dinucleotide (NAD+) is a biomarker of mitochondrial- and age-related disorders as cancer and a balance of NAD+/NADH is important for immune cells to function properly. We hypothesize that the immune cells of CLL and MGUS patients might be less responsive to infection and tumor, which could be related to mitochondrial disruption. The aim of this study is to compare mitochondrial function based on NAD+ levels in peripheral blood mononuclear cells (PBMCs) from patients with MGUS or CLL and healthy controls.
Blood samples were collected from 6 SID patients: 2 MGUS and 4 CLL patients, and 2 healthy controls, to isolate PBMCs. NAD+ levels in PBMCs were quantified using a colorimetric assay. According to our preliminary results, patients with SID due to hematological malignancy have lower NAD+ levels than healthy controls (5,33 pmole/10^6 cells versus 6,96 pmole/10^6 cells; p= 0,429).
In conclusion, our preliminary results suggest that NAD+ levels were diminished in SID to hematological malignancy, which can influence their immune dysfunction. Further studies in larger cohorts are warranted to confirm NAD+ dysfunction, which might point to mitochondria as a target for new therapeutic agents to restore mitochondrial activity and favor the immune response.

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ESPECTRO CLÍNICO, RADIOLOGÍCO Y ANALÍTICO DE LA INMUNODEFICIENCIA COMÚN VARIABLE

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La Inmunodeficiencia Común Variable (IDCV) es un diagnóstico que engloba un conjunto de patologías que son agrupadas por compartir la presencia de hipogammaglobulinemia. Por eso como parte de su presentación clínica los pacientes con IDVC pueden presentar complicaciones que implican un riesgo vital mayor que tener únicamente infecciones de repetición. La desregulación inmune y sobre todo la linfoproliferación implican un peor pronóstico. La definición de marcadores clínicos, radiológicos y analíticos que permitan diferenciar el tipo de IDVC en cada paciente adquiere una relevancia clínica fundamental en este contexto. La correlación de algunos marcadores de activación o linfoproliferanción con las complicaciones en la IDVC ya han sido establecidos, pero la relación existente entre estos marcadores o su relevancia al ser analizados conjuntamente no ha sido aún concretada.

En una cohorte de 18 pacientes con IDVC se recolectaron en una base de datos anonimizada marcadores clínicos (autoinmunidad, linfadenopatías, esplenomegalia, procesos oncológicos, infecciones), analíticos (poblaciones TBNK, T naïve-memoria-efectores, B naïve-memoria-efectoras, activación celular), radiológicos (lesiones en parénquima pulmonar) y puntuaciones en scores pronósticos junto con los niveles de sCD25 sérico (sIL-2Rα) en el momento del diagnóstico y su valor máximo durante su seguimiento hasta hoy. Los pacientes se dividen según 4 fenotipos (Chapel 2012): Solo infecciones, Citopenias autoinmunes, Enteropatía y Linfoproliferación.

El resultado de este análisis pretende comparar y relacionar marcadores de la enfermedad para diferencias los fenotipos clínicos de la IDVC de manera objetiva y poder de esta manera ajustar el tratamiento y predecir la evolución clínica.

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MANAGEMENT OF AUTOIMMUNE THROMBOCYTOPENIA IN PATIENTS WITH COMMON VARIABLE IMMUNODEFICIENCY DURING PREGNANCY, SYSTEMATIC REVIEW.

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Introduction: the presence of peripheral cytopenias, particularly thrombocytopenia (TP), in Common Variable Immunodeficiency (CVID) is a typical autoimmune manifestation of the disease and presents a complicated clinical management that differs from that of non-immunodeficient patients. Pregnancy can lead to the appearance of these alterations, which entails a more difficult approach. Currently, there are no specific guidelines for its management and treatment during pregnancy.

Objectives: to carry out a systematic review of the treatment of TP in patients with CVID and ongoing pregnancy.

Material and Methods: a systematic review of all the articles published since 1990 in different databases of medical articles: Medline, Pubmed and Google Scholar was made.

Results: a total of 8 case-reports were found. 4 of the case-reports do not establish the treatment used. Two cases used high-dose intravenous gamma globulins (IVIg) and platelet transfusions, another case used high-dose IVIg, corticosteroids, platelet transfusions, and WinRho. In one case no treatment was required. Only 2 cases required a cesarean delivery. No alterations were found in the newborn in any case.

There is no data on the use of TPO receptor agonists or splenectomy in these patients. Meanwhile, the meta-analysises for PT in pregnant women without CVID and non-pregnant CVID patients recommends the use of corticosteroids and GGIV at high doses as the first line of treatment and propose the use of rituximab as a second line treatment.

Conclusion: the presence of thrombocytopenia during pregnancy in patients with CVID implies a complicated and individualized management. There are no specific treatment guidelines, neither platelet counts that justify a preterm labor induction. In the case of requiring treatment, the use of IVIg at high doses and corticosteroids is the most widely used first line. The use of rituximab as a second line should be assessed according to its risk/benefit ratio.

Sesión Poster Defensa 2. Pantalla 4. Inflamación + Inmunidad innata (II)

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IL-5 PROMOTES PRO-CACHECTIC GMDSC FUNCTIONS IN CANCER-ASSOCIATED CACHEXIA

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Cancer cachexia is a complex metabolic disorder characterized by the progressive and irreversible loss of cardiac and skeletal muscle, as well as adipose tissue, in response to malignant growth. This wasting disorder is evident in approximately 30% of cancer patients and accounts for 1/3 of all cancer deaths. Management of cachexia is a major challenge, and current treatments typically have shown limited effectiveness. Despite the growing list of implicated cachectic factors, little is known about the potential role of immune cells in this process.
We have characterized two tumor models, pro-cachectic Lewis Lung Carcinoma (LLC), and non-cachectic Colon Adenocarcinoma (MC38) implanted in C57BL/6 mice. This comparison revealed a significant expansion of granulocytic myeloid-derived suppressor cells (gMDSCs) in both the hearts and muscles of cachectic mice. Depletion of gMDSCs with aLy6G antibodies prevented cardiac and skeletal muscle wasting. Luminex multiplexing analysis showed that LLCs produced high quantities of gMDSC-related factors, including IL-5, a Colony Stimulating Factor 2 Receptor Subunit Beta (CSF2RB) ligand. CSF2RB deficiency prevented the development of LLC-induced cachexia, without affecting gMDSC expansion. Neutralization of IL-5 with anti-IL-5 antibodies completely abrogated cachectic cardiac and skeletal muscle loss, further supporting its critical role in LLC-induced cachexia.
Oxidative stress is observed clinically in cachectic patients and implicated in upregulating muscle degradation pathways. Reactive oxygen species (ROS) levels in LLC mice were significantly increased compared to non-tumor controls, but not in LLC-implanted CSF2RB deficient mice. To understand whether NADPH oxidase (NOX), the primary enzyme responsible for ROS production, is involved in cachectic muscle wasting, we treated LLC-implanted C57BL/6 mice with a pan-NOX inhibitor (APX-115). NOX inhibition completely protected against both cardiac and skeletal muscle atrophy. Collectively, our data suggest an important pathway involved in the development of cancer cachexia, specifically the expansion and IL-5/CSF2RB-dependent programming of pro-cachectic gMDSCs.

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CONTROL OF *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS* LOAD WITHIN INFECTED BOVINE MONOCYTE-DERIVED MACROPHAGES IS ASSOCIATED WITH HOST GENETICS

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The mechanisms underlying host resistance and the genetic loci influencing individual resistance to *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection are still largely unknown. In the current study, we evaluated the performance of monocyte-derived macrophages (MDM) isolated from peripheral blood of 75 healthy Holsteins cows and infected *ex vivo* with MAP. Bacterial load within MDM was quantified at 2 h and 7 days p. i. using a Bactec MGIT 960 instrument. In addition, the expression levels of some genes with important roles in the innate immune response including *Epiregulin* (*EREG*), *Complement Component C3* (C3), *Galectin-9* (*Gal9*), and *nitric oxide* (NO^-) were measured in the supernatant of the infected cells. DNA from peripheral blood samples of the animals included in the study was genotyped with the EuroG MD bead Chip (44,779 single nucleotide-polymorphisms, SNPs). After performing a genome-wide association study, the only phenotypes that showed SNPs with a significant association were the bacterial load within MDMs at 2 h (hereditability= 0. 87) and 7 days (hereditability= 0.83) p.i. A total of 6 SNPs, 5 candidate genes, and one microRNA on the *Bos taurus* chromosomes BTA2, BTA17, BTA18, and BTA21 were associated with MAP load at 2 h p.i. Overlap was seen in two SNPs associated with the MAP load at 2 h and 7 d p.i. The identified SNPs had negative regression coefficients, and were, therefore, associated with a low bacterial load within MDMs. Some of the identified SNPs were located within loci previously associated with longevity, reproductive, and udder health traits. The identified candidate genes; *Oxysterol Binding Protein like*, *Serine Rich Nuclear Protein 3*, and the *Coiled-Coil Domain Containing 92* regulate cellular cholesterol trafficking and efflux, apoptosis, and interferon production, respectively. Our results define a heritable and distinct immunogenetic profile designed to limit bacterial load early after infection.

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EL TÁNDEM LEUCEMIA-LEPTOMENINGE COMO RESPONSABLE DE LA NEUROINFLAMACIÓN EN LEUCEMIA LINFOBLÁSTICA AGUDA.

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La leucemia linfoblástica aguda (LLA) es el cáncer pediátrico más común y, aunque actualmente las tasas de curación son superiores al 80%, sigue siendo la primera causa de muerte infantil asociada al cáncer, principalmente debido al fallo en los tratamientos y a la recaída en el Sistema Nervioso Central (SNC) que supone un 2-7% de los casos. Aunque el papel de la inflamación ha sido estudiado en profundidad en diferentes tipos de cáncer, en la LLA infantil existen pocos datos a nivel sistémico y son casi inexistentes en el SNC.
En este trabajo se pone de manifiesto el aumento de citoquinas inflamatorias en suero sanguíneo durante el desarrollo de la enfermedad utilizando un modelo xenogénico de LLA. Además, la expresión de genes relacionados con la inflamación aumenta en el cerebro y en la médula espinal, especialmente en su parte posterior, estos datos correlacionan con lo observado por resonancia magnética y con la sintomatología característica de los animales en fases avanzadas de la enfermedad. En este contexto, el estudio de la infiltración leucémica en el SNC revela que, en paralelo al incremento de la respuesta inflamatoria, tienen lugar la formación de agregados de células leucémicos en el espacio subaracnoideo que favorecen a su vez el reclutamiento de células del sistema inmune como neutrófilos o macrófagos. Además, el análisis de los agregados mediante el establecimiento de organoides leucemia-leptomeninge así como de cultivos organotípicos demuestra que son estas estructuras tridimensionales las responsables de la proliferación tumoral, inflamación e invasividad del tumor.
Por tanto, nuestros resultados indican que el contacto de células leucémicas y leptomeníngeas favorece la formación de estructuras complejas con potencial inflamatorio que podrían ser el origen de la neuroinflamación observada en el SNC y su sintomatología asociada, así como la protección frente a los quimioterápicos que producen las recaídas.

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IMMUNE CHARACTERIZATION FOR TARGETED TREATMENT IN HIDRADENITIS SUPPURATIVA

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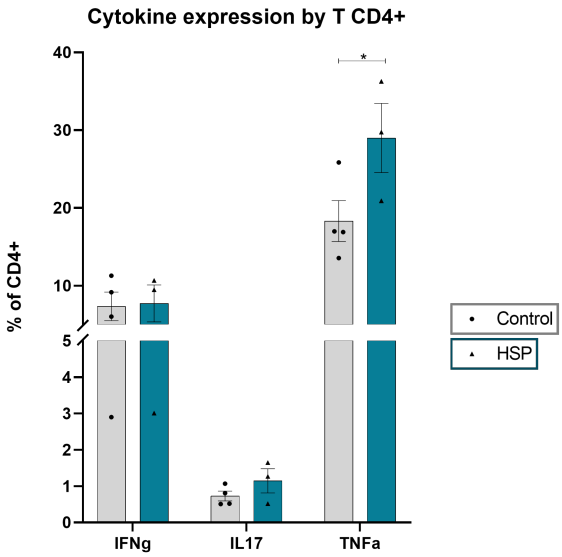
Hidradenitis suppurativa (HS) is an inflammatory chronic disorder that creates painful and pus-discharging cutaneous alterations, typically formed in skin folds. HS's prevalence is thought to be around 1% although it is highly underdiagnosed. Normally, it first appears during early adulthood and strongly impacts patients' quality of life. Although there are some treatments approved, they are limited and not always satisfactory.

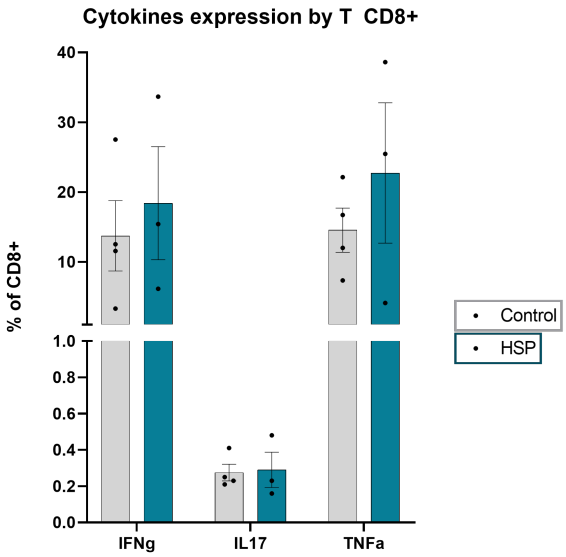
The aim of the study is to exhaustively characterize the inflammatory response of patients suffering from HS to further understand the immune mechanisms underlying the development of skin alterations. Moreover, our results can help dermatologists decide a targeted immunomodulatory treatment according to the type of inflammatory response identified.

An extensive and multiparametric immune system characterization by flow cytometry is being performed. Moreover, we are developing a protocol to assess the neutrophil cytokine expression profile after isolation and stimulation by an automated immune assay (Simple-Plex ELLA^{TM}).

Results are still ongoing but, currently, we have recruited some patients and aged-matched healthy controls. In addition, we also have follow-up samples from patients who have been treated with anti-TNFa. Before treatment, patients appeared to have increased neutrophil counts and increased percentage of TNFa cytokine producing T-CD4+ and T-CD8+ lymphocytes. Two of the patients also showed increase of Th1 and Th17 T-CD4+ lymphocytes compared to healthy controls. Post-treatment samples show a reduction in neutrophil counts although not in all patients a reduction of percentage of TNFa producing lymphocytes was observed despite showing clinical improvements.

HS patients show distinct inflammatory response although neutrophils seem to be a common feature in the few patients we have studied. The increase in TNFa and the clinical improvements of the patients with the treatment suggests that this cytokine plays an important role in the physiopathology.


Cytokine expression by T CD4+
% of CD4+
40
30
20
10
5
4
3
2
1
0
*
IFNg
IL17
TNFa
Control
HSP


Cytokines expression by T CD8+
% of CD8+
40
30
20
10
1.0
0.8
0.6
0.4
0.2
0.0
IFNg
IL17
TNFa
Control
HSP

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NON-INVASIVE DIFFERENTIAL DIAGNOSIS OF COVID19 AND OTHER RESPIRATORY INFECTIONS BASED ON EXHALED BREATH AND SERUM INFLAMMATORY CYTOKINE PROFILE

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Volatile Organic Compounds (VOCs) are gaseous molecules secreted in the exhaled air during breathing as a degradation product of metabolic processes. The interaction of pathogenic viruses with the host during respiratory infection produces a series of changes in the host cellular metabolism result in distinctive VOCs.

Pattern changes in exhaled VOCs associated to specific infections are applied to the diagnosis and evolution of these diseases. This work aims to analyze the profile of VOCs present in the exhaled breath from healthy donors (HDs), COVID-19 and non-COVID-19 patients with other respiratory infections to establish VOC fingerprints with clinical utility for COVID-19 diagnosis and prognosis and also to determine inflammatory cytokines in serum by performing combinatorial diagnostic analysis.

Gas sampling bags were used to collect expired air from patients at hospital admission. VOCs

were concentrated and quantified by solid-phase-microextraction associated with gas chromatography-mass spectrometry. A descriptive statistical analysis of VOC levels was performed. Moreover, multivariate statistical analysis was carried out using the non-parametric Kruskal-Wallis Test. Then, the adjusted *p* value was calculated comparing the populations two by two, using test Dunn as non-parametric multivariate analysis, adjusted by Benjamini-Hochberg FDR including only the most significant compounds found in the previous analysis. Of 27 VOCs analyzed, 21 were detected in the exhaled air samples. The quality of VOCs analysis was confirmed by the absence of outliers in the descriptive analyses. Populations were separated from each other, being the COVID-19 patients the ones that most tend to separate from healthy controls. As a result of the multivariate analysis, we were able to establish a VOC profile that showed significant differences between the groups of COVID-19, non-COVID-19 patients, and HDs.

Several compounds allowed differentiate COVID-19, other respiratory infections and HDs. Based on these findings specific VOC fingerprints were established in healthy, COVID-19 and non-COVID-19 patients.

