IV Jornadas Científicas PTI+ Salud Global

28-29 de octubre 2024

Auditorio Marie Curie – Parc Científic de la Unviersitat de València

Índice

N° I

A dual-antigen MVA-SARS-CoV-2 vaccine candidate provides enhanced protection compared to single-antigen vaccines

Maria M. Lorenzo[1]; Sandra Moreno[1]; Alejandro Brun[2]; Sergio Utrilla-Trigo[2]; Luis Jimenez-Cabello[2]; Aitor Nogales[2]; Javier Ortego[2]; Alejandro Marín[3]; **Rafael Blasco Lozano**[1]

[1] Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA-CSIC)

[2] Centro de Investigación en Sanidad Animal (CISA-INIA-CSIC)

[3] University of Yale

Background. Vaccinia virus (VV), widely known as the smallpox vaccine, provides several advantages as a vaccine vector, including low virulence, a robust immune response, and high physical and genetic stability. These traits make it a versatile vaccine platform for diseases of humans and also for livestock and even wildlife. Furthermore, it can accommodate large segments of exogenous DNA, enabling the introduction of multiple genes into a single vaccine vector. This capability enables the development of recombinant vaccines targeting complex or large antigens.

Methods. We have developed a novel system for generating recombinant VV that facilitates the insertion of two genes into independent genomic loci. This system 1eutrali plaque formation as a selectable trait, leveraging the functions of two viral proteins, A27L and F13L. The system facilitates isolation of VVs capable of expressing multiple exogenous proteins.

Results. To enhance the immune response generated during vaccination against Covid-19, we have developed a panel of vaccine candidates expressing the S protein alone or in combination with other SARS-CoV-2 proteins. Among these combinations, a recombinant VV based on the MVA strain co-expressing the full-length S protein, 1eutralizi in the prefusion conformation, along with a segment of a polyprotein encompassing the nsp1 and nsp2 proteins was evaluated in mouse protection models.

As demonstrated in previous studies, expression of the SARS-CoV-2 S protein alone induced anti-S neutralizing antibodies in mice and was able to confer protection against a lethal dose of SARS-CoV-2. Notably, co-expression of non-structural proteins nsp1/nsp2 provided additional protection, presumably through cell-mediated immunity. Altogether, these results suggest that this dual-antigen vaccine candidate provides more effective protection than previously evaluated vaccines by inducing a robust humoral and cellular immune responses.

Conclusions. The dual recombinant virus induced neutralizing antibodies, similar to the MVA recombinants that express only the S glycoprotein.

It was found that expression of nsp1/2 provides partial protection against the SARS-CoV-2 challenge.

Protection obtained through immunization with the dual MVA-Spf-nsp1/2 was more efficient than that achieved with single recombinant viruses expressing either Spf or nsp1/2 alone, indicating an improvement over previous formulations in protection against SARS-CoV-2 infection.

The dual recombinant virus may offer superior protective potency and broader cellular immunity, as well as benefits derived from the low variability of nsp1/2 compared to the S protein.

N° 2

Heterologous combination of NDV and MVA viral vectors expressing optimized SARS-CoV-2 S proteins as a vaccination strategy against coronavirus infection

Patricia Pérez[1]; Kaijun Jiang; Irene González-Domínguez[2]; Daniel Rodríguez-Martín[1]; Adam Abdeljawad[2]; Cupic, Anastasija; Stefan Slamanig[2]; Sara Flores; María A. Noriega[1]; Pedro J. Sánchez-Cordón[1]; Nicholas Lemus[2]; Tsoi Ying Lai[2]; Peter Palese[2]; Weina Sun[2]; Mariano Esteban[1]; Adolfo García-Sastre[2]; **Juan García-Arriaza[1]**

[1] Centro Nacional de Biotecnologia (CNB-CSIC)

[2] Ichan School of Medicine

Viral vectors are powerful vaccine platforms against emerging viruses like SARS-CoV-2. Effective vaccination strategies quickly adapted to new emerging viruses and capable of inducing robust and broad antigen-specific protective immune responses are necessary. If boosters are required to improve protective immunity, the use of different viral vectors in heterologous prime/boost might lead to higher immunogenicity as compared to the homologous use of the same viral vector. Here, we studied the immunogenicity and efficacy against SARS-CoV-2 infection triggered in transgenic K18-hACE2 mice and hamsters by different prime/boost vaccination regimens (mucosal and systemic) comprising two vaccine candidates based on Newcastle disease virus (NDV) and Modified vaccinia virus Ankara (MVA) vectors expressing a prefusion-stabilized SARS-CoV-2 spike (S) protein (NDV-Hexa Pro (HXP)-S and MVA-S(3P), respectively). Vaccination conferred full protection from weight loss and lung histopathological lesions after SARS-CoV-2 challenge. High binding and neutralizing IgG antibody titers against the ancestral SARS-CoV-2 strain and different variants of concern were detected upon vaccination in both animal models. Moreover, S-specific CD4+ and CD8+ T-cellular immune responses were induced in mice. Among the different combinations tested, sequential intranasal administration of NDV-HXP-S followed by an intramuscular inoculation of MVA-S(3P) presented the highest CD8+T cell activation in mice. In the hamster model, that combination showed complete protection of the upper respiratory tract 5 days postchallenge that prevented SARS-CoV-2 transmission. This research demonstrates the potential of heterologous NDV/MVA prime/boost vaccine strategies for the generation of antigen-specific humoral and T-cellular immune responses, capable of preventing both disease and transmission of a respiratory virus such as SARS-CoV-2, reinforcing its combined use for vaccination against other pathogens.

N° 3

Immune response to orthopoxviruses after recent vaccination with MVA and after past vaccination with the attenuated smallpox vaccine.

Elena Campos-Sánchez[1], Bruno Hernáez[1], Ignacio Ruiz-Fernández[1], Ángela Vázquez[1], Laura L. Gutiérrez-Oliva[1], Andrea C. Méndez[1], Rocío Martín[1], Beatriz Mena[1], Beatriz Tejedor[1], Gloria Castells[2], Miriam Miranda[3], Fernando García Muñoz[1], Rebeca Jiménez[3], Anabel Álvarez-Ciara[4], David Abia[1], Luis C. Antón[1], Marta Bermejo[3], Antonio Alcamí[1] and Margarita Del Val[1]

[1] Centro de Biología Molecular Severo Ochoa (CBMSO-CSIC)

[2] Universitat Autònoma de Barcelona.

[3] Unidad de Vigilancia de la Salud y Medicina del Trabajo CSIC

[4] Farmàcia de Dalt

One challenge in viral immunology is to understand the correlates that mediate protection or vulnerability to infectious diseases. This knowledge is especially important in particularly vulnerable and frail populations, such as the elderly. In Europe the fraction of people over 65 continues to grow and the population over 80 has doubled since 2001, reaching 6% of the total. Ageing has implications for the competence to respond to new infections. Characterizing and understanding the duration of immune protection generated decades ago in life is critical for the implementation of health strategies targeting this segment and for the rational design of vaccines whose protective effects remain in the elderly.

The multi-country outbreak of mpox disease, caused by the monkeypox virus MPXV, has affected over 115 countries since 2022, causing 2,763 new cases this September 2024, out of a total of 109,699, including 236 deaths. Spain is the most affected country relative to population of non-endemic countries. MPXV is an orthopoxvirus, as is the virus responsible for human smallpox, eradicated in 1980 by global vaccination with vaccinia virus (VACV). VACV attenuated variant MVA is currently used to protect against mpox. We analysed the anti-poxvirus adaptive immune memory (neutralizing antibodies (nAb) and cellular CD8 and CD4 T-lymphocyte responses) developed following a current MVA vaccination program in a Spanish adult population (naive or VACV vaccinated). We compared it to a cohort of very old individuals, presumably VACV vaccinated or infected with smallpox in childhood and sampled before the mpox outbreak.

We observed that while naïve individuals required two full doses of MVA vaccination, 66% of VACV-vaccinated volunteers [median age, 53] had pre-existing immunity against poxvirus and developed high nAb levels after one dose. Notably, 60% of the elderly [median age, 87] also had nAb to VACV, at similar levels as the adults with previous immunity. VACV nAb titers correlated with MPXV neutralizing capacity. Furthermore, 75% of the elderly showed cellular immune memory against either VACV or MVA poxvirus, which was mainly CD8 T-cell mediated, which still show polyfunctional activity.

In summary, two full doses of MVA vaccine are required in naïve populations and the human immune memory to historical vaccination has an extraordinary duration.

N° 4

Genomic Epidemiology of West Nile Virus in Andalucia

María José Ruiz López[1]; Jordi Figuerola[1]; Ana Vázquez[2]

[1] Estación Biológica de Doñana (EBD-CSIC)

[2] Instituto de Salud Carlos III

West Nile virus (WNV) is an emerging zoonosis in Europe. Transmitted among birds by mosquitoes, WNV can infect humans and horses, causing severe disease. In Spain, local circulation of WNV has been documented for decades, but a significant outbreak with 77 human cases occurred in 2020, marking a change in its dynamics. Since then, new cases have been reported annually and an expansion of the virus range has culminated in 2024 with the largest outbreak to date of WNV in Spain. We integrated genomic data with geographic observations and phylogenetic and phylogeographic inferences to uncover the spatial and temporal viral dynamics of WNV in Western Andalucia between these two outbreaks. We sequenced 60 new WNV genomes obtained from mosquito pools and compared it to other Spanish and European genomes. All WNV genomes obtained from Culex mosquitoes captured in western Andalucia between 2013 and 2023 belong to lineage 1. From 2013 to 2021, all strains clustered together in WNV linage 1, cluster 2, sub cluster WMed 1.3, except for a 2017 sample. The strain in subcluster W.Med 1.3 caused the human outbreak in 2020. In 2022 and 2023, co-circulation of two WNV strains was observed, including the one responsible for the 2020 outbreak and another strain previously detected in Spain and other countries (France, Italy and Senegal) that belongs to subcluster WMed2. Partial sequences from 2024 indicate that the main circulating strain was WMed2, and might be the one causing the 2024 outbreak in Andalucia. This strain has been associated with the 2022 outbreak in Italy. These results show the complicated dynamics of WNV L1 in the region, with several independent introductions, co-circulation, extinction and re-emergence of variante that can lead to different strains being responsible for outbreaks in humans.

N° 5

Detection of high pathogenicity avian influenza virus in Antarctica

Angela Vazquez Calvo[1]; Begoña Aguado Orea[1]; Ana Isabel Moraga Quintanilla[1]; Rafael Gonzalez-Serrano[1]; Australis Team[2]; **Antonio Alcamí[1]**

[1] Centro de Biología Molecular Severo Ochoa (CBMSO-CSIC)

[2] HPAI Australis Expedition

The current panzootic spread of high pathogenicity avian influenza virus (HPAIV) subtype H5N1 clade 2.3.4.4b has caused unprecedented mortality in wild animals worldwide, and has pandemic potential. In 2022-2023, the virus caused mass mortality of seabirds and marine mammals in South America, which raised concerns about HPAIV spreading to Antarctica.

With the purpose of monitoring HPAIV spread in Antarctica, we participated in the Spanish Antarctic Campaign, setting up a diagnostic laboratory at the Spanish Station Gabriel de Castilla (Deception Island) and sampling various sites around South Shetland Islands from Hesperides. We also participated in the International HPAI Australis Expedition aboard the sailing vessel Australis to investigate the spread and impact of HPAIV at several remote sites with suspected cases at the South Shetland Islands, Trinity Peninsula and Northern Weddell Sea. A molecular laboratory was set up at Gabriel de Castilla Station and on-board the Australis vessel to do state-of-the-art diagnostics for HPAIV, according to protocols and recommendations of the Spanish Reference Laboratory.

We identified the first HPAIV cases in Antarctica in birds, in collaboration with Argentinian scientists that found dead skuas close to Primavera Station, and in marine mammals, in an elephant seal we sampled in Robert Island. Monitorization carried out in the Northern Weddell Sea from the Australis vessel identified four sites with HPAIV-infected skuas, with a large outbreak in the skua colony at Beak Island, and an infected snowy sheathbill in Heroina Island. Further refinement of the RT-qPCR methodology in our laboratory in Madrid identified many more cases of HPAIV-infected samples from penguins and an Antarctic fur seal. Of particular relevance was the identification of mass mortality events in two penguin colonies at Heroina and Beagle Islands.

Our results show that it was possible to perform the diagnoses in Antarctica itself, at research stations or ships, which gives results much faster and avoids the risk of losing sample quality during transport to laboratories outside Antarctica. The finding of many HPAIV-infected animals from different species indicates that HPAIV has reached Antarctica and might have been spreading wider than estimated. Further spread of the virus in the next breeding season may have catastrophic consequences for the Antarctic fauna.

N° 6

UShER for Mycobacterium tuberculosis: an evaluation of pandemic-scale tools capacity to perform transmission surveillance

Lily Karim[1]; **Francisco José Martínez-Martínez**[2]; Iñaki Comas[2,3]; Russ Corbett-Detig[1]; Mariana Gabriela López[2]

[1] UC Santa Cruz Genomics Institute (UCSC)

[2] Instituto de Biomedicina de Valencia (IBV-CSIC)

[3] CIBER Epidemiología y Salud Pública (CIBERESP-ISCIII)

Whole-Genome Sequencing (WGS) of *Mycobacterium tuberculosis* (MTB) is increasingly standard in health services. The growing data amount allows the reconstruction of phylogenies with a broader genomic context, providing a higher resolution for transmission dynamics but also escalating computational demands. UShER revolutionized SARS-CoV-2 phylogenomics by using sample placement to reconstruct massive phylogenies, and it's been now extended to MTB. We tested its capacity to reconstruct large phylogenies and place new sequences to transmissionclusters.

To evaluate the robustness of UShER, we first identified transmission clusters in the Valencian Region, Spain, using a population-based dataset collected between 2014-2019. To recover transmission clusters, we built a regional phylogeny (N=1,455), and another including global strains (N=39,677), and applied a 12 SNPs threshold. To test the accuracy of UShER to assign strains to their corresponding transmission clusters, we removed samples collected in the Valencian Region between 2017-2019 (N=729) from the phylogenies and used phylogenetic placement to reincorporate them.

In the global phylogeny, 95.33% (692/729) of the samples fell at 2 nodes or less from its original neighbour and 88.7% (112/124) of the clusters were perfectly recovered, reaching a Cohen's Kappa value of 0.841. In the regional phylogeny, 95.88% (696/729) of the samples were placed at 2 nodes of less of its original neighbour and 88.8% (111/125) of the clusters were perfectly recovered, with a Cohen's Kappa of 0.939. Global phylogeny reconstruction took 3 hours and each sample was placed in the phylogeny in about 6 seconds.

UShER places new sequences in a pre-existing tree in no time, allowing the update of large phylogenies within minutes or hours where classic tools would take weeks. This tool can be used as a first approach to identify the phylogeographic background of a given dataset from a global context with all the available genomic information. Finally, UShER provides a close inference of actual transmission while maintaining all deposited sequences, which is particularly relevant in international transmission studies.

N° 7

A decision analysis model for colorectal cancer screening

Daniel Corrales Alonso[1]; David Rios Insua[1]

[1] Instituto de Ciencias Matemáticas (ICMAT-CSIC)

Background: Colorectal cancer (CRC) is the third most common type of cancer worldwide, making up for about 10% of all cases and being accountable for around 12% of all deaths due to cancer. Despite this, as an example, only about 14% of susceptible European Union citizens participate in screening programmes. Hence, there is an urgent need for accurate, non-invasive, cost-effective screening tests based on novel technologies to raise further awareness of the disease and its detection.

Methods: We develop a Bayesian network model to facilitate CRC predictions drawing on expert judgment and a large database from an observational study. This network is used to map CRC risks depending on numerous factors. We then embed such network in an influence diagram model through which we support decisions concerning whether and which screening method to consider and/or whether a colonoscopy should be administered. We include comfort, costs, complications, and information as decision criteria integrated within a multi-attribute utility model.

Results: The model can be employed for individual decision support based on different CRC risk factors and for assessing and redesigning current screening programs. Furthermore, it can aid in benchmarking novel screening devices to analyse their potential use.

Conclusions: The use of predictive models embedded in decision tools can be of great importance in screening programs as they offer a more personalised approach to CRC detection. Moreover, these models aid in the design of new, more cost-effective screening approaches and can be used to design incentives to promote the uptake of screening in the population.

N° 8

Seroprevalence in Spanish Population vs Global population: Pre-existing immunity effects on viral infections and vaccinations

Manuel Fuentes García[1]; Javier De Las Rivas[1]; Rafael Gongora[1]; Fernando Corrales[2]; Fermín Sanchez-Guijo[3]; José Ángel Lorente[4]; Ana Nuño-Soriano[1]; Sara Cuesta[5]; David Bernardo[5]; Angela-Patricia Hernandez[1]; Carlota Arias-Hidalgo[1]; Pablo Juanes-Velasco[1]

[1] Instituto de Biología Molecular y Celular del Cáncer (IBMCC-CSIC)

[2] Centro Nacional de Biotecnología (CNB-CSIC)

[3] Hospital Universitario Salamanca

[4] CIBER Enfermedades Respiratorias (CIBERES-ISCIII)

[5] Instituto de Biología Funcional y Genómica (IBFG-CSIC)

The health of the immune system, as partially shaped by past infections or vaccinations, is critical to control and resolve the infection. Infectious diseases are one of the major health issues worldwide, causing high-morbidity, and mortality each year. Virus exposure, through infection or vaccination, can elicit cellular and humoral responses. For many viruses, these immune responses are long-lasting; among cross-reactive pre-existing antibodies can exacerbate microorganisms pathogenesis in some cases. The implication of pre-existing immunity to many pathogens (viruses, bacteria, parasites, fungi…) are not fully understood. The human repertoire, or reactome, is a vast and dynamic network of antibody-antigen interactions that plays a crucial role in health and disease. Understanding the complexity of this network is key to unlocking novel understanding of disease etiologies, new diagnosis, therapeutics, and personalized medicine approaches.

In this study, it is presented a cutting-edge technology for high-throughput and high-resolution antibody reactome profiling in order to evaluate the seroprevalence against 37 microbial antigens prevalence in the Spain Population that reflect each donor´s infection history and previous immunity.

The seroprevalence (IgG & IgM profile) has been evaluated in a Spain cohort (n =450) and compared with an international cohort (n =400) of human Personalized Omics Profiling (hPOP) covering population from 5 continents and different ethnicities.

We delve into how this technology is providing unprecedented insights into the immune system´s response to various disease, including infectious diseases, allergies, immune disorders and vaccines.

Methods: A novel multiplex platform (high-density protein array format) has been designed and developed for the simultaneous immune-detection of IgM and IgG against 37 microbial antigens (Influenza, SARS-Cov-2, salmonella, pneumococo, varicella-zoster, Epstein-Barr, cytomegalovirus, candida albicans, mycobacterium tuberculosis, streptococcus, among others….) prevalent in the world-wide population.

Results: Distinctive serological profiles have been observed when comparing demographic variables such as sex, age range, etchnicity, geographical location…. In addition, differences were observed in the distribution of antigens between two immunoglobulin isotypes; for example: IgM predominant in outer membrane porin of Salmonella typhi.

Conclusions: Knowing in depth the natural variations of these proteins, depending on the different clinical-biological characteristics, could enable us to correlate these patterns with patients presenting different pathologies, the role of pre-existing immunity, cross-reactive immune responses, and vaccine approaches that circumvent pre-existing immunity. In addition, it could reduce mortality or improve

diagnosis and treatment progression in an individualized and personalized manner (precision immunology).

N° 9

Novel method for isothermal amplification of padlock probes for nucleic acid detection and Phi29 DNA polymerase variants

María Isabel Martínez Jiménez[1]; **Alicia Del Prado Díaz[1]**; Luis Blanco Dávila[1]; Miguel De Vega José[1]

[1] Centro de Biología Molecular Severo Ochoa (CBMSO-CSIC)

Nucleic acid amplification has become essential for molecular diagnostics. Although polymerase chain reaction (PCR) is the most extended technique, the technical requirements needed, limit its applicability. A good alternative is the use of isothermal amplification methods, that are highly sensitive, productive, and simpler to use. Due to its robustness and simplicity Rolling Circle Amplification (RCA) has become one of the most promising alternatives to PCR in clinical diagnosis. This technique makes use of the bacteriophage Phi29 DNA polymerase, a well-studied enzyme that amplifies limiting amounts of circular DNA thanks to its excellent fidelity, processivity and strand displacement capacity.

We have developed a novel isothermal primer-less amplification method (PrimPol-primed HRCA), to detect directly viral RNA without a reverse transcription step. A specific DNA padlock probe, complementary in both ends to an adjacent region of the target nucleic acid, is circularized by a ligase when the viral genome is present. The single stranded circular probe is then amplified by RCA, using the 3'-end of the target nucleic acid as an efficient primer. An engineered variant of Phi29 DNA polymerase called Qx5, was specifically designed for this method. This new enzyme has improved thermostability being competent from 30 to 42 ºC. In addition, Qx5 shows an unexpectedly strong 3'-5' exoribonucleolytic activity that generates a 3'-OH end in the RNA/DNA hybrid region which can be used as a primer to start the RCA of the circularized padlock (RCA step). The concatemeric ssDNA generated is further amplified by the combination of Qx5 and a DNA primase that triggers a hyperbranched rolling circle amplification (HRCA). The singular DNA primase TthPrimPol continuously synthesizes DNA primers only in the RCA product, producing a significant amount of DNA that can be directly analyzed and quantitated with a pocket fluorometer. This method pretends to simplify the amplification procedures for RNA identification, which is being patented (PCT/EP2024/07997).

This method has the potential to be used for the detection of any nucleic acid, by only adjusting the padlock probe. We aim to implement the method to also detect double stranded DNA sequences, as the presence of bacteriophages in the dairy industry to avoid failures during the fermentation processes.

