Sociedad Española de Virología - Sociedad Española de Virología

VIROLOGÍA

Publicación Oficial de la Sociedad Española de Virología

XVI CONGRESO NACIONAL DE VIROLOGÍA

6 _ 9 Sept 2022

Málaga

2022, ¿el año del fin de la pandemia?

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Número 1/2022
EXTRAORDINARIO

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Página web del Congreso:
www.congresovirologiasev2022.com

Virología, vol 25, nº1. Publicación Oficial de la Sociedad Española de Virología

BIENVENIDA

Estimados amigos y colegas:

La Sociedad Española de Virología y el Comité Organizador os dan la bienvenida al XVI Congreso Nacional de Virología que se celebrará en Málaga del 6 al 9 de septiembre de 2022. La sede del Congreso será el Hotel NH Málaga, situado en el centro histórico de la ciudad y desde el que se llega caminando en menos de 15 minutos a lugares emblemáticos del corazón de la ciudad, como la Catedral, el Museo Picasso, la Calle Larios o la Plaza de la Merced.

El Comité Organizador, con la ayuda del Comité Científico, ha elaborado un programa científico que esperamos sea atractivo para los asistentes y que dé cabida a la diversidad que caracteriza el estudio de los virus, tanto en su concepto más básico como en sus vertientes aplicadas y biotecnológicas de los ámbitos humano, veterinario y vegetal.

Esperamos que todos los que trabajáis en cualquiera de las ramas de la Virología y habéis enviado vuestras comunicaciones (orales o en formato póster) participéis activamente en este Congreso. Asimismo, hemos hecho el esfuerzo de contar con la presencia de un número de virólogos extranjeros de reconocido prestigio, cuya contribución estimulará la discusión sobre los avances y los retos de la Virología actual. Esperamos haber encontrado un justo equilibrio entre la intensidad que conlleva este tipo de congresos y la distensión necesaria para permitir una comunicación fluida y amena entre los asistentes.

No se nos escapa que este Congreso será especial, tanto por el retraso sufrido en su celebración como por las circunstancias que lo han motivado. Todos hemos padecido y aún seguimos inmersos en una situación en la que, con frecuencia, soportamos un cierto nivel de sobreinformación –e incluso desinformación– en relación a la pandemia de la COVID-19. Por ello, este Congreso debería servir como revulsivo para transmitir a la sociedad española la importancia de la Virología y cómo esta Ciencia puede contribuir a aportar soluciones contra esta pandemia y las que puedan surgir en el futuro. En este sentido, se ha programado una sesión divulgativa destinada al público en general que se celebrará el día 7 de septiembre en el Centro Cultural La Malagueta de la Diputación de Málaga que acercará la SEV y la Virología en general al conjunto de la sociedad malagueña.

Quiero reconocer de manera especial a los miembros del Comité Organizador y del Comité Científico por su predisposición a participar en la preparación del Congreso. Y al presidente, tesorero y resto de la Junta Directiva de la SEV, por la confianza mostrada al invitarnos a su organización y el apoyo incondicional que nos han prestado.

Esta será la primera vez que celebremos nuestro Congreso en Málaga y espero que todos disfrutemos tanto de las actividades científicas como del tan añorado contacto directo con nuestros colegas, sin olvidar el atractivo de una ciudad acogedora que ha sabido reinventarse.

Recibid un cordial saludo en nombre del Comité Organizador,

Jesús Navas Castillo
Presidente del Comité Organizador

Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora" (IHSM-UMA-CSIC)
Consejo Superior de Investigaciones Científicas
Algarrobo-Costa (Málaga)

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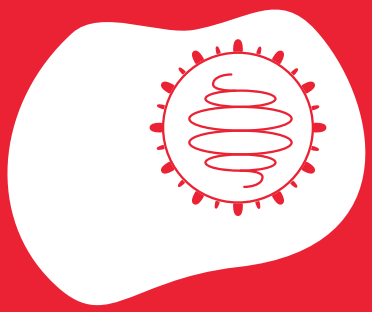

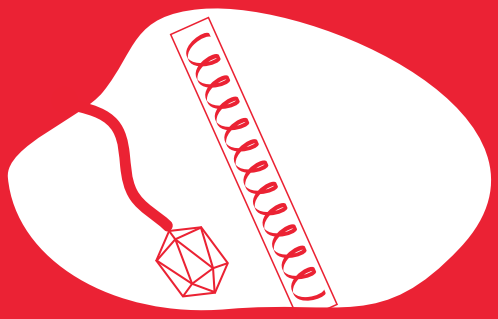

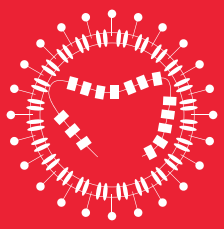

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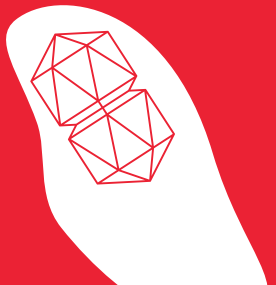

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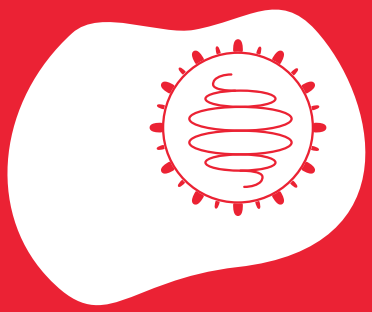
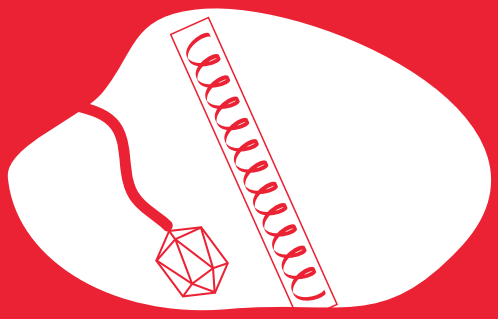
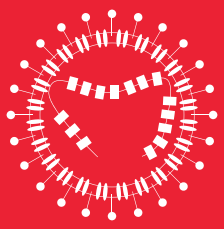
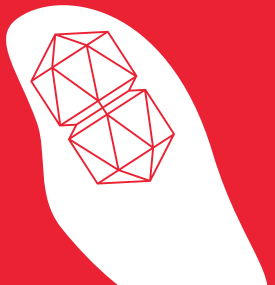






Programa resumido



PROGRAMA RESUMIDO

6 DE SEPTIEMBRE, MARTES

16:00-18:30	Registro de participantes y entrega de documentación
18:30-19:00	Inauguración oficial del congreso
19:00-20:00	Conferencia inaugural **(SPL1)**
20:00-21:00	Conferencia de la ganadora del Premio Virólogo Senior SEV 2022 **(SPL2)**
21:00-22:30	Cóctel de bienvenida. Hotel NH Málaga

7 DE SEPTIEMBRE, MIÉRCOLES

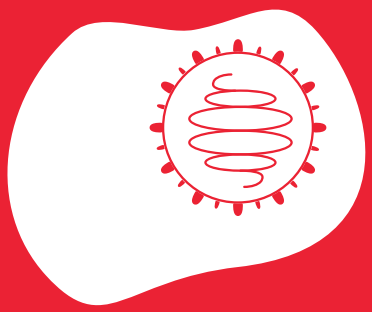
08:00-09:00	Registro de participantes
09:00-11:00	Sesión plenaria **(SPL3)**
11:00-11:30	COFFEE BREAK - Sesión de pósteres
11:30-13:30	Sesiones paralelas **(SP1-SP3)**
13:30-15:00	ALMUERZO
15:00-17:00	Sesiones paralelas **(SP4-SP6)**
17:00-17:30	COFFEE BREAK - Sesión de pósteres
17:30-19:30	Sesiones paralelas **(SP7-SP9)**

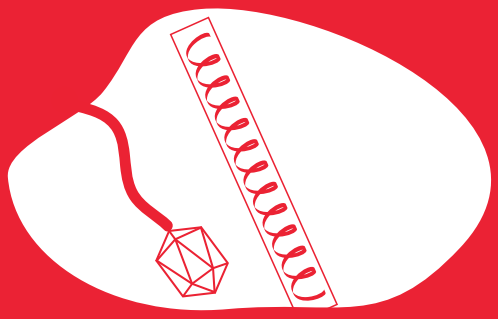
8 DE SEPTIEMBRE, JUEVES

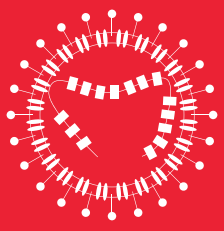
08:30-09:00	Registro de participantes
09:00-11:00	Sesión plenaria **(SPL4)**
11:00-11:30	COFFEE BREAK - Sesión de pósteres
11:30-13:30	Sesiones paralelas **(SP10 - SP12)**
13:30-15:00	ALMUERZO
15:00-17:00	Sesión plenaria **(SPL5)**
17:00-17:30	COFFEE BREAK - Sesión de pósteres
17:30-19:30	Sesión plenaria **(SPL6)**
19:30-21:00	Asamblea de la Sociedad Española de Virología

9 DE SEPTIEMBRE, VIERNES

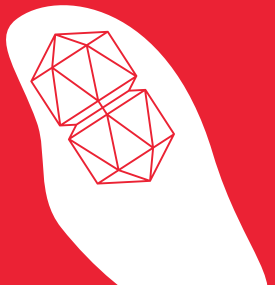
08:30-09:00	Registro de participantes
09:00-11:00	Sesión plenaria **(SPL7)**
11:00-11:30	COFFEE BREAK . Sesión de pósteres
11:30-13:30	Sesión plenaria **(SPL8)**
13:30-15:00	ALMUERZO
15:00-16:00	Sesión plenaria **(SPL9)**
16:00-18:00	Sesión plenaria **(SPL10)**
18:00-18:30	Clausura oficial del congreso y presentación del XVII Congreso Nacional de Virología
21:00-23:30	Cena de clausura y entrega de premios







Programa completo



06 Martes SEPT 22

16:00 – 18:30 Registro de participantes y entrega de documentación

18:30 – 19:00 Inauguración oficial del congreso

19:00 – 20:00 Sesión plenaria 1

Conferencia inaugural
Presentación por parte de Covadonga Alonso, *Vicepresidenta en funciones de la Sociedad Española de Virología*

Miles W. Carroll *(Wellcome Centre for Human Genetics, University of Oxford, Oxford, Reino Unido)*

» **Learning from outbreaks; preparing for pandemics**

20:00 – 21:00 Sesión plenaria 2

Conferencia de la ganadora del Premio Virólogo Senior SEV 2022
Presentación por parte de Albert Bosch, *Presidente de la Sociedad Española de Virología*

Amelia Nieto *(CNB-CSIC, Madrid)*

» **Influenza Virus a simple virus with a complex life: The viral polymerase, a key factor for genome expression and pathogenesis**

21:00 – 22:30 Cóctel de bienvenida

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Los continuos avances tecnológicos, las oportunidades limitadas de formación y el envejecimiento de la población activa amenazan el futuro de la calidad del diagnóstico.

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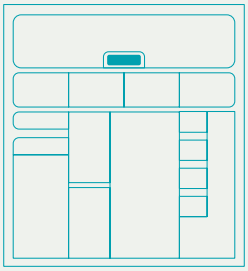

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07 Miércoles SEPT 22

09:00 – 11:00 Sesión plenaria 3

Mesa Redonda - COVID-19 y SARS-CoV-2
Moderadores: Albert Bosch y Sonia Vázquez-Morón

Tomàs Pumarola *(Vall d'Hebron, Barcelona)*

» Surveillance of microbiological emergencies. What have we learned from SARS-CoV-2?

Inmaculada Casas *(Instituto de Salud Carlos III, Madrid)*

» Consolidation of Whole Genome Sequencing *(WGS)* activities for SARS-CoV-2 towards sustainable use and integration of enhanced infrastructure and capacities in Spain

Federico García *(Hospital Universitario San Cecilio, Granada)*

» Diagnosing SARS CoV-2 variation in Andalusia: challenges and opportunities

Susana Guix *(UB, Barcelona)*

» Lessons from the two-year experience of SARS-CoV-2 wastewater monitoring in Spain

11:00 – 11:30 Sesión de pósteres SARS-CoV-2 y COVID-19 y Técnicas de diagnóstico / café

11:30 – 13:30 Sesiones paralelas 1-3

SP1. Técnicas de diagnóstico (Sala plenaria)
Moderadores: Diego Arroyo y Laura Ruiz Palmero

Antonio Martínez-Murcia *(UMH, Genetic PCR Solutions, Orihuela, Alicante)*

» Sequence diversity on public databases and its influence in designing the specificity of PCR primers for pathogen detection: the case of Monkeypox virus

María Dolores Fernández-García *(IMIBIC, Córdoba – ISCIII, Madrid)*

» Whole-genome characterization of Toscana virus detected in patients with

idiopathic meningitis reveals the first evidence of intra-genotype reassortment, Southern Spain, 2015 – 2019: a retrospective study

Ana María Gavilán *(CNM-ISCIII, Madrid)*

» Whole genome sequencing of RNA/DNA viruses from clinical specimens using RNA massive parallel sequencing

Albert Carcereny *(UB, Barcelona)*

» SARS-CoV-2 in sewage: qPCR and NGS techniques comparison based on the detection of signature mutations corresponding to different Variants of Concern

Llilianne Ganges *(CReSA-IRTA-UAB, Barcelona)*

» A novel dendrimeric-based strategy for the detection of the Classical swine fever virus FlagT4G vaccine

SP2. Diversidad genética, metagenómica y evolución (Sala paralela 1)
Moderadores: Santiago F. Elena y Ana Grande-Pérez

Gonzalo Moratorio *(Institut Pasteur, Montevideo, Uruguay)*

» Cornering arboviruses to their arthropod vectors

Guillermo Domínguez-Huerta *(Ohio State University, Columbus, Estados Unidos)*

» Diversity and ecology of Global Ocean RNA viruses

Jorge Lang *(CNM-ISCIII, Madrid)*

» Reemergence of enterovirus D68 in Spain after easing the COVID-19 lockdown

Julia Hillung *(I2SYSBIO-CSIC-UV, Paterna, Valencia)*

» Accumulation dynamics of DVGs during experimental evolution of betacoronaviruses

María Francesca Cortese *(Vall d'Hebron, Barcelona)*

» HDV-mediated inhibition of HBV in a superinfection mouse model: the role of type I Interferon

SP3. Compuestos antivirales I (Sala paralela 2)
Moderadores: José Antonio López-Guerrero y Beatriz Novoa

José Gallego *(UCV, Valencia)*

» Novel SARS-CoV-2 inhibitors discovered by phenotypic screening of viral RNA-binding molecules

Paula Bueno *(CNB-CSIC, Madrid)*

» Discovery and characterization of broad-spectrum antivirals against RNA viruses with dual mode of action against SARS-CoV-2

Sabina Andreu *(CBMSO-CSIC-UAM, Madrid)*

» The valproic acid derivative valpromide inhibits pseudorabies virus infection in swine epithelial and mouse neuroblastoma cell lines

Patricia Mingo-Casas *(INIA-CSIC, Madrid)*

» Synthetic polyphenols inhibit West Nile virus infection though alteration of sphingolipid metabolism

Florencia-Evelin Alonso *(UPF, Barcelona)*

» Novel therapies for rapid responses to pandemic viral threats

13:30 – 15:00 Comida

15:00 – 17:00 Sesiones paralelas 4-6

SP4. Virus de plantas (Sala paralela 1)
Moderadores: Vicente Pallás y Juan A. Díaz-Pendón

Elvira Fiallo-Olivé *(IHSM-UMA-CSIC, Algarrobo-Costa, Málaga)*

» Insights into the biology of deltasatellites, an emerging class of begomovirus-associated DNA satellites

José-Antonio Daròs *(IBMCP-CSIC-UPV, Valencia)*

» Delivery of gene editing CRISPR-Cas reaction components in plants using viral vectors

Izan Melero *(I2SYSBIO-CSIC-UV, Paterna, Valencia)*

» Determinants of the age-dependent responses to viral infection in Arabidopsis thaliana

Sandra Martínez-Turiño *(CNB-CSIC, Madrid)*

» Involvement of homologous and heterologous O-GlcNAc cycling enzymes, OGT and OGA, in the O-GlcNAcylation turnover of the plum pox potyvirus coat protein, during infection

Gabriela N. Condezo *(CNB-CSIC, Madrid)*

» Structure and assembly of Polinton-like virus TsV-N1, a possible ancestor of the PRD1-Adenovirus lineage in eukaryotic hosts

SP5. Virología veterinaria (Sala plenaria)
Moderadores: Fernando Rodríguez y Ana Doménech

Daniel Rodríguez-Martín *(CISA-INIA-CSIC, Valdeolmos, Madrid)*

» Peste des Petits Ruminants *(PPRV)* impairs DC function to suppress T cell activation

David Marín *(CReSA-IRTA-UAB, Barcelona)*

» BA71ΔCD2 intranasal immunization is safe and effective against direct-contact challenge with pigs infected with the African swine fever virus pandemic strain

Luis Jiménez-Cabello *(CISA-INIA-CSIC, Valdeolmos, Madrid)*

» Development of novel vaccine candidates against bluetongue virus based on the induction of humoral and cellular immune responses by Modified Vaccina Ankara vectors expressing VP2, VP7, NS1 and NS2-Nt proteins

Gonzalo Vigara-Astillero *(CBMSO-CSIC-UAM, Madrid)*

» Role of cell factors on African Swine Fever Virus tropism

Álvaro López-Valiñas *(CReSA-IRTA-UAB, Barcelona)*

» Genomic and evolutionary analysis of swine influenza virus H1N1 and H3N2 in vaccinated and nonvaccinated pigs after simultaneous contact infection

SP6. Virus de peces (Sala paralela 2)
Moderadores: Esther García-Rosado e Isabel Bandín

Carlos P. Dopazo *(USC, Santiago de Compostela)*

» Viruses, aquaculture and climate change: what surprises are around the corner

Beatriz Novoa *(IIM-CSIC, Vigo)*

» Zebrafish *(Danio rerio)* as a model for the study of the interaction of virus and immune system

Laura Cervera *(UM, COMU-IEO-CSIC, Murcia)*

» Antimicrobial peptides Hepcidin and Dicentracin produce immunomodulation and confer partial protection against Nodavirus

Sandra Souto *(USC, Santiago de Compostela)*

» Efficient incorporation of a betanodavirus antigen in viral hemorrhagic septicemia virus *(VHSV)* vectors with rearranged genomes

Rocío Leiva-Rebollo *(IBYDA-UMA, Málaga)*

» Immune response of vaccinated juvenile gilthead seabream *(Sparus aurata)* after LCDV-Sa infection

17:00 – 17:30 Sesión de pósteres Diversidad genética y evolución viral, Compuestos antivirales y Virus de plantas / café

17:30 – 19:30 Sesiones paralelas 7-9

SP7. Replicación y expresión génica (Sala plenaria)
Moderadores: Fernando Almazán y Jesús Navas-Castillo

José M. Almendral *(CBMSO-CSIC-UAM, Madrid)*

» A mouse parvovirus infecting human glioblastoma stem cells with patient-specific p53 deregulations

Enara San Sebastián *(CNB-CSIC, Madrid)*

» DNA-dependent protein kinase complex differentially restricts hepatitis B virus and adeno-associated virus gene expression

Marc Talló-Parra *(UPF, Barcelona)*

» CHIKV uses two different tRNA-related strategies to favor viral protein expression

Pablo A. Gutierrez *(UNC, Medellín, Colombia – I2SysBio-CSIC-UV, Paterna, Valencia)*

» Dynamic analysis of the SARS-CoV-2 response using single-cell RNA sequencing data

Pablo Gastaminza *(CNB-CSIC, Madrid)*

» Cryo-SXT imaging of SARS-CoV-2-infected human lung epithelial cells

SP8. Patogénesis viral I (Sala paralela 1)
Moderadores: Dolores Castro y Javier Buesa

Ron Geller *(I2SysBio-UV-CSIC)* **1700 15**

» Insights into picornavirus biology, evolution, and pathogenesis from deep mutational scanning analyses

Carlos García-Crespo *(CBMSO-CSIC-UAM, Madrid)*

» Hepatitis C virus fitness can influence the extent of infection-mediated epigenetic modifications in the host cells

Inés Ripa *(CBMSO-CSIC-UAM, Madrid)*

» Interaction of herpes simplex virus type 1 with autophagy in oligodendrocytes

Mireya Martínez-Pérez *(IBMCP-CSIC-UPV, Valencia)*

» The Arabidopsis m6A readers ECT2, ECT3 and ECT5 restrict infection of alfalfa mosaic virus

Ana-Belén Blázquez *(INIA-CSIC, Madrid)*

» Usutu virus replication evades cellular stress response impairing eIF2α phosphorylation and stress granules formation.

SP9. Nanovirología y otras aplicaciones biotecnológicas (Sala paralela 2)
Moderadores: Carlos Briones y Pilar Domingo-Calap

Fernando Merwaiss *(IBMCP-CSIC-UPV)*

» Production of recombinant plant virus-derived nanoparticles carrying capsid-fused nanobodies for SARS-CoV-2 detection

Javier Arranz-Herrero *(USP-CEU, Madrid)*

» Broad viral inactivation by soda-lime glass and kaolin based materials

Sara Cuadrado-Castano *(Mount Sinai, New York, EE.UU.)*

» Avian paramyxovirus 4 *(APMV-4)* oncolytic virotherapy leads to complete responses and long-term anti-tumor memory in preclinical melanoma, colon carcinoma and lymphoma models

Vicent Tur-Planells *(USP-CEU, Madrid)*

» Infectious bursitis disease virus as a potential oncolytic virotherapy against glioblastoma and other cancer cell tumor models

María Zamora-Ceballos *(CISA-INIA-CSIC, Valdeolmos, Madrid)*

» Design of RHDV VLPS with enhanced mechanical stability and evaluation of their immunogenicity

19:00 - 20:00 Sesión Divulgativa

"Lo que quiere saber de la pandemia y si se atreve a preguntar..." (Centro Cultural la Malagueta. Paseo de Reding, 8)
Moderador: Ana Grande Pérez
Profesora Titular de la Universidad de Málaga e investigadora del Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora" (IHSM-UMA-CSIC)

José Antonio López Guerrero

» Catedrático de Microbiología, director del grupo de Neurovirología de la Universidad Autónoma de Madrid y director del Departamento de Cultura Científica del Centro de Biología Molecular Severo Ochoa *(UAM-CSIC)*

Raquel Rodríguez Merlo

» Responsable de Urgencias y Emergencias Sanitarias del Servicio de Salud del Principado de Asturias

08 SEPT 22 Jueves

09:00 – 11:00 Sesión plenaria 4

Mesa redonda - Hepatitis virales (sesión patrocinada por Roche y Diasorin)
Moderadores: Francisco Rodríguez-Frías y Maria Francesca Cortese

Francisco Rodríguez-Frías *(Vall d'Hebron, Barcelona)*

» Viral hepatitis round table: an introduction

Rosa M. Pintó *(UB, Barcelona)*

» Hepatitis A virus: from new paradigms to new lacunae

Urtzi Garaigorta *(CNB-CSIC, Madrid)*

» Cellular determinants of hepatitis B virus persistence

Carlos Briones *(CAB-INTA-CSIC, Madrid)*

» In vitro selection of RNA and DNA aptamers against hepatitis C virus core protein: applicability to ultrasensitive viral diagnosis and therapy

David Tabernero *(Vall d'Hebron, Barcelona)*

» Is hepatitis delta virus infection underdiagnosed due to suboptimal screening methods? Exposing a hidden problem

Maria I. Costafreda *(UB, Barcelona)*

» Specific plasma microRNA signatures underlying the clinical outcomes of hepatitis E virus infection

11:00 – 11:30 Sesión de pósteres Virología veterinaria, Virus de peces y Replicación y expresión génica / café

11:30 – 13:30 Sesiones paralelas 10-12

SP10. Patogénesis viral II (Sala plenaria)
Moderadores: Pablo Gastaminza y Estanislao Nistal-Villán

José F. Rodríguez *(CNB-CSIC, Madrid)*

» Deciphering the molecular basis underlying IBDV persistent infections

Llilianne Ganges CReSA-IRTA-UAB, Barcelona)

» Into the eye of the classical swine fever cytokine storm: Role of the Erns RNase activity and a poly-uridine insertion in the 3´UTR

José Manuel Honrubia *(CNB-CSIC, Madrid)*

» SARS-CoV-2 pathogenesis mediated by E protein PBM was prevented by modulators of CFTR function

Darío López garcía *(CNB-CSIC, Madrid)*

» The IFN alpha inducible proteins IFI6 and IFI27 are negative regulators of innate immune responses and modulate RIG-I activation

Isabel Alonso-Sánchez *(CBMSO-CSIC-UAM, Madrid)*

» Impact of TNF, LTα and chemokines in the pathogenesis of mousepox

SP11. Invasión y transporte viral (intra- e intercelular) (Sala paralela 2)
Moderadoras: Covadonga Alonso e Isabel García-Dorival

Vicente Pallás *(IBMCP-UPV-CSIC, Valencia)*

» Intra- and inter-cellular transport of plant viruses. Similarities and differences with animal viruses

Isabel García Dorival *(INIA-CSIC, Madrid)*

» Elucidating the interactome of African swine fever virus proteins

Lisa K. Busch *(CiQUS-USC, Santiago de Compostela)*

» The nonstructural protein muNS of Avian Reovirus engages in liquid-liquid phase separation

Susana Guerra *(UAM, Madrid)*

» ISG15 is an important host factor for Vaccinia virus dissemination

Rafael Blasco *(INIA-CSIC, Madrid)*

» Genome-wide CRISPR screen identifies β2-microglobulin as a host factor for vaccinia virus infection

SP12. Compuestos antivirales II (Sala paralela 1)
Moderadores: Beatriz Novoa y José Antonio López-Guerrero

Urtzi Garaigorta *(CNB-CSIC, Madrid)*

» Discovery and characterization of potent and selective SARS-CoV-2 antivirals with pan-coronavirus potential

Carlos García-Crespo *(CBMSO-CSIC-UAM, Madrid)*

» Efficacy decrease of antiviral agents when administered to ongoing hepatitis C virus infections

Samara Martín-Alonso *(CBMSO-CSIC-UAM, Madrid)*

» Novel RNase H inhibitors with multiple targets in HIV-1

Erick De La Torre Tarazona *(H. Ramón y Cajal, ISCIII, Madrid)*

» High concentration of maraviroc does not alter immunological parameters of CD4 T cell subpopulations

Victoria Castro *(CNB-CSIC, Madrid)*

» Persistent hepatitis C virus infection causes permanent transcriptional alterations after virus elimination

13:30 – 15:00 Comida

15:00 – 17:00 Sesión plenaria 5

Virus emergentes
Moderadores: Juan García-Costa y Juan Emilio Echevarría

Rafael Sanjuán *(I2SysBio-UV-CSIC, Valencia)*

» Enveloped viruses show increased propensity to cross-species transmission and zoonosis

Melissa Bello-Pérez *(CNB-CSIC, Madrid)*

» MERS-CoV ORF4b is a virulence factor involved in the inflammatory pathology induced in the lungs of mice

África Sanchiz *(CBMSO-CSIC-UAM, Madrid)*

» SARS-CoV-2 surveillance in the air: a long term study in education centres

Juan Ramón Rodríguez *(CNB-CSIC, Madrid)*

» Lyophilized homodimers of the RBD domain of spike protein as vaccine against SARS-CoV-2

Belén Borrego *(CISA-INIA-CSIC, Valdeolmos, Madrid)*

» Extreme attenuation of a mutagenized variant of Rift Valley fever virus *(RVFV)*: towards a safer live attenuated vaccine

17:00 – 17:30 Sesión de pósteres Patogénesis viral, Nanovirología y otras aplicaciones biotecnológicas, Invasión y transporte viral (intra- e intercelular) y Virus emergentes / café

17:30 – 19:30 Sesión plenaria 6

SARS-CoV2 y COVID-19
Moderadores: Josep Quer e Inmaculada Casas

Juan García-Arriaza *(CNB-CSIC, Madrid)*

» Preclinical characterization of the SARS-CoV-2/COVID-19 vaccine candidate MVA-CoV2-S: Robust immunogenicity and full efficacy against SARS-CoV-2 in mice, hamsters and rhesus macaques

Carolina Campos *(Vall d'Hebron, UAB, Barcelona)*

» Frequency of defective genomes in Omicron differs from that of the Alpha, Beta and Delta variants

Tránsito García-García *(UCO, IMIBIC, Córdoba)*

» Antiviral immune responses, cellular metabolism and adhesion are differentially modulated by SARS-COV-2 ORF7a and ORF7b

Teresa Aydillo *(Mount Sinai, New York, Estados Unidos)*

» Antibody immunological imprinting in COVID-19 patients

María Iglesias-Caballero *(ISCIII, Majadahonda, Madrid)*

» Genetic diversity of SARS-CoV-2 outbreaks in farmed minks in Spain

19:30 – 21:00 Asamblea de la Sociedad Española de Virología (Sala plenaria)

09 Viernes SEPT 22

9:00 – 11:00 Sesión plenaria 7

Mesa redonda – Desarrollo de vacunas contra la COVID-19
Moderadores: Ana Fernández-Sesma y Juan García-Costa

Adolfo García-Sastre *(Mount Sinai, New York, Estados Unidos)*

» A Newcastle-disease virus based vaccine for COVID-19

Luis Enjuanes *(CNB-CSIC, Madrid)*

» RNA replicons as human pathogenic coronavirus recombinant vaccines

Mariano Esteban *(CNB-CSIC, Madrid)*

» Development of the MVA-CoV2-S vaccine by the CNB-CSIC and advantages against COVID-19

Antoni Prenafeta *(HIPRA, Barcelona)*

» Development of the PHH-1V vaccine against COVID-19

11:00 – 11:30 Sesión de pósteres Hepatitis virales y Respuesta inmune / café

11:30 – 13:30 Sesión plenaria 8

Respuesta inmune
Moderadores: Ana Doménech y Javier Ortego

Ana Fernández-Sesma *(Mount Sinai, New York, EE.UU.)*

» Modulation of innate immune responses by arboviruses

Laia Bosch-Camós *(CReSA-IRTA-UAB, Barcelona)*

» Elucidating the complex cellular response associated with cross-protection against African swine fever virus

José M. Rojas *(CISA-INIA-CSIC, Valdeolmos, Madrid)*

» Comprehensive immune profiling of in vivo bluetongue virus infection reveals that the virus mediates an acute immunosuppression of T cells

Elisabet Díaz-Beneitez *(CNB-CSIC, Madrid)*

» Chicken TRIM25 knockout reduces the innate immune response elicited by IBDV and favors viral replication

Yanis H. Bouzaher *(CIMUS-USC-IDIS, Santiago de Compostela)*

» SUMO modulation of the anti-viral and anti-apoptotic activities of ISG15

13:30 – 15:00 Comida

15:00 – 16:00 Sesión plenaria 9

Conferencia de la ganadora del Premio Virólogo Joven SEV 2022
Presentación por parte de Urtzi Garaigorta, *ganador del Premio Virólogo Joven SEV 2019*

Pilar Domingo-Calap *(I2SYSBIO-UV-CSIC, Paterna, Valencia)*

» Phages, protagonists of a post-antibiotic era

16:00 – 18:00 Sesión plenaria 10

Simposio-Homenaje al Dr. Ricardo Flores – Viroides y otros agentes subvirales
Presentación por parte de José Antonio Daròs

Francesco di Serio *(ISPP-CNR, Bari, Italia)*

» Viroids: from tiny pathogens to source of knowledge on RNA biology

Fernando García-Arenal *(CBGP-UPV-INIA-CSIC, Madrid)*

» Plant RNA viruses and satellites: from antagonistic to mutualistic

Antonio Aguilera *(CHUS, Santiago de Compostela)*

» Hepatitis delta: the ignored diagnosis

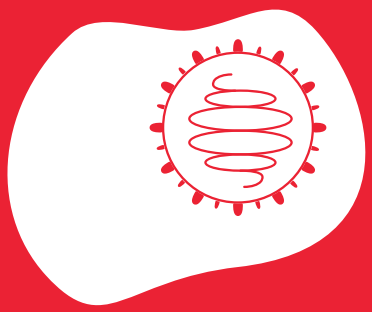
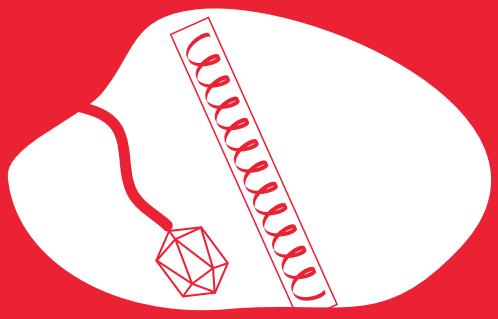
Alba Marín-Moreno *(CISA-INIA-CSIC, Valdeolmos, Madrid)*

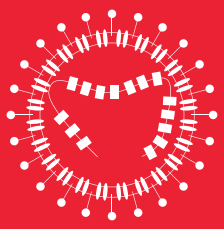
» Molecular elements involved in prion propagation and transmission

18:00 – 18:30 Clausura oficial del congreso y presentación del XVII Congreso Nacional de Virología

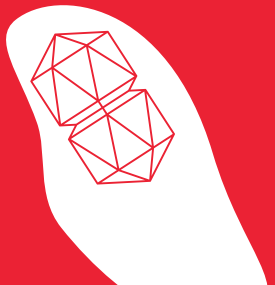
21:00 – 23:30 Cena de clausura

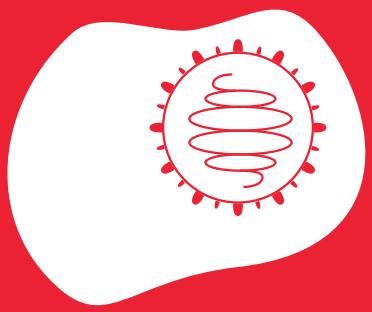
Entrega de premios a las mejores charlas orales y pósteres de investigadores pre-doctorales

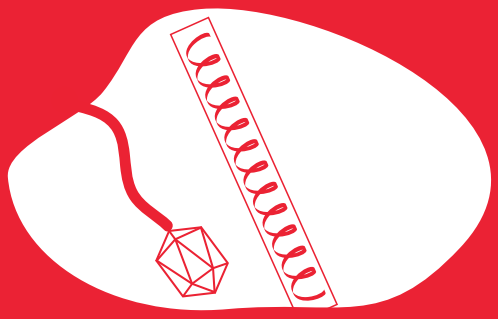



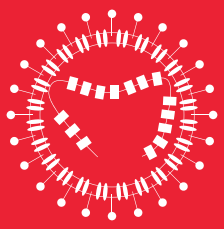


Abstracts









Abstracts

Sesiones plenarias

SPL1

SPL1
Responding to Outbreaks: Preparing for Pandemics

Miles W Carroll[1]
[1]*Pandemic Sciences Institute, University of Oxford, Oxford UK.*

High consequence emerging viruses continually spill over into the human population from their animal reservoirs and cause numerous outbreaks, which can lead to epidemics and even pandemics. Additionally, it is thought that we currently know less than 1% of the total number of viruses in existence. In response to the 2013-2016 Ebola virus disease epidemic the WHO brought together international experts to establish the R&D Road Map of Priority Pathogens. The selected priority pathogens, including a currently unknown Pathogen X, have the potential to cause future epidemics and for which no vaccines or therapeutics currently exist. Much progress in infectious disease "outbreak response" was made during the west African EBOV epidemic including the fields of: molecular epidemiology, diagnostics, therapeutics & vaccine development. We will discuss the key translational research outputs of the EBOV epidemic and how this knowledge has been used in the international response to the COVID-19 and Monkeypox virus pandemics.

SPL2

SPL2
Influenza Virus, a simple virus with a complex life: The viral polymerase, a key factor for genome expression and pathogenesis

Amelia Nieto[1]
[1]*Centro Nacional de Biotecnología-CSIC, Cantoblanco, 28049 Madrid, Spain*

Influenza virus polymerase establishes a network of functional interactions with the host, including translation and transcription factors as well as chromatin remodelers to allow an efficient viral replication. Moreover, during replication the viral polymerase synthesizes besides the complete vRNAs of the different viral segments, defective genomes containing large internal deletions (DVGs), that are packaged into the virions. Virions purified from clinical isolates from infected patients with very severe symptoms or even fatal cases, contain a low proportion of DVGs compared to those present in isolates coming from sentinel patients. Evaluation of the immune response indicates that viruses with few DVGs induce a low antiviral response, according to the ability of the DVGs to be recognized by the interferon system. These data indicate that the low production of DVGs constitutes a pathogenicity factor. On the other hand, during the infection, the host tries to reestablish an efficient interferon response increasing the methylation of lysine 79 histone 3 (H3K79) catalyzed by Dot1L, which specializes in signaling the control of retinoic acid-inducible gene I protein (RIG-I). Interferon-inducible E3 ligase tripartite motif-containing protein 25 (*TRIM25*) expression increases in influenza virus infected cells, but Dot1L inhibition reduces *TRIM25* expression. *TRIM25* overexpression reverses the defective innate response mediated by Dot1L inhibition indicating that TRIM25 is a control point of the RIG-I recognition pathway controlled by Dot1L. Human Influenza A virus (hIAV) infection is associated with cardiovascular complications, although cardiac infection pathophysiology is poorly understood. Mice were infected with strains of different pathogenicity and 3D reconstructions of infected cardiac tissue showed viral proteins inside mouse cardiomyocytes, Purkinje cells, and cardiac vessels, as well as cultured human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). Highly pathogenic recombinant showed faster replication, higher level of inflammatory cytokines in cardiac tissue and higher viral titres in cardiac HL-1 mouse cells and hiPSC-CMs compared with the attenuated virus. Correspondingly, cardiac conduction alterations were especially pronounced in mice infected with the highly pathogenic strain, associated with high mortality rates, compared with the attenuated-infected animals. In summary, human IAV can infect the heart and cardiac-specific conduction system, which may contribute to cardiac complications and premature death.

SPL3 MR-COV

SPL3.1 MR-COV

Surveillance of microbiological emergencies. What have we learned from SARS-CoV-2?

Tomàs Pumarola
Laboratoris Clínics Vall d'Hebron. Hospital Universitari Vall d'Hebron. Barcelona (Spain)

The pandemic caused by SARS-CoV-2 has been one of the main challenges that societies and health systems around the world have had to face in this century. The irruption of an infectious agent from the highly transmissible animal reservoir and with an important pathogenic capacity is one of the phenomena with the greatest disruptive potential at a global level, affecting, in addition to health, the economy, work, mobility, culture, and all areas of activity of the society as a whole. Despite the recommendations of the World Health Organization (WHO), the European Center for Disease Prevention and Control (ECDC) and other international organizations regarding its importance, preparation for pandemics has been insufficient. This lack of preparation, added to the intensity and duration of the current pandemic, has shown the limited validity and effectiveness of existing plans to deal with this type of global crisis. The high genetic variability, the lack of prediction of these changes, the existence of animal reservoirs and the possibility of the appearance of future pandemics make it necessary to establish future plans to deal with annual epidemic outbreaks and future pandemics, based on , among others, the improvement of integrated syndromic, microbiological and epidemiological surveillance of microorganisms within a One-Health perspective.

SPL3.2 MR-COV

Consolidation of Whole Genome Sequencing (WGS) activities for SARS-CoV-2 towards sustainable use and integration of enhanced infrastructure and capacities in Spain

Inmaculada Casas[1], Sonia Vazquez-Morón[1], Maria Iglesias-Caballero[1], Francisco Pozo[1] and RELECOV network members.
[1]National Centre for Microbiology, Institute of Health Carlos III, Madrid, Spain

On January 21, the Public Health Commission of the Interterritorial Council, as the board of foremost and coordination authority among the Autonomous Regions and the Spanish Ministry of Health, approved the document "Strategy for integration of genomic sequencing in the surveillance of SARS-CoV-2". Its objective was the inclusion of the data generated by genomic sequencing for the identification of variants in Spain, as part of the national surveillance. To meet this objective, a National Network of Laboratories for SARS-CoV-2 Genomic Sequencing (RELECOV) was created to cover the sequencing needs and to gather knowledge of SARS-CoV-2 viruses through their genomic analysis. The majority of partners of the RELECOV network worked together from the end of 2020 when started the circulation of variant Alpha. The network is coordinated by the ISCIII, in collaboration with the Ministry of Health, Alerts and Emergencies Coordination Center (CCAES) as National Contact Point. In a total, there are 41 members and in some regions there are nodes of regional networks or consortium well defined. RELECOV were officially structured under the ECDC/2021-Grant/2021/PHF/23776 project "Enhancing whole genome sequencing (WGS) and/or reverse transcription polymerase chain reaction (RT-PCR) national infrastructures and capacities to respond to the COVID-19 pandemic in Spain". After one year of Grant, RELECOV has improved infrastructures and capacities to respond and to increase the knowledge of the circulation of different variants of SARS-CoV-2 in our country. The work has raised new hypotheses and questions about the significance of specific mutations in the SARS-CoV-2 genome for a better understanding of viral evolution. Now, we aim to consolidate RELECOV for a sustainable and collaborate platform to obtain virological information about SARS-CoV-2 variants/lineages.We will enable timely data sharing through a coordinated response, allowing for investigation of variants/lineages with low or wide geographical reach. This will be achieved through integration, adaptation and consolidation of the existing RELECOV network. Conducting a genomic based surveillance of SARS-CoV-2 we will extend to other viral respiratory infections through the update of the network to make it more practical, technically more efficient and

rapidly adaptable to future emerging pathogens and contributing to international pandemic preparedness. ECDC/HERA/2021/024 ECD.12241.

SPL3.3 MR-COV
Diagnosing SARS CoV-2 Variation in Andalucia: challenges and opportunities

Federico García[1]
[1]Servicio de Microbiología, Hospital Universitario Clínico San Cecilio, Granada

SARS CoV-2 is an RNA virus with one of the largest genomes and more than three times as big as HCV or HIV; however, genetic variation in comparison to other RNA Viruses, such as HIV and HCV is very limited, due to the RNA proofreading, exonuclease and N7-MTase activity of the nonstructural protein 14 (NSP-14). However, the huge number of infections worldwide has provided an ideal scenario for genetic variation. In fact, throughout these two years and a half pandemic we have assisted to more than 550 million of new cases, prompting the "ideal" scenario for viral variation, and of course, global spread.

Spain has not been away from the importation of new variants, and has assisted to variant replacement following introduction from other countries. Andalucia, the most populated autonomous community is Spain, with more than 9 million inhabitants, started a SARS-CoV-2 genomic surveillance program from January 2021. The program offered two reference centers for SARS-CoV-2 whole genome sequencing (WGS), located in Hospital Universitario San Cecilio in Granada and Hospital Universitario Virgen del Rocio in Sevilla. Samples from all the provinces of Andalucía were sent weekly to these two centers. FastaQ files were analyzed both locally and at the clinical bioinformatic unit from Fundación Progreso y Salud. Genomic data are integrated to clinical data through the "Base Poblacional de Salud". In addition a real time detection of viral variants through allele specific real time PCR (AS-RtPCR) was installed. By July 2022, more than 25.000 viral genomes have been sequenced and even more patients have been screened by AS-rtPCR, giving the opportunity to be in front of the genomic surveillance of the Spanish autonomous communities, not without having to solve numerous challenges. However, a number of opportunities were brought to clinical microbiology laboratories in Andalucia. Both challenges and opportunities will be discussed during the presentation at the congress in September.

SPL3.4 MR-COV
Lessons from the two-year experience of SARS-CoV-2 wastewater monitoring in Spain

Susana Guix, Rosa M Pintó, Albert Bosch
Enteric Virus Laboratory, Department of Genetics, Microbiology and Statistics, School of Biology, and Research Institute of Nutrition and Food Safety (INSA-UB), University of Barcelona, 08028 Barcelona, Spain

Wastewater based epidemiology (WBE) has traditionally been used to monitor circulation of pathogens including viruses such as polio, and during the last two years has evolved as an applicable tool for SARS-CoV-2 surveillance to inform public health decisions. Today, the increased transmissibility and propensity of some Variants of Concern (VOCs) to potentially escape immune responses and cause more severe disease continue to pose a threat to our response against the virus. Since wastewater testing can provide near real-time data of SARS-CoV-2 viral spread at the community level, most countries around the world have setup WBE surveillance systems to be used as early warning and to monitor emergence and spread of variants in the population. Following the European Commission Recommendation adopted in March 2021, all EU Member States have taken rapid action and today over 1300 wastewater treatment plants are under regular surveillance across the EU territory. Since June 2020, our laboratory has been actively involved in the VATar COVID19 (https://www.miteco.gob.es/es/agua/temas/concesiones-y-autorizaciones/vertidos-de-aguas-residuales/alerta-temprana-covid19/) and SARSaigua (https://sarsaigua.icra.cat/) SARS-CoV-2 surveillance networks, funded by the Spanish and Catalan governments, respectively. During the last 2 years, we have centered our efforts to develop, optimize and validate methods to quantify SARS-CoV-2 genomes and correlate them with the number of cases throughout different waves. The combination of duplex discrimination

Molecular. Para todos

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Kits de RTq-PCR

- **PCR** Multiplex de diferentes dianas víricas con análisis simultáneo.
- Interpretacion automática de resultados.
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- Resultados **rápidos** y **confiables** en menos de 2 horas.
- Presentación **liofilizada** para asegurar la estabilidad y reducir costes de transporte.
- **Nuevo** formato predispensado sin necesidad de preparar ningún reactivo y con placa divisible en tiras (LPD) para mayor comodidad del usuario.

SARS-COV-2 OMICRON SUBVARIANTS REALTIME PCR KIT	**Kit de RT-PCR** en tiempo real para detectar cualitativamente las subvariantes Ómicron más prevalentes o con mayor riesgo de escape inmune BA.4/BA.5/BA.2.1.12 en muestras respiratorias humanas en formato de placas rompibles.
VIRAL MENINGITIS REALTIME PCR KIT	**Kit de RT-PCR** en tiempo real para detectar cualitativamente Herpes simplex 1, Herpes simplex 2, varicella zoster virus, enterovirus, human parechovirus, Toscana virus, West Nile virus en formato de placas rompibles.
ZIKV/DENV/CHIKV REALTIME PCR KIT	**Kit de RT-PCR** en tiempo real para detectar cualitativamente Zika, Dengue y Chikungunya en muestras humanas y en formato de placas rompibles.
HEPATITIS DELTA REALTIME PCR KIT	**Kit de RT-qPCR** cuantitativo según estándar OMS 7657/12 para detectar los genotipos del 1 al 8 del virus de hepatitis delta en muestras de suero y plasma en formato de placas rompibles
MONKEYPOX REALTIME PCR KIT	**Kit de RT-PCR** en tiempo real para detectar ácidos nucleicos del virus de la viruela de los monos en muestras de lesiones cutáneas en formato de placas rompibles.

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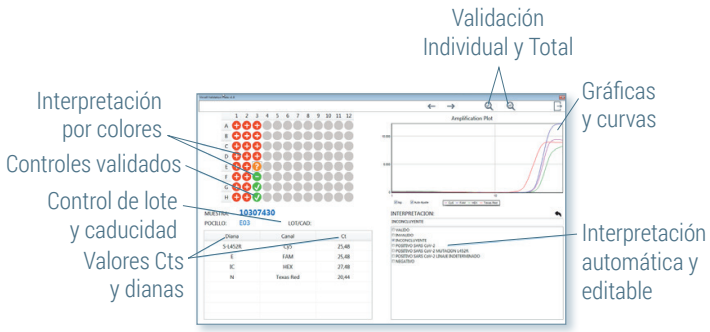
- **Software** para el análisis de datos de los sistemas de amplificación con el **LIS** del laboratorio.
- Permite mantener una **trazabilidad total de todo el proceso**.
- Realiza una **interpretación automática** de los resultados de manera sencilla y directa **(con curvas y valores Cts)**.
- **Integración con el software LIS** del laboratorio.



Vircell, S.L. Parque Tecnológico de la Salud, Avicena 8. 18016 Granada, España. info@vircell.com
Vircell Spain, S.L.U. Pol. Ind. Dos de Octubre, Plaza Domínguez Ortiz 1. 18320 Santa Fe, Granada
Delegación Madrid: 913 457 903. Delegación Barcelona: 933 099 530. www.vircell.com

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RT-qPCR assays targeting specific VOC's signature mutations and amplicon-based NGS approaches have allowed us to detect several VOCs in wastewater before their reporting from clinical studies. We have also characterized the dynamics of VOCs introduction, emergence and spread in the territory, affected mainly by factors inherent to each VOC, differences in the population vaccination status and mobility restriction policies. We observed that Omicron outcompeted Delta significantly faster than the previous outcompetition of Alpha by Delta and B.1.177 by Alpha. Our experience highlights how WBE has been a valuable unbiased non-invasive and cost-effective complementary tool to be used in association with other epidemiological indicators to evaluate the evolution of COVID-19 pandemic. Lessons learnt up to date will also be especially relevant for scenarios where timely clinical testing is reduced or unavailable, as well as to further expand WBE to the surveillance and tracking of other priority pathogens.

SPL4MR-HVI

SPL4.1 MR-HVI
Viral hepatitis round table: an introduction

Francisco Rodríguez-Frías
Liver Pathology Unit, Biochemistry and Microbiology Services of the Vall d'Hebron University Hospital, Barcelona, Spain

Hepatitis can be caused by many etiologies, among which infection by various viruses stands out, especially five of them known as "hepatotropes": Viruses A, B, C, D and E, which selectively infect the liver, by different routes (enteral, parenteral). However, other viruses can also cause hepatitis. The WHO has estimated that around 400 million people in the world suffer from these chronic infections which cause 1.5 million deaths and 3 million new infections each year. In some of these viral infections (B, C, D at different rates) the process becomes a chronic, progressing to cirrhosis and liver cancer. The medical advances made in recent decades have led to the implementation of preventive measures, the development of vaccines (hepatitis A and B) and passive immunization strategies, and, more recently, the development of promising and effective treatments, especially successful and curative for HCV (>95% of cases treated), but highly "inhibitory" for HBV (80%). Despite these very positive data, viral hepatitis remains a global public health problem. However, based on these data, the WHO has proposed a program to eliminate viral hepatitis by 2030, which requires a very significant increase in its prevention, diagnosis, and treatment. But do we explain everything with these five viruses? It seems not. On 5 April 2022, 10 cases of severe acute hepatitis of unknown origin in children under 10 years of age were reported in the UK. As of June 17, 991 similar cases have been reported in 35 countries. In 6% of cases, they have required liver transplantation, presenting almost 3% mortality. The Center for Coordination of Health Alerts and Emergencies of the Ministry of Health reported that the casuistry of these unaffiliated acute hepatitis in Spain was not higher than those that have usually been observed in previous years, although it calls deeply attention to the severity of these cases. Its etiology remains unknown, and several hypotheses are being considered, the most plausible involves a gastrointestinal adenovirus 41F, usually of little relevance and associated with cases of hepatitis in immunosuppressed patients, and perhaps in these tremendous cases associated with additional factors, such as the current SARS-CoV-2 infection.

SPL4.2 MR-HVI
Hepatitis A virus: from new paradigms to new lacunae

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Despite the efficient available vaccines, huge outbreaks of hepatitis A still occur, particularly among the men-having-sex-with-men (MSM) and the home-less/drug-user groups. The hepatitis A virus (HAV) is a unique picornavirus, showing striking genomic, structural, antigenic and biological differences with regard to other members of the *Picornaviridae* family. At the genomic level, HAV has an inefficient IRES, is unable to shut-down the cellular protein

synthesis and has a much-deviated codon usage. Altogether, results in a highly regulated ribosome traffic pace, which might explain the smooth capsid and its exceptional stability. Epitope residues are mostly encoded by highly conserved rare codons, whose role in stalling the ribosome machinery during translation might constraint antigenic variability. Nevertheless, infections in vaccinated people may foster the selection of new variants. HAV exists in a double particle phenotype: as naked capsids shed in stool and responsible for person-to-person transmission and as exosome-entrapped capsids circulating in the blood, responsible for the cell-to-cell transmission. This latter point, however, represents an important interpretation caveat regarding antibody neutralization. Additionally, the mechanisms for the entry and egress of both types of particles in the multipolar hepatocyte still bear many lacunae. While HAV has been previously considered exclusively a human virus, new findings have proven its zoonotic occurrence in many other mammals, and even its codon usage might point to a very ancient origin. In summary, we are in front of a very old pathogen with still many unclarified features.

SPL4.3 MR-HVI
Cellular determinants of hepatitis B virus persistence

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Hepatitis B virus (HBV) represents an important human pathogen causing both acute and chronic hepatitis. It is estimated that over 250 million people are chronically infected and more than 780,000 people die every year due to complications of HBV, including liver cirrhosis and hepatocellular carcinoma (HCC) accounting for more than 50% of all HCC cases worldwide. Despite the availability of an effective vaccine, more than 700 new infections still occur every year in Spain alone. Currently approved therapies for the treatment of chronic HBV are very effective in suppressing virus replication and viremia but they are not curative because they do not eliminate the nuclear long-lasting episomal DNA form of HBV, known as cccDNA. HBV cccDNA is the transcriptional template for HBV gene expression and it re-establishes infection upon interruption of therapy, representing the hallmark of HBV persistence. Therefore, there is an imminent need to develop novel cccDNA-targeting therapies that will help eradicate HBV from chronically infected patients. Despite of our understanding of many aspects of the HBV replicative cycle, details of HBV cccDNA biology including the identity and function of cellular and viral factors regulating its formation, expression, silencing and homeostasis are scarce. The recent identification of the HBV receptor, the establishment of new cell culture HBV infection systems and the development of specific and quantitative methods to analyze HBV cccDNA have been instrumental for undertaking cccDNA-focused studies. In the last years host factors regulating cccDNA formation and expression have been identified. In my talk I will review some of the latest advances in the regulation of cccDNA formation and expression by host factors, including our contributions to the discovery of new HBV restriction factors. This work was supported by SAF2016-75169-R and PID2020-118970RB-100 grants to U.G. and FPU17/03424 fellowship to A.G.M.

SPL4.4 MR-HVI
In vitro selection of RNA and DNA aptamers against hepatitis C virus core protein: applicability to ultrasensitive viral diagnosis and therapy

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Aptamers are short, single-stranded nucleic acids (RNA or ssDNA) that can bind to their targets, from small molecules to cells and tissues, with high affinity and specificity. They are selected by an *in vitro* process termed SELEX. Aptamers have emerged as promising alternatives to antibodies and they currently are key biological recognition elements for biosensing, disease diagnosis and therapy. Hepatitis C virus (HCV) causes chronic hepatitis, which can

progress to fibrosis, cirrhosis and hepatocellular carcinoma. Current diagnostic tests are mainly based on serological assays that detect anti-HCV antibodies produced by the infected patient, and on molecular assays that quantify viral genomic RNA in plasma or serum. However, faster, cheaper and more sensitive analytical tools are needed for viral diagnosis. HCV core is a multifunctional protein whose main function is to form the viral capsid that surrounds and protects the genomic RNA. Moreover, as core is the least variable of all the HCV-coded proteins, it has been proposed as an attractive target for developing new HCV-specific binding molecules, including aptamers. In this work, ssDNA and RNA aptamers have been selected *in vitro* against six variants of the HCV core protein belonging to genotypes 1 to 4. The individual aptamers present in the final, enriched populations were analysed by either Sanger sequencing or Ultra-Deep Sequencing (UDS, Illumina). Their affinity constants (*Kd*) were quantified by colorimetric ELONA and ELONA-qPCR (for DNA aptamers) or ELONA-RTqPCR (RNA aptamers). A detailed bioinformatics analysis showed that the DNA aptamer AptD-1312 was highly represented in all selection processes. This pangenotypic aptamer exhibited one of the highest affinities (with *Kds* in the low nanomolar range) and specificities for the HCV core protein. AptD-1312, together with the high-affinity aptamer AptD-1932, was assayed in Huh-7.5 reporter cell lines infected with HCV, where they decreased both the viral progeny titer and the extracellular HCV RNA level (Torres-Vázquez *et al., 2022, J.Mol.Biol.*). This suggests their clinical applicability as antiviral drugs. In addition, AptD-1312 was used as a biosensing probe in a novel graphene-based aptasensor that detects HCV core protein in human plasma in the attomolar range (Palacio *et al., under review*).

SPL4.5 MR-HVI

Is hepatitis delta virus infection underdiagnosed due to suboptimal screening methods? Exposing a hidden problem

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Hepatitis D is caused by the hepatitis delta virus (HDV), which needs the hepatitis B virus (HBV) surface antigen (HBsAg) to entry into hepatocytes and spread between hosts. HBV-HDV infection is considered the most severe form of chronic viral hepatitis; however, cases with a benign outcome have also been described. Only interferon alpha, which poorly controls this infection, has been recommended as off-label therapy by major scientific societies. The particular characteristics of HDV genome, related to plant viroids, make it difficult to find therapeutic targets against this virus: HDV is the smallest known virus capable of infecting animals, with a circular 1.7-Kb single-stranded negative-sense RNA genome with a high degree of self- complementarity (>70%), leading to a rod-like secondary structure. This genome only shows a single open reading frame encoding 2 forms of the structural HDV antigen (HDAg), S- and L-HDAg. Therefore, this virus is fully dependent on host polymerases, particularly the RNA polymerase II, for replication and transcription. Still, viral genomes coexisting in the same host show a high genetic diversity, giving rise to a quasispecies. In recent years, advances in knowledge of HBV and HDV replicative cycles have allowed development of several new approaches to HDV therapy. However, some unmet needs can hamper the effectiveness of these therapeutic approaches to control this infection. One of the most important needs in clinical management of HDV infection is the development of standardized virological methods to diagnose and monitor the infection. Another important need is the universal testing for anti-HDAg antibodies (anti-HDV) in HBsAg-positive individuals, as recommended by European and Asian-Pacific guidelines on the management of HBV infection, to avoid under-di-

agnosis of HDV infection. The impact of applying universal anti-HDV testing has been recently assessed in a pilot study in hospital and primary care HBsAg-positive population from Barcelona. As a result, during 1 year of study, the anti-HDV detection increased by 5-fold relative to the previous 3-year period, illustrating the need for screening all HBsAg-positive population, to implement successful HDV screening and treatment programs.