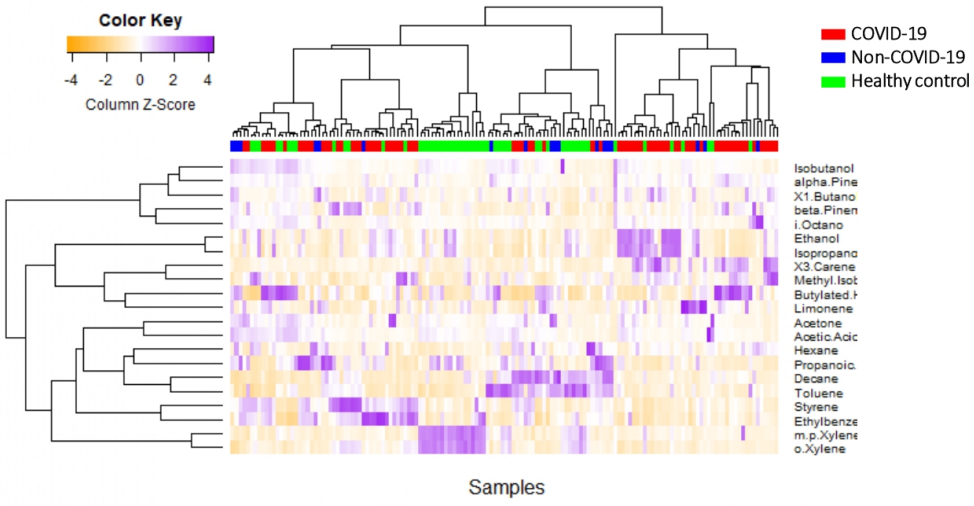


Figure 1. Heatmap with two dendrograms that represents the results of quantification of 21 VOCs from all the samples of the study. The upper dendrogram shows clustering of the three populations (COVID-19 patients, non-COVID-19 patients, and HDs). The dendrogram on the left shows the correlation of the VOCs profile in the samples. Data has been scaled for visualization purposes.

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COLON MACROPHAGE TRANSCRIPTOMES REVEAL MITOCHONDRIAL DYSFUNCTION AND ASSOCIATED MICROBIOTA UNDERLYING ULCERATIVE COLITIS SEVERITY AND RESISTANCE TO TNF INHIBITOR THERAPY

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Background and Aims: Mitochondrial dysfunction plays a key role in ulcerative colitis [UC] severity and treatment response. Here, we aimed to study the impact of altered mitochondria in colon macrophage transcriptome using a mouse model with MCJ deficiency, a natural inhibitor of the respiratory chain Complex I. MCJ expression was primarily linked to macrophages in human colic tissue from IBD patients.

Methods: Colon macrophage transcriptome was conducted from wild type (WT) and MCJ-deficient mice subjected to 3% of DSS for 6 days followed by a recovery period of 2 days with water. Colon macrophages were isolated and RNA extracted for sequencing. Fecal microbial profile was also studied.

Results: RNA-seq analyses showed that MCJ deficiency primarily affected the expression of genes belonging to the FcγR signaling pathway which are linked to an anti-TNF refractory gene signature in UC patients. Non-responding MCJ-deficient mice showed a significantly different microbiota composition compared to WT mice, with an increased abundance of specific members such as *Ruminococcus gnavus*. Macrophage transcriptional signature in MCJ deficient mice was associated with microbial changes which contribute to disease severity and anti-TNF response. Our results suggest that MCJ-deficient microbial environment can be transferred to WT cohoused animals, decreasing treatment efficacy. Conversely, restoring this environment with the commensal bacterium *Akkermansia muciniphila* improves the response to anti-TNF even overcoming mitochondrial dysfunction.

Conclusions: Results implicate mitochondria dysfunction and FcγR signaling-bacteria interplay in UC pathogenesis and non-responders to therapies, opening new research lines to predict disease outcome and the response to anti-TNF therapy at baseline in UC patients. Our data indicate that the study of the gut microbiota composition in patients with defect mitochondrial function might be an invaluable diagnostic and therapeutic tool in healthcare.

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HYPOXIA ELICITS PROINFLAMMATORY FEATURES AND INDUCES EPIGENETIC REPROGRAMMING DURING MACROPHAGE ACTIVATION

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Myeloid cells are derived from hematopoietic stem cells, which eventually differentiate to functionally mature cells via complex epigenetically regulated processes. In the tumor microenvironment, myeloid differentiation is altered. In some solid cancers, tumors eventually lose tissue organization, leading to low oxygen concentration (hypoxia) in the tumor core. Hypoxia can redirect macrophage (MAC) differentiation to tumor-associated macrophages (TAMs), which display unique features. In such contexts, the effect of hypoxia has been thought to associate with opposing features such as hyperinflammation or immunosuppression. These results pose hypoxia as a yet controversial factor regarding MAC immune functions. In this project, we aimed to ascertain the effect of hypoxia during MAC differentiation and under inflammatory activating conditions. Our data has revealed that hypoxia enhances inflammatory features during MAC activation. These features are related to functional, transcriptional, and epigenetic reprogramming acquired in hypoxia. Concomitant to those phenotypic changes, we identified an upregulation of hypoxia-inducible factor (HIF-1a) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) transcriptional activity, which revealed a coordinated and synergistic involvement of both pathways during this process. We performed ChIP-seq of both factors and integrated it with our epigenetic and transcriptomic data, which revealed insights into the molecular cooperation between HIF-1a and NF-kB in the regulation of inflammatory responses in both normoxia and hypoxia. Finally, we identified our *in vitro*-derived signatures in human *in vivo* MAC single-cell RNA-seq data, validating our findings in physiological conditions. Our results provide insights into the role of HIF-1a in regulating MAC activation in normoxia and hypoxia through epigenomic reprogramming. In all, we believe our findings shed light on the understanding of MAC responses in hypoxia and provide new avenues for leveraging the hypoxia signaling pathway as a therapeutic target through the manipulation of MAC molecular profiles, with implications in fields such as myeloid cell-based therapies.

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PLATELETS AS POSSIBLE BIOMARKER CANDIDATES TO DIFFERENTIATE BETWEEN NEURODEGENERATIVE DISORDERS.

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Background:

Alzheimer's disease (AD), Parkinson's disease (PD) and dementia with Lewy bodies (DLB) are complex diseases that usually overlap in their neuropathological manifestations, which makes a challenge for clinicians to establish a correct clinical diagnosis and management of the disease. Nowadays the study of platelets in the context of neurodegenerative diseases is intensifying with increasing evidence of their role in the pathogenesis of neurodegenerative disorders. The aim of this pilot study is to explore the interaction of platelets with other immune cells in neurodegenerative disorders.

Methods:

Twenty-three individuals, seven controls (CTRL), six AD, five PD, and five DLB patients, were included in this pilot study. The percentage of platelets attached to T lymphocytes (CD3+CD4+, CD3+CD8+), B lymphocytes (CD19+), monocytes (CD14+), and their activation grade were measured in peripheral blood by Flow cytometry.

Results:

Higher percentage of platelets attached to CD4+ and CD4+ CD25+ T lymphocytes were observed in PD compared to CTRL (p=0,030; 0,048) and DLB (p=0,031; 0,031). A similar result was obtained with CD19+ and CD19+ CD25+ B lymphocytes attached to platelets in PD compared to CTRL (p=0,030; 0,048) and DLB (p=0,015; 0,015). Moreover, the percentage of platelets attached to CD14+ monocytes was higher in PD compared to DLB (p=0,031) as well. The study of CD8+ and CD8+ CD25+ T lymphocyte subpopulation revealed a similar tendency but with no significant results.

Conclusions:

The percentage of platelets attached to B lymphocytes, CD4+T lymphocytes and monocytes could be a promising biomarker to discriminate between PD and DLB. Further studies with higher number of participants are needed to validate these results.

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ANALYSIS OF THE SIGNALING PATHWAYS OF FOUR POTENTIAL ASTHMA TRIGGERS IN LUNG SAMPLES OF ASTHMATIC AND COPD PATIENTS

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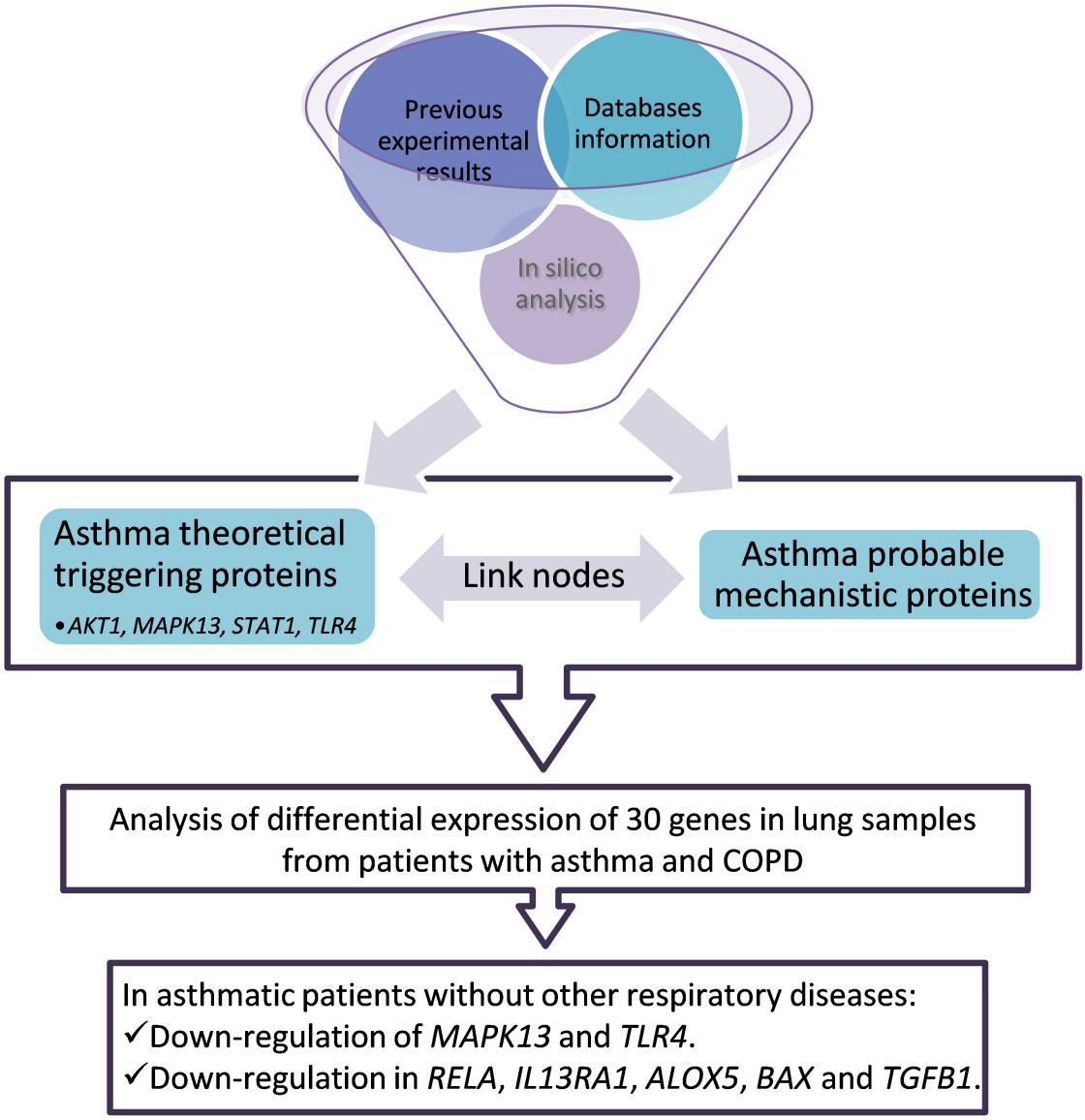
Background. Chronic inflammatory respiratory diseases, like asthma and COPD, are highly heterogeneous, hindering the diagnosis and management of some patients. Besides, current treatments are symptomatic and needed for long periods. We previously theoretically identified, by systems biology, four potential asthma triggers (*AKT1*, *MAPK13*, *STAT1* and *TLR4*), along with intermediate and mechanistic elements of their signaling pathways.

Objective. Analyze the expression of the defined asthma triggers and elements from their signaling pathways in lung samples of patients.

Methods. Lung tissue samples from 10 healthy controls (HC), 9 asthma patients (Ao), 10 asthma patients with other respiratory pathologies (Arp) and 10 COPD patients were obtained from the CIBERES biobank. RNA was extracted using TRIzol method, and differential expression of 30 genes was studied trough RT-qPCR and adjusted p-values were calculated with parametric T-Student test.

Results. All groups showed a down-regulation of the four triggers compared to HC, with statistical significance in *TLR4* ($p=0.009$ and $p=0.02$ in Ao and Arp, respectively) and *MAPK13* ($p=0.02$ in Ao). Respecting elements of their signaling pathways, a reduced expression of all studied genes were found in Ao, while some showed higher expression in Arp (*NFATC1, PTGFR, COL5A1, CCL17, IL10*) and COPD (*CCL17*); compared to HC. Also, significant differences were observed between Ao and HC in *RELA* ($p=0.03$), involved in all signaling pathways; and *IL13RA1* ($p=0.01$), involved in the MAPK13 pathway; having the latter also a significantly lower expression in Arp ($p=0.01$). From the mechanistic proteins studied, the only significant differences ($p<0.05$) were found in *ALOX5*, *BAX* and *TGFB1* between Ao and HC.

Conclusion. The differences found in the gene expression of *AKT1, MAPK13, STAT1* and *TLR4* and elements of their signaling pathways in lung samples of patients with asthma and COPD opens new possibilities in the search of treatments.



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EL *PRIMING* CON CITOCINAS PROINFLAMATORIAS REDUCE LA HETEROGENEIDAD DE LAS MSC AL INCREMENTAR SU CAPACIDAD INMUNOMODULADORA

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Una de las aplicaciones clínicas de las células stem mesenquimales (MSC) con mayor potencial es su empleo en enfermedades de base inmune e inflamatoria. Actualmente existen productos basados en MSC aprobados para el tratamiento de la enfermedad inflamatoria intestinal o la enfermedad injerto contra receptor. Es sabido que *in vivo* las MSC han de activarse por estímulos proinflamatorios para ejercer su acción inmunomoduladora y anti-inflamatoria. Sin embargo, aún queda por resolver si el *priming in vitro* con citocinas proinflamatorias puede incrementar su capacidad inmunomoduladora y anti-inflamatoria.

En el presente trabajo hemos analizado comparativamente las propiedades biológicas e inmunomoduladoras de MSC procedentes de médula ósea y tejido adiposo tras su primado con TNF-a, IL-1b e IFN-g durante 24 horas, respecto a la condición basal.
Nuestros resultados indican que el primado de las MSC no modifica significativamente sus características morfológicas, capacidad de diferenciación y fenotipo. Sin embargo, las MSC pre-

estimuladas, tanto de tejido adiposo como de médula ósea, sobreexpresan diversas citocinas, quimioquinas, moléculas de adhesión y factores inmunosupresores claves en la respuesta inmunológica, así como factores angiogénicos y de reparación, tanto a nivel génico como de proteína.

En cuanto a su capacidad inmunomoduladora, las MSC pre-estimuladas presentan una capacidad significativamente mayor para inhibir la proliferación de las células NK, la diferenciación y función de las DCs, la supervivencia y función de los macrófagos pro-inflamatorios M1 y la proliferación de linfocitos T activados que las MSC en condiciones basales.

Cabe destacar que el primado de las MSC reduce sensiblemente la variabilidad y heterogeneidad entre muestras de distintos donantes y distintas fuentes.

En conjunto nuestros resultados sugieren que la pre-estimulación *in vitro* de las MSC con citocinas proinflamatorias incrementa su capacidad inmunomoduladora y reduce la variabilidad entre muestras de diferentes donantes y de distintos orígenes, así como las diferencias derivadas de la interacción donante-receptor.

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ATYPICAL LATE PRESENTATION IN CRYOPYRIN-ASSOCIATED PERIODIC SYNDROME

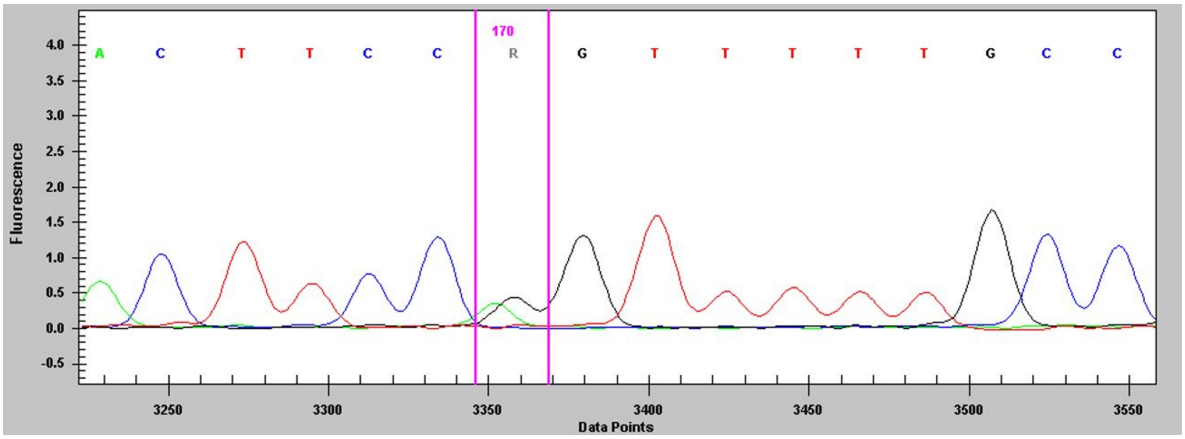
Jurado Orozco, Alicia; Muñoz García, Raquel; Morales García, Carmen; Montes Cano, Marco Antonio; Garrido Puñal, Noemi.