N° 10

Wastewater-based epidemiology (WBE) as a promising approach for monitoring AMR for the identification of seasonal trends

Jesús López Cañizares[1]; Pilar Truchado[1]; Francisco Jose Cobo[2]; María I. Gil[1]; Avelino Alvarez-Ordóñez[2]; Ana Allende Prieto[1]

[1] Centro de Edafología y Biología Aplicada del Segura (CEBAS-CSIC)

[2] Universidad de León

Background. In 2020, the Centre for Disease Control and Prevention (CDC) identified antimicrobial resistance (AMR) as one of the top 10 threats to public health. Wastewater-based epidemiological (WBE) surveillance has been proven to be an effective method for tracking the evolution of various pathogens, including SARS CoV-2 and Mpox. Recently, WBE has gained attention as a valuable method for tracking AMR as an integrated approach able to provide a broader understanding of AMR patterns, facilitating the identification of epidemiological connections between humans, animals, and the environment, to align with the One Health framework. However, it has not been implemented by the competent authorities to fight against AMR. In the present study, WBE was used to investigate changes in prevalence and relative concentrations of antimicrobial resistance genes (ARGs) within three communities in the South East of Spain during September and December 2023.

Methods. Wastewater samples were collected between September and December 2023, corresponding to the autumn and winter seasons, respectively, from three WWTPs located in the North, Center and East of the Region of Murcia. Samples (40 mL) were filtered using a pore size of 0.2 µm and DNA was extracted with DNeasy PowerWater Kit. The libraries of the samples were prepared using the Native Barcoding Kit 24 V14 and were sequenced using the MinION Mk1C. The generated reads were analyzed using a bioinformatics pipeline, where they underwent quality filtering and the removal of adapters and barcodes. High-quality reads (q > 10) were selected for the identification of ARGs using the ResFinder database.

Results. The results showed a higher number of CPMs in samples from the North and Center regions compared to the East, with the North and Center displaying similar values during the autumn sampling period. In contrast, during the winter sampling, all WWTPs experienced a decline in the number of ARGs. The types of ARGs that showed the highest abundance in both samples were those conferring resistance to macrolides, beta-lactams, aminoglycosides, tetracyclines and sulfonamides. Specifically, the ARGs with the highest CPMs were mph(E), msr(E), erm(F), sul1 and tet(Q) in both samplings (autumn and winter).

Conclusions. This study aimed at providing insight into resistome analyses in different locations and seasons. WBE has enabled the resistome analysis facilitating the comprehensive characterization of determinants of antimicrobial resistance in wastewater. Correlations were observed in the variations of ARGs number in the three communities after the seasonal analysis highlighting the potential of WBE as a valuable tool for monitoring regional AMR. The obtained data present potential for integration into AMR surveillance and management programs to provide valuable insights into emerging AMR profiles within a specific population. Acknowledgements: This study is funded by the State Research Agency (AEI) of the Spanish Ministry of Science and Innovation (TED2021-131427B-C2) and AGROALNEXT program (MCIN and NextGenerationEU funds, PRTR-C17.I1).

N° 11

Photocatalytic air filters based on ZnO-CeO2 nanocomposites with antimicrobial properties

Ana Iglesias Juez[1]; Antonio Alcamí[2]; Fernando Fresno[1]; Maite García-Castey[2]; Arturo Martínez-Arias[1]; Gonzalo Sánchez-Domínguez[1]; **Angela Vázquez-Calvo**[2]

[1] Instituto de Catálisis y Petroleoquímica (ICP-CSIC)

[2] Centro de Biología Molecular Severo Ochoa (CBMSO-CSIC)

Indoor-air quality not only stands on air renewal, but also on efficient inactivation of biological agents. Airborne transmission is the main vector of respiratory diseases spread and the probability of infection is much higher in indoor environments. It is necessary to develop innovative health technologies that enable safe environments and can help providing preventive air purification devices to contain the infectious disease transmission.

We propose a continuous aerosol transmission barrier based on novel air purifiers which use photocatalytic filters that inactivate viruses and bacteria by oxidative stress inhibiting their ability to infect cells.

Zinc oxide nanoparticles exhibit antiviral activity due to photoproduction of reactive oxygen species (ROS). However, the recombination rate of photogenerated electron–hole pairs decreases its photocatalytic performance. We have proposed the employment of zinc oxide/cerium oxide nanocomposites since the heterojunction can reduce charge recombination processes and CeO2 also presents inherent redox characteristics that can improve ROS generation. Also, CeO2 electronic properties depend on the exposed facet types, so here we analysed the influence of using different morphologies of ceria in forming the nanocomposites.

The ZnO-CeO2 nanocomposites exhibited high inactivation against several viruses and bacteria. Differences in the antiviral activity were observed between the nanocomposites depending on the amount of CeO2 and their nanoparticle morphology. Bare ZnO showed a low viricidal capacity, requiring long exposure times to light to achieve virus inactivation. Composites with ceria nanocubes and among them those with higher (100) exposition presented the fastest viral inactivation (Figure 1A). The optimum material, ZnO-22%CeO2(NC3), was also tested in an air filter showing high inactivation of several pathogens at short times (more than 3-log reduction at 15 min). We demonstrated the improvement of systems with high inactivation activity. This technological solution is safe and can be easily implemented to prevent pathogen aerosol transmission. In addition to plate assays, which allow for discrimination between systems, optimization of photocatalyst parameters, and determination of operating mechanisms, a chamber has been specifically designed to assess the real viability of viruses in aerosols. The chamber enables bioaerosol generation with viral load, real-time in situ monitoring of suspended particles, and includes a bioaerosol sampling system, making it a suitable tool for studying the efficiency of air purification systems. A small prototype of the air purifier has been constructed and tested within the chamber, using the filter with the most effective photocatalyst, yielding highly promising results. This has required extensive work to fine-tune the entire system, conduct validation tests, and establish reliable testing protocols, among other tasks.

N° 12

Assessment of the biocompatibility and mechanism of action of highly efficient and broadly active antimicrobial surfaces

Raquel Cue López[1]; Antonio Alcamí[2]; Paula Bosch[1]; Alejandro Díez[3]; Fernando García Arenal[4]; Enrique Martínez Campos[1]; Laura Montalvillo Jiménez[1]; Ángela Vázquez[2]; Pedro José de Pablo[5]

[1] Instituto de Ciencia y Tecnología de Polímeros (ICTP-CSIC)

[2] Centro de Biología Molecular Serevo Ochoa (CBMSO-CSIC)

[3] Instituto Geológico y Minero de España (IGME-CSIC)

[4] Centro de Biotecnología y Genómica de Plantas (CBGP UPM INIA/CSIC)

[5] Universidad Autónoma de Madrid

Background. Environmental surfaces may serve as potential reservoirs for virus and bacteria. The rise of antibiotic-resistant microorganisms and pandemics caused by emerging viruses has in-creased the demand for antiviral measures and treatments for clean surfaces, especially in public places. However, not only it is important to prove the efficacy of antimicrobial surface coatings, since the main limitations to the application of antimicrobial materials include issues related to toxicity, antimicrobial resistance and environmental concerns. The excellent antimicrobial activity of our modified surfaces has been previously proven against 9 model microorganisms, employing even complex 3D platforms. Hence, in this work, we have carried out the determination of the cytotoxicity of the materials to rule out whether they could pose a risk to human health. Also, to explain the broad activity and high efficiency of the material, the mechanism of action has been studied at the molecular level.

Methods. We explored whether the contact of relevant cell lines with antimicrobial materials causes adverse effects in terms of their metabolic activity and total DNA content, using fluorescent binding dyes. Also, to give insights into the mechanism of action of the antimicrobial materials we have used five model microorganisms: two coronavirus species, Porcine Transmissible Gastroenteritis Virus (TGEV) and Human Coronavirus 229E (HCoV-229E GFP); one plant virus, Tobacco Mosaic Virus (TMGMV), and two strains of bacteria, Gram-positive Staphylococcus aureus and Gram-negative Escherichia coli. First, we explored whether the contact with the antimicrobial surfaces alters the genomic integrity of viral particles, via RT-qPCR. Also, their structure and mechanical stability at the nanoscale was investigated under an atomic force microscope (AFM). Finally, two strains of bacteria growing over the modified surfaces were used to assess intracellular ROS formation using a fluorescent 2’,7’-diacetate probe.

Results. the specificity of the tested antimicrobial materials has been demonstrated: although the surfaces inactivate pathogenic microorganisms, they have not shown cytotoxicity on relevant cell lines, allowing their proliferation. With regards to the material’s mechanism of action, despite their proven efficacy, the damage to the virus does not reach the genetic material of the viral particles. In addition, no significant increase in intracellular production of ROS is observed in bacteria growing over the coatings, with regards to the control. The nanostructure of both TGV and HCoV-229E viral particles remains comparable between infective and non-infective samples. However, AFM measures point out a higher fragility of the inactivated virus.

Conclusions. The results show that this new generation of antimicrobial materials are safe in contact with human cells. In addition, we have further gained insight into their antimicrobial mechanism of action. In view of the results, our hypothesis is that the interaction of viral particles with the material

surface affects proteins or lipids of the envelope of microorganisms very locally, without damaging their genetic material or overall structure/topology. Efforts to elucidate the mechanism of action could open the door to the generation of broad-spectrum antimicrobial surfaces that meet safety standards for people and for the environment.

N° 13

Latest advances in antimicrobial and more sustainable PPE and air purification materials based on nanofibers

José María Lagarón[1]; Zoran Evtoski[1]; Cristina Prieto[1]; Ana Kramar[1]

[1] Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC)

The current presentation will gather the latest research efforts in prophylactic protection carry out by our research group within the context of the PTI+ Salud Global in cooperation with the CSIC spin-off company Bioinicia Fiber Manufacturing S.L., which translates our research into both intermediate and finished commercial products. In particular, several advances in nanofiber-based materials with improved balance between filtration and pressure drop (breathability), antimicrobial capacity and also in properties related to comfortability (balsamic filters) and sustainability (biodegradable and compostable head bands) will be presented.

N° 14

Pathogen inactivation through catalytic filters

Miguel A. Bañares[1]; Antonio Alcamí[2]; Teresa García Castey[1]; Alicia Gómez López[1]; Paula Llanos[1]; Ana Serrano Lotina[1]; Ángela Vázquez Calvo[2]

[1] Instituto de Catálisis y Petroleoquímica (ICP-CSIC)

[2] Centro de Biología Molecular Serevo Ochoa (CBMSO-CSIC)

Background. Lower respiratory tract infections represent the third leading cause of death in the world. Airborne transmission is the main propagation vector. To avoid infection propagation, due to airborne transmission, different technologies of air purification have been reported[1]. We have developed and patented (P202430200) two families of catalytic filters that can be placed in indoor air-cleaning devices, which effectively inactivate several pathogens (HCoV-229E, SARS-CoV-2, RV-B14, E. coli, S. aureus) at mild temperatures (below 40 ºC). These promising filters will be tested with aerosol using the phage φ-29. First, this virus has been evaluated by plaque assays and, afterward, the built prototypes will be tested in an aerosol chamber (CORINA). The mechanisms that caused the inactivation of pathogens were also studied.

Methods. Ceramic filters were prepared by immersion of pre-shaped discs or monoliths into a solution whereas polymeric filters were prepared by impregnating commercial fan filters using an airbrush. Plaque assays determined the inactivation of bacteriophage φ-29. E. coli cells were characterized by transmission electron microscopy before and after exposure to the filters. On the other hand, the retention virus on the filters was evaluated using HCoV-229E by studying the viral viability and the total virus recovered by virus titration assay and qPCR, respectively.

Two prototypes of the catalytic filters were developed and preliminary tests in CORINA were carried out. The aerosol was generated using a modified Counterfog® SDR-F05A and monitored by a SPS30 sensor of Sensirion.

Results. Good inactivation potentials for φ-29 were observed at mild temperature (37ºC). To understand the mechanism of action over the pathogen, E. coli, before and after exposure to the filters, was evaluated, showing that viable bacteria exhibit the classical rod-shaped morphology with well-preserved outer and cytoplasm membranes. On the other hand, for the killed bacteria, there was an extensive blebbing in the rough-and smooth-type outer membranes; some material leakage and cytoplasmic matrix condensation were also observed.

No retention of HCoV-229E was detected in any filter. However, lower viral RNA copies were detected after treatment which may indicate some RNA damage.

To evaluate the inactivation of bioaerosol-containing pathogens through catalytic filters inside CORINA, we have developed two prototypes of air purification systems. We have currently evaluated the aerosol stability over time when the prototypes are inside the aerosol chamber and some preliminary tests with the filters are ongoing.

Conclusions.

- Both catalytic filters effectively inactivate the bacteriophage φ-29.
- When the virus was exposed to the filters, some RNA damage was detected.

[1] Song L, Zhou J, Wang C, Meng G, Li Y, Jarin M, Wu Z, Xie X. J Hazard Mater. (Pt B) 424: 127429, 2022.

- The death of bacteria was due to the damage to its membrane.
- This aerosol chamber is a useful tool for studying the efficiency of filters present in air purification systems.

Acknowledgements. We gratefully acknowledge the funding from CSIC PTI+ Salud Global, Nextgeneration EU and by "la Caixa" Bank Foundation, with code LCF/PR/HR22/52420032.

N° 15

Preliminary study of Crimean-Congo hemorrhagic fever virus proteins and their role in the interferon response evasion

Ana B. Carlón[1]; Andrés Louloudes-Lázaro[1]; José M. Rojas[1]; **Noemí Sevilla Hidalgo[1]**;

[1] Centro de Investigación en Sanidad Animal (CISA-INIA-CSIC)

Crimean-Congo hemorrhagic fever virus (CCHFV) is a tick-borne pathogen responsible for severe hemorrhagic fever in humans with a high mortality rate. It is an emerging zoonotic virus, with no specific antiviral treatment or licensed vaccines. Despite the expanding range of the vector and the increase of the cases reported, the pathogenesis of the virus is yet poorly understood. CCHFV can only cause severe disease in humans, although it can also infect other animals, such as ruminants, acting as a reservoir. A critical aspect of CCHFV pathogenesis is its ability to modulate the host's innate immune response, particularly type I interferon (IFN), which serves as the first line of defense against viral infections. The innate immune response is triggered when host pattern recognition receptors (PRRs) detect viral components, leading to the activation of downstream signaling pathways. These pathways result in the production of IFN, inducing an antiviral state in host cells. However, many viruses, including CCHFV, have evolved mechanisms to counteract this antiviral response, allowing them to establish infection and propagate within the host. This preliminary study aims to explore the interactions between CCHFV viral proteins and the host's innate immune response. More precisely, we investigate how CCHFV proteins modulate the IFN response. Initial results indicate that certain CCHFV proteins can interfere with the interferon response, suggesting that these viral factors play a crucial role in viral evasion of innate immunity. Understanding how CCHFV manipulates the innate immune system is critical for developing effective antiviral therapies and vaccines. Further studies are needed to characterize the full spectrum of viral-host interactions and to elucidate the precise mechanisms by which CCHFV proteins inhibit interferon signaling. These insights could provide new targets for therapeutic intervention and help mitigate the severe outcomes associated with CCHFV infection.

N° 16

Harnessing the Caenorhabditis elegans/Orsay virus pathosystem as a new model for studying the evolution of host-virus interactions

Santiago Francisco Elena Fito[1]; Victoria García Castiglioni[1]; Esmeralda García Legarda[1]; Dominik Herek1; Susana Martín García[1]; Izan Melero Peris[1]; Juan Carlos Sánchez Muñoz[1]; María José Olmo Uceda[1]

[1] Instituto de Biología Integrativa de Sistemas (I2SysBio-CSIC-UV)

The discovery of the Orsay virus (OrV), the first virus infecting wild populations of Caenorhabditis elegans, has catalyzed studies of viral immunity pathways in this nematode. Given the numerous advantages that C. elegans offers for fundamental research in host-pathogen interactions, this pathosystem holds great potential to serve as a model system for experimental virus evolution studies. However, the evolutionary constraints operating in this pathosystem have received scant exploration.

Here, we delineate a series of evolutionary experiments aimed at probing various aspects of virus-host interactions. Initially, we have subjected OrV to evolutionary pressures within worm populations exposed to diverse environmental stresses such as temperature fluctuations, radiation, and simulated absence of gravity. Our findings indicate that, on the whole, infection mitigates the detrimental effects of these stresses on nematode fitness. Moreover, we have pinpointed candidate genes associated with the virus-induced enhancement of tolerance and susceptibility to abiotic stresses.

Subsequently, we delved into the three-way interplay among nematode development and senescence, the vigor of innate immune responses to infection, and the evolution of virus diversity. Our investigation unveiled differential activation of various immune factors across developmental stages, revealing a damping-wave-like pattern of infection progression. Specifically, we observed an initial acute infection in young larvae transitioning into a persistent low-level infection, punctuated by several rebounds as the animals age and senesce.

N° 17

Unique properties in the viral CC chemokine inhibitor from mpox virus

Samuel Donaire Carpio[1]; Isabel Alonso[1]; Francisco Javier Álvarez de Miranda[1]; Alazne Rubio[1]; Pilar Delgado[1]; Antonio Alcamí[1]; **Bruno Hernáez[1]**

[1] Centro de Biología Molecular Severo Ochoa (CBMSO-CSIC-UAM)

Currently mpox (formerly known as monkeypox) is a clear threat to public health, as we now know that the emerging clades Ib and IIb are adapting to more efficient human-to-human transmission. As poxvirus tropism mainly relies on their ability to counteract the innate host immune response, we aimed to study some selected and unexplored MPXV proteins to better understand the modulation of the immune response by this emerging pathogen. One of these immunomodulatory proteins is the secreted viral CC chemokine inhibitor (vCCi), also known as 35K, which binds CC chemokines with high affinity to impede their presentation to specific receptors in leukocytes, thus preventing migration of these cells to infected areas.

Here, we provide the first insights into the structure and function of MPXV 35K. We have tested the ability of MPXV 35K to associate cell surface through glycosaminoglycans (GAGs) binding and compared with other orthologues from the poxvirus family. This viral protein can bind and neutralize chemokines of the CC family in vitro, preventing leukocyte migration. We have determined the binding parameters for MPXV 35K interaction with human and murine chemokines using surface plasmon resonance (SPR).

We have identified two novel properties in the MPXV 35K: i) the ability to bind cell surface GAGs is unique to this protein compare with orthologues from other poxvirus species such as vaccinia or ectromelia viruses. ii) although 35K is known to bind and neutralize most of the CC class chemokines we show first evidence for additional binding to CXCL12, which represents another class of chemokines.

These findings indicate that 35K from MPXV is unique among orthopoxvirus orthologues. Further studies in a mouse model of MPXV pathogenesis will be conducted to define whether inactivation of the 35K protein contributes to viral pathogenesis and transmission.

In parallel, we have established an animal model of MPXV infection at the CBM using a CAST/EiJ mice colony and a clade IIb MPXV isolated from the skin lesion of a patient. This animal model should be very useful for the future determination of the relevance of this (and others) immunomodulatory protein(s) during the MPXV infection, and also for other research groups interested in antiviral or vaccine studies.

N° 18

T-cells from post-COVID individuals are exhausted in response to SARS-CoV-2 stimulation, but not influenza, regardless of Long COVID status

Marina Perez Mazzali[1]; Jimena Gutierrez Beneitez[1]; Juan Hernández Vázquez[1]; Laura Barrero Santiago[1]; Federico Montero Cuadrado[2]; Juan J. Tellería[1]; Raquel Almansa[1]; Cándido Pérez Segurado[1]; Álvaro Martín Muñoz[1]; Pablo Tellería[3]; Silvia Rojo Rello[3]; José A. Garrote[1]; Eduardo Arranz[1]; Antonio Orduña[3]; José María Eiros[4]; Eduardo Tamayo[4]; David Bernardo[1],,[5]; **Sara Cuesta Sancho[1],**

[1] Instituto de Biomedicina y Genética Molecular (IBGM-CSIC)

[2] East Valladolid Primary care management (Sacyl)

[3] Hospital Clínico Universitario de Valladolid

[4] Hospital Universitario Río Hortega

[5] CIBER de Enfermedades Infecciosas (CIBERINFEC-ISCIII)

Background and Aims: Long COVID emerges as a significant public health concern, affecting around 10% of individuals infected with SARS-CoV-2. This condition is characterized by a range of persistent symptoms that cannot be explained by other diagnoses. To better understand the mechanisms underlying this condition, we aimed to analyze the immune system of affected individuals.

Methods: Peripheral blood mononuclear cells (PBMCs) and plasma were collected from 19 adult patients diagnosed with LC and 22 non-LC controls (with confirmed prior infections), matched by sex, age, and time since initial infection. Humoral and cellular immune memory were evaluated in both groups, together with an unbiased characterization of their immune cell populations using spectral and computational cytometry. T-cell stimulation assays in response to SARS-CoV-2 spike protein and Influenza H1N1/H3N2 proteins were also performed.

Results: LC patients exhibited reduced humoral (IgG anti-N and IgA anti-S, but not IgG anti-S) and cellular memory responses compared to non-LC controls. Following T-cell stimulation assays, LC patients' T-cell subsets showed an exhausted phenotype (with elevated levels of LAG-3, TIM-3, CTLA-4, and PD-1) in response to the spike protein but not influenza peptides, along with a mixed Th1/Th2 response pattern. However, non-LC controls displayed a similar exhausted phenotype, with no significant differences in the type or magnitude of response. Computational cytometry further identified immune cell clusters specifically associated with LC.

Conclusion: LC patients display a reduced humoral and cellular memory response which, however, cannot be explained by a T-cell exhaustion mechanism. Current work in our lab aims to identify whether the T-cell exhaustion induced by spike protein is an innate effect of the protein. Furthermore, we are working to identify the unique immune fingerprint of LC patients, which may help explain the development of this condition.

N° 19

Point mutations at specific sites of the nsp12–nsp8 interface dramatically affect the RNA polymerization activity of SARS-CoV-2

Cristina Ferrer-Orta[1]; **Sergi Vázquez-Monteagudo[1]**; Diego S. Ferrero[1]; Brenda Martínez-González[2]; Celia Perales[3,3]; Esteban Domingo[4]; Nuria Verdaguer Massana[1]

[1] Institut de Biología Molecular de Barcelona (IBMB-CSIC)

[2] Centro Nacional de Biotecnología (CNB-CSIC)

[3] Instituto de Investigación Sanitaria-Fundación Jiménez Díaz, Universidad Autónoma de Madrid (IIS-FJD, UAM)

[4] Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM)

Background. Previous research analysed 30 diagnostic samples from patients infected by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and found 41 amino acid substitutions in the nonstructural protein 12 (nsp12) the main subunit in the RNA-dependent RNA polymerase (RdRp) complex. Eight mutations were chosen for further in vitro characterization of their impact towards the RdRp activity of the complex formed by nsp12 and its co-factors nsp8 and nsp7. The substitutions in nsp12 are placed within the template entry channel (D499G and M668V), the motif B (V560A), and the contact surface between nsp12 and nsp8F (P323L, L372F, L372P, V373A and L527H). Additional mutations were designed based on structural information of the complex to further understand the importance of the interaction between nsp12 and nsp8F (V330S, V341S and nsp8-R111A/D112A) or to study the nsp8-RNA interaction (nsp8-Δ1-76).