SPL4.6 MR-HVI

Specific plasma microRNA signatures underlying the clinical outcomes of hepatitis E virus infection

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Hepatitis E virus (HEV) is a major cause of acute viral hepatitis worldwide due to the large outbreaks and epidemics associated to genotypes 1 and 2 in developing countries, and the emergence of foodborne zoonotic infections caused by genotypes 3 and 4 in industrialized countries. HEV infections have also been associated with extrahepatic manifestations and can induce chronic hepatitis, cirrhosis, and liver failure in immunocompromised patients, but host and viral factors influencing the clinical outcome of HEV infections are unknown. There is increasing evidence that viruses de-regulate the expression and/or secretion of microRNAs, small non-coding RNA molecules that play critical roles in regulating a wide range of cellular functions in both biological and pathological processes. Consequently, viral infections are associated with changes in the abundance of extracellular microRNAs, which may in turn promote viral replication, immune evasion, and pathogenesis. Despite specific microRNA expression profiles have been associated with HEV-induced acute liver failure during pregnancy and chronic infections, miRNA regulation during HEV infection remains poorly understood. Thus, we aimed to identify specific circulating microRNA signatures capable of predicting the clinical outcome of HEV infections. First, we identified potentially HEV-regulated miRNAs by RT-qPCR profiling of 175 miRNAs in plasma of patients with acute hepatitis E (AHE, n=6) and healthy blood donors (BDs, n=6). Then, we measured the abundance of eight differentially expressed microRNAs in plasma of patients with either acute hepatitis E (AHE, n=7) or chronic hepatitis E (CHE, n=6), blood donors with asymptomatic HEV infection (HEV BDs, n=9), and healthy blood donors (HEV RNA-negative blood donors presenting anti-HEV IgG antibodies, n=10; HEV RNA-negative blood donors without detectable anti-HEV antibodies, n=12). The differences observed between HEV-infected groups and healthy controls demonstrate that HEV infection leads to altered microRNA signatures in plasma. Furthermore, the differences observed between the microRNA profiles of HEV patients and asymptomatic HEV BD suggest that different microRNA regulation may influence the clinical outcome of HEV infections and could be used as potential prognosis biomarkers. Unveiling the role of these HEV-regulated microRNAs and their targets during the course of infection will also improve our understanding of HEV-induced pathogenesis.

SPL5 VEM

SPL5.1 VEM

Enveloped viruses show increased propensity to cross-species transmission and zoonosis

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Cross-species viral transmission and zoonosis depend on complex ecological and demographic processes, but also potentially on viral features. Here we exploit newly available virus-host datasets to test the association between the ability to infect multiple host species and general viral features, including the nature of the genetic material (RNA/DNA), genome size, segmentation and polarity, presence of an envelope, mode of entry, and viral replication compartment, controlling for research effort. We found that, across viral families, the presence of an envelope is the feature most strongly associated with the ability of a virus to cross the species barrier and become zoonotic. We discuss possible mechanisms by which viral envelopes might contribute to extending the host ranges of viruses. These findings have implications for the management and predictability of emerging viral diseases.

SPL5.2 VEM
MERS-CoV ORF4b is a virulence factor involved in the inflammatory pathology induced in the lungs of mice

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Middle East respiratory syndrome coronavirus (MERS-CoV) is an emerging virus that causes severe pneumonia in humans. MERS-CoV was first identified in Saudi Arabia in 2012. Since then, MERS-CoV has led to sporadic zoonotic infections transmitted from camels to humans, with the risk to become a pandemic virus. Its high mortality rate (~37%) and prevalence in dromedary camels have created an urgent need for the development of effective vaccines or specific antiviral drugs. MERS-CoV genome encodes five accessory proteins, 3, 4a, 4b, 5 and 8b for which limited information is available in the context of infection. This work describes that gene 4b is a virulence factor *in vivo*, as a deletion mutant of a mouse-adapted MERS-CoV-Δ4b (MERS-CoV-MA-Δ4b) was completely attenuated in a humanized DPP4 knock-in mouse model, resulting in no mortality. Attenuation in the absence of 4b was associated with a significant reduction in lung pathology and chemokine expression levels at 4 and 6 days post-infection, suggesting that 4b protein contributed to the induction of lung pro-inflammatory pathology. The expression of 4b protein in cell nucleus *in vivo* was not relevant to virulence, as deletion of protein nuclear localization signal led to 100% mortality. The presence of 4b protein regulated autophagy in mice lungs, leading to upregulation of BECN1, ATG3 and LC3A mRNA expression. Further analysis in MRC-5 cells showed that during MERS-CoV-MA infection 4b inhibited autophagy, as confirmed by the increase of p62 and the decrease of ULK1 protein levels. Together, these results showed a correlation between autophagy activation in the absence of 4b, and downregulation of an inflammatory response, contributing to attenuation of MERS-CoV-MA-Δ4b.

SPL5.3 VEM
SARS-CoV-2 surveillance in the air: a long term study in education centres

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Aerosols produced by an infected patient, as well as small droplets, have been established as the main transmission pathway of SARS-CoV-2. However, few studies describing reliable detection of viruses in the air, especially in non-hospital places, have been published. Moreover, studies evaluating the transmission level of different variants of SARS-CoV-2 in infants and toddlers are lacking. We present a study performed between June 2021 and May 2022 in 8 centres from 4 different educational levels: nurseries, elementary schools, high schools and university centres. Airborne viruses were captured for a few hours with an air sampler developed by CSIC, consisting of an air pump coupled to filters, in different locations of the education centres. The sampling times were coincident with different waves of incidence in Madrid, Spain. Among the sampled locations, we prioritized classrooms and dining rooms for students and/or teachers. The indoor CO2 concentration was also monitored every 5 minutes, along with tempera-

ture and relative humidity. After RNA isolation from the filter, specific identification of SARS-CoV-2 was performed in triplicate by RT-qPCR specific for the nucleoprotein gene. A total of 1053 air samples have been collected and analysed. Obtained Cq ranged from 27 to 39, and a synthetic SARS-CoV-2 RNA was used for calibration. We observed a relevant increase in the level of copies of viral RNA by m3 of air in January 2022, when SARS-CoV-2 *Omicron* started to be the prevalent variant in Spain. In May 2022, just after the removal of the masks indoors, we also detected high amounts of virus in the air. Detection of SARS-CoV-2 in aerosols was higher in centres with 0-3 and 3-12 years- old students, corresponding to the age range of non-vaccinated people. We were able to detect very relevant levels of SARS-CoV-2 in the environment in a coincident moment of confirmed positive cases of COVID-19 among the centre's staff. Finally, we sequenced the complete genome of SARS-CoV-2 captured from the air in the filters by an amplicon-based protocol (ARTIC). This study illustrates the possible application of airborne virus capture methodology as a tool for monitoring virus presence and anticipate viral outbreaks.

SPL5.4 VEM
Lyophilized homodimers of the RBD domain of spike protein as vaccine against SARS-CoV-2

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The unprecedented research carried out to counteract COVID-19 pandemics, has resulted in the development of efficacious vaccines, most of them based on the full-length spike (S) protein (420 kDa) of SARS-CoV-2 as antigen. However, few approaches have resorted to using the S protein's receptor binding domain (RBD) as a vaccine candidate. The RBD is an essential target to eliciting protective immune responses against SARS-CoV-2. Additionally, its reduced low molecular mass bestows RBD (29 kDa) relevant potential advantages, i.e. reduced large-scale production costs and improved long-term stability for the development of second generation of COVID19 subunit vaccines. Accordingly, we have expressed two RBD versions (RBD1 and RBD2) in insect cells, using the baculovirus system. Both recombinant RBD polypeptides are secreted to the extracellular medium, from where they are efficiently purified applying two subsequent chromatographic steps, i.e. affinity chromatography followed by size exclusion chromatography. Expression of both recombinant proteins results in the production of a combination of dimer/monomer RBD molecular species. Interestingly, the relative abundance of the dimeric species is significantly higher following RBD1 expression. Immunogenicity analyses carried out in mice showed that anti-RBD antibody titer induced by RBD1 is considerably higher than those recorded after immunization with RBD2. This indicates the existence of a positive correlation between RBD homodimerization and immunogenicity. The consistent induction of high SARS-CoV-2 neutralizing antibodies titers in RBD1-immunized mice led us to further explore the potential of this polypeptide as a vaccine candidate. We have developed a pre-industrial process leading to the production of stable, lyophilized, pure RBD1 protein lacking recombinant tags. To analyze RBD-1 protection potential, groups of transgenic K18-hACE2 mice were immunized intramuscularly at 21 days intervals, with three doses of lyophilized RBD1 (50 μg each). 18 days after the last boost, mice were challenged with a lethal dose (2x104) of SARS-CoV-2 (Wuham strain). Immunization with RBD1 induced a complete protection (100%) of challenged animals. Additionally, RBD1-immunzed mice did not show clinical signs of disease nor body weight reduction after virus challenge. This work is supported by PTI Salud Global. grant: SGL2103058.

SPL5.5 VEM

Extreme attenuation of a mutagenized variant of Rift Valley fever virus (RVFV): towards a safer live attenuated vaccine

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Vaccination is the most efficient measure to prevent and control infectious diseases. Live-attenuated vaccines (LAVs) are particularly efficient since they usually induce strong and long-term protective immune responses after a single shot. On the other hand, the risks associated to viral replication may restrain their use in certain conditions. Therefore, it is important to improve the safety of attenuated vaccines while keeping good levels of immunogenicity. Rift Valley fever (RVF) is a good example for improving the existing live-attenuated vaccines. RVF is a zoonotic viral disease affecting several species of domestic and wild ruminants characterized by massive abortions, stillbirths and congenital malformations in the affected flocks. RVF outbreaks cause high economic losses as well as dozens of human deaths in different geographical areas in Africa, where it is endemic. To date the only measure that could help to prevent the disease is vaccination in RVF risk areas, and a few live-attenuated vaccines of veterinary use that are commercially available in Africa constitute excellent candidates for successful immunization programs. However, the risk of reversion and / or the presence of residual virulence limits their use in immune compromised hosts, especially in pregnant sheep. In previous studies we obtained under selective mutagenic pressure a novel RVFV variant that was termed 40Fp8. This virus showed an extremely attenuated phenotype in both wild-type and immunodeficient IFNAR-/- mice, but was still able to induce protective immunity after a single inoculation, including target livestock species (sheep), thus supporting the use of 40Fp8 as a safe, live attenuated vaccine. To further explore the potential of 40Fp8 as a safer vaccine candidate, in this work we have tested its virulence in the extremely sensitive model of IFNAR-/- mice upon intranasal administration, as well as its genetic stability in vivo. Our results confirm the higher attenuation of 40Fp8 compared to other live-attenuated RVF vaccine prototypes, and offer some insights into the dynamics of the potential attenuating mutations in the absence of efficient immune responses.

SPL6 COV

SPL6.1 COV

Preclinical characterization of the SARS-CoV-2/COVID-19 vaccine candidate MVA-CoV2-S: Robust immunogenicity and full efficacy against SARS-CoV-2 in mice, hamsters and rhesus macaques

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Novel vaccines with wide and long-term action against SARS-CoV-2 are needed to control COVID-19 pandemic and the emergence of variants of concern (VoCs). Thus, we generated a COVID-19 vaccine candidate based on the modified vaccinia virus Ankara (MVA) vector expressing a full-length SARS-CoV-2 spike (S) protein (termed MVA-CoV2-S), and we analyzed its immunogenicity and efficacy in several animal models (mice, hamsters and rhesus macaques). In C57BL/6 mice, MVA-CoV2-S induced robust, broad and polyfunctional adaptive and long-term memory S-specific humoral and T-cellular immune responses, even at 6 months after immunization. Remarkably, one

and two doses of MVA-CoV2-S protected K18-hACE2 transgenic mice from a lethal dose of SARS-CoV-2, preventing virus replication in the lungs, reducing lung pathology, and diminishing levels of pro-inflammatory cytokines. High titers of IgG antibodies against S and receptor-binding domain (RBD) proteins and of neutralizing antibodies were induced against parental virus and VoCs, correlating with protection. Similar SARS-CoV-2-specific antibody responses were observed at prechallenge and postchallenge in the two-dose regimen, while the single-dose treatment did not avoid vaccine breakthrough infection. All vaccinated mice survived infection and were also protected from SARS-CoV-2 reinfection. In hamsters, immunization with one or two doses of MVA-CoV2-S also elicited high titers of S- and RBD-binding IgG antibodies and neutralizing antibodies against parental SARS-CoV-2 and several VoCs. After SARS-CoV-2 challenge, vaccinated hamsters showed also a strong reduction of viral RNA, infectious virus and lung histopathology compared to control group. Finally, in rhesus macaques, two doses of MVA-CoV2-S were well tolerated and induced S and RBD-binding IgG antibodies and neutralizing antibodies against SARS-CoV-2 and several VoCs. S-specific IFNγ, but not IL-4, -producing cells were also elicited. After SARS-CoV-2 challenge, vaccinated macaques showed a significant strong reduction of virus loads in bronchoalveolar lavages and decreased levels in throat and nasal mucosa. Remarkably, MVA-CoV2-S also protected macaques from fever and infection-induced cytokine storm. Moreover, computed tomography and histological examination of the lungs showed reduced lung pathology in vaccinated animals. The robust T-cell and humoral immunogenicity and full efficacy induced by MVA-CoV2-S in several animal models supports its use as a potential vaccine for SARS-CoV-2/COVID-19 in human clinical trials.

SPL6.2 COV

Frequency of defective genomes in Omicron differs from that of the Alpha, Beta and Delta variants

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The SARS-CoV-2 Omicron variant emerged showing higher transmissibility and possibly higher resistance to current COVID-19 vaccines than other variants dominating the global pandemic. In a March 2020 study performed in clinical samples, we found that a portion of genomes in the SARS-CoV-2 viral population accumulated deletions at the S1/S2 cleavage site (PRRAR/S) of the *spike* gene, generating a frameshift and appearance of a premature stop codon.

The main aim of this study was to determine the frequency of defective deletions in prevalent variants from the first to sixth pandemic waves in our setting and discuss whether the differences observed might support epidemiological proposals. The complete SARS-CoV-2 spike gene was deeply studied by next-generation sequencing using the MiSeq platform. More than 90 million reads were obtained from respiratory swab specimens of 78 COVID-19 patients with mild infection caused by the predominant variants circulating in the Barcelona city area during the six pandemic waves: B.1.5, B.1.1, B.1.177, Alpha, Beta, Delta, and Omicron. The frequency of defective genomes found in variants dominating the first and second waves was similar to that seen in Omicron, but differed from the frequencies seen in the Alpha, Beta and Delta variants. Our results support the notion cited in epidemiological reports that Omicron did not emerge from continuous evolution of the Alpha, Beta or Delta variant.

SPL6.3 COV
Antiviral immune responses, cellular metabolism and adhesion are differentially modulated by SARS-COV-2 ORF7a and ORF7b

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The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of the ongoing coronavirus disease 2019 (COVID-19) pandemic that is a serious global health problem. There is an urgent need to better understand the molecular mechanisms governing SARS-CoV-2 pathogenesis, as this will have implications for the design of better treatments and vaccines. SARS-CoV-2 possesses eleven accessory proteins encoded in its genome and some of them have been involved in facilitating infection and pathogenesis by their interaction with cellular components. Among these proteins, accessory protein ORF7a and ORF7b functions have not been fully comprehended. In this study, A549 human epithelial cells were transduced to express ORF7a and ORF7b, respectively, to explore more in depth the role of each accessory protein in the pathological manifestation leading to COVID-19. Using transcriptomic combined with bioinformatic analysis and functional assays we showed that individually expressed ORF7a and ORF7b are sufficient to alter lung cells creating conditions more favorable for SARS-CoV-2. Overexpression of ORF7a or ORF7b induce alteration of metabolic cascades via UGT1A9, PTGS2 and CYP1A1; interferon responses via OASL, IFIT1 and IFIT2; inflammation via IL-8, IL-11 and CXCL1 and cell adhesion via ICAM-1, ZO-1 and γ-catenin. Thus, in light of these results, it is plausible to speculate that ORF7a and ORF7b could be targeted by new therapies or used as future biomarkers in this pandemic.

SPL6.4 COV
Antibody Immunological Imprinting in COVID-19 Patients

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There has been a great effort to monitor specific serologic responses after SARS-CoV-2 infection and vaccination, however the level and clinical consequences of pre-existing immunity, immunodominance and antigenic hierarchy of human coronaviruses (HCoVs) remains unclear. In addition to SARS-CoV-2, humans are also susceptible to other six coronaviruses, for which consecutive exposures are frequent. Here, we investigated the role of pre-existing immunity and immunological imprinting on COVID-19 patients' antibody response. A comprehensive longitudinal antibody profiling against SARS-CoV-2 and seasonal HCoVs (alpha- 229E; and beta- OC43) was performed, including characterization of antibodies against full-length spike (S), the receptor binding domain (RBD)/S1 and S2 domain. Our results showed that immune memory recall to conserved regions of the S protein of beta- HCoVs can interfere with the newly induced immune response to SARS-CoV-2 upon infection in adults. This phenomenon –termed immune imprinting– led to delayed antibody responses against variable but immunodominant domains of SARS-CoV-2 S protein, such as the RBD, versus the conserved S2 domain. Next, we explored the role of immune imprinting upon COVID-19 infection according to age in a pediatric cohort. Eighty–eight children were enrolled. Of these, 13 (15%) were infants (0-1 years) and 75 (85%) were children and adolescents (>1 years-19.9 years). All patients developed detectable levels of antibodies against SARS-CoV-2 antigens that remained stable at post-convalescent phase. However, infants showed higher levels of induction compared older children. Antibody profiling against seasonal HCoVs showed preexisting and detectable levels in all pediatric patients in an age-dependent manner. However, a significant back-boosting effect was noted only for the beta- HCoVs OC43 S2 domain upon SARS-CoV-2 infection. Interestingly, no increase was detected overtime for patients <1 year, suggesting that these are maternal antibodies. Importantly, a negative correlation was also found between IgG responses against SARS-CoV-2 antigens and pre-existing immunity to HCoVs-OC43 in children and adolescents. Our results demonstrate that primary infection with HCoVs can occur early in childhood. Infection with beta-HCoV results in imprinting of B cells specific for the S protein and this correlates with reduced induction of specific antibodies against variable domains of the SARS-CoV-2 S protein upon SARS-CoV-2 infection.

SPL6.5 COV
Genetic diversity of SARS-CoV-2 outbreaks in farmed minks in Spain

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After the onset of the pandemic by SARS-CoV-2, mink susceptibility to the virus have been well reported. During season 2020-2021, were received 129 extracts of respiratory samples from 20 farm outbreaks for sequencing and genomic analysis. In order to obtain the complete genome, was performed an in-house approach that combine SISPA amplification with ARTIC protocol. With the aim to complete the sequences of the main antigen, an amplification protocol was performed with specific primers targeting spike protein. The measure of the binding kinetics of the interaction between mink's spike protein and different ACE-2 receptors was obtained using Biacore technology. According to antigenic characterization, the impact of the mink's spikes were measured with pseudovirus neutralization and with a competition for receptor assay. In this work, we've obtained 59 complete SARS-CoV-2 sequences plus 6 complete spikes belonging to 5 different lineages. Phylogenetic analysis of the complete genome sequences from minks supported the clustering of the sequences by lineage. The genomic analysis protein by protein of the sequences revealed changes in two relevant regions: papain-like protease nsp3 in ORF1ab polyprotein and spike protein. The papain-like protease nsp3 presented a high number of non-synonymous mutations, highlighting the

mutations W1190C and G1822R present in several mink genomes but rare in human sequences. These changes had structural impact in the domain that could affect viral replication. Spike's genomic analysis from different outbreaks, showed mutations in relevant antigenic sites as Y453F and Y453F plus M869T in the lineage B.1.1777 or N501T and a combination of three mutations (F486V, N501T, D796H) in lineage B.1. These changes were not previously associated to those lineages and were also present in different variants of concern. Due to the location of these changes in the receptor binding site, was performed a functional analysis of these spikes revealing an increased affinity for mink's ACE-2 receptor, proving the adaptive to host properties of these mutations. According to the antigenic characterization, these spikes hadn't a significant impact in vaccinated people serum. The spread of SARS-CoV-2 in farmed mink is a potential environment where the rapid transmission can facilitate the emergence of new variants.

SPL7 MR-VAC

SPL7.1 MR-VAC
A Newcastle-disease virus based vaccine for COVID-19

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Vaccines against SARS-CoV-2 have been made in a record time. However, although the vaccines that have been deployed have clearly reduced the percentage of severe infections in countries where they have been widely used, many developing countries have problems to afford the purchase and/or distribution of approved vaccines. In addition, current vaccines provide with limited mucosal immunity to appropriately prevent infections. New efficacious and safe SARS-CoV-2 vaccines that could be manufactured locally in developing countries, that would not require ultra-congelation and that would induce infection-preventing mucosal immunity are desirable. We have been working in the development of such a vaccine based on a Newcastle disease virus (NDV) vector. This avian virus is widely used as live and inactivated vaccine in poultry against Newcastle disease, a potentially devastating avian disease. NDV inoculation in mammals results in abortive infection due to the induction of a robust local innate immune response that inhibits further virus replication while potentiating the induction of adaptive immune responses. NDV vaccines are manufactured using the same technology as most of the influenza virus vaccines, making it possible its production in most developing countries. We have generated NDV-based vaccines expressing a stabilized version of the S protein of SARS-CoV-2. These vaccines are stable at 2 degrees and provide protection against SARS-CoV-2 and its variants in animal models. NDV-based vaccines not only express the vaccine antigen in infected cells, but also incorporate the antigen into the virion, making it possible the use of either live or inactivated versions of the vaccine. Results from phase I clinical trials demonstrated lack of severe adverse events and very mild reactogenicity, as well as the induction of a robust T cell and neutralizing antibody response against SARS-CoV-2. Additional clinical trials have been initiated using live intranasal administration which has the potential to generate a more potent mucosal immunity for prevention of not only disease but also asymptomatic infections and transmission.

SPL7.2 MR-VAC
RNA replicons as human pathogenic coronavirus recombinant vaccines

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Coronavirus (CoV) transcription mechanism is based on a high frequency recombination process based on sequence identity what may help the generation of virus variability, facilitating CoV crossing of species barrier and zoonosis. In the case of the Middle East Syndrome CoV (MERS-CoV) and the acute and severe respiratory syndrome CoV-2 (SARS-CoV-2) that emerged in 2012 and 2019, respectively, their pathogenicity mechanism has been clarified,

and the origin of these viruses has already been identified as the camels and the racoon dogs, respectively. The pathogenicity of SARS-CoV-2 has been directly linked to a proteolytic cleavage of its spike (S) protein by furine protease, present in most tissues of human body. The development of a vaccine for pathogenic human CoVs designed in our laboratory is based on the construction of RNA replicons derived from CoV genomes. These vaccines are based in a self-amplifiable RNA propagation defective, highly safe replicon expressing several virus proteins. The replicon was administered through the intranasal route to promote respiratory mucosal immunity. The resulting vaccine candidates elicited sterilizing immunity in humanized transgenic mice susceptible to virus infection. This type of self-replicating RNAs were constructed in our laboratory first for MERS-CoV and then, this concept was applied to SARS-CoV-2, by the insertion of a set of gene deletions in the CoV genome, significantly different from that used in the case of MERS-CoV vaccine candidate. These replicons are now been evaluated in alternative animal models.

SPL7.3 MR-VAC
Development of the MVA-CoV2-S vaccine by the CNB-CSIC and advantages against COVID-19

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The COVID-19 pandemic caused by SARS-CoV-2 is one of the major disasters in human health with more than 6 million deaths worldwide, and still two years later the spread of the virus continues unabated in spite of a major human vaccination program. While vaccines helped to prevent hospitalization and deaths, still there is a major limitation in the availability of those vaccines in poor countries, making it difficult to achieve the virus control. In addition, current vaccines do not prevent primary infection of the respiratory tract and hence transmission occurs with high incidence even in three-dose vaccinated individuals. Moreover, the emergency of variants of concern (VoC) more resistant to current vaccines represents a strategic problem in vaccination and virus eradication. Our group at CNB-CSIC started with the development of a vaccine candidate soon after the complete genome sequence of the SARS-CoV-2 was announced early in January 2020. Within a short time and using the poxvirus platform that we previously applied in the development of vaccine candidates against other pathogens (ebola, chikungunya, zika, hepatitis C, HIV-1, malaria and leishmania), the group generated the vaccine candidate MVA-CoV2-S with the spike (S) protein in two forms, either wild type or prefusion-stabilized. Preclinical studies in three animal models (mice, hamsters and macaques) demonstrated the potent immunogenicity and efficacy of this type of vaccine, with high titers of neutralizing antibodies against VoC, induction of specific T cell responses, reduction of tissue damage and of cytokine storm, all markers of effectiveness of the vaccine. All of these findings have been published in a series of articles, representing one of the most complete preclinical studies of any COVID-19 vaccine, and attest the safety, immunogenicity and efficacy record of the poxvirus MVA platform for further clinical studies. The advantages of this type of vaccine will be discussed.

SPL7.4 MR-VAC
Development of the PHH-1V vaccine against COVID-19

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Despite the efforts of SARS-CoV-2 mass vaccination programs, the situation is far from being controlled. Only 66.7% of the world population, and, more starkly, 20.2% in low-income countries have received at least one dose of a COVID-19 vaccine. Moreover, due to immunity waning with time after both vaccination and/or infection, boost immunization is crucial to control the SARS-CoV-2 infection rate and avoid saturation of health services. Accordingly, access to second-generation vaccines with a broader variants scope and longer duration protection is required. HIPRA has developed a vaccine, PHH-1V, based on a recombinant fusion heterodimer protein with the sequences of the RBD of two SARS-CoV-2 variants. In preclinical studies, the PHH-1V vaccine has shown a good safety profile

and immunogenicity as a primary immunization in different species. In the challenge studies conducted in hamsters, K18-hACE2 mice and cynomolgus macaques, PHH-1V has demonstrated efficacy as a primary immunization. The interim results of Phase IIb clinical trial in humans show that PHH-1V, as a heterologous booster immunization, elicits a non-inferior neutralizing antibody response to SARS-CoV-2 delta variant, a superior neutralizing antibody response against beta variant and, most importantly, a superior neutralizing antibody response against the omicron BA.1 variant of concern (predominant during the development of the trial) at 14 days post-vaccination when compared to the BNT162b2 booster. Results have also shown that PHH-1V induces a significant increase of CD4+ (even higher compared with the BNT162b2 booster) and $CD8^+$ T-cells expressing IFN-γ on day 14 when used as a heterologous booster. Concerning the safety profile, PHH-1V group had less percentage of adverse events compared with BNT162b2 group, with most of mild intensity, and similar percentage of non-severe COVID-19 cases. Preliminary results of the ongoing Phase III clinical trial confirm the boost effect when the PHH-1V vaccine is administered after a primary vaccination with ChAdOx1 or mRNA-1273, eliciting a strong neutralizing antibody response against SARS-CoV-2 variants (including omicron BA.4/BA.5) and a balanced T-cell response. These results indicate that PHH-1V aims to enrich the COVID-19 vaccines portfolio by providing a safe and effective heterologous boost, meaning a significant contribution to the coming immunization programs and campaigns.

SPL8 RIN

SPL8.1 RIN

Modulation of Innate Immune Responses by Dengue Virus in Primary Human Systems.

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Dengue virus (DENV) belongs to the Flaviviridae family and is endemic in more than 120 countries in the world. There are 4 different serotypes that co-circulate and can cause annual epidemics in tropical and subtropical areas of the world and are transmitted by aedes.sp mosquitoes. We and others showed that DENV can efficiently inhibit the generation of innate immune responses in infected cells by blocking both the production and signaling of type I interferons (IFN) in susceptible cells, including dendritic cells (DCs). We found that the DENV protease complex (NS2B3) could mediate the evasion of innate immunity in those cells and others by targeting and inducing the degradation of different innate immune factors, including cGAS and STING, which results in the inhibition of type I IFN production and of antiviral responses. Moreover, we showed that leakage of mitochondrial DNA (mtDNA) induced during DENV infection can trigger the activation of the DNA sensing cGAS/STING pathway of type I IFN production. Recent studies in our lab suggest that other arboviruses, such as chikungunya virus (CHIKV) may also target the cGAS-STING pathway. Using primary human cells, such as PBMCs and dendritic cells (DCs), we have shown that different DENV serotypes can induce different immune profiles in infected primary cells and that infected and bystander cells both contribute to the overall immune response in DENV infection by secreting different cytokines and chemokines. Our multi-dimensional analysis of PBMCs and DCs infected with DENV-2 or DENV-4 revealed distinct infection kinetics and immune profiles in those cells that may contribute to pathogenesis and transmission of these viruses. More recently, we have optimized human tonsillar histocultures (HC) a model system to study modulation of immune responses by DENV infection and vaccination and other pathogens. Funding: NIH/NIAID: 1R01AI07345, R21AI116022, 1U19AI118610; DoD/DARPA: HR0011-11-C-0094.

SPL8.2 RIN

Elucidating the complex cellular response associated with cross-protection against African swine fever virus

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African swine fever (ASF) pandemics is currently causing enormous economic losses to the swine industry worldwide. Lack of treatments hampers its control, and the insufficient knowledge regarding the immunological mechanisms underlying protection hinders rational vaccine design. Despite biosafety concerns have delayed their implementation in the field, live attenuated viruses (LAVs) induce a broad protective immunity against ASF virus (ASFV)

experimental homologous challenges, thus becoming useful tools to analyze ASFV-specific immune responses. We previously generated a LAV vaccine prototype consisting of a deletion mutant lacking the CD2v, namely BA71ΔCD2, which confers protection against both homologous and heterologous challenge infection. Aiming to gain better insight into the immune response involved in protection against ASFV, we characterized systemic and local *in vitro* recall responses induced in circulating and lymph node cells from BA71ΔCD2 intranasally vaccinated pigs. To do so, we performed bulk gene expression analysis and single-cell RNA-sequencing (scRNA-seq), and the most relevant results from transcriptomic data were further validated by flow cytometry. Gene expression analysis of PBMC revealed a robust Th1 response in cells from BA71ΔCD2-vaccinated pigs. Concomitant with this adaptive immune response, we also distinguished an innate immune response marked by the expression of macrophage-related inflammatory genes. Immunophenotyping by flow cytometry demonstrated the presence of polyfunctional CD4+CD8+ memory T cells, and an IFNγ-dependent inflammatory response mediated by TNF-producing macrophages. scRNA-seq analysis of submandibular LN cells validated and extended these results, uncovering an overrepresentation of cytotoxic CD8+ T cells in the vaccinated animals. Indeed, flow cytometry analysis of perforin-expressing PBMC revealed the presence of cytotoxic CD4+CD8+ and γδ memory T cells as well as increased levels of nonspecific cytotoxic CD8+ T and NK cells in vaccinated animals after in vitro stimulation with ASFV. Altogether, this study allowed elucidating the complex cellular response associated with cross-protection against ASFV. Our findings represent a step forward in the understanding of ASF immunology and provide important clues on the functional immune mechanisms that should be considered to more rationally design future ASF vaccines.

SPL8.3 RIN
COMPREHENSIVE IMMUNE PROFILING OF IN VIVO BLUETONGUE VIRUS INFECTION REVEALS THAT THE VIRUS MEDIATES AN ACUTE IMMUNOSUPPRESSION OF T CELLS

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Bluetongue virus (BTV) is the prototypical Orbivirus that belongs to the *Reoviridae* family. BTV is transmitted by the bite of infected *Culicoides* midges and affects domestic and wild ruminants producing great economic losses. The infection induces an IFN response in the host, followed by an adaptive immune response that plays a critical role in disease clearance. There is nonetheless evidence that BTV can impair IFN and humoral responses. The main goal of this study was to gain a more detailed understanding of BTV pathogenesis and its effects on immune cell populations. To this end, we combined flow cytometry and transcriptomic analyses of several immune cells at different times post-infection (pi). Four sheep were infected with BTV serotype 8 and blood samples collected at days 0, 3, 7 and 15pi to isolate peripheral blood mononuclear cells. Transcriptomic analysis of B-cell marker+, CD4+, CD8+, and CD14+ sorted cells showed that the maximum number of differentially expressed genes occurred at day 7pi, which coincided with the peak of infection. KEGG pathway enrichment analysis in B-cell marker+, CD4+, and CD14+ cells indicated that genes belonging to virus sensing and immune response initiation pathways were enriched and day 3 and 7 pi. Concomitant to this pathway enrichment, we observed an increase in the percentage of non-classical CD14+ CD16+ monocytes, indicating that these cells could be important in the antiviral response. ELISpot assays showed that T cell responses to BTV became detectable by day 15pi. Curiously, T cell exhaustion pathway was enriched in CD4+ cells at day 7pi, while CD8+ cells showed downregulated immune response initiation pathways at this timepoint. We therefore assessed T cell functionality during the course of the infection. ELISpot assays and intracellular cytokine staining demonstrated that, at day 7pi, BTV impaired CD4+ and CD8+ T cell responses to the mitogen concanavalin-A and the potent activation cocktail of PMA and ionomycin. These data indicate that BTV produces an acute inhibition of T cell activation at the peak of replication. These findings identify several mechanisms in the interaction between host and BTV, which could help develop better tools to combat the disease.

SP8.4 PVI

The Arabidopsis m6A readers ECT2, ECT3 and ECT5 restrict infection of alfalfa mosaic virus

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Methylation of N^6-adenosine (m^6A) is a post-transcriptional modification that influences the fate of their RNA targets, mainly through the binding of m^6A *readers*. Recent reports have empathized the key role of this post-transcriptional modification in the infection cycle of an increasing number of mammalian viruses. However, research about m^6A in plant viruses is in its infancy and we are now beginning to understand the regulatory mechanisms of this modification. In previous work, we identified the presence of m^6A in the RNAs of two plant viruses and found that the relative abundance of m^6A in alfalfa mosaic virus (AMV) RNAs regulates viral infectivity. Furthermore, we showed that the demethylase activity of ALKBH9B modulates this viral process, probably via its interaction with the AMV CP. Here we analyze the involvement of Arabidopsis m^6A *readers* in the infection cycle of a plant virus. Consistent with the previously observed m^6A-dependent antiviral effect, we find that, in Arabidopsis plants, the absence of the ECT2/ECT3/ECT5 module promotes the systemic infection of AMV. Furthermore, an ECT2 point mutant specifically defective in m^6A recognition loses wild type antiviral activity, suggesting that this effect, at least for ECT2, relies on the capability of this *reader* to interact with m^6A sites. HyperTRIBE (targets of RNA-binding proteins identified by editing) using transgenic plants expressing either WT or point mutant ECT2 corroborates the in vivo interaction between this protein and AMV m^6A-RNAs through its m^6A binding site. Finally, we show that the expression of the ECT2/ECT3/ECT5 module reverts the partial resistance against the infection conferred by the absence of ALKBH9B, and that this hitherto defensive mechanism seems to be genetically uncoupled from the role of ECT proteins in organogenesis. Funding: This research was funded by grant PID2020-115571RB-I00 to F.A and V.P. from the Spanish MCIN/AEI/ 10.13039/501100011033 granting agency. M.M.-P. was recipient of a Short-Term Fellowship from the Federation of European Biochemical Societies (FEBS).

SPL8.5 RIN

SUMO modulation of the anti-viral and anti-apoptotic activities of ISG15.

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Interferon (IFN)-stimulated gene (ISG15) is a 15 kDa protein of the ubiquitin-like family of proteins that is part of the antiviral defense program. However, ISG15 also negatively regulates type I interferon signaling. ISG15 exists in different states: conjugated to target proteins, as a free intracellular molecule and as a secreted extracellular protein.

The mechanisms regulating ISG15 status and activities are unclear. Here we evaluated the hypothesis that status and activities of ISG15 can be modulated through its interaction with small ubiquitin-like modifier (SUMO). Our data demonstrate that ISG15 can interact with SUMO both in a covalent and non-covalent manner. SUMOylation of ISG15 is modulated in response to different stimuli including virus infection, suggesting that this modification can contribute to the modulation of virus replication mediated by ISG15. Inhibition of the covalent or non-covalent interaction of ISG15 with SUMO alters the intracellular distribution of the protein and its secretion. Finally, our results reveal that the antiviral and antiapoptotic activities of ISG15 are also modulated by SUMO. In summary, here we demonstrate that ISG15 subcellular distribution, its interaction with other components of the antiviral innate immune response mediated by type I IFN, and its secretion can be controlled by modulating the ISG15-SUMO interplay. These results indicate that SUMO cooperates with ISG15 in the regulation of the innate immune response and cancer.

SPL9

SPL9
Phages, protagonists of a post-antibiotic era

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The indiscriminate use of antibiotics has led to the emergence of multi-drug resistant (MDR) bacteria. In the absence of available treatments, this represents a major threat and poses a challenge in the search of alternative therapies against MDR bacteria. In this scenario, phages, viruses of bacteria, have been proposed as promising biocontrol tools. Phages are the most abundant biological entities in the biosphere. They present multiple advantages as adaptive treatments, such as their ability to evolve, their multiplicity at the site of infection and their high specificity among others. Thus, phages can be used as therapeutic tools against MDR bacteria, but their valuable properties also allow them to be used in prevention and diagnosis, encompassing a myriad of potential uses. It is noteworthy that MDR bacteria are found in all ecosystems, being an emerging concern in many fields, including clinics, livestock and crops. Therefore, the "One Health" approach to monitoring and integrating bacterial diseases control through a comprehensive approach should be considered. Environmental surveillance to determine the spread of resistance in nature, and new biocontrol strategies to reduce the emergence of MDR bacteria at all levels are required. In this view, phages or phage-derived products can be used as eco-friendly alternatives in global health. In conclusion, phages, are nowadays in the spotlight as promising tools to reduce the impact of MDR bacteria worlwide.

SPL10 HRF

SPL10.1 HRF
Viroids: from tiny pathogens to source of knowledge on RNA biology

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Ricardo Flores, who recently passed away, was responsible for fundamental advances in molecular biology and epidemiology of viroids, making a huge contribution to the current knowledge on these infectious agents and on their interaction with plants. At more than fifty years from their discovery, viroids are well known plant pathogens infecting economically relevant herbaceous and woody hosts. The small (230-430 nt), single stranded (ss), circular, non-protein coding RNA genome of viroids has molecular signals to localize in the nucleus or in the chloroplast, where it replicates and accumulates, and to move from cell to cell and systemically in the host plant. Unable to code for proteins, viroids use catalytic activities endowed in their own RNA (ribozymes) or provided by cellular enzymes, some of which are forced to act on the viroid RNA instead of their physiological DNA template/substrate. During the infectious process, viroid RNAs are targets and triggers of RNA silencing, with this sequence-specific degradation mechanism shown to act as a plant antiviroid defense system and, in certain plant-viroid combina-

tions, as the primary molecular lesion leading to the downregulation of host genes involved in the development of macroscopic symptoms.. Altogether these features. make viroids valuable tools to further investigate biology and potential regulatory functional roles of infectious and cellular RNAs. In the past, as well as in the last few years through high-throughput sequencing approaches, we have identified ribozyme-containing viroid-like RNAs likely not directly associated with plants, thus providing indirect evidence that they may exist in other organisms. The identification of hammerhead ribozymes, the typical self-cleaving ribozymes of some viroids, in a large number of DNA genomes of organisms along the tree of life opens interesting evolutionary questions on the origin of these infectious agents. This point will be specifically addressed also taking into consideration the recent identification of self-cleaving ribozymes in the circular ssRNA genome of certain mycoviruses.

SPL10.2 HRF
Plant RNA viruses and satellites: from antagonistic to mutualistic interactions

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Satellites are subviral entities that depend on a helper virus (HV) for their multiplication and transmission. Satellites may depend on the HV for replication, encoding their own coat protein and forming particles (satellite viruses), or for their replication and encapsidation (satellite nucleic acids). Satellites have been mostly, but not only, reported in association with viruses infecting plants. The parasitic relationship between satellites and HVs may result, or not result, on a detrimental effect on the HV replication, thus varying from antagonistic to neutral or even mutualistic. As parasites of viruses satellites pose interesting questions, including what are the conditions for the invasion of, and maintenance in, the populations of the HV of satellites. Here we explore these conditions and if they depend on the nature, antagonisticn or non-antagonsitic, of the interaction. For this we analyse two different biological systems. The first is the interaction between cucumber mosaic virus (CMV), and its satelliteRNA (CMVsatRNA), a small, linear, non-coding RNA. The presence of CMVsatRNA results in a depression of the multiplication of CMV, and on the modulation of CMV symptoms according to the CMV and CMVsatRNA genotype and the host plant species. Thus, virulence of the satRNA for CMV, and of CMV plus or minus CNVsatRNA for the host plan,t vary in complex ways that affect their transmission. Conditions for invasion and survival of CVsatRNA in CMV populations were analysed from the study of their epidemiology, their interaction under controlled conditions, and modelling. The second system is the satellite virus of tobacco mosaic virus (satellite tobacco mosaic virus, STMV) which is nature is found associated with tobacco mild green mosaic virus (TMGMV) in its wild host *Nicotiana glauca*. The interaction between TMGMV and STMV in this host is neutral, STMV not affecting the multiplication and virulence of TMGMV. For both systems, satellites can invade the HV population only under conditions that result in high transmission rates of the HV. This limitation raises questions about satellite evolution and maintenance in virus populations.

SPL10.3 HRF
Hepatitis delta: the ignored diagnosis

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The availability of new treatments for chronic delta hepatitis will represent a turning point in the possibility of eliminating this disease, which has the highest known rates of progression to cirrhosis and hepatocarcinoma. In the WHO European Region, in order to eliminate viral hepatitis by 2030, each country should set its specific goals according to the local epidemiological context, which should be based on the best available data. Nevertheless, preventive measures, under diagnosis and a great heterogeneity in published studies makes it difficult to have a general overview of the current epidemiological situation of hepatitis D in Spain. The leading International Scientific Societies recommend, in their Clinical Practice Guidelines for the management of hepatitis B, systematic screening

or screening by risk groups for HDV in all HBsAg-positive patients. Spanish recommendations propose to implement one-step strategies for the diagnosis of hepatitis D based on reflex detection of Anti-HDV and HDV-RNA(if positive) in all HBsAg-positive patients. On the other hand, unlike HBV and HCV, HDV viral load does not correlate with any clinical marker of activity or liver disease status; however, its quantification and detection are useful in monitoring antiviral treatment, when indicated, and to verify remission of infection, respectively. In addition, viral load monitoring may be critical for the assessment of response to new treatments, especially if the duration of new treatments ultimately depends in some way on HDV RNA kinetics. So, detection and quantification of HDV RNA, as the only reliable marker of HDV replication, should be an integral part of the diagnosis, management and treatment of infected patients. In summary, the universal implementation of one-step diagnosis of HDV infection in HBsAg+ patients will be a great step forward in the follow-up and therapeutic management of patients with HDV in the era of new therapies. In this sense, clinical microbiology laboratories should participate, taking the lead and not as mere recipients of demands, due to the endorsement provided by our current diagnostic potential for the elimination of viral hepatitis.

SPL10.4 HRF
Molecular elements involved in prion propagation and transmission

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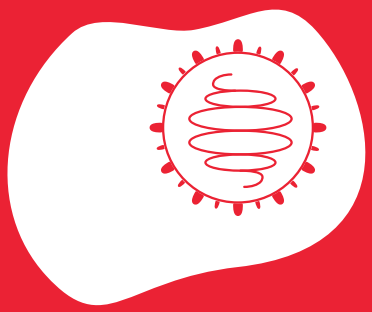
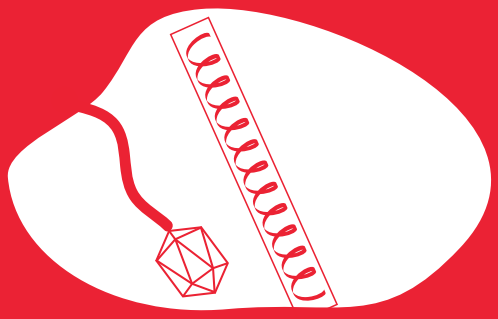
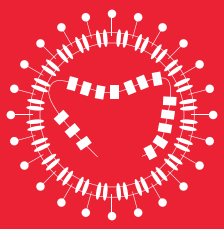
Prions are unique agents that challenge the molecular biology dogma by transmitting information on the protein level. They cause neurodegenerative diseases that lack of any cure or treatment called transmissible spongiform encephalopathies [TSEs]. All TSEs share the same molecular mechanism responsible for disease development. The normal form of the prion protein, also known as cellular form or PrPC, is converted into a disease-associated form known as PrPSc. PrPSc sustains its formation by recruiting PrPC molecules and transforming them into new PrPSc particles in an exponential way. PrPC transformation into PrPSc causes a change in the protein three-dimensional structure characterized by an increase in the β-sheet content. Such conformational change is also reflected in the protein physicochemical features. PrPC is monomeric, soluble in non-ionic detergents and sensitive to protease action whereas PrPSc tends to aggregate, is not soluble in non-ionic detergents and partially resistant to proteases. Indeed, PrPSc treatment with proteinase K produces a protease-resistant core called PrPres that is the most used prion surrogate marker. The function of the normal form of the prion protein, the exact mechanism of prion pathogenesis and prion propagation remains elusive. However, great amount of information known for all these aspects has been achieved thanks to the use of animal models and more precisely to transgenic mouse models. In this presentation, the main contributions of these powerful research tools in the prion field will be revised.

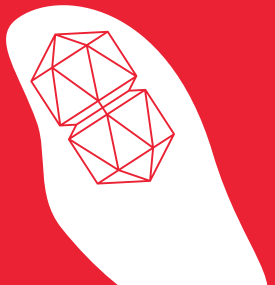






Abstracts

Sesiones paralelas



SP1 TDI

SP1.1 TDI

Sequence diversity on public databases and its influence in designing the specificity of PCR primers for pathogen detection: the case of Monkeypox virus

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The quality, extent, and diversity of the genetic sequence information deposited on public databases are essential aspects that inevitably compromise the design of specific primers and probes for pathogen detection. In addition to some structural requirements of oligonucleotides for a polymerase chain reaction (PCR), a phylogenetic criterium may help at the time of selecting sequence targets. For a decade, GPS™-branded laboratory has shown the capacity to design, produce, calibrate, and validate, qPCR kits for > 350 pathogens, about 140 of them were for viral species. In addition, the implemented development strategy and GPS™ quality system has demonstrated a great agility to respond to the needs arising from epidemiological emergencies. For instance, on January 27, 2020, this laboratory launched a qPCR kit to detect SARS-CoV-2. Last 21st of May 2022, the World Health Organization (WHO) communicated a "Multi-country monkeypox outbreak in non-endemic countries" in Europe, Canada, USA, mainly affecting United Kingdom, Portugal and Spain. At that time, recommended reference PCR methods for detection of Monkeypox virus (MPXV) were not available and the Center for Disease Control (CDC), Atlanta, USA, indicated that a PCR kit for MPXV detection, which may be required to facilitate surveillance, was not commercially available. On May 30, 2022, GPS™ launched a qPCR kit to detect the MPXV. The design was based on a target gene used by the group of Dr I.K. Damon (CDC-Atlanta) but, our primers discriminate a signature sequence that was shown to be inclusive for the 76 sequences of MPXV available at the NCBI (National Center for Biotechnology Information). To verify *in silico* exclusivity, the amplified fragment was compared with all data available focussing the most phylogenetically related viral species according to the current taxonomy of *Orthopoxvirus* (International Committee on Taxonomy of Viruses, ICTV), specifically Cowpox virus (90 sequences), Vaccinia virus (132 sequences), Camelpox virus (15 sequences), Ectromelia virus (12 sequences), Akhmeta virus (6 sequences), and Smallpox virus (73 sequences). Calibration by using ten-fold dilutions ranging from 10 to 10^6 DNA copies was achieved and analytical validation followed guidelines indicated in the norm UNE-EN ISO/IEC 17025. Diagnostic validation is currently underway.

SP1.2 TDI

Whole-genome characterization of Toscana virus detected in patients with idiopathic meningitis reveals the first evidence of intra-genotype reassortment, Southern Spain, 2015 – 2019: a retrospective study

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** These authors jointly supervised this work*

Toscana virus (TOSV) is a phlebovirus (family *Bunyaviridae*) transmitted by infected phlebotomine sandflies. TOSV can cause lymphocytic meningitis in humans and is considered as endemic in multiple regions in the Mediterranean area, with peak circulation during summertime. The virus is rarely considered by physicians in the differential diag-

nosis of meningitis. Although complete genomes records are relatively scarce, two TOSV genotypes have been described with intra-genotype diversity for each of the three segments. Reassortment is believed to play a major role in bunyaviruses evolution, but this process has not been reported yet for TOSV. We conducted a retrospective study on samples from patients hospitalized in Córdoba province between 2015-2019 with idiopathic aseptic meningitis despite routine diagnostic testing for bacteria and viruses (enteroviruses and herpes viruses) at the clinical microbiology laboratory. Untargeted metagenomic next-generation sequencing (mNGS) was performed on cerebrospinal fluid (CSF) samples. Demographic, clinical and CSF data was collected. Among the idiopathic samples, mNGS identified TOSV in 35% of samples (8/23). All TOSV genomes belonged to genotype B, suggesting the exclusive circulation of viruses from this lineage in Spain. mNGS resulted in nearly full-genome TOSV sequences which are the first reported in Spain. Phylogenetic and similarity-based analyses of complete genomes revealed the first evidence of intra-genotype B reassortment in one TOSV genome. All TOSV-positive patients were explicitly asked by physicians about recent insect bites but only one recalled bites. All TOSV-infected patients were male with a median age of 39 years. Length of hospital stay ranged from 2 to 16 days. All these cases were detected in a period comprising July to November in different years. Our study highlights 1) the need to consider the inclusion of TOSV in the differential diagnosis of patients presenting with meningitis and in the routine laboratory-testing at hospitals in southern Spain, regardless of history of insect bites mentioned by the patient and also during autumn months; 2) the benefits of unbiased metagenomics to identify viral pathogens directly in CSF supporting routine diagnosis. The detection of a novel, disease-associated, reassortant virus emphasizes the importance of full-genome surveillance to monitor the spread and evolution of phleboviruses.

SP1.3 TDI
Whole genome sequencing of RNA/DNA viruses from clinical specimens using RNA massive parallel sequencing

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[#]Equal contribution

**Equal contribution as co-senior authors*

Whole-genome sequencing for viruses (V-WGS) is considered an essential laboratory tool due to its growing range of applications on the laboratory surveillance of infectious diseases of interest for Public Health. In addition, a metagenomic approach using massive parallel sequencing (MPS) is essential in the response to emergent diseases. However, these techniques are challenging in terms of sensitivity, accuracy and performance when applied to clinical samples. The aim of this work is the optimization of an amplicon-independent RNA MPS method for the detection and sequencing of full genomes of a wide range of RNA and DNA viruses from clinical specimens. A total of 22 different viruses (Hepatitis A, hepatitis C, hepatitis D, hepatitis E, rubella, measles, mumps, european bat lyssavirus 1, rabies, crimean-congo haemorrhagic fever, poliovirus, rotavirus, norovirus, astrovirus, parechovirus3, parechovirus1, West Nile, chikungunya, dengue, cytomegalovirus, varicella-zoster and human herpesvirus 8) were used to evaluate the performance of a paired-end MPS method using RNA libraries and a shotgun sequencing,

in comparison with the use of two different commercial probe panels (PanViral,TwistBioscence) to capture viral sequences from such libraries. Total RNA was extracted from different clinical specimens (throat swab, salive, urine, feces, serum or plasma) using a commercial kit (Quick-RNAViral kit,ZymoResearch). Libraries were made using the NebNext ultra II directional library kit (NEB) using 5-10 ng of RNA, optimised according to manufacturer instructions. Libraries were sequenced in an Illumina NextSeq Sequencer (NextSeq500 Mid Output (2x75 cycles) for the shotgun approach, whereas captured libraries were sequencing using MiSeq platform (2x150 cycles). Sequencing samples were analysed for viral consensus genome reconstruction using viralrecon pipeline. Despite complete genome were obtained for most viruses (14) by shotgun analysis, a higher number of complete genome sequences (19) were obtained by the capture method using less sequencing reads. As conclusion, an efficient method based on RNA MPS to obtain the whole genome of a wide range of viruses from clinical samples has been developed. The usefulness of this method to the surveillance of known viral diseases and the response to viral emergent diseases is being analysed at this moment in the National Center for Microbiology (CNM, ISCIII).

SP1.4 TDI

SARS-CoV-2 in sewage: qPCR and NGS techniques comparison based on the detection of signature mutations corresponding to different Variants of Concern

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Wastewater based epidemiology (WBE) has been proved to be a valuable surveillance method of utmost importance to monitor the evolution of the SARS-CoV-2 pandemic since it provides the possibility to analyze the epidemiological situation at the community level. RT-qPCR-based methods represent the gold standard since the beginning of the pandemic. Despite its usefulness, these assays are not suitable to discover new uncharacterized variants that may be circulating among the population. Next-generation sequencing (NGS) approaches may contribute to overcome this limitation, enabling to study the evolution of mutations, or combinations of mutations, of interest. The comparison of Illumina with Oxford Nanopore, two of the widely employed NGS platforms, constitutes one of the aims of the present study. Strengths and weaknesses of both systems focusing on the analysis of the S gene of SARS-CoV-2 in sewage will be presented. Combinations of Twist SARS-CoV-2 standards from different variants were prepared and run in both platforms in parallel, as well as real samples, to determine the pros, cons, and limitations of each deep-sequencing method. Additionally, the validity of duplex gene allelic discrimination TaqMan RT-qPCR assays to determine the relative proportion of Alpha, Beta, Gamma, Delta and Omicron variants, in comparison with NGS will be discussed.

SP1.5 TDI

A novel dendrimeric-based strategy for the detection of the Classical swine fever virus FlagT4G vaccine

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Classical swine fever virus (CSFV) causes a highly relevant viral disease that affects domestic and wild swine. Currently, control of the disease in endemic countries is based on live-attenuated vaccines (LAV), which lack the differentiation between vaccinated and infected animals (DIVA concept). The FlagT4G vaccine is a highly promising marker LAV prototype, inducing early and robust immune response. It is based on the CSFV Brescia strain, with a mutation in the E2 glycoprotein and the insertion of a FLAG® sequence. However, it still lacks a reliable DIVA diagnostic test. Here, we describe the development of FlagDIVA, an ELISA test allowing to differentiate FlagT4G-vaccinated animals from those infected with CSFV. FlagDIVA is based on a dendrimeric peptide construct as a capture antigen, which contains two copies of the wild type conserved CSFV epitope in the structural E2 glycoprotein, that has been mutated in the FlagT4G vaccine, accompanied by one copy of an epitope found in the NS2-3. To test the capacity of the FlagDIVA assay to detect FlagT4G antibody response, 15 pigs at six weeks of age were vaccinated with FlagT4G and sera samples were collected weekly until 28 days post vaccination (dpv). Sera samples from these pigs were evaluated for antibodies against CSFV by the FlagDIVA test and a commercial ELISA. FlagDIVA was also used to evaluate the antibody response in 177 samples from a serum collection, including naïve pigs and animals subjected to experimental infection with different field CSFV strains. All these serum samples had previously been tested for antibodies against CSFV by the commercial ELISA. Specific anti-E2 antibodies were detected in all the FlagT4G-vaccinated pigs between 13 and 28 dpv by the commercial ELISA. These samples were negative by FlagDIVA, showing its efficacy as a negative-selection DIVA assay. In the serum collection samples, the FlagDIVA assay showed similar performance to the commercial ELISA test, while outperforming the commercial ELISA in samples of pigs infected with low-virulence CSFV strains. However, detection at early times post-infection remains to be improved. These results show that the FlagDIVA test constitutes a valuable DIVA tool in implementing vaccination with the FlagT4G candidate.

SP2 DEV

SP2.1 DEV

Cornering arboviruses to their arthropod vectors

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The compositional properties of viral genomes are informative about their origin and evolution. Arboviruses (arthropod-borne viruses) replicate in insects that transmit them to vertebrates, their second host. Thus, arboviruses have been exposed to the compositional biases of both hosts from significantly different phyla. Compositional proper-

ties are genomic signatures across a genome and result in different arrays of oligonucleotides. Here, to reduce the high dimensionality of the data and being able to extract meaningful patterns, we applied principal component analysis (PCA) and multidimensional scaling (MDS) approaches to more than 8000 reference viral genomes. Thus, we observed that arboviruses present a dinucleotide under-representation in CpG and UpA, whereas insect-specific viruses (ISVs) were only under-represented in UpA. Using Mayaro virus (MAYV) as a model, we have, by computer design and synthetic biology, rationally altered this dinucleotide frequency balance towards insect-specific viruses (ISVs). Our results show that recoded MAYVs grow as the wild-type virus in insect cells but are significantly attenuated in mammalian cells and in the mouse model. Importantly, the attenuated phenotype of the recoded MAYV can be reverted by targeting the zinc-finger antiviral protein (ZAP). Overall, here we suggest insights about the influence of both arthropod and vertebrate immune systems that shape the genetic composition of arboviruses.

SP2.2 DEV
Diversity and ecology of Global Ocean RNA viruses

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DNA viruses are increasingly recognized as abundant, diverse entities that influence marine microbes and microbe-mediated biogeochemical cycling, and hence as commonly key ecosystem players. However, RNA viruses are insufficiently studied outside disease settings and very little is known about their global diversity and ecology, and ecosystem roles. In this study, we analyzed ≈28 terabases of metatranscriptomic sequencing data derived from plankton collected during the Tara Oceans expeditions to offer a first report on the taxonomy and ecology of Global Ocean RNA viruses. Using new approaches to optimize discovery and classification, we identified RNA viruses that necessitate substantive revisions of taxonomy (doubling phyla and adding >50% new classes) and evolutionary understanding. Two of the novel phyla ("*Taraviricota*" and "*Arctiviricota*") are widespread and dominant

in the oceans. We also uncovered patterns and predictors of marine RNA virus community- and "species"-level diversity and contextualize their ecological impacts from pole to pole. Our analyses revealed four ecological zones, latitudinal and depth diversity patterns, and environmental correlates for RNA viruses. Our findings only partially parallel those of cosampled plankton and show unexpectedly high polar ecological interactions. The influence of RNA viruses on ecosystems appears to be large, as predicted hosts are ecologically important. Moreover, the occurrence of auxiliary metabolic genes indicates that RNA viruses cause reprogramming of diverse host metabolisms, including photosynthesis and carbon cycling, and that RNA virus abundances predict ocean carbon export. These efforts improved our understanding of the RNA virosphere, and provide analytical roadmap for RNA virus ecology and foundational knowledge critical to integrating RNA viruses into ecological models.