Hospital Universitario Virgen del Rocio, Sevilla.

Introduction: Cryopyrin-associated periodic syndromes (CAPS) or cryopyrinopathies are a group of autoinflammatory diseases related to defects in the protein cryopyrin (NLRP3). CAPS consist of Familial Cold Autoinflammatory Syndrome (FCAS), Muckle-Wells Syndrome (MWS) and Neonatal Onset Multisystem Inflammatory Disease (NOMID) or Chronic Infantile Neurologic Cutaneous Articular syndrome (CINCA) . FCAS, MWS and CINCA/NOMID are part of a clinical continuum with shared characteristics that differ in severity, beeing CINCA /NOMID the most severe form. Within the symtomps, it is found cutaneous, musculoskeletal, ocular and central nervous system involvement. Gain-of-function mutations in NLRP3 produces cryopyrin which forms inflammasomes. The defect in this pathway induces an overpro duction of IL 1β, leading to the inflammatory symptoms

Results: A 35-year old woman with recurrent fever, odynophagia, asthenia, lichenoid lesions, members weakn ess, arthalgias, perceptive deafness, oral thrush, livedo reticularis, nausea, vomiting and severe headache. Laboratories studies revealed elevated acute phase reactants, lymphopenia, negative ANAs, antiphospholipid syndrome negative, muscle biopsy negative.
A genetic panel for autoinflammatory diseases were performed using NGS (Next Generation Se quencing), revealing an heterozygous variant c.726G>A in NLRP3 (p. Ser726Gly) which was confirmed by sanger sequencing. Infevers and PolyPen 2 considers this variant of uncertain significance (VUS) and pathogenic respectively.

Conclusion: The patient doesn´t fulfill all clinical criteria for CAPS, especially for CINCA/NO MID, showing a non-typical form with a late onset and less severity. Clinical data with the variant detected in this patient suggests a path ogenic effect that supports the diagnostic.


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A C T T C C R G T T T T T G C C
Fluorescence
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
3250
3300
3350
3400
3450
3500
3550
Data Points

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MODELLING METABOLIC REPROGRAMING OF ALVEOLAR MACROPHAGES AND LUNG RARE DISEASES

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Despite extensive research efforts, rare lung diseases remain a major public health problem since their mechanisms are poorly understood and most of them lack approved treatments. Thus, there is an urgent need to develop novel therapies, being natural products an important source for drug discovery. Furthermore, the heterogeneity of these diseases, their low incidence and unknown etiology as well as the lack of preclinical disease models constitute major challenges for researchers. In the absence of specific target for drug development, phenotypical screening approach offers major opportunities for evaluation of new therapeutic agents. In this model, a characteristic change associated with the disease is used to develop a cell-based assay.
Recent evidences indicate that lung macrophage polarization (mainly M2 macrophages) plays a key role in the development of interstitial lung diseases (ILD). Moreover, a close association between polarization and metabolic state of macrophages has been described. Indeed, some fatty acids are involved in M2 activation, and lipid-laden macrophages or “foam cells” were observed in the lungs of some patients with ILD.
In this study, we describe a cell-based model using the mouse alveolar macrophage cell line MH-S as a potential tool for drug screening. Stimulation of MH-S cells with IL-4 or oleic acid (OA), a monounsaturated fatty acid, was used to induce M2 activation and foam cell formation, respectively.
OA induced lipid accumulation as demonstrated Oil Red O and BODIPY 493/503 staining. Triglyceride and cholesterol esters were also increased. Moreover, OA polarized macrophages to a M2 phenotype as indicated overexpression of M2 markers (CD206, Arg-1). Additionally, the therapeutic potential of a labdane derivative was also determined, showing protective effects in both conditions.

In conclusion, our work describes a cell-based approach, which will constitute a useful tool in preclinical research to test new drugs against several ILD.

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IMMUNE CELLS STIMULATION BY CIS-13-DOCOSENOL FATTY ALCOHOL REVEALS ITS POTENTIAL USE AS A NEW VACCINE ADJUVANT

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Cis-13-Docosenol is an unsaturated long chain alcohol (C22:1) which comes from jojoba oil throughout transesterification reaction and further crystallization steps using organic solvents. Previous studies reported that similar fatty alcohols, from other oils, inhibited pro-inflammatory cytokines production in LPS-stimulated macrophages, pointing out possible anti-inflammatory effects of long-chain fatty alcohols. Nevertheless, different studies investigating other fatty alcohols revealed that they were able to enhance production of pro-inflammatory cytokines, such as TNF-α and IL-1β, decreasing the levels of anti-inflammatory IL-10 cytokine in THP1-derived macrophages. Those results support the use of fatty alcohols as immune stimulators. Here, THP1 monocytes cells and monocyte-derived dendritic cells (MDDC) were treated with LPS and/or different cis-13-docosenol doses to study the effect of this fatty alcohol on immune cells. Results in THP1 cells and MDDC revealed that low doses of cis-13-docosenol are able to increase TNF-α, IL-6 or IL-1β pro-inflammatory cytokines to similar levels as LPS does. Furthermore, similar experiments in lung epithelial cells (A549 cells) treated with 13-cis-docosenol showed absence of pro-inflammatory cytokines. Modulation of T cell stimulation by macrophages or MDDC will be also discussed. These results indicated that Cis-13-Docosenol activates cells of the innate immune system, supporting the idea of further uses as a possible immune stimulator and vaccine adjuvant.

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REVISIÓN DE CASOS CON ELEVACIÓN SÉRICA DE IGG4 Y PUNTUACIÓN SEGÚN CRITERIOS DE ACR/EULAR 2019

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Hospital Fundación Jiménez Díaz, Madrid.

Introducción

La enfermedad relacionada con IgG4 (IgG4-RD) constituye un trastorno fibroinflamatorio inmunomediado que puede afectar a varios órganos. Histopatológicamente se caracteriza por infiltración linfoplasmocítica, fibrosis estoriforme y con frecuencia elevación de los niveles de IgG4 sérica.

Nuestro objetivo principal fue describir cuántos casos con concentraciones séricas elevadas de IG4 estaban diagnosticados o eran sospechosos de IgG4-RD, asignándoles posteriormente un score según los criterios de inclusión descritos en la American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) 2019.

Metodología

Se revisó retrospectivamente un conjunto de muestras provenientes de cuatro hospitales públicos con niveles de IgG4 sérica >172mg/dL (2 veces sobre el límite superior) procesadas entre enero y diciembre de 2019 por el laboratorio de inmunología mediante turbidimetría, analizador Optilite® (Binding Site).

Resultados

Se encontraron 25 casos con elevación de IgG4 según el criterio elegido. Los rangos de IgG4 sérica varían entre 316 a 2420 mg/dl. Del total de historias clínicas revisadas, 5 casos (20%) están diagnosticados formalmente, 4 de ellos tienen un score ≥20 según la ACR/EULAR, uno de ellos tiene un score inferior: tres pacientes son masculinos y dos femeninos; dos casos presentan afectación pancreatobiliar, dos casos afectación de glándulas salivales; incluyendo un síndrome de Mikulicz, un caso presenta compromiso pulmonar/multisistémico.

Encontramos 3 casos (12%) sospechosos en estudio, con un score <20 según la ACR/EULAR.

Conclusiones

El diagnóstico de IgG4-RD continúa siendo un reto, su baja prevalencia, la aparición de clínica compatible de forma progresiva, la necesidad de descartar otros posibles diagnósticos, la falta de correlación entre clínica y niveles de IgG4 sérica y la dificultad para realizar e interpretar

biopsias son los principales limitantes. Consideramos el cribado de casos sospechosos mediante mediciones de IgG4 un método eficiente que junto con el cuadro clínico/radiológico apropiado permiten aumentar la sospecha diagnóstica incluso en ausencia de criterios histopatológicos.

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POLINEUROPATÍA ASOCIADA A GAMMAPATÍA CON DIFERENTE COMPONENTE MONOCLONAL EN SUERO Y ORINA

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Paciente varón de 79 años derivado a la consulta de Hematología por detección de un componente monoclonal en contexto de síndrome tóxico y polineuropatía asociada. Adicionalmente presentaba ensanchamiento de las pareces aórticas con derrame pericárdico y niveles elevados de Troponina I, NT-proBNP y proteínas totales en orina. Tinción rojo Congo negativa y sin signos de inflamación en la biopsia obtenida de nervio subdural.
El estudio inmunológico mostró, mediante inmunofijación, un componente monoclonal sérico IgG kappa en la fracción gamma. Contrariamente, la inmunofijación en orina reveló un segundo componente monoclonal correspondiente a una cadena lambda libre, el cual no fue observado en suero. La cuantificación de la cadena lambda libre en suero mediante turbidimetría fue de 6.24 mg/dL (valores de normalidad 0.6-2.6 mg/dL), apoyando el resultado obtenido en orina.
Un posterior análisis histoquímico identificó la presencia de depósito amiloide en una biopsia subcutánea de grasa. En base al estudio histoquímico e inmunológico y a la afectación renal, cardiaca y nerviosa, se orientó como una amiloidosis sistémica primaria (AL). Para el diagnóstico diferencial con otras gammapatías monoclonales se consideraró el porcentaje de células plasmáticas en médula ósea, la cuantificación del componente monoclonal en suero y orina y los signos clinicos asociados, confirmándose el diagnóstico de AL.
Nuestro paciente presenta una gammapatía biclonal constituida por dos componentes monoclonales: una IgG kappa y una cadena lambda libre. Trabajos previos han sugerido que la presencia de un segundo componente no interfiere en la progresión de la enfermedad. No obstante, es recomendable la identificación de ambos componentes con el objetivo de realizar un correcto seguimiento de la patología. La observación de un componente monoclonal en suero y en orina es dependiente de la técnica utilizada, requiriendo un análisis inmunológico completo.

P-208

UTERINE CYTOTOXIC NK CELLS IN PATIENTS WITH RECURRENT IMPLANTATION FAILURE. A CAUTIONARY TALE

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Failed implantation represents the major limiting factor in assisted reproduction. Despite significant growing new evidence of the involvement of immunological alterations on pregnancy outcome, the supporting evidence of immunological or inflammatory causes of recurrent implantation failure (RIF) has not been re-appraised by the current guideline consensus. RIF was defined as the failure to achieve a clinical pregnancy after transferring at least four good-quality embryos in a minimum of three fresh or frozen cycles in women below 40 years old. NK cells have a crucial role in several pregnancy processes, such as implantation and placentation, remodeling spiral arteries, and promoting trophoblast invasion through the secretion of several angiogenic factors and cytokines. Dysregulation of NK cell function has been associated with unfavorable reproductive outcomes, like recurrent miscarriages (RM), RIF and preeclampsia. Systemic and local inflammatory response at the maternal-fetal interface accounts for some RIF cases, although the exact magnitude of an inflammatory basis remains unknown. This study sought to assay uterine NK cells phenotype's potential role in defining a subgroup with inflammatory RIF. Freshly collected endometrial samples were processed and analyzed by multiparametric flow cytometry using a BD FACS CANTO II. Uterine NK cells were characterized based on CD45, CD56 and CD16 expression. In this study, we evaluated 20 women, 10 with RIF and a group of 10 non-pregnant fertile women was included as a control group (egg donors). The present study results showed that uterine cytotoxic NK cells (CD56+CD16+) were significantly higher in RIF than healthy controls (HC) (6 [3.9-7] vs 2.7 [1.9-3.8]; $p<0.004$). Furthermore, uterine NK cells (CD56bright CD16-) were reduced in RIF patients compared to HC (85.9 [77.5-89.5] vs 64.8 [51.1-74]; $p<0.003$). A more rigorous selection of patients with an inflammatory profile through uterine cytotoxic NK phenotyping may better define patients that could benefit from an immunomodulatory treatment.

P-209

INFLAMMATORY SIGNATURE AND NEUROAXONAL DAMAGE BIOMARKERS IN LONG-COVID SYNDROME

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Introduction: Long-COVID syndrome refers to persistent or late-onset symptoms of SARS-CoV-2 infection. It can have a wide spectrum of neurologic clinical manifestations, including fatigue, brain fog, dysautonomy and headache. A persistent inflammation has been postulated as the cause of this syndrome. The aim of this study was to determine if there is an underlying inflammatory etiology and/or neuronal damage.

Material and methods: We prospectively collected samples from 49 patients with Long-COVID at baseline, 1 month, 3 months and 6 months time points. We studied a total of 183 serum samples and 13 CSF from study participants and 38 sera and 20 CSF from a control group with mild cognitive complaints. We performed a multiplex bead based assay by Luminex technology for the following soluble inflammatory biomarkers: Fractalkine, G-CSF, IL-10, IL-18, IL-1RA, IL-6, IL-8, IL-17a, IL-1b, IFNa2, IFNg, IP10, MCP1, MCP3, MIG and TNFa. Glial fibrilary acidic protein (GFAP) and Neurofilament light (NfL) levels were studied by single molecule array (SiMoA) in a SR-X platform (Quanterix) in serum.

Results: We observed significant lower levels of G-CSF, IP10, MCP1 and MIG in patient's sera, but not an increase in any parameter. Patient's CSF showed significant higher levels of IL-18 (mean 0.36 vs 0.31 pg/ml), IL-17a (mean 0.37 vs 0.4 pg/ml), IL-1b (mean 0.35 vs 0.62 pg/ml) and TNFa (mean 6.4 vs 1.64 pg/ml) and lower levels of IL-10, IL-1RA, IL-8, IP10, MCP1 and MIG. No differences over time were observed in longitudinal serum samples for any studied biomarker. Neuronal damage markers (GFAP, NfL) in serum neither showed differences compared to controls nor substantial variations over time.

Conclusions: No evidence of neuroaxonal damage was observed in Long-COVID patients. Although significant CSF higher concentrations were found in some inflammatory markers, suggesting an inflammatory etiology of long-COVID syndrome, differences were very low.

Sesión Poster Defensa 2. Pantalla 5. Miscelánea (Inmunidad anti-tumoral / Alergias y reacciones de hipersensibiliadad / Vacunas) (II)

P-210

ALVEOLAR MACROPHAGES MODULATE CANCER-ASSOCIATED FIBROBLASTS IN A PRECLINICAL MODEL OF NSCLC.

Garvín Jiménez, Eduardo; Soria, Gonzalo; Munarriz Paños, Mariola; Gómez Parrizas, Mónica; Casanova Acebes, María.

Centro Nacional de Investigaciones Oncológicas, Madrid.

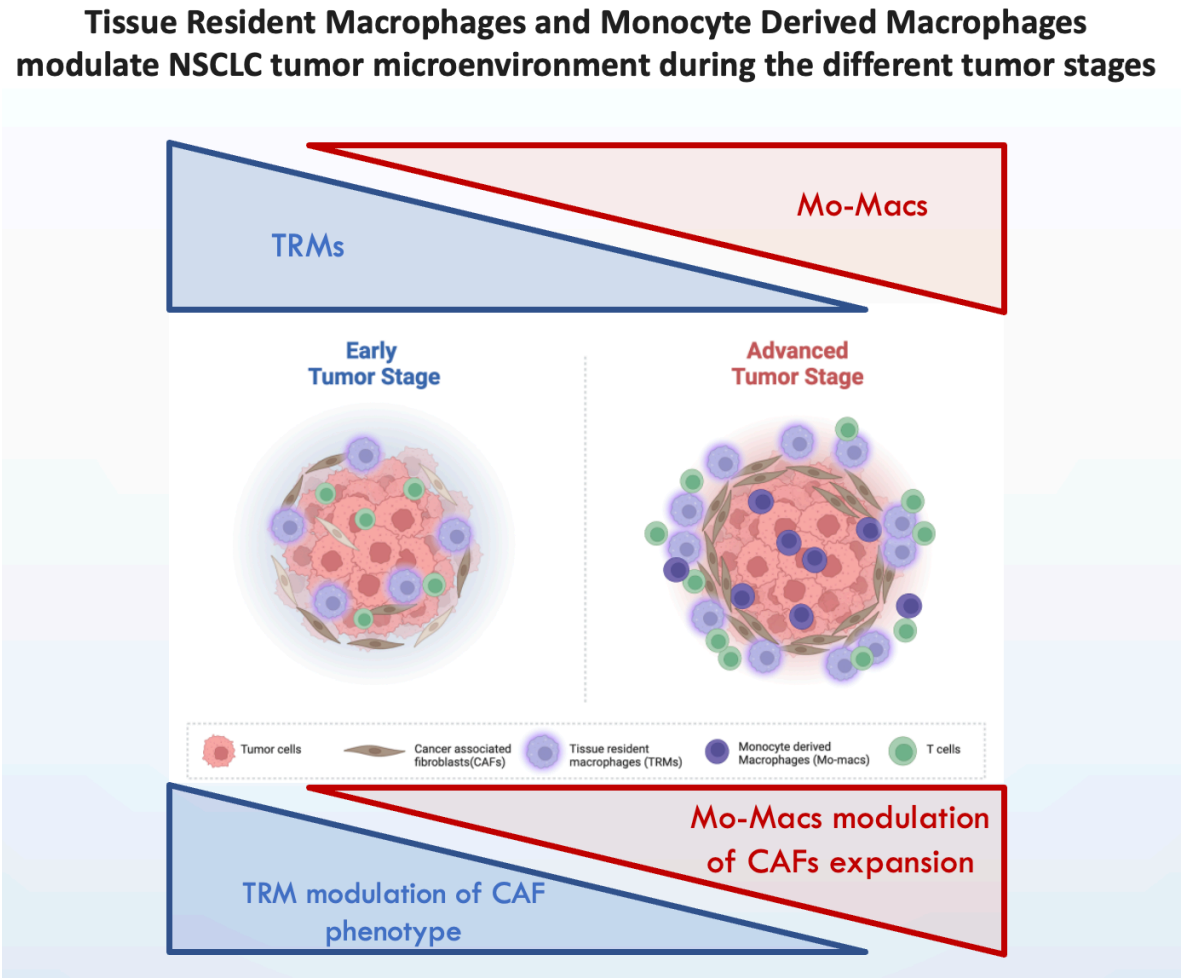
The presence of fibroblasts in tumors is associated with hallmarks of cancer malignancy, such as tumor invasion and metastasis, angiogenesis, and immune evasion. Although the contribution of cancer-associated fibroblasts (CAFs) to cancer progression is well understood, very limited is known about the factors that regulate fibroblast activation at early stages of tumorigenesis and how we can leverage early interventions to modify the tumor microenvironment of lung cancer. Specifically we focus on NSCLC (human non-small cell lung carcinoma), which has been found to clinically account for 82% of all lung cancer diagnoses.