Methods. Mutations were introduced by mutagenesis PCR into a pRSFDuet-1 plasmid coding for the RdRp complex (nsp12-nsp8-nsp7). The wild-type (wt) and the different complex mutants were recombinantly expressed in *E. coli* and purified in 3 consecutive steps. The polymerization activity of each mutant was studied with *in vitro* primer extension assays using different fluorescently-labelled Primer/Template (P/T) duplexes, and different reaction temperatures; the reactions were stopped at different time-points by the addition of quenching buffer and loaded into a TBE/Urea-PAGE to visualize the elongation at each time point. Furthermore, the ability of each of the mutated complexes to bind RNA was studied with the same P/T duplexes through Electrophoresis Mobility Shift Assays (EMSAs).

Results. The nsp12 substitutions located close to the active site (D499G, V560A and M668V) promote little differences in the RdRp activity, when compared to the wt polymerization rate. Meanwhile, mutations involving the binding region of nsp12 with nsp8F were found to produce a significant change in the RdRp activity of the complex. Residues L372, V373 and L527 are involved in a hydrophobic cluster formed between the fingers domain of nsp12 and the long helix of nsp8F. Substitutions that weaken the hydrophobicity of this cluster (L372P, V373A and L527H) reduce the RNA elongation by nsp12, specially as seen in L372P and L527H which greatly impact the hydrophobicity of the region and severely reduce the polymerization. On the contrary, the L372F mutation places a bulkier hydrophobic residue in this patch and displays increased RdRp activity. Mutations designed to disrupt the nsp12-nsp8F interaction (V330S, V341S and nsp8-R111A/D112A) had a considerable effect on polymerization rates, although not as dramatic as L372P or L527H; moreover, the nsp8-Δ1-76 mutant showed extremely low activity.

Conclusions. This study reveals a contact interface involving a hydrophobic cluster in the nsp12 fingers subdomain that is critical for the modulation of the SARS-CoV-2 RdRp activity offering an alternative towards antiviral design by targeting this interaction.

N° 20

Structural heterogeneity analysis of SARS-CoV-2 Spike protein variants Omicron BA2 and ancestor D614G

James Krieger[1]; Carlos P. Mata[1]; Alison Berezuk[2]; David Herreros[3]; Sriram Subramaniam[2]; Carlos Óscar Sorzano Sánchez[3]; **Jose Maria Carazo Garcia**[1]

[1] Centro Nacional de Biotecnologia (CNB-CSIC)

[2] University of British Columbia (UBC)

[3] Centro Nacional de Biotecnología (CNB-CSIC))

SARS-CoV-2 is the causative agent of COVID-19. Infection of cells by SARS-CoV-2 is driven by engagement of the trimeric viral spike protein with the cellular receptor ACE2 via its receptorbinding domain (RBD) and with other co-receptors via the N-terminal domain (NTD), both of which are also targets for neutralizing antibodies. The spike protein is a dynamic trimeric protein assembly, but cryo-EM analyses to date have principally resulted in classification of the structure of the complex into discrete states, and not focused on recovering the dynamic aspects directly from the cryo-EM images. In this work we present a robust, quantitative approach to analyze the conformational spread in the spike protein. We apply this analysis to the BA.2 Omicron variant, contrasting its properties with that of a mutant (D614G) that emerged early in the pandemic and did not yet have the mutations that appeared during the course of the pandemic. Our results show that the BA.2 spike protein is significantly more flexible than the D614G mutant and reveal unique states with greater extents of RBD compaction and closure and the NTD more tightly "locked" onto them as compared to the D614G mutant. We suggest that this difference in conformational spectrum may have been one of the factors that contributed to the more rapid spread of the BA.2 variant and other viral strains that followed the D614G mutant.

N° 21

The gut microbiota's role in the SARS-CoV-2 infection

Verónica Tolosa Enguís[1]; Eva María Medina Rodriguez[1,2]; Alfonso Benítez Páez[1]; María Leonor Fernández Murga[3]; Ron Geller[4]; Ana Isabel Avilés-Alía[4]; María Montoya[5]; Yolanda Sanz Herranz[1]

[1] Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC)

[2] Fundación para el Fomento de la Investigación Sanitaria y Biomédica (FISABIO)

[3] Hospital Arnau de Vilanova

[4] Institute for Integrative Systems Biology (I2SysBio) (UV-CSIC)

[5] Centro de Investigaciones Biológicas Margarita Salas (CIB-CSIC)

Background. In March 2020, the World Health Organization (WHO) declared COVID-19 as a pandemic caused by the virus SARS-CoV-2. The infection mainly affects the respiratory system, but also the gastrointestinal tract and has many different manifestations. Prolonged intestinal dysfunction is seen in many COVID-19 patients. This may be associated with reduced gut microbiota richness and diversity, immune deregulation, and delayed viral clearance in some of the patients. Some studies also suggest that the intestinal tract may be involved in the pathogenicity of the infection because the virus can replicate in intestinal enterocytes. The gut contains the largest number of immunocompetent cells in the body and, together with the microbiota, plays a key role in regulating local and systemic immune responses, including those in response to infection.

Our study aimed to profile the intestinal microbiota in patients hospitalized for COVID-19 and characterize the potential role of the differentially abundant bacteria compared to controls in different in vitro infection models.

Methods. We performed a cross-sectional study in collaboration with the Arnau de Vilanova Hospital, including 63 patients who tested positive for SARS-CoV-2 RT-qPCR and 54 healthy individuals (with no history of SARS-CoV-2 infection). Using a validated method, the patients were classified into four categories according to the severity of their symptoms. Several clinical variables related to SARS-CoV-2 infection were collected (symptoms, severity, duration of symptoms) as well as analytical variables, demographic information, medical history, and biological samples (blood and stools). We performed Nanopore-based rrn operon (16S rRNA gene, ITS region, and 23S rRNA gene) long-amplicon sequencing to study the microbiota profiles in stool samples at the species taxonomic level. We identified bacterial species with differential abundance between COVID-19 patients and healthy controls. To investigate the potential role of the identified bacterial species in the viral cell entry and immune response we performed in vitro experiments using PBMC and Vero6E cells exposed to SARS-CoV-2 peptides and SARS-CoV-2 pseudoviruses and the bacterial cultures.

Results. The microbial composition showed greater diversity and uniformity in the control group compared to the COVID-19 group. The microbiota of patients with COVID-19 also showed a dominance of certain bacterial species and specifically the abundance of Enterococcus faecalis correlated to the infection severity. We also identified 5 bacterial species that were more abundant in controls than in patients. In vitro assays showed that Bacteroides uniformis induces a more important anti-inflammatory response in the presence of virus, but it does not interfere with the virus entry into the cells. In conclusion, the study confirms that the COVID-19 infection and its severity are associated with particularities of the gut microbiota, which could influence the immune response during the disease course.

Abbreviations. SARS-CoV-2, infection, immunity, microbiota, Nanopore sequencing, rrn operon

N° 22

The influence of commonly non-antibiotic drugs and prevalent diseases on the gut microbiome of institutionalized elderly

Cristina Jiménez-Arroyo[1]; Natalia Molinero[1]; Anabel Álvarez[2]; Benjamín Sánchez-Rigau[2]; Santiago Álvarez[2]; Diego Ramiro[3]; Margarita del Val[4]; M. Victoria Moreno-Arribas[1]

[1] Instituto De Investigación En Ciencias De La Alimentación (CIAL-CSIC-UAM)

[2] Farmacia de Dalt

[3] Instituto de Economía, Geografía y Demografía (IEGD-CSIC)

[4] Centro de Biología Molecular Severo Ochoa (CBMSO-CSIC)

Background. Age is a well-established risk factor for the development of several chronic diseases, which are usually managed by prescribing a combination of appropriate medications for each condition, and often results in polypharmacy, commonly defined as the use of 5 or more medications at the same time. Additionally, research has highlighted the role of the gut microbiome in many disorders prevalent in older adults, and, recently, the relation between commonly used drugs (non-antibiotic medications) and the gut microbiome has been proposed. However, interactions between drugs and the microbiome remain understudied in the context of ageing, where polypharmacy and comorbidities co-occur. Therefore, in this study we investigated the associations between drug use and the gut microbiome in a cohort of nursing home (NH) institutionalised older people, considering the intake of multiple drugs at the same time, within the context of multimorbidity, comorbidity and polypharmacy.

Methods. A prospective study was conducted in 18 different NH in Spain, involving 216 residents aged ≥65 years, recruited during 2021-2022. Stool samples were collected from each participant and analysed using a shotgun sequencing approach. Complete information about diagnosed diseases and prescribed medication was recorded. Both diseases and medication were classified according to the International Classification of Diseases Coding System 11th Revision (ICD-11) and the Anatomical Therapeutic Chemical (ATC) Classification. A total of 558 different diagnostics and 306 drugs were classified in 146 and 82 detailed categories, respectively. Only drug and disease categories present in at least 25% of the participants were considered. After shotgun metagenomic analysis of gut microbial composition and functionality, biodiversity was assessed, and linear regression models were constructed to identify key taxa or metabolic pathways in each disease or drug group. Two models were constructed: one that included only one drug/disease category (univariate model), and another that incorporated all drug/disease categories (multivariate model). Statistically significant taxa or metabolic pathways common in both models were considered.

Results. Analysis of the alpha diversity indexes showed that they tend to decreased as the number of prescribed drugs increases; however, this effect was not observed for diseases/comorbidities. Peptic ulcer and gastroesophageal reflux disease drugs were associated with a high number of taxa, while anti-constipation agents were associated with numerous metabolic pathways. Associations were also found for antithrombotic agents, high-ceiling diuretics, ACE inhibitors, other analgesics and antipyretics, antipsychotics, anxiolytics and antidepressants. In the case of diseases, the group of other types of heart disorders was significantly associated with a high number of taxa and metabolic pathways. In addition, metabolic and mental disorders, other degenerative diseases, hypertensive diseases and osteoarthritis also showed significant associations with gut bacteria.

Conclusions. Our study suggests that (poly)medication and common diseases in the elderly population have an impact on the gut microbiome. This effect is different for each drug or disease

category, even in the context of polypharmacy and multimorbidity/comorbidity. Further studies are needed to understand the complexity of the pharmacomicrobiomics interactions.

N° 23

Fitness effect of the isoniazid resistance mutation S315T of the catalase-peroxidase enzyme katG of Mycobacterium tuberculosis

Ugo Bastolla[1]

[1] Centro de Biología Molecular Severo Ochoa (CBMSO-CSIC)

The mutation S315T of the catalase-peroxidase protein of Mycobacterium tuberculosis is the most common mutation that confers resistance to the drug isoniazid, which is present in 74% of the resistant M. tuberculosis strains. Here we reconstruct the evolutionary history of this mutation in 145 whole genome sequences of M. tuberculosis isolates from Russian hospitals (Chernayeva et al. 2021) by inferring the phylogenetic tree and the sequences at ancestral nodes. We inferred the phylogenetic tree with different data and substitution models and verified that the qualitative results are robust. We infer 11 events (from 10 to 12) that caused the first appearance of the mutation katG(S315T). Interestingly, reversions katG(T315S) are also abundant (5 events, from 5 to 7), and their estimated mutation rate is at least 1500 times higher than for non-synonymous or intragenic mutations, which suggests the action of positive selection. This would imply that, contrary to the commonly held view, the mutation katG(S315T) presents a fitness cost, possibly because the mutant M.tuberculosis is less able to cope with oxidative stress in macrophages. Consistent with this hypothesis, the mutant enzyme presents a six-fold reduction in catalase and twofold in peroxidase activity (Wengenack et al. 1997). Applying the torsional network model, we found that the mutant protein shows decreased fluctuations in its thermal dynamics, although the dynamics of the functional site are quite similar to that of the wild type. Of the four internal nodes of the tree where katG(S315T) first arose, two produced many offspring while the other two produced few offspring. We found that these two groups present significantly different reproductive rates, a proxy for fitness, and are differentiated by secondary mutations at the 5'-UTR region of the gene superoxide dismutase A (sodA). Therefore, our results suggest that the resistance mutation katG(S315T) pays a fitness cost, which may be compensated through epistatic interactions with the gene sodA, hinting at possible intervention strategies.

N° 24

In host mutational adaptation of Mycobacterium tuberculosis during TB infection and treatment

Francesc Coll[1]; Naomi Medina-Jaudes[2]; Jody Phelan[2]; Helen Zhang[2]

[1] Instituto de Biomedicina de Valencia (IBV-CSIC)

[2] London School of Hygiene & Tropical Medicine, London, UK.)

Background: Tuberculosis (TB) is the second leading cause of death from an infectious disease worldwide. Mycobacterium tuberculosis (Mtb), the causative bacteria of TB, is becoming increasingly resistant to anti-TB drugs, resulting in poor treatment outcomes. The mutations arising in Mtb strains during infection provide a record of bacterial adaptation and, indirectly, evidence on the selective pressures and conditions that Mtb encountered in host.

Methods: We conducted a meta-analysis of published Mtb genomic datasets in which multiple clinical isolates had been sequenced from the same patient. We applied stringent metadata and genomic QC criteria to keep only clonal isolates. State-of-the-art genomic pipelines were used to characterise mutations accumulated during infection. A convergent evolution approach was applied to identify heavily mutated genes across multiple patients. We estimated the frequency of drug resistance acquisition during treatment for the subset of patients with available drug treatment data.

Results: We kept 5,899 high-quality Mtb genomes from 1,056 TB patients after ensuring clonality of isolate genomes. Limited within-host diversity was identified including 3,296 unique mutations across 501 patients. A total of 24 genes were statistically enriched by mutations compared to the rest of the genome. Among these, genes known to be associated with first-line anti-TB drugs (katG, ahpC, rpoB, rpoC, gid, rpsL, pncA, and embB), second-line, (gyrA, gyrB, alr, ald, ethA, tlyA and thyA) and last-line (Rv0678 and rplC) were found; as well as genes associated with virulence (phoR, mycP1 and eccE1). Less functionally characterised genes including Rv2571c (known to confer resistance to arylamide compounds) and Rv1129c/prpR (involved in the regulation of fatty acids utilization) were also identified. Fluoroquinolone resistance was acquired more frequently during treatment than resistance to any other anti-TB drug (13%, 95% CI: 10 – 17%).

Conclusions: Here we analysed the largest dataset of Mtb within-host diversity and evolution to date. We show that frequently mutated genes in Mtb during infection reveal known and biologically plausible in vivo adaptations, predominantly associated drug resistance, but also in genes involved in virulence. The higher resistance acquisition rates observed for fluoroquinolones may have important clinical relevance.

N° 25

Host-strain compatibility influences transcriptional responses in M. tuberculosis infections.

Mireia Coscollá

[1] Instituto de Biología Integrativa de Sistemas (I2SysBio-CSIC-UV)

N° 26

Phage therapy: from the bench to the bed

Mireia Bernabeu Gimeno[1]; **Pilar Domingo Calap[1]**; Lucas Mora Quilis[1]; Marco Pardo-Freire[1]; Miranda Tomás[1]

[1] Instituto de Biología Integrativa de Sistemas (I2SysBio-CSIC-UV)

Background: Emergence of multi-drug resistant bacteria is a global health problem. In the absence of available treatments, one of the most promising alternatives are phages. In Spain, their clinical use is limited to the context of compassionate therapy, with the Spanish Agency of Medicines and Health Products (AEMPS) being responsible for the authorization of each treatment on an individual case basis.

Methods: After the development of an in-house protocol for the production of phages approved by the AEMPS, we generated therapeutic preparations of phages containing 10^8-1010 viral particles. These vials have been used, so far, in more than a dozen compassionate treatments. To date, different phages have been used for treatments against Pseudomonas aeruginosa, Staphylococcus aureus, Mycobacterium abscessus and Klebsiella pneumoniae. The routes of administration have been nebulization, intravenous or local, depending on the infection.

Results: Patient follow-up includes analysis of clinical indicators, bacterial counts, bacterial susceptibility to phage and antibiotics, phage detection and immune response. No adverse effects have been reported in any case. Many of the patients have improved their quality of life and some of them have eradicated the infection.

Conclusions: This work shows the potential of phage therapy and the need for clinical trials to advance towards its widespread use in our country.

N° 27

Antiviral activity of iron oxide nanoparticles against SARS-CoV-2 and influenza A virus in mice

Marta López de Diego[1]; Yadileny Portilla[1]; Darío López García[1]; Neus Daviu[1]; Laura Villamayor[1]; Paula Vázquez Utrilla[1]; Vladimir Mulens Arias[1]; Sonia Pérez Yagüe[1]; Aitor Nogales[2]; Jesús G. Ovejero[3]; Álvaro Gallo Cordova[3]; Luis Enjuanes[1]; Sabino Veintemillas Verdaguer[3]; M. Puerto Morales[3]; Domingo F. Barber[1]

[1] Centro Nacional de Biotecnología (CNB-CSIC)

[2] Centro de Investigación en Sanidad Animal (CISA-INIA-CSIC)

[3] Instituto de Ciencia de Materiales de Madrid (ICMM-CSIC)

Background: Severe Acute Respiratory syndrome coronavirus 2 (SARS-CoV-2) and Influenza A viruses (IAVs) are among the most important causes of viral respiratory tract infection. IAVs usually cause mild respiratory disease in humans, although the symptoms may be more severe in some individuals and serious complications like bronchitis and pneumonia may arise, even leading to death. SARS-CoV-2 infection can provoke mild symptoms like fever, cough, sore throat, loss of taste or smell, or it may have severe consequences and lead to acute respiratory distress syndrome or death. While treatments for IAV and SARS-CoV-2 infection are available, IAV antivirals often target viral proteins, which facilitates the emergence of drug-resistant viral variants. Furthermore, universal treatments against coronaviruses and IAVs are hard to obtain due to genus differences (in the case of coronavirus) or subtypes (in the case of IAV), highlighting the need for novel antiviral therapies to treat coronavirus and IAV infections.

Methods: Here, we analyzed the antiviral activity of iron oxide nanoparticles (IONPs) coated with the biocompatible dimercaptosuccinic acid, or coated with polydopamine-functionalized silica, against SARS-CoV-2 and IAV infections in vitro and in vivo. Results: These IONPs displayed a prophylactic and therapeutic effect against SARS-CoV-2 and IAV in tissue cultured cells. Moreover, at non cytotoxic doses, the treatment of mice with these IONPs after SARS-CoV-2 and IAV infections, impaired virus replication in the lungs and mildly reduced pro-inflammatory cytokine induction, indicating that these IONPs can serve as COVID-19 and flu therapeutic agents. As an exacerbated inflammatory response to SARS-CoV-2 and IAV is detrimental to the host, weakening this response in mice through IONP treatment may reduce disease severity. Interestingly, our data suggest that IONP treatment affects the oxidative stress and iron metabolism in cells, which may influence SARS-CoV-2 and IAV production.

Conclusions: This study highlights the antiviral activity of IONPs against important human respiratory viruses.

N° 28

Discovery of a novel coronaviral molecular target through characterization of molecules with pan-coronavirus antiviral activity

Paula Bueno[1]; Daniel Cabrera-Torrejón[2]; Victoria Castro[1]; Francisco F. Castro-Navas[2]; Carlota Fernández-Antunez[3]; Jennifer Moya[1]; Javier L. Barrera[2]; Laura Barbado[1]; Álvaro Cobos[2]; Gema Calvo[1]; Santseharay Ramírez[3]; Jens Bukh[3]; Miguel Ángel Martín-Acebes[4]; Urtzi Garaigorta[1]; Marta Gutierrez[2]; Pablo Gastaminza[1]

[1] Antiviral Discovery and Characterization Platform. Centro Nacional de Biotecnología (CNB-CSIC)

[2] Instituto de Química Médica (IQM-CSIC)

[3] Copenhagen Hepatitis C Program (CO-HEP), Copenhagen University Hospital

[4] Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA-CSIC)

Coronaviruses constitute a serious pandemic threat due to their zoonotic and efficient dissemination potential. While the development and implementation of prophylactic vaccines constitutes a major measure to control the spread and the severity of the disease induced by these viruses, the availability of therapeutic options for infected individuals that cannot control infection and/or infection-related pathologies is needed.

Using a phenotypic cell-based screening approach, a new family of antiviral molecules against SARS-CoV-2 has been identified and optimized through a medicinal chemistry program, including structure-activity and structure-property relationship studies for the generation of preclinical candidates with optimal therapeutic window in different cell culture models. This family of compounds showed selective activity against human coronaviruses such as SARS-CoV-2, hCoV-229E and MERS-CoV, without any measurable activity against other RNA viruses like West Nile and dengue viruses, suggesting a potential pan-coronavirus antiviral activity. Time of addition as well as subgenomic replicon transfection experiments indicate that these compounds strongly inhibit infection at a post-entry step and that they impair accumulation of subgenomic viral RNAs in single cycle infection experiments.

The genetic resistance profile of lead compounds has been determined by directed evolution in cell culture. These studies revealed that the selective pressure imposed by the compounds cluster together in a non-structural region of SARS-CoV-2 genome responsible for the formation of double membrane vesicles, the putative viral RNA replication compartment. Individual candidate mutations, and combinations thereof, were evaluated by reverse genetics in subgenomic SARS-CoV-2 replicons, confirming the requirement of two mutations in nsp4 and one mutation in nsp6 to confer full resistance to these antivirals, reinforcing the notion of a novel mechanism that could be exploited for combination therapies with drugs targeting well-established viral molecular targets.