SP2.3 DEV
Reemergence of enterovirus D68 in Spain after easing the COVID-19 lockdown

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The more than 100 different types of enteroviruses (EV) that can infect humans belong to *Enterovirus genus*. EV-D68, from EV species D, went unnoticed since its discovery in 1962 but in the last years has been causing numerous outbreaks of respiratory diseases worldwide, with cases developing neurological complications. In autumn of 2021, re-emergence of EV-D68 was reported in several European countries. This study aims to investigate EV-D68 circulation after COVID-19 pandemic in Spain and to describe clinical, virological and epidemiological characteristics of the infections. A total of 171 EV-positive respiratory samples from patients admitted in four Spanish hospitals from January-2021 to April-2022 were sent for genotyping to the CNM. Genotyping was performed by PCR amplification of partial VP1 region of genome and subsequent sequencing. A phylogenetic analysis was conducted. Clinical information of patients was also analyzed. EV-D68 was identified in 68 out of 171 samples analyzed (39.76%), 67 associated with respiratory diseases and one with fever and convulsions. EV-D68 circulated from October-2021 to April-2022 with a higher detection frequency in November-December (42/68, 61.76%). In contrast to previous studies, in almost half of EV-D68-positive samples (31/68), a coinfection with other respiratory virus was detected. All EV-D68 infections but one were in children (98.52%) with a mean age of 3.14 years (range, 21 days-8 years). Most common symptoms were difficulty breathing (54/67, 80.59%), cough (51/67, 76.12%) and fever (29/67, 43.28%). About 70% of children (47/67) were hospitalized (mean of 3.4 hospitalization days), and 23.4% of them (11/47) required ICU admission. One patient subsequently suffered from limb paralysis. Finally, 32 children (47.76%) had underlying diseases, mainly recurrent wheezing episodes or asthma (22/32, 68.75%). Phylogenetic analysis performed with 50 EV-D68 VP1 sequences showed that all Spanish sequences from 2021 to 2022 clustered in subclade B3 together with strains detected in France, USA and Spain during 2014, 2016 and 2018 outbreaks, but forming two new lineages. In conclusion, this study evidences the re-emergence of EV-D68 in Spain after the pandemic along with the rest of Europe. Infections were associated with moderate/severe respiratory diseases in children. Circulating EV-D68 strains belonged to the subclade B3 described previously.

SP2.4 DEV
Accumulation dynamics of DVGs during experimental evolution of betacoronaviruses

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Replication of RNA genomes by virus-encoded RdRPs often results in hypermutated or in truncated versions of the genome (DVG), an especial case of the latter being the defective interfering particles (DIPs). Coinfection with a helper virus that provides the missing protein(s) in trans may result in amplification and transmission of DIPs. DIPs are an important part of the multicomponent viral system.

Here, we aimed to characterize the dynamics of formation and evolution of DVGs in evolution experiments with betacoronaviruses and as long-term goal, to select DIPs with optimized interfering activity. Human coronavirus OC43 (HCoV-OC43) were used to perform more than 30 serial passages at low and high multiplicity of infection (MOI) and in two susceptible cell lines, BHK-21 and HCT-8. High-throughput sequencing was performed at several evolutionary passages to identify DVGs. The variability of DVGs in BHK-21 at high MOI increased first to significantly decrease after passage 23, while at low MOI no significant changes in the number of DVGs were detected. Some DVGs were persistently observed at high MOI, while others had a transitory existence. In HCT-8, the tendency was similar, though less drastic. Regarding the abundance of DVGs, significant MOI-dependent changes were only found in the first-intermediate stages of the evolution in both experimental hosts. Comparing the differences among passages, at high MOI the virus increases the abundance of DVGs in both cell lines, but especially in BHK-21. At low MOI the number of DVG counts was not increasing in HCT-8 and slightly decreasing in BHK-21. Next, we looked at deletions as they may be of special interest for positive strand RNA viruses. The most abundant DVGs were deletions. Their size distribution at low MOI was bimodal, with short and long DVGs being common, and showed almost no changes during the evolution. In samples at high MOI, the size distribution was multimodal and shifted over time to longer DVGs. Hot-spot regions for deletions were found. They were similar to the ancestral strain, but density increased along with evolutionary passages. In conclusion, we showed the pervasive formation of DVGs during HCoV-OC43 evolution, with the dynamics being MOI- and host-dependent.

SP2.5 DEV
HDV-mediated inhibition of HBV in a superinfection mouse model: the role of type I Interferon

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Background: More than 5% of the Hepatitis B virus (HBV)-infected patients is co-infected with Hepatitis Delta Virus (HDV), which strongly activates the type-I Interferon pathway and inhibits HBV replication. The HBV X gene (*HBX*) encodes a protein that is essential for viral replication. The aim of this study is to inspect the role of IFN-I in *HBX* variability upon HDV superinfection in a novel HBV- expressing transgenic (HBVtg) mouse model knock out for the

IFNalpha/beta-receptor (HBVtg x IFNα/βR-KO). Method: HBVtg (n=7) and HBVtg x IFNα/βR-KO mice (n=6) were infected with $5x10^{10}$ viral genomes of an adeno-associated vector (AAV) expressing luciferase (Luc) or HDV. HBV expression had been weekly monitored by quantifying circulating viral DNA, whereas the intrahepatic expression was analyzed at 21 days after AAV injection (dpi). At this timepoint, HBX gene quasiespecies (QS) of the intrahepatic HBV RNA was explored by next-generation sequencing (NGS). QS Complexity and variability was studied quantifying the Rare Haplotype Load (RHL) and analyzing the Single Nucleotide Variations (SNVs). Results: The presence of HDV strongly reduced HBV expression at circulating and intrahepatic level. The inhibition of IFN-I pathway strongly limited this trend. At 21dpi of HDV-AAV, HBX QS in superinfected IFNα/βR-WT mice showed a higher RHL, mainly at the 5' end. SNVs were mainly C□T transitions and presented a higher average of frequency per position in HDV/HBVtgxIFNα/βR wild-type group related to superinfected KO mice. Different SNVs with higher relative frequency were observed in HDV/HBVtg x IFNα/βR-WT mice, especially in the *HBX 3'*-end encoding for the transactivating domain of the homonymous protein. Conclusion: The administration of HDV strongly hindered HBV expression. When knocking out the IFNα/βR, this restraining effect was limited, suggesting that type I IFN is essential for HDV-mediated HBV inhibition. The increased *HBX* variability in superinfected WT mice could contribute to viral inhibition, especially in the 3'*HBX* which encodes for the transacting domain of HBx protein. The moderated variability observed in HDV/HBVtg x IFNα/βR-KO mice suggests that IFN might play a role in this process. Funding: Instituto de Salud Carlos III (grant PI18/01436), co-financed by the European Regional Development.

SP3/SP12 CAV

SP3.1 CAV

Novel SARS-CoV-2 inhibitors discovered by phenotypic screening of viral RNA-binding molecules

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Novel antiviral agents are needed for the treatment of SARS-CoV-2 infection. Here we report the identification and evaluation of RNA-binding molecules that inhibit SARS-CoV-2 replication. The compounds were detected by screening a small library of antiviral molecules previously shown to bind HIV-1 or HCV RNA motifs with a live-virus cellular assay detecting inhibition of SARS-CoV-2 replication. These experiments allowed detection of eight compounds with anti-SARS-CoV-2 activity in the sub-micromolar to micromolar range and wide selectivity indexes. Examination of the mechanism of action of three selected molecules indicated action on the replication stage of the viral life cycle, and recognition by two of the compounds of conserved RNA motifs of the SARS-CoV-2 genome, including the highly conserved S2m hairpin located in the 3'-untranslated region of the virus. These results may be used for developing novel RNA-targeted therapeutic agents against coronaviruses. We acknowledge the support of La Caixa Banking Foundation of Spain (Caixaimpulse grant LCF/TR/CD20/527000).

SP3.2 CAV

Discovery and characterization of broad-spectrum antivirals against RNA viruses with dual mode of action against SARS-CoV-2

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COVID-19 pandemic has evoked the need for increasing our preparedness towards emerging viral infections. While pandemic contention is achieved through vaccination of the general population, effective antiviral therapies may be essential to improve clinical outcome of the infection in infected individuals. To increase the antiviral armamentarium against SARS-CoV-2, we screened a large collection of small molecules with the aim of identifying novel compounds with antiviral potential through an unbiased phenotypic screening approach. A family of compounds with antiviral activity in the low micromolar range was identified. These compounds were effective at interfering with SARS-CoV-2 infection after the infection had been initiated and reduced viral spread by several orders of magnitude. These compounds displayed comparable antiviral activity not only against other coronaviruses but also against distant RNA viruses, such as vesicular stomatitis or West Nile viruses, suggesting broad-spectrum antiviral activity. Structure-activity relationship carried out with dozens of analogs, led to identification of lead compounds that shared structural features with a drug with clinical potential and well characterized mode of action on a cellular

biosynthetic route. The clinical candidate showed antiviral activity which was rescued with a metabolite downstream of the inhibition point. Interestingly, infection could only be rescued with the same metabolite in some of the newly discovered compounds, arguing for a different mode of action in some of the new antivirals. Next, we took advantage of chemical proteomics approaches to identify host cell pathways alteration of which may result in reduced viral spread. As comparison, we studied in parallel the impact of the clinical candidate to determine whether the newly discovered family shared not only structural similarities but also similar proteomic alterations. The results of this analysis suggest that both the clinical candidate and the newly discovered family modulated similar cellular pathways. However, cells treated with the newly discovered compounds show an additional cluster of genes altered by the treatment in a host pathway often exploited by RNA viruses, suggesting a potential dual mode of action against SARS-CoV-2. This work was funded by the European Union – NextGenerationEU, PTI+ Salud Global and CSIC (CSIC-COVID-153; PIE 202080E221; PIE 202020E079).

SP3.3 CAV

The Valproic Acid Derivative Valpromide Inhibits Pseudorabies Virus Infection in Swine Epithelial and Mouse Neuroblastoma Cell Lines

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Pseudorabies virus (PRV) infection of swine can produce Aujeszky's disease, which causes neurological, respiratory, and reproductive symptoms, leading to significant economic losses in the swine industry. Although humans are not the natural hosts of PRV, cases of human encephalitis and endophthalmitis caused by PRV transmission from animals to workers have been reported. Currently, a lack of specific treatments and the emergence of new PRV strains against which existing vaccines do not protect makes the search for effective antiviral drugs essential. As an alternative to traditional nucleoside analogues such as acyclovir (ACV), we studied the antiviral effect of valpromide (VPD), a compound derived from valproic acid, against PRV infection in the PK15 swine cell line and the neuroblastoma cell line Neuro-2a. First, the cytotoxicity of ACV and VPD in cells was compared, demonstrating that neither compound was cytotoxic at a specific concentration range after 24 h exposure. Furthermore, the lack of direct virucidal effect of VPD outside of an infected cell environment was demonstrated. Finally, VPD was shown to have an antiviral effect on the viral production of two strains of pseudorabies virus (wild type NIA-3 and recombinant PRV-XGF) at the concentrations ranging from 0.5 to 1.5 mM. Considering that VPD is already licensed for clinical use by the EMA (European Medicines Agency), the time required to approve its new use for the treatment of alphaherpesvirus infections should be shorter than for all-new drugs. Further research is needed to unravel the detailed mechanism responsible for the antiherpetic activity of VPD, but this research suggests that VPD could be a suitable alternative to nucleoside analogues as an antiherpetic drug against Aujeszky's disease.

SP3.4 CAV

Synthetic polyphenols inhibit West Nile virus infection though alteration of sphingolipid metabolism

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Polyphenols are promising lead compounds for new therapeutic agents as they can exert multiple pharmacological activities, including the alteration of lipid metabolism. The evaluation of our own collection of polyphenols against West Nile virus (WNV) led to the identification of two hits with significant antiviral activity and no toxicity. These compounds also inhibited the multiplication of other flaviviruses, exhibiting lower or undetectable antiviral activity against other non-related RNA viruses. The mechanism underlying their antiviral activity against WNV involved the alteration of sphingolipid metabolism. These results unveil the potential of novel antiviral strategies based on the modulation of the cellular levels of sphingolipids for the control of flavivirus infection.

SP3.5 CAV
Novel therapies for rapid responses to pandemic viral threats

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Humanity faces an almost unlimited pool of viral threats. To handle and contain upcoming and unpredictable outbreaks before they spread worldwide it is fundamental to rapidly generate specific antivirals or to count with broad-spectrum antivirals that can be stockpiled. Neither of these options are functional today. We have addressed these two needs by generating a novel circular RNA (circRNA)-based antiviral platform. Artificial circRNAs were designed to hybridize and interfere with highly-conserved RNA structures within RNA viral genomes that are essential for their translation and/or replication. Using an in-house developed software, as a proof of principle we designed circRNAs that highly inhibited HCV, DENV, WNV or SARS CoV-2 in cell culture. CircRNA-based with broad-spectrum activities were also generated by including sequences targeting different viruses. Importantly, as predicted by the lack of free 5´or 3´ ends accessible to exonucleases for degradation, circRNAs were highly stable in cell culture and in mice models without the need of including any RNA modification. Moreover, circRNA-based therapies are predicted to hamper the emergence of resistant mutants, a major problem of traditional antivirals, because they target highly conserved structures and multiple structures can be targeted with the same circRNA molecule. In sum, the high stability and versatility of circRNA-based antivirals together with the in-house developed methods for their designed and low-cost production point at circRNAs as a novel RNA-based strategy for ultrarapid generation of antivirals against novel viral threats.

SP12.1 CAV

Discovery and characterization of potent and selective SARS-CoV-2 antivirals with pan-coronavirus potential.

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The current COVID-19 pandemic, caused by SARS-COV-2 coronavirus infection, is generating a strong social and economic burden worldwide. While global virus spread is being contained through vaccination campaigns, there is a need for development of antiviral drugs that may contribute to improve the clinical outcome of infected individuals and our overall preparedness for future coronavirus outbreaks. Using an unbiased phenotypic screening approach, a family of novel antiviral molecules was identified. SAR studies enabled optimization of the antiviral compounds and selection of lead compounds with optimal activity and therapeutic window. The identified family was strongly selective against human coronaviruses, since no activity was detected against any of the tested RNA viruses. In contrast, evaluation of human coronavirus 229E revealed a very strong antiviral activity, suggesting a potential pan-coronavirus activity. Time of addition experiments indicate that these compounds strongly inhibit infection in a post-entry step and that accumulation of subgenomic viral RNAs is strongly reduced in cells treated with the compounds and infected at high multiplicity of infection, arguing for inhibition of aspects of the replication cycle leading to viral RNA replication. Directed evolution studies are currently being carried out to define the genetic resistance profile of this family of molecules in order to gain insight into the viral target under selective pressure and the overall mode of action. Additional target deconvolution in vitro studies have ruled out that these compounds target the main viral protease (Mpro). Proteomic analysis of viral and cellular ligands of the antiviral compounds will be carried out to define their molecular target. This work was funded by the European Union – NextGenerationEU, PTI+ Salud Global and CSIC (CSIC-COVID-153; PIE 202080E221; PIE 202020E079).

SP12.2 CAV

Efficacy decrease of antiviral agents when administered to ongoing hepatitis C virus infections

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The efficacy of antiviral agents in preclinical trials in cell culture is usually tested by adding the antiviral agents at the time of infection. Theoretical models predict that delaying inhibitor addition relative to the infection onset should

decrease antiviral efficacy. Experimental quantifications of this effect have not been reported. Hepatitis C virus (HCV) replicating in human hepatoma (Huh-7.5) cells is an adequate system to quantify effects of delayed inhibitor administration, since well characterized anti-HCV agents are available. We report a quantification of the decrease of effectiveness of antiviral agents directed to HCV, when the agents are added during an ongoing infection in cell culture. Major determinants of the decrease of inhibitory activity are the time post-infection of inhibitor administration, and viral replicative fitness. The efficacy decrease has been documented with antiviral assays involving the combination of the direct acting antiviral agents daclatasvir and sofosbuvir, as well as the combination of the lethal mutagens favipiravir and ribavirin. The results suggest that strict antiviral effectiveness assays in preclinical trials should involve the use of high fitness virus populations, and the delayed administration of the agents, relative to infection onset.

SP12.3 CAV
Novel RNase H inhibitors with multiple targets in HIV-1

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Human immunodeficiency virus (HIV) reverse transcriptase (RT) is a major target for AIDS treatment due to its key role in the viral replication cycle. Although current antiretroviral therapies can be effective in reducing viral loads to undetectable levels, long-term drug toxicity, adverse side effects, and an increased prevalence of drug resistance among circulating HIV strains remain as the biggest short-term and long-term threats to therapy success. Current efforts focus on the development of novel antiretroviral drugs acting on unexploited targets, such as the ribonuclease H (RNase H). This enzymatic activity is essential for reverse transcription. The RT's RNase H catalytic site shares structural homology with the active site of HIV-1 integrase (IN). In this study, inhibitory activities of selected RNase H active site inhibitors containing different pharmacophores as chemical scaffolds have been determined in RNase H, RNA-directed DNA polymerase (RDDP) and IN strand transfer activity assays. In addition, RNA-dependent DNA synthesis reactions under strand displacement conditions were performed using RNA/DNA template-primers including an additional displaced oligonucleotide. Our results showed that most of the RNase H inhibitors tested were also HIV-1 IN inhibitors, while all compounds caused defective RNA-directed strand displacement DNA polymerization in reactions catalyzed by HIV-1 RTs. Dual inhibitors of the HIV-1 RT's RNase H and RDDP activities, containing a 7-hydroxy-6-nitro-2H-chromen-2-one pharmacophore (coumarin derivatives DW3 and K04-9) were the most potent strand displacement inhibitors while copying RNA templates. Enzymatic assays carried out with these compounds revealed two non-overlapping binding sites at the HIV-1 RT. One of them was located at the RNase H catalytic site where coumarin derivatives competed with β-thujaplicinol in inhibiting RNase H activity. On the other hand, the RDDP inhibitory activity of coumarin derivatives was not influenced by representative mutations at the RT's NRTI and NNRTI binding sites. Molecular docking predictions suggested that these inhibitors bind at the RT p66/p51 interface with a potential binding site around Thr^{139}. In agreement with this prediction, mutant T139I conferred low-level resistance to DW3 in nucleotide incorporation assays. These findings might be helpful in the design of novel compounds targeting multiple activities in the HIV replication cycle.

SP12.4 CAV
High concentration of Maraviroc does not alter Immunological Parameters of CD4 T cell Subpopulations

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Background: Maraviroc (MVC) is an antiretroviral drug able to bind to CCR5 co-receptor and block human immunodeficiency virus (HIV) entry into cell targets, which are primarily CD4 T cells. Also, MVC can activate NF-kB and induce viral transcription in HIV-infected cells, and it has been proposed as a latent reversal agent (LRA) on the HIV cure strategy called "kick and kill". Several LRAs have been tested in clinical trials, showing a limited effect to decrease HIV reservoir and some of them able to affect immunological markers. In this work, in order to analyze more in detail the antiviral and/or latency reversing capacity of MVC on CD4 T cells, we evaluated immunological and metabolic parameters of CD4 T cell subpopulations. Methodology: We obtained peripheral mononuclear cells (PBMC) and isolated CD4 T cells by negative selection. CD4 T cells were cultured in the absence or presence of MVC (at the concentration of 5 μM). After the culture, we evaluated the frequency, levels of HIV infection and activation markers on CD4 T cell subpopulations by flow cytometry. Also, we evaluated metabolic rates of CD4 T cells by Seahorse Analyzer. Results: Our results indicate that this high concentration of MVC does not induce significant changes on the frequency of memory CD4 T cell subpopulations ($p>0.1$). Also, MVC inhibits HIV infection more efficiently on memory CD4 T cells ($p<0.05$). Likewise, MVC did not increase the levels of activation markers (HLA-DR, CD69, Ki67, PD-1 and CD25) in total CD4 T cells ($p>0.4$). Also, slightly elevated CD25 levels in naïve and central memory CD4 T cell subpopulations were observed ($p<0.05$). Finally, MVC does not modify both glycolytic and oxidative metabolic rates in CD4 T cells ($p>0.1$). Conclusions: This data supports that MVC is a good LRA candidate, because it does not induce significant changes on immunological and metabolic parameters that could affect the immune response against HIV infection.

SP12.5 CAV

Persistent hepatitis C virus infection causes permanent transcriptional alterations after virus elimination.

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Hepatitis C virus infection (HCV) is a major biomedical problem worldwide. Chronic HCV infection causes liver inflammation and fibrosis, which can lead to development severe liver disease, such as cirrhosis or hepatocellular carcinoma (HCC). Approval of direct acting antiviral (DAA) drug combinations has revolutionized antiviral therapy against HCV. These drugs enable virus eradication in >98% of the treated patients, regardless of the genotype and liver disease status. The high efficacy of these antiviral molecules is such, that some models suggest that elimination of infected cells by reactivated immune responses may be dispensable for virus eradication, in contrast to interferon-based therapies. It is thus formally possible that patients that are declared cured, indeed carry formerly infected cells that display irreversible alterations due to prolonged chronic HCV infection. We used two different cell culture models for persistent infection, in which HCV infection causes profound alterations of host cell transcriptional profile in proliferating and growth-arrested, partially differentiated cell models. These transcriptional alterations are a manifestation of the infected cell to regain homeostasis in the context of intracellular membrane rearrangements, interference with metabolic processes and persistent stress conditions permitting cell survival even under conditions where the virus has colonized the host cell. In this context, we asked the question of whether all transcriptional alterations are back to their original expression levels in the formerly infected cells after DAA treatment-mediated virus eradication. Using persistent infection models, we determined alterations in the cellular transcriptome due to persistent HCV infection. Our results show that persistently infected cells were virologically cured by DAA-treatment

and that the vast majority of the HCV-regulated host genes return to baseline expression after treatment completion. However, we observed a number of transcripts that remain altered several weeks after treatment completion and treatment withdrawal. Comparison of the results obtained in proliferating and growth-arrested cell culture models suggest that permanent transcriptional alterations may be established by several mechanisms. These results indicate that persistent HCV infection causes permanent molecular sequels in cell culture, some of which may be also found in liver biopsies from cured patients. This work was funded by SAF2014-52400R, BES-2015-074920, SAF2017-87846-R and CIBERehd.

SP4 VPL

SP4.1 VPL

Insights into the biology of deltasatellites, an emerging class of begomovirus-associated DNA satellites

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Deltasatellites are small non-coding circular DNA satellites associated with begomoviruses (genus *Begomovirus*, family *Geminiviridae*) which have been recently recognized as a new class of subviral agents. They have even given a taxonomic status, constituting the genus *Deltasatellite* in the family *Tolecusatellitidae*, the first family of nucleic acid satellites officially recognized by the ICTV. In recent years our laboratory has been involved in the molecular and biological characterization of a number of deltasatellites both from the Old World and the New World that naturally infect plants of the families *Malvaceae*, *Convolvulaceae* and *Fabaceae* and the results obtained will be summarized in this presentation. Main results include the demonstration that: i) these DNA molecules are true satellites as shown by agroinoculation of infectious clones in the presence of the helper begomoviruses both in the model plant *Nicotiana benthamiana* and the natural host plants, ii) some deltasatellites are transreplicated by a range of begomoviruses and even by a curtovirus (genus *Curtovirus*, family *Geminiviridae*), iii) most deltasatellites did not affect the symptomatology caused by the helper viruses, and its effect on viral DNA accumulation depended on the helper virus–host plant combination, and iv) deltasatellites are transmitted by the whitefly *Bemisia tabaci*, the vector of their helper begomoviruses. To decipher the role of deltasatellite features in begomovirus infection, several deltasatellite mutans were constructed for which molecular and biological analyses showed somehow unexpected results.

SP4.2 VPL

Delivery of gene editing CRISPR-Cas reaction components in plants using viral vectors

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Genome editing based on the clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) proteins from prokaryotes have revolutionized research in biology and biotechnology. CRISPR-Cas technology is particularly attractive to crop science because of the potential straightforward generation of new cultivars that can be more productive and nutritive, resistant to pests and pathogens, and better adapted to changing environmental conditions. In addition, ethical issues related to off-target effects are negligible in plants. However, delivery of CRISPR-Cas reaction components, basically a Cas nuclease or derivative and one or more guide RNAs, is not easy in plants and usually requires stable genetic transformation. While some plants species show recalcitrant regeneration, those in which transformation is possible frequently require time-consuming and labor-intensive protocols based on tissue culture techniques that can generate undesired genetic or epigenetic variations. Plant viruses constitute a much faster and efficient altenative to deliver CRISPR-Cas reaction components into plant cells. Our

research focuses on developing plant virus-derived vectors able to self-replicate and move systemically through the plant as well as efficiently deliver multiple guide RNAs to plant cells. We particularly aim to reach the plant germline cells for heritable genome editing. Interestingly, the fusion of RNA mobile signals to guide RNAs, such as a 5' fragment of *Flowering locus T* mRNA, promotes the migration of the guide RNA to germline cells while the virus that served as a vector is usually unable to penetrate this barrier. As a consequence, edited progeny can be obtained avoiding plant regeneration from adult tissue. On the other hand, although size of Cas nucleases makes this goal really challenging, we are also developing strategies to deliver this reaction component into plant cells. We will discuss our current advances using vectors derived from potato virus X (PVX; *Potexvirus*), tobacco rattle virus (TRV; *Tobravirus*), cucumber green mottle mosaic virus (CGMMV; *Tobamovirus*) and several potyviruses, such as tobacco etch virus (TEV), watermelon mosaic virus (WMV) or zucchini yellow mosaic virus (ZYMV). With these vectors, we are currently developing strategies for easy and efficient gene editing of some solanaceous and cucurbit cultivars.

SP4.3 VPL

Determinants of the age-dependent responses to viral infection in Arabidopsis thaliana

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Gene expression patterns differ throughout an organism's lifetime, since biological needs and goals change as individuals grow and age. These changes on gene expression may imply changes in immune defense responses, and therefore organisms may present different susceptibilities to pathogen infection depending on the life moment they are infected at. In plants, some studies support the notion that infections are less severe in aged than in younger individuals, which is consequence of a plant defense response known as age related resistance (ARR). However, little is known about whether ARR is also effective against viruses. To characterize how developmental stages may affect the outcome of a viral infection and study the host's differential strategies and responses to it, we used the pathosystem *Arabidopsis thaliana* - turnip mosaic virus (TuMV) at three different host developmental stages: juvenile vegetative, bolting (transition from vegetative to reproductive) and mature reproductive. For that, we inoculated and experimentally evolved two viral strains of TuMV: one naïve and other well-adapted to *A. thaliana* on each developmental stage. For both viral strains, we observed that the infection was faster and more severe as hosts grew older. After the evolution, we also observed a link between virulence and age: while all evolved viruses caused a significant reduction on seed progression, hosts infected on a stage of reproductive growth produced significatively more offspring than those infected on the other two developmental stages. Next, we characterized the hosts' transcriptional responses to viral infection on all developmental stages, finding that even though the biological processes involved on the response against all evolved viruses were similar, hormone levels in conjunction with the regulation of a particular set of genes ought to be the reason responsible for this age-dependent susceptibility to viral infection. Specifically, we hypothesize that abscisic acid may be a key component on these processes. Overall, our study contributes to understand the impact of host age upon the responses to viral infection and tries to shed light on the molecular mechanisms behind it.

SP4.4 VPL

Involvement of homologous and heterologous O-GlcNAc cycling enzymes, OGT and OGA, in the O-GlcNAcylation turnover of the plum pox potyvirus coat protein, during infection

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Post-translational modifications (PTMs) expand the functional repertoire of proteomes allowing rapid dynamic signalling and regulation processes, something especially valuable for RNA viruses with limited genome-coding capacity. *Plum pox virus* (PPV) (genus *Potyvirus*, family *Potyviridae*), the causal agent of sharka, a devastating disease of stone fruit trees, has a positive single-stranded RNA, encapsidated by multiple subunits of the virus coat protein (CP). The N-terminal protrusion of PPV CP is targeted by at least two types of PTMs, *O*-GlcNAcylation and phosphorylation, whose opposite action would modulate the protein susceptibility to degradation. While phosphorylation has been studied extensively in animals and plants, research on *O*-GlcNAcylation of plant proteins and, by extension its role in pathogenesis, lags far behind that of other eukaryotic organisms. In animals, the highly regulated O-GlcNAc cycle is governed by two enzymes, an O-linked N-acetylglucosamine transferase (OGT) and an O-GlcNAcase (OGA). In plants, the addition of O-GlcNAc groups is handled by homologous of SEC, the *Arabidopsis thaliana* OGT, which also modifies PPV CP. However, no plant enzyme with OGA function has been formally identified so far. In this study, we look further into plant OGT functioning by determining the endogenous levels of SEC and their deviations due to virus infection. Quantification of SEC mRNA revealed that PPV infection positively impact on SEC transcriptional levels, mainly at the early times of infection. Putative OGA function of the *A. thaliana* β-N-acetylglucosamidases, HEXO1, HEXO2 and HEXO3, was also evaluated. Analysis of PPV CP *O*-GlcNAcylation status by mass spectrometry showed that all three hexosaminidases can remove O-GlcNAc groups from the viral protein, when ectopically overexpressed in *N. benthamiana* along with PPV. These results would support the putative reversibility of SEC-imposed CP *O*-GlcNAcylation in the context of PPV infection. Additionally, a combination of biochemical, confocal microscopy, and proteomic approaches were used to analyse the behaviour of heterologous cycling O-GlcNAc enzymes, human OGT (hOGT) and OGA (hOGA), in plants. We demonstrated that hOGT can restore PPV CP *O*-GlcNAcylation in SEC-deficient plants, although resulting O-GlcNAcylation pattern emulates that imposed by SEC only partially. Evidence on hOGA function and hOGT/hOGA subcellular co-localization in plants are also provided.

SP4.5 VPL

Structure and assembly of Polinton-like virus TsV-N1, a possible ancestor of the PRD1-Adenovirus lineage in eukaryotic hosts.

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Polintons are large eukaryotic dsDNA transposons encoding a protein-primed DNA polymerase (POL) and a retroviral-like integrase (INT). Most of them also include a DNA-packaging ATPase and a maturation protease similar to those found in viruses of the PRD1-Adenovirus (AdV) lineage. They also encode genes that could translate into orthogonal jelly roll proteins, suggesting that in certain conditions they could form icosahedral capsids. These observations prompted the hypothesis that Polintons may have evolved from a PRD1-like ancestor (encoding capsid proteins, POL, and ATPase), which entered a proto-eukaryotic host with a bacterial endosymbiont and acquired

the protease and integrase genes by recombination with a transposon. Subsequent evolution would have resulted in the "polintovirus" elements splitting into two different ways of life: the transposable, capsid-less integrating elements, and the bona fide viruses. Recently, marine metagenome analyses have revealed a group of putative polinton-like viruses (PLVs) in eukaryotes. PLV genomes contain genes for single and double jelly roll proteins and a packaging ATPase, but lack the protease and integrase genes. Therefore, PLVs could represent a minimal version, or a first ancestor, of the PRD1-AdV lineage in eukaryotic hosts. We are analyzing the structure of the only isolated virus belonging to this newly defined group: *Tetraselmis striata* virus N1 (TsV-N1). The cryo-EM capsid structure at 5.2 Å resolution corroborates the placement of TsV-N1 in the PRD1-AdV viral lineage. The relation between capsid and genome size indicates that TsV-N1 could have one of the most tightly packed genomes in nature. Observing cellular sections in electron microscopy, we describe the organelles and structures in the *T. striata* cell (algae host) and how they are remodeled or lost upon infection. We also analyze the appearance of virus particles in the cell to establish an initial model for TsV-N1 assembly.

SP5 VVE

SP5.1 VVE

Peste des Petits Ruminants (PPRV) impairs DC function to suppress T cell activation

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Viruses have evolved numerous strategies to impair immunity so that they can replicate more efficiently. Among those, the immunosuppressive effects of Morbillivirus infection can be particularly problematic as it allows secondary infections to take hold in the host worsening disease prognosis. In the present work we hypothesized that the highly contagious morbillivirus Peste des Petits Ruminants virus (PPRV), closely related to measles, rinderpest and canine distemper virus, could target monocytes and dendritic cells (DC) to contribute to the immunosuppressive effects produced by the infection. Monocytes isolated from healthy sheep donor, a natural host of the disease, could be infected by PPRV and this impaired the differentiation and phagocytic ability of immature monocyte-derived DC (MoDC). We also assessed PPRV capacity to infect differentiated MoDC. Ovine MoDC could be productively infected by PPRV, and this drastically reduced MoDC capacity to activate allogeneic T cell responses. Transcriptomic analysis of infected MoDC indicated that several tolerogenic DC signature genes were upregulated upon PPRV infection. Furthermore, PPRV-infected MoDC could impair the proliferative response of autologous $CD4^+$ and $CD8^+$ T cell to the mitogen concanavalin-A, which indicated that DC targeting by the virus could promote immunosuppression. These results shed new light on the mechanisms employed by Morbillivirus to suppress the host immune responses.

SP5.2 VVE

BA71ΔCD2 intranasal immunization is safe and effective against direct-contact challenge with pigs infected with the African swine fever virus pandemic strain

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African swine fever (ASF) is today the number one threat for the porcine industry worldwide. No commercial vaccine is available against the ASF virus (ASFV), and control of the disease relies on early diagnosis followed by stamping out policies, which are not always easy to implement. So far, experimental vaccines based on inactivated ASFV and subunit vaccine formulations have failed to induce solid protection. Conversely, and despite the inherent biosafety issues, live attenuated viruses (LAVs) have demonstrated solid protection against experimental ASFV challenge. We previously generated a recombinant LAV by deleting the CD2v gene (encoding the ASFV hemagglutinin), namely BA71ΔCD2 (genotype I). Despite intramuscular administration of BA71ΔCD2 confers protection against homologous and heterologous challenges, including the genotype II pandemic virus, a proportion of vaccinated pigs showed low albeit detectable viremia and nasal shedding. Several strategies have been followed aiming to improve BA71ΔCD2 safety, including the generation of double-deletion mutants, the use of adjuvants and/or different inoculation routes. Here we show that intranasal inoculation with BA71ΔCD2 resulted safer for the animals, demonstrated by the fact that neither viremia nor shedding was observed at any time after vaccination, independently of the vaccine dose tested. This reality, together with the fact that intranasal vaccination with an optimal dose of BA71ΔCD2 resulted extremely effective against a direct-contact challenge with pigs infected with the ASFV pandemic strain, opened new expectation for its further field implementation.

SP5.3 VVE

Development of novel vaccine candidates against bluetongue virus based on the induction of humoral and cellular immune responses by Modified Vaccina Ankara vectors expressing VP2, VP7, NS1 and NS2-Nt proteins

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Bluetongue (BT) is an important arthropod-borne livestock disease caused by Bluetongue virus (BTV), the prototype member of the genus *Orbivirus* (family *Sedoreoviridae*). Since 1998, it has expanded to non-endemic areas, producing important outbreaks in the Mediterranean Basin and devastating incursions in Northern and Western Europe. Live-attenuated and inactivated vaccines have permitted to control BT. However, besides their low safety profile, these vaccines do not simultaneously protect against the 36 serotypes of BTV, as the protection induced is mediated by serotype-specific neutralizing antibodies against VP2. Recently, the highly conserved nonstructural protein NS1 of BTV has been identified as a potential antigen able to confer multiserotype protection against BTV. Moreover, its combination with the N-terminal end of NS2 induced an antigen-specific cell-mediated response that protected sheep from developing clinical disease. In this work, we have design Modified Vaccinia Ankara (MVA) viral vectors that expressed VP2 or VP7 of serotype 4 along with NS1 and NS2-Nt as well as MVAs that expressed VP2, VP7 or NS1 and NS2-Nt. Cloning of VP2 and VP7 was conducted in the *F13L* locus of MVA whereas NS1 and NS2-Nt were cloned in the *TK locus* as a fusion protein including the "ribosomal skipping" linker 2A from foot-and-mouth disease virus into the fusing point. To study the protection conferred by these MVAs, IFNAR(-/-) mice were immunized with one dose or following a prime-boost strategy and challenged with a lethal dose of BTV-4. Immunization with two doses of MVA-NS1-2A-NS2-Nt protected mice from BTV infection by the induction of an antigen-specific

T cell immune response. Inclusion of VP7 in the vaccine formulation served to amplify the cell-mediated response induced by NS1 and NS2-Nt. In this sense, survival rates after challenge were equal for mice immunized with two doses of MVA-VP7-NS1-2A-NS2-Nt or MVA-NS1-2A-NS2-Nt although the upsurge of viraemia was significantly cushioned in the first group. Incorporation of VP2 led to the induction of neutralizing antibodies in immunized animals and improved the protection observed in MVA-NS1-2A-NS2-Nt immunized mice, with almost undetectable viraemia levels in the prime/boost MVA-VP2-NS1-2A-NS2-Nt immunization group. Further experiments are being conducted to study the multiserotype protective capacity of these vaccine candidates.

SP5.4 VVE
Role of cell factors on African Swine Fever Virus tropism

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African Swine Fever virus is a large cytoplasmic dsDNA causing African Swine fever, a devastating pig disease without so far effective vaccine, which produces severe losses on the pig industry worldwide. ASFV tropism is limited to the porcine myeloid lineage cells, more specifically monocytes and macrophages, which represents a technical problem to grow the virus for research purposes and for LAV vaccine candidates scaling up. Several immortalized pig cell lines like PK15 or ST cells have been described as non-susceptible for ASFV infection, whereas other pig cell line, like WSL, becomes infected by ASFV and produce new virions. Interestingly, SV40 T large antigen (TL Ag)-transformed epithelial monkey COS-1 cell line, has been widely used for ASFV studies and virus production, whereas the parental COS-1 cell line CV-1, which does not express TLAg, is non-susceptible to ASFV infection. To determine the mechanisms underlying the ASFV tropism, we have focused on cellular codified viral restriction factors that may be implicated on it. Specifically, we are exploring the role of three cellular restriction factors: FAM111A, SAMD9 and SAMHD1. FAM111A is a protein described as a host restriction factor for SV40 infection by interacting to TL Ag, playing an important role on SV40 tropism. SAMD9 is a protein implicated in viral restriction via de formation of RNA granules and has been described as a restriction factor in poxviral infection, neutralized by poxviral C7L protein family. SAMHD1 is implicated in restriction of several viral family replication via the regulation of the dNTP pool in host cells, specifically in macrophages and is antagonized by Vpx HIV-2 protein. Our data show that both attenuated and virulent ASFV strains can modulate the expression of these restriction factors on cells permissive to ASFV infection, implicating still undetermined viral mechanisms, which are being currently approached in our lab. This knowledge is key for the generation of new cell lines that support ASFV infection, both for research of virus-host interactions and future ASFV vaccine development.

SP5.5 VVE
Genomic and Evolutionary Analysis of Swine Influenza Virus H1N1 and H3N2 in Vaccinated and Nonvaccinated Pigs After Simultaneous Contact Infection

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Swine influenza viruses (SIV) produce a highly contagious and worldwide distributed disease which poses a continuous threat to both, human and animal health. There are two main mechanisms that shape SIV evolution, the accumulation of point mutations and the genome segment reassortment among different SIV subtypes. Trivalent vaccine application could potentially favour the evolution of SIV as it does not provide sterilizing immunity and the virus has an extraordinary ability to adapt to new host environments. Herein, to deeply understand the evolutionary dynamics of SIV under immune pressure, two groups of pigs, vaccinated and nonvaccinated, were challenged by contact with 4 pigs inoculated with H1N1 and 4 pigs with H3N2 SIVs subtypes. Nasal swab samples were daily collected and bronchoalveolar lavage fluid (BALF) was also collected at necropsy day of each pig, for SIV detection. In the present work 39 whole SIV genome sequences were obtained by NGS, 20 collected from vaccinated animals and 19 from nonvaccinated ones. Furthermore, a genomic and evolutionary analysis was carried out to detect both, genomic reassortments and single nucleotide variants. Our results showed a complex dynamic in viral population, with animals infected by one or another subtype and animals in which an alternation of subtypes is seen over time. Likewise, genomic reassortments were detected, mainly in M and NS segments, and PB1 to a lesser extent. These reassortments were mainly observed in viral samples collected from nonvaccinated animals. Regarding the point mutations, 1716 and 1267 SNV were reported in H1N1 and H3N2 subtypes, respectively. Four relevant nonsynonymous substitutions were found with an allelic representation greater than 50% in H1N1 subtype, HA A148S in Sa epitope region, HA S274P in fusion domain, NA V379I in the head domain and PB2 G77A. Meanwhile in H3N2 subtype, most notable nonsynonymous substitutions were allocated in NS, NP, PA and PB2 protein, the most of them in viruses collected from nonvaccinated animals. Once again, the vast evolutionary capacity of SIV is demonstrated, with different patterns in vaccinated and nonvaccinated animals.

SP6 VPE

SP6.1 VPE
Viruses, Aquaculture and Climate Change: What surprises are around the corner

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Climate change (CC) is a fact, nobody doubts it. For Society, Its most obvious effects are those directly affecting humans: world warming, catastrophic weather events, and the rise of oceans level; and as a side effect, how all these phenomena affect natural marine animal populations. In aquaculture, CC is a concern for farmers since the raise in surface water temperature may force them to change the species traditionally cultured for others better adapted to the new conditions. For instance, as demonstrated by the NASA, in Galicia, in the last 2 decades the coastal water temperature has increased 1°C with respect to the last 20 years of the 20th century. Therefore, in some areas the water temperature is getting close to the limit of tolerance of certain species as turbot or some crustacea. And predictions by the IPCC point to an average rise of 4°C at the end of this century if extreme measures are not implemented immediately. The change of water conditions is also a concern for experts in microbial diversity, but few virologists have realised their importance on the distribution of fish viruses and their effect on the hosts. Some viruses have adapted to cold-water (≤ 20°C) fish, others affect warm-water fish (>28°C), and cool-water (20-28°C) fish viruses have an intermediate tolerance. Cool-water viruses were rarely detected in cold-water areas; however, recently certain cool-water fish viruses are being isolated from asymptomatic fish at temperatures below 18°C. Where did those viruses come from? Migration of virus-carrying fish fleeing the high temperatures to cooler regions could be one of the explanations. This is the first scenario: exotic viruses invading areas with naïve fish populations. A second scenario would be local viruses not being able to produce disease in their natural host, because the water temperature is above their tolerance limit. In the third one, highly adaptative viruses could generate variants able to survive in their hosts at higher temperatures, which might lead to new epidemics. In this presentation, all these scenarios will be addressed as the consequence of the effect that the CC will have on viral diseases in aquaculture.

SP6.2 VPE

Zebrafish (Danio rerio) as a model for the study of the interaction of virus and immune system

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Zebrafish (Danio rerio), largely used as a model for studying developmental processes, has also emerged as a valuable system not only for other fish viral diseases with importance in aquaculture but also for modeling human viral diseases. Zebrafish possesses a complex immune system comparable to those of mammalian models. Their advantages such as the transparency of early stages that allows live imaging of cells, the significant number of mutants and transgenic lines, the use of larvae without ethical restrictions, etc, facilitate the study of the molecular basis of the virus infection process clarifying the relevance of genes or pathways.

In our work, we show how the real-time imaging and the use of the whole animal are excellent tools to visualize and characterize the in vivo interaction of a virus with the immune system. Using the rhabdovirus SVCV and genomic tools as the RNA-seq, we were able to identify new key players in the antiviral response and also to establish a screening platform to identify antiviral and anti-inflammatory compounds in a whole organism model.

SP6.3 VPE

Antimicrobial peptides Hepcidin and Dicentracin produce immunomodulation and confer partial protection against Nodavirus

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Aquaculture is an economic sector which is continuously growing. However, natural outbreaks of pathogens constitute an important difficulty that this sector must face. Nodavirus (NNV) is one of the most prevalent pathogens in the Mediterranean Sea, causing viral encephalopathy and retinopathy to more than 170 fish species. One of the most affected species is European sea bass (*Dicentrarchus labrax* L.), which is greatly important for the Spanish aquaculture sector. Antimicrobial peptides (AMPs) are short aminoacidic sequences, cationic and amphipathic with lytic activity against a broad spectrum of pathogens including viruses. Until now, scare studies have focused on AMPs antiviral activity in fish but with positive prospects. Therefore, we aimed to evaluate the potential stimulatory effects of AMPs on the European sea bass immune system, as well as their potential as a prophylactic agent against NNV. Thus, Hepcidin (Hamp) and Dicentracin (Dic) antimicrobial peptides were chemically synthesized and intramuscularly administered to European sea bass juveniles. Head-kidney (HK) and muscle were removed after one day from the treatment to evaluate the immunomodulatory capability of the AMPs. In that moment, fish were infected with NNV and sampled two days after infection, where HK, muscle and brain were removed. In all samplings, serum samples were taken in order to perform functional analysis. Mortality and clinical signs of infection (ranked according to the severity) were recorded daily. Hamp and Dic administration resulted in a local recruitment of immune cells such as macrophages, neutrophils and T and B-cells to the site of injection. Moreover, the levels of AMPs were modulated both systemic and locally. Regarding the disease pattern upon NNV infection, AMPs administration reduced the transcription levels of proinflammatory gene markers in brain, leading to the prevention of neuronal damage and ameliorating the mortality rates. In addition, Hamp is also able to prevent, to a certain extent, NNV mortalities conferring partial protection against this disease. To conclude, the use of AMPs as European sea bass could be a valuable tool to prevent NNV disease in fish farms. Funded by MCIN/AEI 10.13039/501100011033 (grants RTI2018-096625-B-C33, PID2019-105522GB-100 and PRE2020-093771 to L.C) and “Fundación Séneca” (grant 19883/GERM/15).

SP6.4 VPE

Efficient incorporation of a betanodavirus antigen in viral hemorrhagic septicemia virus (VHSV) vectors with rearranged genomes

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Viral hemorrhagic septicemia virus (VHSV) and Nervous necrosis virus (NNV) are two major pathogens of fish belonging to the genera *Novirhabdovirus* and *Betanodavirus*, respectively.

In this study, the genome of a salmonid VHSV isolate has been engineered to develop a bivalent vaccine against these two important viruses of aquaculture. By reverse genetics, we have generated nine attenuated recombinant VHSVs (rVHSVs) bearing an expression cassette encoding the major protective antigen domain of NNV capsid protein. Non-segmented negative-strand RNA viruses are subject to a transcription gradient dictated by the order of genes along their genomes, which plays a critical role in viral replication. As an approach for constructing stably attenuated virus, the order of the VHSV genes has been rearranged. In addition, the NNV specific antigenic domain has been fused to the signal peptide and the transmembrane domain derived from novirhabdovirus glycoprotein to enable trafficking of the antigen to the surface of infected cells and incorporation into viral particles. All recovered rVHSV, termed NxGyCz according to the respective positions of the genes encoding the nucleoprotein (N) and glycoprotein (G) genes as well as the expression cassette (C) along the genome, have been fully characterized in vitro for NNV epitope expression in cells and incorporation into VHSV virions. The suitability of the rVHSVs was assessed by immunization and challenge in juvenile trout and sole, the susceptible host species for VHSV and NNV, respectively. Following bath immersion administration of the various rVHSVs to juvenile trout, some of the rVHSVs were attenuated and protective against VHSV challenge. Results indicate that rVHSV N2G1C4 is safe and protective against VHSV challenge in trout. In parallel, juvenile sole were injected with rVHSVs and challenged with NNV. The rVHSV N2G1C4 is also safe, immunogenic, and protective in sole against NNV, thus presenting a promising starting point for the development of a live attenuated bivalent vaccine candidate for the protection of these two commercially valuable fish species against two major pathogens of aquaculture. This work was supported by the Xunta de Galicia (Consellería de Cultura, Educación y Universidad), postdoctoral grant nº ED481D-2022-024.

SP6.5 VPE

Immune response of vaccinated juvenile gilthead seabream (Sparus aurata) after LCDV-Sa infection

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Lymphocystis disease is one of the main viral pathologies affecting cultured gilthead seabream (*Sparus aurata*) in the Mediterranean area. Although the mortality rate of affected fish is low, the economic impact for hatcheries is remarkable due to the impossibility of marketing affected specimens showing the characteristic external lessions. In our group, a DNA-vaccine has been developed based on the major capsid protein (MCP) of the Lymphocystis Disease Virus 3 (LCDV-Sa). The aim of the present study is the evaluation of immune-related gene expression in vaccinated fish after viral infection to identify immunogenes involved in the vaccine-induced protection. To fulfil this objective an OpenArray® platform has been developed to study 49 genes related to the immune response. Reference and viral genes were also evaluated. Gilthead seabream specimens (5 g mean weight) were distributed into 3 experimental groups, inoculated with the vaccine at 0.1 μg/g fish dose, the empty plasmid at the same

dose or PBS. Thirty days post-vaccination, fish were intramuscularly injected with the virus at 10^6 $TCID_{50}$/fish dose. Samples of head-kidney, spleen, intestine and caudal fin from 6 fish were individually collected at 1, 2 and 3-days post-injection in all groups. The quantification of viral DNA in fins of fish challenged with LCDV-Sa were carried out by a qPCR assay targeting a viral structural gene (putative myristoylated membrane protein, MMP) alternative to the *mcp* gene contained in the vaccine. The results obtained showed an increase of genes deregulated within the haematopoietic organs between vaccinated and non-vaccinated fish. However, in the intestine and fin, the results showed the opposite trend. The global effect of fish vaccination was a diminished immune response compared to non-vaccinated fish, being 83 and 99 genes differentially expressed through the experiment, respectively. Moreover, viral replication decreased in groups of fish previously vaccinated. The modulation of the immune response provoked by the vaccination trial seems to control the progression of the disease. This research was funded by the Junta de Andalucia and FEDER under Grants P12-RNM-2261 and UMA20-FEDERJA-076.

SP7 REG

SP7.1 REG

A mouse parvovirus infecting human glioblastoma stem cells with patient-specific p53 deregulations

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Glioblastoma multiforme (GBM) remains a major type of cancer with no current effective therapy. On the other hand, multiple studies support that the efficacy of cancer therapies must be primarily shown against the cancer stem cells driving tumourigenesis. We have therefore attempted to develop a GBM therapy using two strains of the Minute Virus of Mice (MVMp and MVMi), a non-pathogenic parvovirus with evidenced oncolytic capacity and well characterized tropism determinants. To this aim, primary glioblastoma stem cells (GSCs) isolated from GBM patients were challenged with the MVM strains and the system was comprehensively studied in neurospheres. GSC neurospheres accumulated assembled capsids, but restrict viral NS1 cytotoxic protein expression by an innate PKR/eIF2α-P response. Inter- and intra-patient GSC heterogeneity was dissected by their diverse innate responses and the ratio between structural and non-structural viral gene expression. Further, viral infection triggered a comprehensive DNA-damage response involving cell cycle arrest, neurosphere disorganization, and bystander disruption of GSC-derived brain tumour architecture in rodent models. Notably, the MVM infection preferably targeted those GSC subpopulations within patients showing weak innate responses, and harbouring p53 gain-of-function mutants and/or p53-Ser15 phosphorylation. This study provides a molecular foundation for personalized biosafe viral therapies against devastating glioblastoma and other cancers with weakened innate responses and /or deregulated p53 signalling.

SP7.2 REG

DNA-dependent protein kinase complex differentially restricts hepatitis B virus and adeno-associated virus gene expression.

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Hepatitis B virus (HBV) is an hepatotropic virus that causes acute and chronic infections. It is estimated that more than 250 million people are chronically infected and 780.000 die every year from hepatic complications, such as

cirrhosis and hepatocellular carcinoma. Current treatments for chronic infection reduce HBV replication and spread efficiently, but fail to eliminate the infection. This is because they do not completely eradicate eliminate the covalently closed circular DNA (cccDNA) that serves as the viral transcriptional template and constitutes the hallmark of HBV persistence. *cccDNA* formation relies on the repair of the viral genome, in a process mediated by cellular factors. This study focuses on the role of DNA-dependent protein kinase (DNA-PK) as a restriction factor for HBV infection. This nuclear complex is formed by the regulatory heterodimer (ku70 and ku80) and the catalytic subunit (DNA-PKc), which triggers the repair of DNA double strand breaks by the non-homologous end joining repair pathway. Using shRNA expressing lentiviruses we observed that reduction of endogenous Ku70 and ku80 protein accumulation leads to an increase on HBV parameters (i.e. intracellular core protein (HBVcore) and extracellular e antigen (HBeAg)) upon infection. This phenotype was extended to a GFP expressing adeno-associated viral vector (AAV3b.AAT.GFP), in which the formation of concatemeric circular genomes is essential for a proper transduction. Using this system, a 36-fold increase in GFP expression was observed in Ku-deficient cells compared to control cells. Overexpression of tagged-Ku70 in Ku-silenced cells decreased AAV-driven GFP expression, ruling out the possibility that the phenotype was related to off-target effects. Interestingly, chemical inhibition of the DNA-PKc enzymatic activity did not affect AAV-driven GFP expression while it produced a small (2- to 2.5-fold) but reproducible increase in HBVcore and HBeAg accumulation upon HBV infection. Collectively, these results suggest that the underlaying mechanisms responsible for the effects observed on HBV and AAV-driven gene expression are different. We are currently working on elucidating these molecular mechanisms. This work was supported by grants SAF2016-75169-R and PID2020-118970RB-100 to U.G. and FPU17/03424 fellowship to A.G.M and RTI2018-101936-B-I00 to GGA.

SP7.3 REG
CHIKV uses two different tRNA-related strategies to favor viral protein expression

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Viruses are obligatory intracellular parasites that intimately adapt to the host they infect. How mosquito-borne viruses such as chikungunya virus (CHIKV) expand so efficiently in humans and mosquitoes, two organisms one million years apart in evolution, and why they kill the human cells but chronically infect the mosquito ones remain unsolved fundamental questions. Previously, we showed that CHIKV alters the human tRNA epitranscriptome to adapt the host translational machinery to the viral RNA genome enriched in sub-optimal codons in such a way that they become optimal for viral gene expression. Here, we address whether this mechanism is conserved in mosquito-infected cells. By using RNA-seq and ribosome profiling we obtained a high-resolution time course analyses of the transcriptome and translatome in CHIKV-infected mosquito cells. CHIKV-infection did not induce changes in codon optimality but increased the translational capacity of the cell by favouring translation of host genes related to ribosomal RNA biogenesis and to tRNA function, including tRNA maturation enzymes and aminoacyl tRNA synthetases, that are enzymes that attach the appropriate amino acid onto its corresponding tRNA. Consequently, the viral RNAs can be efficiently translated without majorly affecting the host expression programme, reaching the virus-host equilibrium required to establish chronic infection. Our results demonstrate that CHIKV uses two different tRNA-mediated strategies to favour viral protein expression in human and mosquitoes.

SP7.4 REG
Dynamic analysis of the SARS-CoV-2 response using single-cell RNA sequencing data

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Single-cell RNA sequencing (scRNA-seq) has become a powerful technique to study the dynamic changes in the transcriptome of a cell a population subjected to an external stimulus. This method can also be used to characterize the progression of a viral infection if virus accumulation is used as proxy of time or pseudotime. In this study, we analyzed publicly available scRNA-seq datasets to extract common features of the SARS-CoV-2 response. We compared data from *in vitro* inoculation studies of human bronchial epithelial cells, colon, and ileum organoids, using scaled transcriptional profiles of individual genes. Our analysis revealed that about 90% of genes exhibited a transcriptional response characterized by a triphasic pattern comprising and two down-regulatory phases and one intermediate up-regulatory phase. Additionally, a correlation network analysis revealed a distinct correlated response of mitochondrially-encoded genes and genes involved in translation. Finally, we used the transcriptional time profiles genes to model the progression of infected cells using a network of protein-protein interactions.

SP7.5 REG
Cryo-SXT imaging of SARS-CoV-2-infected human lung epithelial cells.

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A common feature among positive strand viruses is that they alter cellular membranes to generate replication complexes. Although the origin, nature and structure of these membranous compartments are not identical, they constitute a characteristic feature of these viruses and are observed in yeast, plants and higher eukaryote (+)-strand RNA viruses. Thanks to recent advances in 3D reconstruction of cellular volumes by electron tomography, regardless of the virus or the host, membranous (+)-strand RNA virus replication compartments have been structurally classified into double-membrane vesicles (DMV), such as those observed in coronavirus infections, or invaginated vesicles (IV), such as those observed in dengue virus infection, among others. In this study we have performed full-field cryo soft X-ray tomography (cryo-SXT) in the water window photon energy range to investigate in whole, unstained cells, the morphology of the membranous rearrangements induced by SARS-CoV-2 infection in near-native conditions. Our results are comparable to those obtained by others using electron and cryo-tomography which suggests that SARS-CoV-2 infected cells display DMV structures similar to those found in other coronaviruses or hepatitis C virus infection. Our studies provide a wider cellular context in which these membranous alterations occur and point at the formation of compact perinuclear structures where viral antigens are concentrated by constriction within intermediate filaments, as determined by confocal microscopy. This perinuclear structure is formed by a tightly juxtaposed tubular membranous network reminiscent of a highly modified endoplasmic reticulum. This structure is virtually devoid of normal mitochondria and adjacent mitochondria display clear ultrastructural signs of stress. Finally, late stages of the infection indicate deformation of the cell nucleus is areas close to the viral factory and an overall cytoplasmic retraction of the infected cell. Overall, our studies provide whole cell volumes of SARS-CoV-2 infected cells at 30nm resolution that contribute to document the context in which observations with higher resolution techniques are made. This study was funded by were founded by ALBA Synchrotron standard proposals 2021024899. AJPB, GC, VC, EP and PG are part of the CoCID Consortium, which has received funding from the European Union's Horizon 2020 research and innovation programme under Grant No. 101017116.

SP8/SP10 PVI

SP8.1 PVI

Interaction of herpes simplex virus type 1 with autophagy in oligodendrocytes

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Autophagy is a highly conserved catabolic process among eukaryotes consisting of the degradation of intracellular components into lysosomes to ensure metabolic homeostasis. Autophagy may act as an antiviral mechanism that promotes virions degradation and viral antigen presentation. Herpes simplex virus type 1 (HSV-1) is a neurotropic virus that stablishes latency in the trigeminal ganglia and, after reactivation, might spread upstream to the CNS. Due to the high adaptability of HSV-1 to its hosts, the virus has developed several mechanisms to get away from autophagy. Although the relation HSV-1- autophagy has been extensively studied in neurons, there are no studies in oligodendrocytes, the myelin- forming cells of the CNS, even when HSV-1 infection of oligodendrocytes has been related to demyelinating diseases, such as multiple sclerosis. We observed that, in differentiated human oligodendroglioma (HOG) cell line, autophagy is not induced during infection, not even with UV- irradiated HSV-1, suggesting that autophagy may not be an important antiviral mechanism in these cells. However, HSV-1 inhibits autophagy in later stages of infection, a previously described HSV-1 defence strategy in certain cell types. Besides, later in infection, HSV-1 triggers a new form of autophagy known as nuclear envelope-derived autophagy (NEDA), which is independent on classical autophagy or macroautophagy and whose effects on viral infections remain mainly unknown. To analyse the effect of autophagy on oligodendrocyte infection, we blocked the pathway with pharmacological autophagy inhibitors. Interestingly, lower levels of autophagy resulted in infection reduction. We also generated HOG cells knock-out for the autophagy-essential gene *atg5* by CRISPR/Cas9. HSV-1 infection was highly impaired in these deficient-autophagy cells, suggesting that a basal level of autophagy is necessary for HSV-1 replication in oligodendrocytes. *Atg5* knock-out cells treated with autophagy inhibitors showed a significant decrease in infection compared to non-treated cells, indicating that the antiviral role of these inhibitors is mostly due to its nonspecific effects. We argue that macroautophagy may not be a main antiviral mechanism in oligodendrocytes, and other types of autophagy could play a more relevant role. Furthermore, basal autophagy could be exploited by HSV-1 to promote infection.