Our laboratory has identified a population of macrophages that, although residing in the lung (tissue resident macrophages or TRMs, also known as alveolar macrophages), are found in the periphery of NSCLC tumors. TRMs promote epithelial-mesenchymal transition in cancer cells and enhance the function of regulatory T cells in lung NSCLC tumors, promoting tumor escape from the immune system. Furthermore, TRMs interact closely with a subset of CAFs, leading us to hypothesize whether TRMs may contribute to the modulation and malignant conversion of NSCLC CAFs.

Using multiparametric flow cytometry, confocal imaging and transcriptomics of FACS-sorted NSCLC-CAFs, we demonstrated that ablation of specific TRMs is sufficient to control CAFs activation by PDGFRa, podoplanin (PDPN) and other inflammatory marker controlled expresión in the tumor microenvironment. Interestingly, tumors without TRMs showed increased T-cell activation and infiltration, suggesting that in addition to mediating fibroblast activation, they also play a role in the encapsulation of tumors, shielding neoplasic tissue from T cell surveillance.

Based on our preliminary data, we aim to dissect how mechanistically TRMs modulate CAFs,

and how this influences T cell infiltration and activation in order to design novel therapeutic strategies that boost TRM-CAF immunomodulatory activity.



P-211

*HLA-B*53:01* ALLELE CORRELATES WITH REDUCED OVERALL SURVIVAL IN PATIENTS WITH ACUTE MYELOID LEUKAEMIA

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Background

Acute myeloid leukaemia (AML) is an aggressive haematologic neoplasm derived from the accumulation of undifferentiated myeloid precursors in the bone marrow and peripheral blood, representing the most common acute leukaemia in adults. Allogeneic stem cell transplantation (allo-SCT) and conventional chemotherapy are the best therapeutic options in fitted patients. AML onset remains unknown, so it may be of great importance to further investigate the role of the MHC-KIR axis. We studied the *HLA* and *KIR* genes in AML and its performance with clinical outcome.

Material and methods

KIR and *HLA* genes were typed in 312 AML patients, eligible for allo-SCT diagnosed between 2010-2020, and 1164 healthy controls. SSOP-PCR and NGS-PCR methodologies were used for KIR and HLA typing respectively. Data were collected and analysed using the IBM-SPSS® Statistics 25. Case-Control analysis was performed by applying Xi2 and Bonferroni correction. Kaplan-Meier was used for survival estimations. A p-value <0.05 was considered statistically significant.

Results

*HLA-C*12:02* (5.2% vs 1.8%, Pc=0.016), *DRB1*14:01* (2.3% vs 0.3%, Pc=0.002), and *DQB1*06:01* (4.4% vs 1.8%, Pc=0.045), but not *B*53:01*, alleles exhibited an increased AML risk compared to controls (Table 1).

Regarding survival analysis, *HLA-B*53:01* carriers showed significantly lower 3y overall survival (Figure 1, 31.3% vs 62.4%, Log-rank<0.001). There were no relevant differences of concern with other *HLA* alleles or *KIR* genes.

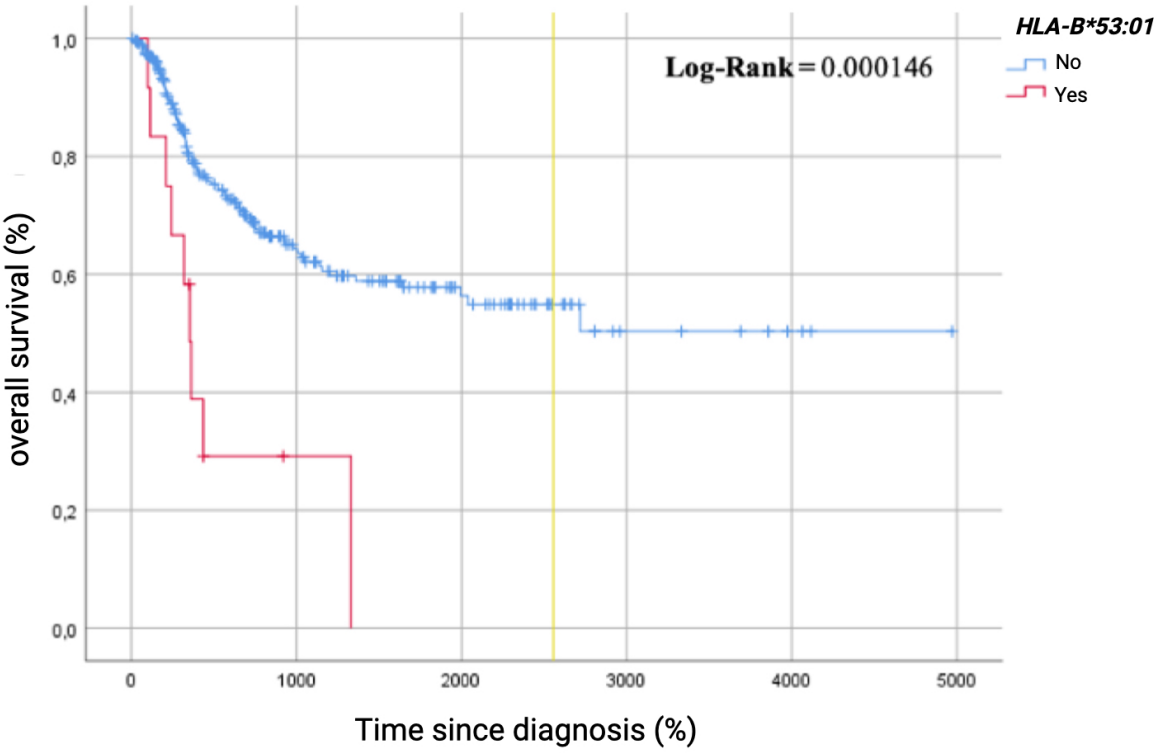
Table 1. *HLA* phenotypic frequencies in AML patients and controls

***HLA* genes**	**Controls (N=1164)**	**AML Patients (N=312)**	***Pc***	**OR (95% CI)**
*B*53:01*	23 (2%)	13 (4.2%)	0.725	2.164 (1.083-4.323)
*C*12:02*	21 (1.8%)	16 (5.2%)	0.016	2.962 (1.826-5.747)
*DRB1*14:01*	3 (0.3%)	7 (2.3%)	0.002	8.873 (2.281-34.515)
*DQB1*06:01*	20 (1.8%)	12 (4.4%)	0.045	2.560 (1.236-5.303)

HLA, *Human Leukocyte Antigen*; **AML**, Acute myeloid leukaemia; ***Pc***, corrected *p*-value

Conclusion

The *HLA-B*53:01* allele could have a relevant role in AML prognosis amongst other known clinical and biological variables affecting patient outcome



OS	1sty	2ndy	3rdy	4thy	5thy
*HLA-B*53:01* Neg (n=279)	78.6% (n=167)	68.6% (n=121)	62.4% (n=83)	59.2% (n=66)	58.1% (n=47)
*HLA-B*53:01* Pos (n=12)	41.7% (n=4)	31.3% (n=3)	31.3% (n=1)	0% (n=0)	0% (n=0)

P-212

FISH FOOD ALLERGY: SEEKING SUITABLE PEPTIDES FOR ALLERGEN-SPECIFIC IMMUNOTHERAPY

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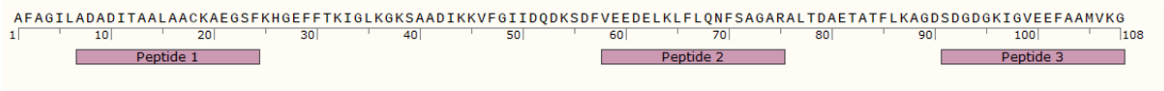
[1]Centro de Investigacións Biomédicas (CINBIO), Vigo; [2]Instituto de Investigaciones Marinas (CSIC), Vigo.

Fish is one of the most frequent causes of immunoglobulin E (IgE)-mediated food allergy (Type I hypersensitivity). Currently, there is no cure, and sensitized individuals must practice strict, life-long avoidance of fish products in their diets. The allergen-specific immunotherapy (AIT), based on the administration of repeated doses of allergen extracts to induce immunological tolerance, is a promising therapeutic approach. However, the effectiveness and safety profile of this strategy varies, and there is a need for more effective AIT approaches. The use of synthetic peptides representing T-cell epitopes of the main allergens is a good alternative.

Parvalbumins beta (β-PRVBs) are considered the major fish allergens. These proteins are present in elevated quantities in the white muscle of fish species. We have developed an animal model for fish allergy as a previous step for preclinical testing of AIT. We sensitized BALB/c mice using the allergen Mer mr 1 (European hake β-PRVB). A Th2-like immune response was induced with production of β-PRVB specific IgE and IgG1. Here, we use the IEDB (Immune Epitope Database) platform to predict T-cell epitopes of Mer mr 1 sequence. We combined the predictions for the $H2^d$ haplotype present in BALB/c mice with the predictions for an array of 27 HLA alleles that cover for more than 99% of the human population. Three peptides were selected:

- ADADITAALAACKAEGSF
- VEEDELKLFLQNFSAGAR
- SDGDGKIGVEEFAAMVKG

which are in well-conserved areas of the protein and there is a good degree of correlation with the predictions for other fish species. The immunomodulatory capacity of these peptides are being evaluated to fully validate these predictions.



Beta-Parvalbumin (Mer mr 1)

Merluccius merluccius

P-214

PLASTICITY OF CYTOKINE-INDUCED MEMORY-LIKE (CIML) NK CELLS IN RESPONSE TO TGF-β

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Natural killer (NK) cells play a key role in the defence against tumour cells and viral infections. Traditionally, NK cells have been considered part of the innate immune system. However, they can acquire immune memory-like properties after stimulation with a combination of interleukin (IL)-12+IL-15+IL-18 for a short period of time. These pre-activated NK cells are known as cytokine-induced memory-like (CIML) NK cells, and due to their proven enhanced anti-tumor capacity they are being used in clinical trials. On the other hand, NK cells have a high plasticity, being able to change their phenotype and effector functions depending on external signals, such as the immunosuppressive factors in tumour microenvironment. *In vitro* and *in vivo* studies have demonstrated that exposure to tumor-derived factors, such as transforming growth factor-β (TGF-β), can drive the conversion of circulating NK cells into a decidual-like phenotype. Decidual NK cells (dNK cells) are characterised as proangiogenic and less cytotoxic, which is crucial for foetal growing. However, in a cancer context, NK cells with decidual-like phenotype could promote tumour progression and growth. Thus, the aim of our present study was to analyse the influence of TGF-β on CIML-NK cells and to test if they could also acquire a decidual-like phenotype. For that, PMBCs from healthy donors were isolated and incubated with IL-12+IL-15+IL-18 for 18h. After a resting period of 7 days, those pre-activated NK cells were exposed to either TGF-β alone, IL-15 alone or TGF-β + IL-15. Flow cytometry studies showed that TGF-β alone is able to induce the expression of CD9, a marker of dNK cells. Furthermore, the combination of TGF-β and IL-15 also induced the expression of other surface markers associated to dNK cells, such as CD103 and CD49a. Our data suggest that CIML-NK cells are sensible to the conversion to a decidual-like phenotype.

P-215

THERAPEUTIC EFFICACY OF MTBVAC AGAINST ASTHMA: FROM ANIMAL MODELS TO HUMAN PBMCS

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[1]Universidad de Zaragoza, Zaragoza; [2]Fundación Jiménez Díaz, Madrid.

Asthma, a prevalent pulmonary disease worldwide, is associated with exacerbated type 2 inflammation that leads to high economic burden in public health. Allergen-specific Th2 lymphocytes produce cytokines responsible for clinical symptoms. Targeting asthma-associated type 2 responses has shown high promise in therapy.

MTBVAC, a live attenuated tuberculosis vaccine under clinical evaluation, has shown a Th1 response-promoting activity. Intradermal MTBVAC has been extensively tested in preclinical and clinical trials and is expected to commence efficacy trials in newborns in 2022. The pulmonary and intravenous administration of MTBVAC has revealed strong therapeutic efficacy in animal mouse models, in terms of reducing eosinophil profile and Th2 cytokines. Here we show the effects of MTBVAC incubation on peripheral blood mononuclear cells (PBMCs) from asthmatic patients. Results suggest that MTBVAC reduces the clonal proliferation of allergen-specific cells and increases the expression of pro-Th1 genes, as well as their production on human PBMCs.

In a context of established disease, our data demonstrated the therapeutic effectiveness of MTBVAC in animal models, and aimed to study their potential in human PBMCs.

P-216

EVALUATION OF SPECIFIC CELLULAR AND HUMORAL RESPONSE AFTER THE THIRD DOSE OF ANTI-SARS-COV-2 RNA VACCINE IN PATIENTS WITH IMRDs ON BIOLOGIC THERAPY

Mohamed Mohamed, Kauzar; Álvarez Hernández, Paula; Jiménez García, Carlos; Guevara Hoyer, Kissy; Freites, Dalifer; Martínez Prada, Cristina; Pérez Sancristobal, Inés; Fernández Gutierrez, Benjamin; Mato, Gloria; Garcia Bravo, Laura; Guerra Galán, Teresa; Mansilla Ruíz, María Dolores; Villegas Mendiola, Ángela; Pereiro Rodríguez, Alejandro; Palacios Ortega, María; Rodero, María; Rodríguez De La Peña, Antonia; Mulero, Teresa; Bravo, Cecilia; Toledano, Esther; Culebras Lopez, Esther; Mediero Valeros, Beatriz; Sánchez-Ramón, Silvia; Candelas Rodríguez, Gloria.

Hospital Clínico San Carlos, Madrid.

Objective

Data on cellular and humoral immunogenicity after the third dose of anti-SARS-CoV-2 vaccines in patients with immune-mediated rheumatic diseases (IMRDs) are scarce. Herein, we evaluated the adaptive immune response in IMRDs patients treated with different biological therapies after the booster of anti-SARS-CoV-2 vaccine to determine whether any drug reduces the vaccine's response.

Methods

A single-center prospective study was conducted including patients presenting IMRD and healthy controls (HC). Specific anti-SARS-CoV-2 interferon-gamma (IFN-γ) production was evaluated between 8-12 weeks after the third dose of the SARS-CoV-2 vaccine. In addition, anti-Spike IgG antibody titers were measured by an immunoassay against the receptor-binding domain (RBD).