N° 29

Synthetic heparan sulfate mimics based on chitosan derivatives show broad-spectrum antiviral activity

Alfonso Fernández Mayoralas[1]; **Ron Geller[2]**; Miguel Ángel Martín Acebes[3]; Julia Revuelta[1]; Ana Belén Blázquez[3]; Clara Frances Gomez[2]; Alfonso Gutiérrez Adán[3]; María Gómez Martínez[4]; Susana Iglesias[1]; Acsah Konu Paramban[4]; Jordi Llop[4]; José Luis LLácer[5]; María Luisa López Redondo[5]; Oscar Moreno[4]; Luciana Rusu[2]; Elena Trapero[1]

[1] Instituto de Química Orgánica General (IQOG-CSIC)

[2] Instituto de Biología Integrativa de Sistemas (I2SysBio-CSIC-UV)

[3] Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA-CSIC)

[4] Centro de Investigación Cooperativa en Biomateriales CIC biomaGUNE

[5] Instituto de Biomedicina de Valencia (IBV-CSIC)

Enveloped viruses enter cells by binding to receptors present on host cell membranes, which trigger internalization and membrane fusion. For many viruses, this either directly or indirectly involves interaction with membrane-anchored carbohydrates, such as heparan sulfate, providing a potential target for a broad-spectrum antiviral approach. Based on this hypothesis, we screened a library of functionalized chitosan sulfates that mimic heparan sulfate in cellular membranes for inhibition of SARS-CoV-2 and respiratory syncytial virus (RSV) entry. An array of compounds blocking SARS-CoV-2 and RSV were identified, with the lead compound displaying broad-spectrum activity against multiple viral strains and clinical isolates. Mechanism of action studies showed the drug to block viral entry irreversibly, likely via a virucidal mechanism. Importantly, the drug was non-toxic in vivo and showed potent post-exposure therapeutic activity against both SARSCoV-2 and RSV. Together, these results highlight the potential of functionalized carbohydrates as broad-spectrum antivirals targeting respiratory viruses.

N° 30

3D in vitro skin model as broadly available and comprehensive platform for antibacterial therapies

Simona Villata[1]; Désirée Baruffaldi[1]; Paula Bosch[2]; Raquel Cue López[2]; Francesca Frascella[1]; Andrea Mario Gio Vannozzi[3]; Enrique Martínez Campos[1]; Lucia Napione[1]; Camilla Paoletti[1]; Fabrizio Candido Pirri[1]

[1] Politecnico di Torino

[2] Instituto de Ciencia y Tecnología de Polímeros (ICTP-CSIC)

[3] Istituto Nazionale di Ricerca Metrologica

Background: One of the largest worldwide emergencies is for sure the spreading of skin infections. In the last decades, moreover, the rise of antibiotic-resistant bacteria has led to the ineffectiveness of most antibacterial strategies and antibiotics, with terrible consequences on life quality and survival possibilities of patients. For this reason, lots of effort is made to design and develop new and innovative antibacterial therapies. The first and more common step to validate these new therapies is to test them on traditional 2D cell culture and planktonic bacteria culture, but these methodologies can be obsolete due to their losses in resembling the complex infection environment. Consequently, 3D in vitro skin models can be a powerful tool.

Methods: In this work, a 3D in vitro skin model has been developed: the dermis compartment was obtained embedding human fibroblasts into gelatin methacryloyl matrix, while to obtain the epidermis, human keratinocytes were seeded on top of the dermal part. After a first submerged culture period, constructs were cultured in Air-Liquind Interface configuration for 31 days. Models showed impressive development, in particular in terms of barrier building and extracellular matrix remodelling. To further test the model, a controlled wounding was performed, using a 3mm diameter biopsy punch. Next, either Staphylococcus aureus (gram positive bacteria) or Escherichia coli (gram negative bacteria) were inoculated into the wounded and unwounded samples, to simulate infection. After 24 hours of infection, antibiotic treatment was performed using a mix of penicillin-streptomycin at 5% v/v. This antibiotic mixture was chosen to validate the infected model, as it is the most used antibiotic mixture in cell culture. After 24h of treatment, samples were analysed both in terms of bacterial survivor and in terms of skin model response.

Results: Results were remarkable: differences between wounded and unwounded samples underlined the barrier effect: indeed, the bacterial proliferation was lower in unwounded samples, for both bacterial strains, meaning that the developed epidermis was able to partially stop the bacterial proliferation. Moreover, the 3D skin model was able to react to both wound and infection in a complete and complex way in terms of extracellular matrix deposition and remodeling, inflammatory response, antimicrobial peptides production and change in cellular behaviors, from epithelial to mesenchymal and from fibroblasts to myofibroblasts. Another important outcome was the change in skin response during infection, showing the ability of both bacteria, in different ways, to impair the model immune response. Also, the antibiotic interacted with the model, modulating some markers, giving some evidences of the release of endotoxins with Escherichia coli death.

Conclusions: In conclusion the developed 3D in vitro skin model has the potential to become a future landmark as platform for infection investigation and novel therapies testing. Indeed, it demonstrated to be able to behave in a complex and complete way despite being easily producible and low cost. These properties put it in a prominent position for future standardization of platforms to test antibacterial therapies and strategies.

N° 31

A new generation of cell culture supports: customized thermosensitive hydrogels for biomedical applications

Enrique Martínez Campos[1]; Carlos Elvira[1]; Alberto Gallardo[1]; Ana García Crespo[1]; Pedro Liz Basteiro[1]; Helmut Reinecke[1]; Juan Rodriguez Hernandez[1]

[1] Instituto de Ciencia y Tecnología de Polímeros (ICTP-CSIC)

Background. In regenerative medicine, certain complex pathologies require the application of next-generation biomaterials to provide new therapies. In this sense, particular skin diseases (such as psoriasis) or cartilage damage (due to osteoarthritis or other injuries) are defined by ineffective standard medical treatments that are expensive for the healthcare system.

Our group is developing new cell therapies based on smart hydrogels (iFABCell technology) to improve tissue regeneration in these clinical indications. For this goal, these supports present thermosensitivity, which allows both controlled drug release and cell harvesting capacity, providing an optimal platform to control cell behavior. Moreover, our hydrogels can be used as a direct carrier to transplant cell sheets to the injured area with a temperature decrease, avoiding the implantation of the biomaterial. In order to first assess the efficacy of our platforms in an animal model, partial-thickness osteochondral defects in rabbits have been treated using primary cultures of mesenchymal stem cell sheets.

Nevertheless, the requirements of major injuries (e.g., full-size osteochondral defects or several burns) are more demanding, and require the implantation of tailor-made scaffolds into the target tissue. For this aim, drug encapsulation and 3D bioprinting technology is being implemented to achieve customized microenvironments optimal for improving tissue regeneration in relevant clinical models, such as skin or cartilage.

Methods. Hydrogels based on poly-vinylcaprolactam (PVCL) has been synthesized by photopolymerization. 3D samples have been obtained both through SLA (Zrotrax) and bioprinting (DomoTekBio) using different bioinks (alginate and gelatine) for the latter. Precision and mechanical properties (rheology) have been analyzed. Icariin-loaded PVCL nanoparticles were obtained by supercritical CO2 SAS technology. Commercial cell lines (C166-GFP, MC3T3) and bone-marrow rabbit mesenchymal stem cells have been used to biological evaluation. Articular knee cartilage (rabbit) has been used as a preclinical model of study (partial thickness defects) and paraffin sections have been obtained in order to evaluate osteochondral regeneration.

Results. Customized thermosensitive hydrogels have been obtained using several 2D and 3D techniques. Hydrogels were activated with nanoparticles containing icariin, a small molecule involved in bone regenerative processes. These supports have demonstrated their efficacy as controlled drug release platforms to stimulate osteoblastic proliferation and differentiation, while retaining their ability to detach monolayers by lowering temperature. Finally, in rabbit osteochondral partial defect regeneration, thermosensitive supports carrying mesenchymal cell sheets accelerated tissue healing increasing chondrocyte activity and matrix secretion.

Conclusions. In order to design optimal samples for surgical purposes, a multidisciplinary point of view should be applied. First, both the physical and chemical properties of the cell support could be adjusted, including composition, synthesis method or topography. Second, biological implications such as tissue behavior, signaling or other complex processes (vascularization, immune response) should

be considered. Thus, new successful advanced therapies can be developed and transferred to the society.

N° 32

Harnessing Rho GTPase activity to preserve human endothelial barrie function during systemic inflammation

Jaime Millán[1]

[1] Centro de Biología Molecular Severo Ochoa (CBMSO-CSIC)

Rho GTPases are molecular targets of bacterial toxins that modulate their enzymatic activity. RhoA, RhoB and RhoC are almost identical and regulate common effectors that generate actomyosinmediated contractile forces and hyperpermeability in the endothelium during inflammation. Here, we demonstrate that the specific activation of these three Rho GTPases with a chimeric recombinant toxin does not induce cell contraction but stabilizes adherens junctions and preserves microvascular endothelial barrier function in response to pathological inflammatory challenges in vitro and in vivo. This pro-barrier effect is specifically mediated by RhoC, whose activity is increased by cell confluence. The uniqueness of RhoC relies on an arginine 188 within its hypervariable region, which determines its junctional localization, high homeostatic activity, and barrier-protective function. Quantitative proteomics revealed that RhoC regulates the expression of myosin light chain proteins and the junction-stabilizing actomyosin. Hence, harnessing the activity of RhoC represents a potential therapy for strengthening endothelial barriers during pathological inflammation.

N° 33

Optimization of the interaction between tubulin and drugs from the colchicine site: in vitro and in vivo evaluation

Daniel Lucena-Agell[1]; María José Guillén Navarro[2]; Ruth Matesanz Rodríguez[1]; Beatriz Álvarez-Bernad[1]; Rafael Hortigüela Mecerreyes[1]; Pablo Avilés[2]; Marta Martínez-Díez[2]; Gema Santamaría-Núñez[2]; Julia María Contreras[3]; Iván Plaza Menacho[3]; Juan Francisco Giménez Abián[1]; Marian Oliva[1]; Carmen Cuevas Marchante[2]; José Fernando Díaz Pereira[3]

[1] Centro de Investigaciones Biológicas Margarita Salas (CIB-CSIC)

[2] PharmaMar

[3] Centro Nacional de Investigaciones Oncológicas (CNIO-ISCIII)

Over the past sixty years, microtubule-targeting agents have demonstrated a great success in their employment in cancer treatment, both alone and in combination with other drugs. However, two significant drawbacks limit their clinical application. Firstly, their toxic side effects, primarily neurotoxicity and neutropenia that block the use of these drugs in several cycles of chemotherapy. Secondly, the rise of resistances through different mechanisms like the overexpression of membrane pumps and, the differential expression of specific tubulin isotypes, which urge to find new compounds capable to override these problems.

In addition to their relevance in cancer biology and chemotherapy, microtubules are used by certain viruses as intracellular platforms to facilitate their viral life cycle. Based on this, we proposed the use of these compounds as antiviral agents in a study published in 2022. From that study, ligands that bind to the colchicine site emerged as potential candidates to inhibit de infectivity of some viruses, indicating that the colchicine site's mechanism of action on tubulin is relevant for this purpose.

Here, we present a collaborative effort with the pharmaceutical company PharmaMar to develop PM534, a colchicine site binder with potent antitumor properties. Through cell biology, mouse xenografts, biochemical assays, and structural analysis, we have characterized the relevance of this compound as a microtubule-targeting agent. PM534 binds to the full colchicine site in tubulin, occupying four of the five centers in the pharmacophore model. Its nanomolar affinity, extended dwelling time, and ability to overcome the two distinct mechanisms of resistance underscore its exceptional potential. Furthermore, PM534's significant growth-inhibitory effects over non-small cell lung cancer xenografts in mice demonstrate high efficacy in preclinical evaluation.

In summary, PM534 is a promising new drug currently undergoing its first human Phase I clinical trial.

N° 34

Near clinical validation of screening models in large-scale deployment

Francisco Albiol[1]; Alberto Albiol[2]; Roberto Paredes[2]; Salvador Tortajada[1]

[1] Instituto de Física Corpuscular (IFIC-CSIC)

[2] PRHLT Research Center-Universidad Politécnica de Valencia

Background. Machine Learning (ML) models are trained for various purposes, with screening and diagnosis serving distinct roles. Screening focuses on identifying individuals at higher risk of developing diseases, while diagnosis confirms or evaluates a condition. In breast cancer, two screening approaches are explored: real screening, which involves systematic detection in asymptomatic populations, and opportunistic screening, where patients are identified during unrelated clinical encounters. Both strategies are being developed with high Technology Readiness Levels (TRL) to ensure scalability for clinical use. This work presents a methodology to facilitate the deployment of ML models in these screening contexts, ensuring their reliability, reproducibility, and regulatory compliance.

Methods. To ensure that ML models for healthcare, particularly medical imaging, meet the necessary requirements for clinical integration. To achieve reproducibility, maintainability, compatibility, and usability, we implement software isolation tools, which enable consistent execution across different environments. We use explainable ML techniques for visual interpretability and/or for textual explanations, thus enhancing transparency in decision-making. Integration with existing medical systems is streamlined through the use of DICOM formats and/or HL7/FHIR normalization, facilitating standardization, scalability, and seamless deployment in clinical settings and focusing on regulatory compliance, ensuring future readiness for certification processes. This approach guarantees that the ML models not only deliver reliable outcomes but also fit within the stringent operational and regulatory frameworks of the healthcare industry.

Results. Our methodology has been successfully tested in real clinical environments, demonstrating its efficacy in achieving the expected outcomes for ML model deployment in medical imaging (TRL 7). The models exhibited consistent performance across varied settings, validating their reproducibility and reliability. Additionally, we have established collaborations with prominent hospital groups that have shown strong interest in adopting and integrating the developed technologies into their clinical workflows. These partnerships underline the potential impact and scalability of our approach, marking an important step towards the broader clinical application of AI in healthcare.

Conclusions. Our approach for the clinical implementation of ML models ensures that key features such as reproducibility, transparency, and regulatory compliance are achieved, enabling smooth integration into medical environments. The consistent performance across different systems, combined with increased transparency and adherence to industry standards, establishes guidelines for implementing ML models in clinical practice and enabling innovation. This approach lays a foundation for the broader adoption of adaptive ML in medical imaging and other healthcare fields, potentially transforming clinical workflows and enhancing patient outcomes.

N° 35

Inflammation cellular platform (INCEPLAT) for testing anti-inflammatory compounds for SARS-CoV-2

Blanca Dies López-Ayllón[1]; Laura Mendoza-Garcia[1]; Ana De Lucas-Rius[1]; Tránsito Garcia-Garcia[2]; Raúl Fernández-Rodríguez[2]; Antonio Romero-Guillén[2]; Judit Serrat[3]; Sonia Zuñiga[4]; Natalia Redondo[1]; Mar Siles-Lucas[3]; Juan J. Garrido; María Montoya[1]

[1] Centro de Investigaciones Biológicas Margarita Salas (CIB-CSIC)

[2] Universidad de Córdoba.

[3] Instituto de Recursos Naturales y Agrobiología de Salamanca (IRNASA-CSIC)

[4] Centro Nacional de Biotecnologia (CNB-CSIC)

Background. From the early days of the COVID-19 pandemic, an excessive release of proinflammatory cytokines, such as IL6, was detected in serum from patients. As a consequence, several anti-inflammatory drugs, such as Dexamethasone (a strong corticoid), were used to counteract such cytokine storm occurring during severe disease. By contrast, pro-inflammatory interleukin 11 (IL11), a member of the IL6 family, was detected in respiratory tissues from infected patients and in experimental epithelial cellular models.

Methods and Results. In this work, human A549 lung epithelial cells were individually transduced with SARS-CoV-2 open reading frames (ORFs), resulting in a IL11 increase, which was significantly decreased after Dexamethasone treatment. The use of this cellular platform allowed us to screen for new possible anti-inflammatory compounds from Fasciola hepatica. Our results highlighted the ability of FhNEJ (Fasciola hepatica newly excysted juvenile flukes) somatic extract to decrease IL11 levels in ORF-transduced cells.

Conclusions. These results emphasized the role of IL11 in lung epithelial inflammation, making it a potential target for future treatments of lung inflammation which occurs in COVID-19, and validate the use of these ORF-expressing cells as a cellular platform to test anti-inflammatory compounds for COVID-19 disease.

N° 36

Presentación del comité de emergencias del CSIC

Ana Allende Prieto[1]

[1] Centro de Edafología y Biología Aplicada del Segura (CEBAS-CSIC)

N° 37

The impact of viruses that infect marine animals in global health

Carolina Johnstone[1]

[1] Centro Oceanográfico de Málaga (IEO-CSIC)

Global health depends on the health of marine living resources and ecosystems. Viruses that infect marine animals can impact global health in different ways. Firstly, food security relies on increasing the production of protein-rich food through fisheries and aquaculture. Viruses such as nervous necrosis virus that infect fish species worldwide affect the aquaculture industry. The identification of wild reservoirs and the understanding of the antiviral host response are necessary to control outbreaks and develop treatments for aquaculture. Secondly, viral infectious diseases can threaten the conservation of marine animal species. Such is the case of herpesviruses associated to tumours in seaturtles, iconic species that provide important ecosystem services. Lastly, preparedness for future pandemic events demands research on panzootic viruses that infect marine higher vertebrates. An example is the present H5N1 influenza panzootic that has caused deaths in seabirds and marine mammals in different continents. In conclusion, widening our knowledge on viral infections in wild marine animal species is important from the global health perspective, particularly in the present climate change scenario.

N° 38

From Short-Term Strain to Lifelong Burden: A Dual Perspective on Studying Health Impacts of Heatwave Exposure in Spain

Dariya ORDANOVICH[1]; Diego RAMIRO FARIÑAS[1]

[1] Instituto de Economía, Geografía y Demografía (IEGD-CCHS-CSIC)

Background. The rise in global temperatures is a growing concern, particularly in Mediterranean regions like Spain, where climate change has severe implications for public health. With predictions indicating a continued increase in extreme weather events by the end of the 21st century, this contribution aims to assess the short-term and long-term mortality impacts of heatwave exposure in Spain. Specifically, it investigates the immediate effects of heatwaves by age and sex across Spanish provinces over the past 45 years and explores long-term cumulative exposure to heat across birth cohorts. By combining both perspectives, we seek to provide a comprehensive understanding of heatwave-related mortality risks.

Methods. This research integrates two complementary approaches to analyze mortality risks from heatwave exposure. The first study utilizes daily individual mortality data from the National Institute of Statistics (INE) and air temperature estimates from the ERA5 global reanalysis dataset, focusing on 48 Spanish provinces from 1980 onwards. A quasi-Poisson time-series regression model, employing a distributed lag non-linear model (DLNM) with a 10-day lag, is used to estimate both the main and added effects of heatwaves on mortality, controlling for trends and day of the week. The second study adopts a cohort-based approach, leveraging the same mortality statistics to analyze health disparities across birth cohorts from 1975 onwards. Cumulative heat exposure is measured using climate reanalysis data and urbanization indicators derived from cadastral data on human settlement patterns since 1900. The birth cohorts are stratified by demographic and geographic characteristics, allowing for an in-depth exploration of long-term environmental exposure.

Results. The results of the first study reveal that while heatwaves have a significant added effect on mortality in a limited number of provinces, the dominant factor influencing mortality is temperature itself. The highest overall risks were observed in northern and central-southern Spain, with less impact seen along the Mediterranean coast and in the northwest. Vulnerability to heatwaves was particularly pronounced among non-married women of advanced ages, especially in provincial capitals. Over time, the number of provinces exhibiting significant heatwave-related mortality effects increased until 2009, after which a decline was noted. However, the mortality attributable fractions generally remained below 1%. The preliminary results of the analysis performed in the second study include a clear increase in cumulative heat wave exposure across more recent birth cohorts, reflecting the increasingly harsh climate conditions over the past decades. We anticipate that older age groups, particularly those exposed to extreme temperatures later in life, will show higher vulnerability to heat-related mortality. Additionally, the analysis is likely to reveal gender and socio-economic disparities, with women and lower-income individuals disproportionately affected by cumulative heat wave exposure due to factors such as housing conditions and access to resources. These findings will highlight the differential impacts of climate change on mortality across demographic groups and locations.

Conclusions. This dual-perspective analysis demonstrates the critical role of both short-term and long-term heatwave exposure in shaping health outcomes in Spain. Immediate effects of heatwaves

pose significant risks to most vulnerable populations, particularly the elderly, women, and individuals of lower socioeconomic status. At the same time, cumulative exposure to heat over the life course—especially during critical developmental windows—creates substantial disparities in mortality outcomes across regions and generations. These findings underscore the need to consider both the acute and accumulated health impacts of extreme heat in understanding the adaptive mechanisms and shaping public health responses to climate change.

N° 39

Development of an Interactive Application with Shiny for the Analysis and Visualization of COVID-19 RT-qPCR Data

Paula Martínez García[1]; Manuel Ramos Alvarez-Buylla[1]; Sandra González De La Fuente[1]; Miriam Miranda García[1*]; Rosa Maria Moreno Alonso[2]; Rebeca Jimenez Peral[2]; Juncal Sánchez Alonso[2*]; Marta Bermejo Bermejo[2]; Margarita Del Val Latorre[1]; Begoña Aguado Orea[1]

[1] Centro de Biología Molecular Severo Ochoa (CBMSO-CSIC)

[2] Unidad de Vigilancia de la Salud y Medicina del Trabajo CSIC

[*] Former Member

Background. During the first year of the COVID-19 pandemic and the following two, extensive follow-up was carried out on numerous patients who presented symptoms consistent with COVID-19 or who had been in direct contact with positive cases. This monitoring included periodic RT-qPCR tests, as well as the detailed documentation of symptoms, infection circumstances, and vaccination status of the patients.

The main motivation for this work arises from the challenge of analyzing such a volume of data in a way that allows for proper correlation to draw clear conclusions. The **traditional use of graphs and static images is limited**, since this type of analysis usually requires constant adjustments of parameters and variables to obtain the desired information, adapting the presentation results to the specific needs of each researcher. However, **the use of interactive applications offers a solu-tion to these limitations**, allowing a more dynamic and flexible exploration of the data. This improves researcher´s ability to extract valuable information and optimizes the analysis and decision-making process.

Methods and Results. For this project, we have developed, with the Shiny[1] package of the R[2] programming language, an interactive application that analyzes data from SARS-CoV-2 RT-qPCR for the diagnosis of COVID-19. This tool allows users to quickly and efficiently select, based on the combined visualization of the data rather than subjective decisions, the Cq threshold from which an RT-qPCR is considered negative, as well as the determination of the number of days that should elapse between two con-secutive samples from the same patient to consider them as different infections or part of the same infection. In this way, the data generated by RT-qPCRs have allowed us to study, define and distinguish cases of viral persistence and patients with multiple infections. Currently, ongoing studies involve the correlation of these data with others, as those already mentioned above, and additional data obtained from parallel studies with the same patients.