SP8.2 PVI

Hepatitis C virus fitness can influence the extent of infection-mediated epigenetic modifications in the host cells

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Cellular epigenetic modifications occur in the course of viral infections. Hepatitis C virus (HCV) can affect the course of the infection itself, and the development of post-infection sequels such as hepatocellular carcinoma (HCC), often linked to progression of liver cirrhosis. We previously documented that HCV infection of human hepatoma Huh-7.5 cells results in a core protein-mediated decrease of Aurora kinase B (AURKB) activity and of phosphorylation of Serine 10 in histone 3 (H3Ser10ph), with an affectation of inflammatory pathways. Viral fitness, defined as the capacity of a viral population to produce infectious progeny, can have profound effects in the virus-host relationship, infection outcome, and response to antiviral treatments. The possible role of HCV fitness in infection-derived cellular epigenetic modifications is not known. Given the multiple consequences of HCV fitness on host interactions, we approach this question using HCV populations that display up to 2.3-fold increase in general fitness (infectious progeny production), and 45-fold increase of the exponential phase of intracellular viral growth rate, relative to the parental HCV population. We show that infection results in an HCV fitness-dependent decrease of the levels of H3Ser10ph, AURKB, and histone 4 tri-methylated at lysine 20 (H4K20m3). In contrast, high HCV fitness did not enhance the intracellular levels of histone deacetylase 1 (HDAC1) nor did it contribute to a modest increase of DNA methyl transferases 1 and 3a (DNMT1 and DNMT3a). Remarkably, the decrease of H4K20m3, which is a hallmark of cellular transformation, was significant upon infection with high fitness HCV but not with basal fitness virus. Thus, HCV fitness can differentially impact cellular epigenetic markers. A prolonged replication of a human RNA virus in a constant cellular environment which results in an increase replication capacity, can modify the type and intensity of some (but not all) host cell epigenetic modifications. The results point towards the possibility that infection-mediated differential epigenetic changes may influence liver disease and liver cancer in HCV infections in vivo. Emphasis is made in the possibility that HCV-mediated HCC may be favored by prolonged HCV infection of a human liver, a situation in which viral fitness is likely to increase.

SP8.3 PVI
Insights into picornavirus biology, evolution, and pathogenesis from deep mutational scanning analyses

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Background: RNA viruses have the highest mutation rates in nature, which facilitate their adaptation to changing environments and complicate their clinical management. However, most mutations are deleterious to protein function, raising the question of how RNA viruses contend with such high mutation rates and how these mutations affect viral biology, evolution, and pathogenesis. Methods: To address these fundamental questions, we performed the largest analysis of mutational fitness effects in a picornavirus to date, encompassing >92% of all possible single amino acid mutations across two-thirds of the viral protein-coding region, including both structural and non-structural proteins. We integrate the results of this analysis with functional, structural, and evolutionary information to gain insights into the features that influence mutational fitness effects in these diverse viral proteins. In addition, we evaluate how different environments alter mutational fitness effects to better understand how the environment influences evolutionary processes. Finally, we map how this virus escapes neutralization by polyclonal sera to gain insights into the role of diversity in host-pathogen interaction. Results: Mutations were overall deleterious yet the distribution of mutational fitness effect differed between different viral proteins and was significantly altered by environmental conditions such as temperature and cellular stress. Different structural, evolutionary, and functional attributes correlated with mutational fitness effects across all proteins but were different for distinct environmental conditions. Polyclonal sera result in distinct escape profiles depending on the donor, and differed significantly between human and mice. Conclusion: Our results provide a comprehensive understanding of mutational fitness effects across multiple picornavirus proteins and protein classes, and the different functional, evolutionary, and structural attributes that correlate with them. In addition, we provide insights into how different environments can shape mutational fitness effects and, as a result, viral evolution. Finally, we reveal for the first time how a picornavirus is targeted by polyclonal antibody responses in natural infection conditions.

SP8.4 PVI

The Arabidopsis m6A readers ECT2, ECT3 and ECT5 restrict infection of alfalfa mosaic virus

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Methylation of N^6-adenosine (m^6A) is a post-transcriptional modification that influences the fate of their RNA targets, mainly through the binding of m^6A *readers*. Recent reports have empathized the key role of this post-transcriptional modification in the infection cycle of an increasing number of mammalian viruses. However, research about m^6A in plant viruses is in its infancy and we are now beginning to understand the regulatory mechanisms of this modification. In previous work, we identified the presence of m^6A in the RNAs of two plant viruses and found that the relative abundance of m^6A in alfalfa mosaic virus (AMV) RNAs regulates viral infectivity. Furthermore, we showed that the demethylase activity of ALKBH9B modulates this viral process, probably via its interaction with the AMV CP. Here we analyze the involvement of Arabidopsis m^6A *readers* in the infection cycle of a plant virus. Consistent with the previously observed m^6A-dependent antiviral effect, we find that, in Arabidopsis plants, the absence of the ECT2/ECT3/ECT5 module promotes the systemic infection of AMV. Furthermore, an ECT2 point mutant specifically defective in m^6A recognition loses wild type antiviral activity, suggesting that this effect, at least for ECT2, relies on the capability of this *reader* to interact with m^6A sites. HyperTRIBE (targets of RNA-binding proteins identified by editing) using transgenic plants expressing either WT or point mutant ECT2 corroborates the *in vivo* interaction between this protein and AMV m^6A-RNAs through its m6A binding site. Finally, we show that the expression of the ECT2/ECT3/ECT5 module reverts the partial resistance against the infection conferred by the absence of ALKBH9B, and that this hitherto defensive mechanism seems to be genetically uncoupled from the role of ECT proteins in organogenesis.

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SP8.5 PVI

Usutu virus replication evades cellular stress response impairing eIF2α phosphorylation and stress granules formation.

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Usutu virus (USUV) is a mosquito-borne member of the genus flavivirus closely related to West Nile, Japanese encephalitis, Zika, and dengue viruses. The virus was isolated for the first time in Africa, in 1959, where it regularly circulates. Since its emergence in 1996 in Europe, USUV has quickly spread across the continent causing a high number of bird deaths and varied neurological disorders in humans, including encephalitis, meningoencephalitis, or facial paralysis, thus warning about USUV as a potential health threat. USUV replication takes place on the endoplasmic reticulum (ER) of infected cells, inducing ER stress and resulting in the activation of stress-related cellular pathways collectively known as the integrated stress response (ISR). The alpha subunit of the eukaryotic initiation factor eIF2 (eIF2α), the core factor in this pathway, is phosphorylated by stress activated kinases: protein kinase R (PKR), PKR-like endoplasmic reticulum kinase (PERK), heme-regulated inhibitor kinase (HRI), and general control non-repressed 2 kinase (GCN2). Small changes in eIF2α phosphorylation can derive in a high suppression of downstream protein

synthesis, and even disable the cellular translation machinery, with accumulation of discrete foci in the cytoplasm termed stress granules (SGs). USUV infection eludes cellular stress response reducing eIF2α phosphorylation and SGs assembly induced by treatment with the HRI activator ArsNa. Our results indicated that USUV can take advantage of cellular stress mechanisms to counteract cell stress responses, promoting an appropriate environment for viral multiplication. This protective effect was related with oxidative stress responses in USUV-infected cells. Overall, these results provide new insights into the complex connections between the stress response and flavivirus infection in order to maintain an adequate cellular environment for viral replication.

SP10.1 PVI
Deciphering the molecular basis underlying IBDV persistent infections

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Members of the *Birnaviridae* family exhibit a dual behavior, causing acute infections often followed by the establishment of lifelong asymptomatic persistent infections. Indeed, persistently infected specimens might represent undetected virus reservoirs, hence playing a chief role on virus dissemination. In spite of the paramount importance of this biological trait, information about mechanisms supporting birnavirus persistency is as yet negligible. Our work has focused on the establishment of persistent infections with infectious bursal disease virus (IBDV), the best characterized birnavirus and a major avian pathogen causing both acute and persistent infections in domestic chickens. Here, we describe the establishment of long-term, productive persistent IBDV infections in DF-1 cells (immortalized chicken embryonic fibroblasts). Long-term maintenance of persistently infected cultures leads to selection of clonal cell populations unable to respond to type I interferon (IFN). High-throughput genome sequencing revealed that this is due to mutations affecting the IFN-α/® receptor subunit 2 (IFNAR2) gene, resulting in the expression of IFNAR2 polypeptides harboring large C-terminal deletions, and then, abolishing the signaling capacity of IFN receptor complex. Indeed, ectopic expression of a recombinant chicken IFNAR2 gene efficiently rescues IFN responsiveness. Prolonged treatment with 7-deaza-2-C-methyladenosine (7DMA), a potent inhibitor of the IBDV RNA-dependent RNA polymerase, eradicates viral populations from persistently infected DF-1 cells. 7DMA-cured DF-1 cells exhibit a drastically enhanced susceptibility to establishing new persistent IBDV infections. In addition, work performed with HeLa IFNAR2 knockout cells fully recapitulate data gathered with DF-1 cells. Our results indicate that the inactivation of the JAK-STAT signaling pathway largely ablates the apoptotic response triggered by the infection, hence increasing the susceptibility to establishment IBDV persistent infections. In addition, we have found that IBDV progeny yields in persistently infected cells are exceedingly lower (<4 $\log_{10}$) than those recorded in acute infections, therefore suggesting that persistency also entails a major downregulation of IBDV replication. We are currently applying NGS-based analyses to try and unveil mechanisms responsible for this intriguing phenomenon. This work was supported by grant PID2020-112847RB-I00 funded by MCIN/AEI/10.13039/501100011033.

SP10.2 PVI

Into the eye of the classical swine fever cytokine storm: Role of the Erns RNase activity and a poly-uridine insertion in the 3´UTR

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The understanding of attenuation mechanisms in low virulence classical swine fever virus (CSFV) strains remains scarce, despite its potential contribution to vaccine and diagnosis development. Previous studies revealed that the unique uninterrupted poly-uridine (poly-U) sequence found in the 3'UTR of the low virulence CSFV field isolate Pinar de Rio (PdR) is a virulence factor that decreased CSFV replication and attenuated virulence in piglets. Moreover, the loss of pathogenicity and replication capacity in pigs when the Erns RNase function was abrogated in this PdR strain has been reported. The present study aimed to assess the synergistic role of the Erns RNase activity and the poly-U insertion in the 3'UTR of the PdR strain in the innate and adaptive immunity regulation, viral replication and their relationship with CSF pathogenesis in two age ranges of pigs. A double mutant with an inactivation of the Erns RNase function and 5 uridines instead of 36 in the 3'UTR was constructed (vPdR-H30K-5U). A group of nineteen 5-day-old piglets and another of six 3-week-old pigs were infected with vPdR-H30K-5U. Severe clinical manifestations were observed in both infected groups, with higher mortality in the 5-day-old piglets, indicating the important role of pig's age for CSFV susceptibility and the pathogenesis modulation by the synergistic effect of the 3´UTR and the lack of the Erns RNase activity. Pigs infected with vPdR-H30K-5U showed high viral replication in tonsils but low viremia and viral excretion, likely due to a modulation of viral replication by the 3'UTR and the host innate immunity by the Erns RNase activity. IFN-α and IL-12 were highly elevated in the two groups. High IL-8 levels were found in the newborn but not in the older pigs. This points towards a role of these cytokines in the outcome of disease, with age-related differences. In addition, infection with vPdR-H30K-5U resulted in the reduced adaptive immune response from the pigs, which correlated with the viral replication and innate immune response. These results give a better understanding of the relationship between CSFV virulence and viral replication and host immunity, providing new directions for the study of the natural CSFV attenuation molecular determinants.

SP10.3 PVI

SARS-CoV-2 PATHOGENESIS MEDIATED BY E PROTEIN PBM WAS PREVENTED BY MODULATORS OF CFTR FUNCTION

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Coronaviruses (CoVs) of genera ⟨, ®, © and ™ encode proteins that have a PDZ-binding motif (PBM) consisting of the last four residues of the E protein (PBM core), which may bind over 400 cellular proteins containing PDZ domains, making them relevant for the control of cell function. Three highly pathogenic human CoVs have been

identified to date: SARS-CoV, MERS-CoV and SARS-CoV-2. The relevance of their E protein PBMs in virulence has been studied. First, recombinant variants of these viruses missing each of E protein PBMs were generated and their pathogenicity was analyzed in mice. The PBMs of these three CoVs were virulence factors. A collection of SARS-CoV mutants in which the E protein PBM core was replace by the E protein PBM core from virulent or attenuated CoVs was constructed and their virulence analyzed. A gradient of virulence, depending on whether the alternative PBM core introduced was derived from a virulent or an attenuated CoV was observed. The gene expression patterns associated with the different PBM motifs in lungs of mice infected with SARS-CoV was analyzed by deep sequencing of the mRNAs expressed in lungs of infected mice, and it was observed that the E protein PBM motif of SARS-CoV and SARS-CoV-2 dysregulated the expression of genes related to ion transport and cell homeostasis. Specifically, a decrease of the mRNA expression of the cystic fibrosis transmembrane conductance regulator (CFTR), which is essential for edema resolution, was observed. The reduction of CFTR mRNA levels was associated with edema accumulation in lungs of mice infected with SARS-CoV. The effect of compounds that modulate the expression and activity of CFTR on the replication of SARS-CoV and SARS-CoV-2 was studied in cell cultures and it was observed that these compounds drastically reduced the production of SARS-CoV-2 and protected against its infection in mice model. These results showed the high relevance of the PBM motif in the replication and virulence of CoVs, and have allowed the identification of cellular targets for the selection of antivirals.

SP10.4 PVI

The IFN alpha inducible proteins IFI6 and IFI27 are negative regulators of innate immune responses and modulate RIG-I activation

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Innate immune responses mediated by interferons (IFNs), IFN-stimulated genes (ISGs), and inflammatory cytokines are essential to establish an antiviral response. As such, retinoic acid-inducible gene I (RIG-I) is a key sensor of virus infections, mediating the transcriptional induction of IFNs and inflammatory proteins. However, these responses need to be highly regulated since exacerbated responses could be detrimental to the host. In this work, we show, for the first time, that knocking-down or knocking-out the expression of IFN alpha inducible proteins 6 and 27 (IFI6 and IFI27, respectively) increases IFN, ISGs, and pro-inflammatory cytokine expression after influenza A virus (IAV), Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), and Sendai virus (SeV) infections, or after poly(I:C) transfection. Even more, we describe how the overexpression of IFI6 and IFI27 produces the contrary effect, even in vivo, indicating that these proteins negatively modulate the induction of innate immune responses. In addition, we show that knocking-down or knocking-out the expression of IFI6 and IFI27 decreases the production of infectious IAV and SARS-CoV-2, most likely through its effect on the antiviral responses. Remarkably, we report a novel interaction of IFI6 and IFI27 with double-stranded (ds)RNA, and an interaction of both proteins with RIG-I, being this interaction most likely mediated through binding to dsRNA. We describe that IFI6 and IFI27 affect RIG-I activation, providing a molecular mechanism for the effect of these proteins on modulating innate immune responses. Importantly, these new functions for IFI6 and IFI27 could be exploited to treat diseases associated to excessive innate immune responses and to combat several viral infections, including IAV and SARS-CoV-2.

SP10.5 PVI

Impact of TNF, LTα and chemokines in the pathogenesis of mousepox

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Tumor necrosis factor (TNF) is an inflammatory cytokine produced in response to viral infections that promotes the recruitment and activation of leukocytes to sites of infection. This TNF-based host response is essential to limit virus spreading, thus poxviruses have evolutionarily adopted diverse molecular mechanisms to counteract TNF antiviral action. One of them is the secretion of viral proteins from infected cells with the ability to bind key host cytokines before engagement with cellular receptors, thus acting as decoy receptors. Among these proteins, we find four viral TNF receptors (vTNFRs): cytokine response modifier B (CrmB), CrmC, CrmD and CrmE. It is worth noting that these soluble receptors are differentially distributed among poxvirus species. A strictly human poxvirus like variola virus only expresses CrmB as functional vTNFR and the mouse-specific ectromelia virus (ECTV) expresses only CrmD. Unlike CrmC and CrmE, both CrmB and CrmD have the ability to bind lymphotoxin α (LTα) and contain a C-terminal domain (SECRET domain) with the ability to bind with high affinity and block the activity of a particular set of chemokines (CKs) involved in mucosal and skin inflammation. In the present work, we have assessed the impact that blocking TNF, LTα and CKs have on viral pathogenesis, using the mousepox infection model, a disease caused by ECTV in susceptible mice and the surrogate animal model for smallpox. In this model, an ECTV lacking CrmD (anti-TNF, anti-LTα and anti-CKs) is highly attenuated and allows us to test whether expression of other vTNFRs reverts this attenuation. An ECTV expressing CrmB from cowpox virus instead of CrmD has full virulence, demonstrating the critical role of CrmB in viral pathogenesis. In order to assess the importance of blocking LTα, we generated a recombinant ECTV expressing CrmC (anti-TNF) instead of CrmD (anti-TNF and anti-LTα), keeping intact the SECRET domain. Expression of CrmC-SECRET did not fully restore ECTV virulence, emphasizing the relevance of blocking LTα for infection progression. In summary, this model allows us to dissect the specific contribution of TNF, LTα and CKs to poxvirus pathogenesis.

SP9 NAB

SP9.1 NAB

Production of recombinant plant virus-derived nanoparticles carrying capsid-fused nanobodies for SARS-CoV-2 detection

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The use of viruses as expression vectors to produce recombinant proteins in plants has been extensively characterized in biotechnology. Viral nanoparticles (VNPs) are a new class of virus-based formulations that have recently attracted increasing attention because they can be used as building blocks for novel materials to support a range of functions of potential interest in medicine and nanotechnology. For instance, the use of VNPs presenting monoclonal antibodies (mAbs) on their surface is becoming a very promising tool for therapeutic and diagnostic purposes. An interesting new alternative to mAbs are the nanobodies or VHHs. These are the variable domains of heavy-chain antibodies from animals belonging to the family *Camelidae*, which have several properties that make them attractive therapeutic molecules, such as their small size, simple structure, and high affinity and specificity. In this work, we describe the production of VNPs derived from two different flexuous rod-shaped plant viruses as platforms for SARS-CoV2 nanobody presentation in *Nicotiana benthamiana* biofactories. On the one hand, we produced multivalent VNPs derived from potato virus X (PVX), whose use as a scaffold for peptide presentation has been previously described. On the other hand, we developed for the first time recombinant VNPs derived from tobacco etch virus

[TEV], which belongs to the largest family of RNA viruses, *Potyviridae*. For both viral expression systems, correct assembly of recombinant VNPs was successfully observed by transmission electron microscopy. Moreover, the functionality of the CP-fused nanobodies was demonstrated by enzyme-linked immunosorbent assays. We are currently working in the expression of viral antigens following the same strategy. With this aim, we have designed and produced VNPs expressing different SARS-CoV-2 antigenic regions in order to analyze their efficacies as peptide presenting platforms. In sum, in this work we successfully produced *in planta* VNPs derived from both PVX and TEV, decorated with functional nanobodies, which could be an interesting tool for therapeutic and diagnostic purposes.

SP9.2 NAB
Broad viral inactivation by soda-lime glass and kaolin based materials

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Inorganic materials can provide a set of tools to decontaminate solid, liquid or air containing viral particles. The use of disinfectants can be limited or not practical in scenarios where continuous cleaning is not feasible. Physicochemical differences between viruses raise the need for effective formulations for all kind of viruses. In the present work we describe two types of antimicrobial inorganic materials: i) a novel soda-lime glass [G3], and ii) kaolin containing metals nanoparticles [Ag or CuO], as materials to disable virus infectivity. Strong antiviral properties can be observed in G3 glass, and kaolin-containing nanoparticle materials showing a reduction of viral infectivity close to 99%. in the first 10 minutes of contact of vesicular stomatitis virus [VSV]. A potent virucidal activity is also present in G3 and kaolin containing Ag or CuO nanoparticles against all kinds of viruses tested, reducing more than 99% the amount of HSV-1, Adenovirus, VSV, Influenza virus and SARS-CoV-2 exposed to them. Virucidal properties could be explained by a direct interaction of materials with viruses as well as inactivation by the presence of virucidal elements in the material lixiviates. Kaolin-based materials guarantee a controlled release of active nanoparticles with antiviral activity. Current coronavirus crisis highlights the need for new strategies to remove viruses from contaminated areas. We propose these low-cost inorganic materials as useful disinfecting antivirals in the actual or future pandemic threats.

SP9.3 NAB

AVIAN PARAMYXOVIRUS 4 (APMV-4) ONCOLYTIC VIROTHERAPY LEADS TO COMPLETE RESPONSES AND LONG-TERM ANTI-TUMOR MEMORY IN PRECLINICAL MELANOMA, COLON CARCINOMA AND LYMPHOMA MODELS.

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Avulaviruses represent a diverse subfamily of non-segmented negative strand RNA viruses infecting avian species worldwide. Among the 22 different serotypes identified, only APMV-1, also known as Newcastle disease virus (NDV), has been extensively characterized due to its relevance for the poultry industry and, more recently, its inherent oncolytic activity and potential as a cancer therapeutic. To date, the oncolytic potential of other closely related avulaviruses remains unknown. We have examined the in vivo anti-tumor capability of prototype strains of APMV serotypes -2, -3, -4, -6, -7, -8 and -9 in syngeneic tumor models. Our studies have identified APMV-4 Duck/Hong Kong/D3/1975 virus as a novel oncolytic agent with greater therapeutic potential than one of the NDV clinical candidate strains, La Sota. Intratumoral administration of the naturally-occurring APMV-4 virus significantly extends survival, promotes complete remission, and confers protection against tumor rechallenge in lymphoma, colon carcinoma and melanoma preclinical models. In addition, as a single agent, APMV-4 virotherapy has demonstrated abscopal effect or elimination of distant untreated tumors. Lastly, we have designed a plasmid rescue strategy that allows us to develop recombinant APMV-4-based viruses. The infectious clone rAPMV-4 preserves the extraordinary antitumor capacity of its natural counterpart, paving the way to a promising next generation of viral therapeutics with broad applicability in the clinic. Financial support: this work has been partly supported by NCI grant R01CA229818 to AG-S.

SP9.4 NAB

INFECTIOUS BURSITIS DISEASE VIRUS AS A POTENTIAL ONCOLYTIC VIROTHERAPY AGAINST GLIOBLASTOMA AND OTHER CANCER CELL TUMOR MODELS.

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Glioblastoma is considered the most aggressive type of central nervous system (CNS) cancer. Current therapies such as temozolomide (TMZ) do not offer a life expectancy greater than 15 months, while other cancers such as colon carcinoma still do not have a consolidated immunotherapy. The use of oncolytic viruses (OVs) is an immune-related therapeutic alternative based on the selective destruction of cancer cells lacking or with limited anti-viral innate immune mechanisms, leaving non-tumoral cells as barrier controlling virus growth and non-tumoral cell damage. In this work, it is proposed the potential oncolytic use of infectious bursitis disease virus (IBDV). IBDV is a bird pathogen causing the Gumboro disease, but non-pathogenic in humans. IBDV oncolytic potential has been tested in several tumor models *in vitro* and *in vivo* comparing the oncolytic potential with the oncolytic reference Newcastle disease virus (NDV). Results indicate a potential oncolytic activity of IBDV in several cancer types such as glioblastoma (CT-2A and GL261), melanoma (B16-F10) and colon carcinoma (CT26). In all cell lines tested, IBDV has the ability to infect, replicate and destroy cancer cells as well as stimulating pro-inflammatory cytokines that may potentially trigger the activation of the immune system against both, the virus and the the tumor.

SP9.5 NAB
DESIGN OF RHDV VLPS WITH ENHANCED MECHANICAL STABILITY AND EVALUATION OF THEIR IMMUNOGENICITY

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Virus-like particles (VLPs) are protein complexes which mimic virion well-defined geometry, while being devoid of genetic material. They are outstanding and safe immunogens combining a highly ordered and particulate nature with lack of replication ability. Rabbit Hemorrhagic Disease Virus (RHDV) VLPs represent a promising platform for multimeric antigen display due to their intrinsic characteristics: i) RHDV VLPs are composed of a single capsid subunit (protein VP60), easing the production and engineering of chimeric VLPs; ii) they are highly immunogenic, inducing complete protection of rabbits against RHDV lethal challenge; iii) RHDV is restricted to lagomorphs (rabbits and hares), hence no pre-existing immunity is expected in humans or livestock species, avoiding potential detrimental effects of anti-carrier immunity; iv) RHDV VLPs have proven to be very tolerant, accepting simultaneous insertion of target epitopes at different insertion sites, as well as incorporation of tandem copies of foreign epitopes at surface-exposed loops; and v) chimeric RHDV VLPs displaying foreign epitopes have been shown to be excellent immunogens, inducing cellular immune responses against inserted target cytotoxic T-cell epitopes, as well as potent protective humoral responses against foreign B-cell epitopes in the mouse and pig models. One important feature of VLPs intended to be used for biotechnological applications is the physical stability of the assembled particles. Generating VLPs with increased physical stability might be desirable for the development of improved biotechnological nanodevices (i.e. particles able to resist relatively harsh physical and/or chemical conditions during production processes and/or increased storage duration). Currently, no information is available on the mechanical properties of the RHDV capsid. Here we report the analysis of the mechanical properties of RHDV capsid protein (virions and VLPs) using atomic force microscopy (AFM), and the generation of rationally modified VLPs with increased mechanical stiffness and rupture force. These engineered VLPs were subjected to immunogenic evaluation in the mouse model.

SP11 ITV

SP11.1 ITV

Intra- and inter-cellular transport of plant viruses. Similarities and differences with animal viruses

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While in animal cells, extracellular matrix components offer a platform for virus recognition and entry, the rigidity of plant cell walls constitutes a physical barrier that makes virus entry difficult. Thus, plant viruses cannot exploit any of the membrane fusion-based routes of entry described for animal viruses. To overcome the rigidity of the cell wall, plant viruses normally take advantage of the way of life of different biological vectors to enter and replicate within plant cells. Once inside the cell, plant viruses hijack endogenous host transport machinery to aid their intracellular spread. In addition, taking advantage of the characteristic symplastic continuity of plant cells, viruses need to remodel and/or modify the restricted pore size of the plasmodesmata (channels that connect plant cells). In a successful interaction for the virus, it can reach the vascular tissue to systematically invade the plant. We use one of the simplest multicomponent transport systems identified to date, such as the *Carmovirus* genus, which is extremely reduced in the case of Melon necrotic spot virus (MNSV), as a model to study intra- and inter-cellular movement of plant viruses. The MNSV genome codes for five functionally characterized proteins and two small proteins are directly involved in the virus movement, p7A and p7B. p7A shows RNA-binding capabilities whereas p7B has a single-TMD domain, which allows protein insertion into the ER-derived microsomal membranes obtained in vitro. By the other hand, MNSV CP localizes to both mitochondria and chloroplasts in ectopic expression and during MNSV infection. By using different experimental approaches, we will show results that collectively demonstrate that a functional Golgi-mediated secretory pathway is essential for the intra- and intercellular movement of a plant virus and that a specific region/domain of MNSV CP can act as an ambiguous transit peptide driving dual targeting of MNSV CP to mitochondria and chloroplasts.

SP11.2 ITV

Elucidating the interactome of African swine fever virus proteins

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There is little information regarding the cellular interactome of African swine fever virus (ASFV) proteins, especially for those proteins potentially involved in early stages of the infection such as entry and viral replication. ASFV starts its infection cycle with the viral adsorption and entry into the host cell. Then, it is internalized in endosomes to be incorporated into the endocytic pathway. This is a dynamic process, in which endosomal maturation entails changes in the characteristics of these vesicles, including luminal acidification. The acidic pH of endosomes dissolves the outer capsid of the virion, exposing the inner viral membrane and specific viral proteins, in order to interact with the limiting membrane of the late endosome, for viral fusion facilitating the egress to the cytoplasm and ultimately, the start of viral replication. In this work we have studied the cellular interactome of viral proteins related to entry and viral replication by proteomics. The knowledge of these targets would enlighten the mechanisms initiated at endosomes that ASFV requires for infection. We studied ASFV proteins with a potential role at the entry/fusion

stages by affinity purification followed by mass spectrometry, in order to build the cellular interactome network for these proteins. For example, ASFV protein, E199L at the exposed inner viral membrane, interacted with endosomal receptor Niemann-Pick C type 1 (NPC1). Then, the relevance of this interaction was analyzed as the absence of NPC1 impaired viral infection in CRISPR NPC1 knock-out cells. In these null cells, virion entry and progression were arrested, and viral cores retained at late endosomes. Our results provide new insights for the virus targets at early stages of infection. Funding from ICRAD, an ERA-NET co-funded under European Union's Horizon 2020 research and innovation programme under Grant Agreement n°862605

SP11.3 ITV
The nonstructural protein muNS of Avian Reovirus engages in liquid-liquid phase separation

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Avian reovirus (ARV), a prototype fusogenic virus, is a naked, double-stranded RNA virus with a segmented genome. Like many virus, ARV replicates in large cytoplasmic structures termed viroplasms or viral factories, where viral proteins and genomic RNA are concentrated to presumably facilitate the formation of infectious particles. The non-structural protein muNS appears to play a critical role in the formation of viroplasms as it is the only viral protein able to form viral factory-like inclusions in transfected cells and is capable of recruiting several other ARV proteins to these inclusions. Recently the viroplasms of several viruses, including rabies and measles virus, VSV, and rotavirus have been shown to behave as biological condensates based on their ability to engage in liquid-liquid phase separation. We present data that muNS inclusions behaves as biological condensates based on their ability to form spherical structures that can fuse and reversibly deform in living cells. Additionally, muNS condensates recover from photobleaching and hypotonic shock. We are currently studying our panel of muNS structural mutants to determine the features required to support this behavior and how the addition of other ARV proteins affect the condensate dynamics.

SP11.4 ITV
ISG15 is an important host factor for Vaccinia virus dissemination

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Viruses have developed many different strategies to counteract immune responses, and *Vaccinia virus* (VACV) is one of a kind in this aspect. To ensure an efficient infection, VACV undergoes a complex morphogenetic process resulting in the production of two types of infectous virus forms: intracellular mature virus (MV) and extracellular enveloped virus (EV). Each VACV form has specific features, being the mechanism of dissemination one of them. While MVs are released after cell lysis, the generation of EVs has been shown to be dependent on intracellular membrane transport and cytoskeleton dynamics, similar to the production of multivesicular bodies. Here we show that the ubiquitin-like modifier ISG15 participates in the control of VACV dissemination. Infection of *Isg15-/-* mouse embryonic fibroblasts (MEF) with VACV IHD-J and WR strains resulted in reduced actin tail formation, concomitant with the abolition of comet-like plaque formation and decreased EV production, comparing with *Isg15+/+* MEF. Transmission electron microscopy revealed accumulation of intracellular virus particles in the absence of ISG15, consistent with altered virus egress. Last, the generation of a recombinant IHD-J expressing V5-tagged ISG15 (IHD-J-ISG15) allowed us to identify several viral proteins as potential ISGylation targets, highlighting the proteins A34 and

A36, essential in EV formation. Altogether, our results indicate that ISG15 is an important host factor in the regulation of virus spread during VACV infection. This work was funded by the Spanish State Research Agency (Agencia Estatal de Investigación, AEI).

SP11.5 ITV

Genome-Wide CRISPR Screen Identifies β2-microglobulin as a Host Factor for Vaccinia Virus Infection

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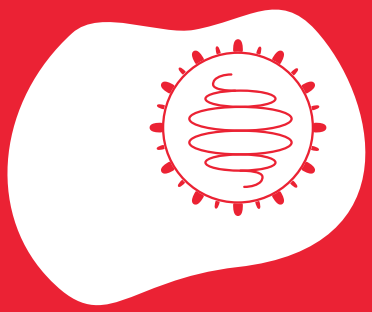
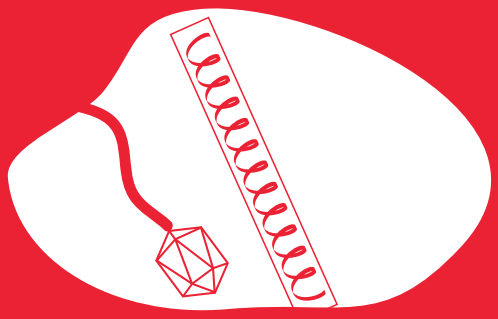
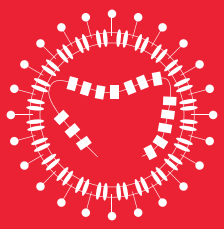
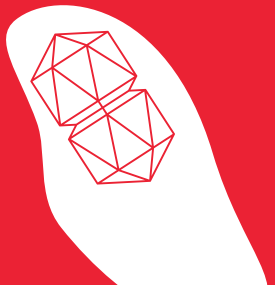
Vaccinia virus is a large, complex dsDNA virus whose cell entry is accompanied by the activation of various cellular pathways, being fluid-phase endocytosis its main mode of entry. According to current models, initial binding to extracellular matrix proteins, internalization and finally to fusion of the viral membrane with cell membranes either at the cell surface or in the endosomal compartment. During the process the virus leads to activation of particular cellular pathways and remodeling of cytoskeletal structures. Although the process is only partially understood, several proteins and extracellular matrix components have been described as players in Vaccinia virus binding and internalization. In an attempt to identify additional factors in Vaccinia virus infection, we carried out a loss-of-function, Genome-Wide genetic screen in HeLa cells. For this, pooled cells containing single gene-inactivation were infected with Vaccinia virus, in the hope that some gene inactivations would make cells resistant to infection. Regular infection resulted in no surviving cells, suggesting that no single gene knock-out was able to provide complete resistance to Vaccinia virus and thus allow cells to survive infection. In the absence of an absolute infection blockage, we explored if some gene KO could provide partial protection leading to a decreased probability of infection. Experiments using modified screening procedures involving lower multiplicities of infection and replication-restricted viruses led to define a list of multiple genes that potentially increase resistance to infection and therefore enhanced cell survival. As expected, significant gene hits were related to proteins known to act in virus entry, such as ITGB1, AXL and genes of their downstream related pathways. Additionally, we consistently found β2-microglobulin, encoded by the B2M gene, among the screening top hits. Inactivation of B2M resulted in reduced VV infection efficiency in HeLa and HAP-1 cell lines. In the absence of B2M, while virus binding to the cells was unaffected, virus internalization and early gene expression were significantly diminished. These results point to β2-microglobulin as a relevant factor in the Vaccinia virus entry process.



Abstracts

Pósteres



P1 COV

P1.1 COV

Temporal dynamics of SARS-CoV-2 variants

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BACKGROUND: Genomic surveillance of SARS-CoV-2 is a critical public health function whose objective is to serve as a basis for decision-making on social, health, diagnostic, treatment and vaccination measures. Given the serious impact that the appearance of new variants can have, it is necessary to continue knowing the sequences and therefore mutations of circulating SARS-CoV-2 strains. OBJECTIVE: Descriptive analysis of the distribution of SARS-CoV-2 variants in the province of Cáceres (Extremadura). METHODS: From September 26, 2021 to May 8, 2022 have been analyzed 795 samples for the identification of SARS-CoV-2 variants in our laboratory (Hospital San Pedro de Alcántara) using Next-generation sequencing (n=726) (IonTorrent TermoFisher) and Sanger sequencing (n=69). TorrentSuite software has been used to data analysis. To obtain the lineages and clades, the Pango lineage and Nextclade tools have been used. The samples studied include confirmed cases of SARS-CoV-2 selected in a targeted manner for epidemiological surveillance, suspected cases of reinfection and random screening by clinical diagnosis. RESULTS: From epidemiological week 39 (Sep 26 – Oct 2, 2021) to week 19 (May 9-15, 2022) a total of 772 positive samples for SARS-CoV-2, have been sequenced. From primary care 71.5% and hospitals 28.5%. In 89.4% the screening was random. Of the total number of sequences, in 5.7% no sequence was obtained (insufficient amplification product). Until the week 48 a total of 155 samples were sequenced, being 100% Delta variant and its sublineages (7.7% AY.43). In week 51, the Omicron variant stood at 61.8% while the Delta variant fell to 23.5%. In week 3, the Delta variant and its sublineages completely disappear. In week 5 the first sequences of the BA.2 sublineage appear (27.5%). As of week 14, practically all the sequences were BA.2 sublineage (75.5% BA.2, 7.2% BA.2.9). Throughout the study period, the Omicron variant was the most sequenced (68.3%); being the most frequent sublineages: 41% BA.2, 38.8% BA.1, 10.1% BA.1.1. CONCLUSIONS: Genomic sequencing is essential to follow the appearance and effect of variants of interest (VOI) and concern (VOC) as well as to understand the local epidemiological situation.

P1.2 COV

A single dose of an MVA vaccine expressing a prefusion-stabilized SARS-CoV-2 spike protein neutralizes variants of concern and protects mice from a lethal SARS-CoV-2 infection

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To control the COVID-19 pandemic, caused by coronavirus SARS-CoV-2, novel safe, immunogenic, and single-dose effective vaccines are needed. Therefore, we have generated an optimized COVID-19 vaccine candidate based

on the modified vaccinia virus Ankara (MVA) vector expressing a full-length prefusion-stabilized SARS-CoV-2 spike (S) protein, termed MVA-CoV2-S(3P), containing 3 mutations in the furin cleavage site (R682G, R683S, and R685S) and three proline substitutions in the S2 region (A942P, K986P, and V987P) for improved stability, and we analyzed its immunogenicity and efficacy in mice in comparison to a previous MVA-based vaccine candidate expressing a non-stabilized full-length S protein. The S(3P) protein was expressed at higher levels than the non-stabilized S in cells infected with the corresponding recombinant MVA viruses. Importantly, one single dose of MVA-CoV2-S(3P) induced higher IgG and neutralizing antibody titers against parental SARS-CoV-2 and variants of concern than MVA-CoV2-S in wild-type C57BL/6 and in transgenic K18-hACE2 mice. In immunized C57BL/6 mice, two doses of MVA-CoV2-S or MVA-CoV2-S(3P) induced similar levels of SARS-CoV-2-specific B- and T-cell immune responses. Remarkably, a single administration of MVA-CoV2-S(3P) protected all K18-hACE2 mice from morbidity and mortality caused by SARS-CoV-2 infection, reducing SARS-CoV-2 viral loads, histopathological lesions, and levels of pro-inflammatory cytokines in the lungs. These results demonstrated that expression of a novel full-length prefusion-stabilized SARS-CoV-2 S protein by the MVA poxvirus vector administered as a single dose enhanced immunogenicity and efficacy against SARS-CoV-2 in animal models, further supporting MVA-CoV2-S(3P) as an optimized vaccine candidate for clinical trials.

P1.3 COV

Intranasal administration of a single dose of MVA-based vaccine candidates against COVID-19 induced local and systemic immune responses and fully protects mice from a lethal SARS-CoV-2 infection

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To control the COVID-19 pandemic caused by SARS-CoV-2 coronavirus, novel safe, immunogenic and effective vaccines are needed. Moreover, new ways to try to enhance the vaccine immunogenicity and efficacy are desired, being the route of administration, an important vaccination parameter that can significantly modify the quality of the response. Here, in this study we evaluated in mice the immunogenicity and efficacy of modified vaccinia virus Ankara (MVA)-based vaccine candidates against COVID-19 administered as a single dose by intranasal route. Results showed that in C57BL/6 mice MVA-based vaccine candidates elicited high levels of S-specific IgG and IgA antibodies and neutralizing antibodies against parental SARS-CoV-2 and variants of concern (VoC), as well as robust and polyfunctional S-specific CD4+ and CD8+ T-cell immune responses locally (in lungs) or systemically (in spleen). Remarkably, a single intranasal administration of the MVA-based vaccine candidates protected K18-hACE2 transgenic mice from morbidity and mortality caused by SARS-CoV-2 infection, being the MVA-CoV2-S(3P) vaccine candidate, expressing a full-length prefusion-stabilized SARS-CoV-2 S protein, the more efficacious vaccine candidate. SARS-CoV-2 viral loads were undetectable in lungs and nasal washes, correlating with high titers of S-specific IgG antibodies and neutralizing antibodies against parental SARS-CoV-2 and VoC. Moreover, lower histopathological lung lesions and levels of pro-inflammatory cytokines in the lungs and nasal washes were observed in vaccinated animals. These results demonstrated that intranasal inoculation of one single dose of MVA-based vaccine candi-

dates against COVID-19 is highly immunogenic and confers full protection in animal models, opening the way to be used as a better administration route in future clinical trials.

P1.4 COV

Construction and generation of SARS-CoV-2 candidate vaccines carrying S, M, and N proteins by ARV-derived IC-tagging methodology

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The COVID-19 pandemic has led to an unprecedented global effort to develop safe and efficacious vaccines, mostly based on the induction of neutralizing antibodies directed to the SARS-CoV-2 spike (S) protein. However, alternative SARS-CoV-2 antigens should be explored to enhance the immunogenicity of marketed vaccines. Here, we have applied the methodology to produce avian reovirus muNS-Microspheres (MS) loaded with diverse SARS-CoV-2 antigens through their C or N-terminal tagging with the so-called IC-Tag. Several different microspheres carrying the RBD or the S1 domains of the S protein, or prefusion stabilized full-length S, N or M proteins of SARS-CoV-2 were generated. The correct expression of all these antigens as well as their appropriate incorporation into avian reovirus muNS-Mi MS were confirmed. Serum from convalescent patients were used to determine whether these potential subunit vaccines could be recognized by naturally induced SARS-CoV-2 specific antibodies. Their expression and stability were compared, and the best candidates chosen for mice immunization. Specific immune responses were obtained against S and N proteins in BALB/c mice. The presence/absence of correct response has led to the development of new rational approaches for improving the MS formulations. This work received funding from Instituto de Salud Carlos III, Fondo Supera-CoviD, Banco de Santander-CRUE, UROVESA and Grupo Zendal.

P1.5 COV

High PLAUR Expression Levels in the Upper Respiratory Tract Are Associated With COVID-19 Severity

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The mucosal immune response of the upper respiratory tract is crucial for the initial control of SARS-CoV-2 replication and clearance and the progression of COVID-19. Urokinase-type plasminogen activator (uPA) and its receptor

(uPAR) are involved in many physiological processes, including inflammation, cell proliferation, cell migration, and activation of immune cells. uPAR is a membrane receptor that lacks a signal transducer cytoplasmic domain and associates with plasma membrane receptors, such as integrins, to activate intracellular signaling pathways. In addition, a soluble form of uPAR (suPAR) has many roles in chemotaxis and immune response regulation independent of the uPA/uPAR system. We aimed to quantify the ARN expression of different components of the uPA/uPAR system in the upper respiratory tract of patients infected with SARS-CoV-2 and evaluate its association with the severity of COVID-19. Gene expression was analyzed by quantitative RT-PCR in 159 nasopharyngeal samples distributed in two study groups: i) 80 patients infected with SARS-CoV-2 with mild symptomatology and ii) 79 patients infected with severe symptomatology. The differences between groups were assessed using the Mann-Whitney U test. The genes studied were *PLAU* and *PLAUR* encoding uPA and uPAR, respectively, and *ITGB1*, *ITGB2*, and *ITGA5* encoding integrins. The results showed that severe patients had significantly higher *PLAUR* levels compared to mild patients ($P < 0.05$), the same was not observed for the *PLAU*, *ITGB1*, *ITGB2*, and *ITGA5* genes ($P > 0.05$), where the differences between groups were not significant. These results suggest that uPAR may have a role in SARS-CoV-2 infection independent of the uPA/uPAR system and point to *PLAUR* as a potential nasopharyngeal biomarker to predict COVID-19 progression.

P1.6 COV

Potency and Breadth of Neutralization against SARS-CoV-2 VoC after 3 doses of mRNA vaccines in COVID-19 Convalescents and Naïve individuals

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The implementation of mRNA vaccination against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been a main tool for control of the pandemics. However, the emergence of the different SARS-CoV-2 variants of concern (VoC), particularly Omicron, related with increased transmission efficiency and neutralizing escape, have represented an important challenge to achieve high levels of protection. The objective of our study was to investigate the neutralizing response after the third mRNA vaccine dose against SARS-CoV-2 VoC in a cohort of COVID-19 convalescent patients in comparison with only-vaccinated naïve individuals. The neutralization activity was tested by using recombinant SARS-CoV-2 pseudotyped rVSV-luc system in VeroE6 cells. COVID-19 convalescents and naïve individuals were tested for neutralizing activity against SARS-CoV-2 VoC 614G, Alpha, Beta, Gamma, Delta, Omicron BA1 and Omicron BA2 at 2 months after two doses of the Pfizer-BNT162b2 and 1.5 months after third dose of the Moderna mRNA-1273 vaccine. The neutralization assay included as a control an external calibrant WHO SARS-CoV-2 Serology International Standard, 20/136 (Frederick National Laboratory for Cancer Research). Neutralizing titers in naïve individuals after prime-boost vaccination with Pfizer-BNT162b2 against all SARS-CoV-2 VoC were significantly lower as compared to COVID-19 convalescent individuals. This was particularly pronounced in case of Omicron BA1 and BA2 variants since naïve individuals had very low or no neutralizing activity detected against those variants. However, the third dose of Moderna mRNA 1273 increased the levels of neutralization potency in only-vaccinated naïve individuals against all VoC achieving comparable levels to the convalescent group. In contrast, COVID-19 convalescent patients had a great neutralizing response with a wide coverage against all SARS-CoV-2 VoC including Omicron after the first two doses. The results of our study indicate that maximum levels of neutralizing response with a wide breadth against SARS-CoV-2 VoC are achieved after repeated events of immune stimulation either by natural infection and/or vaccination. The number of stimulation events is likely limited to three, since a third dose of an mRNA vaccine did not significantly increase the neutralizing levels reached after two doses in COVID-19 convalescent patients.

P1.7 COV
muNS-derived, ER-protein microspheres (secMS) as candidate SARS-CoV-2 vaccines

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The viral protein muNS from avian reovirus (ARV) has been extensively studied by our group. In the infected cells, this protein is responsible to form the matrix of the viral factories and can attract other components into them in an orderly manner. Around 75% of muNS sequence can be removed from the N-terminal, while maintaining the capacity to generate spherical intracellular inclusions. This functional minimal portion of muNS was named muNS-Mi (muNS-minimal) and the spherical inclusions microspheres (MS). Due to the affinity of the MS with intercoil (IC) domains, an IC labelling methodology was developed. This method base on IC-tagged proteins being recruited to the MS, generating MS in vivo that contain any protein of interest tagged with the IC domain (IC-Tagging technology). These MS are easily purified, and we have proved that the incorporated proteins maintain their correct folding, even when they are oligomers. MS were successful inducers of humoral and cellular immunity against BTV and AHSV in the absence of adjuvants and is being used in our group to develop candidate vaccines against SARS-COV-2. We used a variant of the IC-tagging method (sec) that functions through the endoplasmic reticulum, allowing the production of MS loaded with fully glycosylated proteins. Basing on this IC-Tagging technology, we produced and characterized MS loaded with fully glycosylated S protein of SARS-CoV2 in pre-fusion in order to evaluate its ability to induce neutralizing immune response in mice.

P1.8 COV
SARS-CoV-2 sequence analysis using EPISEQ SARS-CoV-2

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BACKGROUND: EPISEQ® SARS-COV-2 is a new application online to identify SARS-CoV-2 variants, it is compatible with three major sequencing platforms (Illumina, Oxford Nanopore, ThermoFisher). The application permits the export of viral genome assemblies and mutations in order to facilitate reporting to national public health authorities and for epidemiology studies. OBJETIVE: Comparative study of the EPISEQ SARS-CoV-2 analysis result with the routine analysis performed in our laboratory. MATERIAL AND METHODS: From December 6, 2021 to May 15, 2022 a total of 448 sequences for SARS-CoV-2 were analyzed by our system (platform Ion Torrent TermoFisher, Torrent Suite software). These generated fastaq were in turn analyzed by the EPISEQ SARS-CoV-2 application (BioMérieux) for the comparative study. RESULTS: Four hundred and forty-eight fastaq were analyzed by EPISEQ, obtaining clade and lineage results in 448 (93.9%). Of the 27 samples that did not obtain a result, 19 were identified by our routine method. The concordance of results with respect to our system was 90.1%. Four differences were found regarding the sublineage of the Delta variant and 20 in the Omicron variant. The clade distribution was: 45.8% Clade 21K (Omicron), 38.2% Clade 21L (Omicron), 15.9% Clade 21J (Delta). The most frequent sublineages of clade 21J were: 40.3% AY.43 (n=27), 22.3% AY.4 (n=15), 7.5% AY.122 (n=5). Clade 21K lineages were: 72% BA.1 (n=139), 9.3% BA.1.17 (n=18) and Clade 21L: 83.2% BA.2 (n=134), 8% BA.2.9 (n=13). CONCLUSION: Most of the observed differences were due to reclassifications in sublineage nomenclature and software version updates. Therefore, EPISEQ SARS-CoV-2 is a genomic software solution for identification and reporting from raw sequencing data related to SARS-CoV-2 variants. It is an easy-to-use, fast and reliable tool for Clade and lineage assignment by any microbiology lab without bioinformatics knowledge or computing resources.

P1.9 COV

Preclinical efficacy, safety, and immunogenicity in cynomolgus macaques of the PHH-1V COVID-19 vaccine candidate based on a recombinant RBD fusion heterodimer of SARS-CoV-2

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Despite the success in SARS-CoV-2 mass vaccination programs, the situation is far from being controlled. Accordingly, accessible second-generation vaccines are required. HIPRA has developed an adjuvanted vaccine candidate, PHH-1V, based on a recombinant fusion heterodimer protein consisting of the RBD domain of two SARS-CoV-2 variants. Challenge studies of vaccine candidates conducted in animals provide information of vaccine efficacy that cannot be measured in human trials. A challenge study in cynomolgus macaques (*Macaca fascicularis*) has been performed in order to assess efficacy, immunogenicity and safety of a prime-boost immunization scheme with PHH-1V vaccine. Twelve cynomolgus macaques (6 females and 6 males) were vaccinated intramuscularly on day 0 and day 21 with 40 μg of PHH-1V vaccine or PBS (control item) and challenged on day 36 with SARS-CoV-2 (strain D614G). The animals were monitored for six days post-infection. On day 42 all animals were euthanized for organ collection. Samples from upper and lower airways and faecal material were taken at different points of the study, as well as blood samples. PHH-1V vaccine has been shown to be safe as no adverse reactions have been observed in this study and neither differences in rectal temperatures nor body weight evolution in comparison with the placebo group were observed. The efficacy of the vaccine candidate was assessed in terms of protection against the infection and its consequences. Vaccination prevented the infective viral load in the lungs and oropharyngeal swabs upon the experimental challenge with SARS-CoV-2. On the contrary, infectious virus was detected in the lungs and oropharyngeal swabs in most of the animals in the control group. PHH-1V vaccination induced high levels of IgG RBD binding and neutralizing antibodies from 14 days after the second vaccination against some of the most relevant SARS-CoV-2 variants of concern, as well as a balanced Th1/Th2 cellular immune response with the activation of $CD4^+$ and $CD8^+$ T cells after re-stimulation. Importantly, vaccination induced IgA secretion in the respiratory airways after SARS-CoV-2 infection. These results highlight the potential use of the PHH-1V vaccine in humans, currently undergoing Phase III clinical trials.

P1.10 COV

Nanobodies protecting mice from lethal SARS-CoV-2: Selection, characterization, humanization and evolution toward escaping virus variants

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The emergence of SARS-CoV-2 variants that escape from immune neutralization is challenging vaccines and antibodies developed to stop the COVID-19 pandemic. Thus, it is important to establish therapeutics directed toward multiple or specific SARS-CoV-2 variants. We selected a panel of camel nanobodies (Nbs) and engineered heavy chain antibodies (hcAbs) with human IgG1 Fc domain that targeted the receptor-binding domain (RBD) of the envelope SARS-CoV-2 spike (S). These hcAbs bound to S with very high affinities and some inhibited virus cell entry, neutralized SARS-CoV-2 infection in vitro and prevented the progression to lethal COVID-19 in infected humanized mice. S-binding cross-competition and structures of S-Nb complexes identified two distinct Nb-binding regions, which either covered or laid outside receptor binding motifs. We also evaluated mono and bispecific hcAb binding to the RBD of SARS-CoV-2 variants, identified variant-specific and molecules that recognized all virus variants of concern but omicron. Our current research efforts focus on directed evolution of Nbs for binding escaping variants of concern like omicron. In addition, more potent neutralizing Nbs have been fully humanized and are being tested in vitro and in vivo. Our work demonstrated the high potential of Nbs as therapeutics to prevent SARS-CoV-2 infections.
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P1.11 COV

Non-replicative antibiotic resistance gene-free DNA vaccine based on the S and N protein genes from SARS-CoV-2 virus induces full protection in the murine animal model.

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COVID-19 vaccines have helped control the SARS-CoV2 pandemic. However, the emergence of variants for the spike glycoprotein (S) can reduce the efficacy of vaccine strategies solely based on this viral target. We described here a synthetic DNA-based vaccine (pPAL-DNA-CSIC) that lacks the antibiotic resistance genes typically used for manufacturing selection and encodes for two of the coronavirus structural proteins, the spike glycoprotein (S) and the nucleocapsid protein (N). Both viral proteins are expressed *in vitro* in pPAL-DNA-CSIC transfected cells. Vaccination with these DNA vaccines elicited a potent humoral and cellular immune response in mice. Electroporation delivery of pPAL-DNA-CSIC fully protected K18-hACE2 mice against a lethal dose (105pfus) of SARS-CoV-2 virus. Moreover, viral load was drastically reduced in lungs, brain and heart of vaccinated animals. Importantly, pPAL-DNA-CSIC vaccine is also able to protect K18-hACE2 mice against a lethal dose of SARS-CoV2 Delta variant. These data indicate that vaccinated animals are not only able to control the viral disease, but also to limit viral replication. p-PAL-DNA-CSIC is therefore a promising DNA vaccine candidate for protection against the disease in the murine animal model.

P1.12 COV

A new Aerosol Chamber: a tool to assess the stability of viruses in aerosols

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Although airborne infectious disease transmission events are documented in the veterinary and agricultural biosafety, clinical medicine and public health, the COVID-19 pandemic has highlighted the dangers of airborne pathogen transmission. Viruses such as measles virus, influenza virus, respiratory syncytial virus, human rhinovirus (hRV), severe acute respiratory syndrome coronavirus (SARS-CoV) and SARS-CoV-2 are known to be transmitted through aerosols, but little is known about the dynamics of these aerosols. To better understand the stability of viruses in bioaerosols, an aerosol chamber has been designed and constructed. This aerosol chamber uses dynamic fog cones constituted of liquid nanometric droplets (Counterfog® technology) to generate bioaerosols. In addition, the aerosol chamber has a suspended particle sensor (SPS30, Sensirion) that allows monitoring of particles of different sizes over time. After the generation of virus aerosols, a filtration system through 1 μm polytetrafluorethylene (PTFE) filters connected to the aerosol chamber and a vacuum pump allows bioaerosol recovering. From the PTFE filters, the presence of the virus can be evaluated by viral titration and PCR. In this study, we evaluated the effect of different pressures (11 or 5 bar) to generate the bioaerosol over viral viability. The stability of the bioaerosol generated inside the aerosol chamber and the viability of the recovered virus over time was also evaluated. The viruses employed in this report were bacteriophage ϕ29, human coronavirus 229E (hCoV-229E) and hRV-14. This aerosol chamber is a useful tool for studying the viability of airborne viruses in BSL2 and BSL3 facilities. Moreover, it could be used to evaluate the efficiency of different filters for capturing biological samples in air and air purification systems.

P1.13 COV

Exploring the accessibility of adaptive pathways of the SARS-CoV-2 spike protein by in vitro experimental evolution.

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High mutation rates, short generation times and large population sizes fuel up the ability of RNA viruses to explore adaptive landscapes, resulting in fitness increases. Such evolvability is particularly relevant for zoonotic viruses, as it is the case of SARS-CoV-2, because they are still engaged in adaptation to their new hosts. During the ongoing COVID19 pandemic, a large number of SARS-CoV-2 variants have been identified. Some of these variants have been categorized by the WHO as VOC or VOI (variants of concern or of interest, respectively), due to the presence of mutations in the spike glycoprotein (S) that could be potentially important for its function. However, not all of them have been equally relevant according to their incidence in the population or to the severity of symptoms. One could expect that this relevance might only reflect differences in biological fitness of the variants, yet other factors could affect the epidemiological fate of these variants (e.g., demographic, economical, sanitary, social, political...). Our aim is to explore and describe the topography of the adaptive fitness landscape of the SARS-CoV-2 S protein in susceptible human cells, describing the number of potential fitness peaks, and evaluating the number of accessible adaptive walks. To achieve this goal, we have started focusing in the most prevalent mutations (present in >75% of the sequences) in the VOC and VOI identified by September 2021. Using maximum likelihood methods, we

have inferred the ancestral amino acid sequences at each node of the diversification tree of the S protein. These sequences depict a set of potentially accessible evolutionary pathways followed by SARS-CoV-2 S protein along the pandemic. Fitness for all these S variants was assayed using pseudo-typed vesicular stomatitis virus (VSV), with the S protein in their envelope, by head-to-head competition assays in human (HEK293) or monkey (VeroE6) cells expressing the ACE2 receptor with their respective ancestors and descendants. To further evaluate the evolvability of all these variants and the likelihood of selection to find different evolutionary solutions, serial passages of the pseudoviruses carrying different S forms were performed.