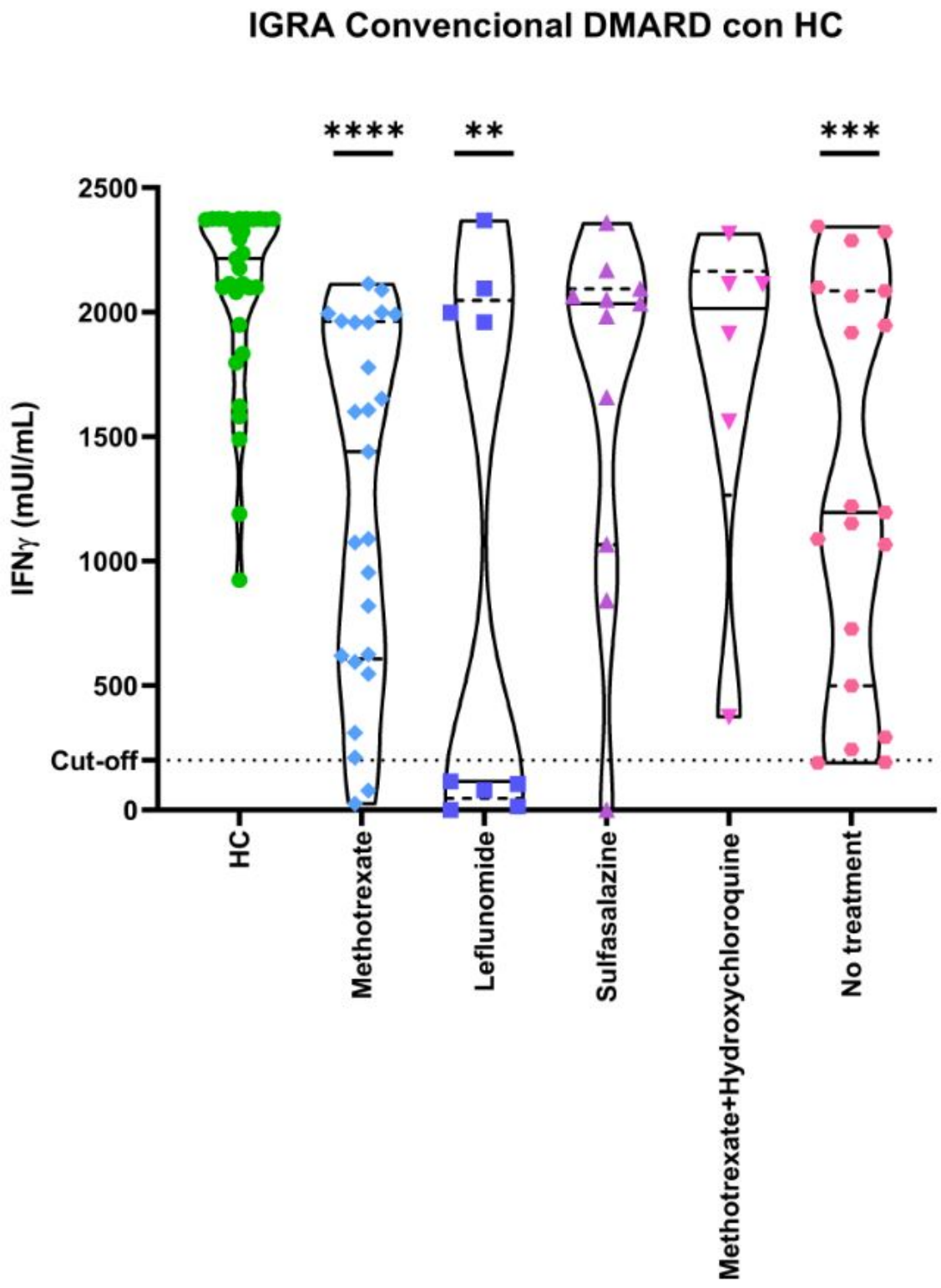
Results

Samples were obtained from 79 IMRDs patients (51 women, 28 men; mean age 57±11.3 years-old): 43 rheumatoid arthritis (RA), 10 psoriatic arthritis (PsA), 14 ankylosing spondylitis (AS), 10 undifferentiated spondyloarthritis (USpA) and 2 IBD-associated spondyloarthritis (IBD-SpA). Thirty-one HC (mean age 50.9±13.1 years-old, 67.7% women) were included in the study. Post-vaccine results displayed positive T cellular immune responses in 68 out of 79 (86.1%) in IMRDs patients (82.3% of those without prior COVID-19). MTX and leflunomide

were associated with lower titers of IgG and IFN-γ responses. Among bDMARDs, adalimumab, etanercept and guselkumab are related with reduced cellular response.

Conclusion

Our data show that the majority of patients with IMRDs were able to mount an adequate specific adaptive response after the booster vaccination, emphasizing the relevance of vaccination in this group. However, the magnitude of specific responses was dependent on the immunosuppressive therapy administered. Specific vaccination protocols and personalized decisions of booster are essential for these patients.


IGRA Convencional DMARD con HC
****
**
***
IFNγ (mUI/mL)
2500
2000
1500
1000
500
Cut-off
0
HC
Methotrexate
Leflunomide
Sulfasalazine
Methotrexate+Hydroxychloroquine
No treatment

P-217

NOVEL SARS-COV-2-STABILIZED SPIKE PROTEINS WITH IMPROVED PRODUCTION AND PROTECTIVE ACTIVITY AGAINST SARS-COV-2-INDUCED DISEASE IN ANIMAL MODELS

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[1]Institut de Recerca de la Sida (IrsiCaixa), Badalona; [2]Centre de Recerca en Sanitat Animal (CReSA), Bellaterra; [3]Barcelona Supercomputing Center (BSC), Barcelona.

Background

Most SARS-CoV-2 vaccines are based on the Spike (S) glycoprotein and include a double mutation to stabilize its prefusogenic conformation (K986P/V987P, S-2P). These mutations improve stability, yield, and immunogenicity. However, S-2P still shows certain instability degree, and it is poorly produced. Here, we designed novel stabilized S glycoproteins, with greater production levels than S-2P that could be used in COVID-19 vaccine development.

Methods

A set of S mutations were selected *in silico* by extensive computational modelling. Mutated S variants were produced by transient transfection in Expi293 cells. Yield and RBD exposure were analyzed by ELISA. Immunogenicity and protective activity of selected S variants were tested in K18-hACE2 transgenic mice and golden Syrian hamsters (GSH). Animals were vaccinated using a prime-boost protocol, and challenged with either SARS-CoV-2 D614G or beta variants. Vaccine-induced humoral responses were analyzed by ELISA and neutralization assays, and protection against SARS-CoV-2-induced disease was evaluated by analyzing clinical signs, viral load, and tissue damage in biological samples (oropharyngeal swab, nasal turbinate, lung, and brain).

Results

We identified several mutations that improved from two- to five-fold the S glycoprotein production. Immunogenicity of these S variants was equivalent to S-2P in K18-hACE2 mice

and GSH. These animals developed vaccine-induced antibody responses with neutralizing activity against Wuhan-1, Beta, and Delta variants, and gained neutralization activity against Omicron after viral challenge. Interestingly, two of our S variants, but not S-2P, protected 100% SARS-CoV-2-immunized K18-hACE2 transgenic mice from severe disease.

Conclusion

Overall, we described a set of mutations that increased S glycoprotein production, maintaining the immunogenicity elicited by the S-2P variant. Relevantly, our newly-developed S mutants showed a higher degree of protection against SARS-CoV-2-induced disease in mice and GSH than S-2P. Thus, these novel S proteins provide an alternative to S-2P that could be used in future SARS-CoV-2 vaccine development.

P-218

EL EFECTO ANTITUMORAL DEL SISTEMA INMUNE MEDIADO POR RECEPTORES KIR EN PACIENTES TRATADOS CON CETUXIMAB ES DEPENDIENTE DE Fc Y Fab CONJUNTAMENTE

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Introducción: La supervivencia del cáncer colorrectal (CCR) mejoró gracias al uso de anticuerpos monoclonales como cetuximab. Esta molécula actúa por dos mecanismos: su fragmento Fab se une a EGFR y bloquea su acción y gracias a un mecanismo de inmunomodulación por Fc. Ambos serían efectivos en pacientes sin mutación de KRAS. En mutados, sólo sería efectivo a través de Fc, al estar constitutivamente activada la vía de EGFR. En el presente trabajo se considera que la comparación del efecto en CCR con y sin mutación de KRAS, sería un modelo "in vivo" ideal para poder discriminar el efecto de cetuximab dependiente de bloqueo de EGFR, de la inmunomodulación a través de Fc o por ambos. Para discriminar entre estos posibles mecanismos, el estudio se centrará en el análisis de los genes KIR.

Pacientes y métodos: Se realizó un estudio observacional retrospectivo con 69 pacientes con CCR KRASm y 55 sin mutación. El ADN se extrajo mediante MagCore SUPER, el tipaje de HLA-A, B y C por LIFECODES HLA-A,B SSO y LIFECODES HLA-C eRES SSO Typing Kits y el genotipaje KIR mediante KIR Ready gene of Inno-train.

Resultados: No se observa un patrón común en ambos tipos de tumores que pudieran confirmar que el efecto de la modulación del sistema inmune es dependiente sólo de Fc. De alguna manera, las consecuencias derivadas del bloqueo de Fab en KRAS no mutados, provocan que el perfil de respuesta KIR sea diferente en su conjunto y no permite diferenciar efectos independientes derivados de Fab y Fc. Así, en KRASm mejora la supervivencia la presencia de 2DL5 y genotipos Bx. La presencia de 2DP1/2DL1 sin 2DL5 empeora el pronóstico. En no mutados, sin embargo, sólo se asocia a mejor supervivencia la combinación 3DS1/Bw4.

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ENZYME-LINKED IMMUNOSORBENT ASSAY FOR THE DETECTION OF EGG IN FOODS BASED ON A SCFV-ANTIBODY OBTAINED BY PHAGE DISPLAY

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Hen's egg allergy is the second most common food allergy in infants and young children. The prevalence of egg allergy in young children ranges from 0.5 to 2.5 %. Egg white proteins are the main cause of allergenicity, with ovomucoid being the most dominant allergen due to its stability against heat and proteinase digestion. The possible presence of undeclared egg in foodstuffs poses a significant risk for sensitized individuals. Therefore, reliable methods for the detection and quantification of egg allergens are needed to ensure compliance with food labelling and to guarantee consumer protection. In this study, an ELISA methodology based on a recombinant antibody fragment against ovomucoid was developed for the detection of egg in foods. Firstly, a single-chain variable fragment (scFv) library was constructed from mRNA isolated from the spleen of an ovomucoid-immunized rabbit. After successful construction of the library, it was screened by phage display through four consecutive rounds of selection using both, ovomucoid and egg white extract, as target antigens. From the different clones analysed by phage-ELISA for the specific detection of ovomucoid, the SR-G1 clone exhibited the best recognition characteristics. The gene encoding the anti-ovomucoid scFv SR-G1 was subcloned into the plasmid pET-scFv and expressed in E. coli BL21(DE3). The obtained scFv fragment was purified by IMAC resulting in a yield of 1 mg/L from the culture medium. The performance of the purification process was verified by visualization of a protein band at the expected molecular weight of the scFv (29 kDa) by SDS-PAGE and Western Blot. Based on the purified SR-G1 antibody, an indirect ELISA was developed for the detection of ovomucoid resulting in a LOD and LOQ of 43 and 79 ng/mL, respectively. Finally, the developed method was successfully employed for the detection of ovomucoid in different food samples.

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BACTERIORUBERIN: A NOVEL THERAPEUTIC MOLECULE AGAINST CANCER

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In recent years, oxidative stress has been catalogued as one of the possible causes of the appearance and progression of many pathologies, including cancer. However, this can be counteracted through the use of antioxidant molecules, which could prevent and cure the development of the disease. Among the natural antioxidants, carotenoids stand out, which can be found in plants, algae, microorganisms and some animals. In favor of the therapeutic use of carotenoids against cancer, a multitude of epidemiological studies have described how the intake of carotenoids correlates with a lower incidence of different types of tumors. In addition, it has been shown that carotenoids suppress the appearance and progression of cancer by different mechanisms, such as the induction of apoptosis and the reduction of cell proliferation. In turn, they also have an important antitumor role mediated through the immune system, acting as potent immunomodulators.

In this context, the bacterioruberin is a rare carotenoid from extremophilic haloarchaea which is little studied and has a much higher antioxidant capacity than conventional carotenoids such as β-carotene. This suggests that this compound could have a greater antitumor capacity than conventional pigments. Our main goal is to study the effect of bacterioruberin in the viability, proliferation, apoptosis and cell cycle in K562 myeloid leukemia cell line. We have cultivated these cells with different concentrations of bacterioruberin during different time intervals. Cell proliferation (CFSE), cell cycle (propidium iodide) and apoptosis (annexinV-FITC) were determined by flow cytometry (FACSCanto, BD Bioscience) and viability was estimated using the MTT assay.

The group's preliminary results to date indicate that this molecule can decrease the proliferation of K562 cells in a dose dependent-manner, affecting their viability and altering the cell cycle. In turn, we have observed that this carotenoid is able to induce cell death by apoptosis in these tumoral cells.

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DERMATOPHYTID IN A PATIENT WITH TINEA CRURIS CAUSED BY *TRICHOPHYTON RUBRUM*. REGARDING A CASE

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Introduction

Dermatophytid or "ide" reaction is a delayed hypersensitivity-mediated inflammatory reaction that manifests in areas distant from the site of primary dermatophyte infection during the acute phase of the infection or within a few days after initiation of treatment. It is unusual and is observed in 5% of patients with dermatophytosis. After primary sensitization and diffusion of the mycotic antigens through the epidermis, the proliferation of lymphocytes that recognize these antigens and the release of cytokines and other inflammatory mediators will trigger the reaction pattern. Histologically, it is characterized by a perivascular infiltrate with a predominantly lymphohistiocytic and surrounding eosinophils.

Case Description

A 64-year-old male who presented erythematodecamative plaques in the right groin with extension to the contralateral groin **(Figure 1 A)**. Due to the suspicion of tinea cruris, the patient began taking terbinafine, which resulted in the appearance of new maculopapular lesions **(Figure 1 B)**. Due to the suspicion of drug-related symmetrical intertriginous flexural exanthema (SDRIFE), hospital admission was decided for treatment with intravenous corticotherapy pulses, dexchlorpheniramine and itraconazole. Skin culture was negative and skin biopsy showed perivascular and superficial interstitial dermatitis of lymphocyte predominance with neutrophils and eosinophils. Furthermore, specific IgE against *Trychophyton rubrum* was requested and its result was positive (0.63IUa/ml), supporting the diagnosis of dermatophytid. Loratadine and topical methylprednisone was prescribed.

The patient progressed satisfactorily after the prescribed treatment, with complete remission of the lesions in approximately one month.

Conclusions

We present a case of a patient with tinea cruris and dermatophytid in whom the presence of sensitization to *Trychophytum rubrum* and a compatible biopsy allowed a diagnosis. We must highlight the usefulness of the determination of specific IgE in doubtful or suspicious cases of other diagnoses such as toxicodermia in order to adequately supervise the treatment of the patient.

A



Figure 1 A. Erythematodesquamative plaques with circinate borders in the right groin with extension to the contralateral groin.

B



Figure 1 B. Extension of the initial lesion with the appearance of maculopapular lesions in antecubital, axillary and abdominal folds.

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CÓCTELES PRE-MEZCLADOS DE ANTICUERPOS CONSERVADOS A -20ºC PARA ESTANDARIZAR LOS ESTUDIOS DE INMUNOFENOTIPO POR CITOMETRÍA DE FLUJO

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Con el aumento del número de marcadores utilizados en un mismo panel para el análisis de poblaciones celulares, aumenta la complejidad técnica y analítica de los estudios de inmunofenotipo realizados por citometría de flujo (CMF). Considerando los potenciales errores humanos que pueden ocurrir durante la preparación del cóctel de un panel amplio de anticuerpos y el tiempo necesario que se requiere para esta etapa pre-analítica, junto al elevado número de muestras que se procesan a diario, la utilización de cócteles pre-mezclados puede aportar grandes ventajas para la estandarización de la técnica.

En este trabajo se evalúa la estabilidad de cócteles pre-mezclados conservados a -20ºC frente a un marcaje realizado con los mismos anticuerpos sin congelar. Para ello se testó un mismo panel con cócteles congelados durante 29, 22, 16, 8 y 3 días con una misma muestra de sangre periférica, y se comparó con una tinción manual en fresco. El panel consistió en 16 anticuerpos monoclonales analizados en un citómetro FACS-Lyric de 12 fluorescencias. Panel de "Linfoma-screening": CD3-BV711, CD4-APC, CD5-PECy7, CD8-FITC, CD10-APC, CD19-BV786, CD20-BV605, CD23-VB510, CD38-APCr700, CD43-Per-C5.5, CD45-APC-H7, CD56-PECy7, cB1-PE, FMC7-V450, Lambda-FITC y Kappa-PE.

Los resultados muestran que la congelación de los cócteles durante 29 días no altera la tinción de la muestra, ni los resultados observados tanto en el porcentaje de las poblaciones leucocitarias principales (linfocitos, monocitos, neutrófilos y eosinófilos) como en las subpoblaciones linfocitarias (CD3, CD4, CD8, CD19, CD20, etc). Tampoco se alteró la intensidad medida de fluorescencia (MFI) de ninguno de los marcadores. Las pequeñas variaciones observadas en la MFI de algunos fluorocromos fueron achacables al cambio de lotes de los reactivos.

Por tanto, la utilización de cócteles pre-mezclados de anticuerpos monoclonales conservados a -20ºC para el estudio de inmunofenotipo es fiable, reproducible y estandarizable.

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LAS CIFRAS DE LINFOCITOS T CD8 EN EL LAVADO BRONCOALVEOLAR APORTAN VALOR PRONÓSTICO EN LA SUPERVIVENCIA DE PACIENTES CON CÁNCER

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El sistema inmunitario juega un papel primordial en la inmunovigilancia frente al cáncer. Las cifras de linfocitos T que infiltran tumores, especialmente TCD8, aportan valor pronóstico independiente y complementan al pronóstico histopatológico en diversos tipos de cáncer. El "inmunoscore" cuantifica los linfocitos que infiltran el tumor y describe una relación directamente proporcional con la supervivencia de los pacientes.

En este trabajo se han analizado mediante citometría de flujo multiparamétrica las células del lavado broncoalveolar (LBA) en pacientes con carcinoma de pulmón (n=55) y otros tipos de cáncer (n=153), predominantemente hematológicos, que presentaron patologías pulmonares mayoritariamente infecciosas. Las cifras de dichas células se compararon con las de LBAs realizados a pacientes sin patología pulmonar (habitualmente SAHS, n=107). Así mismo se comparó la supervivencia de los pacientes con cáncer en relación a las cifras de linfocitos TCD8 del LBA.