Conclusions. The development of interactive applications with Shiny has proven to be a powerful tool for the analysis and visualization of clinical and omics data. These applications allow researchers to modify variables and parameters in real time, facilitating a deeper and more detailed exploration of the data, making it more accessible and comprehensible. This not only improves the interpretation of results, but also optimizes the decision-making process, allowing for more precise and efficient conclusions, which in turn enhances the quality and speed of scientific research and, in the medical field, contributes to more informed and personalized care for patients.

---

[1] BorgesB (2024). shiny: Web Application Framework for R. R package version 1.8.1.9001,https://github.com/rstudio/shiny, https://shiny.posit.co/

[2] R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

N° 40

Are our homes ready for teleworking?

Teresa Cuerdo Vilches[1]; Miguel Ángel Navas Martín[2]

[1] Instituto de Ciencias de la Construcción Eduardo Torroja (IETcc-CSIC)

[2] Escuela Nacional de Sanidad, Instituto de Salud Carlos III

Background. The COVID-19 pandemic and the precautionary measures applied globally (lockdowns and curfews) impacted homes, including work. Working from home (WFH) has emerged as a growing trend in the post-pandemic era. The research question was: Are our homes ready for teleworking?

Methods. A national prospective mixed approach was launched for Spanish households during the spring 2020 lockdown, using two online questionnaires, one quantitative and the other qualitative. Through an online survey, photos and narratives, the study assessed the perceived adequacy of telework spaces and their specific features, the availability of digital resources and the internet.

Results. A total of 1800 surveys and over 200 images and testimonies linked to telework settings were obtained. The results suggest that the adequacy of these spaces was insufficient for more than a quarter of the homes. Also, strong relations between the perceived workspace adequacy and a social status or stability of homes were shown and validated, despite other sociodemographic features, the home composition or habitat were not related. Some other variables statistically significant were occupation regime, type and surface of dwellings; their indoor environmental quality; the availability of exclusive spaces for teleworking; quality of digital resources; and the specific space features. The analysis was completed with qualitative insights through photos and texts. Conclusion: Telework, lived in this context as an experiment, invites to reflect on the environment, resource-availability, and ergonomics. Moreover, the study suggests the need of stablishing a representative study, for the whole national territory, where a validated questionnaire on indoor environmental quality and socio-economic features are a further analysis to be led to taxonomize the teleworkers' housing stock, and evaluate how suitable it is for this new mass domestic task.

N° I

AI-driven de-novo design and synthesis of non-toxic DYRK1A inhibitors for alzheimer's disease

Pablo Varas Pardo[1,2]; Eduardo González[3]; Pedro González Naranjo[4]; Eugenia Ulzurrun[1,3]; Concepción Pérez[4]; Juan Antonio Páez4; Simón Rodríguez Santana[5]; Nuria E. Capillo[3]

[1] Instituto de Ciencias Matemáticas (ICMAT-CSIC)-UAM-UC3M-UCM

[2] Altenea Biotech, S.L.

[3] Centro de Investigaciones Biomédicas Margarita Salas-CSIC

[4] Instituto de Química Médica (IQM-CSIC)

[5] ICAI-Universidad Pontifícia de Comillas

Alzheimer's disease (AD) is the most prevalent form of dementia, accounting for 60-80% of cases. Currently, there is no cure, and existing medications are palliative. It is a complex, multifactorial disease whose pathophysiological mechanisms are not fully understood. In recent years, the enzyme DYRK1A has emerged as a promising therapeutic target in AD, as it is involved in multiple biological functions. Specifically, studies have shown that alterations in DYRK1A, such as the phosphorylation of proteins like TAU and APP, correlate with AD progression. Therefore, DYRK1A is a highly promising target for designing new drugs to treat AD. Our objective in this work is to use predictive and generative AI tools to design non-toxic DYRK1A inhibitors.

We present a successful strategy for integrating AI-based methods in the de-novo generation of molecules within a binary target drug discovery framework. The generative process is detailed, demonstrating its effectiveness through in-vitro studies focused on candidate molecules designed to inhibit DYRK1A kinase. This approach was conducted under a small-data regime, utilizing various AI techniques to develop an optimal model for generating viable candidates. The most promising candidate, with a novel structure, has been synthesized along with a family of derivatives. All of these compounds have been evaluated enzymatically against DYRK1A, showing nanomolarlevel activity, as well as antioxidant and anti-inflammatory potential. Thanks to the synergy between AI, computational techniques such as docking, and organic chemistry, we have developed a family of DYRK1A inhibitors with a highly promising pharmacological profile.

N° 2

A C-2/C-7 doubly arylated tryptophan tetramer exhibits selective activity against ómicron SARS-COV-2

Ron Geller[1]; José Luis Llácer[2,3]; Alberto Marina[2,3]; Miguel A. Martín-Acebes[4]; María-Jesús Pérez-Pérez[5]; Vicente Rubio[2,3]; **Ana Rosa San-Félix García**[5]; Anmol Adhav[2]; Ana Avilés[1]; Ana Isabel Avilés-Alía[1]; Ana-Belén Blázquez[4,2]; Ana Esteban[4]; Alicia Forcada-NadaL[2,3]; Nereida Jiménez de Oya[4]; Maria Luisa López-Redondo[2]; Olaia Martí-Marí[5]; Marta Moreno[5]

[1] Instituto de Biología Integrativa de Sistemas (I[2]SysBio UV-CSIC)

[2] Instituto de Biomedicina de Valencia (IBV, CSIC)

[3] CIBER Enfermedades Raras (CIBERER-ISCIII)

[4] Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA, CSIC)

[5] Instituto de Química Médica (IQM-CSIC)

Background. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has evolved since the beginning of the COVID-19 pandemic. Successful variants generally exhibit enhanced transmission or immune escape. The Omicron form (B.1.1.529) in its several subvariants (including BA.1 to BA.5) has become predominant worldwide. We recently developed a new class of SARS-CoV-2 entry inhibitors with antiviral potential, of which the prototype was the tripodal tryptophan derivative ASF-071[1]. This compound inhibited cellular entry of SARS-CoV-2 through interaction with the viral spike (S) protein. Unhappily, ASF-071, although effective with the initial SARS-CoV-2 strain (Wuhan-Hu-1), had little antiviral potency against Omicron. Now we report that a tetrapodal compound, ASF-006, which was little active against the Wuhan-Hu-1 strain, is highly effective against Omicron, acting through a mechanism similar to ASF-071.

Methods. Members of our in-house collection of multivalent derivatives were tested in an HTS assay in Vero E6 and A549-hACE2 cells using VSV pseudoparticles expressing the spike of SARS-CoV-2 variants[2]. Thermofluor (Tf) assays were used to detect the interaction of ASF-006 with the Receptor Binding Domain (RBD) of protein S[1], and microscale thermophoresis (TH) was utilized to quantify the affinity of this interaction with the RBD or S proteins, and to monitor the effects of ASF-006 on the affinity of these proteins for their ACE2 cellular receptor[1]. Single-particle cryo electron microscopy (cryoEM[1]) was used to determine the structure of S in the presence of ASF-006. Humanized ACE-2 mice were used for testing the efficacy of intranasally administered ASF-006 against omicron (BA.1) infection.

Results. In the HTS assay, trimer ASF-071 had no significant antiviral activity on VSV pseudoparticles exhibiting the S protein of Omicron variants (BA.2 and BA.4.5 tested), while tetramer ASF-006 was active at submicromolar concentrations against Omicron variants, without cellular toxicity. A new synthetic route was developed for multigram efficient ASF-006 production, preparing this compound in salt form for improved water solubility. TF assays showed that ASF-006 binds to the RBD of Omicron (tested with variant BA.1), and TH assays confirmed such binding and showed ASF-006 binding to Omicron BA.1 S protein with a KD ≈ 5 μM. Furthermore, ASF-006 hampered binding of BA.1 RBD or S to ACE2, supporting that the antiviral effect on Omicron variants was due to inhibition of viral entry. Cryo-EM experiments on the Omicron BA.1 S protein in the presence of ASF-006 revealed a predominance of "3 RBD-down" conformation, suggesting that decreased RBD exposition could be an

[1] Gargantilla, M. et al. J.Med.Chem.2023;66:10432-10457

important factor in Omicron viral entry inhibition of ASF-006. In mice infected with Omicron (BA.1), intranasal treatment with ASF-006 significantly reduced viral load in lungs (determined by qPCR) and was not toxic.

Conclusions. Thanks to a mechanism of viral entry inhibition, ASF-006 appears a promising prototype for developing drugs for preventing SARS-CoV-2 Omicron infection.

Acknowledgements. Supported by grants from the NextGenerationEU fund (EU2020/2094) through CSIC's PTI Salud Global+; by Plan Estatal de I+D+I, Projects PID2020-120322RB-C21 (to VR) and PID2020-116880GB- I00 (to JLL) from the Spanish Government and by CSIC, PIE 202340E18 (to MAM-A).

N° 3

Thioaryl imides, a novel familiy of potential SARS-COV-2 entry inhibitors identified through a high thoughput pseudotyped assay

Ernesto Quesada del Sol[1]; Carla Lozano-Morcillo[1]; Ana Isabel Avilés-Alía[2]; Víctor Fernández Cabello[1]; Mónica Marlasca[1]; María José Camarasa Rius[1]; María-Jesús Pérez-Pérez[1]; Ron Geller[2]

[1] Instituto de Química Médica IQM-CSIC

[2] Instituto de Biología Integrativa de Sistemas (I[2]SysBio UV-CSIC)

Background. Although the WHO declared the end of the COVID-19 emergency in May 2023, the disease remains as a global public health threat and complete eradication of the causative agent, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), remains an elusive goal in the short- to medium term[1]. Fortunately, this lethal pandemic has been controlled by the emergence of vaccines capable of inducing neutralizing antibodies that efficiently block infection. These vaccines were developed using revolutionary mRNA technical breakthroughs based on harmless fragments of the spike-shaped S protein (S-Protein). It must be noted that it is well established that SARS-CoV-2 infects cells through the interaction of the S-protein located on the surface of the virus with the angiotensin-converting enzyme 2 (ACE2) cell receptors.

In spite of these success, the main concerns regarding the contemporary clinical landscape of the disease are the emergence of new variants, particularly focused on mutations in the S-protein. Since the identification of the original Wuhan strain, there have been multiple variants of concern, like the Omicron variants, which evolved into five lineages, among them the XBB1.5, the currently most prevalent subvariant due to its enhanced immune evasion from protection provided by prior infections or vaccines. This emphasize the importance of identifying and developing new chemotherapeutics, including small molecules, to combat COVID infection by targeting the entry of the virus into the host cells as a still attractive strategy whether the antivirals under investigation are adapted to the prevalent S-protein variants[2].

Methods. High-throughput screening (HTS) assays based on pseudotyped vesicular stomatitis viruses expressing the currently prevailing S-protein of SARS-CoV-2 (VSV-S) can be considered as simple, reliable platforms for the rapid identification of novel compounds with potential as entry inhibitors (hits)[3]. We report herein the preliminary evaluation of a small chemical library on VSV-S pseudoparticles harboring the XBB.1.5 S protein in Vero E6 cells expressing the TMPRSS2 entry factor.

Results. A library of 30 synthetic thioaryl imides provided by IQM were assayed by I2SysBio in this HTS platform. Evaluated compounds proved a noticeable ability to inhibit the virus attachment to the host cells, showing IC50 values in the range 0.2-7.0 μM whereas keeping selectivity indexes >10.

Conclusions. The screening campaign allowed to identify a series of potential SARS-CoV-2 entry inhibitors (XBB.1.5). The most active molecule showed significant capability to selectively block the interaction between ACE2 and pseudotyped VSV-S (IC50=0.2 μM). Moreover, thioaryl imides are a largely unexplored family of chemicals. These promising results could encourage us to explore focused

[1] WHO report, COVID-19 epidemiological update 167, 17 May 2024

[2] European Centre for Disease Prevention and Control, SARS-CoV-2 variants of concern 31 May 2024, (https://www.ecdc.europa.eu/en/covid-19/variants-concern)

[3] Gargantilla, M. et al. J. Med. Chem., 2023, 66, 10432

research aimed at providing a candidate with enhanced activity and improved properties for further evaluation.

Ackowledgements. The Spanish Ministry of Science and Innovation AEI/10.13039/501100011033 (grants PID2022-136307OB- C21 and PID2022-137372OR-C22 (MICINN/AEI/10.13039/501100011033/ FEDER, UE) and NextGeneration (EU 2020/2094) CSIC Global Health Platform (PTI Salud Global) are acknowledged for financial support.

N° 4

Host factor PLAC8 is required for pancreas infection by SARS-CoV-2

Marta López de Diego[1]; Lesly Ibarguren-González[2,3]; Sandra Geller[4]; Darío López-García[1]; Alba M Gómez-Valero[2,3] Thomas Fe Barth[5]; Patricia Gallego[3]; Israel Fernández-Cadenas[6]; Sayoa Alzate-Piñol[6]; Catalina Crespi[2]; Julieth A Mena Guerrero[2,3]; Eugenia Cisneros-Barroso[2,7]; Alejandro P Ugalde[8]; Gabriel Bretones[8]; Charlotte Steenblock[9,10]; Alexander Kleger[4]; Carles Barceló[2,3]

[1] Centro Nacional de Biotecología (CNB-CSIC)

[2] Health Research Institute of the Balearic Islands (IdISBa)

[3] Hospital Universitario Son Espases

[4] Institute of Molecular Oncology and Stem Cell Biology (IMOS)

[5] Ulm University Hospital

[6] Sant Pau Biomedical Research Institute

[7] Son Llàtzer University Hospital

[8] Instituto Universitario de Oncología (IUOPA), Universidad de Oviedo

[9] Hospital Carl Gustav Carus

[10] Technische Universität Dresden

Background. Although mounting evidence demonstrated that pancreas is infected by Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) after COVID-19 severe disease, the severity and pathophysiology of pancreatic SARS-CoV-2 infection are still unclear.

Methods. Here, we investigated the consequences of SARS-CoV-2 infection of the pancreas and the role of Placenta-associated protein-8 (PLAC8). We analyzed plasma levels of pancreatic enzymes and inflammatory markers in a retrospective cohort study of 120 COVID-19 patients distributed in 3 severity-stratified groups. We studied the expression of SARS-CoV-2 and PLAC8 in the pancreas of deceased COVID-19 patients as well as in non-infected donors. We performed pseudovirus infection experiments in PLAC8 knock-out PDAC and human beta cell derived cell lines and validated results with SARS-CoV-2 virus.

Results. We found that analysis of circulating pancreatic enzymes aided the stratification of patients according to COVID-19 severity and predicted outcomes. Interestingly, we found an association between PLAC8 expression and SARS-CoV-2 infection in postmortem analysis of COVID-19 patients both in the pancreas and in other bona-fide SARS-CoV-2 target tissues. Functional experiments demonstrated the requirement of PLAC8 in SARS-CoV-2 pancreatic infection by using lentiviral particles pseudotyped with the SARS-CoV-2 spike protein. Furthermore, using full SARS-CoV-2 infectious virus inoculum from Wuhan-1 and BA.1 strains, we demonstrated that PLAC8 is necessary for productive infection of PDAC cell lines. Finally, we observed an overlap between PLAC8 and SARS-CoV-2 immunoreactivities of the pancreas of deceased patients.

Conclusions. Our data confirm the human pancreas as a SARS-CoV-2 target, at least in the most severe cases, and demonstrate the requirement of PLAC8 for SARS-CoV-2 pancreatic infection, thereby opening new target opportunities for COVID-19-associated pancreatic pathogenesis.

N° 5

Combination studies of antivirals against monkeypox virus: vaccinia virus as a model

Patricia de León[1]; Bruno Hernáez[1]; María J Bustos[1]; Margarita Sáiz[1]; Francisco Sobrino[1]

[1] Centro de Biología Molecular Severo Ochoa (CSIC-UAM)

Background. The rise of zoonotic mpox (formerly monkeypox) disease, which is caused by the double-stranded DNA monkeypox virus (MPXV), a member of the Orthopoxvirus (OPXV) genus, has turned into a worldwide concern. Although there isn't a specific drug approved for treating human mpox infection, smallpox direct-acting antivirals (DAAs) such as cidofovir, brincidofovir (BCV), and tecovirimat are currently being used to manage mpox. An alternative strategy to DAAs, that interfere with viral components, is to control the spread of infection with host-targeted antivirals (HTAs), either as sole treatment or in combination with DAAs or vaccines. Our previous results demonstrated the wide-ranging effectiveness of two HTAs, lauryl gallate (LG) and valproic acid (VPA), in inhibiting different viruses and revealed a synergistic effect between them against SARS-CoV-2, prompting us to investigate their combined effect on OPXVs, along with the DNA Polymerase inhibitor BCV, a DAA with a proven effect against OPXV multiplication.

Methods. The inhibitory effect of LG, VPA or BCV, or the combination of two or the three of them was assayed on cells infected with either vaccinia virus (VACV) or MPXV. Synergy analyses based on data from antiviral combination trials were performed using CompuSyn software and/or SynergyFinder web application.

Results. A consistent decrease of 2 to 3 logarithmic units in virus yields in VACV-infected Hela cells or MPXV-infected BSC-1 cells was found in the presence of non-toxic concentrations of each antiviral. The combined effect of LG and VPA was evaluated, together with BCV, searching for possible synergistic effects, at non-toxic concentrations of combined drugs. A strong synergy was found for the combined use of LG and VPA against VACV, after data analysis with Compusyn software. The synergistic effect was confirmed by analysis with SynergyFinder web application, obtaining a delta score above 10 for all applied reference models (ZIP, Bliss, Loewe, and HSA). The results were very similar when analyzing the combination data of LG and VPA against MPXV-infected BSC-1 cells. However, when the BCV compound was combined with the HTAs against VACV infection, a clear synergistic effect was only found between VPA and BCV, while the combination of LG and BCV was additive or slightly antagonistic.

Conclusions. Our results show the potent synergistic effect that two HTAs, LG and VPA, can exert against VACV or MPXV infection. The combination of HTAs and DAAs is an effective antiviral strategy, as they act on different targets. We have found that when BCV is combined with VPA, a strong synergy occurs, unlike when combined with LG. Furthermore, given the broad spectrum of action of these antivirals and the high homology of the OPXVs studied, VACV could be considered as a good model for testing antivirals against MPXV and other highly pathogenic viruses of this genus.

N° 6

Transparent surfaces with excellent virucidal activity: preparation, characterization and inactivation mechanism

Raquel Cue López[1]; Adrián Quintana Trápaga[1]; Helmut Reinecke[1]; Laura Montalvillo-Jiménez[1]; Ángela Vázquez[2]; Antonio Alcamí[2]; Carolina García Sánchez[1]; Enrique Martínez Campos[1]; **Paula Bosch Sarobe**[1]

1 Instituto de Ciencia y Tecnología de Polímeros (ICTP-CSIC)

[2] Centro de Biología Molecular Severo Ochoa (CBMSO-CSIC)-UAM

Background. The long persistence of viruses and bacteria (hours to days) onto surfaces calls for an urgent need for effective surface disinfection strategies, to intercept virus transmission and disease spread. There is growing consensus that contaminated fomites play a critical role in the spread of viruses, so there is an increased need for innovative antimicrobial materials that are able to kill or inhibit the growth of pathogenic microorganisms. Polystyrene is one of the most versatile and widely used plastics, due to its excellent properties of low cost, light weight, high transparency and durability. This material can be used to manufacture screens, used by healthcare professionals as part of their personal protective equipment (PPE), as partitions in public spaces, and shields to protect receptionists, cashiers, etc. Also, biomedical devices made of polystyrene are essential in the healthcare system. Hence, in this work we present a strategy to modify transparent polystyrene surfaces to obtain materials with excellent virucidal activity, without degrading the material or affecting its transparency. The functional group is very active, non-toxic and it has a rechargeable activity. The antimicrobial activity has been tested on two strains of bacteria, gram+ and gram-, and with two models of human coronaviruses. Cytocompatibility of the surfaces has been also studied.

Methods. We have modified transparent rigid sheets of polystyrene by immersion in reactive solutions during very short periods of time (1min to 30min). Full characterization of the materials has been done by conventional techniques (FT-IR, TGA, UV-Vis, XPS, SEM-EDX and tritration). To give insights into their antimicrobial activity four model of microorganisms have been used: two strains of bacteria, Gram-positive Staphylococcus aureus and Gram-negative Escherichia coli, and two coronavirus species, HCoV-229E GFP and SARS-CoV-2. Antibacterial assays was measured using LIVE/DEAD BacLight Bacterial Viability Kit using Propidium Iodide and SYTO® 9 stainings. Antiviral activity was studied by infecting HuH-7 or Calu3 cells, and virus yield was determined by plaque assay. To determine the cytocompatibility of the surfaces, cell assays were performed using C166-GFP mouse endothelial cell line (ATCC CRL-2583™, USA), and metabolic activity of the cells and DNA quantitation were measured through Alamar Blue and FluoReporter® Blue Fluorometric dsDNA Quantitation Kit fluorescent staining, respectively.

Results. Transparent polystyrene sheets have been modified rapidly via immersion in reactive aqueous solutions. Degree of functionalization is easily controlled and transparency is maintained in the range in which the materials show a high antimicrobial activity. They have not shown cytotoxicity on relevant cell lines, allowing their proliferation.

Conclusions. We have developed an economical, easy and scalable method to modify transparent polystyrene surfaces with a very active, non-toxic and reactivable antimicrobial group, without losing their most essential physical properties, especially transparency. In addition, the results show that this new generation of antimicrobial materials are safe in contact with human cells. These findings make this process to be very reliable in the fabrication of materials for healthcare or general purpose applications, to prevent the spread of infections.

N° 7

De novo design of protein-protein interactions modulators

Eduardo González-García[1]; **Eugenia Ulzurrun**[1,2]; Celia Miró[3]; Daniel Muñoz-Reyes[3]; Sara Pérez-Suárez[3]; Mª José Sánchez-Barrena[3]; Roi Naveiro-Flores[4]; Nuria E. Campillo[1]

[1] Centro de Investigaciones Biológicas Margarita Salas (CIB-CSIC)

[2] Instituto de Ciencias Matemáticas (ICMAT-CSIC)-UAM-UC3M-UCM

[3] Instituto de Química Física Blas Cabrera (IQF-CSIC)

[4] CUNEF Universidad

The process of drug development is inherently lengthy and costly, often taking over a decade and requiring substantial financial investment. Deep learning models offer a promising solution by enhancing both the exploration of the chemical space with generative models and the identification of potential drug candidates with property predictors. This work focuses on generating modulators of protein-protein interactions (PPIs), which are important therapeutic targets due to their central role in numerous biological processes and their potential in treating various diseases.