P1.14 COV

A combined microRNA and proteome profiling to investigate the effect of SARS-CoV-2 ORF7a on lung epithelial cells

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The coronavirus SARS-CoV-2 is the cause of the ongoing pandemic of COVID-19. Since there are no effective treatments for SARS-CoV-2, a molecular understanding of how the virus affects host cells is urgently required. The SARS-Cov-2 genome encodes 31 proteins, including 11 accessory proteins that have been implicated in infection and pathogenesis through their interaction with cellular components. In the present study, we investigated the SARS-CoV-2 accessory protein ORF7a, whose role in the viral pathogenicity is poorly understood. ORF7a is a type-I transmembrane protein of 121 amino acid residues that possesses an Ig-like ectodomain containing an integrin binding site. In this study, we have used a cellular model of lung epithelial A549 cells expressing SARS-CoV-2 ORF7a (A549-ORF7a) to analyse alterations in the proteome and microRNA-omic profiles. Label-free quantitative proteomic analysis revealed that 244 proteins were differentially expressed in A549-ORF7a. A significant number of proteins were detected in A549-ORF7a cells, but not in control cells, indicating that the expression of ORF7a causes higher abundance of these proteins. The miRNA expression profiling identifies 150 miRNAs with statistically significant expression changes. The study found that 59 miRNAs were up-regulated, while 91 miRNAs were down-regulated in A549-ORF7a cells. We demonstrated through proteomic analysis and bioinformatics that ORF7a, individually expressed, can cause host pathway deregulation, specifically protein folding, RNA processing, cell adhesion, and metabolism pathways, such as glycolysis or pentose phosphate pathway. Integration of miRNA and proteome expression data with miRNA target prediction algorithms generated a potential regulatory network consisting of 112 miRNAs and 148 proteins. The miRNA-protein network analysis suggests that miRNAs are involved in immune response by controlling TRIM25 and TKFC mediated cellular antiviral response. In addition, the results indicate that miRNAs play a relevant role in the promotion of metabolism reprograming by modulating the expression of critical proteins related to glycolysis/glyconeogenesis and pentoses phosphate pathway such as DLD, PFKL or TKT. Together, these results provide important new information on the role of accessory proteins in SARS-CoV-2 pathogenesis, representing an important step towards developing new therapies and biomarkers.

P1.15 COV

Low frequency Delta, Iota and Omicron mutations in vaccine-breakthrough infections with SARS-CoV-2 in samples of April 2021 in Spain

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RNA viruses replicate as complex and dynamic distribution of mutants that are present at different frequencies within an infected host. In this study we examined by ultra-deep sequencing the mutant spectra of amplicons from the spike-coding (S-coding) region of five nasopharyngeal swabs recollected during April 2021. All samples were from fully vaccinated patients (2 doses of BNT162b2, Pfizer-BioNTech) that were nevertheless infected and were diagnosed with COVID-19. The analyses revealed that all patients were infected with the Alpha variant, but that their mutant spectra included mutations that correspond to phylogenetically distinct variants such Delta Plus, Iota and Omicron that become epidemiologically relevant months after the vaccine-breakthrough infections. Thus, mutant spectra are rich reservoirs of mutants, some of which may acquire epidemiological relevance at later epidemic waves.

P1.16 COV

SARS-CoV-2 accessory proteins involvement in inflammatory and pro-fibrotic diseases through IL11 and WNT5A dysregulation signaling

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SARS-CoV-2, the cause of the COVID-19 pandemic, possesses eleven accessory proteins encoded in its genome. Their roles during infection are still not completely understood and several of them have been mutating into the different variants of the virus. WNT5A dysregulation signaling has been implicated in the development of various pathological conditions in humans such as inflammation and fibrosis. Interleukin-6 (IL6) family members induce WNT5A expression in various cell types, highlighting a critical role for WNT5A in immune responses. Expression of Interleukin-11 (IL11), a member of IL6 cytokine family, correlates with the extent of fibrosis and its signaling induced

fibroblast activation via TGFβ. In this study, A549 were transduced with lentivirus expressing individual viral accessory proteins ORF6, ORF8, ORF9b or ORF9c from SARS-CoV-2 (Wuhan-Hu-1 isolate) and their interaction with cellular responses was analyzed. Firstly, the transcriptomic analysis revealed that both WNT5A and IL11 were significantly upregulated in all transduced cells. Some IL11 signaling-related genes, such as STAT3 or TGFβ, were differentially expressed. IPA software analysis showed that both WNT5A and IL11 were involved in pulmonary fibrosis idiopathic disease. Subsequently, bioinformatics and functional assays revealed that these four accessory proteins were implicated in both inflammatory and fibrotic responses. While overexpression of ORF8 and ORF9c appear to trigger a STAT3-dependent cellular response mediated by IL11, ORF6 and ORF9b seem to provoke a cell profibrotic response mediated by TGFβ through WNT5A. Our results suggest that ORF6, ORF8, ORF9b and ORF9c could be involved in inflammatory and fibrotic responses in SARS-CoV-2 infection. Thus, these accessory proteins could be targeted by new therapies for COVID-19 disease.

P1.17 COV
SARS-CoV-2 mutant spectra reveal differences between COVID-19 severity categories

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RNA virus populations are composed of complex mixtures of genomes that are termed mutant spectra. SARS-CoV-2 replicates as a viral quasispecies, and mutations that are detected at low frequencies in a host can be dominant in subsequent variants. We have studied mutant spectrum complexities of SARS-CoV-2 populations derived from thirty nasopharyngeal swabs of patients infected during the first wave (April 2020) in the Hospital Universitario Fundación Jiménez Díaz. The patients were classified according to the COVID-19 severity in mild (non-hospitalized), moderate (hospitalized) and exitus (hospitalized with ICU admission and who passed away due to COVID-19). Using ultra-deep sequencing technologies (MiSeq, Illumina), we have examined four amplicons of the nsp12 (polymerase)-coding region and two amplicons of the spike-coding region. Ultra-deep sequencing data were analyzed with different cut-off frequency for mutation detection. Average number of different point mutations, mutations per haplotype and several diversity indices were significantly higher in SARS-CoV-2 isolated from patients who developed mild disease. A feature that we noted in the SARS-CoV-2 mutant spectra from diagnostic samples is the remarkable absence of mutations at intermediate frequencies, and an overwhelming abundance of mutations at frequencies lower than 10%. Thus, the decrease of the cut-off frequency for mutation detection from 0.5% to 0.1% revealed a increasement (50- to 100-fold) in the number of different mutations. The significantly higher frequency of mutations in virus from patients displaying mild than moderate or severe disease was maintained with the 0.1% cut- off frequency. To evaluate whether the frequency repertoire of amino acid substitutions differed between SARS-CoV-2 and the well characterized hepatitis C virus (HCV), we performed a comparative study of mutant spectra from infected patients using the same bioinformatics pipelines. HCV did not show the deficit of intermediate frequency substitutions that was observed with SARS-CoV-2. This difference was maintained when two functionally equivalent proteins, the corresponding viral polymerases, were compared. In conclusion, SARS-CoV-2 mutant spectra are rich reservoirs of mutants, whose complexity is not uniform among clinical isolates. Virus from patients who developed mild disease may be a source of new variants that may acquire epidemiological relevance.

P1.18 COV
SARS-CoV-2 and hRSV inactivation based on non-ionizing UV type C LED radiation

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After the 2020 pandemic situation, new and more effective sterilization systems have been shown as a worldwide requirement to mitigate and control different sorts of pathogens. Moreover, the power efficiency and inactivation time are key parameters to achieve an applicable solution for future wearable air filtering devices and surface disinfection. On one hand, Light Emitting Diodes (LED) technology has been used for decades as sustainable and stable light source with low power consumption and reduced size, on the other hand, Ultraviolet type C (UVC) rays (200–280 nm wavelength) are widely used for pathogens inactivation; therefore, both combined could be an enabler for new purifying technologies and proper solution to get ready for present and future bio hazards. We evaluated the antiviral activity of an experimental setup designed by Airbus-Crisa; combining light radiation simulation with experimental results in order to calculate the total dose needed to inactivate RNA based virus using 275 nm UVC LED strips (4 LED) powered by steady driver. For that purpose, two different viruses were tested, Human Respiratory Syncytial Virus (hRSV) and SARS-CoV-2. Both are respiratory enveloped viruses with RNA genomes that can be easily transmitted through contaminated surfaces. Viral samples were irradiated with different times of exposure (0.5, 1, 1.5 and 2 seconds) and the recovered virus was quantified by plaque assays and compared with non-irradiated samples. The results showed a great inactivation capacity for both hRSV and SARS-CoV-2, achieving UVC radiation doses that can reduce virus concentration from 5 to 6 log-reduction after 2 seconds exposure time, improving the time-reduction ratio previously published. This project has been funded by Airbus Crisa and CDTI (Centro para el Desarrollo Tecnológico Industrial).

P1.19 COV
Improving neutralization methods for SARS-CoV-2.

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Two months into the pandemic, it was decided to develop antigenic characterization techniques for the phenotypic study of SARS-CoV-2. Containment measures were taken that WHO had recommended for cultivation of SARS-CoV-2 in BSL3 facilities. The aim of this assay was to develop techniques for virus isolation, virus quantification by titration, virus growth monitoring by immunofluorescence visualisation of infections and finally the development of a system for virus neutralisation. A virus archive of different SARS-CoV-2 variants quantified by knowning titre has been obtained for use in the study by viral neutralisation with a panel of positive patient sera. Isolation procedure consisted of infection of different types of cell lines with positive clinical respiratory samples with different Cts values. The effect of adding trypsin to the culture medium and growth in CO2 and non-CO2 incubators was tested. Viral isolation was effectively achieved using the VERO-E6 cell line with respiratory clinical samples with Ct values < 25. Trypsin was not required and viral growth was optimised in CO2 incubators. Visualisation of the cytopathic effect was achieved between 3 and 7 days in the first pass and 72 hours in successive passes. For immunofluorescence, different types of antibodies and virus isolates with different titres were tested. The immunofluorescence was performed with a rabbit polyclonal produced in March 2020 at the CNM-ISCIII and a commercial anti-human IGG/FITC antibody (Sigma). Virus titration was performed in 96-well plates, with cells in suspension and with plates seeded 24 hours before, with and without cell counting, CPE observation after 24-72 hours. Neutralisation was performed by incubating different dilutions of the human sera panel with the virus on a plate seeded with cells, with different incubation and fixation times. Neutralisation plates were fixed in the BSL3 laboratory and visualization was developed with a rabbit polyclonal produced in March 2020 and a peroxidase labelled anti-rabbit. International NIBS-standard was used in order to optimise the assay.

P1.20 COV
Enhancing SARS-CoV-2 molecular surveillance in Spain

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Since the beginning of SARS-CoV-2 coronavirus pandemic, the frantic race started to sequence this virus. This has led to the production of millions of whole genome sequences to date. However, the massive generation of data is not only important, but also a good quality data and the establishment of adequate surveillance are relevant to contribute to meaningful surveillance in public health. In this context, a network of Spanish laboratories for molecular surveillance of SARS-CoV-2 was created (RELECOV). Objective: RELECOV network goal is promoting actions to detect, analyse and evaluate variants of SARS-CoV-2 at national level. Methods: Databases were created to study and monitoring SARS-CoV-2 viruses from September 2021 to March 2022 with information from RELECOV and public databases. Moreover, a QC was performed including ten RNA lyophilized samples containing different SARS-CoV-2 viruses isolated from VERO E6 culture. A matrix with 739 reference SARS-CoV-2 sequences were obtained, from sequences available in GISAID and after exhaustive reviewing, using MAFFT, MEGA 7 and Bioedit Programmes. Finally, phylogenetic analyses were performed using FASTREEE and IQ-TREE programs Results: We observed a reduction in the GISAID upload gap of 42% comparing the two quarters. In addition, there was a 36% increase in sequence deposit. Analysis of lineage-defining mutations showed 5 mutations present in all Delta lineages [S (T19R, T478K,

and D614G), M (I82T), and ORF3a (S26L)] and 14 in Omicron lineages [ORF1a (P3395H); ORF1b (P314Land I1566V); S (D614G, H655Y, N679K, P681H, D796Y, Q954H and N969), E (T19I); M (A63T), and N (R203K and G204R)]. A low hit rate for lineage assignment was observed relative to variant for the full QC panel. Tree reference obtained showed the clusters corresponding to different variants. Conclusions: RELECOV actions to date have made it possible to improve the surveillance of the viruses that circulate in Spain, through sequences analysis, virus genome changes monitoring and the performance of QC SARS-CoV-2 sequencing at national level. In addition, the implementation of a phylogenetic analysis tool to characterize SARS-CoV-2 allows us to increase the knowledge about the viruses that circulate in Spain in an independent and quickly way in order to assist public health.

P1.21 COV

Covid-19 vaccine candidates based on vaccinia MVA confer efficient protection in the mouse and hamster disease models.

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Its past use as Smallpox vaccine, together with the development of improved genetic modification techniques, have expanded the use of Vaccinia virus as a vaccine vector not only for humans but also for farm animals or even wildlife. As a vaccine platform, it offers many advantages including low virulence, good immune response, good genetic stability and temperature resistance (it is not necessary to maintain the cold chain). In addition, it allows the introduction of large portions of exogenous DNA, making it possible to introduce multiple genes in a single vaccine vector. We developed a novel system for the isolation of recombinant vaccinia virus, starting from a double mutant virus (ΔA27L-ΔF13L), which is unable to be transmitted from cell to cell. Re-introduction of the A27L and F13L genes acts as a powerful selection to mediate dual insertions into the viral genome. This selection system facilitates the combined expression of multiple foreign proteins from a single recombinant virus. We have applied this system to the MVA strain of Vaccinia virus and generated a panel of vaccine candidates against SARS-CoV-2 that express protein S separately or in combination with other SARS-CoV-2 proteins, in order to broad the immune response to vaccination. We have characterized the viruses with the different combinations and tested the response in a mouse and hamster model in order to select vaccine candidates. Prefusion-stabilized S protein of different SARS-Cov-2 variants were expressed as complete proteins that were correctly transported to the cell membrane. Mice vaccinated with the recombinants induced anti-S neutralizing antibodies and were able to induce protection against a lethal dose of SARS-CoV-2. Similarly, Syrian hamsters were protected against SARS-CoV-2 infection by vaccination, which prevented lung pathology, a variety of clinical signs and, importantly, virus dissemination in the brain. As a whole, those results point to these vaccine candidates as inducers of a robust immunity. Notably, this vaccine platform facilitates the fast isolation of new vaccine candidates. Results obtained by co-expression of additional SARS-CoV-2 genes will be presented.

P1.22 COV

Assessment of SARS-CoV-2 neutralizing antibody titers in breastmilk from convalescent and vaccinated mothers

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Breastmilk contains antibodies that could protect breastfeeding infants from infections. In this work, we examined if the antibodies in breastmilk could neutralize SARS-CoV-2 in >100 samples from women that were either vaccinated (Pfizer, Moderna, or AstraZeneca), infected with SARS- CoV-2, or both infected and vaccinated. The neutralization capacity of these sera was tested using pseudotyped vesicular stomatitis virus carrying either the Wuhan, Delta, or Omicron spike proteins. We find that neutralization correlates positively with levels of IgA in breastmilk. Moreover, natural infection resulted in higher neutralizing titers. Finally, significant differences in the capacity to produce neutralizing antibodies were observed between the mRNA-based vaccines and the AstraZeneca vaccine. Overall, our results indicate that breastmilk from naturally infected women or those vaccinated with mRNA-based vaccines induces SARS-CoV-2 neutralizing antibodies that could potentially provide breastfeeding infants protection from infection. Funding: the authors would like to acknowledge the support from LaMarató-TV3 COVID grant (MilkCORONA, ref. 31/109/202106) and funding from European Commission NextGenerationEU fund (EU 2020/2094), through CSIC's Global Health Platform (PTI Salud Global).

P1.23 COV

Association between humoral response to seasonal human coronaviruses with acquisition and severity of COVID-19

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Background: Susceptibility to SARS-CoV-2 infection and degrees of COVID-19 severity among individuals have been recognized. It has been proposed that immune response generated by previous infections with seasonal human coronavirus (H-CoV) could influence on SARS-CoV-2 infection and/or COVID-19 progression. Methodology: Case-control study among healthcare workers (HCW) at a University Hospital. Cases were HCW with confirmed SARS-CoV-2 infection by PCR in nasopharyngeal swabs. Controls were HCW with no SARS-CoV-2 infection (no previous symptoms suggestive of COVID-19, no antibodies against SARS-CoV-2 in a recent blood sample, and no

positive PCR). Cases and control were matched by age (± 5 years), sex, and degree of exposure to the virus. As all selected HCW had mild to moderate disease, we added a group of individual who died from COVID-19 related complications. Antibody titers against the nucleocapsid (N) of the four seasonal H-CoV (NL63, HKU1, 229E and OC43) and the spike (S) of SARS-CoV-2 were measured in all the participants, using enzyme-linked immunoassay methods. Results: A total 221 participants (92 cases, 84 controls, 45 subjects who died from COVID-19) were included. Median age 41 years, 88% women, 87% high degree of exposure to SARS-CoV-2. No differences in the antibody titers against any of the H-CoV were detected between cases and controls (p>0.1 for all the comparisons). Participants who had severe disease had titers of antibodies against H-CoV not statistically different to those found in participants with mild to moderate disease (p>0.1 for all the comparisons). Participants with sever disease had significantly higher antibodies titers against SARS-CoV-2 than participants with mild to moderate disease (1256 AU/ml vs 471 AU/ml, p<0.01). Conclusions: Humoral response against seasonal H-CoV does not seem to influence the risk of infection by SARS-CoV2 or the severity of COVID-19.

P1.24 COV

Development of a MoA-based Bioassay Platform using Virus-like Particles for Safe and Rapid Measurement of Viral Entry and Neutralization

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Research into viral attachment and entry does not require the full, infectious virus. Instead, recombinant viral particles can substitute. In this context, luciferase enzymes are an important tool for making recombinant reporter viruses. In 2012, a new reporter enzyme called NanoLuc® Luciferase (NLuc) and its detection reagent, Nano-Glo® Luciferase Assay System, were introduced. NLuc was molecularly evolved from a 19kDa deep sea shrimp luciferase to increase stability and improve brightness. Recently, a short 11-amino acid bioluminescent protein tag (HIBiT) was introduced that uses structural complementation to achieve a functional Nluc luciferase (Nanoluc Binary Technology or NanoBiT®), which also can be applied to viral research. We have demonstrated the use of HiBiT protein tagging to enable luminescent detection of a protein-of-interest (POI) via complementation with LgBiT to form a functional NanoBiT® luciferase enzyme in live cells. We can also use HiBIT to create Pseudotyped Virus Like Particles (HiBiT-PsVLP technology), which provides a fast, convenient, and biologically-relevant method for assaying viral entry and neutralization, and permits rapid assessment of SARS-CoV-2 entry inhibitors. Also, the flexible HiBiT-PsVLP platform can be adapted for other priority viral pathogens.

P1.25 COV

Validation of a passive sampler as an affordable and easy-to-use tool for wastewater based epidemiology

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Wastewater-based epidemiology (WBE) has raised as an important tool for the prevention and early warning of infectious disease outbreaks within a community. Most of the published WBE studies use active sampling (by means of autosamplers) to collect composite samples, from the inlet of municipal wastewater treatment plants (WWTP). Although autosamplers collect flow dependent samples over 24-h, their use in smaller settings can be limited by their cost, the need of a power supply and, in some cases, limited access to the sampling area. Passive samplers have been purposed as affordable and easy-to-use devices for virus surveillance in smaller scale scenarios. In this study, we validated a passive sampling method, for the detection and quantification of a diversity of viruses, in a large urban WWTP and in an aged care facility. A total of 15 passive samplers, containing 2 electronegative membranes, were deployed, and retrieved 24h later. Using a traditional autosampler, 24-h composite samples were collected in parallel in both settings. Electronegative membranes were used for direct nucleic acid (NA) extraction using a Qiagen RNeasy Power Microbiome Kit. Twenty-four-hour composite samples were concentrated using the automatic CP-Select™ device with ultrafiltration tips and NA were extracted with QiAamp Viral RNA Kit. The detection and quantification of several viruses (SARS-CoV-2 (N1 and N2 assays), JC Polyomavirus, Human adenovirus, Rotavirus, Norovirus genogroup I and II and Enterovirus) was assessed using specific qPCR and massive sequencing using a Targetted Enrichment Approach. Both methods were also compared as for their performance in subsequent massive viral sequencing. The results indicated no differences in the detection of JCPyV and HAdV in the WWTP by applying any of the sampling-methods. Although active sampling demonstrated to have greater sensitivity over the WWTP samples, the proposed passive sampling protocol performed equally at the building level. In the event of new epidemic outbreaks, the use of passive samplers can be used for targeted actions or to track back specific facilities with vulnerable population (e.g. schools, universities or aged care facilities).

P2 TDI

P2.1 TDI

A novel, rapid molecular method for the diagnosis of avian metapneumovirus by nanoprobe

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Early diagnosis of active viral infections is crucial to prevent transmission among the population, as we have seen during the present COVID-19 pandemic. Single-stranded RNA viruses, including SARS-CoV-2 and avian metapneumovirus (aMPV), are currently diagnosed using molecular methods, such as the gold standard qRT-PCR. These techniques require expensive equipment and specialized training, which limits their use. aMPV is the cause of turkey rhinotracheitis (TRT) and swollen head syndrome (SHS) in chickens, both severe emerging diseases that produce important economic losses in avian industry. TRT is a notifiable disease to the World Organization for Animal Health (WOAH), and in 2018, the Strategic Agenda for Animal Health Research of the Vet+i Foundation under the Ministry of Agriculture, Fisheries, and Food identified the need to improve its diagnosis as a priority in poultry production. We are developing a point-of-care (POC) diagnostic system for aMPV detection based on a novel, fast and simple method that can be performed from sample collection to final reading on a farm in an integrated manner. This avoids the costs associated with sample transport and the previously mentioned limitations of qRT-PCR-based techniques, but without losing sensitivity or specificity in the assay. In this system, the amplification by LAMP of the viral RNA and subsequent recognition by the DNA probe triggers a change in the aggregation of gold nanopar-

ticles, which leads to a change of color, visible with the naked eye. In this study, a panel of 50 positive samples provided by CESAC reference laboratory has been analysed. Negative samples included avian infectious bronchitis, infectious laryngotracheitis, and Newcastle disease virus. Our results show high levels of specificity and sensitivity after 90 min, which could be reduced to 40 min with further optimization. Upon *in vivo* validation, a marketable and cheap diagnostic kit will be implemented in the animal health system as a routine method for assaying TRT. Funded by Spanish Ministerio de Ciencia e Innovación (PID2020-114956GB-I00) and Comunidad de Madrid (IND2019/BIO-17124).

P2.2 TDI

Differential expression of tRNA-derived small RNAs in COVID-19 patients enables the identification of severe SARS-CoV-2 infection

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Small RNAs (sRNAs) are a class of non-coding RNAs that play regulatory roles in many specific physiological and pathological processes. It is well established that alterations in the host sRNAs profiles offer unique insight into cellular pathways associated with virus pathogenesis. In addition, the characterization of host sRNAs responses to virus infection constitutes a valuable source of potential biomarkers that could improve disease detection and evaluation. In this study, we investigate by high- throughput sequencing the sRNAs alterations associated with Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease (COVID-19). Specifically, sRNAs extracted from nasopharyngeal swabs obtained from twenty SARS-CoV-2–infected patients with moderate or severe symptoms at two different infection time points were analyzed. Samples derived from 10 uninfected individuals were also sequenced. Cleavage of double-stranded RNA is described as an evolutionary conserved host defense mechanism against viral infection, however, alignment of the obtained sequences against the SARS-CoV-2 genome, shows the absence of virus- derived sRNAs in the samples obtained from infected patients. Regarding host sRNA-population, we observed that the COVID-19 infection induces the differential expression of diverse micro RNAs (miRNAs), mainly characterized by a significant decrease in patients that exhibit severe symptoms at both analyzed times and a slight miRNA increase associated with moderate SARS-Cov-2 infection. The miRNAs are the predominant type of sRNAs in Eukaryotes. In general, miRNAs function by interacting with the 3′ untranslated region of target transcripts to induce degradation and translational repression. Interestingly, the levels of a special group of tRNA-derived small RNAs (tsRNAs) were found to be upregulated in infected patients, particularly in those with severe symptoms. TsRNAs are classified (based on their biogenesis and length) into two major classes: tRNA halves (30–45 nt) and smaller tRNA fragments (tRFs) (14–30 nt). Specifically, the predominant over accumulating tsRNA corresponded to a 30 nt in length sequence (tRNA halves) derived from the tRNA-GluCTC. Moreover, we found that this RNA-biomarker can be detected quantitatively without prior amplification using a suitable enzymatic system, which allows patients with mild or severe symptoms to be differentially recognized.

P2.3 TDI

Development of an indirect ELISA for the detection of anti-myxoma virus antibodies based on recombinant viral proteins.

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Myxoma virus (MYXV), a member of the *Poxviridae* family, produced early cases of myxomatosis on the American continent and was later released in Australia and Europe in the1950s as a biological pest control. As MYXV causes a virulent disease in the European rabbit (*Oryctolagus cuniculus*), its introduction had a devastating impact on rabbit populations reaching mortality rates up to 95-99%. Over the years, there has been an evident coevolution in which

virus and host have adapted to each other and nowadays MYXV is not only widely distributed but also considered an endemic disease in Europe. Several vaccines are currently available, but there are still annual MYXV outbreaks affecting both wild and farm rabbits. Routine serological monitoring procedures to indicate its prevalence in wild rabbit populations and thorough surveillance of the serological status after vaccination on rabbit farms are required in order to help establish suitable control measures. Current serological tests use indirect enzyme-linked immunosorbent assay (ELISA) based on whole virus extracts as antigen. The production of whole virus extracts is costly and must be carried out under appropriate biological safety conditions. Here we describe the development of a cost-effective indirect ELISA serological test that could be used for the detection of anti-MYXV antibodies, based on recombinant proteins. For that, a selection of full-length recombinant protein candidates were produced using heterologous expression systems and screened in order to find the best requirements for expression and purification to be used for rabbit antibody detection. After optimization, the indirect ELISA was tested and compared with a commercial test indicating that the use of recombinant antigens is a sensitive and safer alternative for serologic evaluation of vaccinated farm animals and for performing myxomatosis seroepidemiologic surveys.

P2.4 TDI

Development of a method for obtaining rubella virus full-length genomes to be used in the characterization of strains associated to persistence

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Rubella virus (RuV) is the etiologic agent of rubella, which is usually a mild exanthematous disease. The primoinfection of a pregnant woman lead to miscarriage or congenital rubella syndrome, a disease with high relevance in Public Health. RuV has a single-stranded positive RNA of 9762 nt. Rubella was declared eliminated in Spain in 2016, but persistent cases continue to appear, such as recurrent uveitis after infection in childhood and rubella vaccine-induced granulomas in congenital immunodeficient individuals. The aim of this work was to develop a method to obtain the RuV full-length genome from clinical samples of persistent infections. Five samples from patients with different forms of persistence were used. Eight RuV isolates of different genotypes and a clinical specimen with RuV(2B) were used as controls. Total RNA was extracted, cDNA was obtained and a nested-PCR to amplify the nearly-full genome in 9 overlapping segments (650 pb-1650 pb) was developed. Primers were designed using PerlPrimer(v1.1) and all the RuV genomes from GenBank aligned(MAFFTv7). Sequences were obtained by Sanger method and assembled by SeqManProv15. In parallel, a massive parallel sequencing (MPS) protocol was used. RNA libraries (NebNext ultra II directional library kit,NEB) were prepared and captured with DNA probes (PanViralv2,TwistBioscience) to be finally sequenced in a Illumina MiSeq sequencer (2x150 cycles). Sequencing samples were analysed for viral consensus genome reconstruction using viralrecon pipeline. All fragments from the different genotypes of RuV used as controls were amplified and sequenced, but only some in the persistence samples. The RuV full-genome was obtained from the clinical specimen used as control by MPS and partially from the persistence samples. The low sensitivity in persistence samples could be related to the RNA degradation associated to previously handling for diagnosis and the long time since collection. As conclusion, two methods to obtain full or nearly-full genome from clinical samples of any of the RuV circulating genotypes have been developed. The Sanger sequencing method could be implemented in any laboratory of rubella surveillance. The use of fresh samples from persistence infections could improve the sensitivity in order to obtain the RuV full genome and search for any special features.

P2.5 TDI

Diagnostic procedures for the diagnosis of Monkeypox virus in Spain: comparison of methodology

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Monkeypox virus (MPXV) is a zoonotic virus belonging to the *Orthopoxvirus* genus. It has been causing sporadic cases in Africa although in 2003, 47 human cases were reported after the infection of pets (prairie dogs) through infected wild animals coming from Africa. The number of cases has been increasing in endemic countries in recent years so the virus is considered as an emerging virus and Public Health Systems have been preparing for the apparition of sporadic cases in travelers coming from endemic regions. However, since the detection of two autochthonous cases in UK on the 12th of May this year a multi country epidemic has been declared affecting more than 19 non-African countries. The ongoing epidemic in non-African countries has pushed virologists, clinicians and other Public Health professionals to design and implement protocols for diagnostic, treatment and control of infected cases. One key aspect in this process is the early diagnostic of the cases that requires specific, sensitive and validated methods. In the National Center of Microbiology (NCM) two methods were available for the detection and characterization of the virus. Both of them are generic PCRs for *Orthopoxvirus* genus and are well validated through different external quality controls. From the apparition of cases at middle May many companies have developed different molecular methods. In this poster, we report the assays performed to test some of the methods available in order to compare sensibility and specificity.

P3 DEV

P3.1 DEV

Proposal of two new tentative norovirus genotypes in genogroup I

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Noroviruses are genetically diverse viruses, which are the most common cause of outbreaks of nonbacterial gastroenteritis. They are classified into 10 distinct genogroups (GI-GX), which are further subdivided into 49 genotypes. Most human infections are caused by genogroups GI and GII. The goal of this study was to conduct a molecular surveillance of noroviruses genogroup I causing sporadic cases of acute gastroenteritis (AGE) in Valencia from 2016 to 2020. Norovirus RNA was detected by RT- PCR in stool specimens from patients with AGE attended at the Hospital Clínico Universitario of Valencia, and samples collected from AGE outbreaks. In order to genotype and characterize recombinant strains, RT-PCR and sequencing of the ORF1/ORF2 junction region was performed. RNA- polymerase and capsid gene sequences were obtained from 49 GI norovirus strains including one unassigned (NA) capsid type (isolate 3718) close to GI.3 genotype. The whole genome of this virus was determined by NGS and a phylogenetic analysis of the VP1 amino acid sequence including all GI genotypes was performed. Three defined clusters (GI.3, GI.NA1 and GI.NA2) were identified. Although these three clusters grouped in a monophyletic branch, the analysis of patristic distances demonstrated that they are different genotypes. Many non-synonymous amino acid changes were detected, although most of them occurred within a specific region (335 aa to 400 aa) of VP1, including major evolutionary changes such as insertions (threonine at 372 aa) and deletions (373 aa) in the GI.NA1 and GI.NA2 genotypes, respectively. Hence, according to 2XSD criteria we propose that the sequences within GI.NA1 and GI.NA2 (including our isolate 3718) clusters should be re-defined as new genotypes within genogroup I, that must be

assigned as GI.10 and GI.11, respectively. This study was supported by a grant from the Spanish Ministry of Science and Innovation, Carlos III Health Institute (grant PI16/01471) and by a research grant to NN-L from the Conselleria d'Educació, Cultura i Esports, Generalitat Valenciana (grant ACIF/2020/076).

P3.2 DEV

Changing molecular epidemiology of rotavirus infection with predominance of equine-like G3 strains and multiple rotavirus vaccine breakthrough cases, season 2021/2022, Madrid, Spain.

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Group A rotavirus (RVA) is a leading cause of acute gastroenteritis (AGE) worldwide. In Spain two rotavirus vaccines are licensed for use but not introduced into the national immunisation programme. It is crucial to screen the natural and/or vaccine-induced changes in RVA circulating genotypes. In Spain, both, G1P[8] and G12P[8] were the most prevalent genotypes in season 2019/2020. In December-2021, a RVA seasonal upsurge was detected. Here we aim to describe the molecular epidemiology of RVA on the current season to monitor the prevalence and distribution of RVA genotypes. RVA-positive stool samples were collected from December-2021 to April-2022 from patients with AGE attending four public hospitals in the Madrid region. For RVA detection, three hospitals used commercially available immunochromatographic methods and one hospital the Seegene-Allplex™ Gastrointestinal Panel. RVA G and P genotypes were determined by RT-PCR and further characterized by Sanger sequencing and phylogenetic analysis. A total of 301 RVA-positive samples were collected. The overall number of RVA-positive samples exceeds what was reported by the same 4 hospitals the same months in previous seasons (37 in 2019/2020 and 28 in 2020/2021). Majority of RVA-positive cases were younger than 3 years (263/301; 87%). Among the 266 cases with data on vaccination against RVA, 135 (51%) were vaccinated, either with RotaTeq (43%) or with Rotarix (57%). About 58% (45/78) of cases with data on vaccination and coinfections were RVA vaccine breakthrough cases. Of the cases with data on hospitalisation, 44% (119/272) were admitted, of whom 31% were vaccinated. RVA genotypes were determined for 92% (276/301) of samples. The most prevalent genotype was G3P[8] (64.5%), followed by G1P[8] (14.5%), G2P[4] (10%), G9P[4] (5%), G9P[8] (4%), G4P[4] (0.4%) and G8P[8] (0.4%). No G12P[8] was detected. Four samples were vaccine-derived strains. Phylogenetic analysis of G3 strains showed predominance of equine-like strains (84%). Our study has detected a major replacement in the dominant genotypes from G1P[8] and G12P[8] to G3P[8]. The spread of the equine-like G3P[8] strains (a human-equine reassortant) contributing significantly to the seasonal wave including a majority of breakthrough strains emphasizes the need for molecular RVA surveillance to monitor the effectiveness of current vaccines.

P3.3 DEV

Variability and genetic heterogeneity of betanodavirus quasispecies in European sea bass (Dicentrarchus labrax) and gilthead sea bream (Sparus aurata)

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European sea bass (*Dicentrarchus labrax*), which is widely farmed in the Mediterranean Sea, is severely affected by nervous necrosis disease, caused by nervous necrosis virus (NNV). NVV genome is composed of two single-stranded positive-sense RNA molecules, RNA1 and RNA2. The most virulent NNV species to sea bass is red-spotted grouper nervous necrosis virus (RGNNV), with a high mortality rate of larvae and juveniles. Studies on the variability of RGNNV within the host are scarce. In this work, we analysed RGNNV whole genome quasispecies *in vivo* in sea bass and sea bream (*Sparus aurata*) by NGS. Sea bass and sea bream juveniles were infected with wild-type RGNNV and sea bass were also infected with a less virulent virus harbouring a mutation in amino acid 270. At 1 and 5 dpi, viral replication was examined by qRT-PCR and mutant spectra of RNA1 and RNA2 were characterized. Mutant viruses in sea bass and wild-type viruses in sea bream showed impaired replication and migration to the brain at 5 dpi. In wild-type RGNNV populations, RNA2 had higher mutation frequency and nucleotide diversity, but lower haplotype diversity than the RNA1 segment. Principal component analysis revealed that the non-virulent populations (mutant virus in sea bass and wild-type virus in sea bream) showed more similarities in terms of genetic variability and diversity than the virulent quasispecies (wild-type virus in sea bass). In the RNA2 segment, mutant quasispecies showed a decrease in Ts/Tv ratio as well as mutation and recombination frequency from 1 dpi compared to wild-type populations, suggesting that mutation at amino acid 270 altered the genetic composition and variability of nodavirus quasispecies. These changes may underlie the modifications in serological properties and the consequent decrease in virus virulence in sea bass. In addition, characteristic recombination patterns were observed in all viral populations at the 5' and 3' ends of RNA1 that could suggest circular RNA formation. However, we do not know their implication in the virulence of RGNNV.

P3.4 DEV
Dissimilar conservation pattern in hepatitis C virus mutant spectra, consensus sequences, and data banks

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The influence of quasispecies dynamics on long-term virus diversification in nature is a largely unexplored question. Specifically, whether intra-host nucleotide and amino acid variation in quasispecies fit the variation observed in

consensus sequences or data bank alignments is unknown. Genome conservation and dynamics simulations are used for the computational design of universal vaccines, therapeutic antibodies and pan-genomic antiviral agents. The expectation is that selection of escape mutants will be limited when mutations at conserved residues are required. This strategy assumes long-term (epidemiologically relevant) conservation but, critically, does not consider short-term (quasispecies-dictated) residue conservation. We have calculated mutant frequencies of individual nucleotides and amino acids from mutant spectra of hepatitis C virus (HCV) populations from deep-sequencing data from a cohort of 220 infected patients, as well as from a high-fitness HCV evolved during 200 passages in cell culture. We have compared it with what we observed in their consensus sequences, and in an alignment of the HCV Los Alamos databank. Nucleotide or amino acid conservation in consensus sequences of the same populations, or in the Los Alamos HCV data bank did not match residue conservation in mutant spectra. Interestingly, the comparative analysis of consensus sequences and viral quasispecies did reveal positions that do not vary (which we have called superconserved) that should be taken into account for the design of universal vaccines or pan-genomic antiviral agents. The results relativize the concept of sequence conservation in viral genetics and suggest that residue invariance in data banks is an insufficient basis for the design of universal viral ligands for clinical purposes. Our calculations suggest relaxed mutational restrictions during quasispecies dynamics, which may also contribute to higher calculated short-term than long-term viral evolutionary rates, a largely unsolved question in the evolutionary dynamics of viruses.

P3.5 DEV

Co-infection with two strains of the parvovirus Minute Virus of Mice selected chimeric viruses with enhanced tropism toward human glioblastoma cells.

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Tumor cells provide conditions that facilitate the replication of some oncolytic viruses. Virotherapy in cancer relies on the natural capacity of some viruses to interfere with tumor progression by cell lysis (oncolytic viruses) or by eliciting a secondary immune response. This is a field of intensive research with more than 40 active clinical trials and two licensed recombinant viruses. Rodent Parvoviruses are being investigated as oncolytic agents in several preclinical and clinical trials. Our group is exploring the oncolytic potential of the parvovirus Minute Virus of Mice (MVM) against high-grade glioblastomas, the deadliest form of brain cancer. Here we show preliminary attempts to enhance MVM oncotropism toward glioblastoma cell lines U87 and U373. Our targeted-evolution strategy consisted in producing genetically diverse populations by forcing genetic recombination between the MVMp and MVMi strains. Emerging viruses were then subjected to serial blind-passages in glioblastoma cell lines. A high multiplicity of coinfection with these strains produced viruses that did not propagate in U87 cells, but rapidly adapted to U373 cells developing extensive cytopathic effects in a few passages. Sanger sequencing of molecular clones from passages #5 and #7 revealed a complex quasispecies of chimeric viruses dominated by genomes with NS genes of MVMi and structural genes of MVMp. The enhanced oncolytic potential of some of these chimeric viruses will be discussed. Interestingly, mutants with deletions in a region with predicted strong DNA-secondary structure progressively accumulated. Transfection of U373 or highly permissive NB324K cells with an engineered infectious plasmid harbouring one of these deletions failed to express viral proteins, suggesting a defective nature. Our results reveal that emerging defective interfering genomes may hamper targeted-evolution strategies based on repeated blind-passages on culture. Importantly, recombination between natural MVM strains may give rise to viruses with enhanced oncotropism.

P3.6 DEV

Genome stability of the recently emerged myxoma virus affecting the Iberian hare.

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Endemic since its release in Europe in 1952, myxoma virus (MYXV) causes myxomatosis in the European rabbit (*Orytolagus cuniculus*). Myxoma virus is a leporipoxvirus (family *Poxviridae*) with a genome of 160 kb and contains 171 ORFs. Historically MYXV is considered a rabbit specific pathogen, with reports of myxomatosis affecting hares being of sporadic cases dating back to the 1950s and 1960s, with one more recent report occurring in the UK in 2014. However, in 2018 the first large scale outbreak of myxomatosis was described affecting the Iberian hare (*Lepus granatensis*). This was the first evidence that MYXV had jumped species and a new MXYV capable of causing large scale mortalities in the Iberian hare had emerged. Sequencing of MYXV genomes from affected hare samples collected in 2018 demonstrated that while the virus detected was 99% similar to MYXV it contained an additional 2.8 kb region of unknown origin containing 4 novel genes present within the M009 ORF. The 2.8 kb region interrupts the M009 ORF, additionally, genes M036, M150 and M152 contain mutations with respect to traditional MYXV sequences. These mutations would lead to the truncation of ORFs that may play a role in the pathogenicity in hares. We hypothesized that sequence analysis of these regions from a samples collected in the years following emergence may provide clues as to the mechanism that has allowed this species jump to occur and determine if these mutations are ha-MXYV specific. Therefore, in this study partial sequences of approximately 80 ha-MYXV samples collected from wild hares from different geographic locations from 2018-2022 and a limited number of rabbit samples were analyzed. The data generated indicate the importance of the detected mutations in maintaining the virus specificity in hares and may give clues as to how this species jump has occurred. Analysis of the data allows the correct design of robust sequence based diagnostic techniques for improved detection of this emergent pathogen. KPD and JMMA acknowledge funding from the Spanish Ministry for Science Innovation and Universities (REF: PID2020-120349RB-100). IGB acknowledges funding from the Spanish Ministry for Science Innovation and Universities (REF: PID2019-111080RB-C21).

P3.7 DEV

Metatranscriptomics to unmask marine mollusca virome

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The high abundance of microorganisms that inhabit the sea is a potential threat to marine metazoans. For instance, it is estimated that 10^{23} viral infections occur every second in oceans. Therefore, marine metazoans must show viral entities naturally constituting their microbiome. In the last years, the use of metatranscriptomics as an unbiased method to describe microbial variability, has gained interest. Due to their filter-feeding behaviour that force most molluscs to be in touch and even accumulate potential pathogens, in the present work, about 60 SRA datasets be-

longing to molluscs from 16 geographical origins and 17 different tissues were analyzed looking for viral sequences. Among the results, viruses typically identified in molluscs such as Abalon herpesvirus type 2 Taiwan/2007, Wenzhou gastropodes virus 2, Mytilus mediterranean mussel adintovirus or Southern pygmy squid flavivirus were found. Moreover, we could identify some others that are relatively new and remain unclassified such as Barns Ness bread-crumb sponge aquatic picorna-like virus 2, Beihai picorna-like virus 56 and Wenzhou picorna-like virus 51. Finally, the studied samples also showed in some cases viral sequences belonging to mammalian viruses or plant viruses.

P3.8 DEV

Targeted evolution of parvovirus Minute Virus of Mice towards human glioblastoma cells selects mutants at the receptor binding site with improved oncotropism

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Glioblastoma is the deadliest form of brain cancer (5% of survival rate at five years). Due to its high resistance to current clinical treatments, several potential alternative therapies are being assessed. Virotherapy based on oncolytic viruses are one of the most promising alternative strategies, a field of intensive research with more than 40 active clinical trials and two licensed recombinant viruses. Rodent Parvoviruses show natural preference for proliferative cells, elicit a strong secondary immune response and are safe for humans in clinical trials. The parvovirus Minute Virus of Mice (MVM) infects target cells via recognition of sialic acid (SIA) residues exposed on the cell surface. This interaction takes place in a small depression at the 2-fold axis of symmetry of the capsid called "dimple". We have previously demonstrated that amino acids surrounding this region are key determinants of tropism and pathogenicity. Tumor cells show important glycan surface alterations including a glycocalix richer in SIA residues and several tumor-marker SIA forms. Here we attempt to enhance MVM oncotropism by retargeting its capsid to SIA glycans specifically expressed on glioblastoma cells. Two libraries of 10,000 and 8,700 MVM mutants at the SIA binding domain of the capsid (1-2 coding changes) were obtained by error-prone PCR-based mutagenesis of the MVMp and MVMi strain genomes, respectively. Targeted evolution was addressed by serial blind-passages in the U87 and U373 human glioblastoma cell lines. Neither of these libraries of mutants propagated in U87 cells. In contrast, they rapidly adapted to U373 cells, developing extensive cytopathic effects in a few passages. Sanger sequencing of molecular clones from the viruses selected after five passages in U373 cells revealed the rapid selection of mutants at VP2 residues 321 and 396 of MVMi, and 317, 361, 377 and 396 of MVMp. These amino acid changes were located nearby the SIA contact site in the capsid. Recombinant viruses engineered with some of these mutations showed improved oncotropism toward human glioblastoma cells.

P3.9 DEV

A new Respiratory Syncytial Virus B cluster emerged in Spain during 2021-2022 season.

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This study characterize the RSVs viruses circulating during 2021-2022 season in Spain. 250 positive samples for RSV were sequenced using two different approaches. First, with the aim to obtain the sequences of the main antigens was performed an amplification protocol with specific primers targeting attachment (G) and fusion (F) proteins.

The second approach was based in whole genome sequencing (WGS) methods. Complete genome using WGS methods was obtained in 26 samples. The F and G protein sequences were achieved in 180 of 250 samples analyzed. The 82% of the sequences obtained belonged to RSVB type. As RSVB predominated over RSVA our analysis was focused in this group. According to our sequences analysis, the RSVB viruses characterized in this study were a well-differentiated group of viruses among RSVB type. High bootstrap values supported the cluster (0,996) which differentiate these viruses from previous circulating RSVs. Three amino acid changes in RSVB F protein were identified in the majority of sequences: S190N, S211N and S389P. This polymorphism, that has not been previously described, contain changes with potential antigenic impact since are located at the apex of F trimer, target of high neutralizing antibodies. This work detected new strains in 2021/2022 season in Spain. The description of amino acid changes that can affect the effectiveness of vaccines and treatments available showed the importance of tracking these changes and its impact in pediatric patients.

P3.10 DEV
Impact of codon volatility in an RNA virus under constrained evolution conditions

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The volatility of a codon is defined as the probability that a random point mutation in the codon generates a non-synonymous change. To study the impact of genomic volatility under constrained evolution we recoded the structural region (117 codons in the P1 region) of the genome of the human enterovirus Coxsackievirus B3 (CVB3). Thus, we genetically engineered two mutants with different genomic volatility according to a mathematical framework. Hence, one mutant bears the most volatile synonymous codons of Serine and Leucine (MoreV). On the contrary, the second mutant was designed to use only the lowest volatile codons of these two amino acids (LessV). In this study, we performed plaque-to-plaque passages in tissue culture of both mutants and the Wild Type virus (WT). This approach was carried out during ten passages using three lineages for each virus. During the evolution, we saw that infective particles recovered from plaques were decreasing in titers, as expected. Moreover, we evaluated the impact of the mutations fixed in passages three, six, and ten by measuring the relative fitness. We then sequenced all these passages using Nanopore and we related every genotype to the relative fitness and a plaque size phenotype. Our results suggest that the WT and LessV viral populations lost their fitness due to the action of the Muller's ratchet but the MoreV population had the most chaotic behavior (in virus titer and plaque phenotype) after the subsequent bottlenecks imposed.

P3.11 DEV
The molecular chaperone Hsp70 buffers against the deleterious effects of mutations and can shape viral evolutionary trajectories

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Proteins must fold to their native 3-dimensional structure in order to function. This folding process is highly complex, often necessitating the support of cellular protein folding factors (e.g. Hsp70). As a result, most mutations are deleterious to protein function. RNA viruses have extreme mutation rates and are therefore expected to be highly dependent on chaperones for maintaining the functionality of their proteins. To directly test this hypothesis, we evaluated whether a key cellular protein-folding factor, Hsp70, can influence the fitness of mutations in a viral capsid protein as well as its evolutionary trajectory. Specifically, we evaluated the effects of thousands of mutations on viral fitness under normal conditions or those of Hsp70 inhibition. In addition, we assessed whether serial passaging in

the presence of an Hsp70 inhibitor can alter the distribution of mutations maintained in the viral populations. Our results indicate that Hsp70 inhibition results in a large increase in cost to mutations. Moreover, we find that growth in the presence of Hsp70 inhibitors can alter which mutations arise during short-term experimental evolution. Overall, our results support a key role for Hsp70 in buffering against the deleterious effects of mutations to viral fitness, which has important implications for viral evolution and pathogenesis.

P3.12 DEV
Genetic and antigenic characterisation of the circulating influenza viruses in Spain during the 2021-2022 season.

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Influenza surveillance remains the first line of defence for detection of new influenza virus strains with pandemic potential. During the 2021-2022 influenza season in Spain, influenza A was the dominant type being H3N2 the most prevalent influenza virus detected (81%). Clade assignation of circulating viruses was performed by analysing the sequences of the hemagglutinin HA1 subunit resulting from whole genome sequencing methods or specific PCRs, depending on the viral load in clinical specimens. A total of 791 H3N2 virus were characterised and almost all belonged to the 'Bangladesh-like' subgroup (3C.2a1b.2a.2), represented by the A/Bangladesh/4005/2020 virus. Only two viruses were not 'Bangladesh-like' and belonged to the 3C.2a1b.1a subgroup, represented by A/Denmark/3264/2019(H3N2). In accordance with the already published by ECDC and WHO, 'Bangladesh-like' viruses have proven to be antigenically different to the virus used in the composition of this year's vaccine (A/Cambodia/e0826360/2020), which belongs to the 3C.2a1b.2a subgroup. During the evolution along the season, 'Bangladesh-like' viruses formed different well-separated clusters, defined by specific HA1 amino acid substitutions, with one cluster (D53G) substituting the dominant one (D53N) amidst the season. Fewer viruses H1N1pdm09 were characterised, in comparison to H3N2, being all 29 viruses 'Guangdong-like', [A/Guangdong/SWL1536/2019(H1N1)]. Nevertheless, a new subgroup started to emerge in the 'Guangdong-like' group defined by two amino acid substitutions (P137S and G155E), first and predominantly detected in the Canary Islands but also in the Balearic Islands and Murcia. In accordance with ECDC and WHO reports, 'Guangdong-like' viruses have proven to be antigenically different to the component H1N1pdm09 vaccine virus (A/Victoria/2570/2019 IRV-215). Three influenza B viruses, belonging to the Victoria lineage, were characterised, two of them included in the subgroup defined by B/Washington/02/2019 and one included in B/Austria/1359417/2021. No influenza B virus from the Yamagata lineage was detected this season. No amino acid substitution associated with reduced or highly reduced inhibition to oseltamivir and zanamivir was found in 354 influenza viruses analysed and normal inhibition to these antivirals was confirmed using the MUNANA technique in 14 viruses. However, the amino acid substitutions S31N or S31D in the matrix protein associated with resistance to adamantanes were detected in all viruses analysed.

P3.13 DEV
Isolation and characterization of novel highly lytic phages against Mycobacterium abscessus

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The emergence of multidrug-resistant bacteria is a global health threat. Among these bacteria, *Mycobacterium abscessus* is an opportunistic bacterium that causes serious complications, especially in patients with cystic fibrosis and bronchiectasis. Phages, viruses of bacteria, are emerging as promising biomedical tools to treat these infections

due to their specificity and safety. Here we isolated novel highly lytic phages against *M. abscessus* using *Mycobacterium smegmatis mc2 155* as an intermediate host. For this purpose, we analyzed environmental samples from wastewater, soil and plants, among others. Using an in-house phage hunting protocol, we succeeded in isolating a total of 31 phages from *M. smegmatis*. Of these, at least 5 phages showed strong lytic activity in clinical strains of *M. abscessus* from 20 patients admitted to the Hospital Universitari i Politècnic La Fe (Valencia, Spain). Transmission electron micrographs showed the typical morphotype of *Siphoviridae* with isometric heads and long flexible tails. Genomic characterization of the phages is a work in progress. The emergence of phage-resistant mutants in vitro to determine mechanisms involved and directed evolution of the phages will help us to create a phage cocktail for compassionate use against *M. abscessus*.

P3.14 DEV

Two different perspectives to design broad-range phage cocktails to treat Klebsiella pneumoniae infections

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Klebsiella pneumoniae is an opportunistic nosocomial encapsulated pathogen, considered a worldwide threat due to the emergence of multidrug-resistant high-risk clones. Bacteriophage (phage) therapy has recently been rediscovered as one of the most promising tools to combat infections caused by these bacteria. Here, we design broad-spectrum phage cocktails against *K. pneumoniae* from two different perspectives. In the first, we focus on the wide variety of capsular types of *Klebsiella*, which is known as a major virulence factor and may determine the susceptibility to phage infection. We isolated >80 environmental phages from the 77 reference capsular types of *Klebsiella* (collection acquired from the Staten Serum Institute, Copenhagen, Denmark), and explored their host range by spot tests to construct a cross-infection matrix using the reference strains. We obtained very diverse infection ranges, as 40% of the isolated phages were capsular dependent, and only 6 of them were able to infect at least 10 capsule types, and hence considered broad-spectrum phages. All phages were phenotypically and genomically characterized, showing enormous diversity. Using the cross-infection matrix and the Phage-Cocktail Cytoscape application, we designed a cocktail combining 12 phages with an expected host range ≈ 70 % of the capsular types. The observed infectivity, tested *in vitro* using spot tests, was reduced to 56 % of capsular types, suggesting potential phage-phage interactions, as we have demonstrated synergistic and antagonistic interactions between phages. In a second pipeline, we are developing phage training by directed evolution to develop a broad-spectrum cocktail. To this end, we are using phages isolated directly from high-risk clones of carbapenemase-producing *K. pneumoniae* in Spain. By working with antibiotic-resistant clinical strains, we will also be able to explore phage-antibiotic synergy as a therapeutic alternative. We have already isolated 114 new *Klebsiella* phages using this strategy and are conducting cross-infection assays. Both cocktails will be tested on a collection of clinical isolates of *K. pneumoniae*. Interestingly, the results are encouraging, as the first broad-range phage cocktail attempt infected 68% of the high-risk clones tested (17/25), suggesting that phage therapy against carbapenem-producing *K. pneumoniae* could be a promising therapeutic solution.

P4 CAV

P4.1 CAV

High-throughput screening to identify new inhibitors directed against the early steps of the cytomegalovirus infectious cycle

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CMV is a major cause of morbidity and mortality in immune-compromised individuals. Although the current anti-CMV treatments are effective at preventing and limiting CMV-associated disease, they are associated with undesirable side effects (nephrotoxicity) and selection of resistance mutations in addition to the high cost. Consequently, strong efforts are necessary to search for new therapeutic drugs. In order to search for new compounds targeting CMV entry and the early steps of infection, we developed a high- throughput screening of the Prestwick Chemical Library consisting of 1520 drugs. For the inhibition assay, GFP-AD169-infected ARPE-19 epithelial human cells were exposed to increasing concentrations of the compounds. We identified 32 compounds that decreased at least 80% cell-infection. Extensive secondary assays using both ARPE-19 and MRC-5 infected cells confirmed the inhibitory effect of epirubicin and auranofin with low cell cytotoxicity. The selected compounds likely target various cellular processes involved in the early steps of infection including capsid transport, chromatin remodelling, and viral gene expression.

P4.2 CAV

Molecular determinants of human rhinovirus infection, assembly and conformational stability at capsid protein interfaces

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Human rhinovirus (HRV) holds singular clinical and economical importance as one of the most frequent human pathogens. HRV is the major causative agent of common colds, and is also associated to severe respiratory diseases including asthma and chronic obstructive pulmonary disease. However, no anti-HRV vaccine or antiviral drug are available to date. Although protein-protein interfaces in virus capsids have been increasingly recognized as promising targets for antiviral drugs, the structural elements and amino acid residues responsible for the biological role and biochemical functions of these regions are largely unknown. In this study we have carried out a comprehensive mutational analysis to determine which specific residues along the interpentameric interfaces of the HRV capsid are relevant for viral infection and the steps in the viral cycle in which they play a role. Most of the residues analyzed, irrespective of type or position at the interface, proved to be critical for infectivity. A representative subset of virus mutants with reduced infectivity were analyzed for virion assembly and stability against thermal inactivation. Most of them were found to be critical for virion morphogenesis and/or conformational stability. Together, the results indicate that the HRV interpentameric interface constitutes a quasi-continuum of biologically relevant residues. The entire interface may constitute a potential target for the design of antiviral drugs that could interfere with virion assembly and/or resistance to virion-inactivating conformational transitions.

P4.3 CAV
Coronavirus inhibition by cell-targeted antiviral drugs

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The coronavirus disease pandemic [COVID-19], which is caused by severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2], has triggered the search for multiple antiviral compounds. An alternative strategy to conventional antiviral drugs that interfere with viral components is the identification of compounds targeting cellular functions. Following this approach, we have focused our studies on lauryl gallate [LG], an ester derivative of gallic acid widely used as an antioxidant, and valproic acid [VPA], a branched short-chain fatty acid with a known therapeutic role against neurological diseases. Our previous results, which demonstrated the efficacy of LG and VPA in inhibiting different viruses, led us to test their effect on human coronaviruses. The inhibitory effect of LG or VPA was assayed on Huh7 cells infected with HCoV-229E-GFP or HCoV-OC43, and on Calu-3 2B4 infected with SARS-CoV-2. A consistent decrease of 2 to 5 logarithmic units in virus yields in susceptible coronavirus-infected cell lines was observed in the presence of LG for HCoV-229E-GFP, HCoV-OC43 and SARS-CoV-2, with an average IC50 value of 1.6 μM. VPA inhibition resulted in a 2-3 logarithmic unit reduction on virus production, with an average IC50 value of 7.2 mM, when tested against these three human coronaviruses. By evaluating the effect of the time of addition of the antiviral during infection, similar levels of inhibition were obtained when adding the drug before [-3 hpi], at the time [0 hpi] or after [2 hpi] infection, supporting a post-virus entry mechanism of action. Specificity of LG among other gallic acid derivatives [propyl gallate, octyl gallate, gallic acid and epicatechin gallate] was also studied. Finally, the combined effect of these compounds was also evaluated, together with remdesivir, a virus-targeted drug with a proven effect against coronaviruses multiplication, searching for possible synergistic effects. Our findings reinforce the interest of testing the effect of these cell-targeted compounds as a first line of disease defense or as a vaccine complement to minimize the gap in antibody-mediated protection evoked by vaccines, either in the case of SARS-CoV-2 or for other possible emerging viruses.

P4.4 CAV
Eeyarestatin I, an inhibitor of the valosin-containing protein, exhibits potent virucidal activity against the flaviviruses

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Cellular responses to stress generally lead to the activation of the endoplasmic reticulum-associated protein degradation [ERAD] pathway. Several lines of study support that ERAD may be playing a proviral role during flaviviral infection. A key host factor in ERAD is the valosin-containing protein [VCP], an ATPase which ushers ubiquitin-tagged proteins to degradation by the proteasome. VCP exhibits different proviral activities, such as engaging in the biogenesis of viral replication organelles, and also facilitating flavivirus genome uncoating after the viral particle enters the cell. To investigate the possible antiviral value of drugs targeting VCP, we examined two VCP inhibitors: eeyarestatin I [EEY] and xanthohumol [XAN]. Both compounds were highly effective in suppressing Zika virus [ZIKV] and Usutu virus [USUV] replication during infection in cell culture [selectivity indexes >13 for XAN and >34 for EEY]. Further analysis revealed an unexpected virucidal activity for EEY connected to its antiviral effect, but not for XAN.