Las cifras de linfocitos TCD8 estaban incrementadas en los pacientes con cáncer de pulmón (6.4%±1.1, $p<0.016$) y otros cánceres (12.7%±1.02, $p<0.000$) comparadas con los LBAs control (2.8%±0.27). Mientras que cifras de linfocitos TCD8>7% del total de células del LBA se asociaban a tasas de supervivencia global más elevadas en pacientes con otros tipos de cáncer (54.8% vs. 30.8%, $p<0.006$), en pacientes con carcinoma de pulmón lo hacían a tasas de supervivencias más reducidas (10.0% vs. 26.7%, $p=0.248$). La mediana de supervivencia en función de cifras de linfocitos TCD8 mayor o menor al 7% fue de 41.0±8.1 y 63.5±11.4 meses ($p=128$) en carcinoma de pulmón y de 105.9±9.2 y 73.8±11.5 meses ($p<0.03$) en otros cánceres. Por tanto, aunque cifras elevadas de linfocitos TCD8 en el LBA parecen ofrecer un pronóstico favorable en pacientes con patología pulmonar asociada a diversos tipos de cáncer, no parecen

proteger, incluso parecen aportar riesgo, en los pacientes con carcinoma de pulmón. Estos resultados deberían confirmarse en series más amplias.

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INMUNOCAP INHIBICIÓN EN EL DIAGNÓSTICO DE ANAFILAXIA INTRAOPERATORIA POR TIMOGLOBULINA® (ANTICUERPOS DE CONEJO)

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Varón de 47 años con anafilaxia intraoperatoria (hipotensión refractaria y broncoespasmo), coincidiendo con la infusión de Timoglobina®. Los antecedentes son el uso del inmunosupresor en un trasplante previo, sensibilización a seroalbúmina (gato, cerdo y conejo).

Material y Metodos

Determinación in vitro de IgE total, triptasa e IgEs por FPIA, Instrumento Phadia™ 1000, (Thermo Fisher Scientific) y Prick Test.

Ensayo de inhibición: muestra suero (paciente (***P***) y control (***C***)), inhibidor (i) (Albúmina humana®, Timoglobulina®, suero conejo y cerdo (100ml suero + 200mL i)). El control es suero de un varón con Síndrome Gato-Cerdo (anafilaxia a morcilla, sensibilización a seroalbúminas y epitelio de gato).

Calculo de inhibición: 1 – (IgEs inhibida/IgEs basal)*100

Resultado

Triptasa seriada: 41,5 mg/L intraoperatoria, 57,4mg/L a las 4h de la infusión, 25,1 mg/L a las 8h y 10 mg/L valor basal.

Valores basales de IgEs > 0,35 kU/l a epitelio de cerdo (e83), caspa de gato (e1), suero de conejo (e206), Albumina sérico gato (e220), carne conejo (f213) y Prick + a Timoglobulina a carne de conejo, cerdo y epitelio de conejo y gato.

ESTUDIO INHIBICION	PACIENTE (kU/l)	Inhibición (%) Albúmina + P	CONTROL+ suero humano (kU/l)	Inhibición Albúmina (%) + C	Inhibición % Timoglobulina P+	Inhibición % Timoglobulina C+	Inhhibición % Conejo + C	Inhibición % Cerdo + C
e82	0.08	**50**	5,58		**50**		**95,12**	
e83	0.05	**33**	1,66		**33**		33,33	
e206	0.33	**10**	27,3		**19**		**86,31**	
e211	0.00		2,66	**22.13**			**94,26**	
e204	0.02		0,26				12,50	
e220	0.73		58				26,34	
e222	0.06	**33**	2		**33**	**33**	21,78	**70,30**

Conclusiones

La triptasa seriada confirma la anafilaxia (activación mastocitaria). Sin embargo, los resultados del CAP inhibición no son concluyentes. La ausencia de exposición al alérgeno primario, no permite valorar la inhibición (IgEs <1 kU/l).

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RESPUESTA INMUNITARIA TRAS LA INMUNIZACIÓN HETERÓLOGA CON UNA VACUNA EXPERIMENTAL PARA COVID-19 y BNT162b2

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El objetivo de este trabajo es evaluar la repuesta inmunitaria tras 6 meses de la administración de una vacuna experimental para COVID-19 y posterior vacunación con pauta completa de BNT162b2.

Los análisis se llevaron a cabo 6 meses tras la administración de la vacuna experimental y antes de la primera dosis de la vacunación con BNT162b2 (t0), tres semanas después de la primera dosis (t1), dos semanas después de la segunda dosis (t2) y 6 meses (t3) después. Se determinó en células CD4+ la expresión del marcador de activación CD154 y en células CD8+ la expresión de CD137/CD69 tras la estimulación con un pool de péptidos de la proteína S, así como la producción de IL-2, IFN-γ y TNF-α mediante citometría de flujo.

Los resultados de la producción de citoquinas en las subpoblaciones naive (CD27+CD45RA+), memoria efectora (CD27-CD45RA-), memoria central (CD27+CD45RA-) y TEMRA (CD27-CD45RA+), revelaron que la mayor respuesta se observó en células CD4+ memoria central en los individuos vacunados con BNT162b2 dos semanas después de recibir la segunda dosis. También se apreció respuestas en la subpoblación memoria efectora, aunque ésta varió entre individuos. La detección de células T específicas se observó tras la primera dosis con BNT162b2, aumentando significativamente tras la segunda dosis y disminuyendo 6 meses después. La respuesta humoral se determinó mediante la cuantificación de anticuerpos específicos. A los 6 meses de la inmunización con la vacuna experimental, los niveles de anticuerpos anti-proteína S fueron muy bajos. Tras la vacunación heteróloga con BNT162b2, con la primera dosis se observó un aumento significativo, aumentando tras la segunda dosis y disminuyendo a los 6 meses.

En conclusión, la vacunación heteróloga con BNT162b2 indujo un aumento significativo en la respuesta inmunitaria específica tras previamente haber sido inmunizados con una vacuna experimental, la cual se mantuvo al menos 6 meses.

Sesión Poster Defensa 2. Pantalla 6. Inmunología Básica

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TH17-DERIVED MIR-721 AS A KEY PLAYER IN INFLAMMAGING AND METABOLIC IMBALANCE

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MicroRNAs (miRNAs) are small, non-coding RNAs that regulate gene expression at a post-transcriptional level and can tightly regulate a wide array of processes. Our laboratory focuses on mmu-miR-721, which is produced by Th17 lymphocytes and has been observed in autoimmune myocarditis. However, little is known about the role of this regulator in other contexts. Its target genes suggest a multifaceted activity: Meox2, a negative regulator of angiogenesis; Ppar-gamma, a regulator of insulin metabolism; and Nos2, involved in nitric oxide metabolism. Our goal is to better understand the metabolic processes and mechanisms that Th17 cells and miR-721 are involved in mediating.

The novel *miR-721*$^{-/-}$ mice generated in our lab showed slower weight gain, especially after reproductive senescence. These animals develop a reduced age-related white adipose tissue accumulation and glucose resistance compared to wild-type mice and displayed a lower Th17 signature, known to contribute to the process of inflammaging. This was accompanied by a normal progression of age-related insulin resistance, pointing towards a pancreatic effect rather than difference in tissue tolerance. To address the contribution of the lymphocytes in this process, chimeras lacking miR-721 in the lymphoid compartment were generated and fed a high-fat diet over 8 weeks. The metabolic phenotyping revealed a similar protection against glucose resistance and weight increase, with no differences in insulin resistance or brown adipose tissue activity in comparison to WT chimeras. Further, a lower liver immune infiltrate was observed, suggesting an involvement of the recruitment of these cells in the development of lipidic imbalance.

These data not only presents miR-721 as a potential target for addressing metabolic disorders but also highlights the importance of lymphocytes in the development and progression of metabolic diseases as seen in an aging or western diet models.

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RETINOIC ACID-DEPENDENT EPIGENOMIC AND TRANSCRIPTOMIC REMODELLING LEADING TO IMMUNOSUPPRESSIVE DENDRITIC CELLS

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All-trans retinoic acid (RA), a vitamin A metabolite, is an important determinant of intestinal immunity, and has been shown to play a role in the immunosuppressive properties of the tumor microenvironment (TME), acting as a major barrier to immunotherapy. In the presence of RA, monocytes reaching the TME preferentially differentiate into immunosuppressive dendritic cells (DCs), although the mechanism regulating this change in differentiation remains unclear. In this study, we investigated the transcriptomic and epigenomic remodelling associated with the differentiation and activation of monocyte-derived DCs (moDCs) in the presence of RA. Our analysis confirmed the immunosuppressive phenotype along with reduced secretion of proinflammatory cytokines such as TNFa, IL-12, and IL-1b, but also decreased levels of anti-inflammatory cytokine IL-10. We also observed decreased expression of *IRF4*, a key transcription factor (TF) for DC differentiation, and increased expression of *IRF8* and *MAFB*, TFs relevant for macrophage differentiation. Our DNA methylation analysis showed a significant block in the demethylation process of genes related to the immune response, both during differentiation and activation in the presence of RA. This blockade in demethylation correlates with a decrease in the expression of nearby genes. We also observed a specific demethylation of chronic inflammation genes together with an increase of their expression. The genomic regions displaying DNA methylation changes are accessible regions in monocytes, and present an enrichment of retinoic acid X receptor alpha (RXRa) binding motifs. Furthermore, to predict potential additional TFs involved in the acquisition of the specific transcriptome, we performed a Discriminant Regulon Expression Analysis (DoRothEA). This analysis revealed that liver X receptro alpha (LXRa), wich interacts with RXR, were enriched in DC treated with RA. Our results indicate that DNA methylation and the RXRa dimers, together with LXRa, orchestrate the epigenomic and transcriptomic remodelling that defines immunosuppressive DCs in TME.

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PODOCALYXIN ENHANCES THE INTERACTION OF ANTIGEN PRESENTING CELLS WITH CD4+ AND CD8+ T CELLS

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Podocalyxin (PODXL) is a cell surface protein belonging to the CD34 family of sialomucins that has been detected in diverse types of normal and malignant cells. PODXL interacts with the actin cytoskeleton, allowing it to regulate cell morphology, polarity, adhesion and migration. Both anti-adhesive and adhesive properties have been attributed to PODXL. We have previously showed that the differentiation of human monocytes to immature dendritic cells u*sin*g IL-4 and GM-CSF induces total and cell surface expression of PODXL. However, the functional significance of PODXL expressed by antigen presenting cells on the immune system remains virtually unknown. In this study, we demonstrated that total expression levels of PODXL decayed considerably following maturation using pro-inflammatory stimuli in human dendritic cells. Moreover, we found that IL-4 induced PODXL expression through MEK/ERK and JAK3/STAT6 signaling pathways in THP-1 myeloid cell line. In addition, using Raji B cell line as a model of antigen presenting cells, we observed that overexpression of PODXL enhanced its interaction with CD4+ T cells and CD8+ T cells and decreased the expression of MHC-I, MHC-II, and the costimulatory molecule CD86. Confocal microscopy assays revealed that PODXL polarized at the contact zone during the interaction of antigen presenting cells with CD4+ T cells, partially colocalizing with F-actin cytoskeleton. Markedly, PODXL expressed by antigen presenting cells reduced the repositioning of CD4+ T-cell centrosome toward the contact site, a process required for proper T-cell effector function. Our results suggest a role for PODXL expressed by antigen presenting cells in the regulation of immune responses, thereby representing a therapeutic target for manipulating the immune response to treat cancer and infectious diseases.

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EFFECT OF T CELL SPECIFIC-PI3K-P110A INACTIVATION IN CELL DEVELOPMENT AND IN ANTIGEN-SPECIFIC RESPONSE.

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Class I PI3K enzymes are critical for essential and varied cellular processes, including metabolism, proliferation, inflammation and carcinogenesis. Although PI3Kδ and PI3Kα are activated by the TCR and T cell costimulatory receptors (CD28, ICOS), the involvement of PI3K-p110α isoform in the maintenance of effective immunity is poorly understood.
P110α is an ubiquitous catalytic isoform in body cells, with a high mutation rate in cancer. To study the role of p110α in T lymphocytes, we have developed a new mouse model with Cre-dependent functional inactivation of p110α (Kinase dead) in T lymphocytes (p110αKD-T).
P110αKD-T mice show some alterations in cell content and T cell subpopulations in the thymus and spleen. The *in vitro* differentiation of follicular T helper subpopulations (Tfh) showed a lower yield of Tfh cell number and IL-21 production in p110αKD-T cultures versus control. In addition, a decrease in *in vitro* $CD4^+$ Treg differentiation and proliferation yield is observed in p110αKD-T cultures.
When the specific *in vivo* response to a protein antigen (KLH) is studied in the p110α-KD-T model, the proportion of germinal center B (PNA^+) cells is increased. The surface ICOS expression in p110αKD-T $CD4^+$ cells or $CD8^+$, freshly obtained or after 24h stimulation in the presence of native KLH protein or anti-CD3 Ab, is increased. When $CD4^+$Treg are studied in the KLH-immunized p110αKD-T mice, differences in ICOS expression are observed in freshly obtained Treg or after KLH- or anti-CD3-*in vitro* stimulation of the cells. In addition, lower Ki67 expression in p110αKD Treg from KLH-immunized mice upon *in vitro* re-stimulation with KLH may denote a defect mediated by p110α in cell proliferation mechanisms. Further studies are in progress to elucidate the effect of T cells-p110α inactivation in the T-B cell cooperation to produce Ag-specific antibodies.

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EFFECTS OF SULFORAPHAN ON THE INFLAMMATORY IMMUNE RESPONSE

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Background: Sulforaphane (SFN), an isothiocyanate of vegetable origin found in cruciferous, such as broccoli, presents potent antioxidant and immunomodulatory properties. These pleiotropic activities derive from their ability to influence multiple signaling pathways, highlighting the Nrf2/HO-1 pathway. Specifically, the induction of this signaling pathway in dendritic cells (DCs) produces their polarization towards a tolerogenic phenotype.
Objective: The aim of this work was the study the immunomodulatory effect of the SFN in an inflammatory microenvironment on monocyte-derived dendric cells (moDCs).
Methods: We characterized the immune and anti-inflammatory and immunoregulatory response to SFN in moDC from healthy subjects (N=9). Lipopolysaccharides (LPS, 100ng/mL) was added to induce the inflammatory environment cell. After that, the immune and anti-inflammatory response in presence of SFN (at 10µM) was analyzed. Thus, the SFN internalization and cytotoxicity by moDCs, moDCs maturation (CD83, CD86, …etc) and T-lymphocytes proliferation were evaluated by flow cytometry and ROS assay.
Results: Results demonstrated that SFN was up taken and processed by moDCs, exerting a low cytotoxic effect on them. Moreover, SFN significant reduced CD83 and CD80 maturation markers in moDCs and their ability to stimulate T-lymphocyte proliferation in a context of chronic inflammation. In addition, a decrease in the cytoplasmic levels of Nrf2 and pNrf2 expression with a significant increase in the HO-1 expression were observed moDCs, indicating the important role of the SFN in the prevention of inflammation.
Conclusion: Our findings indicate that the use of this nutraceutical capable of inducing a decrease moDCs maturation state and an increase in the HO-1 enzyme opens a new perspective in the treatment of pathologies with an inflammatory and/or autoimmune component.

P-231

PROTECTIVE ROLE OF CORTISTATIN IN THYMIC INVOLUTION AND IMMUNOSENESCENCE

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Immunosenescence defines the decline in immune function that occurs with aging, leading to an ineffective response to pathogens/vaccines, cancer and autoimmunity. Age-related regression of the thymus plays a key role in this process, associated with a decreased output of naïve T-cells and less capacity to sustain immune competence. Moreover, thymus undergoes acute involution following insults such as infections, stress(glucocorticoids) or chemotherapy. The responsible mechanisms for both types of thymic regression remain to be fully identified, wherein various cytokines/chemokines, growth factors and neuropeptides/hormones appear to be involved. Among them, the neuropeptide cortistatin emerged as a potential factor participating in this process. Although its immunomodulatory actions are widely described, the effect of cortistatin in thymus function is mostly unknown. This study investigated its role in thymic involution by using cortistatin-deficient mice exposed to both acute and chronic scenarios. We initially confirmed the expression of cortistatin in murine thymus, particularly in DP, SP and DN thymocyte populations. Cortistatin-deficient mice exposed to glucocorticoids or bacterial toxins showed exacerbated signs of acute thymic decline, including reduced organ weight and cellularity, changes in thymocyte subpopulations and increased thymocyte apoptosis, compared to wild-type mice. Interestingly, cortistatin deficiency significantly accelerated thymic involution with aging (6-24 months), affecting various thymocyte populations. Noteworthy, 6 month-old cortistatin-deficient and 12 month-old wild-type mice showed comparable thymic weight/cellularity. RNA-sequencing analysis of cortistatin-deficient and wild-type thymocytes of different ages showed that 450-900 genes are differentially expressed. The early thymic involution due to cortistatin-deficiency could be associated with an impaired response to immune challenges. Thus, 12 month-old cortistatin-deficient mice showed increased mortality and clinical signs when subjected to experimental polymicrobial sepsis. Moreover, cortistatin pretreatment for 5-weeks of aged mice before sepsis induction improved thymic cellularity and significantly protected from subsequent polymicrobial infection. Overall, cortistatin appears to play a beneficial role in acute and chronic thymic involution.