We present a comprehensive framework for the de novo generation of PPI modulators, structured around three main pillars. First, we employ a diverse set of state-of-the-art molecular generators to effectively explore the chemical space, ensuring a wide range of molecular diversity. Second, we adapt existing research to develop a novel PPI bioactivity regressor, designed to predict the affinity of molecules to specific PPIs. This regressor guides the generative process toward compounds with high therapeutic potential. Third, we incorporate various auxiliary property filters, focusing on predicting toxicity, blood-brain barrier penetration capability, drug-likeness, synthetic accessibility, molecular similarity to known compounds, and the binding affinity to specific protein pockets. These filters are essential for eliminating low-quality compounds and refining the selection of viable candidates.

This framework is applied to generate modulators for two specific PPIs, NCS-1/Ric8A and NCS- 1/D2R. The resulting compounds are compiled into two virtual chemical libraries, which are currently undergoing further testing to identify new and effective modulators. This work highlights the potential of deep learning in drug discovery and proposes a robust methodology for developing targeted PPI modulators.

N° 8

A biosafety level 2 chimeric virus to study chikungunya glycoproteins

Ron Geller[1]; **Brayan Grau Argente**[1]

[1] Instituto de Biología Integrativa de Sistemas (I2SysBio) (UV-CSIC)

Chikungunya Virus (CHIKV) is an enveloped Togaviridae family member that is encoded by a positive-sense RNA genome. CHIKV, classified as level 3 risk virus, is spread by the bite of an infected mosquito causing in humans mild (fever, headache, muscle pain, or rash) to severe symptoms (joint swelling and pain). In some cases, after acute infections, symptoms can persist and lead to chronic arthritis. To date, just one vaccine is commercialized in USA, being only palliative treatments of the symptoms accessible to the rest of the world. Due global warming, the mosquito species that spread CHIKV (Aedes aegypti and A. albopictus) are now present in several countries, including Spain, which enhances the possibility of rapid outbreaks in non-endemic areas.

The need to utilize biosafety level 3 facilities to study CHIKV presents several obstacles, including limited access to such facilities, high cost, and increase biosafety concerns. To overcome these limitations, we have developed a chimeric biosafety level 2 system where the structural genes of CHIKV are used to replace those of a related togavirus, sindbis virus, which is among the least pathogenic togaviruses known. Two additional genetic modifications known to restrict the pathogenicity of sindbis were included. The system enables the recovery of recombinant viruses via direct DNA transfection and yields authentic CHIKV particles. A reporter gene is included to facilitate detection of viral replication. In sum, the reporter system provides a convenient platform to study the biology of CHIKV glycoproteins as well as high-throughput screening of antivirals and neutralizing antibodies under biosafety level 2 conditions. Moreover, the ability of the virus to spread can enable assessing the effects of mutations in the viral glycoproteins on fitness and the selection of drug-resistant or neutralization-evading viruses.

N° 9

Air sampling as a non-invasive method to monitoring influenza A virus in different environments

Ángela Vázquez Calvo[1]; Ana Isabel Moraga-Quintanilla[1]; Teresa García-Castey[1]; Cristina del Álamo[2]; Juan Sánchez García-Casarrubios[3]; José Luis Pérez-Díaz[2]; Antonio Alcamí[1]

[1] Centro de Biología Molecular Severo Ochoa (CBMSO-CSIC)-UAM

[2] Escuela Politécnica, Universidad de Alcalá

[3] COUNTERFOG, S.L.

Background. Influenza A virus is a paradigm for an emerging virus. The virus belongs to the family Orthomyxoviridae, composed of enveloped viruses with a segmented ssRNA (-) genome. A large and antigenically diverse reservoir of influenza A viruses exits in wild aquatic birds that can also infect a range of host species including a large variety of mammals. Occasionally, influenza A viruses of animals infect humans (zoonoses), which could cause a pandemic. Flu pandemics happen when a new influenza A virus emerges that is able to infect people easily and spread from person to person in an efficient and sustained way, and to which most of the world's population do not have immunity. Moreover, influenza A viruses, especially the highly pathogenic avian influenza (HPAI) virus can have a high impact on both farm animals and wildlife. For these reasons, it is important to monitor the presence of the virus in different environments as a prevention measure. Here we describe a new air sampling system to monitor influenza A virus.

Methods. Air samples were collected in different environments using a nanofiber filter coupled to an air pump. Then, the presence of influenza A virus in these filters was determined by RTqPCR.

Results. Sampling protocols have been defined for the detection of influenza A virus in air samples. We have been able to detect this virus in a pig farm and in different penguin colonies.

Conclusion: Air sampling is an efficient method to monitor influenza A virus in different environments. This method has some advantages: it samples simultaneously many animals, not single animals (cost effective) and it is less invasive because samples are collected at a distance from the animals, increasing animal welfare and reducing exposure of personnel to the virus. Moreover, this type of sampling could be used to detect pathogens relevant to animal and human health.

N° 10

A Profile of elderly people in Spain 2024

M. Pilar Aceituno Nieto[1]; **Ana Belén Castillo Belmonte**[1]; Diego Ramiro Fariñas[1]

[1] Instituto de Economía, Geografía y Demografía (IEGD-CSIC)

Background. This report focusses on statistical, demographic, social, health and aging issues. Demographic, health, economic and social indicators of the elderly population in Spain are offered, as well as the changes they have experienced in recent years.

Methods & Data: Data on demographic, health, economic and social characteristics are compiled from National and international sources.

The estimates are selected for their relevance to represent the elderly population in Spain.

These indicators are chosen based on their periodicity and temporal continuity.

Results. 63.6% of the population aged 65 and over reports having some long-term illness or health problem.

Conclusions. Increasing the years of healthy life is one of the main objectives of the European health policy. The analysis of the available data indicates that the increase in life expectancy is not clearly translated into an increase in the number of years of healthy life among the elderly.

N° 11

Devising a novel approach to comprehensively define recombination-altering mutations in a human picornavirus

Cristina Vidal Verdú[1]; Brayan Grau Argente[1]; Beatriz Álvarez Rodríguez[1]; Ron Geller[1]

[1] Instituto de Biología Integrativa de Sistemas (I2SysBio) (UV-CSIC)

RNA recombination plays a crucial role in shaping viral diversity, being central to viral evolution and pathogenesis with significant evolutionary and medical consequences. Recombination in positive-sense, single-stranded RNA ([+]ssRNA) viruses primarily occurs by a "template switching" mechanism of the virus-encoded RNA-dependent RNA polymerase during replication, producing hybrid genomes. This process emerges as a key adaptive mechanism for viruses by bringing beneficial mutations into the same genome and purging deleterious mutations, which also arise from the error-prone nature of the viral polymerase. Despite the development of several approaches to study recombination in (+)ssRNA viruses, the underlying mechanism and determinants involved remain poorly understood. To advance this field, here we present the design of a novel experimental approach to comprehensively assess the impact on RNA recombination of a large number of mutations previously introduced across the polymerase of the human picor navirus coxsackievirus B3 (CVB3). In order to address this challenging project in the future, in this work we have developed the first CVB3-specific recombination assay, adapting its design to detect recombination by fluorescence methods and to allow the system to handle the huge mutational diversity engineered into the polymerase of this virus as well as the specific selection of polymerase variants driving recombination. Although much work remains to be done, our results bring us one step closer to achieving a new approach to globally define how mutations alter recombination. Successful implementation of this new methodology could increase our understanding on this important biological process, and consequently, contribute to the development of new antiviral therapies.

N° 12

Comparative analysis of detection methods and assessment of the infectious potential of Avian Influenza Virus H5N1 in pasteurised and raw milk

Gloria Sánchez Moragas[1]; Pablo Puchades Colera[1]; Azahara Díaz Reolid[1]; Inés Girón Guzmán[1]; Alba Pérez Cataluña[1]

[1] Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC)

Background. The ongoing panzootic caused by Influenza A virus (IAV) subtype H5N1 clade 2.3.4.4b has spread across wide geographical areas, leading to significant economic losses and posing a serious threat to both public and animal health. Following the confirmation of an outbreak in dairy cows in the United States, and the demonstrated ability of IAV H5N1 to replicate in mammary glands, with milk serving as a primary transmission route, surveillance through pooled milk tank analysis has proven to be an effective early warning tool for farms. Furthermore, to address the potential risk of human exposure and related food safety concerns, dairy products are currently undergoing analysis. However, there is a current lack of rapid methods to infer the infectivity of viral particles. Therefore, our study aims to validate a rapid procedure to detect and quantify IAV H5N1 in milk, and, in terms of food safety, develop a molecular method to infer the infectious potential of IAV H5N1 based on capsid integrity in dairy products.

Methods. Pasteurized and raw milk concentrates, obtained using the aluminum adsorption-precipitation method (AlCl3), were inoculated with IAV subtype H3N2 (ATCC VR-1680) and pre-treated with three intercalating agents: propidium monoazide (PMAxx), Crosslinker (Promega), and platinum chloride (PtCl4). Nucleic acid extraction was performed with the Maxwell® RSC kit, using the Maxwell® RSC Pure Food GMO and Authentication kit (Promega), and supplemented with Plant RNA Isolation Aid (ThermoFisher). Detection was carried out using generic IAV RT-qPCR. Subsequently, purified IAV H5N1 RNA from cell cultures was used to evaluate the effectiveness of these intercalating agents, in combination with three RT-qPCR assays, in eliminating the RT-qPCR signal. Finally, viability PCR was optimized using heat-inactivated IAV H5N1/Larus ridibundus/Spain/CR4063/2023 in pasteurized and raw milk.

Results. The detection limits for IAV in raw and pasteurised milk was calculated as 2.3x105 and 6.5x104 genome copies (gc)/L, respectively. Preliminary results indicated that a fivefold dilution of milk concentrates was necessary to achieve a signal reduction between live and inactivated IAV H3N2, with a 7.74 Ct with PtCl4. Depending on amplicon size and detection limits, IAV H5N1 specific RT-qPCR was most effective at distinguishing between infectious and inactivated forms. Similar to IAV H3N2, PtCl4 resulted in >6 Ct in both raw and pasteurised milk when using generic and specific RT-qPCR assays.

Conclusions. Pathogen detection through milk surveillance, from a One-Health perspective, can help to monitor endemic and emerging diseases at farm, regional or national level, thereby supporting disease surveillance and enabling informed decision-making for farm management. From food safety perspective, the results of the capsid integrity assay indicate its potential to assess and infer IAV H5N1 infectivity using rapid molecular methods. Future research includes optimisation of viability assays on food surfaces.

N° 13

Large-scale viral intra-patient evolution reporting and analysis

Miguel Álvarez-Herrera[1,2]; Alejandro Sanz-Carbonell[2]; Fernando González-Candelas[1,2,3]; Mirella Coscolla[1,2]

[1] Instituto de Biología Integrativa de Sistemas (I2SysBio) (UV-CSIC)

[2] Fundació per al Foment de la Investigació Sanitària i Biomèdica de la Comunitat Valenciana (Fisabio)

[3] CIBER Epidemiología y Salud Pública (CIBERESP-ISCIII)

Background. While chronic SARS-CoV-2 infections in immunocompromised patients are hypothesized to be a breeding ground for variants of concern (VOC), current workflows lack the ability to comprehensively analyze intra-patient viral evolution. To address this, we developed VIPERA (Viral Intra-Patient Evolution Reporting and Analysis), a modular, scalable tool that integrates phylogenetic and population genetic analyses of genome sequencing data. This allowed us to characterize the complete viral population within a single host and the evolutionary trajectories throughout the course of the infection. The application of VIPERA to multiple serially sampled viral sequences would provide a critical missing piece in understanding the dynamics of SARS-CoV-2 evolution within infected individuals.

Methods. We investigated a dataset containing more than 50,000 SARS-CoV-2 samples using an adapted and extended workflow built upon VIPERA. After grouping chronologically-ordered samples by patient and confirming the serial nature of viral sampling across patients, this workflow allowed us to execute the original VIPERA analysis in parallel for all target intra-patient datasets within the larger group. Analysis of the evolutionary trajectories within each patient allows for the identification of patterns and trends in viral evolution over time. Additionally, this workflow incorporates new data summaries and data visualizations. As a results, variations in viral dynamics across different patients can be explored, providing broader population-level perspectives on intra-host SARS-CoV-2 evolution.

Results. First, we identified above 200 infections associated with the same patient with at least two time points. Using our expanded VIPERA workflow, we verified for each separate intra-patient sample set that it originated from a single infectious event by examining their phylogeny and lineage admixture. Then, the within-host nucleotide variants and evolutionary trajectories were studied. We also assessed their evolutionary and substitution rate. These findings shed light on the intricate mechanisms by which SARS-CoV-2 adapts within infected individuals, providing crucial insights that can inform our understanding of the emergence of VOC.

Conclusions. We have adapted the VIPERA workflow to analyze massive sequence datasets. By analyzing a a large-scale dataset of SARS-CoV-2 sequences, we have provided a major large-scale exploration of intra-patient viral evolution. Additionally, the identification of selection footprints and the dynamic shifts in sublineage populations offer valuable clues towards understanding VOC emergence. Our work underscores the power of VIPERA as a tool for dissecting intra-host viral evolution and paves the way for future investigations into this critical area of SARS-CoV-2 research.

N° 14

Restoration of the duodenal immunome in gluten-free diet treated coeliac patients despite the presence of persistent mucosal inflammation

David Bernardo[1]; Aida Fiz-López[1]; Ángel de Prado[1]; Alejandro G. del Hierro[1]; Elisa Arribas-Rodríguez[1]; Sandra Izquierdo[2]; Álvaro Martín-Muñoz[1]; Daniel Corrales-Cruz[2]; Sara Cuesta Sancho[1]; José A. Garrote[1]; Eduardo Arranz[1]; Luis Fernández-Salazar[2]

[1] Instituto Biomedicina y Genética Molecular de Valladolid (IBGM-UVA)-CSIC

[2] Hospital Clínico Universitario de Valladolid

Background. Current treatment for coeliac disease (CD) is a life-long restricted gluten free diet (GFD). However, some treated patients have reported mucosal lesion despite years into the diet. Hence, we hereby aimed to study the effect of the GFD on the mucosal immunome from CD patients.

Methods. Duodenal biopsies were collected from non-CD controls (n=6), CD patients at diagnose (n=6), and GFD-treated patients (n=19). The profile of intraepithelial lymphocytes (lymphogram) was determined in each patient, while the lamina propria immune infiltrate was assessed by a 40plex panel using spectral and computational cytometry.

Results. CD patients at diagnose had an expansion of total mucosal NK cells, innate lymphoid cells, B-cells, Tregs and Tγδ cells, all of them expressing higher levels of Fas, together with Integrin α4 and β7. Of note, and although all GFD-treated patients had negative serology, 68.4% of them had villous atrophy (Marsh score 3) despite having followed the GFD for 7±6 years. Nevertheless, they did not display any major alteration of the immunome composition or function referred to the control cohort. Besides, duration of the GFD, but not the persistence of mucosal atrophy, correlated with an increased expression of migration and inflammation markers related to effector T-cells on these patients.

Conclusions. Whereby have unveiled the mucosal immune infiltrate in CD patients at diagnose and proved how it normalizes after the GFD irrespectively of the mucosal architecture. The immunological basis for persistent villous atrophy in celiac patients on a gluten-free diet remains elusive.

N° 15

Genomic epidemiology and evolution of Candida auris clade III

Irving Cancino Muñoz[1]; **Fernando González-Candelas**[1]; Concepción Gimeno Cardona[2]; Remedios Guna[2]; Nuria Tormo Palop[2]; Carme Salvador[2]; Juan Vicente Mulet[2]

[1] Instituto de Biología Integrativa de Sistemas (I2SysBio) (UV-CSIC)

[2] Consorcio Hospital General Universitario de Valencia

Background. The number of infections caused by Candida auris in Europe has tripled between 2016 and 2022. According to the ECDC,Spain is responsible for 76% of those cases and the Comunitat Valenciana (CV), in the east of the country, is the region with the largest prevalence. Since 2016, several intrahospital outbreaks have been reported. However, there has been no detailed genomic analysis of these and this is the main goal of this work. For this, we have sequenced complete genomes from 35 isolates from the CHGUV, where more than 550 cases have been reported since 2017.

Methods. 34 clinical, sampled in 2017-2021, and one environmental isolates were subjected to complete genome sequencing using Illumina MiSeq standard methodology. Phylogenetic (ML, IQTree) and dating (Bayesian, BEAST) analyses were performed with these and additional sequences downloaded from public databases.

Results. All the outbreak isolates belong to clade III of C. auris and are non-susceptible to fluconazole (mutation ERG:VF125AL). One was also resistant to echinocandins (FSK1:S639Y). After removal of hypervariable regions (3.8% of the 12.7 Mb genome), outbreak isolates were between 0 and 12 SNPs apart. These isolates share a most recent common ancestor dated on May 2015 (95% HPD 11/2014–12/2015). The comparison with 334 database genomes revealed 4 additional sequences closely related (1-8 SNPs) to this outbreak, 3 from another hospital in CV and one from Austria (sampled in 2021). A joint analyses of dated genomes from clade III revealed an evolutionary rate of 2.88 SNPs/year, which provided an estimate for the emergence of fluconazole-resistant subcluster of C. auris clade III around December 2010 (95% HPD 4/2010-6/2011).

Conclusions. These results are congruent with the initial detection of C. auris in the CV in 2016, and its subsequent spread to other hospitals in the region and even abroad. Despite the adoption of stringent control measures, the outbreak is still active at the CHGUV and the pathogen is evolving. Further genomic surveillance is necessary to monitor its spread at local, national and international levels.

N° 16

IL-27 as predictor of severity and outcome in COVID-19

Carola Zurita Palomo[1]; M. Ángeles Rodríguez Hernández[1]; David Carneros Trujillo[1]; Matilde bustos de Abajo[1]

[1] Instituto de Biomedicina de Sevilla (IBiS)

Background. Interleukin (IL)-27, a member of the IL-6 cytokine family, exerts a broad spectrum of functions, including pro-inflammatory and anti-inflammatory properties, with notable antiviral activity. The potential role of IL-27 in COVID-19 (Coronavirus Disease 2019) has been studied with controversial results. Additionally, there is an urgent need to identify biomarkers in order to predict disease progression in patients infected with SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) and to help guide rational targeted immunomodulatory therapeutic strategies. The aim of this study was to explore the role of IL-27 in the context of COVID-19.

Methods. Serum samples from patients infected with SARS-CoV-2 at different stages of severity were used, and clinical and biochemical data were collected. Cytokine profiling in serum samples was established by ELISA. Statistical analyses and logistic regression models were constructed to predict disease progression and outcome. Lung tissue samples (autopsies) were used to localize IL-27 and macrophages by immunohistochemistry. The role of IL-27 in macrophages was assessed using a monocyte cell line (THP-1).

Results. We report that serum levels of IL-27 had the ability to act as predictors of disease progression in severe patients (ICU) along with clinical data. The analysis of lung tissue from SARS-CoV-2 infected patients (autopsies) revealed an increased number of macrophages (CD68+), M2 polarized macrophages (CD68+/CD163+) and IL-27, together with collagen bounds. In vitro experiments showed that IL-27 activated Signal Transducer and Activator of Transcription (STAT)-1/STAT-3 signalling pathway in macrophages, without inducing M1/M2 polarization. Interestingly, we demonstrated that IL-27 primes macrophages for IL-4-dependent M2 polarization.

Conclusions. Our study suggests that IL-27 could play a relevant role in the immune response in COVID-19, and that the screening of serum levels of IL-27 combined with clinical data can identify patients at risk of fatal COVID-19. This strategy may constitute a helpful tool for the early identification, stratification of patients at hospital entry, and prediction of the disease outcomes, with clear implications in treatment and clinical decision-making.

N° 17

Magneto-assay for early diagnosis of HIV based on aptamers and electrochemical detection

Eva Balada[1]; César Fernández Sánchez[2]; África Holguín[3]; Ana Valadés[3]

[1] Instituto de Química Avanzada de Cataluña (IQAC-CSIC)

[2] Instituto de Microelectrónica de Barcelona (IMB-CNM-CSIC)

[3] Instituto Ramón y Cajal de Investigación Sanitaria (IRyCIS-Hospital Universitario Ramón y Cajal)

Background. Early HIV detection is crucial for timely treatment and transmission prevention. Current techniques cannot detect HIV within the first ten days of infection. DNA-aptamers are single-stranded oligonucleotides that can recognize proteins with high affinity and specificity. They offer promise for new molecular assays enabling early virus detection. The aim of this work was the development of a biosensor platform to quantitatively detect HIV proteins by combining magnetic nanoparticles (MNPs), functionalized with a pioneering DNA-aptamer targeting a highly-conserved peptide across >150 HIV variants in HIV integrase (IN), together with a rapid electrochemical detection approach for decentralized rapid and sensitive detection of HIV infection.

Approach. For the detection of the recombinant HIV-1 IN, a sandwich magneto-assay was developed. This assay includes the use of a label, that is a peroxide enzyme (HRP), whose reaction was carried out in the presence of a redox mediator that facilitates the electrochemical transduction of the magneto-assay [ACS Sens. 2023, 8, 3032−3042]. MNPs were coupled to anti-HIV-1/2 IN aptamer Apt2-IN, which previously showed high affinity and sensitivity for recombinant HIV-1 IN. Apt2-IN recognized a highly conserved and exposed IN peptide, being 100% conserved in 113/4 HIV-1/HIV-2 variants. The same aptamer could be used for detecting the protein when coupled to HRP. Different assay strategies were assessed looking at shortening the analysis time and enhancing assay sensitivity. The analytical response was recorded by chronoamperometry following the HRP reaction in a solution containing H2O2 and ferrocenomethanol redox mediator. A compact, low-power instrumentation was used for recording the analytical signal.

Results. The combination Apt2-IN/Apt2-IN as capture/detection aptamers was the one utilized in the magneto-assay. After optimizing the protocol steps, the best results were obtained by using streptavidin-functionalized MNPs coupled with biotinylated-Apt2-IN and PEG6000 or PVP40 as blockers, together with an anti-digoxigenin-poly-HRP, which selectively reacted with the Apt2-IN detection aptamer modified with digoxigenin. We used a concentrated stock of functionalized and blocked MNPs to reduce the incubation times and washing steps in subsequent magneto-assays. The overall analysis time was 75 min.