Preincubation of ZIKV or USUV with EEY before inoculation to cells resulted in significant decreases in infectivity in a dose- and time-dependent manner. This decay in infectivity was never observed in the absence of drugs or in the presence of XAN. Viral genomes in virus samples previously treated with EEY were more sensitive to exposure to propidium monoazide, with 10- to 100-fold decreases observed in viral RNA levels, further supporting that EEY affects viral particle integrity. These results suggest that EEY is a strong virucide for the flaviviruses, encouraging further studies to investigate its potential therapeutic use in the treatment of flaviviral infection, or the development of improved derivatives with this aim. Funding information: This research was funded by Ministerio de Ciencia e Innovación, grant number PID2019-106068GB-I00. AA is supported by a Beatriz Galindo Senior Fellowship [BEAGAL18/00074], CB has been supported by a Beca CRIB Iniciacion 2021 [Universidad de Castilla-La Mancha].

P4.5 CAV

Design of non-immunogenic peptides that prevent the activation of SARS-CoV-2 nsp14 and nsp16 proteins

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The emergence of new SARS-CoV-2 variants less sensitive to monoclonal antibodies has reduced the efficacy of vaccines and led to new outbreaks. Therefore, there is a need to develop antiviral strategies that complement the use of vaccines to treat SARS-CoV-2 infections. The nsp10 protein of SARS-CoV-2 is a cofactor that interacts with the nsp14 and nsp16 proteins, activating their proofreading [ExoN] and 2´-O-methyltransferase [2´-O-MTase] activities, respectively. In this study, we have designed peptide inhibitors at the interfaces of the viral nsp10 interacting with nsp14 and nsp16. For peptide design, the crystal structures of SARS-CoV and SARS-CoV-2 were used to determine the nsp10-nsp16 and nsp10-nsp14 interaction interfaces and to select suitable nsp10-binding fragments according to peptide length, secondary structure, and interaction energy. Using computational protein design based on virtual mutagenesis, inhibitory peptides were derived from wild-type peptide sequences of nsp10. The designed peptides were ranked according to the interaction energy, or the existence of intramolecular interactions. From clinical samples, quasispecies were analyzed by Illumina and mutations in nsp14 and nsp16 were identified to estimate whether they affected the binding of peptides to viral proteins. Bioinformatic analysis revealed levels of genetic complexity in nsp14 and nsp16 that were not observed in the consensus sequences. Mutation frequency was estimated to be between 10-4 and 10-3 mutations/nt in both regions. The analysis of polymorphisms revealed nucleotide substitution biases in the genomic regions towards transversions G→T and C→A, which contrasts with consensus sequences studies, where C→T transitions significantly predominate. Most of the mutations present in samples did not modify the action of the peptides and even mutations that increased the binding efficiency of the peptides to the proteins were detected. However, some mutations did decrease the binding efficiency of the peptides, such as the amino acid changes Leu38Ser and Gly202Val on the nsp14 protein. As a result, short non-immunogenic peptides of 9 or 12 amino acids were obtained, targeting the ExoN proofreading and the 2´-O-MTase activities of SARS-CoV-2 that may alter the interaction of nsp10 with nsp14 and nsp16. The inhibitory capacity of these peptides will be tested in future cell culture assays.

P4.6 CAV

Exploration of natural compounds uncovers Honokiol and ⟨-Mangostin as potential antivirals against Mayaro and other arboviruses

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Arboviruses have provoked large epidemics in the Americas, including those of the Chikungunya (CHIKV) and Zika (ZIKV) viruses. Endemic and emerging arboviruses, such as Mayaro virus (MAYV), show increasing circulation across the region. Despite the challenges these viruses present to local health systems, there are no approved treatments to combat these pathogens. Thus, there is an urgent need to identify potential broad-spectrum antivirals. Natural products are a rich source of molecules with diverse biological activities, including antiviral drugs. Honokiol and ⟨-Mangostin are natural compounds isolated from the *Magnolia* and *Garcinia* plant genera, respectively. These compounds have demonstrated various pharmacological activities, including antioxidant, anti-inflammatory, antitumoral, antibacterial, antifungal and antidepressant effects, as well as antiviral activity against certain viruses. However, Honokiol and ⟨-Mangostin have not been studied extensively in the context of arboviruses. In the present study, we analyzed the potential antiviral activity of Sanguinarine, Shikonin, Fisetin, Honokiol, Tanshinone IIA, and ⟨-Mangostin against Mayaro and other arboviruses. The compounds' cytotoxicity was assessed for different cell lines using the MTT method. A cell protection assay was conducted with MAYV-infected Vero-E6 cells that were treated or untreated with the natural compounds and evaluated using an inverted microscope. Viral progeny production in various cell lines infected with different MAYV strains, Una virus (UNAV), CHIKV or ZIKV was assessed using plaque-forming assays. Moreover, viral protein expression was evaluated using immunofluorescence and Western blot. Sanguinarine and Shikonin showed significant cell toxicity, whereas Fisetin, Honokiol, Tanshinone IIA and ⟨-Mangostin were well-tolerated at doses of 5 or 10 μM. Honokiol and ⟨-Mangostin protected Vero-E6 cells from MAYV-induced damage and resulted in a dose-dependent reduction in viral progeny production for each of the MAYV strains tested. This reduction was observed for all the human cell lines tested. Similarly, Honokiol and ⟨-Mangostin decreased the viral progeny yields of the UNAV, CHIKV, and ZIKV viruses. Finally, these compounds downmodulated the expression of E1 and nsP1 viral proteins from MAYV, UNAV, and CHIKV. Taken together, these results indicate that Honokiol and ⟨-Mangostin present potential broad-spectrum activity against important members of the *Alphavirus* and *Flavivirus* genera. Funding: grant numbers 19911.012, MEF; 23-2021, SNI-SENACYT, Panama.

P4.7 CAV

Antiviral activity of cold atmospheric plasma-activated water against SARS-CoV-2 and influenza viruses

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Atmospheric Plasma is the fourth state of matter. On Earth, matter mainly comes in three forms: Solid; Liquid; Gaseous. But in space, a fourth state prevails: the plasma. The generation of Cold Atmospheric Plasma (CAP), and its derivative Plasma Activated Media (PAM, or water PAW), may be used effectively against viruses because they can deliver Reactive Oxygen and Nitrogen Species (RONS) on a living tissue with negligible damage on health cells. In the present study, the capability of Plasma Activated Media (PAW) to inactivate SARS-CoV-2 and PR8 H1N1 influenza virus with negligible damage on healthy cells is demonstrated. MDCK cells treated with PBS-buffered PAW reduced significantly the PR8 infection by 53%. On the other hand, infected A549 cells treated with PR8 and subsequently treated with PAW completely reduced PR8 nucleoprotein transcription. Furthermore, the treatment of A549 lung cells at different times with buffered PAW did not increase interleukin 8 expression, showing that PAW did not induce inflammation. Moreover, PAW also reduced SARS-CoV- 2 infectivity when directly incubated and subsequently titrated in Vero E6 cells. In addition, we studied the effect of ultrasonic nebulization of PAW on SARS-CoV-2 infectivity. SARS-CoV-2 adsorbed on Vero E6 cell monolayers was nebulized with PAW for 10 min. Subsequent assessment showed a statistically significant reduction in infectivity when the SARS-CoV-2 adsorption time was short. Overall, PAW acted by both virus detaching and diminished replication. These results open a new research field by using PAW to the development novel treatments for COVID-19, influenza, and other respiratory diseases.

P4.8 CAV
Impact of NEDD8 in the replication of coronavirus CoV 229E

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NEDDylation is a post-translational modification in which the neural precursor cell expressed developmentally downregulated protein 8 (NEDD8) is conjugated to lysine residues of target proteins. It can affect gene regulation, cell survival, stress response, or innate immune response. Thus, NEDDylation has been reported to contribute to NF-κB activity or to play a role in type I interferon production. In addition, conjugation of NEDD8 to specific virus proteins has been shown to reduce its stability affecting to the virus replication. However, the ubiquitin-dependent proteasome pathway can also be hijacked by certain viruses to facilitate replication. Neddylation might then exert distinct roles in the infection of different viruses. Thus, whereas the NEDDylation inhibitor MLN4924 prevents adenovirus, influenza virus or herpesvirus genome amplification, blockage of NEDDylation restricts the replication of the human immunodeficiency virus or Rift Valley fever virus. Here we have studied the impact of NEDD8 on CoV 229E. Our results demonstrate that CoV 229E modulates NEDDylation. In addition, our results reveal that the NEDDylation inhibitor MLN4924 exhibited pronounced antiviral activity against CoV 229E replication. Finally, studies carried out with the NEDDylation inhibitor MLN4924 point to the involvement of NEDD8 in the endocytosis of the virus. In summary, here we identified NEDD8 as a potential therapeutic target against Cov 229E infections.

P4.9 CAV

IN VITRO MODEL OF THE ANTIVIRAL ACTIVITY OF THE HRA2pl FUSION PEPTIDE AT THE ATTACHMENT OF HUMAN ORTHOPNEUMOVIRUS, HUMAN METAPNEUMOVIRUS AND HUMAN ORTHORUBULAVIRUS-2

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Acute respiratory infections (ARIs) are a group of diseases caused by viruses, bacteria and/or parasites that mainly affect children under 5-year-old and immunocompromised older adults. In Mexico these kinds of infections are the main cause of morbidity with more than 26 million cases reported. The most frequent viral agents are *human Orthopneumovirus (hOPV)* causing 70% of the total cases of bronchiolitis and 25% of the total cases of pneumonia; *human Metapneumovirus (hMPV)* with 5-20% of hospitalizations, and finally *human Orthorubulavirus-2 (hORUV-2)* with 2-17% of hospital admissions. Currently, Palivizumab (mabaF fusion protein) is the specific treatment of choice against hOPV. In the other hand, this viral protein is considered a therapeutic target for the design of antiviral peptides that inhibit the fusion of the virus with its host cell. According with the information, we propose to study the antiviral activity of the HRA2pl peptide, obtained by transient expression system in tobacco plant. The effect of the fusion peptide was evaluated by an attachment assay and we determinate the viral titer with or without the peptide treatment and the size of the syncytium. Our results demonstrated the effect of the HRA2pl peptide over the virus attachment. The attachment assay showed a decrement in the viral titer until 5 log between the viral strains treated versus none treated. With these data, we decided to study the effectiveness of the HRA2 pl over viral isolates from respiratory clinical samples. Our results showed a reduction in the size of the syncitium until of 50%. Conclusion. The HRA2pl peptide showed antiviral activity by decreasing the viral attachment and the viral particles production of hOPV, *hMPV*, and *hORUV-2*, and in the case of the viral isolates the reduction of the syncytium size.

P5 VPL

P5.1 VPL

Virus host jumping can be boosted by adaptation to a bridge plant species

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Understanding mechanisms involved in emergence of viral diseases, in particular those events engaging cross-species pathogens spillover, is becoming increasingly important in virology. Species barrier crossing has been extensively studied in animal viruses, and the critical role of a suitable intermediate host human pandemics generated by animal viruses is highly topical. However, studies on host jumping of viruses in plants have been focused on intraspecies shifting, leaving aside the putative role of "bridge hosts" in facilitating interspecies crossing. Here, we approach this subject by analyzing the differential behaviors of several VPg mutants, derived from a chimeric construct of the potyvirus *Plum pox virus* (PPV), in three herbaceous species. Our results showed that two VPg mutations in a *Nicotiana clevelandii*-adapted virus, emerged when it was propagated in the bridge-host *Arabidopsis thaliana*, prompted partial adaptation to *Chenopodium foetidum*. Although both changes are expected to facilitate productive interactions with eIF(iso)4E, polymorphism detected in PPV VPg and the three eIF(iso)4E studied, extrapolated to a recently published VPg:eIF4E structural model, suggested that two adaptation ways could be operating. Remarkably, we found that VPg mutations driving the expansion of the host-range of the virus to the two non-related species, not only did not cause trade-off constraints in the original host, but also improved fitness on it.

P5.2 VPL

Analysis of gene expression during Tomato chlorosis virus (ToCV) infection of susceptible tomato plants

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Tomato chlorosis virus (ToCV, genus *Crinivirus*, family *Closteroviridae*) is an emergent plant virus that infects tomato plants leading to economic losses in many countries. To study the dynamic transcriptome changes in host gene expression after infection with ToCV we sampled susceptible tomato "Moneymaker" plants and performed RNA-Seq at 2, 7 and 14 days post-infection (dpi). Compared with uninfected control samples, a total of 2422 (1191 up and 1231 down), 914 (333 up and 581 down) and 6918(3197 up and 3721 down) differentially expressed genes (DEG) were identified after ToCV infection in the three considered time points, revealing a complex scenario with many alterations occurring late during the infection process. Gene Ontology (GO) category and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis revealed that many of these DEGs were associated with multiple biological functions, including defense responses, chaperone activity, transport and other processes potentially relevant for the development of symptoms. In particular, we found that the expression of the Hsp90 (heat shock protein 90) and its co-chaperone Sgt1 (suppressor of the G2 allele of Skp1) were up-regulated after ToCV infection. Interestingly, these two genes are known to positively regulate disease resistance conferred by many Resistance (R) proteins, and indeed their specific silencing using a Tobacco rattle virus (TRV)-based system resulted in increased ToCV accumulation, suggesting that they might contribute to the plant response to viral infection. Our results provide new insights into the molecular responses occurring in ToCV-infected tomato plants and they might represent a step toward identifying potential genes useful for designing future disease control strategies. [IO was supported by Contract BES-2017-080808, and funding was provided by project PID2019-105692RB-100].

P5.3 VPL

Adaptation of RNA virus populations to the host's epigenetic-regulated defense responses

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In this study we have explored how RNA viral populations evolve, interact and adapt to plant epigenetically-regulated defense pathways by using the pathosystem of turnip mosaic potyvirus (TuMV) and *Arabidopsis thaliana*. We have evolved five independent TuMV lineages in a set of genotypes (apart from that of the wild type) carrying mutations that affect key components of different plant epigenetic pathways (DNA methylation and histone modification). These epimutants include *dcl2/3/4* (DICER deficient), *ddm1* (a remodeler involved in heterochromatin stability), *ibm1* (a H3K9-histone demethylase), *jmj14* (a H3K4-histone demethylase), and *polV* (involved in the canonical RNA-directed DNA methylation pathway). At each passage, several traits related to viral fitness and virus-host interactions were quantified for each evolving lineage: infectivity, disease progression, severity of symptoms, and viral load. Overall differences for these traits were observed along evolution, with the viral populations infecting the *ibm1* mutant, followed by those of *dcl2/3/4*, *jmj14*, and *ddm1* showing the lowest fitness at the beginning of the evolution experiment. All evolved viral lineages showed adaptation to epigenetically-regulated responses through significant increases in infectivity, virulence and viral load (apart from *polV*) although the magnitude of the improve-

ments strongly depended on the plant genotype. The dynamics of evolution for these traits were biphasic, being faster in the earlier passages and flatting off in the later ones. Virulence was positively correlated with viral load, though the allometric relationship between these two traits was different among ancestral and evolved viruses. High-throughput sequencing was used to evaluate the viral diversity of each evolving lineage as well as identifying and characterizing the nature of the mutations fixed, evolutionary convergences and potential targets of TuMV adaptation. Regarding the host epigenome-virus interactions, we have compared the transcriptomes (RNA-seq) of plants infected with the ancestral TuMV and the evolved lineages to tackle two relevant questions: which epigenetically-regulated host genes have changed as a result of virus adaptation? And to which functional classes and regulatory epigenetic pathways or networks do they belong?

P5.4 VPL

Development of an expression system based on tomato chlorosis virus: GFP expression in Nicotiana benthamiana plants

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Tomato chlorosis virus (ToCV, genus *Closterovirus*, family *Closterovidae*), as all other closterovirids, is phloem-limited, non-mechanically transmissible, and semipersistently transmitted in nature by hemipteran insects. ToCV is transmitted specifically by the whiteflies (Hemiptera: Aleyrodidae) *Bemisia tabaci, Trialeurodes vaporariorum* and *T. abutilonea*. Symptoms observed in ToCV-infected tomato plants first develop on lower leaves and then advance towards the upper part of the plant, including interveinal yellowing and thickening of leaves; bronzing and necrosis of the older leaves are accompanied by a decline in vigor and reduction in fruit yield. In addition to tomato, ToCV has been found to infect plants belonging to 25 botanical families, including economically important crops as pepper and potato. Present in around 40 countries and territories, ToCV constitutes a paradigmatic example of an emergent plant pathogen. Most crinivirus genomes consist of two positive-sense single-stranded RNA molecules separately encapsidated in long, flexuous, rod-like virions, RNA1 and RNA2. RNA1 contains four open reading frames (ORFs) encoding proteins responsible of virus replication. RNA2 contains nine ORFs encoding proteins associated with virus encapsidation, movement and whitefly transmission. We have previously developed a system of agroinfectious clones with the aim to carry out reverse genetic studies, understand the virus–vector interactions and facilitate breeding programs to incorporate resistance against ToCV in commercial cultivars. In this work, the ToCV RNA2 infectious clone has been engineered to introduce the GFP gene in two different positions with the objective of developing an expression system. Accumulation of ToCV and GFP expression was assessed in *Nicotiana benthamiana* plants agroinoculated with each of the two RNA2 clones constructed together with the RNA1 clone. No fluorescence was observed by eye under UV light in the agroinoculated plants although they were shown to be infected and the presence of the GFP gene was confirmed. Nevertheless, GFP expression was observed in leaf petiole sections under fluorescence microscope, fluorescence being located only in scattered phloem cells. These results are promising although at the same time show the limitations of working with closterovirids, a group of viruses whose study has been hindered by the complex genome organization and expression strategies.

P5.5 VPL

Toward characterization of the molecular determinants governing the transmission of the crinivirus tomato chlorosis virus by the whiteflies Bemisia tabaci and Trialeurodes vaporariorum

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Tomato chlorosis virus (ToCV) (genus *Crinivirus*, family *Closterovidae*) causes an important emergent viral disease of tomato in addition to infect other cultivated and wild plants. Symptoms caused by ToCV in tomato include interveinal yellowing and thickening of lower leaves that advance towards the upper part of the plant. Criniviruses have a bipartite genome of positive-sense single-stranded RNA. RNA1 contains four open reading frames (ORFs) which encode proteins related to virus replication, and RNA2 contains nine ORFs encoding proteins associated with virus encapsidation, movement and whitefly transmission. Both RNAs are encapsidated separately in flexuous virions with the typical body-tail (rattlesnake) structure of closterovirids. The body is composed of a single coat protein (CP) whereas the tail is composed of at least four proteins including the minor coat protein (CPm), suggested to be involved in transmission by insect vectors. ToCV is transmitted in a semipersistent manner by whiteflies belonging to two genera: *Bemisia tabaci*, *Trialeurodes vaporariorum* and *Trialeurodes abutilonea*. In order to know the molecular basis involved in the atypical transmission of ToCV, nine deletion mutants in the CPm gene were constructed using an infectious ToCV RNA2 clone. The viral progeny of the mutant clones was assayed for infectivity in *Nicotiana benthamiana* and tomato plants as well as for transmission by B. tabaci and *T. vaporariorum*. Results have revealed a candidate region in the CPm protein that could be involved in the specific transmission of ToCV by *T. vaporariorum,* in agreement with predictions based on in silico analysis of the CPm proteins of criniviruses.

P5.6 VPL

Deciphering the sweepovirus-deltasatellite-plant host interactions: expanded helper virus range and effect dependence on virus-host combination

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Sweepoviruses are begomoviruses (genus *Begomovirus*, family *Geminiviridae*) that infect sweet potato and other species of the family Convolvulaceae. They cluster phylogenetically separate from the rest of the begomoviruses, representing one of the earliest points of divergence among the genus. Deltasatellites (genus *Deltasatellite*, family *Tolecusatellitidae*) are small non-coding circular ssDNA satellites associated with begomoviruses, including sweepoviruses. In this study, the genetic diversity of deltasatellites associated with sweepoviruses infecting blue morning glory (*Ipomoea indica*) plants was analyzed by further sampling the populations where the deltasatellite sweet potato leaf curl deltasatellite 1 (SPLCD1) was initially found, expanding the search to other geographical areas in southern continental Spain and the Canary Islands. The sweepoviruses present in the samples coinfected with deltasatellites were also characterized by sequencing in order to define the range of viruses that could act as helper viruses in nature. Additionally, experiments were performed to assess the ability of a number of geminiviruses (the monopartite begomovirus tomato leaf deformation virus, the bipartite begomoviruses Sida golden yellow vein virus and tomato leaf curl New Delhi virus, and the curtovirus beet curly top virus) to transreplicate SPLCD1 in their natural hosts or the experimental host *Nicotiana benthamiana*. The results showed that SPLCD1 can be transreplicated by all the geminiviruses assayed in *N. benthamiana* and by tomato leaf curl New Delhi virus in zucchini. The

presence of SPLCD1 did not affect the symptomatology caused by the helper viruses and its effect on viral DNA accumulation depended on the helper virus-host plant combination.

P5.7 VPL

Increasing diversity of begomovirus-deltasatellite complexes: the first deltasatellite found infecting legumes

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Legumes play an important nutritional role in the diets of millions of people, mainly in developing countries, but their productivity is seriously affected by a variety of pathogens including viruses. Begomoviruses (genus *Begomovirus*, family *Geminiviridae*) and associated DNA satellites are involved in pathosystems that include many cultivated and wild dicotyledonous plants and the whitefly vector *Bemisia tabaci*. A survey of leguminous plants, both cultivated and wild, was conducted in Venezuela to determine the presence of begomoviruses. Molecular analysis identified the presence of bipartite begomoviruses in 37% of the collected plants. Four of the six begomoviruses identified constituted novel species, and two others had not been previously reported in the country. Furthermore, a novel deltasatellite (genus *Deltasatellite*, family *Tolecusatellitidae*), cabbage leaf curl deltasatellite (CabLCD), was found to be associated with cabbage leaf curl virus (CabLCV) in several plant species. CabLCD is the first deltasatellite found to infect legumes and the first found in the NewWorld to infect a cultivated plant. Agroinoculation experiments using *Nicotiana benthamiana* plants and infectious viral clones confirmed that CabLCV acts as a helper virus for CabLCD. The begomovirus–deltasatellite complex described here was also found in wild legume plants, suggesting the possible role of these plants in the emergence and establishment of begomoviral diseases in the main legume crops in the region. These results illustrate the increasing complexity faced by researchers and breeders looking to develop control strategies against emerging pathogens, stressing the need for a profound knowledge of unrevealed novel actors like the begomovirus-deltasatellite complexes.

P5.8 VPL

Sweet potato symptomless virus 1: First detection in Spain and generation of an infectious clone

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Sweet potato (*Ipomoea batatas*) is affected by many viral diseases. In 2017, complete genome sequences of sweet potato symptomless virus 1 (SPSMV-1) (genus *Mastrevirus*, family *Geminiviridae*) isolates from seven countries were reported, although a partial genome sequence had previously been identified by deep sequencing of of small RNAs in Peru. To assess the presence of this virus in Spain, sweet potato leaf samples collected in Málaga (southern continental Spain) and the Spanish Canary Islands of Tenerife and Gran Canaria were analyzed. SPSMV-1 was detected in samples from all the geographical areas studied, as well as in plants of several entries obtained from a germplasm collection supposed to be virus free. Sequence analysis of full-length genomes of isolates from Spain showed novel molecular features, i.e. a novel nonanucleotide in the intergenic region, TCTTATTAC, and a 24 nucleotide deletion in the V2 open reading frame. Additionally, an agroinfectious clone was developed and infectivity assays showed that the virus was able to asymptomatically infect *Nicotiana benthamiana*, *Ipomoea nil*, *I. setosa*, and sweet potato, confirming previous suggestions derived from observational studies. To our knowledge, this is the first report of the presence of SPSMV-1 in Spain and Europe and the first agroinfectious clone developed for this virus.

P6 VVE

P6.1 VVE

BLUETONGUE VIRUS NS3 PROTEIN INDUCES cGAS DEGRADATION AND INTERFERES WITH TYPE I INTERFERON INDUCTION

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Bluetongue virus (BTV) is a double-strand RNA virus transmitted by midges that belongs to the *Orbivirus* genus of the *Reoviridae* family. Upon viral infection, pathogen-associated molecular patterns (PAMPs) are detected by the host cell pattern recognition receptors (PPRs), inducing the synthesis of Type I interferon (IFN-I) and other proinflammatory cytokines that lead to the establishment of an antiviral state of infected and neighboring cells as part of the innate immune response. However, viruses have developed strategies to evade the innate antiviral response. More precisely, BTV has been shown to antagonize both INF-I induction and signaling. Although BTV antagonizes IFN signaling through the viral NS3 protein by reducing STAT1 phosphorylation and inducing autophagic degradation of STAT2, the mechanism by which BTV interferes with IFN-I induction has not been well described. Typically, RNA viruses are sensed by PRRs such as toll- like receptor 3 (TLR3), TLR7, RIG-I and MDA-5, which leads to IFN-I induction. Cyclic GMP- AMP synthase (cGAS) is a DNA sensing PRR that was canonically understood to respond to pathogen and host derived DNA. Nonetheless, cGAS activation has also been described during infection of RNA viruses from the *Flaviviridae* and *Coronaviridae* family due to the release of DNA to the cytosol during the infection. In the present study we describe that BTV NS3 protein interferes with IFN-I induction by interacting with cGAS, inducing its degradation. Additionally, NS3 ubiquitination appears to be required for the degradative process. Thus, this study identifies a new mechanism by which BTV antagonizes IFN-I induction through the DNA-sensor cGAS.

P6.2 VVE

Adenoviral delivery of soluble ovine CD70 or OX40L costimulatory molecules improves adaptive immune responses to a model antigen in sheep

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The tumour necrosis factor superfamily OX40L and CD70 and their receptors are costimulatory signalling axes critical for adequate T and B cell activation. In this work we inoculated groups of sheep with human recombinant adenovirus type 5 (Ad) expressing *Ovis aries* (*Oa*)OX40L or *Oa*CD70 or a control adenoviral vector to determine whether they could improve the immune response to the model antigen OVA. PBMCs and serum samples were obtained for analysis of the adaptive immune response to OVA at days 0, 15, 30 and 90 post-inoculation (pi). Recall responses to OVA were assessed at day 7 and 30 after the second antigen inoculation (pb) at day 90. Administra-

tion of these immunomodulatory molecules did not induce unspecific PBMC stimulation. *Oa*OX40L administration mainly increased TNF-α and IL-4 in PBMC at day 15pi concomitantly with a slight increase in antibody titer and the number of IFN-γ producing cells. We detected greater effects on adaptive immunity after *Oa*CD70 administration. Ad*Oa*CD70 inoculation improved antibody titers to OVA at days 30 and 90 pi, and increased anti-OVA-specific IgG-secreting B cell counts when compared to control. Moreover, higher IFN-γ production was detected on days 7 pi, 7 pb and 30 pb in PBMCs in this group. Phenotypic analysis of T cell activation showed an increase in effector $CD8^+$ T cells ($CD8^+$ CD62L- CD27-) at day 15 pi in AdOaCD70 group, concurrent with a decrease in early activated cells ($CD8^+$ CD62L- $CD27^+$). Moreover, recall anti-OVA $CD8^+$ T cell responses were increased at 7 pb. Ad*Oa*CD70 administration could therefore promote $CD8^+$ T cell effector differentiation and long-term activity. This work characterizes the *in vivo* adjuvant potential of *Oa*OX40L and *Oa*CD70 delivered by non-replicative adenovirus vectors on the adaptive immune response to a model antigen. We present data highlighting the potency of these molecules as veterinary vaccine adjuvant.

P6.3 VVE
Study of the immunogenicity against PPRV induced by different vaccination regime based on MVA and adenovirus recombinant expressing F and H PPRV proteins

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Peste des petits ruminants virus (PPRV) is the causative agent of Peste des petits ruminants (PPR), a highly contagious disease affecting goats and sheep. The live attenuated vaccines that are currently in use to control the disease have several drawbacks. Therefore, it is necessary to optimize the vaccination strategy to combat the disease. Adenoviral vector-based vaccines have shown great potential as vaccine candidates, while the use of recombinant modified Vaccinia Ankara virus (MVA) showed better immunization results when used as booster doses. F and H proteins from PPRV are highly immunogenic and have been used in different vaccination strategies to induce protective responses against the virus. In this work we studied the immunogenicity against PPRV induced by an initial immunization based on adenoviral vectors and a subsequent booster dose using MVA-based vectors expressing PPRV F and/or H proteins. We designed different antigenic expression regimes as vaccine strategies to be studied: a mono-antigenic regime consisting of [Ad-H+MVA-H] or [Ad-F+MVA-F]; or a bi-antigenic protein expression regime delivered either separately [(Ad-H+Ad-F) + (MVA-H+MVA-F)] or as a single fused protein boost [(Ad-H+Ad-F) + (MVA-H/F)]. Our data suggest that this Ad-MVA heterologous vaccination strategy is able to induce good humoral and cellular responses. The mono-antigen immunization with either H or F produces the highest titers of PPRV-specific IgGs. However, the antigenic combination, expressing the proteins separately, induces the highest neutralizing antibody titers. The mono-antigen strategy induced a better immune response against H, whereas immunization against F is improved with bi-antigenic vaccination. The current work presents a promising alternative vaccination strategy for PPRV.

P6.4 VVE
Study of the interaction of Peste des petits ruminants virus (PPRV) with goat dendritic cells

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Peste des petits ruminants virus (PPRV), a morbillivirus of the *Paramyxoviridae* family, is the causative agent of Peste des petit ruminants (PPR). PPR is an OIE (World Organisation for Animal Health) notifiable disease affecting sheep and goats, with a major economic impact, especially in developing countries. PPRV infection induces significant

immunosuppression, which can cause animal death due to opportunistic infections. In general, the disease is more severe in goats than in sheep, but the mechanisms that explain this phenomenon are still unknown. Dendritic cells (DCs) are the main antigen presenting cells, with a relevant role as a link between the innate and acquired immune response. Other studies have described the interaction of PPRV with ovine DCs, with a massive infection of DCs, which inhibits their function. In order to determine the possible role of DCs in the differential PPRV pathogenicity described in sheep and goats, we deep on the effects of PPRV infection in goat monocyte-derived DCs (MoDC). In this study we were able to establish a protocol for the obtention of goat MoDC expressing the classical MoDC markers (MHCI, MHCII, CD1, CD1w2, CD80, CD86, CD40, CD209, CD11b, CD11c and CD172a). We are able to detect PPRV infection in goat MoDCs and moreover, this infection induced a partial maturation of MoDCs, not as potent as that of treatment with the TLR7/8 agonist R848. The capacity of goat MoDC to present antigens to T lymphocytes and the phagocytic capacity of MoDC were also studied. These results contribute to understand the pathogenicity of PPRV in goats.

P6.5 VVE
Molecular determinants of ASFV hemadsorption

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African swine fever virus (ASFV) is a dsDNA virus causing African swine fever (ASF), a devastating disease affecting domestic pigs and wild boards. ASFV is currently spreading through Asia, Oceania, Europe and Dominican Republic threatening the pig industry worldwide since no fully safe commercial vaccine is available. Differences in virulence among ASFV strains vary from 100% of mortality in acute infection, to low or no mortality induced by attenuated strains. One of the main features associated with virulence is the hemadsorption (HAD) which induces “rosette” formation, representing erythrocytes bound to infected cells. However, the molecular mechanisms connecting HAD and virulence are still unknown. Regarding to the viral factors involved, ASFV EP153R and CD2v proteins (EP402R) were reported to be responsible of HAD. Here, we demonstrate by ectopic expression and ASFV mutants that HAD relied exclusively on CD2v, and more specifically, on its Nt domain, whereas no role for EP153R has been found. We show here that within the CD2-Nt domain, the predicted signal peptide is essential for HAD function. On the other hand, CD2v-Nt is highly glycosylated, and inhibition of glycosylation prevented HAD. In addition, we identified several key residues within the multiple predicted glycosylation sites within CD2v-Nt. Interestingly, CD2v-Nt mutants lacking these residues did not induce hemadsorption, although the molecule still presented a highly glycosylation pattern. Finally, we investigated the CD2v-Nt status of the attenuated, non-HAD NH/P68 strain. The NH/P68 CD2v-Nt was expressed and glycosylated and key residues for HAD were identified. However, no HAD was observed, in agreement to what we observed during NH/P68 infection. A role of the signal peptide, which is present in CD2v from virulent strains, but absent in NH/P68, is currently under study.

P6.6 VVE
Molecular epidemiology of swine influenza in Spain

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Swine Influenza surveillance worldwide is mostly carried out in classical industrial intensive swine herds. Spain is the first swine producer and exporter in Europe accounting for more than 30M pigs in 2019, 10% of which belong to

Iberian pig breed that, in high proportion, are bred in a free-range system. Wild boar is other member of *Sus scrofa* species which is susceptible to swine influenza viruses. In this study, we aim to investigate the epidemiology of influenza infection and the characteristics of circulating influenza viruses in industrial farms of white pigs, in free-ranging Iberian pigs and in wild boars. Overall, a higher number of distinct genotypes (n = 16) were observed in swine in Spain than in any other European country over the period 2009-2021. Four subtypes were detected (H1N1, H1N2, H3N1, and H3N2). The virus population in Spain was dominated by avian-like Eurasian H1 viruses, which tend to be dominant across European swine populations. But lower-frequency HA variants were also detected, including H1 viruses (human seasonal H1-like and human-pandemic H1-like), as well as two human-origin H3 viruses. Most notably, 6 reassortant H3N1 viruses emerged in Spanish white pigs and wild boars that have avian-like Eurasian NA and internal gene segments but a novel H3 of human origin. As a conclusion, the many reassortment events identified in the virus isolates and the variety of co-circulating genotypes may pose a potential public health risk. This work was supported by the Center for Research on Influenza Pathogenesis and Transmission (CRIPT), a National Institute of Allergy and Infectious Diseases (NIAID) Center of Excellence for Influenza Research and Response (CEIRR).

P6.7 VVE

Identification of a potential African swine fever virus entry fusion complex based on sequence alignments against poxviral Vaccinia virus

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Poxviruses are large, enveloped, double-stranded DNA viruses that infect arthropods, humans, and other vertebrates. They belong to the Nucleocytoplasmic Large DNA Viruses (NCLDV) family and share common phylogeny and replication pathways. Poxviruses, such as vaccinia virus (VACV) were considered unique in having an elaborate entry-fusion complex (EFC) that is comprised of 11 highly conserved proteins integrated in the membrane of mature virions. Its architecture is being increasingly described elucidating diverse connections between EFC proteins through new methodological techniques. African swine fever virus (ASFV) also belongs to this family of complex nucleocytoplasmic large DNA virus (NCLDV). This virus causes an acute, highly contagious, and deadly infectious disease of *suids* that is causing massive financial losses to the world farming industry because its current pandemic extension. Very little is known about the internalization of ASFV in the host cell despite its immediate necessity. To date, there are still no effective commercial vaccines or antiviral drugs for the prevention of ASF. Here we describe an approach to the elucidation of a potential ASFV entry fusion complex, that resulted in finding ten candidate proteins with possible structural similarities with the VACV EFC that could achieve crucial functions in the virus infection cycle due to the similarities between both virus families.

P6.8 VVE

Phylogenetic and Genomic Study of a New Dependoparvovirus and an Adenovirus in cloacal swabs from Monk Parakeet (Myiopsitta monachus) from urban areas of Madrid

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Monk parakeet (*Myiopsitta monachus*) is a bird belonging to the Psittacidae family that is originally distributed throughout South America and has been introduced in many countries worldwide. It has been estimated that the

population of *M. monachus* in Madrid increased by 33% between 2016 and 2019 and there is a growing concern to understand the transmission of pathogens to native birds or humans by this invasive species. Metagenomics approach by deep sequencing is used to discover new viruses. We are especially interested on those which have zoonotic potential. In this study, we investigated by metagenomics the virome of cloacal swabs of 28 monk parakeets captured in different urban areas in Madrid and characterized the complete genome of a new adeno-associated dependoparvovirus and the fragments of an adenovirus. Posterior procedures included phylogenetic inferences and genomic characterization. The complete genome of the dependoparvovirus consists of a ssDNA of 5.4 kb, composed of two ORFs. The first ORF encodes a nonstructural protein (NS1) and the second ORF encodes a structural protein (VP1). These genes had a length of 2175 and 1962 nucleotides, respectively. Identity analysis reveals that the amino acid sequences of NS1 and VP1 shared 58% and 66%, respectively, with other dependoparvovirus sequences, suggesting that it could be a new species. The phylogenetic study situated this new dependoparvovirus with another unpublished dependoparvovirus sequence from a Chinese psittacid. Likewise, we found fragments of the genome of an adenovirus encoding the pVIII, fiber-1 and fiber-2 genes. Amino acid sequence identity of these proteins ranged between 40-70% with other adenovirus sequences isolated from Psittacidae in a metagenomics study in Brazil and an outbreak associated to avian chlamydiosis and human psittacosis in China. Besides, the phylogenetic exploration suggests that this adenovirus is within the genus *Atadenovirus.* The identification of a new dependoparvovirus and adenovirus distantly related to other currently known viruses indicate the poor understanding of the avian virome and emphasizes the need for further elucidation. Furthermore, this is the first scientific documentation of the presence of dependoparvovirus in urban monk parakeets. Funded by PID2020-114956GB-100

P6.9 VVE
SEROLOGICAL AND MOLECULAR DETECTION OF RHDV IN WILD RACOON SAMPLES

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Rabbit hemorrhagic disease virus (RHDV) which emerged in the 1980s is the prototype species of the Lagovirus genus within the Caliciviridae family. The disease caused by RHDV is highly contagious, usually fatal in adult rabbits and is a major threat to domestic and wild European rabbit (*Oryctolagus cuniculus*). In 2010 a new RHDV genotype named *Lagovirus europaeus* / GI.2 (RHDV2), with a distinctive pathogenic profile, emerged in France from an unknown origin. This virus rapidly spread through the rabbit range worldwide, replacing the former circulating RHDV classical strains, GI.1. Several recombination events have been reported involving GI.2 genotype, with pathogenic and benign lagoviruses, generating virus diversity which might be beneficial for the adaptation to new hosts and environments. Indeed, the lagovirus GI.2 exhibits a distinguishing ability to cause disease in both, rabbits and certain hare species (*L. europaeus, L. timidus, L. capensis and L.mediterraneus*). Furthermore, RHDV2-specific antibodies and viral genomic RNA have been detected in sympatric wild animals such as rodents, badgers or wild Tasmanian devil. The role of these species as reservoir host for RHDV2 is currently unknown. In regard of the potential relevance of new RHDV hosts for the epidemiology of the disease, in this study we analysed the presence of antibodies against RHDV in serum samples collected between 2017 and 2022 from feral racoons in the region of Madrid. An initial panel of 70 samples was collected and tested by ELISA against RHDV. We performed ELISAs based on the use of recombinant virus-like particles (VLPs) derived from genotypes GI.1 and GI.2 as antigens, previously validated in sero-surveys among wild rabbit populations. More than 60% of the sera tested were positive to RHDV and around 30% of samples showed high antibody titers (ODs> 2). Antibodies were detected against both genotypes but the antibody titers were higher against GI.2 than GI.1.This result suggests that Lagovirus GI.2 was the responsible of the antibody response. In order to confirm the RHDV GI.2-infection of feral racoons, we tested faecal samples from 14 animals by RT-PCR, using primers amplifying a fragment of 325 bp of the capsid protein VP60. 9 out 14 samples were positive, exhibiting amplicons closely matching the expected size. Further molecular and antigenic analyses are ongoing to confirm these results. This work was supported by Comunidad de Madrid fund (P2018/BAA-4370 PLATESA

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P6.10 VVE
Characterization of a novel Alphaherpesvirus (TuHV-1) in common blackbird (Turdus merula) from Madrid.

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Herpesvirus form a large group of viruses that affect a variety of hosts. Some of the avian herpesviruses, included in the subfamily *Alphaherpesvirinae*, have great economic and health importance. Such is the case of infectious laryngotracheitis virus (*Gallid alphaherpesvirus 1*, GaHV-1) in poultry, or Pacheco's disease virus (*Psittacid alphaherpesvirus 1*, PsHV-1) that affects psittacines. GaHV-1 causes a highly contagious acute respiratory syndrome while PsHV-1 causes necrosis of the intestines, crop, pancreas, and liver. In previous studies, we found a 417 bp fragment of a novel herpesvirus in cloacal swabs from common blackbirds which had an identity to the terminase gene of PsHV-1 of 91%, and of GaHV-1 of 70%. Through phylogenetic analysis, the DNA fragment was assigned to the *Alphaherpesvirinae* subfamily within the genus *Iltovirus*. Using specific primers for this gene the virus was detected by PCR in a cloacal swab of a wild adult male *Turdus merula* captured in Madrid which showed signs of paralysis prior to death, dying shortly after. No external lesions were observed. After the necropsy, several internal organs resulted positive to PCR, including the kidney, the duodenum, and the cloaca. Sequencing of the PCR amplicons confirmed herpesviral presence, suggesting a systemic infection, similar to what happens with PsHV-1. The pathological analysis has been inconclusive for the moment. Subsequently, the isolation of the virus in DF-1, a cell line of avian fibroblasts, was carried out. The study was extended to more specimens to find out more about this new virus that may threaten the population of common blackbirds. Funded by PID2020-114956GB-100.

P6.11 VVE
Evaluation of Cytotoxicity Induction by a Recombinant Newcastle Disease Virus Expressing Human IL-12β in Human Prostate Cancer Cells In Vitro

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Introduction. The Newcastle disease virus (NDV) is an avian avulavirus that has been demonstrated to possess oncolytic activity against cancer cells, due to replicating specifically in tumor cells and inducing toxic effects leading to apoptosis. To elicit a greater anti-cancer immune response, it is believed that the incorporation of immunostimulatory genes such as Interleukin-12 (IL12) could enhance its oncolytic properties. Objectives. This study aimed to explore the cytotoxicity effects of recombinant rNDV (rFLCF5nt) that expresses interleukin-12 chain β (rFLCF5nt-IL12β) in DU145 prostate cancer cells in vitro. Methods. We developed a new recombinant NDV (rFLCF5nt), with five mutations into the F gene to enhance replication without proteases exogenous. To enhance its properties oncolytic, the gene IL12β was inserted between Phosphoprotein (P) and M (Matrix) genes to generate a new recombinant

(rFLCF5nt-IL12β). The expression of IL12β was determined in cells infected with rFLCF5nt-IL12β by immunofluorescence assay (IFA) and Western blotting. The cytotoxicity effect of rFLCF5nt-IL12β against cancer cell line DU145 was determined by MTS assay. The apoptosis was measured with Annexin V FITC by flow cytometer analysis. Results. The rFLCF5nt-IL12β, induce apoptosis of DU145 cells in vitro as revelated in the Annexin V FITC analysis and significantly inhibited the growth of DU145 cells as compared to cells not treated. Conclusions. These results suggest that the expression of IL12β could be an ideal approach to enhance the antitumor ability and cytotoxicity of NDV in DU145 cells. Funding. This research was co-financed by CONCYTEC-FONDECYT Peru grant under contract N° 143-2017-FONDECYT.

P7 VPE

P7.1 VPE

Chemically-free extracted red seaweed polysaccharides show antiviral activity against viral haemorrhagic septicaemia virus (VHSV)

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The rhabdovirus viral haemorrhagic septicaemia virus (VHSV) is the causative agent of viral haemorrhagic septicaemia (VHS), the most important viral disease affecting European rainbow trout farming. VHSV genome encodes five structural proteins and among them a transmembrane glycoprotein (G) is responsible for attachment to the cell membrane and entry into the cell. At present no vaccines are commercially available for VHS prevention and therefore effective antivirals are necessary to control the disease. In this study, we have assessed the ant-VHSV activity of different polysaccharide extracts, including carrageenan and carrageenan oligomers, obtained from the red seaweed *Chondrus crispus*. Carrageenans and other sulphated marine polysaccharides have demonstrated to have antiviral activity against enveloped human and veterinary pathogenic viruses as Herpes simplex virus, Dengue virus or Rabies virus. Because polysaccharide extraction conditions may affect their properties, a chemical-free extraction process based only on pressurized hot water was used (heating up to temperatures in the range 120-200 ºC during non-isothermal operation). After cooling, the suspension was separated by filtration and the polysaccharides were precipitated with ethanol (1.5:1, v/w ratio) and freeze dried. In an initial test, four samples (AH120, 140, 160 and 200) showed a significant percentage of VHSV inhibition and they were chosen for further analysis. A time-course study was performed to assess the inhibitory effect at different steps of the viral cycle. Polysaccharide fractions were added to Epithelioma papulosum cyprini (EPC) cells simultaneously with VHSV or at different time intervals post virus inoculation. In addition, to study the putative prophylactic properties of the polysaccharide fractions pre-treatment of EPC cells was assayed. In all experiments, treated and untreated VHSV was quantified by plaque formation and TCID50 assay as well as by RT-qPCR. No significant protective effect on EPC cell monolayers against VHSV infection was observed. However, a high inhibitory effect was shown when either high molecular weight carrageenans (AH120) or low molecular weight carrageenans (AH200) were added to cells together with the virus, indicating that red seaweed polysaccharides can block viral adsorption to EPC cells probably by binding to the G protein.

P7.2 VPE

Hepcidin and dicentracin increase the viral clearance via autophagy in European sea bass

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Autophagy is an intracellular degradation process playing an important role in homeostasis. Viral infections can modulate autophagy and, when induced, can be host-beneficial. To date evidence points to the interest of investigating strategies that upregulate autophagy as fish virus preventive treatments. Nodavirus (NNV) is a great threat for Mediterranean aquaculture as it produces severe natural outbreaks leading to great economic losses to the industry. Regarding autophagy, the NNV capsid protein is able to induce autophagic processes. Antimicrobial peptides (AMPs) are short proteins with lytic activity against a broad range of pathogens, and exert great regulatory effects on the immune response. Recently, in insects, a positive relationship between the AMP and autophagy-related genes has been successfully established. Thus, the objective of this work was to analyze whether the administration of synthetic AMPs might regulate the NNV-induced expression of autophagy-related genes. To accomplish our objective, hepcidin and dicentracin peptides were intramuscularly administered to European sea bass juveniles and a day later infected with NNV. Brain, the NNV target tissue, and muscle, the site of infection, were removed in order to ascertain NNV replication in both tissues as well as analyze the transcription levels of autophagy-related genes. Mortality rates were recorded daily. Our data show that NNV replicates in both studied tissues and AMPs administration resulted in lower mortality rates. The transcription profile observed in brain suggests that the treatment with AMPs prior to NNV infection induces autophagy in the main target site of the virus. Therefore, the autophagy induced by AMPs could be an effective mode of viral clearance and could contribute to the increase of survival during NNV infection. Funded by MCIN/AEI 10.13039/501100011033 (grants RTI2018-096625-B-C33, PID2019-105522GB-100 and PRE2020-093771 to L.C) and "Fundación Séneca" (grant 19883/GERM/15).

P7.3 VPE
The impact of welfare in the susceptibility of shi drum to betanodavirus infection

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Spain leads the aquaculture production in the European Union; however the appearance of new competitors in the Mediterranean area resulted in lower prices for most cultured species. In this framework shi drum (*Umbrina cirrosa*) has attracted attention due to its good growth rates and great adaptability to culture conditions together with a great flesh quality. An inadequate welfare state results in stress, poor health and increased severity of infectious diseases, including those caused by viral pathogens. The betanodavirus (NNV) is the causative agent of viral encephalopathy and retinopathy and infects more than 170 fish species. Nowadays, NNV is a great concern due to its high prevalence in the Mediterranean basin. In addition, several kind of physiological and induced stress conditions have been related with increased mortalities and NNV spread. The diversification of species in aquaculture requires the continuous study of the physiological process of stress to determine appropriate animal welfare parameters for each species together with the study of the susceptibility to pathogens. Shi drum is susceptible to the four genotypes of NNV, although with different clinical signs and replication rates. To increase the knowledge on the biological aspects of fish welfare in general and of shi drum in particular, we have studied different rearing densities and their impact on the response to stress and to NNV infection. Our data show an increase in stress behavior in the highest density that in turn affects the physiological performance of fish. This study was financed by the project OWI TWO (Operational welfare indicators in two aquaculture species) cofunded by the European Maritime and Fisheries Fund (EMFF) and IEO-CSIC, and by MCIN/AEI 10.13039/501100011033 (grants RTI2018-096625-B-C33 and PID2019-105522GB-100) and "Fundación Séneca" (grant 19883/GERM/15).

P7.4 VPE
Epidemiological survey of NNV prevalence in wild fish populations in the Atlantic Ocean

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Viral Encephalopathy and Retinopathy (VER) is a serious disease affecting marine farmed and wild fish. It is caused by the nervous necrosis virus (NNV), a positive-sense and bisegmented RNA virus. Segment 1 (RNA1), codes for the RNA polymerase RNA-dependent (RpRd) and contains the region responsible for temperature adaptation, while RNA2 encodes the capsid protein and holds the T4 region, used for the current classification into four genotypes: Barfin flounder-, Redspotted grouper-, Striped jack-, and Tiger puffer nervous necrosis virus (BFNNV, RGNNV, SJNNV, and TPNNV, respectively). Moreover, VER outbreaks in Southern Europe led to the detection of genetic reassortment between RGNNV and SJNNV (RGNNV/SJNNV and SJNNV/RGNNV). BFNNV is commonly detected in cold water fish from Japanese and Northern European and American waters, SJNNV has been identified in fish from Japan and the Iberian Peninsula at mild temperatures, RGNNV is frequently reported in Mediterranean, Asian, and Australian waters, around 23-30ºC, and TPNNV was only found on one occasion in Japan. However, NNV prevalence could be increased due to global warming, highlighting the importance of conducting frequent epidemiological surveys. Most of these studies have been performed in the Mediterranean area and have led to the identification of RGNNV-type strains mainly. This prompted us to assess NNV prevalence in wild fish caught in the Galician Atlantic coast. A total of 1277 fish were analyzed by RT-qPCR and 1,72 % tested positive for NNV, including two fish species in which the pathogen had not been detected to date. The reassortant RGNNV/SJNNV was detected in 55 % of these individuals, while the remaining 45 % contained SJNNV type genome. Moreover, 4 reassortant strains were isolated in cell culture: three from pilchard and one from mackerel. These isolates carried several amino acid substitutions in both the RdRp and capsid protein when compared with the reference parental strains, affecting regions linked to temperature adaptation, host specificity and virulence. Whereas some of the changes are shared by all the reassortants, others seem to be exclusive of these new isolates. The potential role of these amino acids in the colonization of new hosts and environments is yet to be determined.

P7.5 VPE
NNV horizontal transmission to sea bass larvae through the live food

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The Nervous Necrosis Virus (NNV), the causal agent of Viral Encephalopathy and Retinopathy (VER), is an important aquatic pathogen responsible for major losses in marine fish hatcheries worldwide. NNV spreading can occur vertically, and horizontally, through the water column or by contact between infected or asymptomatic carriers with susceptible healthy animals. In addition, marine invertebrates, including rotifers and the crustacean *Artemia* sp used as live food in marine hatcheries, have been demonstrated to act as NNV reservoirs. Therefore, this study was aimed to investigate whether rotifers, can transmit the NNV to sea bass (*Dicentrarchus labrax*) larvae, following the oral route. To this end, larvae were fed on rotifers previously inoculated with NNV. For comparative purposes, a positive control group consisting of larvae water-borne infected with the same strain and a negative control group were also set up. NNV replication kinetics was monitored in larvae sampled up to the end of the experiment, by RT-qPCR and titration in striped snakehead (SSN-1) cell line, and antigen localization was also tested by immunohistochemistry staining (IHC). Data from this challenge revealed that a single dose of rotifers previously exposed to NNV is sufficient to induce clinical signs and 100% cumulative mortality in sea bass larvae, in a similar way to

the bath-challenge, demonstrating rotifers role as potential NNV mechanical vectors. However, the course of the oral infection was delayed about 10 days with regard the bath-challenged larvae, probably because only a few larvae were infected in the first days, as evidenced by IHC and viral genome quantification. In addition, we have also demonstrated that rotifers can internalise NNV particles due to their filtering nature and maintain virus viability, since viral particles were detected within rotifers' body by immunofluorescence assay (IFA), IHC and cell culture. However, viral quantification data suggested that rotifers are not permissive to NNV replication, and that the virus is quickly cleared. To conclude, this work highlights the importance of establishing strict routine controls on live food, to prevent NNV entry into a hatchery. This research was funded by the European Union's Horizon 2020 and grant agreement Nº 731014 (VetBioNet project).

P7.6 VPE

Betanodavirus reassortants replicate and produce mortality in gilthead seabream larvae

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Among the pathogens that most affect Mediterranean aquaculture, betanodavirus (NNV), a non-enveloped icosahedral RNA virus with a genome composed of two single-stranded positive-sense RNA segments known as RNA1 and RNA2, stands out. Apart from the traditional NNV genotypes, there are two reassortant genotypes called RGNNV/SJNNV and SJNNV/RGNNV, due to the origin of the RNA genome. It has been confirmed that gilthead seabream, a species resistant to traditional NNV genotypes, is susceptible to developing NNV disease in larval stages when infected by the RGNNV/SJNNV genotype, bringing a new focus to the prevention of infection in aquaculture farms. Therefore, the main objective of this research was to examine the susceptibility of 37 and 86 days post-hatching (dph) seabream larvae against RGNNV/SJNNV an SJNNV/RGNNV reassortants and the infective capacity of both viruses. Our results confirmed that both genotypes induced mortality in seabream larvae of both ages, being higher for RGNNV/SJNNV genotype and in 36 dph larvae. For both ages, the mortality rates were related to the replicative capacity of the virus. In 36 dph larvae, the high mortality can be explained by the high mRNA levels of viral genes from the beginning of the infection, increasing exponentially until the end of the infection, being always higher for RGNNV/SJNNV. In addition, we were able to recover viral particles for both genotypes, again higher for RGNNV/SJNNV. In contrast, the low, but statistically significant, mortality rate for 86 dph larvae might be due to the low replicative capacity of both viruses at this age. This issue together with a low expression level of viral genes and the failure in recovering infective particles seems to indicate that, even when the virus is initially competent to infect cells, its growth is dismissed or even blocked by different immune mechanisms and, therefore, the virus loses its pathogenic capacity. Further studies are needed to understand the host-viral interactions for NNV reassortants since they are threatening marine larviculture. Funded by Ministerio de Ciencia e Innovación-Agencia Estatal de Investigación [MCIN/AEI/10.13039/501100011033, grant PID2019-105522GB-I00 to AC], Ministerio de Economía y Competitividad and FEDER [grant RTI2018-096625-B-C33 to ECP and MA] and Fundación Séneca, Grupo de Excelencia de la Región de Murcia [19883/GERM/15].

P7.7 VPE

Immune gene expression in gilthead seabream after nervous necrosis virus (NNV) challenge

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Viral nervous necrosis (VNN) is a disease that affects farmed fish worldwide. Its etiologic agent is the nervous necrosis virus (NNV), genus *Betanodavirus*, family *Nodaviridae*. NNV are small and non-enveloped viruses with a genome consisting of two molecules of positive-sense single-stranded RNA, RNA1 and RNA2, which encode the RNA-dependent RNA polymerase and the capsid protein, respectively. The betanodaviruses have been classified into four species: *Striped jack nervous necrosis virus* (SJNNV), *Tiger puffer nervous necrosis virus* (TPNNV), *Red-spotted grouper nervous necrosis virus* (RGNNV), and *Barfin flounder nervous necrosis virus* (BFNNV). In Southern Europe, natural reassortants between RGNNV and SJNNV have been isolated from Senegalese sole (*Solea senegalensis*), gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) associated to VNN outbreaks. Immune response against betanodavirus infections has been poorly studied in gilthead seabream. In this study, fish were challenged by intramuscular (im) injection or by immersion, using a reassortant strain containing RGNNV-type RNA1 and SJNNV-type RNA2 segments. Head kidney and brain samples were collected at 24, 48 and 72 h post-challenge (pc) for the injection experiment, while in the bath challenge sampling was performed at 48 and 72 h pc. The immunogen expression analysis was carried out using the platform OpenArray®. In the im-injected fish, 21 differentially expressed genes (DEGs) were identified in head kidney samples at 24 h pc, whereas a lower immune response was detected at 48 and 72 h pc (11 and 9 DEGs, respectively). In brain samples, a delayed response was observed, with 32 DEGs recorded at 72 h pc. Regarding the bath-challenged fish, fewer immunogenes were differentially expressed although all of them were up-regulated. This research was funded by the Ministerio de Ciencia, Innovación y Universidades (MCIUI) and FEDER under Grant RTI2018-094687-B.

P7.8 VPE

Booster vaccination against Nervous necrosis virus (NNV) improves immunity and protection in Senegalese sole

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Nervous necrosis virus (NNV), the causative agent of viral encephalopathy and retinopathy (VER), is one of the most widespread fish viruses. NNV is a small non-enveloped virus member of the genus *Betanodavirus* within the family *Nodaviridae*. Its genome is composed of two single stranded RNA segments named RNA1, which codes for the RNA-dependent RNA polymerase, and RNA2, encoding the capsid protein. Based on a variable sequence of RNA2 betanodaviruses are classified into four genotypes: barfin flounder-, red spotted grouper-, stripped jack- and tiger puffer nervous necrosis virus (BFNNV, RGNNV, SJNNV and TPNNV, respectively). However, the emergence in Southern Europe of natural reassortants between the RGNNV and SJNNV genotypes has been reported as a threat for Senegalese sole and gilthead seabream farming. Viral transmission occurs both horizontally and vertically and the most affected growth stages are larvae and early juveniles with mortality rates up to 100%. Different strategies

have been implemented to avoid NNV infection in young fish including selection of NNV-free broodstocks and disinfection of fertilised eggs, but vaccination is the best prevention tool. Our group has previously reported an increased immune response and survival rate in juvenile sole after intraperitoneal injection with an inactivated vaccine, prepared with a reassortant strain and binary ethylenimine (BEI). In the present study, a re-immunization programme including a booster injection at 30 or 45 days post vaccination (booster I and II, respectively) was assessed. Our results demonstrate that booster I improves vaccination performance because a significant increase in survival (relative percent survival 77 vs 55) was observed when compared with prime vaccination. In addition, a clear decrease in the viral replication in brain was recorded in the re-immunized group. Finally, although during the immune induction period almost no differences were observed between prime vaccinated and re-immunized fish, the immune response to viral infection after challenge was clearly enhanced in the re-immunized group which showed high antibody production and overexpression of immune-related genes. This research was funded by the Ministerio de Ciencia, Innovación y Universidades (MCIUI), the Agencia Estatal de Investigación (AEI) and FEDER under Grant RTI2018-094687-B

P7.9 VPE
Preliminary study of an NNV attenuated vaccine

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The Nervous Necrosis Virus (NNV) is a non-enveloped aquatic virus, whose genome is composed by two molecules of ssRNA, RNA1 and RNA2, that code for the viral polymerase and the capsid protein, respectively. The NNV is the etiological agent of Viral Encephalopathy and Retinopathy (VER), a neurological disease, which affects mainly larvae and juveniles of different fish species farmed in the Mediterranean area such as sea bass, gilthead sea bream and sole. During the infection course, NNV triggers the vacuolization and necrosis of the nervous tissues, especially the central nervous system and the retina, which causes abnormal swimming behavior and finally results in the death of the individual. Vaccination seems to be a promising strategy to prevent VER outbreaks although only two formaline- inactivated vaccines are authorized in Mediterranean aquaculture for sea bass. Therefore, the aim of this study was to design an NNV attenuated strain that could be used for sole vaccination. The attenuated strain, known as mut93/08-12, harbors point mutations in 3'-NCR regions of both RNA1 and RNA2 molecules of the wild type (*wt*) strain. Infection trials showed that mortality in fish challenged with the mutant strain was significantly lower than that of the *wt* (31 % *vs* 90 %). Similar replication kinetics was observed in fish inoculated with both strains, although NNV load was lower in fish challenged with mut93/08-12 strain. Furthermore, we have also quantified the expression of immune related genes in fish brain and head kidney. Both strains produced a significant induction on the expression of different immune-related genes including *mx, mhc ii, isg15* and *rtp3*, in brain (around 15-fold change) at 7 days post infection, while up-regulation in head kidney was more subtle. Vaccination assays are in progress to analyze the protective response elicited by the attenuated strain in sole juveniles. This work was supported by the Ministerio de Ciencia, Innovación y Universidades (MCIUI), the Agencia Estatal de Investigación (AEI) and FEDER RTI2018-094687-B-C21.