P-232

XBP1 MODULATES THE MCH CLASS I ANTIGEN PRESENTATION UNDER UNFOLDED PROTEIN RESPONSE ACTIVATION.

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The presentation of peptides by major histocompatibility complex class I (MHC-I) proteins is critical for adaptive immunity. The generation of peptides and their loading on MCH-I proteins, mainly occurs in the endoplasmic reticulum (ER) and is mediated by presenting machinery. Alterations in protein homeostasis lead to ER stress activating the unfolded protein response (UPR) pathway, and increasing the expression of genes involved in the folding machinery. Our aim was to determine the influence of UPR pathway on the presenting machinery and MHC-I antigen presentation.

Human tubule epithelial cell line, HK-2, was treated with serum deprivation and with specific UPR branches inhibitors (STF083010, ISRIB or AEBSF), small interfering RNAs (siRNA XBP1, ATF4, ATF6), or subjected to knockdown of XBP1 by CRISPR/cas9 technology. The antigen presentation efficiency was measured by a SIINFEKL-based system. The binding of XBP1 to target genes was analyzed by ChIP assay.

Treatment of HK-2 cells with specific inhibitors or gene silencing of the UPR transcription factors, XBP1, ATF4 and ATF6, reveals that only the blockage of XBP1 significantly reduces the expression of ERP57, ERAP1, SEC61 and calreticulin molecules. Moreover, we show that under UPR activation, XBP1 binds to the promoter region of ERAP1 inducing its expression and with it, the enhance of SIINFEKL peptide-MHC-I complex. As consequence, cytotoxicity assays performed with OT1-CD8^{+}cells in HK2-H2kb/SINFEKL cells shows that XBP1 inhibition reduces OTI-CD8+ cell-mediated cytotoxicity.

XBP1 could modify the peptide repertoire presented by MHC class I molecules and subsequently, the immune response triggered by tubular cell under UPR activation.

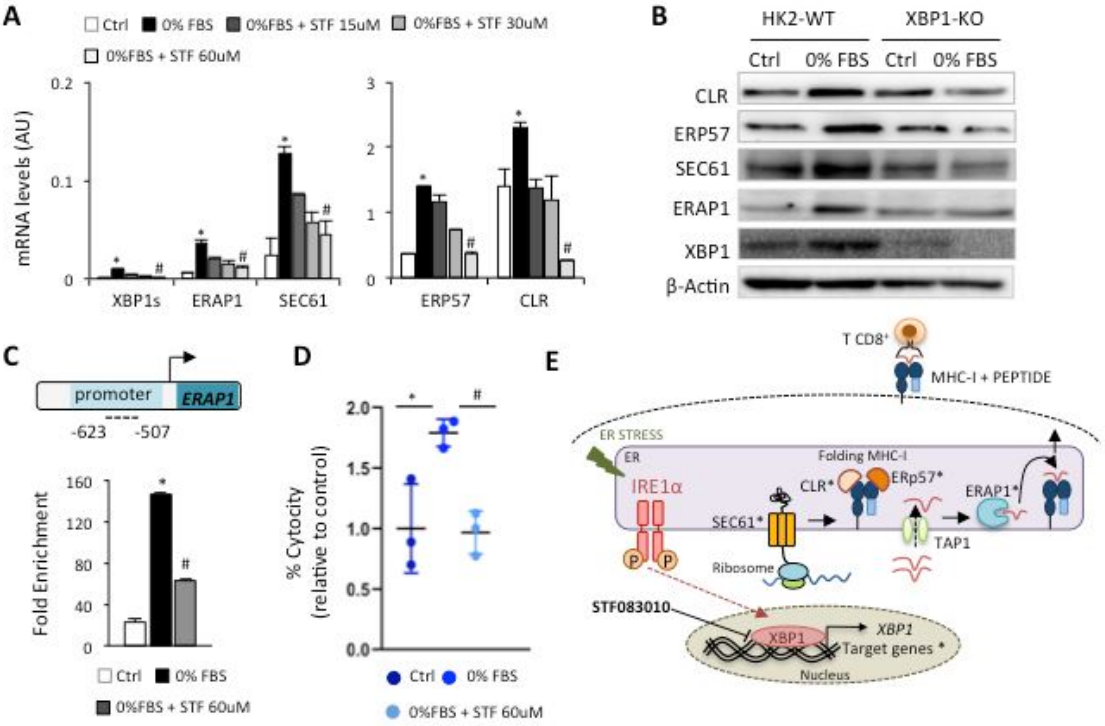


Figure 1.- XBP1 regulates the loading machinery complex and the MCH class I antigen presentation. A. HK-2 cells were treated with serum deprivation or XBP1 inhibitor at different doses (STF083010; 24 h). XBP1, ERAP1, SEC61, ERp57 and CLR gene expression were analyzed by RT-PCR. **B.** Western Blot of XBP1, ERAP1, SEC61, ERp57 and CLR in HK2-Wild Type (HK2-WT) and HK2 XBP1 Knock out (XBP1-KO) under serum deprivation conditions 24 h. **C.** ChIP assay were performed with a specific antibody against XBP1, and the region of interest of *ERAP1* gene was amplified by RT-PCR. **D.** Cytotoxicity assays were performed in HK2-H2kb/SINFEKL cells treated with serum deprivation or XBP1 inhibitor with OT1-CD8+ cells. **E.** Schematic representation of the results obtained in that study.

P-233

TOLL-LIKE RECEPTOR 4 EXPRESSION IN HUMAN DECIDUAL STROMAL CELLS ASSOCIATED WITH PREGNANCY COMPLICATIONS

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Introduction: The Preeclampsia (PE) affects 8% of pregnant women, being one of the main causes of maternal and fetal morbidity and mortality in the world. The decidua is the maternal tissue that plays an important role in the immunological regulation of pregnancy and its main cellular component is the decidual cells. These cells are in close contact with trophoblast cells and participate in innate immune regulation during infection, protecting the fetus against gram-negative bacteria and danger signals released during severe inflammation with or without infection.

Objective: to determine the expression of the Toll-like receptor 4 in DSC cells in vitro and to analyze cytokines associated with the in vitro immunomodulatory behavior of DSC from PE.

Materials and methods: analytical study of cases and controls. DSCs were isolated and cultured from placental tissue, from patients with uncomplicated vaginal delivery (control n=6) and cesarean delivery with preeclampsia (PE n=6). The phenotypic characterization of the DSCs was carried out using flow cytometry. TLR4 mRNA expression was quantified by real-time RT-PCR. Cytokine determination was performed by means of ELISA from DSC cell culture supernatants**.**

Results: the expression of characteristic antigens was similar in the DSC studied (CD10, CD29, CD44, CD73 and CD90). The DSCs expressed TLR4, but there wasn't significant difference (p=0.136). Control DSCs secreted a higher amount of IL-6 (mean= 3631.7pg/mL) compared to PE DSCs (760pg/mL). The secretion of IL-10 presented a significant difference (control= 4pg/mL, PE= 11.2pg/mL p=0.0108).

Conclusions: the control and PE DSCs do not show significant differences in the expression of TLR4, suggesting a protective role at the placental level.

P-234

MONOCLONAL ANTIBODIES FOR THE DETECTION OF *FUSOBACTERIUM NUCLEATUM*, A CRC BIOMARKER

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CIC bioGUNE, Derio.

Fusobacterium nucleatum is a pathogen involved in colorectal cancer (CRC) development. Accumulating evidence shows a significant positive relationship between *F. nucleatum* abundance and poor overall survival of patients with CRC. High levels of *F. nucleatum* are observed in preneoplastic and neoplastic tissues, as well as in fecal samples of patients with CRC and premalignant lesions. Thus, *F. nucleatum* is a solid candidate as an early diagnostic and prognostic biomarker for CRC. Nevertheless, *F. nucleatum* genome has an AT content of more than 70%, which hinders the use of traditional DNA-based detection methods. We are developing an alternative antibody-based method for *F. nucleatum* detection with the aim of effectively screen for this CRC biomarker. Employing hybridoma technology, monoclonal antibodies have been developed against a *F. nucleatum* tannase, an extracellular enzyme with the capacity of detoxifying gallotannins ingested in the diet, which therefore constitutes a survival mechanism for the microorganism in phenolic rich environments. Since the product of the reaction, gallic acid, has also been associated with oncogenic activity in some contexts, global tannase detection is also desirable. The obtained monoclonal antibodies were tested against purified tannases and complex murine and human samples such as histological and stool CRC samples, and the most promising ones were characterized. Two antibodies, 153B and 456A, were specific anti-*F. nucleatum* tannase and global tannase detectors, respectively, which can be used as an alternative method for the screening of microbial-related CRC risk factors and biomarkers, leading the way into reducing CRC related mortality by early detection and targeting of microbes associated to tumor proliferation. This approach might be complementary to and use the same samples as the widely used occult blood testing in stool.

P-235

NATURAL KILLER (NK) CELL-DERIVED EXOSOMAL MICRORNAS PROMOTE TH1 RESPONSES

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Natural killer (NK) cells play a key role as innate immune effectors, capable of recognising and killing cells undergoing different types of stress. However, NK cells are also capable of modulating immune responses, in particular they have been shown to regulate T cell function. Small RNA deep sequencing of resting and activated NK cells and their derived small extracellular vesicles (EVs) led to the identification of a specific signature of NK-EV-associated microRNAs, bearing specific post-transcriptional modifications.

Moreover, NK-EV-microRNAs promote T cell activation, skewing Th1-like responses both directly, by inducing $CD4^+$ T cell polarization via GATA-3 down-modulation and subsequent T-bet de-repression and also indirectly, by enhancing monocyte-derived dendritic cells co-stimulatory and presenting functions.

Additionally, gold-nanoparticle based delivery of EV-microRNAs *in vivo*, namely miR-10b, miR-92a and miR-155, efficiently boost T cell responses in immunocompetent mice, opening new avenues for therapy in immune-related diseases.

P-236

ISG20L2: AN RNA ENZYME REGULATING T CELL ACTIVATION

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ISG20L2, a 3' to 5' exoribonuclease previously associated with ribosome biogenesis, is identified here in activated T cells as an enzyme with a preferential interaction for uridylated substrates. This enzyme is upregulated upon TCR and IFN I stimulation, and appears to be involved in regulating T cell function. ISG20L2 absence leads to an increased basal expression of CD69. Additionally, ISG20L2 deficiency induces greater IL2 secretion. However, ISG20L2 silencing impairs CD25 upregulation, CD3 synapse accumulation and MTOC translocation towards the antigen-presenting cell. The dysregulation observed in these key molecules for T cell responses may support a role for this exonuclease acting as a new RNA-based regulator of T cells. ISG20L2 could be exerting its function through modulation of immunoregulatory molecules, such as AHR, NKG2D, CTLA-4, CD137, TIM-3, PD-L1 or PD-1, which show increased levels upon ISG20L2 knockdown.

P-237

CYTOKINES PROFILE FOR HOST-DIRECTED THERAPIES IN MALARIA DRUG DISCOVERY

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GSK, Tres Cantos Madrid.

Malaria is a parasitic vector-borne disease that affects over 200 million people every year, with around 100 countries officially considered malaria endemic regions. *Plasmodium* parasites are transmitted by the bite of infected female mosquitoes of the genus *Anopheles*. Blocking or reducing malaria transmission rates constitutes one of the main strategies to achieve global malaria eradication.

Plasmodium berghei mice model is the standard surrogate used for Malaria preclinicals studies. In this work, we describe the setting up of HDT assays for this parisitic disease. We used two stages of the parasite: asexual and liver stages.

In the assay we used a compound with immunomodulatory effect in combination or not with standard antimalarial drugs. During the treatment an in vivo cytokine profile at different timepoints was performed aiming at Th1, Th2 and Th17 immune response in C57BL/6 (liver stages) and CD1 mice (asexual stages).

The immunomodulatory effect of the compound was assessed as the reduction of the parasitemia in mice after the treatment. The cytokines expression was monitored throughout the assay comparing with vehicled treated mice.

Several cytokines profile expression was affected 4 hours after the immunomodulatory compound administration, with different impact on parasite growth.

"All animal studies were ethically reviewed and carried out in accordance with European Directive 2010/63/EU and the GSK Policy on the Care, Welfare and Treatment of Animals."

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DECIPHERING THE ROLE OF PEROXISOMES IN B CELLS

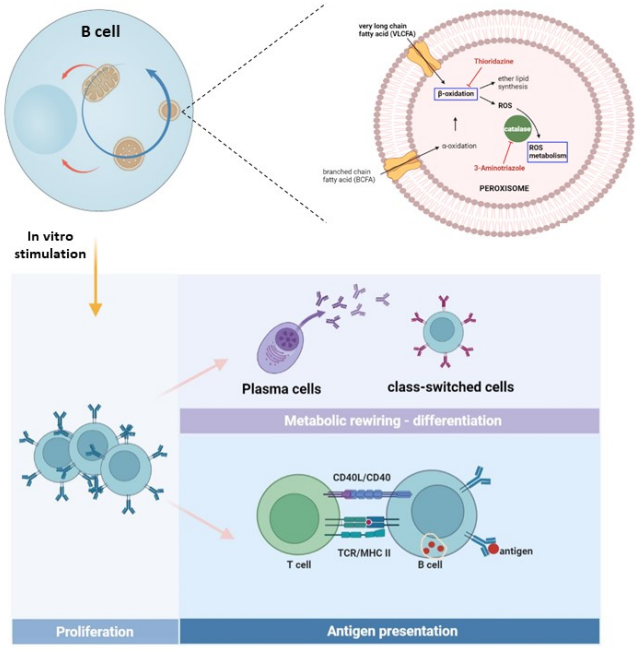
Seco Estrada, Belén; Martínez Martín, Nuria.

Centro de Biología Molecular Severo Ochoa (CBMSO), Madrid.

B lymphocytes are key elements of an adaptive immune response, producing antibodies and generating a memory cell compartment after pathogen encounter. Metabolism is well-known to be important after immune cells' activation, shaping cell fate and function. During the last decade, mitochondria has been considered as the central metabolic hub of immune cells. However, peroxisomes have recently been proposed as additional metabolic platforms due to their function in lipid and reactive oxygen species (ROS) metabolism. The role of peroxisomes in B cells has been almost neglected until now. Our team is aiming to dissect the role of peroxisomes in B cells in vitro, and more precisely, we are evaluating the role of peroxisomal fatty acid oxidation (FAO) and ROS detoxification using specific inhibitors -Thioridazine and 3-Aminotriazole (3-AT), respectively-.

We have seen that peroxisome content is tightly controlled in each type of B cell, and interestingly, each peroxisomal function seems to play specific roles during their activation and differentiation. Precisely, peroxisomal ROS detoxification seems to be fundamental for controlling ROS homeostasis shaping proliferation after B cell stimulation, while peroxisomal FAO appears to impact directly on mitochondrial activity affecting B cell differentiation maybe by modulating B cell's metabolic profile after stimulation. Additionally, we have unveiled a new role of peroxisomal FAO in B cells: antigen presentation to CD4 T cells.

Overall, our work demonstrates the relevance of peroxisomes in B cells, suggesting their impact on B cell proliferation and differentiation. These results in vitro encourage us to study the peroxisome function in vivo, aiming to prove their potential as a new therapeutic target for immune related diseases.


B cell
very long chain fatty acid (VLCFA)
Thioridazine
β-oxidation
ether lipid synthesis
ROS
catalase
ROS metabolism
α-oxidation
3-Aminotriazole
branched chain fatty acid (BCFA)
PEROXISOME
In vitro stimulation
Plasma cells
class-switched cells
Metabolic rewiring - differentiation
CD40L/CD40
T cell
TCR/MHC II
B cell
antigen
Proliferation
Antigen presentation

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VALOR DE HEVYLITE DURANTE EL DIAGNOSTICO Y SEGUIMIENTO DE PACIENTES CON GAMMAPATIAS MONOCLONALES CON PROTEINA MONOCLONAL DEL TIPO IgA

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Hospital de la Santa Creu i Sant Pau, Barcelona.

Introducción: Las gammapatías monoclonales se monitorizan en suero a través de la cuantificación de la proteína monoclonal (PM) mediante electroforesis (EPS) y la detección por inmunofijación. Sin embargo, un 40% de los casos con PM de tipo IgA migran en la región beta junto a otras proteínas, impidiendo su correcta cuantificación.
Objetivo: En este estudio se evalúa Hevylite® como método alternativo para el seguimiento de la PM de tipo IgA que migra en la región beta del proteinograma.
Métodos: Se incluyeron 49 muestras pertenecientes a 6 pacientes. En estas muestras se cuantificó la IgA total, la PM por EPS y la IgAκ e IgAλ por Hevylite®, calculándose posteriormente la ratio IgAκ/IgAλ (rHLC) indicativa de clonalidad.
Resultados: La validación de Hevylite® se realizó mediante análisis de regresión lineal Passsing-Bablock. Se observó una elevada correlación entre la suma IgAκ+IgAλ y la IgA total (n=49; y=0,08+1,25x; r^2=0,91) y entre la concentración de la PM medida por EPS y la cuantificada por Hevylite® (n=30; y=1,22+1,27x; r^2=0,85). Diez de 49 muestras mostraron una PM no cuantificable por EPS (4/19 muestras de GMSI, 1/12 de MMq y 5/18 de MM) y en 9/49 no se detectó PM en la EPS (3 muestras de GMSI, 3 de MMq y 3 de MM) pero si banda monoclonal por inmunofijación. En total, 19/49 muestras no se pudieron cuantificar por el método de referencia y solo se pudo monitorizar la cuantificación de la PM por Hevylite®. En 9/49 muestras se observó una rHLC normal, todas pertenecientes a pacientes MM en tratamiento.
Conclusión: Hevylite® presentó una elevada correlación con los métodos convencionales para la cuantificación de la IgA total y de la PM. En aquellos pacientes en los que la EPS fue negativa o la PM no se pudo cuantificar, Hevylite® representó una alternativa fiable para la monitorización de la enfermedad.