The HRP label catalyzed the reduction of H2O2 using the ferrocenemethanol as electron donor, generating in-situ the ferrocinium-methanol cation. This cation was reduced back to ferrocenemethanol at the electrochemical cell, and the recorded cathodic current was directly proportional to the concentration of IN in the sample. The electrochemical anti-HIV apta-biosensor showed a linear range of 0 to 1000 ng/mL of recombinant-HIV-1 IN, with a limit of detection (3σ) of 260 ng/mL (26 ng/well), being similar to the sensitivity obtained by the sandwich-ELONA.

Conclusions. We develop the first prototype of an aptamer-based magneto-assay with electrochemical detection of recombinant HIV-IN, but theoretically able to recognize most HIV-1/2 variants. After optimizing the sandwich magneto-assay, the results demonstrate that the

electrochemical measurement is feasible for the detection of HIV-1 IN. Assay optimization is ongoing to enhance sensitivity. Assays with clinical samples will eventually be carried out in an attempt to show the potential of our cost-effective, user-friendly, and timely electrochemical platform to be implemented at the point-of-care (POC) for decentralized HIV molecular diagnosis.

N° 18

Artificial intelligence-based haplotype mapping reveals early functional diversification of SARS-CoV-2

Soledad Delgado[1]; **Pilar Somovilla**[2]; Cristina Ferrer-Orta[3]; Brenda Martínez González[4]; Sergi Vázquez-Monteagudo[3]; Javier Muñoz-Flores[5]; María Eugenia Soria[2,6]; Carlos García-Crespo[2]; Ana Isabel de Ávila[2]; Antoni Durán-Pastor[4]; Ignacio Gadea[6]; Cecilio López-Galíndez[7]; Federico Morán[8]; Ramón Redondo Lorenzo[9]; Nuria Verdaguer Massana[3]; Celia Perales[4,6]; Esteban Domingo[2]

[1] E.T.S. de Ingeniería de Sistemas Informáticos (ETSISI-UPM)

[2] Centro de Biología Molecular Severo Ochoa (CBMSO-CSIC)-UAM

[3] Instituto de Biología Molecular de Barcelona (IBMB-CSIC)

[4] Centro Nacional de Biotecnología (CNB-CSIC)

[5] 7GMS Management Solutions, S.L.

[6] Instituto de Investigación Sanitaria (IIS-FJD)-UAM

[7] Centro Nacional de Microbiología (CNM-ISCIII)

[8] Universidad Complutgense de Madrid (UCM)

[9] Center for Pathogen Genomics and Microbial Evolution, Feinberg School of Medicine, Northwestern University

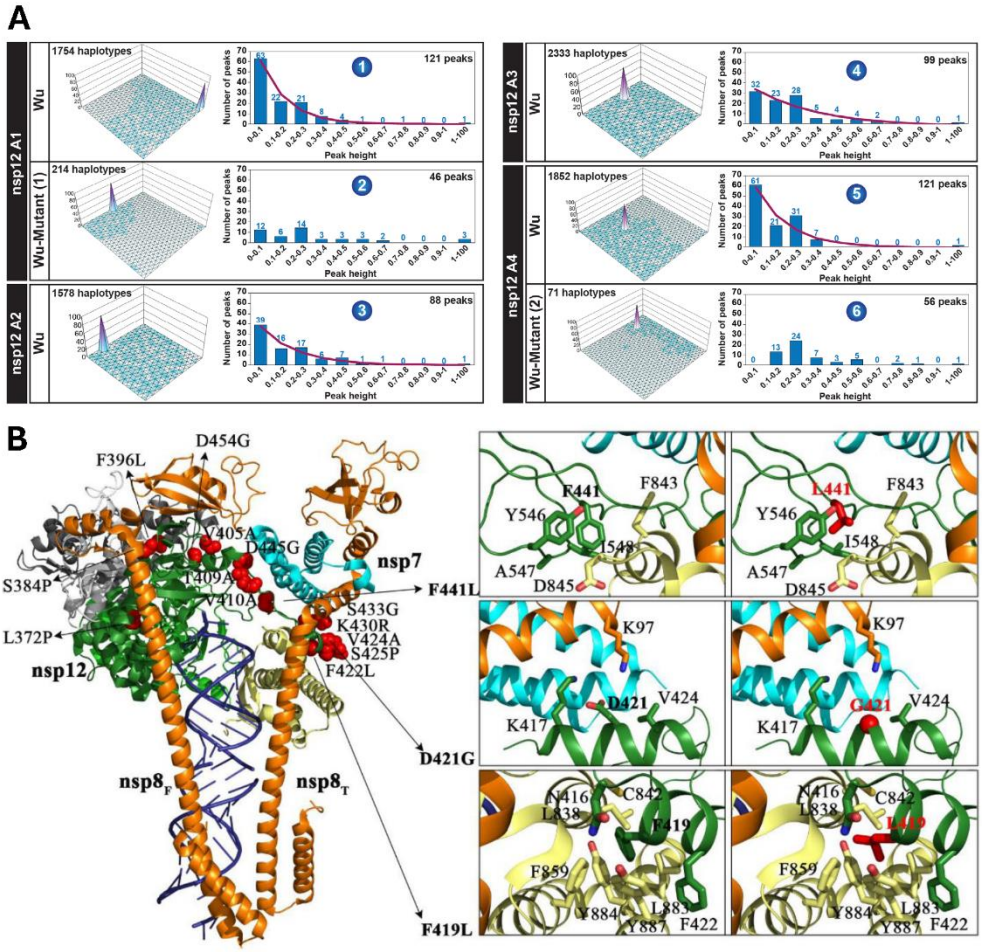
Background. SARS-CoV-2, an RNA virus responsible for the COVID-19 pandemic, is evolving rapidly, leading to the emergence of viral variants. Although mutation has been widely studied at the population level, little is known about the diversification of the virus within infected individuals. Our study aims to unravel the early evolutionary steps of the virus within hosts, using advanced computational methods to understand how this early diversification can influence the virus's replication and transmission capacity.

Methods. We used deep sequencing data obtained from diagnostic nasopharyngeal swab samples of 30 individuals collected during the first wave of the pandemic in Madrid, between April 3 and 29, 2020. We applied a three-dimensional self-organizing map (3D SOM) neural network model, an artificial intelligence (AI) approach, to map and analyze viral haplotypes in detail. This approach allowed us to identify early mutations within hosts and their role in the diversification process. In addition, structural analyses and RNA synthesis biochemical assays with the viral polymerase were conducted to assess the functional effects of the observed mutations.

Results. Our results revealed significant early diversification of SARS-CoV-2 within hosts, even in the initial stages of infection. Through haplotype mapping using neural networks, we identified several mutations that could enhance viral replication efficiency. Our approach allowed the detection of mutations that might go unnoticed with traditional genomic methods, providing a more detailed understanding of intra-host evolution. Biochemical assays corroborated that certain mutations alter the RNA synthesis in vitro. Specifically, kinetics data showed that F419L, D421G, and F441L substitutions clearly altered the RNA synthesis activity of the polymerase complex at both 37℃ and 33℃, with D421G resulting in an almost inactive enzyme.

Conclusions. This study highlights the importance of monitoring the diversification of SARS-CoV-2 within hosts to predict its evolutionary trajectory. Early detection of functional mutations can inform public health interventions and vaccine development, identifying variants with modified replicative capacity. Our haplotype mapping using neural networks is a novel and effective tool to facilitate the visualization of relationships between mutant spectra, aiding in real-time tracking of RNA virus evolution and, with applications beyond SARS-CoV-2. Through this methodology combined with

biochemical assays, we have provided a more detailed understanding of how the early stages of intra-host evolution can influence virus adaptation and pandemic potential. Substitutions in the nsp12 protein (viral polymerase) in the mutant cloud marking early diversification within infected individuals have functional consequences, and similar conclusions can be drawn for substitutions in the spike (S) protein.



Graphical abstract: Self-organized haplotype maps (SOMs) corresponding to four amplicons from the nsp12-coding region of SARS-CoV-2, and the location of amino acid substitutions in the Wu-Mutant SOM master neurons. (A) SOMs indicate the coding region, amplicon number, SOM class (Left), number of haplotypes, neurons, and peak size distribution (Right). (B) The Left panel presents a cartoon of the nsp12-nsp8-nsp7-RNA complex (PDB 6YYT), with amino acid substitutions in red spheres, labeled. The right panels highlight nsp12 substitutions (F419L, D421G, F441L), selected for biochemical assays.

N° 19

Genomic analysis of an epidemiological outbreak of Legionella affecting a prison for 20 years

Irving Cancino-Muñoz[1]; Juana María González-Rubio[2]; Fernando González-Camacho[2]; Mireia Coscolla[1]

[1] Instituto de Biología Integrativa de Sistemas (I2SysBio) (UV-CSIC)

[2] Centro Nacional de Microbiología (CNM-ISCIII)

Introduction. Legionella pneumophila is an intracellular bacteria, usually found in natural aquatic environments such as rivers, lakes and water reservoirs. However, it also has the ability to colonize artificial environments such as cooling towers, pools and water pipes, which can lead to clinical infections and global outbreaks. A key characteristic of L. pneumophila is its ability to form persistent biofilms. In this study, we analyzed an epidemiological outbreak that has affected a prison in Aragon for more than 20 years, with the aim of identifying genetic markers responsible for this persistence.

Methods. We performed whole genome sequencing to analyze 26 isolates from the outbreak of environmental and clinical origin obtained between 2004 and 2022. Furthermore, hybrid assemblies (combining short and long reads) were generated from three samples, which were used as reference genomes for each of the clusters identified within the outbreak. Using these assemblies, we performed pangenome analysis to detect genes associated with virulence and resistance in biofilms. We then carried out experimental assays to phenotypically validate the identified genes.

Results. Genomic analysis revealed the presence of two genetically distinct populations coexisting within the outbreak, termed Cluster-A and Cluster-B. Cluster-A included 22 samples, while Cluster-B had 4 cases. Phylogenetic analysis showed a high genetic closeness within each cluster, with a mean distance of 7 SNPs in Cluster-A and 3 SNPs in Cluster-B. Since Cluster-A predominated, genomic differences between the two clusters were investigated. Out of 404 virulence-associated genes, 27 were unique to Cluster-A, mostly related to type IV Secretion System proteins. A combination of two genes associated with virulence and persistence was also identified in both clusters. When this combination was compared in a database of 5,500 global isolates, it was observed to predominate in isolates genetically related to Cluster-B, suggesting horizontal gene transfer between Cluster-B and Cluster-A. Phenotypic assays confirmed that Cluster-A strains had a greater ability to form biofilms, which could explain their greater persistence.

Conclusions. Our results suggest that the identified gene combination, together with the genetic background of Cluster-A, may be responsible for its evolutionary success and persistence over time in this specific setting.

Nº 20

Targeting carbohydrate-lectin interactions: glyconanomaterials for combating antibiotic resistance

Noureddine Khiar El Wahabi[1]; Mohyeddin Assali[2]; Elena Romero-Ben[1]; Ralf Wellinger[3]

[1] Instituto de Investigaciones Químicas (IIQ-CSIC)-US

[2] Faculty of Medicine & Health Sciences An-Najah National University

[3] CABIMER

Background. The resistance of bacteria to antibiotic treatment represents a global threat to human health. A key step in the initial phase of infection by many pathogens is the interaction between bacterial lectins and host cell carbohydrates, which facilitates bacterial adhesion. Anti-adherent therapy, which aims to prevent this adhesion, is a promising approach to combat bacterial resistance. However, the development of such therapies is hampered by the structural complexity of carbohydrates and their weak binding affinity to proteins. Therefore, new materials capable of enhancing the affinity of protein-carbohydrate interactions are urgently needed. This study aims to design, synthesize, and evaluate glyconanomaterials with controlled size and shape for their potential in selectively agglutinating bacterial cells and inhibiting bacterial proliferation.

Methods. Our approach is based on the supramolecular self-assembly of diacetylenic-based glycolipids in solution and onto carbon-based nanomaterial surfaces. Glyconanomaterials with varying shapes and sizes, were synthesized and their ability to mediate bacterial agglutination was assessed. Structural and functional characterizations were performed using electron microscopy and binding affinity assays to study the interaction between these glyconanomaterials and bacterial lectins.

Results. The glyconanomaterials demonstrated selective agglutination of bacterial cells, with notable differences in efficacy depending on the material's size and shape. The carbon-based nanomaterial surfaces significantly enhanced the interactions between the glycolipids and bacterial lectins, resulting in stronger and more stable binding. This high-affinity binding overcomes the weak protein-carbohydrate interactions typically observed in natural systems, suggesting a novel mechanism of bacterial inhibition. Additionally, the glyconanomaterials effectively inhibited bacterial proliferation.

Conclusions. The designed glyconanomaterials exhibit promising properties for anti-adherent therapies by targeting the crucial carbohydrate-lectin interactions that initiate infection. Their ability to selectively agglutinate and inhibit bacterial proliferation offers a new avenue for developing alternative treatments against antibiotic-resistant bacteria.

Nº 21

Reducción y contención de riesgos: tratamientos superficiales antimicrobianos para la transmisión indirecta

Ana Conde del Campo[1,2]; Marina Medel Plaza[3]; Juan Damborenea González[1,2]; John J. Aguilera Correa[3,2]; Jaime Esteban Moreno[2,3]; María de los Ángeles Arenas Vara[1,2]

[1] Centro Nacional de Investigaciones Metalúrgicas (CENIM-CSIC)

[2] CIBER Enfermedades Infecciosas (CIBERINFEC-ISCIII)

[3] Instituto de Investigación Sanitaria (IIS-FJD)

La adherencia y pervivencia de microorganismos en las superficies inanimadas las convierte en importantes vehículos de transmisión de infecciones, especialmente en zonas con gran afluencia de público. La adherencia de los patógenos en las superficies depende de diversos factores: tipo de microorganismo; tipo de material y sus características superficiales -topografía, rugosidad, composición química-, condiciones ambientales -T y RH-, tiempo de exposición y dosis inoculada. El tiempo de supervivencia de los microorganismos, especialmente de las bacterias, puede ser muy elevados dependiendo de la disponibilidad de nutrientes en la superficie. Por tanto, el desarrollo de superficies antimicrobianas constituye una estrategia complementaria a las prácticas de limpieza y desinfección habituales para prevenir la transmisión de infecciones.

El acero inoxidable es uno de los principales fómites dada su extensiva utilización en multitud de aplicaciones. La incorporación de F en la superficie de estas aleaciones metálicas les proporciona propiedades antimicrobianas. Aunque la incorporación de F en la superficie del acero inoxidable, mediante el proceso de anodizado, presenta importantes retos científico-técnicos, en el presente trabajo se han obtenido superficies compuestas de fluoruros de hierro y de cromo sobre AISI 304L y 316L a partir de una solución de etilenglicol con fluoruro de amonio a 5 ℃, durante 15 minutos aplicando un voltaje constante.

Los ensayos de adherencia de enteropatógenos -Campylobacter jejuni ATCC33560, Escherichia coli ATCC25922, Listeria monocytogenes ATCC15313 y Salmonella enterica subsp. enterica ATCC13311- demostraron que el AISI 304L anodizado presentaba una disminución de la adherencia bacteriana mayor del 95% para E. coli (Ec), L. monocytogenes (Lm), y S. enterica (Se), y una disminución en el recuento del número de agregados adheridos, superior al 90% de Ec, Lm y Se. Contrariamente, el recuento bacteriano de la C. jejuni (Cj) aumentó de forma notable. Finalmente, la concentración de bacterias planctónicas Cj, Ec, Lm y Se se redujo en más de un 92%.

Los ensayos de adherencia de bacterias ambientales gramnegativas presentes en entornos hospitalarios -Pseudomona Aeuroginosa ATCC27853, Stenotrophomona maltophilia ATCC13637, Acinetobacter baumanii ATCC19606, Bulkhoderia cepacia ATCC25416- demostraron que el AISI316L anodizado presentaba una disminución de la adherencia bacteriana mayor del 52% para P. aeruginosa (Pa) y A. baumanii (Ab) y y una disminución en el recuento del número de agregados adheridos, superior al 54% para P. aeruginosa (Pa), S. maltophilia (Sm); y mayor del 68% para B. cepacia (Bc) y A. baumanii (Ab). Finalmente, la concentración de bacterias planctónicas Pa, Ab y Sm se redujo en más de un 92%.

Estos resultados confirman las propiedades antibacterianas de las muestras anodizadas del acero 304L. La notable reducción en el recuento bacteriano y en el área cubierta de E. coli, L. monocytogenes y S. enterica subsp. enterica, demuestran las propiedades antiadherentes, así como la disminución en las unidades formadoras de colonias por mililitro (UFC/mL) en el sobrenadante sugiere un efecto

bactericida de la capa anódica en todos los casos. En el 316L, el anodizado presenta un efecto bactericida muy fuerte para P. aeruginosa (Pa), A. baumanii (Ab) y S. maltophilia (Sm); un fuerte efecto disgregante para S. maltophilia (Sm) y B. cepacia (Bc).

Por tanto, la realización de este tipo de tratamiento superficial puede ayudar a prevenir infecciones en superficies de acero inoxidable expuestas al contacto diario con un gran número de personas tanto en entornos sanitarios como en instalaciones públicas de uso general.

N° 22

Anti-infectives screening at the IPBLN: Drug discovery for tropical and viral diseases

Blanca Martínez-Arribas[1]; Rosario Díaz-González[1]; Giomar Pérez-Moreno[1]; Cristina Bosch-Navarrete[1]; Carlos Cordón-Obras[1]; Olga Genilloud[2]; Amy Dounay[3]; Luis Miguel Ruíz-Pérez[1]; María C. Ramos[2]; Marco Motinelli[4]; Lori Ferrins[4]; Dolores González-Pacanowska[1]

[1] Instituto de Parasitología y Biomedicina López-Neyra-CSIC

[2] Fundación MEDINA, Parque Tecnológico de Ciencias de la Salud

[3] Colorado College

[4] Pharmaceutical Sciences, Northeastern University

Background. Throughout the years, the IPBLN anti-infective screening facility has conducted extensive research in drug discovery for neglected tropical diseases and malaria, developing high throughput assays with diverse target organisms, primarily focusing on protozoa. Recently, we have effectively improved various assays for identifying and characterizing novel antivirals to prevent future viral pandemics.

Methods. To date we have developed high-throughput screening methodologies for five different pathogens: Plasmodium falciparum, Trypanosoma brucei, T. cruzi spp, Leishmania donovani and HCoV-OC43. For HCoV-OC43 and T. cruzi we have developed immunofluorescence-based high content imaging screening methodologies. Additionally, we have established a series of protocols to study cidality, rate of kill and the mechanism of action of hit compounds, including in vitro assays for some kinases. A series of mammalian cell lines are available for cytotoxicity and selectivity determinations.

Results. In collaboration with Northeastern University we have screened approximately 700 compounds against each pathogen from a library of more than 1000 kinase inhibitor chemotypes. We have promising hits that have advanced into hit-to-lead optimization studies to improve selectivity over the human host, and selected hits are being tested in secondary assays to determine mode of action.

Conclusions. The IPBLN anti-infective screening facility offers a wide variety of cellular models of infection and a series of assays oriented towards drug discovery. We have been able to identify a series of hit compounds for different pathogens that are undergoing further optimization following specific work-flows, dictated by the therapeutic area, to progress compounds towards preclinical evaluation.

N° 23

Genomic surveillance reveals different transmission patterns between third generation cephalosporin and carbapenem resistance in Klebsiella pneumoniae in the Comunidad Valenciana (Spain), 2018-2020

Neris García González[1]; NLSAR[2]; Fernando González-Candelas[3]

[1] Instituto de Biomedicina de Valencia (IBV-CSIC)

[2] Networked Laboratory for the Surveillance of Antimicrobial Resistance of the Valencian Community

[3] Fundació per al Foment de la Investigació Sanitària i Biomèdica de la Comunitat Valenciana (Fisabio)

Introduction. The emergence and spread of third generation cephalosporins (3GC) and carbapenem resistant Klebsiella pneumoniae pose a global critical challenge. Understanding the transmission dynamics within and between hospital environments is crucial to develop effective control strategies.

Methods. From 2017 to 2019, we conducted a genomic surveillance program in eight hospitals of the Comunitat Valenciana, Spain, collecting and sequencing 1,768 3GC and carbapenem-resistant isolates. We quantified the overall transmission using core genomes and assessed the contribution of national and global isolates to the spread of AMR in the region by including 11,967 database genomes in the analysis.

Results. The local collection was highly diverse, involving 188 lineages, including global high-risk clones such as ST307 and ST11, and 3GC and carbapenem resistance determinants. Half of the isolates were involved in transmission, with 70.5% occurring within hospitals.

Discussion. Different transmission patterns characterized the spread of 3GC and carbapenem-resistance in the region. While inter-hospital transmission played a significant role in the spread of 3GC-resistance, this was only sporadic for carbapenem-resistance. Moreover, the factors behind inter-hospital spread for each type of resistance differed: while 3GC-resistance likely disseminated between hospitals through intermediate steps, carbapenem-resistance was driven by more direct transmission routes. The burden of national and global cases on the ongoing regional AMR dissemination was low. Moreover, we revealed the rapid expansion in the region and globally of lineage ST307 carrying the blaCTX-M-15 gene, a main driver of local transmissions, providing a deeper understanding of the successful spread of this high-risk clone.

N° 24

Klebsiella pneumoniae in preterm neonates: what risk do they represent?

Manuel Bernabeu Lorenzo[1]; Irving Cancino Muñoz[2]; Iñaki Comas[3]; Fernando González-Candelas[4,5]; María Carmen Collado[1]

[1] Instituto de Agroquímica y Tecnología de los Alimentos (IATA-CSIC)

[2] Instituto de Biología Integrativa de Sistemas (I2SysBio) (UV-CSIC)

[3] Instituto de Biomedicina de Valencia (IBV-CSIC)

[4] Universidad de Valencia

[5] Fundació per al Foment de la Investigació Sanitària i Biomèdica de la Comunitat Valenciana (Fisabio)

Background. Multidrug-resistant Klebsiella pneumoniae poses a significant threat to human health. Infections caused by antibiotic-resistant pathogens are notoriously challenging to treat, and the neonatal microbiome is more susceptible to disturbances from external factors than the established microbiome in later life. While certain strains of K. pneumoniae are highly pathogenic, other strains can also be part of our commensal microbiota, serving as an important reservoir of resistance genes. In this study, we present the genomes of K. pneumoniae isolates obtained from healthy premature infants and explore how important factors, such as breastfeeding or type of delivery, influence their repertoire of resistance genes.