P7.10 VPE
Inhibition of the infection of several fish viruses of distinct families with probable serine- and low-pH-dependent entry by NK-lysin

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NK-lysins are a family of antimicrobial peptides conserved in all vertebrate groups that stand out for their antitumor and broad-spectrum antimicrobial activity. The tertiary structure of these peptides shows helical regions stabilized by disulfide bridges that exhibit a lysine-rich facet responsible for its interaction with anionic lipid membranes. In our previous papers, the peptide corresponding to such region in the turbot (*Scophthalmus maximus*) NK-lysin ortholog (NkI_{71-100}) showed membrane-disrupting activity against the parasite *Philasterides dicentrarchi*, as well as inhibition of the pH-dependent fusion step of the entry phase of the spring viremia of carp virus (SVCV). Biophysical data showed that NkI_{71-100} preferentially interacts with anionic phosphatidylserine lipid bilayers at low pH conditions. In the present study, the susceptibility of a diverse range of fish viruses to NkI_{71-100} has been determined *in vitro*. In particular, SVCV and tilapia lake virus (TiLV) showed high sensitivity to the NkI_{71-100} treatment, while the inhibitory effect on cyprinid herpesvirus 3 (CyHV-3) was only present at higher concentrations and common carp paramyxovirus (CCPV) infectivity was not affected by the peptide. In conclusion, the fusion step of the viral entry phase is postulated as a promising target for the treatment of some viruses with high economic impact on aquaculture by means of molecules such as NK-lysins. Funding: Grant RTI2018-101969-J-I00 funded by MCIN/AEI/10.13039/501100011033 and by "ERDF *A way of making Europe*"; Project *MetDisFish* funded by the Ministry of Agriculture, Fisheries and Food (MAPA) and European Maritime, Fisheries and Aquaculture Fund (EMFAF); project 426513195 funded by the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG).

P7.11 VPE
Optimization of droplet digital pcr assays for detection and quantification of the viral haemorrhagic septicaemia virus (VHSV)

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The viral haemorrhagic septicaemia virus (VHSV) is the causative agent of an important disease in a wide range of fish species, including both fresh and marine environments. Officially, its diagnosis relies on the isolation of the virus in cell culture and its identification by serological procedures or by means of polymerase chain reaction (PCR). In the last decade, the real time quantitative PCR (rt-qPCR) has replaced traditional PCR in the diagnosis of this virus due to the advantages of the former, and because of its capacity of quantification, which provides additional relevant information. Droplet digital PCR (ddPCR) is a novel, sensitive, accurate methodology that combines microfluidics technology with TaqMan-based PCR, and provides absolute quantification not requiring a standard curve or normalisation to reference genes. In addition, this technology also improves the detection capacity, reaching levels of sensitivity unthinkable with qPCR. In the present study, we have developed, optimized and validated a ddPCR procedure for detection and quantification of VHSV using the QX200™ droplet digital PCR system, and a comparative analysis with qPCR has been carried out. The procedure has been tested against crude virus and with a plasmid with an insert of 1049 nc corresponding to the nucleoprotein viral gene (strain Fr0771). The level of detection (LOD) and the level of quantification (LOQ), the dynamic range, repeatability and reproducibility have been evaluated. In addition, the specificity was tested against isolates from the 4 genotypes. The LOD was determined as 1.25 copies/reaction (cps/rct) with plasmid and 1.84 cps/rct with crude virus, corresponding to 0.2cps/rct and 1.4 cps/rct, respectively, as calculated based on the nucleic acid concentration. The LOQ was in all cases 1 log higher, with a dynamic range of 5 Log10 (R2 = 0.999 with plasmid, and 0.997 with crude virus). Comparison with the qPCR procedure showed that ddPCR clearly improves the capability of diagnosis and quantification of this virus.

P7.12 VPE

Droplet digital PCR assay for detection and quantification of the viral nervous necrosis virus (VNNV)

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The nervous necrosis virus (NNV) is a threat to fish aquaculture worldwide, especially in Mediterranean countries. Fast and accurate diagnosis and viral quantification are essential to predict the level of risk of new viral detections in field samples. Although, at present cell culture isolation followed by immunostaining or molecular identification is still considered to be the gold standard for diagnostic purposes by the World Organization for Animal Health, reverse transcription real-time polymerase chain reaction and traditional polymerase chain reaction are the most used methods by diagnostic laboratories due to their speed, sensitivity, reproducibility, and quantification capacities. Droplet digital PCR (ddPCR) is a novel, sensitive, accurate methodology that combines microfluidics technology with TaqMan-based PCR and, provides absolute quantification performed directly on each sample, without external calibration or normalisation to reference genes. In addition, it may be less sensitive to inhibition and suboptimal PCR efficiency due to the end-point detection approach. The aim of this study was to develop, optimize, and validate a ddPCR procedure for detection and quantification of VNNV using the QX200™ droplet digital PCR system. In addition, a comparative analysis with RT-qPCR has been carried out. RGNVV and SJNVV crude virus and plasmid with an insert corresponding to RNA2 from SJNNV and RGVNV genotype were used. The level of detection (LOD) and the level of quantification (LOQ), the dynamic range, specificity and repeatability and reproducibility were evaluated. The LOD was determined as 0.97 and 2.05 copies/reaction (cps/rct) with plasmid RGNNV and SJNNV inserts, respectively. With crude virus, LOD were 0.8 cps/rct with RGNNV and 16.2 cps/rct with RGNNV. The LOQ was in all cases 1 log higher, with a dynamic range of 5 Log10. Comparison of the results with TaqMan real-time PCR (qPCR) using the same primers and probes showed an improvement between 1-2 Log_{10} respect qPCR.

P8 REG

P8.1 REG

Analysis of essential oil compounds as an alternative treatment against cytomegalovirus infection

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CMV is a high prevalent virus that causes severe illness and death in individuals with an immature or dysfunctional immune system. Although the antiviral drugs to treat CMV infection have been improved during the last years, there are still some issues associated with the use of the available antivirals such as undesirable side effects and selection of resistance mutations. The aim of this study was to analyze whether essential oil compounds (EOCs) has antiviral activity as new therapeutic alternatives against CMV infection. Thymol, eugenol and vanillin either free or immobilised onto amorphous silica particles were used. In general, functionalised silica particles, especially those functionalised with vanillin, had less associated cytotoxicity compared with free compounds in cultured ARPE-19 and MRC-5 cells. To analyse the inhibitory effect of EOCs, increasing concentrations of the compounds were added to cells before and after inoculation with the GFP-AD169 CMV strain. Our results suggest that both free and immobilised vanillin have a protective effect on ARPE-19 and MRC-5 cells, preventing infection, while free thymol was more effective against the free virion, likely preventing virus attachment or entry.

P8.2 REG
Role of RAB GTPases in HAV egress from hepatocytes

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HAV can be released from infected cells through exosomes, forming quasi-enveloped particles (eHAV). The packaging and secretion of exosomes remains unclear and deserves further attention. RAB GTPases proteins are thought to play a key role in syndecan–ALIX–mediated exosome release, however their role is still incompletely understood. In this work, we have studied the expression pattern of several RAB genes coding for proteins potentially involved in exosome release in the hepatocyte-derived Huh7–AI cell line. The gene expression pattern did not significantly differ between non-polarized and polarized hepatocytes, showing a higher expression of RAB11, followed by RAB35 and RAB7. Two HAV strains differing in their replication capacity were used to assess the impact of replication on gene expression: the HM175 strain (L0) and the fast-replication HM175-HP (HP). During infection, expression of RAB7A and RAB35 increased, especially with HP, independently of the polarization status. Confocal microscopy was performed to identify any potential co-localization between HAV capsids and RAB proteins. HP-capsids clearly co-localized with RAB35, and to a lesser extend with RAB11A and RAB7A. Contrary, L0 capsids preferentially co-localized with RAB7A and to a lesser extent with RAB11A. These results suggest that RAB35 and RAB7 are preferentially used for eHAV egress in HP and L0-infected cells, respectively. In polarized cells, RAB7A, RAB11A and RAB35 co-localized with markers of both the basolateral and apical membrane, but interestingly, RAB35 was preferentially located at the basolateral membrane. We hypothesize that RAB35 would be involved in the fast traffic pathway occurring at the basolateral membrane, which in turn could explain the more efficient release of the HP strain through this membrane.

P8.3 REG
Replication of cell culture-adapted RVWa strain and clinical isolates human rotaviruses in relevant culture models

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Rotavirus (RV) is the leading cause of acute gastroenteritis in children under five worldwide, and several studies have demonstrated that histo-blood group antigens (HBGAs) play a role in its infection process. The presence of HBGAs on the gut epithelial surfaces is essential for the susceptibility to many RV genotypes. In the present study, 10% human RV stool filtrates were inoculated onto differentiated Caco- 2 cells (dCaco-2), in order to determine whether such clinical viral strains had the ability to replicate in cell cultures. The cell culture-adapted human rotavirus Wa strain (RV_{Wa}) was used as a control. A time course was conducted in dCaco-2 at 1, 24, 48, 72, and 96 hours. The replication of three clinical isolates and RV_{Wa} were further assayed on different continuous cell lines (MA104, undifferentiated Caco-2 cells (uCaco-2), HT29, HT29M6) and monolayers of differentiated human intestinal enteroids (HIEs). The results showed that the RVWa strain replicates better in MA104 cells than in any other cell line. Also, this virus replicated more efficiently in uCaco-2 cells than in dCaco-2. Interestingly, when clinical viruses were used, the highest virus yields were obtained in dCaco-2 cells and in HIEs. It was observed that the RV_{Wa} strain did not bind to HIEs. We speculate that those differences between clinical viruses and RV_{Wa} could be a consequence of the different HBGA contents on the surface of the cell lines employed. dCaco-2, HT29, HT29M6 cells and HIEs display HBGAs on their surfaces, while MA104 and uCaco-2 cells do not present HBGAs on their cytoplasmic membranes. These

results point out the relevance of using non-cell culture-adapted human rotaviruses to investigate the first steps of rotavirus interaction with the host cell. This work is part of the Grant PID2020-115403RB-C22 funded by the Spanish Ministry of Science and Innovation (MICIN). Nazaret Peña is the recipient of a predoctoral grant from the Valencian Government ACIF/2020/085.

P8.4 REG
Viral vectors adapted for molecular farming in different plant species

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There is a growing demand for organic-based compounds, many of them being very scarce from the original natural sources, or difficult for mass production using conventional practices. To address this demand, we aim to test and improve already available production systems based in the use of viral vectors to produce a variety of compounds in plants, a process usually called molecular farming. In our research, we are currently using different viral vectors in *Nicotiana benthamiana* and planning to develop a new one adapted to edible plants like lettuce (*Lactuca sativa*). A first vector was based on the tobacco mosaic virus (TMV; *Tobamovirus*) that retains their replicase-related gene products and also the movement protein (MP) but replaces the coat protein (CP) with the sequence of the protein of interest that is expressed under a subgenomic promoter. This vector can move cell-to-cell and produce large amounts of different proteins that will be redirected to certain subcellular localizations by the addition of signaling elements. The second vector is the pEFF system, which combines the replication machinery of the potato virus X (PVX; *Potexvirus*) and elements of different viruses, like the enhancer region of the alfalfa mosaic virus (AMV; *Alfamovirus*) and the silencing suppressor of the grape vine leaf curl associated virus two (GLRaV2; *Polerovirus*). This vector cannot move in the plant, and the expression is restricted to agroinfiltrated tissues. Despite their differences, both vectors served to produce equivalent yields of proteins in N. *benthamiana* plants, but only the pEFF production system is able to transiently produce heterologous proteins in lettuce. Thus, we are testing the conditions for optimal use of pEFF vectors in this host. Finally, we are exploring designs for a new vector based on the lettuce virus X (LeVX; *Potexvirus*) that we expect to be better adapted to its natural host, as a way to overcome limitations found in N. *benthamiana* by using a robust and rapid growing crop plant that is also considered a GRASS (generally regarded as somewhat safe) organism. Work at CRAG was funded by RTI2018-101115-B-C22 and PID2019-105692RB-I00; work at IBMCP funded by PID2020-114691RB-I00.

P8.5 REG
Senataxin restricts incoming DNA expression

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HBV and HIV are important human pathogens with 250 million and 37.7 million people chronically infected worldwide, respectively. Infection by these viruses lead to the integration of viral DNA into chromosomal cellular DNA and to the production of long lasting nonintegrated covalently closed circular DNAs (cccDNA for HBV and 1-LTR and 2-LTRs for HIV). In fact, HBV cccDNA is a hallmark of its persistence in infected patients. Currently approved therapies for the treatment of chronic HBV and HIV efficiently inhibit replication and virus production, but they are not curative because they do not completely eliminate their reservoirs. Loss-of-function genetic screening experiments performed in our laboratory identified Senataxin (SETX) as a restriction factor of HBV infection and lentivirus-driven gene expression. SETX silencing increased the accumulation of intracellular HBV mRNAs (3.5-fold) and core protein (4.5-fold) and the extracellular e antigen (5-fold) in an HBV cell culture infection system. Similarly, SETX knockdown

produced a significant increase in GFP reporter gene expression upon Integration Deficient Lentivirus (IDLV; 40-fold) and Integration Competent Lentivirus (ICLV; 7-fold) transduction. Since SETX interacts with cellular RNA polymerase II and it is involved in transcription termination, in situ RNA-labeling experiments, using ethyl uridine click technology, were performed to analyze the effect of SETX silencing on cellular transcription. Unlike the effect observed on HBV and HIV transcription, SETX silencing did not increase cellular transcription, suggesting a selective effect on episomal DNAs. To demonstrate this hypothesis a GFP-expressing plasmid was transfected in a stably mCherry-expressing cell line and the expression of both reporters was determined upon SETX silencing. GFP expression increased 3-fold while integrated mCherry expression remained unchanged in SETX silenced cells. Collectively these results suggest a restrictive role of SETX on incoming DNA expression. RNA-Seq analysis and chromatin immunoprecipitation experiments are underway to unravel the molecular mechanism(s) behind the regulation of episomal DNA expression by SETX. This work was supported by grants SAF2016-75169-R and PID2020-118970RB-100 to U.G. and FPU17/03424 fellowship to A.G.M and RTI2018-101936-B-I00 to GGA.

P8.6 REG
Cellular replication protein A1 restricts hepatitis B Virus and adeno-associated virus gene expression

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Hepatitis B virus (HBV) infection is the major cause of acute and chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. About 250 million people are chronically infected and 780.000 people die every year due to its complications worldwide. The HBV genome (rcDNA) is a partially double-stranded DNA molecule of around 3.2 kb in length with distinct structural features reminiscent of its complex DNA replication. Key steps of HBV life cycle e.g., transformation of genomic viral DNA (rcDNA) into transcriptionally active cccDNA take place in the nucleus of infected cells and strongly depends on cellular enzymatic activities. Identification of cellular proteins regulating the formation, metabolism, and expression of cccDNA may provide new therapeutic approaches for the cure of HBV infection. In this study we identified host nuclear single strand DNA binding protein replication protein A1 (RPA1) as a restriction factor of HBV infection. Silencing of RPA1 increased HBV infection efficiency, as determined by measuring intracellular core antigen and extracellular e antigen accumulation, compared to control cells. This phenotype was extended to an adeno-associated viral vector (AAV) transduction system, requiring the formation of circular DNA genomes for an efficient transduction. Using this system, reduction of RPA1 protein levels upon lentivirus shRNA transduction increased AAV-mediated GFP expression by 7-fold. Furthermore, exogenous overexpression of shRNA-resistant tagged-RPA1 protein restored AAV-driven GFP expression in endogenous RPA1 silenced-cells to that of control levels, demonstrating that the phenotype was related to RPA1 expression levels and not to off-target effects from shRNAs. Not only that, but, overexpression of a mutant RPA1 protein, unable to localize into the nucleus, failed to restore AAV-driven GFP expression despite the comparable accumulation levels achieved by both wild-type and mutant RPA1 proteins in these experiments. All these data support the notion that nuclear localization of RPA1 restricts AAV vector derived reporter expression. These results could have important implications in AAV-based gene therapy. We are currently performing experiments in other viral and non-viral systems to characterize the molecular mechanism behind these observations. This work was supported by PID2020-118970RB-100 grant to U.G. and FPU17/03424 fellowship to A.G.M and RTI2018-101936-B-I00 to GGA.

P8.7 REG

The combination of Gapmers and/or siRNA as a potential hepatitis B virus gene therapy strategy against hepatitis B virus: preliminary in vitro results

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Background&Aims: Hepatitis B virus (HBV) infection cannot be eradicated due to the persistence of its covalently closed circular DNA, which supports HBV proteins expression, contributing to hepatocellular carcinoma development. Gene silencing could be a valuable strategy and HBX gene an optimal target. We present a gene therapy strategy based on antisense locked nucleic acid Gapmer (GP) and siRNA targeting HBX hyper-conserved regions. Method: HepG2-NTCP cells were treated with DMSO 2.5% at least 14 days and later infected with HBV at 500 multiplicities of genome equivalents by centrifugation at 37ºC for 1h and incubating overnight. Infected cells were treated with GP (GP1 and/or GP4 at 50nM, Qiagen) and/or siRNA (50nM, Sigma-Aldrich) using TransIT-X2 (Mirus Bio) at 48h or 5d post infection (pi). A scrambled control GP was used in each experiment. Cells and supernatants were collected after 72h of treatment. Pregenomic RNA (pgRNA) was extracted from cells through RNAeasy Plus mini kit (Qiagen) and quantified by RT-qPCR using TaqMan probe (Roche, Lightcycler®480). HBsAg and HBeAg were quantified in supernatants by chemilumiscent enzyme assay (Roche, COBAS®8000). Results: Early after infection (48hpi), GP1 treatment allowed an inhibition of more than 70% for pgRNA, HBeAg and HBsAg (respectively 75.5±9.9;74.7±6.4;72.4±7.7%). Differently, GP4 showed an inhibition between 7.4 and 13-fold lower that of GP1 (62±17;66.1±11.2;64.7±12% for respectively pgRNA, HBeAg and HBsAg). When GP were introduced at late timepoint (5dpi), the inhibition of pgRNA decreased for both GP1 and GP4 (respectively 53.5±29% and 58.5±17%). The efficiency was lower if considering viral proteins expression (less than 48% for both GP). Of note, the combination of both GP or GP with a siRNA increased the inhibition efficiency of pgRNA (69.1±16.5%;66.7±12.2%;63.8±21% for respectively GP1+GP4;GP1+siRNA;GP4+siRNA), just partially improving viral proteins inhibition. Conclusion: Gapmers seem to be valuable molecules to inhibit HBV expression in vitro. Their limited efficiency after 5dpi could be improve by combining GP with each other or with siRNA targeting the same hyper-conserved region. Further experiments are required to confirm these results and other delivery systems should be tested to improve treatment efficiency. Funding: Instituto de Salud Carlos III (grant PI18/01436), co-financed by the European Regional Development Fund (ERDF).

P8.8 REG

Vaccinia Virus Attenuation by Codon Deoptimization of the A24R Gene for Vaccine Development

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Poxviruses have large DNA genomes, and they are able to infect multiple vertebrate and invertebrate animals, including humans. The family poxviridae includes multiple viruses of medical and veterinary relevance, including

Smallpox, Moluscum contagiosum and Monkeypox viruses. After the eradication of smallpox, poxvirus infections still remain a significant public health concern. Vaccinia virus (VV) is the prototypic member in the poxviridae family and it has been used extensively for different therapeutic applications, including the generation of vaccines against multiple infectious diseases and/or for oncolytic treatment. Many attempts have been pursued to develop novel attenuated forms of VV with improved safety profiles for their implementation as vaccines and/or vaccine vectors. In some RNA viruses, it has been shown that codon-deoptimization of viral genes is a novel strategy to generate attenuated, immunogenic viruses. The modified viruses constitute stable vaccine candidates which are able to protect, upon a single administration, against challenge with parental viruses. In this study, we employed the same experimental approach based on the use of misrepresented codons for the generation of a recombinant VV encoding a codon-deoptimized A24R gene, which is a key component of the viral RNA polymerase. Similar to previous studies with RNA viruses, the A24R codon-deoptimized VV (v-A24cd) was highly attenuated in vivo but able to protect, after a single intranasal dose administration, against an otherwise lethal challenge with parental VV. These results indicate that poxviruses can be effectively attenuated by synonymous codon deoptimization and open the possibility of using this methodology alone or in combination with other experimental approaches for the development of attenuated vaccines for the treatment of poxvirus infection, or to generate improved VV-based vectors. Moreover, this approach could be applied to other DNA viruses.

P9 PVI

P9.1 PVI

UL44 protein as vaccine candidate due to their role in cellular and humoral immune response against cytomegalovirus

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Due to the severity of CMV infection and the high number of associated deaths, the development of a vaccine is a worldwide priority. Great efforts have been made to develop a vaccine against CMV however, no candidate has yet been approved for clinical use. We designed a proteomic approach to find, select and characterize CMV antigens that induce a strong CMV-specific immune response, using serum samples from patients that have developed a protective CMV-specific response after transplantation, both cellular and humoral that could be used as a vaccine candidates. Serum samples from 28 kidney transplant recipients that exhibit a high neutralizing antibody response and CMV-specific cellular immune response collected at the University Hospital 12 Octubre in Spain were tested at two time-points (baseline and post-immunization). By using serum samples from immunized patients as primary antibodies in western blot (WB), we selected CMV antigens that were specifically recognized by the post-immunization serum compared with the baseline serum. Proteins were further characterized and identified using liquid chromatography coupled with tandem mass spectrometry. To prove the recognition of the identified protein, the open reading frames were cloned and expressed in HEK 293T cells and further detected by WB using serum samples from immunized patients as primary antibodies. Using this approach we identified UL44 protein detected using 23 out of 28 patient serum samples in WB. UL44 is an essential protein for viral infection, playing a role as a polymerase accessory protein is a tegument protein, and recent studies have demonstrated that UL44 migrates to the cellular membrane and form a complex with other cellullar proteins. UL44 construct was used to immunized BALB/c mice and serum samples collected from immunized mice were used for neutralization assays in ARPE-19 cells. The splenocytes isolated from the immunized mice were stimulated with a UL44 peptide-mix and the INF-γ activation by ELISA determination. Our results indicate that UL44 is highly immunogenic inducing antibodies that are able to neutralize viral entry in epithelial cells and CMV-specific cellular response. All together our results suggest that UL44 may potentially be an ideal candidate for vaccine development.

P9.2 PVI

Isolation and characterization of monoclonal antibodies against CMV from peripheral blood B-cells obtained from immunized transplant recipients.

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Cytomegalovirus (CMV) infection is a major cause of morbidity and mortality, especially in individuals with an immature or dysfunctional immune system. During the last decade, many studies have demonstrated the role of both T-cells and neutralizing antibodies in controlling CMV replication and dissemination *in vivo*. However, despite the improvements in the available treatments, antivirals are still associated with adverse effects such as high toxicity, selection of resistance mutations and cost. Therefore, it is necessary to continue developing new alternative therapies. In this sense, the development of immunotherapies is an attractive option that may offer an alternative to current antivirals, improving the treatment and prognosis of patients with complications related to CMV infection. To this end, doublets of specific antibody-producing B cells that recognize CMV-infected ARPE-19 cells were isolated by sorting from CMV seropositive patients that had developed a strong neutralizing antibody response. Genes encoding the immunoglobulin variable regions were then amplified by PCR and cloned into a eukaryotic expression vector. HEF 293F cells were transfected and used to express the isolated antibodies that were further characterized by Western-Blot (WB), neutralization and antibody-dependent cytotoxicity (ADCC) assays. Up to date, we have been able to clone, express and purify a functional antibody able to detect a CMV-specific transmembrane protein of 100 KDa by WB. Our results demonstrate that this method is effective for the isolation and *in vitro* production of CMV-specific monoclonal antibodies. This methodology could also be extended to isolate monoclonal antibodies against other pathogens.

P9.3 PVI

Studies on the interplay of foot-and-mouth disease virus and the RNA interference pathway

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RNA interference (RNAi) mechanisms can influence infection through both viral- and host-derived small RNAs. Antiviral RNAi starts with the recognition of dsRNA forms generated during viral infection by the protein Dicer to generate small interfering RNAs (siRNAs) which are responsible of the sequence-specific degradation of the viral RNA. RNAi also regulates gene expression through microRNAs (miRNAs) by Dicer cleavage of precursor miRNAs (pre-miRNAs). Additionally, other RNAi mechanisms can influence infection through both viral- and host-derived small RNAs involved in fine-tuning of viral replication or inhibition of antiviral mechanisms in infected cells. Though the role of RNAi in mammalian cells with an active IFN system has been controversial, a number of viral suppressors of RNA silencing (VSRs) have been identified suggesting that mammals, like invertebrates and plants, can use RNAi for antiviral immunity. Here, we have analyzed the integrity and functionality of Dicer during foot-and-mouth disease virus (FMDV) infection in swine kidney cells and the effect of the virally-encoded proteases in Dicer activity. Our results unveil new virus-host interactions and suggest that RNAi is compromised during FMDV infection. The impact of these interactions on viral replication are currently under study.

P9.4 PVI

Interaction of foot-and-mouth disease virus with the cGAS/STING signaling axis

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The cyclic GMP-AMP (cGAMP) synthase (cGAS) is a cytosolic DNA sensor. Upon DNA binding, cGAS catalyzes the synthesis of the second messenger cGAMP which then binds an endoplasmic reticulum (ER)-localized protein stimulator of IFN genes (STING), leading to type I IFN production. Though the cGAS-STING pathway has been considered crucial for the antiviral response induced during infection by DNA viruses, its relevance for the antiviral immunity against RNA viruses is being increasingly unveiled. Recent studies have reported a number of positive-stranded RNA viruses that are targeted by this pathway. Some RNA viruses have also evolved mechanisms for evading innate immune responses by targeting the cGAS/STING pathway. Here, we analyze the role of cGAS/STING during foot-and-mouth disease virus (FMDV) infection. Interestingly, our results suggest that FMDV has evolved mechanisms to antagonize the cGAS/STING pathway through the activity of the Leader and 3C proteases. The impact of FMDV infection on cGAS/STING levels and integrity has been studied as well as the effect of specific inhibitors of the pathway on viral replication in cell culture.

P9.5 PVI

Relevance of SARS-CoV-2 accessory genes to virulence in a transgenic mouse model of infection

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Until May 2022, Severe acute respiratory syndrome coronavirus (SARS-CoV-2), the etiological agent of COVID-19, has been associated with more than 500 millions of infections and six million of deaths. Coronavirus pathogenesis is largely related to the expression of virus virulence factors that induce a dysregulated innate immune response and inflammation. Coronavirus accessory genes are highly variable among different viruses. They are not required for virus replication in cell cultures, although they are involved in the interference with the innate immune response. The SARS-CoV-2 genome encodes six accessory proteins (3a, 6, 7a, 7b, 8 and 9b), for which limited information is available on their contribution to pathogenesis in the context of in vivo infection. This work showed that the deletion of each of ORFs 6, 7a or 7b, using a reverse genetics system, did not affect virus virulence in humanized K18-hACE2 transgenic mice. In contrast, deletion of ORF8, either alone or in combination with ORF6, partially attenuated SARS-CoV-2, resulting in lower clinical signs of disease and 40% less mortality. Importantly, the attenuation of these mutants was associated with a significant increase in the interferon (IFN) and pro-inflammatory responses in the lungs of mice at early times post infection (2 and 3 dpi). The combined deletion of the four accessory genes further attenuated the virus, resulting in a reduction of clinical signs in the lungs of infected mice and 80% survival. This significant reduction in virulence was associated with a decrease of IFN and pro-inflammatory responses in mouse lungs compared to native virus. Together, our results showed that ORF8 was a main determinant of SARS-CoV-2 pathogenesis, although the other ORFs 6, 7a, and 7b also contributed to virulence.

P9.6 PVI

Effect of OAS genes on SARS-CoV-2 infection and the induction of innate immune responses

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Severe Acute Respiratory Syndrome 2 (SARS-CoV-2) infections cause different clinical symptoms ranging from asymptomatic patients to patients suffering severe respiratory disease leading to death in some of them. Genetic and functional studies have shown inborn-errors of interferon (IFN)-related genes in severe COVID-19 patients explaining why some young patients devoid of co-morbidities succumbed to infection. We have sequenced the whole genome of around 400 patients who required hospitalization after SARS-CoV-2 infection, and we have found ultrarare mutations in 2´5´-olygoadenylate synthetase genes (OAS), being these genes IFN-stimulated genes (ISGs). Upon double-stranded (ds)RNA binding, the OAS1, OAS2, and OAS3 proteins synthetize 2´-5´olygoadenylates which activate the endonuclease RNAseL. This endonuclease degrades viral and cellular RNAs, inhibiting viral replication. We have analyzed the effect of OAS1 and OAS3 genetic variants identified in our patients, and found that some of them impair the RNAseL activation. In addition, by using OAS1 and OAS3 knock-out cells generated in our laboratory and performing overexpression experiments, we have shown that OAS3 negatively modulates pro-inflammatory responses induced by immune challenges, and that the activation of the RNAseL activity seems necessary for this function. In addition, by using OAS3 knock-out mice infected with SARS-CoV-2, we have shown that OAS3 curbs viral replication and counteracts the induction of innate immune responses in the mouse infected-lungs. Our results show the antiviral activity of OAS1 and OAS3 against SARS-CoV-2 infection and the negative regulatory effect of OAS3 against the subsequent inflammatory responses. Given the contribution of exacerbated inflammatory responses to COVID-19 disease severity, our results suggest that OAS1/OAS3 could play a role limiting the severity of the clinical symptoms after SARS-CoV-2 infection.

P9.7 PVI
Deciphering the relevance of glycans in human and animal rotavirus B and C infections

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Rotaviruses are the leading cause of severe gastroenteritis in humans and animals, infecting the mature intestinal epithelium. Rotavirus attachment to host cell glycans is mediated through the N-terminal VP8* domain of virion spike protein (VP4). Recent studies have shown that different rotavirus A genotypes interact with histo-blood group antigens (HBGAs) as receptors, although little is known regarding non A rotaviruses. The goal of the present study was to study the interactions of glycans, HBGAs and gangliosides, with rotavirus B (RVB) and C (RVC) species, and to determine their relevance in viral infections. cDNA sequences encoding the VP8* proteins from human RVB genotype P[2] and human RVC genotype P[2], animal genotypes P[3], P[4], P[5], P[10], P[11], P[12] were obtained as synthetic genes. They were cloned into the pGEX-2T vector and expressed in *E. coli* BL21 (DE3) strain. VP8* proteins were purified by affinity chromatography using GSTtrap columns. The interaction of purified VP8* proteins against a panel of synthetic glycans was evaluated. A broad range of glycan-binding profiles was found. Our results show that genotype P[2] of human rotavirus B interacts weakly with the entire panel of glycans assayed. However, P[2] human rotavirus C binds strongly to A trisaccharide (A_{tri}), with double intensity than the positive control (P[14] rotavirus A). Surprisingly, animal RVC genotypes (bovine P[10] and canine P[11]) also recognize A_{tri} glycan in a specific manner, providing valuable information on rotavirus-glycans interactions. Moreover, P[3] bovine RVC genotype interacts with the H2 antigen, and P[4] and P[12] porcine genotypes do not bind to any glycan in the panel. We conclude that human P[2], bovine P[10] and canine P[11] rotavirus C genotypes bind A_{tri}. These findings can help to identify strain-specific host ranges or species barriers between humans and animal rotavirus genotypes. Further investigations are needed to clarify the different host-specificity of rotavirus species. This study was supported by a

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P9.8 PVI
Analysis of the variability of transcription binding sites (TBS) in endogenous avian leukosis virus (ALV-E) from different chicken breeds

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The retrovirus avian leukosis virus (ALV) infects chicken and related avian species. Nowadays, it is still a cause of concern in third-world countries and China, causing economic losses. After integration, proviruses are flanked by Long Terminal Repeats (LTR). The U3 region of the LTR5' contains the viral promoter and Transcription Binding Sites (TBS) and is, consequently, an important factor related to retroviral pathogenesis. ALV-E is an endogenous subtype of ALV present in the genome of modern chickens as remnants of ancient infections. ALV-E LTRs can affect the pathogenesis of exogenous ALV and may regulate other loci close to the provirus. Thus, our work focused on characterizing TBS in the U3 region of ALV-E LTR5'. GenBank sequences of ALV-E LTR5' grouped in two clusters: a) most exogenous sequences, and b) endogenous and endogenous-recombinant viruses. We designed oligonucleotides to amplify this region (274 bp) and sequenced 25 chicken DNA samples of different breeds. Some positions presented Single Nucleotide Polymorphisms (SNP) suggesting more than one integration event. The analysis of these sequences by the software Alggen identified TBS that react to hormones, cytokines or cell growth factors (C/EBP, c-ets, GATA, sp1, NF-AT, GR-alpha, IRF, STAT5A, PU-box and AP-1). We identified 10 SNPs that affected TBS abundance or size in the different LTRs. In order to study the expression controlled by ALV-E LTR5', the LTR5' from chicken G-24 (Moorish chicken), selected due to its high number of SNPs, was ligated to vector TOPO PCR4.0. The most representative sequence was subcloned into the luciferase reporter vector pGL4.14. This construct, pGL4.14-LTR5', is subjected to site-directed mutagenesis to reproduce some of the SNP detected. Cell lines 293T (human kidney fibroblasts) and DF-1 (chicken fibroblasts) are transfected with all the constructs and pGL4.14-empty vector using Lipofectamine, and transcription inductors (chicken IFN-alpha and TNF-alpha, LPS, Sox2, ...) added to the culture media. Differences in the expression of luciferase, driven by the ALV-E LTR5', compared to controls and to constructs with different SNPs, will help unveil how host cell factors interact with the retroviral TBS. Funded by PID2020-114956GB-I00.

P9.9 PVI
ISG15 modulates lipid metabolism in macrophages during Vaccinia virus infection

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The interferon-stimulated gene 15 (ISG15) is a well-known antiviral molecule against a wide range of viruses. ISG15 exerts its antiviral function directly, modifying viral proteins, or indirectly, through the regulation of numerous cellular pathways, ranging from genome replication to energy metabolism. Previous work from our group identified ISG15 as a modulator of mitochondrial metabolism and dynamics in IFN-stimulated bone marrow-derived macrophages (BMDM). Our recent work presents ISG15 as a regulator of lipid metabolism during infection. We reported a dysregulation of the macrophage lipid profile in the absence of ISG15, highlighting a significant reduction in neutral lipids (NL), in line with reduced lipid droplet number and size. These observations were consistent with upregulation of

proteins involved in fatty acid oxidation (FAO) and lipolysis, what correlated with increased expression of PGC-1α and PPARγ. *Vaccinia virus* (VACV) infection altered the lipid profile of BMDM, increasing NL, mainly cholesterol esters. Interestingly, such alterations in the lipid content were exacerbated in *Isg15-/-* BMDM, suggesting a role of ISG15 restraining the effects of VACV on lipid metabolism. Altogether, our results broaden the functions of ISG15 and highlight its relevance as an immunometabolic regulator during viral infections. This work was funded by the Spanish State Research Agency (Agencia Estatal de Investigación, AEI).

P9.10 PVI
Isolation and characterization of bacteriophages for biocontrol of the major plant pathogen Xylella fastidiosa

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Xylella fastidiosa (Xf) is a worldwide important plant pathogen of the family *Xanthomonadaceae*, which can cause serious diseases in strategic crops with devastating effects. Its host range exceeds 600 plant species. Xf lives in the xylem of the plant, where it can form biofilms and obstruct sap flow, and also in the foregut of xylem-feeding insects that act as vectors. This bacterium presents a great genetic diversity that results in different subspecies and sequence types (ST). In Europe there are several outbreaks, most of them located in the Mediterranean basin, affecting different host plants, but mainly olive and almond trees, depending on the subspecies and ST of the pathogen present in each area. Since there are no effective therapeutic measures and antibiotics are forbidden in Europe, the control of *Xf* is mainly based on the eradication of infected plants and the use of chemical compounds against insect vectors, but this is not enough. Efficient alternative strategies that are environmentally friendly are needed. Biological control using bacteriophages can be a viable and sustainable tool in an integrated management of Xf diseases. Due to the inherent difficulty in culturing Xf under laboratory conditions, *Xanthomonas* spp. strains were used as surrogate hosts for phage hunting in plant, water and soil samples from areas with active Xf outbreaks, and also in wastewater samples. A total of 22 bacteriophages were isolated and amplified, and their lytic activity was tested against more than 50 strains of *Xanthomonas* spp. and also on strains of other phytopathogenic bacterial species. All bacteriophages were phenotypically and genomically characterized and four of them with lytic activity against different strains of Xf were selected for *in vivo* assays. Infected plants treated with phages showed less symptoms than control plants. These results are very promising and suggest that some of the selected phages could be used for control of Xf diseases.

P10 NAB

P10.1 NAB
Iron oxide and iron oxyhydroxide nanoparticles impair SARS-CoV-2 infection of cultured cells

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Coronaviruses usually cause mild respiratory disease in humans but as seen recently, some human coronaviruses can cause more severe diseases, such as the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the global spread of which has resulted in the ongoing coronavirus pandemic. In this study we analyzed the potential of using iron oxide nanoparticles (IONPs) coated with biocompatible molecules like dimercaptosuccinic acid (DMSA), 3-aminopropyl triethoxysilane (APS) or carboxydextran (FeraSpin™ R), as well as iron oxyhydroxide nanoparticles (IOHNPs) coated with sucrose (Venofer®), or iron salts (ferric ammonium citrate -FAC), to treat and/or prevent SARS-CoV-2 infection. At non-cytotoxic doses, IONPs and IOHNPs impaired virus replication and transcription, and the production of infectious viruses *in vitro*, either when the cells were treated prior to or after infection, although with different efficiencies. Moreover, our data suggests that SARS-CoV-2 infection affects the expression of genes involved in cellular iron metabolism. Furthermore, the treatment of cells with IONPs and IOHNPs affects oxidative stress and iron metabolism to different extents, probably influencing virus replication and production. Interestingly, some of the nanoparticles used in this work have already been approved for their use in humans as anti-anemic treatments, such as the IOHNP Venofer®, and as contrast agents for magnetic resonance imaging in small animals like mice, such as the FeraSpin™ R IONP. Therefore, our results suggest that IONPs and IOHNPs may be repurposed to be used as prophylactic or therapeutic treatments in order to combat SARS-CoV-2 infection.

P10.2 NAB
Modulation of the self-assembly pathway and architecture of the mature HIV-1 capsid through mutations in protein-protein interfaces

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The mature human immunodeficiency virus 1 (HIV-1) capsid is made of CA protein subunits. CA consists of two domains (NTD and CTD) connected by a flexible linker. Within the capsid lattice, each subunit is associated with three other subunits through two NTD-NTD interfaces and one CTD- CTD interface. The mature HIV-1 capsid is pleomorphic due to the high flexibility of the CA protein itself and its ability to form both hexamers and pentamers. The overall shape is largely dependent on the particular locations where CA pentamers are introduced, as pentamers may allow a higher local curvature than hexamers. Understanding the structural elements that influence the capsid assembly pathway and shape may help the design of antiviral drugs that favor alternative pathways leading to aberrant capsids, and the engineering of nanostructured materials. In a previous study we engineered a thermally and mechanically stabilized HIV-1 capsid protein lattice through the introduction of cysteines that removed electrostatic repulsions and, under nonreducing conditions, established intersubunit covalent bonds between CA subunits. In this study we have determined the effects of those mutations in both the kinetics of CA self-assembly *in vitro* and the final architecture of the capsid-like structures formed. Introduction of a cysteine at the CTD-CTD interface (CA^{1m} mutant) slowed down the rate of assembly, whereas the introduction of a cysteine at the NTD-NTD interface, in the absence (CA^{2m}) or presence of the cysteine at the CTD (CA^{3m}), accelerated the rate of assembly under reducing conditions. The assembly rate was further increased under nonreducing conditions where intersubunit disulfide bonds were formed. Moreover, both CA and CA^{1m} self-assembled as tubes (devoid of pentamers), whereas CA^{2m} and CA^{3m}

assembled as a mixture of tubes and highly curved structures (spherical or cone-shaped particles) under reducing conditions, and CA^{3m} formed exclusively highly curved structures under nonreducing conditions. The results suggest that stabilisation of the NTD-NTD interface by removing electrostatic repulsions and introducing covalent bonds both speed up the capsid assembly reaction and facilitate the introduction of pentamers, thus favoring the assembly pathway leading to authentic capsids instead of aberrant assemblies devoid of pentamers.

P10.3 NAB
Bluetongue viruses expressing reporter genes for in vitro and in vivo studies

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Bluetongue virus (BTV), the prototype member of the genus *Orbivirus* (family *Sedoreoviridae*), is the causative agent of the important livestock disease Bluetongue (BT), which is transmitted via biting midges of the genus *Culicoides*. BT causes severe economic losses associated with its considerable impact on health and trade of animals. The study of BTV requires the use of secondary approaches to detect the presence of virus in infected cells. By reverse genetics, we have designed and rescued BTV-1-based viruses expressing Nanoluciferase (Nluc) or the fluorescent reporter genes iLov or Venus. To generate these viruses, we custom synthesized a modified genome segment 5 encoding NS1 protein with the reporter genes located downstream and separated by the Porcine teschovirus-1 (PTV-1) 2A autoproteolytic cleavage site. As the reporter genes were fused to NS1, fluorescent signal or luciferase activity are only detected after cell entry, virus replication and expression of nonstructural proteins. Fluorescence signal was detected in cells infected with the recombinant viruses rBTV-1/Venus and rBTV-1/iLov. Moreover, marking of NS2 protein confirmed that these reporter genes were only expressed in BTV-infected cells. Growth kinetics of rBTV-1/Nluc, rBTV-1/iLov and rBTV-1/Venus in Vero cells showed a replication rate comparable to that of rBTV-1. Regarding the stability of these viruses, rBTV-1/Venus and rBTV-1/iLov were stable for at least three passages and rBTV-1/NLuc was stable up to passage six in Vero cells. Infectivity studies of these recombinant viruses in IFNAR (-/-) mice showed a higher lethal dose for BTV-1/Nluc, BTV-1/iLov and BTV-1/Venus than for BTV-1 indicating that viruses expressing the reporter genes are attenuated *in vivo*. Interestingly, luciferase activity was detected in the plasma of viremic mice infected with rBTV-1/NLuc. Furthermore, luciferase activity correlated with viremia levels of infected mice throughout the infection. By eliminating the need for secondary labeling of infected cells, BTV expressing reporter genes provide an ideal tool in the ongoing fight to better characterize BTV and identify new therapies for Bluetongue.

P10.4 NAB
Construction and Evaluation of Turkey Herpesvirus vectored Newcastle Disease Vaccine that Expressing the F Protein Genotype XII Generated by NHEJ-CRISPR/Cas9 and Cre-LoxP Systems.

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Introduction. The Newcastle disease (ND) is an important avian disease caused by the Newcastle disease virus (NDV). The circulating of a velogenic strain (genotype XII/class II) has been reported in South America (Colombia and Peru). The classical ND vaccines (genotype I and II) have demonstrated limited efficacy in controlling virus shedding, possibly due to higher genetic distance between vaccine antigens and field challenge viral strains. Therefore, we developed a recombinant vaccine in which the fusion (F) gene from NDV (genotype XII) was inserted into the Turkey herpesvirus (HVT). Objectives. Evaluate the viral shedding and efficacy against the genotype XII challenge in specific pathogen-free (SPF) chickens. Methods. The F gene was inserted into the HVT genome by a clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9 gene-editing technology via a non-homologous end joining (NHEJ) repair pathway. An indirect immunofluorescence assay (IFA) and Western blotting detected the F protein expression. An ELISA detected the NDV-specific antibodies. The efficacy and viral shedding were monitored. Results. This rHVT-F, was capable of eliciting NDV-specific antibodies. This recombinant vaccine showed fully protection in SPF chickens vaccinated and significantly reduced virus shedding in oral at 5 dpc against a genotype XII challenge. Conclusions. This vaccine candidate can fully protect SPF chickens and reduce viral shedding against a genotype XII.

P11 ITV

P11.1 ITV
Determination of Norovirus-interacting bacteria in human clinical samples

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Intestinal microbiota plays an important role in the infection of gut viruses, either favoring or impairing it. *Norovirus* (NoV) belongs to the *Caliciviridae* family and the GII.4 Sydney 2012 genotype is known to be the main responsible for worldwide outbreaks. Aiming to determine the bacterial species interacting with NoV during infection in humans, NoV-interacting bacteria were isolated from 9 clinical samples using protein A magnetic beads coated with a mouse anti-NoV antibody. After 16S rDNA sequencing, *Rhodococcus* and *Lactobacillus* were identified as the bacterial genera bound to NoV in the stool samples, with a 4.5- and 3.2-fold increase respectively compared with control (beads coated with an isotype control antibody). Six *Rhodococcus* strains (CECT) were assayed in binding experiments *in vitro* using NoV GII.4 Sydney 2012 virus like particles (VLPs) produced in baculovirus. The VLPs attached to each strain was shown and quantified by western blot. To assess the presence of histo-blood group antigens (HBGAs) in the surface of the different bacterial strains anti-HBGAs antibodies were used. The results showed that *Rhodococcus* express HBGA-like substances in their surface. These HBGAs could be facilitating the binding since they have been proposed as NoV co-receptors in humans. Furthermore, a competitive displacement assay showed higher adhesion to HT29M6 cell line when VLPs were added together with these bacteria. Future infection experiments in human intestinal enteroids that support NoV replication will allow us to assess whether *Rhodococcus* are favoring or impairing the NoV infection. This work is part of the Grant PID2020-115403RB-C22 funded by the

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P11.2 ITV
Multitasking roles of the coat protein of melon necrotic spot virus in mitochondria and chloroplasts

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Plant defense against melon necrotic spot virus (MNSV) is triggered by the viral auxiliary replicase p29 that is targeted to mitochondrial membranes causing morphological alterations, oxidative burst and necrosis. Here we show that MNSV coat protein (CP) was also targeted to mitochondria and mitochondrial-derived replication complexes [viral replication factories or complex (VRC)], in close association with p29, in addition to chloroplasts. CP import resulted in the cleavage of the R/arm domain previously implicated in genome binding during encapsidation and RNA silencing suppression (RSS). We also show that CP organelle import inhibition enhanced RSS activity, CP accumulation and VRC biogenesis but resulted in inhibition of systemic spreading, indicating that MNSV whole-plant infection requires CP organelle import. We hypothesize that to alleviate the p29 impact on host physiology, MNSV could moderate its replication and p29 accumulation by regulating CP RSS activity through organelle targeting and, consequently, eluding early triggered antiviral response. Cellular and molecular events also suggested that S/P domains, which correspond to processed CP in chloroplast stroma or mitochondrion matrix, could mitigate host response inhibiting p29-induced necrosis. S/P deletion mainly resulted in a precarious balance between defense and counter-defense responses, generating either cytopathic alterations and MNSV cell-to-cell movement restriction or some degree of local movement. In addition, local necrosis and defense responses were dampened when RSS activity but not S/P organelle targeting was affected. Based on a robust biochemical and cellular analysis, we established that the mitochondrial and chloroplast dual targeting of MNSV CP profoundly impacts the viral infection cycle. Funding: M.S. was recipient of a Predoctoral Contract PRE-2018-84130 from the Subprograma FPI-MINECO (Formación de Personal Investigador–Ministerio de Economía y Competitividad). This work was supported by Grant BIO2017–88321-R from the Spanish Agencia Estatal de Investigación (AEI) and Fondo Europeo de Desarrollo Regional (FEDER).

P11.3 ITV
In vitro Modification on mesenchymal stem cells by Human Metapneumovirus infection

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Lower respiratory infection remains one of the mayor global causes of death and morbidity, after the recent pandemics the importance of respiratory virus as an etiological agent is evident. Now the world is focus on virus like Coronavirus and Influenza, we must not stop researching about another important virus, Human Metapneumovirus is one of them. This is a single negative strand RNA virus first identified in 2001, is a frequent virus that causes lower respiratory infections in children under age of 5. Metapneumovirus infections in early life is associated with an increased likelihood of subsequent recurrent wheezing and asthma, and susceptibility to other viral infections. Asthma, wheezing, and respiratory infections share pathologically characterizes airway inflammation and airway remodeling. First airway resident stem cells act in tissue remodeling and keep the functional structure, airway inflammation is suppressed due secretion of stem cells, not only resident, but also peripheral stem cells can also migrate to inflammatory sites. After that if the stem cells are susceptible to be infected by the virus the modulation of inflammation and airway remodeling can be affected. Throughout our investigation in our laboratory, we demon-

strated mesenchymal stem cells are susceptibility to be infected by Human Metapneumovirus, we demonstrated the morphological and physiological changes in the infected stem cells. During the investigation we observed how the migratory capacity of infected stem cells has been affected, and how the differentiation capacity is knock down. Our research is focus on cytoskeleton changes due the implication in motility, vesicle liberation and differentiation. Our study not only can approximate what happen with the airway stem cells, and peripheral stem cells, if mesenchymal stem cells can be infected and lose the immunomodulatory capacity all the therapies that use these cells to treat airway inflammatory diseases can be affected by the presence of the virus.

P12 VEM

P12.1 VEM
MOV10 helicase interacts with coronavirus nucleocapsid protein and has antiviral activity

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Coronaviruses (CoVs) are emergent pathogens that may cause life-threatening respiratory diseases in humans. One of them, Middle East respiratory syndrome coronavirus (MERS-CoV), emerged in 2012 and has caused more than 2,650 cases with 37% fatality rate. A novel human CoV (SARS-CoV-2) emerged in 2019 in China, causing more that 518 million infections and more than 6 million deaths worldwide. To date, there are few approved antivirals to treat human CoVs infections. Understanding of CoV-host interactions may help to identify novel therapeutic targets. MOV10 is an RNA helicase involved in different steps of cellular RNA metabolism. Both MOV10 antiviral and proviral activities have been described in a limited number of viruses but this protein has not been previously associated to CoVs. We found that during MERS-CoV infection, MOV10 aggregated in cytoplasmic structures co-localizing with viral nucleocapsid (N) protein. MOV10-N interaction was confirmed by endogenous MOV10 co-immunoprecipitation and the presence of other cellular proteins was also detected in MOV10 complexes. MOV10 silencing significantly increased both N protein accumulation and virus titer, with no changes in the accumulation of viral RNAs. Moreover, MOV10 overexpression caused a 10-fold decrease in viral titers. These data indicated that MOV10 has antiviral activity during MERS-CoV infection. We postulated that this activity could be mediated by viral RNA sequestration and, in fact, RNA-immunoprecipitation data showed the presence of viral RNAs in the MOV10 cytoplasmic complexes. Expression of wild-type or of a MOV10 mutant without helicase activity in MOV10 knock out cell lines, developed by CRISPR-Cas technology, indicated that the helicase activity of MOV10 was required for its antiviral effect. The interaction between MOV10 and N protein was conserved in other mild or highly pathogenic human CoVs, including the recently emerged SARS-CoV-2. Interestingly, MOV10 antiviral activity was found in highly pathogenic CoVs, such as MERS-CoV and SARS-CoV-2, but not in HCoV-229E, suggesting a role of MOV10 in modulation of human CoVs pathogenesis. The present study uncovers a complex network of interactions between viral and cellular RNAs and proteins modulating the antiviral response against CoVs.

P12.2 VEM
Arbovirosis: a case of meningitis due to Toscana virus

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INTRODUCTION: Infectious diseases caused by arboviruses are a global health problem. Spain is a country that has the ideal characteristics to have autochthonous circulation of some of these viruses. CASE REPORT: We report the first microbiological diagnosis of Toscana virus (TOSV) in Cáceres (Extremadura). On August 24, 2021, a 19-year-old male was admitted to the emergency department of Cáceres Hospital. He had suffered from a severe headache

since the previous day. On admission, the patient had a fever reaching 38.3°C, vomiting and nausea. Clinical examination showed no stiff neck or skin lesions. Computed tomography was normal. Suspecting meningitis, a cerebrospinal fluid(CSF) sample was taken and empirical antibiotic therapy was administered. CSF analysis showed 713 leukocytes/μL (80% lymphocytes, 20% neutrophils), and glucose and protein levels of 59 mg/dL and 153 mg/dL, respectively. Blood and CSF cultures were bacteriologically sterile. The patient was admitted to the neurology service and received Acyclovir for one day. Allplex™ Meningitis Assay (Seegene) of CSF was also negative. Serum contained no antibodies to *Brucella, Coxiella burnetii, Rickettsia conorii, Borrellia burgdoferi,* Herpes simplex virus and HIV. Serum and CSF samples collected during the acute phase were tested for IgM and IgG antibodies to West Nile virus and Toscana virus and polymerase chain reaction(PCR) assays were performed on the National Center for Microbiology. PCR assay of CSF for TOSV and anti-TOSV IgM were positives (index 4,6). The patient recovered after 7 days without sequelae. DISCUSSION: Toscana virus is an emerging sandfly-borne virus within the *Phlebovirus* genus, which is endemic in Mediterranean countries. TOSV infection is often asymptomatic, but it can cause neuroinvasive disease in humans mainly during summer; however, it remains a neglected pathogen and is seldom included in the diagnostic algorithm for central nervous system infections. This illness is generally self-limiting and the evolution is usually favorable with symptomatic and supportive treatment. TOSV should be suspected in cases of meningoencephalitis without identified causal pathogen. In addition, since the competent vector and the virus are found in our territory, surveillance studies are needed to assess potential high-risk areas for TOSV infections and to establish global measures for his control.

P12.3 VEM
Distinct immunogenicity and efficacy of poxvirus-based vaccine candidates against Ebola virus expressing GP and VP40 proteins

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Zaire (EBOV) and *Sudan* (SUDV) *Ebolavirus* causes a zoonosis called Ebola virus disease, which affects humans and non-human primates and is characterized by a high mortality rate. Several vaccine candidates have been evaluated through clinical trials, but it is essential to develop new, more effective, safer and cheaper vaccines that provide long-term protection. In this work, we have generated and studied the immunogenicity and efficacy of five vaccine candidates against EBOV (termed MVA-EBOVs) and SUDV (termed MVA-SUDVs) based on the modified vaccinia virus Ankara (MVA) vector expressing the viral glycoprotein (GP) or GP and viral protein 40 (VP40) simultaneously and forming virus-like particles. GP and VP40 antigens expressed from the different vaccine candidates showed distinct levels of expression and secretion in HeLa cells, and GP expression was also detected early in macrophages and dendritic cells isolated from the lungs of infected BALB/c mice. In a human monocytic cell line, these recombinant MVAs triggered robust innate immune responses, with production of interferon beta, proinflammatory cytokines, and chemokines. Furthermore, several populations of innate immunity, such as dendritic cells, neutrophils, and natural killer cells, were differentially recruited into the peritoneal cavity of mice inoculated with MVA-EBOVs. On the other hand, the immunogenicity of the different vaccine candidates in BALB/c and C57BL/6 mice has been evaluated using homologous (MVA/MVA) and heterologous (DNA/MVA) prime/boost immunization protocols. The recombinant MVAs triggered different immunogenicity profiles characterized by the induction of specific CD4+ and CD8+ T cells, CD4+ T follicular helper cells and germinal center B cells, together with specific IgG antibodies and neutralizing antibodies against EBOV. In particular, the MVA-GP-2A-VP40 Zaire vaccine induced higher antibody responses and specific CD4+ T cells, while the MVA-GP-VP40 Zaire vaccine elicited higher levels of specific CD8+ T cells, with a more balanced CD4/CD8 response. Finally, the efficacy of MVA-EBOVs has been evaluated in chimeric

C57BL/6 mice susceptible to developing the disease after a lethal dose with EBOV, and the results showed that MVA-GP-VP40 Zaire conferred a higher level of protection compared to the other vaccine candidates.

P12.4 VEM

Assessment of innate immunity generated by dendritic cells transfected with two mRNA vaccines against Viral Haemorrhagic Fever viruses

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Viral haemorrhagic fevers (VHF) are a group of zoonotic emerging diseases caused by distinct families of RNA viruses. After infection of human beings, they can affect multiple organs and systems of the body and cause severe life-threatening diseases. Among the *Bunyavirales* order, Crimean-Congo Haemorrhagic Fever virus (CCHFV) is a major causative agent of VHF. CCHFV is a zoonotic pathogen causing Crimean-Congo Haemorragic fever (CCHF), a severe fever disease transmitted by ixodid (hard) ticks. It has a wide distribution, and it is endemic in several areas around the world. High fatality rates, dispersion of the disease, and no approved vaccines, makes CCHFV a threat to global health. In recent years, mRNA vaccination has emerged as a promising tool to control viral diseases. mRNA vaccines have several advantages as they are non-integrating and non-infectious, they lead to protein expression with high efficiency, and small doses can induce robust immune responses. Stability and translation of the mRNA is crucial for a successful RNA vaccine. In this process, specific sequences and secondary structures formed by mRNA may be recognized by a number of innate immune receptors, which can lead to translation inhibition. In the current study, we designed and synthetized two mRNAs encoding for the Gc or the Gn glycoproteins of CCHFV as potential vaccines. To assess the activation of the innate immune response triggered by both mRNAs, mouse bone marrow derived dendritic cells (DCs) were transfected *in vitro* in the presence of lipofectamine with the vaccine candidates. After 24 hours, DCs were stained for the evaluation of IFN-α, TNF-α and IL-12 production by flow cytometry. Both modified single-stranded mRNAs were translated into DCs, while maintaining a low activation of innate immunity after transfection. More studies are needed to check for the specific immune response generated *in vivo* by the vaccine candidates.