P-241

LA INMUNOPRECIPITACIÓN COMO TÉCNICA PARA EL DIAGNÓSTICO DIFERENCIAL ENTRE MIELOMA MÚLTIPLE DE CADENAS LIGERAS "OCULTAS" VS ENFERMEDAD DE CADENAS PESADAS.

Ballester González, Rubén; Rita, Claudia Geraldine; Iturrieta Zuazo, Ignacio Maria; Martín Moro, Fernando; Rodríguez Martín, Eulalia; Espiño Martínez, Mercedes; Roldán Santiago, Ernesto; Villar Guimerans, Luisa Maria.

Hospital Universitario Ramón y Cajal, Madrid.

Introducción

El mieloma múltiple (MM) IgD de cadenas ligeras (CL) "ocultas" es una entidad extremadamente infrecuente de la que existen pocos casos documentados. En estos pacientes la interpretación clínica de la inmunofijación (IF) puede resultar un reto diagnóstico.

Objetivo

Presentar un caso clínico de MM IgD con CL "ocultas" y describir un método de inmunoprecipitación que ayude a la interpretación de la IF.

Paciente y métodos

Varón caucásico de 72 años con presencia de lesiones osteolíticas y proteinuria Bence-Jones sin disminución del filtrado glomerular. Ante la sospecha de MM, se realizó un estudio diagnóstico mediante IF, citomorfología y citometría de flujo (CF).

Resultados

La IF sérica reveló una banda monoclonal IgD sin cadena libre asociada y una segunda banda monoclonal lambda migrando a una altura diferente de la IgD (Figura 1A). Los niveles de CL lambda libres totales fueron 103 mg/L.

Se llevó a cabo una inmunoprecipitación incubando cuatro alícuotas de suero con antisueros anti-lambda, anti-kappa, anti-IgD y anti-IgG durante 48h a 4ºC. Posteriormente se centrifugó la muestra a 1100 x g, 15 min. Con los sobrenadantes obtenidos se realizó una IF en gel de agarosa Hydragel IF 2/4 utilizando un Hydrasis 2.0 (Sebia).

Tras la inmunoprecipitación con el antisuero anti-lambda, no se detectó la presencia de banda monoclonal lambda ni IgD. En el caso de la inmunoprecipitación con antisuero IgD, únicamente desapareció la banda de IgD (Figura 1B).

El estudio del aspirado de médula ósea (MO) identifico un 26% de células plasmáticas (CP) clonales mediante CF (Figura 1C) y una infiltración masiva por CP de pequeño tamaño por citomorfología (Figura 1D).

Conclusiones

Esta técnica permite realizar el diagnóstico diferencial de MM IgD de CL lambda "ocultas" y descartar que se trate de una enfermedad de cadenas pesadas. Su uso podría extenderse a otros casos de MM con CL "ocultas".

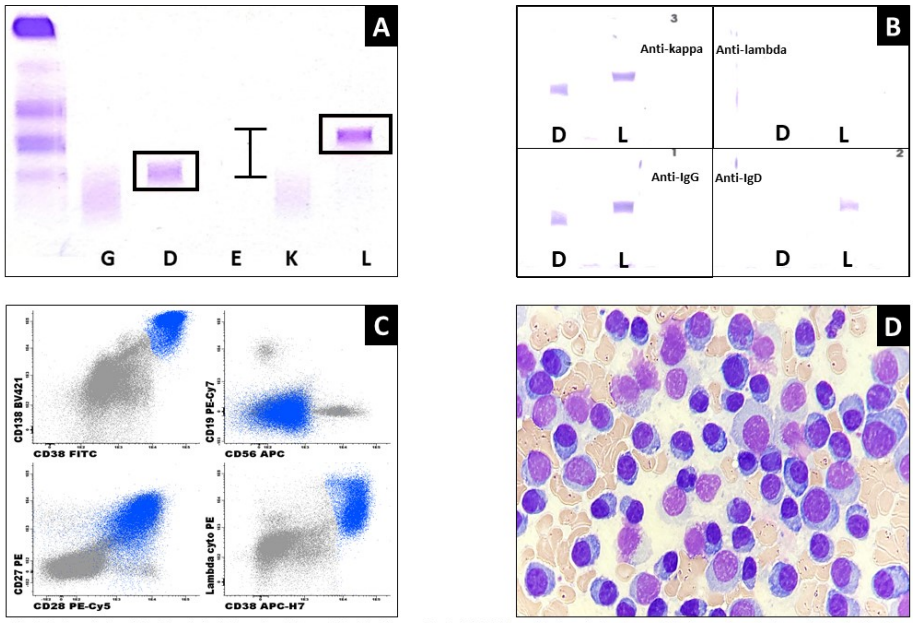


Figura 1: Mosaico con imágenes de las distintas técnicas de laboratorio realizadas para el diagnóstico. A) Imagen del resultado de la IF del suero del paciente, donde se observa que la banda perteneciente a la cadena pesada y la cadena ligera han migrado a diferentes alturas en el gel. B) Imagen de la IF realizada con los sobrenadantes obtenidos tras la inmunoprecipitación con anti-kappa (usado como control negativo), anti-lambda (donde se aprecia desaparición de la banda IgD y lambda), anti-IgG (usado como control negativo) y anti-IgD (donde se aprecia desaparición de la banda IgD y persistencia de la banda lambda). C) Mosaico con imágenes de las células plasmáticas clonales CD38+/CD138+/CD19-/CD56-/CD27+/CD28+/cylambda+ (azul) obtenidas por CF en el análisis del aspirado de MO. D) Imagen de la citomorfología del aspirado de MO, con infiltración masiva de células plasmáticas.

P-242

ANALYSIS OF THE IMMUNOMODULATORY EFFECT OF QUINTON® ISOTONIC SOLUTION IN ASC/PBMCS CO-CULTURES.

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Immunonutrition is gradually developing as a scientific discipline and, in this sense, we are exploring how the isotonic solution Quinton®, based on seawater characterized by containing some minerals of recognized immunomodulatory capacity and necessary for the proper functioning of cells (including Zinc, Copper, Silicon, among others), could present potentially interesting effects both in healthy individuals and in patients with Psoriatic Arthritis (PA). The aim of this study was to evaluate the effect of this solution on human PBMCs, co-cultured with adipose-derived mesenchymal stem cells (ASC). For the proliferation study, co-cultures of ASCs were performed with PBMCs from healthy volunteers and from individuals presenting PA, in the presence of Quinton® isotonic solution, where PBMCs were previously labeled with CFDA-SE. After 5 days of culture, cells were analyzed by flow cytometry (FACSCanto, BD Bioscience). On the other hand, the modulatory capacity of ASCs on the regulatory T cell population in ASC co-cultures with PBMCs from healthy individuals was also studied by flow cytometry. Preliminary results obtained with this solution showed an immunomodulatory effect on proliferation of PBMCs co-cultured with or without ASCs, both in healthy individuals and in PA patients. Similarly, an increase in both the percentage of $CD4^{+}CD25^{+}FoxP3^{+}$cells and $CD4^{+}CD25^{high}FoxP3^{+}$cells were also observed in healthy individuals in the presence of the solution, both without and in presence of ASCs. We can conclude that Quinton® isotonic solution seems to exert immunomodulatory effects, both in healthy individuals and in patients with Psoriatic Arthritis.

P-243

Unveiling the role of the polyamine pathway in B cell immunometabolism

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Polyamines are metabolites involved in several cellular processes such as proliferation, processing and stability of nucleic acids and proteins, signalling, transcription and hypusine-dependent translation. Their pleiotropic effect is due to their capability to bind negatively charged molecules. Polyamine pool is regulated by their metabolism, i.e. synthesis, catabolism and transport. Synthesis is controlled by two rate-limiting enzymes: ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase (AdoMetDC), establishing two synthesis axes. Noteworthy, their dysregulation has been associated with tumorigenesis.
Diffuse large B cell lymphomas (DLBCLs) are non-Hodgkin lymphomas that were thought to be a unique type, but recent evidence shows they are a heterogeneous group – MCD, BN2, N1 and EZB –. Interestingly, according to the information compiled in The Cancer Genome Atlas Consortium and the International DLBCL Rituximab-CHOP Consortium Program Study databases, the two polyamines synthesis axes are differently involved in lymphomagenesis in DLBCLs. More precisely, we have found that while alterations in AdoMetDC were present in EZB group, modifications in the ODC1 gene were enriched in MCD and BN2 subgroups. We believe that each axis of PA synthesis may play a different role in B cell activation and differentiation, explaining this dichotomy in DLBCL development.
To understand better the role of polyamines in lymphomagenesis, we aim to characterize this pathway in B lymphocytes, discerning between intrinsic polyamine functions and hypusination-dependent processes. Our preliminary results show first, that both ODC and hypusination seem to be upregulated in the germinal centre. Second, we have seen that polyamines are essential for a proper proliferative response after stimulation, controlling ATP, ROS and lysosomal homeostasis, whereas hypusination determines someway differentiation of B cells.
This knowledge will be important for a better understanding of B cell immunometabolism and therapeutic approaches in diseases such as DLBCLs.

P-244

B CELL TRANSCRIPTOMIC CHANGES INDUCED BY EXPRESSION OF *CD5* VARIANTS

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CD5 is a lymphocyte surface receptor from the Scavenger Receptor Cysteine Rich (SRCR) superfamily and found expressed on all T cell subsets and a B cell subset (B1a) mainly involved in the production of natural polyreactive antibodies. CD5 is also expressed by some T and B-cell malignancies, including B cell chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL). CD5 is a signal transducing receptor physically associated to the clonotypic receptors of T (TCR) and B (BCR) cell, where it contributes to the downregulation of their intracellular signals delivered upon specific antigen recognition. Two functionally relevant allelic variants of CD5 have been described, which differ by a single amino acid position at the cytoplasmatic tail (Ala471>Val). This polymorphism confers different signaling capabilities to CD5 and holds prognostic value in autoimmune (i.e. psoriasis and systemic lupus erythematosus) and neoplastic (i.e melanoma, prostate cancer or CLL) disorders. To better understand the functional consequences of CD5 polymorphism at the transcriptomic level, the gene expression profile of Daudi B cells (a Burkitt's lymphoma-derived B cell line) lentivirally transduced and selected for stable expression of CD5 Ala471 or Val471 variants was analyzed. RNA-seq analyses showed that Daudi cells expressing the CD5 Ala471 variant

upregulated gene sets associated to glucose metabolism, amino acid transport and several signaling pathways, whereas those expressing Val471 upregulated gene sets related to histones, DNA repair and cholesterol synthesis. When viewed as a whole, data suggests that the Ala471 variant confers a gene expression profile resembling that of lymphocyte activation whereas the Val 471 variant confers a profile closer to that of lymphocyte steady state. Moreover, these results strongly support the CD5 receptor as a molecule deeply involved in lymphocyte functionality and warrant further transcriptomic validation studies involving CLL patients homozygous for either variant.

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HARNESSING THE LIPSTIC MOUSE MODEL TO GENERATE A MODEL OF *IN VIVO* DEPLETION OF INTERACTING IMMUNE CELLS

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Antigen presentation is a key event in the initiation of adaptive immune responses. During this event, intimate interactions between dendritic cells (DCs) and T cells occur. Novel tools of enzymatic intercellular labelling, such as the 'Labelling Immune Partnerships by SorTagging Intercellular Contacts' (LIPSTIC) system, allow the identification and isolation by flow cytometry of interacting cells. However, no tools are available for the evaluation of the physiological impact and functions of interacting cells in an *in vivo* system. Here, we used the LIPSTIC mouse model to generate an *in vivo* depletion system to remove DC and T cells that underwent antigen presentation in the context of intradermal immunization. To do so, we exploited the biotinylated surface of LIPSTIC interacting cells as target. First, we used saporin, a cytotoxic drug, conjugated to streptavidin to deplete biotin+ cells *in vivo*. However, unspecific effects of saporin were observed. Modification of the dose and injection regime did not avoid the unspecific depletion of non-biotinylated cell populations. However, we identified experimental conditions in which the specific effect of saporin-mediated depletion could be evaluated. Alternatively, we used a monoclonal anti-biotin antibody. The differential ability to bind biotinylated cells was first validated. Different experimental approaches were used to test the depleting ability of the anti-biotin antibody and saporin. These were based on (1) *in vivo* biotinylation using a biotynylated antibody, (2) *in vivo* biotinylation of adoptively transferred SrtA-PDGFR OTII CD4+ T cells and, and (3) transfer of $CD40^{G5}$ and WT DCs that are biotinylated following the LIPSTIC labelling protocol. Results indicated a mild but specific depletion activity of the anti-biotin antibody compared to a strong but unspecific activity of streptavidin-saporin. This model will allow the evaluation of the physiological role of interacting DC and T cells by assessing the impact of the depletion in other immune cell populations.

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FACS SORTING STRATEGY FOR PLASMATIC CELLS DETECTION IN HOST DIRECTED THERAPIES FOR MALARIA

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Malaria is a parasitic vector-borne disease that affects over 200 million people every year, with around 100 countries officially considered malaria endemic regions. Plasmodium parasites are transmitted by the bite of infected female mosquitoes of the genus Anopheles. Blocking or reducing malaria transmission rates constitutes one of the main strategies to achieve global malaria eradication.

Immunization protocols for the obtaining of Plasmatic Cells after Malaria infection is tedious and long lasting. The Malaria parasite eludes the imnune system and the generation of antibodies and memory cells is poor when the parasite goes through asexual stages and a little enhanced when the infection starts in liver stages. These experiments requires at least two months of experiments to obtain few plasmatic cells.

In Host Directed Therapies assays, where differences in the expression and synthesis of determined immune cells populations is observed after compound treatment. For this reason, it is necessary to set up FACS panels for the detection of different immune cells populations.

In this work we show the setting up of a sorting panel for the detection of Plasmatic cells, to be used to evaluate the antibodies production after Malaria immunization protocols and treatment with HDT compounds.

For the setting up we have used CD19, CD20, CD38 and IgG, to obtain plasmatic cells that produce IgGs antibodies to evaluate the immunization against the malaria parasite in murine in vivo models for Plasmodium berghei after infection with sporozoites.

“The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents”

“All animal studies were ethically reviewed and carried out in accordance with European Directive 2010/63/EU and the GSK Policy on the Care, Welfare and Treatment of Animals.”

P-247

PHOSPHATASES OF REGENERATING LIVER (PRLS) REGULATES THE SUBCELLULAR DISTRIBUTION AND ACTIVATION OF LCK.

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Phosphatases of regenerating liver (PRLs: PRL-1, PRL-2 and PRL-3) have been implicated in cancer progression. PRL-1 and PRL-3 have an activating role in Src kinases promoting hematopoietic tumours. One of these Src kinases, Lck, promotes the development of acute lymphoblastic leukemia (T-ALL) and has an important role for T-lymphocyte activation. Experimental evidence from the group supports a regulatory role of PRLs in the organized actin cytoskeleton at the immune synapse (IS) and the interaction of these phosphatases with Lck. We have also described the regulation of the trafficking of active Lck species by phosphorylation of the tyrosine residue 394 to the activation sites. Therefore, our hypothesis is that PRLs could modulate intracellular traffic of LCK and its activity. The regulation of the subcellular distribution and the phosphorylation state of Lck by the PRLs was then investigated, both in resting or in antigen-stimulated cells. Likewise, due to the described function of the recycling compartment in trafficking of Lck and the delivery to the IS of PRLs in subcellular vesicles, we have also evaluated whether the PRLs regulate the dynamics of recycling endosomes. We expect that the understanding of the interaction of PRLs and Lck could help in the development of therapies for the treatment of leukemias such as T-ALL or other pathologies of the immune system.

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SSH-1 REGULATES THE PHOSPHORYLATION LEVELS OF TCR SIGNALLING MOLECULES AND INTERLEUKINE-2 SECRETION.

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Slingshot-1 phosphatase (SSH-1) is an important regulator of cofilin activity through its dephosphorylation in the serine 3. Cofilin is an actin-disassembling factor which is implicated in the correct depolymerization of the actin cytoskeleton. The latter is essential for accurate assembly of the immunological synapse (IS) and proper T-cell activation. Nevertheless, the role of SSH-1 in this context has not been stablished yet.

In this work, we have performed Western Blot assays to quantify the phosphorylation levels of TCR signalling molecules in SSH-1 KO antigen-stimulated cells. A decrease in activating molecules such as ZAP70, PLCγ1 or ERK1/2 has been found in SSH-1 Knockout cells indicating that this phosphatase regulates the phosphorylation of TCR downstream signalling molecules. This defect in signalling correlated with a reduced induction of the early activation marker CD69 and IL-2 secretion in antigen-stimulated cells.

Taken together, these data indicate that SSH-1 regulates early signalling contributing to T-cell activation and in effector function.

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