P12.5 VEM

The combined vaccination protocol of DNA/MVA expressing Zika virus structural proteins as efficient inducer of T and B cell immune responses

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Zika virus (ZIKV) is a is a mosquito-borne pathogen with public health importance due to the high risk of its mosquito vector dissemination and the severe neurological and teratogenic sequelae associated with infection. Vaccines with broad immune specificity and control against this re-emerging virus are needed. Here, we described that mice immunized with a priming dose of a DNA plasmid mammalian expression vector encoding ZIKV prM-E antigens (DNA-ZIKV) followed by a booster dose of a modified vaccinia virus Ankara (MVA) vector expressing the same prM-E ZIKV antigens (MVA-ZIKV) induced broad, polyfunctional and long-lasting ZIKV-specific CD4+ and CD8+ T-cell immune responses, with high levels of CD4+ T follicular helper cells, together with the induction of neutralizing antibodies. All those immune parameters were significantly stronger in the heterologous DNA-ZIKV/MVA-ZIKV immunization group compared to the homologous prime/boost immunizations regimens. Collectively, these results provided an optimized immunization protocol able to induce high levels of ZIKV-specific T-cell responses, as well as neutralizing antibodies and reinforce the combined use of DNA-based vectors and MVA-ZIKV as promising prophylactic vaccination schedule against ZIKV.

P12.6 VEM

Susceptibility and transmissibility of SARS-CoV-2 in transgenic mice expressing the cat angiotensin-converting enzyme 2 (ACE-2) receptor.

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SARS-CoV-2 emerged in 2019 causing the Covid-19 pandemic and more than 6 million deaths around the world so far. Its zoonotic nature has pointed to animals as the origin of the outbreak in humans. Indeed, it is speculated that a circulating ancestral lineage in bats jumped to humans, although other mammalian hosts have also been suggested as possible sources of transmission to humans. Host jumping of SARS-CoV-2 between different mammalian species is made possible by the presence of the relatively highly conserved protein angiotensin-converting enzyme 2 (ACE2). In fact, throughout the pandemic, different species of mammals, including domestic species (pets and livestock) have tested positive for SARS-CoV-2, although their role as possible transmitting bridges of the virus to humans has not yet been solidly determined. Furthermore, during the pandemic, the virus has evolved giving rise to variants with different pathogenicity in the human host. For all these reasons, the availability of animal models to determine the susceptibility of the different mammalian species to SARS-CoV-2, and the transmissibility and adaptation of the different variants is necessary. In this work, we have obtained, characterized, and validated 6 new lines of transgenic mice that express the cat ACE2 protein receptor under the control of the K18 gene promoter, necessary for its expression in epithelial cells for which the virus has a high tropism. The two lines with the highest expression of cat ACE2 in the lung and trachea were selected to study the susceptibility to infection, and the pathogenicity and transmissibility of the virus, the results of which will be presented and discussed.

P12.7 VEM
The role of DC-SIGN as a transreceptor in infection by MERS-CoV

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Dipeptidyl-peptidase 4 (DPP4) is known as a direct receptor for MERS-CoV and it plays an important role in the virus infection cycle. However, there could be other molecular mechanisms involved in the spread of the virus yet to be discovered. The C-type lectin DC-SIGN recognizes a wide variety of pathogens and it was predicted to participate in the dissemination of the virus at the first moments of the infection. In this study we have explored the usage of DC-SIGN by MERS-CoV in the process of direct infection (cis-infection), as well as its utility in capturing and subsequent transferring of the viral particles to susceptible cells (trans-infection). We have used an rVSV-luc recombinant virus pseudotyped with MERS-CoV, SARS-CoV, SARS-CoV-2 and Ebola virus (EBOV) envelope. Cis-infection was performed on Jurkat cells (T-lymphocyte cell line) expressing DC-SIGN and on dendritic cells (DCs) derived from monocytes from 4 healthy donors. For trans-infection studies, Jurkat DC-SIGN or DCs, were incubated with recombinant viruses and the infection was measured in susceptible VeroE6 cells. The results obtained in cis-infection on Jurkat DC-SIGN showed that the infection by both MERS-CoV and EBOV depends exclusively on the presence of DC-SIGN. In contrast, there was no infection detected in case of SARS-CoV and SARS-CoV-2. Additionally, MERS-CoV could infect DCs at very high levels, however, this infection was not supressed by ant-DC-SIGN antibody. This could indicate that there are other possible receptors present on the surface of DCs, included DPP4, which are responsible for the infection of these cells and the role of DC-SIGN in this process could be minimal. As in case of Jurkat stable cell line, there was no direct infection in DCs with SARS-CoV or SARS-CoV-2. Trans-infection results showed that Jurkat DC-SIGN and DCs could bind MERS-CoV, Ebola virus, SARS-CoV, and SARS-CoV-2 being capable of capturing and transmitting viral particles to susceptible VeroE6 cells. In conclusion, DC-SIGN function as trans-receptor for MERS-CoV, EBOV, SARS-CoV, and SARS-CoV-2 being a potential route of dissemination for the virus. Ebola virus can use effectively DC-SIGN as a direct receptor. Nevertheless, the role of DC-SIGN as direct receptor for MERS-CoV needs further investigation.

P12.8 VEM
Pathogenesis of an emerging Pestivirus in ovine and swine: old foes or new threats?

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Pestiviruses, a member of the Flaviviridae family, are an ever-expanding viral genus. A newly reported Pestivirus species, ovine pestivirus (OVPV), was isolated in 2017 from aborted lambs in northern Italy. OVPV showed high ho-

mology with classical swine fever virus (CSFV), one of the most important pathogens for the swine industry. However, the capacity of OVPV to induce disease had not been established. This study aimed to elucidate the origin of OVPV, stablish its role as a pathogenic agent in pregnant ewes and swine and evaluate the induction of immune response in both species. Eight ewes at 68 days of gestation were infected with OVPV. The virus caused abortions and stillbirths after infection in seven pregnant ewes and showed high capacity for trans-placental transmission as well as the birth of lambs suffering OVPV-persistent infection. The OVPV infection induced early antibody response in the ewes, detected by the specific ELISA against CSFV. The neutralizing antibody response was similar against genotype 2 CSFV strains and OVPV. These viruses showed high identity in the B/C domain of the E2-glycoprotein. Additionally, the molecular diagnostic test for CSFV showed cross-reactivity between CSFV and OVPV. Twelve piglets were OVPV infected either by intranasal or intramuscular route. OVPV generated only mild clinical signs in the piglets, including wasting and polyarthritis. The virus was able to replicate for at least 5 weeks. Viral replication activated the innate and adaptive immunity, evidenced by the induction of interferon-alpha levels early after infection and cross-neutralizing antibodies against CSFV, including humoral response against CSFV E2 and Erns glycoproteins. Close antigenic relation between OVPV and CSFV genotype 2.3 was detected. To determine the protection against CSFV, the OVPV-infected pigs were challenged with a highly virulent strain. Strong clinical, virological and immunological protection was generated in the OVPV-infected pigs. Our findings show, for the first time, the OVPV capacity to infect swine, activate immunity, and the robust protection conferred against CSFV. The phylodynamic analysis showed that CSFV and OVPV emerged from a common ancestor as the result of an inter-species jump of Tunisian sheep virus (TSV) from sheep to pigs, likely favored by human intervention.

P12.9 VEM
Seroprevalence of West Nile virus in wild birds in Central Spain

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West Nile virus (WNV) is a re-emerging virus of the *Flaviviridae* family maintained in nature in an enzootic cycle involving avian hosts and mosquito vectors. WNV affects sporadically humans and horses, causing neuroinvasive disease and even death. In 2004, the first case of WN fever was notified in Spain. Since then, several cases were reported in Andalusia, Castilla-La Mancha, Extremadura, Castilla y León and Catalonia, but to our knowledge, WNV has not been reported from the Community of Madrid. This retrospective study was carried out throughout a year (August 2020 - August 2021) and aims to estimate the seroprevalence of WNV in wild birds in central Spain. Ethic clearance was obtained from Alfonso X University Bioethics Committee (Decision 2022_1/117). WNV seroprevalence was determined by analyzing 159 serum samples collected from wild birds admitted to a wildlife rescue center located in Madrid, using a competition enzymatic assay (Ingenasa, Spain). Thirteen (8.2%) birds showed positive results in the cELISA, while 9.4% of the birds showed doubtful results. Many positive samples were found among migratory birds, indicating that they could introduce the virus to Central Spain. On the other hand, detection of antibodies in fledgling birds and residential species would suggest that those birds could have been exposed to WNV or other related flaviviruses in the Community of Madrid. ELISA test for IgM detection and microneutralization test against different flaviviruses are currently being performed to verify WNV infection and to rule out cross-reactivity with other flaviviruses. This work was supported by Alfonso X el Sabio University Foundation - Banco Santander [project code nr.1.010.716].

P12.10 VEM

Sero-detection of antibodies to Avian Influenza virus in wild birds in Central Spain

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Avian influenza virus (AIV) A has caused major epizootics worldwide. Belonging to the *Orthomyxoviridae* family, AIVs can be divided into two categories based on their ability to cause disease and mortality in poultry: low (LPAI) and high pathogenicity (HPAI). The virus is known to infect a broad range of hosts (mainly poultry and wild birds), causing respiratory disease, and can be fatal in humans. Infection in birds is caused by any type A AIV, mostly belonging to H5 or H7 subtypes. In the 2021-2022 season, 36 outbreaks of HPAI (H5N1) have been reported in different provinces in Spain, including Community of Madrid. This retrospective study aims to estimate the seroprevalence of AIV in wild birds in central Spain. 92 serums samples were collected from August 2020 to August 2021 from wild birds admitted to a wildlife rescue center in Madrid. Ethic clearance was obtained from Alfonso X University Bioethics Committee (Decision 2022_1/117). Seroprevalence of type A AIV was evaluated in wild birds by using a competition assay (Ingenasa, Spain). Two (2,1%) and one (1%) of the birds tested showed positive and doubtful results, respectively, for AIV. Migratory birds could carry disease to different locations, infecting domestic birds and leading to human transmission. Thus, active and passive surveillance and implementation of sanitary precautions are needed to detect and prevent the spread of AIV in Madrid. Further analysis will be performed to assess AIV subtype detected and to estimate the risk of AIV transmission to local birds. This research work was supported by Alfonso X el Sabio University Foundation - Banco Santander [project code nr.1.010.716].

P12.11 VEM

Human microglia cells and brain microvascular endothelial cells are susceptible to Mayaro virus infection

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Mayaro (MAYV) and Una (UNAV) are emerging and poorly studied arboviruses belonging to the *Alphavirus* genus within the *Togaviridae* family. MAYV causes an arthritogenic disease with non-specific symptoms and there is limited information about UNAV infection in humans. Earlier reports have revealed that MAYV infects different human cell lines, including synovial and dermal fibroblasts, chondrocytes, osteoblasts, astrocytes and pericytes, as well as neural progenitor cells. Although MAYV is not associated with neurological sequelae, investigating the virus' capacity to infect specific human brain cells is of particular interest. Microglia are immune cells in the central nervous system (CNS) and play an important role during viral infection and in inflammatory diseases. In addition, brain microvascular endothelial cells are key elements in the blood-brain barrier, a highly selective and semipermeable barrier that prevents free passage of solutes or blood cells into the CNS. The aim of the present study was to evaluate the susceptibility of human microglia cells (HMC3) and brain microvascular endothelial cells (HBEC-5i) to MAYV or UNAV infection. Cytopathic effects, cell viability, viral progeny production and the presence of E1 and nsP1 proteins in HMC3 and HBEC-5i cells infected with different MAYV or UNAV strains were assessed using an inverted microscope, MTT assay, plaque-forming assays and immunofluorescence or immunoblot, respectively. Lastly, the expression of immune response genes was analyzed using qRT-PCR. MAYV and UNAV demonstrated strong cytopathic effects in HMC3 cells and significantly reduced cell viability. Moreover, these alphaviruses efficiently infected HMC3 cells

regardless of the virus strains tested, and E1 or nsP1 viral proteins were detected for all the strains studied. In contrast, only MAYV appeared to infect HBEC-5i cells, and minimal effects on cell morphology or viability were observed. Furthermore, the MAYV titers and viral protein levels were substantially lower in the infected HBEC-5i cells when compared to those of the infected microglia cells. Finally, unlike UNAV, MAYV elicited a strong expression of specific interferon-stimulated genes in microglia cells, along with transcription factors and pro-inflammatory cytokines implicated in the immune response. Collectively, these findings demonstrate that MAYV and UNAV are capable of infecting relevant human brain cells.

P12.12 VEM

Evaluation of replication kinetics in vitro as a method to determine virulence in new Spanish isolates of West Nile Virus

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West Nile virus (WNV) is a zoonotic and neurotropic mosquito-borne flavivirus whose geographical distribution and incidence in hosts (horses, birds, humans) and vectors (*Culex spp.*) have increased significantly in recent decades. In Europe, two different lineages (lineage 1 and lineage 2) are currently circulating, associated with disease outbreaks. In Spain, since 2010 there have been continuous outbreaks in horses in the south-west, together some sporadic cases in humans. In 2020, there was an unprecedented outbreak of WNV meningoencephalitis with 77 human cases and 8 deaths in Andalusia and Extremadura, where only circulation of WNV lineage 1 has been detected. In Catalonia, WNV lineage 2 has been circulating in goshawks and mosquitoes since 2017, but no human cases have been reported. Determination of viral kinetics *in vitro* could be used as a first estimation of virulence, avoiding or reducing the use of animal models. The objective of this work is to determine the correlation between replication *in vitro* and pathogenicity in *in vivo* animal models. An *in vitro* replication kinetics experiment was performed in Vero cells infected at a MOI of 0.01 of WNV isolates from the year 2020: two WNV lineage 1 isolates from a horse and a vulture from Andalusia and two lineage 2 isolates from goshawks from Catalonia. In addition, in a previous *in vivo* mouse model assay carried out in our laboratory, was determined that the two lineage 1 isolates used showed a high virulence, with high mortality and pathogenicity while lineage 2 isolates from Catalonia were considered as of moderate virulence. The moderate virulent strain B956 of lineage 2 was selected as control. The *in vitro* analysis showed that all the Spanish isolates analysed had significantly higher replication kinetics than the strain B956, but without significant differences between them. It was not possible to correlate the results obtained *in vitro* and *in vivo*. Consequently, the replication kinetics assay used is not useful to determine differences in virulence, at least in the Spanish isolates analysed. Further researches are suggested to determine the virulence factors that could be associated with the differences observed. Acknowledgements: Study funded by PID2020-116768RR-C2.

P12.13 VEM

Metagenomics' tools to identify infectious agent genomes in Amazonian Culex sp. and sensitivity study

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Background: Nowadays, humans are exposed to a wide range of infectious diseases, partially caused by the emergence of unrecognized viruses. Specifically, the interaction between humans and animals can facilitate the transmission of viruses, leading to the replication in the human host. Metagenomics based on Next-Generation-Sequencing (NGS) is a powerful methodology which allows the identification of microbial communities and characterization of infectious agents. It has been published that metagenomics allows identifying all pathogens in a natural isolate, but minority genomes are underrepresented or even lost. The study aimed to develop high-throughput metagenomic tools using *Culex* sp. samples by performing deep-sequencing metagenomics, implementing a study of sensitivity, and developing a bioinformatics pipeline for data analysis. Methods: *Culex* sp. mosquitoes captured in the Peruvian Amazonian region were crushed and pooled (25 individuals/pool), amplified using random hexamers and deep-sequenced using the NextSeq2000 platform. A sensitivity study was performed using an Hepatitis C Virus (HCV) clone, which was diluted to $3x10^6$, $1.5x10^5$, $3x10^5$, $3x10^4$, $3x10^3$ molecules, and mixed with duplicates of the pooled mosquitoes. Low quality, short reads and overrepresented sequences were removed with Trimmomatic. Then, reads were normalized and mapped to the host genome for removal of unwanted contamination. Finally, we performed taxonomic classification with kaiju. Results: A total of 9,687,846 reads per pool were obtained and 2,081,199 of them matched with a known genome. The majority of the matched reads belonged to mosquito (C. *pipiens* and C. *quinquefasciatus*) and human, showing animal-human interaction. 19% of the reads belonged to bacteria and 2.6% to viruses. Interestingly, 45,483 reads were classified as Bunyavirales, in which we found *Nairoviridae*, which has been associated with hemorrhagic fever in humans, among others. The sensitivity study confirmed that minority genomes are clearly underrepresented after random priming amplification and NGS sequencing, as we were able to see losses from 0.08% ($3x10^6$ molecules) to 0.00008% ($3x10^3$ molecules). Conclusion: Metagenomics allows to classify highly diverse genomic entities present in a natural isolate and identify potentially zoonotic viruses. In addition, the sensitivity study had proved that minority genomes are underrepresented in the final results, suggesting that these minority genomes cannot be trusted quantitatively.

P12.14 VEM

HPV presence in environmental and clinical samples: a possible transmission through water and food contamination?

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Many viruses that infect humans are excreted in feces, urine or through skin desquamation and could be present in wastewater and potentially be transmitted to the population by water and food contamination. Human papillomavirus (HPV) infects the skin and mucosal epithelia, with effects ranging from benign lesions to malignant carcinomas. Its occurrence has been described before in wastewater and river waters, including high and low-risk oncogenic HPV types. In fact, HPV excretion in the feces of patients with diarrhea has been described, suggesting transmission through fecal shedding of a virus that was believed to be transmitted by direct contact and, therefore, pointing to a possible transmission through contaminated water. The objective of this study was to study and characterize the presence of these viruses along the oral-fecal route and the potential of this via for HPV transmission. For this, wastewater, irrigation water sources, fresh produce and colorectal polyps were analyzed for the presence of HPV by PCR-based methods. Urban wastewater, irrigation water, lettuce, strawberry and parsley samples were analyzed by nested-PCR and amplicon deep sequencing (ADS), whereas colorectal polyp formalin-fixed paraffin embedded (FFPE) samples were analyzed by nested- PCR and Sanger sequencing in PCR positive samples. Wastewater, irrigation water, fresh produce and colorectal polyps showed a prevalence of HPV. ADS demonstrated to be a suitable tool for HPVs detection and in-depth characterization of the diversity of these viral pathogens in fresh produce and water samples. A large diversity of HPV types was observed by ADS in samples from the whole year of observation. All HPV types detected in food samples were also detected in irrigation water sources, indicating that irrigation water is the most probable source of viral pathogens in fresh produce samples analyzed in this study. In colorectal polyps, HPVs were detected in 34,4% of the samples analyzed and the types detected were HPV-6, HPV-66, HPV-20, HPV-21, HPV-23 and HPV-105. The high-risk types HPV-8 and HPV-66 were detected in water samples. These preliminary results suggest that environmental fecal contamination could be a source of viruses with oncogenic potential, which should be confirmed in further studies.

P12.15 VEM

Flavivirus and Phlebovirus analysis in vectors captured from two different areas in South-West Madrid: detection of phleboviruses in sandflies

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Madrid province is one of Spanish areas with an endemic circulation of Toscana virus (TOSV) where acute neurological human cases has been reported. TOSV (genus *Phlebovirus*) has been detected in phlebotomine sandflies in this region, but recently another phleboviruses have been described. West Nile virus (genus *Flavivirus*) (WNV) is the other neurological arbovirus endemic in Spain, which has not been detected in humans in Madrid until now, but WNV circulated in asymptomatic equine populations was described in 2012. No studies about the presence of flavivirus in vectors has been carried out until now. In this study, we collected insects for four stations situated in two different municipalities from South-West Madrid. CDC light traps were using during 2020–2021 summer seasons. Screening for flavivirus and phlebovirus were carried out using two generics RT-Nested-PCRs designed on

the NS5 gene and L-segment of the genome respectively. From the positive pools, the isolation on Vero cells was also attempted. We analyse sixty-nine pools. No flavivirus were detected, but ten phlebovirus positive pools were detected of a total of thirty-five pools of *Phlebotomus perniciosus*. Phylogenetic analysis allowed the identification of two groups of phleboviruses belonging to different serological complexes. One sequence grouped related to Arbia virus (ARBV) from Salehabad complex and nine samples were strongly related to Granada-Massilia-Arrabida like viruses group (GRAV-MASV-ARRAV) belonging to the Sand fly Fever Naples complex and previously described from Spain, France and Portugal respectively. Three isolates from the second group were achieved after inoculation of the sandfly pools homogenates in Vero E6 cells. Our study confirms the presence and high circulation of two phleboviruses in the studied area: Arbia-like and GRAV-MASV-ARRBV-like viruses in a 2.9% and 25.7% respectively of the analysed sandflies. GRAV was detected for the first time in 2003 in Spain and seroprevalence studies revealed that could infect humans with most cases being asymptomatic. A molecular characterization in the M-segment and complete genome is necessary to carried out an in-deep classification of the detected viruses. The implications in human health until now is unknown and seroprevalence studies in human population in this region are necessary.

P12.16 VEM
Monkeypox in Spain: management of the epidemic at the National Center of Microbiology

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Monkeypox virus (MPXV) is an orthopoxvirus that circulates in some African countries. Most of the African cases have been a result of spillover from animal reservoirs to human. Sporadically infections have been described in travellers returning from endemic regions. In 2003, the importation of infected reservoirs was the cause of the biggest outbreak described outside Africa until now with 47 human cases. In 2022 UK reported an imported case from Nigeria on the 6th of May. On the 12th they confirmed two cases without any epidemiological link with the imported case or with MPXV endemic areas. On the 16th, 4 additional cases without any link to the rest of cases and without travelling history to endemic regions were reported. In Spain, seven cases were suspected on the 17th of May. Samples from the patients were sent to the National Reference Laboratory for zoonosis. A generic real- time PCR for orthopoxviruses was used. The sequence of the amplicon obtained with a conventional nested-PCR was analyzed to confirm that the virus was MPXV belonging to the West African clade. From then, 525 samples have been assayed corresponding to 471 patients. A positive result was obtained for 257 samples corresponding to 239 patients. Samples from skin lesions were sent as the best choice for diagnostic. In 24 patients with a positive result, more than one sample was tested. In 17 patients, two lesions were sent. We have two patients whose result turned to negative 2 and 3 days apart from the first sample and two patients whose result were negative and turned to positive 4 and 6 days later, respectively. The rest were positive with a maximum separation of 8 days between samples. Sera samples were sent in 5 cases and nasopharyngeal swab in 2. Sera samples were positive in 3 cases and nasopharyngeal swabs were all negative. Although more studies are needed, this study confirms that the best choice for diagnostic are samples from skin lesions where high virus load are detected.

P12.17 VEM
Detection of Dengue virus genome in dried blood samples

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Dengue virus (DENV) is an emerging flavivirus transmitted by *Aedes* mosquitoes that can establish urban cycles because humans are amplifiers hosts. The virus is endemic in tropical and sub-tropical areas and around 390 millions of people are infected every year. The clinical picture varies from subclinical infection to severe cases that can cause the death of the patient. One of its main vectors is *Aedes albopictus* that is established in many regions in Europe. In Spain it was detected in 2004 in Barcelona and now it is spread in all the Mediterranean regions although it has been detected also in many regions far away from the coast. The first authochthonous cases in Europe were detected in Croatia and France in 2010 and, since then, France has been reporting cases almost every year. In Spain the first detection of non travelled related circulation of DENV was reported in 2018 with 5 cases affected in Murcia. Later, one case in 2018 and another one in 2019 were reported in Barcelona. This facts point out the necessity of good surveillance protocols. Currently, following Spanish national surveillance protocols, the detection of a viremic case of dengue, chikungunya or zika in an area where *Ae albopictus* is established, triggers entomological and epidemiological surveillance.. We have developed a method to support the surveillance of this infection since dried blood samples can be easily obtained. A method for the molecular detection of DENV using dried blood samples have been optimized and validated with clinical samples. The developed protocol has been used in the surveillance of cases and contacts in Barcelona.

P12.18 VEM

Characterization of the antiviral activities of Mayaro virus NSP2 protein and its regulation

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Mayaro virus [MAYV] is an emerging alphavirus causing Mayaro virus disease in humans. MAYV is transmitted by mosquitoes, and is currently expanding geographically due to the spread of its vector. The Mayaro virus disease is similar to that caused by dengue or chikungunya virus and it is characterized by chronic polyarthritis, neurological complications, hemorrhages, myocarditis and even death. There are no licenced vaccine or clinically proven therapies available, and the cellular and molecular mechanisms of the disease are unclear. MAYV genome encodes for a polyprotein that is cleaved to produce four non-structural proteins: NSP1-NSP4. Mayaro NSP2 is the protease required for the processing of the polyprotein and it is considered a key target for the design of strategies against the virus. Therefore, characterization of Mayaro NSP2 might lead to development of prophylactics or therapeutic means against Mayaro infections. Here we characterized the subcellular distribution of NSP2 and identified its post-translational regulation through its conjugation to the small ubiquitin-like protein SUMO. In addition, we identified two novel functions of the protein: the inhibition of the cGAS/STING signalling pathway and the control of SUMOylation. In summary, we have identified novel functions for Mayaro NSP2 and a mechanism for regulating the protein that can be used for the design of antiviral inhibitors against the virus.

P13 HVI

P13.1 HVI

Enveloped and non-enveloped hepatitis E virus in faeces and plasma

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Hepatitis E virus [HEV] has been known as the leading cause of acute human viral hepatitis worldwide being common in low- and middle-income countries, and with increasing incidence in industrialized countries. In most cases,

hepatitis E infection causes and acute self-limiting and asymptomatic infection that resolves within 2–8 weeks. HEV has a linear, positive-sense, single stranded RNA genome of around 7.2kb in length. HEV was considered a nonenveloped (neHEV) icosahedral spherical virus with a diameter of approximately 27–34 nm from the discovery in 1983 and the cloning and genome sequencing in 1991 until the finding of plasma circulating virions associated with a membrane "quasienveloped" (eHEV). In our study, paired plasma and faeces samples from two HEV chronic patients who were previously treated with rivabirine were collected. Iodixanol gradient technique was applied to isolate the two viral forms by density, obtaining 11 fractions from each sample. Viral RNA was purified and viral load assessed from each fraction. Finally, a segment of the open-reading frame 2 (Orf2) was amplified and sequenced using next-generation sequencing (NGS) MiSeq Illumina platform. Results showed that both type of samples presented virions with different densities, carrying plasma mainly low-density HEV while feces high-density ones. Surprisingly, we have detected a peak of low-density viral particles in feaces, that has not previously reported. Regarding phylogenetic and quasispecies analysis, 30% of low-density HEV virions isolated from plasma and feces paired samples were identical, suggesting that they have a common origin and we discuss that they might have been produced outside of the liver. This study could contribute to change the way HEV is approached and analyzed but further research is needed.

P13.2 HVI

Normalization of circulating plasma levels of miRNAs in HIV-1/HCV co-infected patients following direct acting antiviral-induced sustained virologic response at week 12

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Individuals with hepatitis C virus (HCV) infection who achieve a sustained virological response (SVR) on direct acting antivirals (DAAs) still need to be monitored for signs of liver disease. Therefore, the identification of disease progression biomarkers is needed. We recently identified a signature of three plasma circulating microRNAs (miRNAs) (miR-100-5p_iso3p:-2, miR-122-5p and miR-192-5p) that highly correlate with liver fibrosis progression in human immunodeficiency virus type 1 (HIV-1)/HCV co-infected patients. Herein, we aimed to explore whether circulating levels of these three miRNAs could be linked to liver disease progression in HIV-1/HCV co-infected patients who have achieved HCV SVR at week 12 after DAA treatment. A total of 81 chronic HIV-1/HCV co-infected patient were enrolled for a longitudinal study performed at baseline (T0) of DAA therapy and after 12 weeks (T12) of concluding treatment. Patient clinical parameters and liver fibrosis stage were controlled at T0, T12 and after two years of achieving SVR. After DAA therapy 77 subjects (95%) reached SVR. Transient elastography at T0 showed that most of the study subjects were in an advanced stage of liver fibrosis (F0-1 9%, F2 11%, F3 32%, F4 48%). At T0, significantly higher levels of miR-100-5p_iso3p:-2 and miR-122-5p ($p=0.0119$ and $p=0.0008$, respectively) were observed in individuals carrying the patatin-like phospholipase domain-containing 3 (PNPLA3) I148M variant polymorphism. At T12, SVR was significantly associated with a reduction of the number of circulating miR-100-5p_iso3p:-2, miR-122-5p and miR-192-5p ($p<0.0001$, $p<0.0001$ and $p=0.0003$, respectively) in the overall cohort and in subjects with F3-4 liver fibrosis ($p<0.0001$, $p<0.0001$ and $p=0.0011$, respectively). Two years after SVR, HCV cured patients significantly reduced their liver stiffness ($p<0.0001$). Liver fibrosis reduction was not detected in the group of not responders ($p=0.5000$). Remarkably, at T12 no significant reduction of miRNA levels was observed in individuals that did not achieve SVR ($p=0.8750$, $p=0.1250$ and $p=0.1260$, respectively). Our results demonstrate that DAA-induced SVR at week 12 is linked with a significant reduction in circulating levels of liver disease-associated miRNAs. Circulating miRNA levels may provide a valuable surrogate biomarker of liver fibrosis progression in HIV-1/HCV co-infected patients following HCV cure with DAAs.

P13.3 HVI

Amino acid substitutions associated with treatment failure of hepatitis C virus infection

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Despite the high sustained virological response rates achieved with current directly-acting antiviral agents (DAAs) against hepatitis C virus (HCV), around 2% to 5% of patients do not achieve such a response. Identification of amino acid substitutions associated with treatment failure requires analytical designs, such as subtype-specific ultra-deep sequencing (UDS) methods for HCV characterization and patient management. By deep sequencing analysis of 220 subtyped HCV samples from infected patients who failed therapy, collected from 39 Spanish hospitals, we determined amino acid sequences of the DAA-target proteins NS3, NS5A and NS5B, by UDS of HCV patient samples, in search of resistance-associated substitutions (RAS). Using this procedure, we have identified six highly represented amino acid substitutions (HRSs) in NS5A and NS5B of HCV, which are not *bona fide* RAS. They were present frequently in basal and post-treatment virus of patients who failed therapy to different DAA-based therapies. Contrary to several RAS, HRSs belong to the acceptable subset of substitutions according to the PAM250 replacement matrix. Coherently, their mutant frequency, measured by the number of deep sequencing reads within the HCV quasispecies that encode the relevant substitutions, ranged between 90% and 100% in most cases. Also, they have limited predicted disruptive effects on the three-dimensional structures of the proteins harboring them. The information on HRSs that will be gathered during sequencing should be relevant not only to help predict treatment outcomes and disease progression but also to further understand HCV population dynamics, which appears much more complex than thought prior to the introduction of deep sequencing.

P14 RIN

P14.1 RIN

A new screening method to search for HCMV antigens that may be useful for vaccine development.

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The development of a vaccine against HCMV is as a worldwide priority. Most of the vaccine formulations used until date have mainly used the envelope proteins gB, gH and the tegument proteins pp65 and IE1 obtaining partial protection against HCMV infection. Given the high tropism of HCMV that include fibroblasts, endothelial cells, epithelial cells, and cells of the myeloid lineage and the use of multiple entry mechanisms, it is likely that other additional proteins may be involved in viral entry, and it may be necessary to be targeted in order to elicit a complete protection against infection. To address this issue, our group have used bioinformatic analysis using the genome from 9 different HCMV strains, and have identified 39 proteins with putative transmembrane domains (TM) as new potential viral envelope proteins that may be involved in virus-cell interaction during infection. The open reading frame of 18 putative TM proteins were cloned in a eukaryotic vector and expressed in HEK 293T cells, and cell lysates were used in western blot using as primary antibody serum samples from solid organ transplant (SOT) patients who have developed HCMV specific antibodies. Five of the proteins analysed (UL6, UL10, UL57, UL100 and UL132) were recognized by several patient serum samples. While UL6 and UL10 are TM proteins with unknown function, UL100 is the envelope glycoprotein M, UL132 is essential for the formation of the HCMV assembly compartment and efficient production of infectious particles, while UL57 participates in the initiation of the viral replication. Our results demonstrate that this is an optimal approach for the identification of immunogenic HCMV proteins that are able to elicit an antibody response *in vivo*. Further mice immunization is ongoing to analyse their potential as vaccines candidates.

P14.2 RIN

Swine T-cells and specific antibodies evoked by peptide dendrimers displaying different FMDV T-cell epitopes

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Dendrimeric peptide constructs based on a lysine core that comprises both B- and T-cell epitopes of foot-and-mouth disease virus (FMDV) have proven to be a successful strategy for the development of FMD vaccines. Specifically, B_2T dendrimers displaying two copies of the major type O FMDV antigenic B-cell epitope located on the virus capsid [VP1 (140-158)], covalently linked to a heterotypic T-cell epitope from either non-structural protein 3A [3A (21-35)] or 3D [3D (56-70)] named B_2T-3A and B_2T-3D, respectively, elicit high levels of neutralizing antibodies (nAb) and IFN-γ-producing cells in pigs. To assess whether the inclusion and orientation of T-3A and T-3D T-cell epitopes in a single molecule could modulate immunogenicity, dendrimers with T epitopes juxtaposed in both possible orientations, i.e., constructs B_2TT-3A3D and B_2TT-3D3A, were made and tested in pigs. Both dendrimers elicited high nAb titers that broadly neutralized type O FMDVs, although B_2TT-3D3A did not respond to boosting, and induced

lower IgG titers, in particular IgG2, than B_2TT-3A3D. Pigs immunized with B_2, a control dendrimer displaying two B-cell epitope copies and no T-cell epitope, gave no nAb, confirming T-3A and T-3D as T helper epitopes. The T-3D peptide was found to be an immunodominant, as it produced more IFN-γ expressing cells than T-3A in the *in vitro* recall assay. Besides, in pigs immunized with the different dendrimeric peptides, $CD4^+$ T-cells were the major subset contributing to IFN-γ expression upon *in vitro* recall, and depletion of $CD4^+$ cells from PBMCs abolished the production of this cytokine. Most $CD4^+$IFN-γ^+ cells showed a memory ($CD4^+2E3^-$) and a multifunctional phenotype, as they expressed both IFN-γ and TNF-α, suggesting that the peptides induced a potent Th1 pro-inflammatory response. Furthermore, not only the presence, but also the orientation of T-cell epitopes influenced the T-cell response, as B2TT-3D3A and B_2 groups had fewer cells expressing both cytokines. These results help to understand how B_2T-type dendrimers trigger T-cell populations, highlighting their potential as next-generation FMD vaccines.

P14.3 RIN

Evaluation of the Immune Crosstalk between West Nile Virus and a Live-Attenuated Zika Virus Vaccine Based on Modified Vaccinia Virus Ankara

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Zika virus (ZIKV) is a flavivirus transmitted primarily by *Aedes* mosquitoes. Symptoms, usually mild, include fever, skin rashes, conjunctivitis, muscle and joint pain, malaise, and headaches. Most infected people are asymptomatic, but infection during pregnancy can cause microcephaly and other congenital malformations, referred as Congenital Zika Syndrome. The infection is also associated with other pregnancy complications, such as premature birth and miscarriage. In children and adults, the infection is associated with an increased risk of neurological complications, such as Guillain-Barré syndrome, neuropathy, and myelitis. The first local ZIKV infections in Europe have recently been described, together with the expansion of one of its vectors (*Aedes albopictus*), alerting us to be focused to future ZIKV outbreaks in this continent. In the development of ZIKV vaccines, there is significant concern for what is known as flavivirus-disease exacerbation by antibody-dependent enhancement (ADE) of infection, which could be induced by immune cross-reactivity when multiple flaviviruses circulate and it is due to the antigenic similarities of these flaviviruses. Until now, West Nile virus (WNV) is the main medically relevant flavivirus circulating in the Mediterranean basin. Therefore, anticipating the potential scenario of ZIKV emergency vaccination in areas of Europe where WNV is endemic, we have evaluated the cross-reactivity between WNV and our previously developed ZIKV vaccine candidate based on modified vaccinia virus Ankara (MVA) vector expressing ZIKV structural proteins (MVA-ZIKV). To this end, mice were first immunized with MVA-ZIKV, subsequently challenged with WNV, and then tested for ZIKV- and WNV-specific immune responses and protection against WNV. Our results indicate low cross-reactivity between the MVA-ZIKV vaccine candidate and WNV and the absence of ADE, supporting the safety of this ZIKV vaccine candidate in areas where the circulation of WNV is endemic.

P14.4 RIN

Immune responses against Crimean-Congo Hemorrhagic Fever Virus after immunization with CCHFV-nucleoprotein delivered by muNS-Mi nanospheres and modified vaccinia virus Ankara

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The Crimean-Congo Hemorrhagic Fever Virus (CCHFV) presents a problem for public health due to its high mortality in humans, the form of transmission and the difficulty of treatment, in addition to the lack of vaccines. This negative sense RNA virus has 3 chains that are named according to their size as L (large), M (medium) and S (small), the last one being the one that codes for the nucleocapsid N protein (Np). In our laboratory we have developed a molecular labeling system (IC-tagging) derived by the muNS protein of avian reovirus. This methodology allows us to produce protein microspheres (MS) or nanospheres (NS), that are easily purifiable that can be loaded with different proteins of interest. The particulate material formed by the MS are able to produce adjuvant-free immune response, as has been demonstrated previously in studies with Bluetongue Virus (BTV) and African Horse Sickness Virus (AHSV). In this study, we use the IC-tagging system to express and purify nanospheres (NS) in a bacterial expression system that we loaded with the CCHFV nucleoprotein (Np). Finally, we have studied the immune responses induced by BALB/c mice immunized with NS loaded with the CCHFV-Np and the effect of adding a booster dose with a modified vaccinia Ankara strain (MVA), encoding the same nucleoprotein construct. We analyzed the antibody response after each immunization and evaluated the T cellular immune response elicited by ex vivo intracellular cytokine staining (ICS) assays against 5 peptides of the nucleoprotein. Both immunization strategies generated a specific antibody response with an increase in the magnitude of the antibody responses after MVA boosting. We also detected a CD8+ IFNγ+ T-cell specific response in the presence of several peptides in mice immunized with the prime-boost strategy. In conclusion, these results confirm the efficacy of an immunization regime based in on the use of NS-Np and MVA viral vectors to elicit a humoral and cellular responses and their potential use as a subunit vaccine candidate against CCHFV.

P14.5 RIN

Oral vaccine based on Ebola-VLP particles induces humoral immunity in mice

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Several vaccine candidates against ebolavirus have been developed based on different platforms for the expression of ebolavirus antigens, such as DNA, recombinant proteins, VLPs, and replicating and nonreplicating viral vectors. VLPs (Virus Like Particles) depend on the natural ability of the viral proteins to self-associated and form particles that are morphologically similar to the live virus. In this pilot study, we generated and characterized the immunogenicity of a novel vaccine against EBOV based on VLP that only contains the full-length GP, VP40 and NP proteins of Ebola virus strain Mayinga (no adjuvant was used), and compared the immune response of C57BL/6 mice with

intramuscular or oral vaccine administration (PROEX 225.1/21). For these purpose, two groups of female C57BL/6 mice (8 week-old) were immunized intramuscularly (n=4) or orally (n=8) with 30 or 300μg/dose of Ebola-VLP respectively. A total of three VLP doses were administered with an interval of 2 weeks between each dose. Blood was taken at 0, 14, 28, 48 and 49 days from each animal and tested for the presence of immunoglobulins and for viral neutralizing antibody. Specifically we analized the production of total IgG antibodies anti full-GP; deltaMucin-GP; NP or VP40- from animal serum along time by ELISA sensitizing the plate with recombinant Ebola proteins produced in our laboratory. Neutralization assays were carried out using viral pseudotypes based on VSV with Ebola-Mayinga virus envelope and a luciferase reported gene, mixed with serial dilutions of serum samples (1:25 to 1600) on VERO E6 cells. Neutralizing antibody titer was calculated as the highest dilution that inhibits 50% of the luciferase signal. Our results indicate that total levels of IgG against Ebola GP and NP proteins increased significantly along the immunizations, being more pronounced from the third immunization, both in mice immunized via intramuscular and orally. In contrast, no significant increase in IgG levels against the VP40 protein was observed in none of the two groups. Specific neutralizing titer also increased along the immunization in both groups of oral and intramuscular vaccination. Oral VLP vaccination against Ebola virus infection is a promising strategy for rapid protection against emerging pathogens.

P14.6 RIN

Progression of the viral defense response in the C. elegans-Orsay virus pathosystem

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All living organisms are infected by viruses, the most abundant biological entities on the planet. To protect themselves against viruses most organisms have developed some type of immune system. The innate immune system, present in all multicellular organisms, allows cells to recognize pathogen derived molecules and trigger rapid defense responses. Viruses try to avoid the host's defense by evolving mechanisms to avoid or inactivate various steps of the defense response. The race between viruses and their host have led to the evolution of sophisticated host-virus interactions and defense mechanisms. Here we aimed to shed light into host-virus interactions by following the defense response to viral infection over time. The pathosystem used for these studies was the *C. elegans*-Orsay virus (OrV) pathosystem. OrV, the only identified natural virus of *C. elegans*, is a rapidly-evolving positive-strand RNA virus that replicates inside *C. elegans* intestinal cells. Upon inoculation of *C. elegans* with OrV we observed that the progression of the viral infection could be differentiated into four different stages: a pre-infection phase, a phase of exponential viral replication, a phase of host-pathogen conflict where the accumulation of virus was stalled and, for most worms, a phase of resolved infection. Moreover, we observed that the amplification of RNA2, which contains the capsid protein, was delayed to the amplification of RNA1, which contains the RNA-dependent RNA polymerase. In order to identify genes involved in the defense response along the different infection phases we analyzed the transcriptomic response to infection over time. We selected twelve timepoints, spanning across all larval stages and the four phases of the infection. As a result, we obtained a rich time-sensitive transcriptomic landscape of the defense response, of great use to determine the role of different proteins in the defense response. Finally, we were able to characterize the short-term evolution of Orsay virus, shedding light into the evolution of the virus.

P14.7 RIN

Infection with an attenuated virus promotes the emergence of resistant cells and allows systematic analysis of antiviral pathways

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The identification of antiviral genes is an important goal with fundamental and practical implications. This has been frequently addressed by measuring transcriptional changes in infected cells, as well as by performing siRNA or CRISPR screens. Here, we propose an alternative strategy based on infection with an attenuated virus, which allows cells to undergo a transcriptional reprogramming that confers resistance to infection. Using an attenuated vesicular stomatitis virus (VSV-D51) as a selection agent, we readily obtained stable resistances in both melanoma (B16) and carcinoma (CT26) mouse cell lines, whereas no resistances could be rescued in cells infected with wild-type VSV. RNA-seq analyses demonstrated that resistance was sustained by an expression shift in over 1000 genes. Overexpression of a large number of interferon-regulated genes (IRGs) indicated that the main mechanism involved was constitutive activation of cellular innate immunity. Although many of the top-upregulated genes have been previously associated with antiviral roles, we found overexpression of other that have not, suggesting possible undescribed functions for these genes. Interestingly, the selected cells were also resistant to wild-type VSV, and the nature of the activated genes suggests general mechanisms of resistance that should be also effective against different types of viruses.

P14.8 RIN
European sea bass defensin beta genes are modulated upon NNV infection and vaccination

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Aquaculture is one of the most prosperous economic sectors. Nevertheless, the natural outbreaks of several infectious diseases make the sector to deal with important economic losses. One of the most important pathogens in the Mediterranean Sea is nodavirus (NNV). NNV is the agent causing viral encephalopathy and retinopathy in more than 170 fish species including some of the most impact in Spanish hatcheries as European sea bass (*Dicentrarchus labrax*). Antimicrobial peptides (AMPs) are short aminoacidic sequences which constitute important mediators of the innate immune response in teleost fish. AMPs can kill directly a broad range of pathogens such as bacteria or viruses and modulate the host immune response leading to a more effective clearance of pathogens. These properties along with the world emergency in antimicrobial resistance (AMR) make AMPs good candidates to replace traditional antimicrobials. Therefore, we aimed to analyze the molecular regulation of European sea bass defensin beta genes upon NNV infection and after NNV vaccination. To achieve our objective, European sea bass were infected with NNV and samples of head-kidney (HK), brain and gonad were taken. In addition, gonadal cells from healthy males were *in vitro* stimulated with NNV. Then, ovary samples of control females or vaccinated against NNV (pBAD vaccine) were taken, as well as fertilized eggs and larvae from the same groups. To support our data, we also analyzed in silico the potential antiviral activity of the protein encoded by the studied genes. Our results show that defensin beta 1 gene is up-regulated upon in vivo NNV infection even if in the *in silico* study showed the lesser predicted activity. Interestingly, the *in vitro* NNV challenge resulted in no variation of the defensin beta 1 gene, while defensin beta 2.1 and 2.2 genes were blocked upon this stimulus. Strikingly, females vaccinated with pBAD greatly down-regulate defensin beta 2.2 expression. Moreover, larvae from vaccinated mother up-regulate all defensin beta genes. In conclusion, defensin beta 1 gene seems to be involved in the defense against viruses while defensin beta 2 genes appear to possess a more specific function in gonad.

P14.9 RIN
Bacterial coinfections meta-analysis in influenza patients

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Increased morbidity and mortality after Influenza virus infections are leading concerns in public health. Clinical complications associated with influenza have been attributed to factors such as age, pregnancy, immunosuppression, or underlying respiratory diseases. However, influenza severity is often associated with bacterial coinfections or superinfections. Studies that integrate bacterial complications in the influenza context require an actualization. Here, we performed a systematic review and a meta-analysis of the characteristics and risk factors associated with bacterial infections in influenza patients. In this study, 132 studies with data from more than 48000 influenza hospitalized patients were included. Of them, more than 5000 patients were reported to have bacterial coinfections. These findings have been validated afterwards using an independent TrinetX database cohort of almost 4 million patients. Above all risk factors, our analysis indicates that hematological diseases and coinfection pose around a threefold higher risk of mortality compared to influenza infection alone. Although asthma has been considered a high-risk morbidity/mortality factor associated with influenza, the analysis presented here unexpectedly reveals that asthma is a mortality protective factor in influenza infections. Analysis also indicates a negative correlation between asthma and bacterial coinfection rates, indicating a protective role of asthma against coinfections and their associated complications. Similarly, influenza virus vaccination showed to significantly decrease both bacterial coinfection and death up to one year. Our study allows a better understanding of the clinical implications during coinfections compared to other comorbidities in influenza patients, which are critical to further addressing influenza complications and in predicting influenza severity at early stages of the disease.

P14.10 RIN
The spatiotemporal dynamics of the type I IFN response determine viral infection outcomes

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A fundamental unresolved issue in host-virus interaction is when and how the fate of a viral infection is determined. Here we show that type I Interferons (IFN-I) play a role. Using the lymphocytic choriomeningitis virus (LCMV) infection model of mice, we found that acute-infected mice generate a 2-waves IFN-I response while chronic-infected mice generate only a 1-wave response. Temporal analyses revealed that the 2nd IFN-I response is produced by CD169+ macrophages in the splenic marginal zone and the early disapperance of these cells accounts for the single-wave IFN-I response in chronic infection. We characterize a polyfunctional role for the 2nd IFN-I response, bridging innate and adaptive immunity: it induces inflammatory macrophage and virus-specific T cell responses, finally leading to virus clearance. In chronic infection, these ordered events are deregulated: CD8 T cell-mediated destruction of the splenic marginal zone disrupts the IFN-I response and the subsequent IFN-I-dependent events. Altogether this work illustrates the complexity of the sequential events that act coordinately in response to virus infection and unmasks early events that drastically influence the infection outcome.

P14.11 RIN
Towards a universal therapeutic vaccine against chronic virus infection

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Chronic virus infections like those with Human Immunodeficiency Viruses (HIV) and Hepatitis B (HBV) and C (HCV) viruses continue to threaten global health. A common feature of these infections is the persistence of virus antigen and the associated exhaustion of virus-specific T lymphocytes. Although the latter reduces immune-cell-mediated pathology, it is associated with a reduction of virus control that enables antigen persistence and has per se pathological consequences. Aim of our project is to identify universal immunological ways for shifting the dynamic virus - host immune system balance of a chronic virus infection into a state in which the virus is sufficiently controlled without causing pathology. To achieve this aim, we are testing diverse immunological regimen for their impact on virus control in the mice model of chronic lymphocytic choriomeningitis virus (LCMV) infection. Until now, we have shown that anti-PDL1-mediated reinvigoration of exhausted T cells and viral load reduction can be improved by the addition of the TLR7/8 and TLR3 agonists R848 and Poly I:C, respectively. Analyses with further agonists are in progress. This work is supported by a grant from the La Caixa banking foundation [HR17_00199].

P14.12 RIN

Cross-protection among porcine enteric coronavirus

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Porcine epidemic diarrhea virus (PEDV) causes a highly contagious enteric disease. Recently, we have demonstrated that *Swine enteric coronavirus* (SeCoV), a chimeric virus from PEDV and another porcine coronavirus, was circulating in Spain, and that, currently, one of the most PEDV frequent variant is a recombinant virus from PEDV and SeCoV (rPEDV-SeCoV). In farms, PEDV recurrent infections are common, due to 1) Entries of variants/virus other than those detected on the farm, against which there would be no cross-protection or, 2) Existence of negative subpopulations (due to the entry of new animals without immunity, or to the presence of animals that were infected at an early age and developed immunity -or that acquired it via colostrum- and have lost it over time). To assess how long homologous immunity lasts and the degree of heterologous protection among different variants of enteric coronavirus two studies were developed. Clinical signs, viral shedding, and immune responses were evaluated. First Study: 75 piglets were orally inoculated (0 days post-inoculation; dpi) with PEDV. On 154 dpi, animals were homologous challenged. All inoculated animals seroconverted for neutralizing antibodies (NA). Despite that, PEDV was detected in feces of all pigs, indicating that the homologous immunity did not prevent the reinfection. Second study: 48 piglets were orally inoculated (Group 1: control; Group 2: PEDV; Group 3: SeCoV; and Group 4: rPEDV-SeCoV). On 20 dpi, all groups were challenged with rPEDV-SeCoV: Group 1 challenged for first time (control-rPEDV-SeCoV); Group 2 and 3 heterologous challenge (PEDV-rPEDV-SeCoV and SeCoV-rPEDV-SeCoV, respectively): Group 4: homologous challenge (rPEDV-SeCoV-rPEDV-SeCoV). After the first challenge, the three viral strains induced an undistinguishable mild-to-moderate clinical disease. After the second challenge, pigs subjected to homologous challenge did not show clinical signs or lesions and viral shedding was only detected in a single animal. Homologous protection was probably due to NA. In contrast, prior exposition to PEDV or SeCoV only provided partial cross-protection (lower for SeCoV), allowing for rPEDV-SeCoV replication and viral shedding in feces. Taken together, the results indicated that sterilizing immunity exist but in a short term, and that cross-protection did not prevent infection and viral shedding.
Project: E-RTA2015-0003-C02-01/02.

P14.13 RIN

Early and long-term HIV-1 immunogenicity induced in macaques by the combined administration of DNA, NYVAC and Env protein-based vaccine candidates: The AUP512 Study

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To control HIV infection there is a need for vaccines to induce broad, potent and long-term B and T cell immune responses. With the objective to accelerate and maintain the induction of substantial levels of HIV-1 Env-specific antibodies and, at the same time, to enhance balanced CD4 and CD8 T cell responses, we evaluated the effect of concurrent administration of MF59-adjuvanted Env protein together with DNA or NYVAC vectors at priming to establish if early administration of Env leads to early induction of antibody responses. The primary goal was to assess the immunogenicity endpoint at week 26. Secondary endpoints were (i) to determine the quality of responses with regard to RV144 correlates of protection and (ii) to explore a potential impact of two late boosts. In this study, five different prime/boost vaccination regimens were tested in rhesus macaques. Animals received priming immunizations with either NYVAC or DNA alone or in combination with Env protein, followed by NYVAC + protein or DNA + protein boosts. All regimens induced broad, polyfunctional and well-balanced CD4 and CD8 T cell responses, with DNA-primed regimens eliciting higher response rates and magnitudes than NYVAC-primed regimens. Very high plasma binding IgG titers including V1/V2 specific antibodies, modest antibody-dependent cellular cytotoxicity (ADCC) and moderate neutralization activity were observed. Of note, early administration of the MF59-adjuvanted Env protein in parallel with DNA priming leads to more rapid elicitation of humoral responses, without negatively affecting the cellular responses, while responses were rapidly boosted after repeated immunizations, indicating the induction of a robust memory response. In conclusion, our findings support the use of the Env protein component during priming in the context of an heterologous immunization regimen with a DNA and/or NYVAC vector as an optimized immunization protocol against HIV infection.

P14.14 RIN
Counteraction of the cGAS/STING pathway by the KSHV protein LANA2 requires its interaction with SUMO

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cGAS detects intracellular DNA signals through the adapter protein STING to trigger antiviral response to DNA viruses. Several viruses prevent the activation of the cGAS/STING pathway by different mechanisms. Oncogenes of DNA tumor viruses such as E7 from human papillomavirus (HPV) or E1A from Adenovirus have been identified as inhibitors of the cGAS/STING pathway. Inhibition of this pathway requires the LXCXE domain, involved in the inhibition of the pocket proteins by E1A and E7. Our lab has demonstrated that the KSHV protein LANA2 also contains an LXCXE domain and that this motif is required to interact with and to inhibit the pocket proteins. Here we analyzed whether LANA2 can also inhibit the cGAS/STING signaling pathway. Our results reveal that LANA2 antagonizes the CGAS/STING pathway. However, this inhibition does not require the LXCXE domain in LANA2 but it involves the non-covalent interaction of LANA2 with SUMO. Our results point that SUMO may act as a bridge between LANA2 and STING.

P14.15 RIN

Generation of the MVA-ISG15 vector as a potential adjuvant in HIV immunization protocols.

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The first line of defense against viral infection is the immunity system, the host virus infected cells secreted cytokine mediator know as Interferon type I (IFN-I) and one of the IFN stimulated genes (ISGs) is the IFN-stimulated gene 15 (ISG15). ISG15 is a small ubiquitin-like protein which plays a central role in the antiviral response of the host organism but its role as an immunomodulator in the vaccine field remains to be defined. ISG15 exists in several forms: either intracellular, covalently, and non-covalently conjugated to target proteins, or released into serum. We showed that ISG15 exerts an immunomodulatory role in Human Immunodeficiency virus (HIV) vaccines using a DNA prime/MVA boost immunization protocol, with an increase in the potency and the quality of the HIV-1 Env-specific CD8 T cell response. Moreover, the amount of gp120 vector used to immunize mice could be reduced 5-fold when combined with the ISG15 plasmids without affecting the potency and quality of the HIV-1 Env-specific immune responses. These results highlight the necessity to generate a novel ISG15-based vaccine, the MVA ISG15 that could elicit an improved viral antigen presentation to the immune cells resulting in the development of a new optimized vaccine candidate. This work was funded by the Spanish State Research Agency (Agencia Estatal de Investigación, AEI).

P14.16 RIN

Potency and durability of T and B cell immune responses with high broadly neutralizing antibody recognition after prime/boost combinations with recombinant VSV-GP, DNA or NYVAC vectors expressing the novel HIV-1 Env clade C membrane-bound trimeric gp140:G Δ6 ConCv5 KIKO protein

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The generation of a vaccine against HIV-1 able to elicit long-lasting protective immunity remains a major challenge in the HIV field. Here, we have designed and selected new HIV-1 Env immunogens with specificity against broadly neutralizing antibodies (bnAbs) and expressed these antigens from non-viral and viral platforms. DNA-, VSV-GP- and

NYVAC-based recombinant vectors were generated expressing stabilized HIV-1 clade C sC23v4 or ConCv5 KIKO trimers after optimization of the soluble gp140 proteins for membrane-bound expression. In cultured cells, the different recombinant vectors express the HIV-1 gp140:G Δ6 KIKO trimeric proteins at the cell membrane of transfected (DNA) or infected (VSV-GP and NYVAC) cells in a conformation that is differentially recognized by human bnAbs, with ConCv5 KIKO trimer adopting a more closed and native-like gp140:G Δ6 than sC23v4 KIKO construct. To define to what extent the best-in-class vector improves T and B cell responses, we evaluated in mice the immunogenicity profile induced by HIV-1 gp140:G Δ6 ConCv5 KIKO protein when expressed from DNA, VSV-GP or NYVAC vectors, followed by two consecutive boosts with the soluble trimeric gp140:G Δ6 protein. In immunized mice, the prime/boost combination of VSV-GP-ConCv5/NYVAC-ConCv5 vectors is the most effective regimen for the induction of HIV-1 Env-specific CD4 T cells in the absence of a protein boost. The subsequent administration of trimeric Env protein during boosts maintained the T cell activation levels between groups. The analysis of the HIV-1-specific humoral immune responses elicited by the different immunization groups following protein boosts, showed that the prime/boost protocols induced preferentially Th1-associated IgG2a subclass and that these antibody levels remained high during the memory phase. In summary, our data demonstrated that the novel HIV-1 gp140:G Δ6 ConCv5 KIKO protein should be taken into consideration as an optimized immunogenic component against HIV-1, and when expressed from DNA, VSV-GP or NYVAC vectors.

P14.17 RIN

Enhancement of the HIV-1-Specific Immune Response Induced by an mRNA Vaccine through Boosting with a Poxvirus MVA Vector Expressing the Same Antigen

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Development of a vaccine against HIV remains a major target goal in the field. The recent success of mRNA vaccines against the coronavirus SARS-CoV-2 is pointing out a new era of vaccine designs against pathogens. Here, we have generated two types of mRNA vaccine candidates against HIV-1; one based on unmodified vectors and the other on 1-methyl-3′-pseudouridylyl modified vectors expressing a T cell multiepitopic construct including protective conserved epitopes from HIV-1 Gag, Pol and Nef proteins (referred to as RNA-TMEP and RNA-TMEPmod, respectively) and defined their biological and immunological properties in cultured cells and in mice. In cultured cells, both mRNA vectors expressed the corresponding protein, with higher levels observed in the unmodified mRNA, leading to activated macrophages with differential induction of innate immune molecules. In mice, intranodal administration of the mRNAs induced the activation of specific T cell (CD4 and CD8) responses, and the levels were markedly enhanced after a booster immunization with the poxvirus vector MVA-TMEP expressing the same antigen. This immune activation was maintained even three months later. These findings revealed a potent combined immunization regimen able to enhance the HIV-1-specific immune responses induced by an mRNA vaccine that might be applicable to human vaccination programs with mRNA and MVA vectors.

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