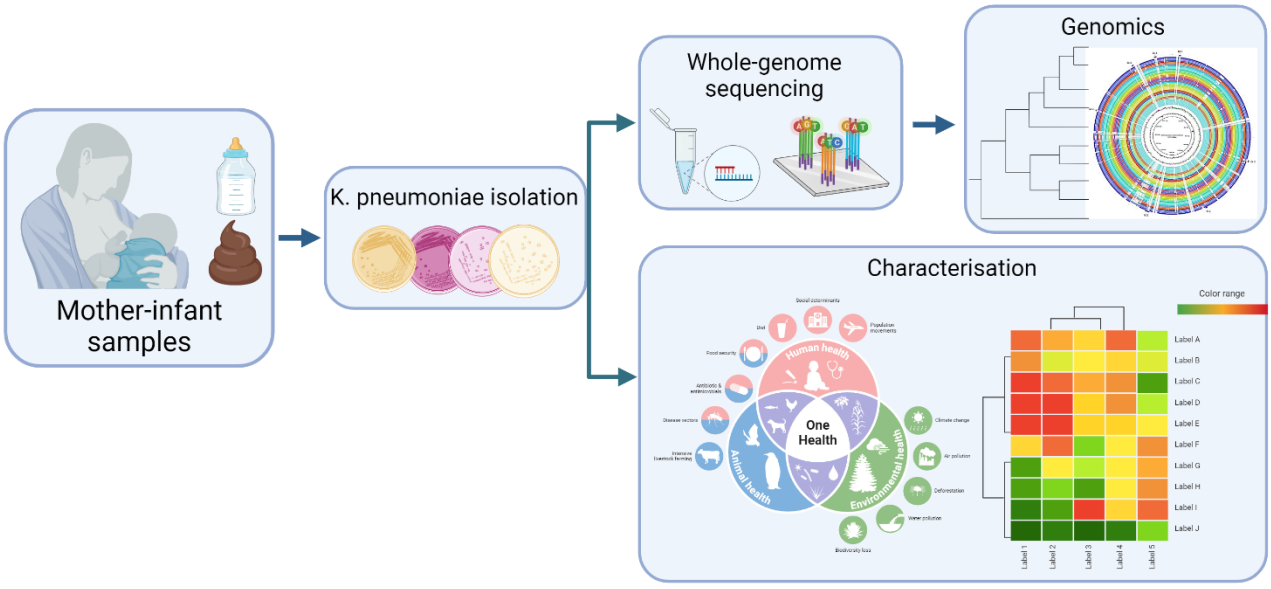
Methods. Isolates have been obtained from stool samples of mothers and premature babies from the prospective longitudinal mother-infant birth MAMI cohort by using MacConkey agar plates. DNA was extracted and, after confirmation by 16s sequencing, whole-genome sequencing of K. pneumoniae isolates was done. Databases were used to characterize the isolates and determine the presence of resistance and virulence genes.

Results. Our results have enabled us to study 32 K. pneumoniae isolates from the samples of healthy premature neonates. These isolates exhibit genetic diversity, with up to 10 sequence types, notably differing from those found in clinical isolates. Furthermore, nearly all strains carry at least one plasmid, enhancing their genetic repertoire. Comparative analysis of the assembled genomes against existing databases reveals that, on average, these isolates have 4 antibiotic resistance genes, with some containing as many as 9. Additionally, when considering external factors, we have confirmed the importance of breastfeeding, type of birth, and prematurity. For example, the type of feeding influences the total number of resistance genes. Specifically, isolates from infants receiving fortified formula tend to have a higher gene count, and the presence of genes such as SHV and TEM beta-lactamases is significantly associated.

Conclusions. The isolated Klebsiella pneumoniae strains represent a significant reservoir of resistance and virulence genes within our microbiota. However, external factors such as breastfeeding can modulate this genetic load, highlighting once again the crucial role of diet in shaping our microbiota, particularly during the early stages of life.

N° 24

Grafical Abstact.



N° 25

Spatial statistics applied to tuberculosis in the Valencian Region

Paula Sinisterra-Sebastián[1]; Miguel Ángel Martínez-Beneito[2]; Iñaki Comas[1,3]; Mariana G. López[1]

[1] Instituto de Biomedicina de Valencia (IBV-CSIC)

[2] Universidad de Valencia (UV)

[3] CIBER Epidemiología y Salud Pública (CIBERESP-ISCIII)

Background. Tuberculosis (TB) incidence and mortality rates vary greatly between different world regions. The Valencian Region (CV, in its Spanish acronym) is a region with a low TB burden. However, since 2014 there has been a slowdown in the incidence rate decrease of this disease. Transmission is the factor with the greatest impact on the global incidence of TB, even in low incidence regions the transmission rate is very high. Our objective is to study the geographical distribution of TB incidence and transmission and to evaluate whether they are homogeneous phenomena in the CV or there are regions that represent a greater problem. Understanding the impact and distribution of this disease will allow for defining efficient control programs to reduce the TB incidence in the CV.

Methods. The incidence risk and the probability of TB transmission were estimated by fitting bayesian hierarchical models that allow obtaining accurate estimates of each variable in small areas, specifically BYM models were used. Given that incidence and transmission have different statistical distributions, models based on Poisson and Binomial distributions were fitted, respectively. Besides, the probability of transmission was estimated both from the values obtained through genomic epidemiology and from those generated by contact studies, provided by the Dirección General de Salud Pública.

Results. The geographical distribution of TB risk, as well as the probability of transmission, are not homogeneous in the CV. Albocàsser, Massamagrell and Muro de Alcoy are the municipalities with the highest risk of TB. Furthermore, 4 of the 5 municipalities with penitentiary centers in the CV present high TB risks.

Regarding transmission, the results obtained from the genomic data show that Gandía, Oliva and Castellón de La Plana have an excess probability of transmission. Finally, the comparison of the transmission analysis carried out with genomic data and contact studies shows a low correlation.

Conclusions.

1) Neither the risk of TB incidence nor the probability of transmission are homogeneously distributed in the CV.

2) The application of this study at the municipal level reflects the effect of penitentiary centers on the incidence of TB.

3) Three outbreaks with greater excess transmission were detected in the provinces of Valencia and Castellón.

4) The results of the transmission analysis carried out with genomic data and contact studies show a low correlation. Genomic approach reveals higher levels of transmission. This is largely because genomics allows us to identify both familial and community transmission.

N° 26

Past and present Influenza A virus infections reported in cetaceans

Teresa Pérez-Sánchez[1]; José Carlos Báez[1]; Carolina Johnston[1]

[1] Centro Oceanográfico de Málaga (IEO-CSIC)

Background. Cetaceans are marine mammals that provide important ecosystem services. Conservation of these iconic species is threatened by factors such as climate change and accidental by catch. Infectious diseases are another threat as mass stranding events in cetaceans can be caused by viral infections, mostly by Morbillivirus. Influenza A viruses (IAV) include avian influenza viruses (AIV) which have a wide host range and infect a high number of wild animal species. In the current 2020s decade a panzootic high pathogenicity avian influenza virus of subtype H5N1 (clade 2.3.4.4b) is circulating in farmed and wild animals, also rarely infecting humans. The virus has reached marine mammals causing very high mortality rates in pinnipeds. This study reviews the past and present knowledge concerning cetacean infection by IAV in order to establish knowledge gaps that need to be addressed for panzootic preparedness.

Methods. A paired review of scientific literature in PubMed was performed in September 2024 combining several keywords (influenza virus and cetaceans, whales, porpoises or dolphins). Information related to the species and number of infected animals, localization and year, virus subtype, or diagnostic technique, was extracted from approximately 50 eligible publications or documents obtained from online resources.

Results. To date (September 2024) a total of four variants of IAV have been reported to infect cetaceans. The first cases were reported in two whales in the 1970s and 1980s and were caused by subtypes H1N3, H13N2 and H13N9. Since 2020s all cases reported are related to HPAIV H5N1 clade 2.3.4.4b. In total almost 30 cases of IAV infection of cetaceans have been reported using both direct and indirect diagnostic techniques. IAV infection has been reported in common minke whale (Balaenoptera acutorostrata), pilot whale (Globicephala melaena), beluga (Delphinapterus leucas); Dall's porpoise (Phocoenoides dalli); harbor porpoise (Phocoena phocoena); Burmeister's porpoise (Phocoena spinipinnis); common dolphin (Delphinus delphis); Atlantic white-sided dolphin (Lagenorhynchus acutus), bottlenose dolphin (Tursiops truncatus), Chilean dolphin (Cephalorhynchus eutropia).

Conclusions. The reviewed data suggest IAV infection of cetaceans is rare, although the available information is limited in space and time. No mass stranding events of cetaceans related to IAV infections have been reported to date. Continuous surveillance of IAV infections require monitoring cetaceans, although diagnostic protocols and collection of biological samples need refinement and standardization.

Acknowledgements. This study has been funded by the subproject ALMA in the framework of the complementary plan on biodiversity, financed by the NextGenerationEU Recovery and Resilience Mechanism, and coordinated under the agreement between Junta de Andalucía and Universidad Pablo De Olavide on behalf of the participant entities.

N° 27

Insights from the MILKCORONA study: Impact of SARS-CoV-2 infection and vaccination on the human milk immunoglobulinome, metabolome and microbiota

Christine Bäuerl[1]; Karla Rio-Aige[2,3]; Blanca Grases-Pinto[2,3]; Maria J Rodríguez-Lagunas[2,3]; Raúl Cabrera-Rubio[1]; Marta Selma-Royo[1]; Anna Parra-Llorca[4,5]; Desirée Mena-Tudela[6]; Laia Aguilar-Camprubi[7]; Elia García-Verdevio[8]; Laura Martínez[9,10]; Victoria Fumadó[11]; Francesca Crovetto[12]; Gerardo Rodríguez[13]; Gemma Ruiz-Redondo[14]; Cecilia Martínez-Costa[9,10]; Carles Lerin[11]; Francisco J Pérez-Cano[2,3]; Maria Carnen Collado[1]

[1] Instituto de Agroquímica y Tecnología de los Alimentos (IATA-CSIC)

[2] Facultad de Farmacia y Ciencias de la Alimentación-UB

[3] Instituto de Investigación en Nutrición y Seguridad Alimentaria (INSA-UB)

[4] Instituto de Investigación Sanitaria La Fe

[5] Hospital Universitario y Politécnico La Fe

[6] Universidad Jaume I

[7] LactApp Women Health

[8] Hospital Universitario Dr. Peset

[9] Hospital Clínico Universitario, Universidad de Valencia

[10] INCLIVA

[11] Instituto de Investigación San Joan de Déu, Hospital Sant Joan de Déu

[12] BCNatal Fetal Medicine Research Center (Hospital Clínic, Hospital Sant Joan de Déu, UB y CIBER Enfermedades Raras)

[13] University of Zaragoza, Hospital Clínico Universitario Lozano Blesa, Instituto de Investigación Sanitaria Aragón (IIS Aragón), Red de Salud Materno-Infantil y del Desarrollo (SAMID)

[14] Hospital Clínico Universitario San Cecilio-Parque Tecnológico de Ciencias de la Salud (PTS)

Background. Mothers develop antibodies against SARS-CoV-2 either via vaccination or natural infection with the virus. Newborns can acquire immunological protection against it through the antibodies present in human milk. However, concerns have arisen regarding potential short and long-term adverse effects, particularly regarding vaccine-induced changes in the human milk metabolome, microbiota, and the composition of immunoglobulins and cytokines.

Methods. Human milk samples were collected from 64 mothers who were infected with SARS-CoV-2 and 98 vaccinated lactating mothers (25 received the mRNA-1273 vaccine, 32 ChAdOx1, and 40 Comirnaty). Furthermore, samples from control group of 63 women not exposed to SARS-CoV-2 from pre-pandemic times were included. Samples were obtained prior to vaccination and 21 days after both the first and the second doses. The concentration of immunoglobulins (IgA, IgM, IgE, IgG1, IgG2, IgG3, IgG4) and 18 cytokines in breast milk was quantified by ELISA and ProcartaPlexTM Multiplex immunoassay, and metabolome analysis was performed applying ultrahigh-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) using the Biocrates MxP® Quant 500 kit. Microbiota composition was determined by 16S rRNA amplicon sequencing.

Results. SARS-CoV-2 infection led to a significant increase in total IgG and IgM concentrations and a significant shift to Th2 associated antibodies, while total IgA and IgE concentrations were not affected. Vaccination raised total IgA and IgG levels which remained elevated after the second dose in the case of IgA, whereas IgG levels decreased again. The metabolomic profile differ between control and COVID-19 women, however, no separation between any of the times after vaccination and the pre-

vaccination time was observed suggesting that vaccination had no major impact on the human milk metabolome. Differences in beta-diversity and in some specific genus as Streptococcus spp but not in alpha-diversity indexes were found in human milk microbiota between Control (prepandemic) and infected women. Additionally, vaccination did not induce changes in the microbiota composition in any of the time points analysed.

Conclusions. Overall, our results indicate that both SARS-CoV-2 infection and vaccination affected the human milk immunological and cytokine profile. However, COVID-19 vaccination has no effect on the human milk microbiota and metabolome profiles, which remain stable after vaccination, suggesting no adverse effect on the initial shaping of the early life microbiota.

Acknowledgements. This work was supported by a research grant from LaMarató-TV3 (Milk-CORONA, ref. 202106). IATA-CSIC team members are part of the CSIC's Global Health Platform (PTI+ Salud Global). IATA-CSIC is a Centre of Excellence Severo Ochoa (CEX2021-001189-S) and INSA-UB is a Maria de Maeztu Unit of Excellence (CEX2021-001234-M) funded by MICIN/AEI/FEDER, UE.

N° 28

Uncovering the potential role of human milk oligosaccharides and Bifidobacterium spp. in combating antibiotic resistance in early life

Anna Samarra Mas[1]; Lars Bode[2]; Maria Carnen Collado[1]; Andrei Osterman[2]; Simone Renwick[2]; Dmitry Rodionov[2]

[1] Instituto de Agroquímica y Tecnología de los Alimentos (IATA-CSIC)

[2] UCSD

Introduction. The impact of Bifidobacterium on modulating infant antibiotic resistance load remains unknown. Bifidobacterium can metabolize human milk oligosaccharides (HMOs), the third major solid component in human milk. This study aimed to explore the association between Bifidobacterium-dominated infant gut microbiota capable of utilizing HMOs and the antibiotic-resistant gene load in neonates at 7 days and 1 month old.

Methodology. 50 infants followed from birth up to 1 years from MAMI cohort were included. Maternal-infant clinical data, including mode of birth, intrapartum antibiotic use, and breastfeeding practices, were available. Gut microbiota profiling was performed using 16S rRNA and microbiome using Illumina shotgun sequencing. Antibiotic resistance genes (ARGs) were quantified by targeted qPCR and resistome analysis using ResFinder. Genome sequencing of the Specific Bifidobacterium isolates (n=55) were performed on the Illumina platform. Prediction of HMO pathways were validated by growing the isolates in presence of HMOs and glycoprofiling was analyzed by HPLC-FL. R statistical software was employed for data clustering, analysis, and plotting.

Results. Infants were categorized into low-Bifidobacterium (LB) and high-Bifidobacterium (HB) groups, displaying distinct beta diversity in their microbiota. LB infants exhibited higher antibiotic-resistant gene levels, with species like Enterococcus and Clostridium positively correlating with ARG abundance. Microbiota profiles were influenced by delivery mode and Bifidobacterium dominance, impacting ARG carriage. Bifidobacterium species demonstrated diverse growth patterns under HMOs, revealing different cases based on their response to HMOs and glucose presence or absence. B. bifidum is the species with higher capacity to degrade HMOs and lower carriage and phenotype of resistance to antibiotics.

Conclusions. The Bifidobacterium genus was associated with a specific early microbiota profile that directly influences antibiotic resistance in neonates. Species-specific HMO metabolism capacity and antibiotic resistance provided knowledge on the role of breastfeeding in reducing antibiotic resistance. Further research is needed to understand the influence of perinatal factors on the acquisition of potentially resistant bacteria and to develop strategies to prevent microbial infections.

N° 29

On the lookout for CVB3 mutants that do not disrupt trafficking

Ron Geller[1]; Mireya Martínez Pérez[1]

[1] Instituto de Biología Integrativa de Sistemas (I2SysBio) (UV-CSIC)

Viruses have evolved to manipulate host cell pathways for their own benefit. Disruption of membrane trafficking during infection has been described for several viruses, and picornaviruses are no exception. Major histocompatibility complex (MHC) class I is one of the molecules whose uptake/release process is affected by several infections. MHC-I is involved in the cellular immune response through antigen presentation to CD8+ T-cells, and by downregulating surface MHC-I, viruses evade part of the cellular immunity. Although some of the proteins responsible for this manipulation have been described, a general screen analyzing the potential influence of each viral protein and the underlying detailed mechanisms are lacking. Here, we use Coxsackievirus B3 (CVB3) as a model to study the effect of picornaviruses on membrane trafficking and, in particular, on the expression of MHC-I during viral infection. The CVB3 genome consists of a single-stranded positive RNA that produces a unique ORF containing three major regions: P1 (capsid proteins), P2 and P3 (non-structural proteins). Deep Mutational Scanning (DMS) approaches are extremely useful to obtain a general picture of the involvement of each individual genomic site in a specific step of viral infection. Therefore, surface MHC-I expression was compared between cells infected with GFP-expressing CVB3 libraries containing single mutations in each genomic site of P2 and P3. CVB3 mutants that failed to downregulate MHCI were selected and identified by NGS. To validate our DMS experiment and bioinformatic analysis, infectious clones of a representative set of individual mutants were generated and MHC-I membrane expression was compared between cells infected with wild-type or mutant CVB3. Taken together, our data not only provide a comprehensive analysis of the effect of picornaviruses on cellular trafficking, but also shed light on the strategies used by viruses to evade the immune system.

N° 30

Deep mutational scanning of a model picornavirus: understanding the effects of mutations across all viral proteins and their role in innate immunity

Sebastian Velandia Álvarez[1]; Ron Geller[1]; Beatriz Álvarez Rodríguez[1]

[1] Instituto de Biología Integrativa de Sistemas (I2SysBio) (UV-CSIC)

RNA viruses have the highest mutation rate in nature. This, combined with their rapid replication and relatively large population sizes, allows them to rapidly evolve and adapt to new environments. However, while the high mutation rate is key to successful adaptation, it also plays an important role in the generation of non-viable viruses, as most mutations tend to be deleterious for protein functions. How the viruses and their proteins contend with this process and adapt under different selective pressures, such as immune responses is unclear. Deep Mutational Scanning (DMS) can help answer such questions, as it assesses the effect of all possible single amino acid mutations in a protein. This method, combined with the application of a selective pressure, can reveal crital mutations for counteracting the effects of this force.

We used DMS to define single amino acid mutations across the full proteome of a model picornavirus, Coxsackievirus B3, that confer resistance or sensitivity to interferon. Several mutations with these phenotypes were identified and validation was carried out by individually constructing mutant viruses. To determine the step of the viral cycle that was affected by the interferon effector pathway, we assessed entry, replication, and translation of the mutants individually. Additionally, 20 ISGs known to affect enteroviruses were overexpressed using a lentiviral system to determine the mechanisms by which these mutants are more sensitive or resistant than the wildtype virus. Further analyses are being carried out to couple the data of different DMS selections to develop a novel live-attenuated vaccine candidate.

N° 31

Poxvirus MVA-based vaccine candidates expressing SARS-CoV-2 prefusion-stabilized S proteins of the Wuhan, Beta or Omicron BA.1 variants protect transgenic K18-hACE2 mice against Omicron infection and induce potent and broad specific humoral and T-cellular immune responses

Patricia Pérez[1]; David Astorgano[1]; Gillermo Albericio[1]; Sara Flores; Cristina Sánchez-Corzo[1]; María A. Noriega[1]; Pedro J. Sánchez-Cordón[2]; Nuria Labiod; Rafael Delgado; José M. Casasnovas[1]; Mariano Esteban[1]; Juan García-Arriaza[1]

[1] Centro Nacional de Biotecnología (CNB-CSIC)

[2] Centro de Investigación en Sanidad Animal (CISA-INIA-CSIC)

Despite the decrease in mortality and morbidity due to SARS-CoV-2 infection, the incidence of infections due to Omicron subvariants of SARS-CoV-2 remains high. The mutations acquired by these subvariants, mainly concentrated in the receptor-binding domain (RBD), have caused a shift in infectivity and transmissibility, leading to a loss of effectiveness of the first authorized COVID-19 vaccines, among other reasons, by neutralizing antibody evasion. Hence, the generation of new vaccine candidates adapted to Omicron subvariants is of special interest in an effort to overcome this immune evasion. Here, an optimized COVID-19 vaccine candidate, termed MVA-S(3P_BA.1), was developed using a modified vaccinia virus Ankara (MVA) vector expressing a full-length prefusion-stabilized SARS-CoV-2 spike (S) protein from the Omicron BA.1 variant. The immunogenicity and efficacy induced by MVA-S(3P_BA.1) were evaluated in mice in a head-to-head comparison with the previously generated vaccine candidates MVA-S(3P) and MVA-S(3Pbeta), which express prefusion-stabilized S proteins from Wuhan strain and Beta variant, respectively, and with a bivalent vaccine candidate composed of a combination of MVA-S(3P) and MVA-S(3P_BA.1). The results showed that all four vaccine candidates elicited, after a single intramuscular dose, protection of transgenic K18-hACE2 mice challenged with SARS-CoV-2 Omicron BA.1, reducing viral loads, histopathological lesions, and levels of proinflammatory cytokines in the lungs. They also elicited anti-S IgG and neutralizing antibodies against various Omicron subvariants, with MVA-S (3P_BA.1) and the bivalent vaccine candidate inducing higher titers. Additionally, an intranasal immunization in C57BL/6 mice with all four vaccine candidates induced systemic and mucosal S- specific CD4+ and CD8+ T-cell and humoral immune responses, and the bivalent vaccine candidate induced broader immune responses, eliciting antibodies against the ancestral Wuhan strain and different Omicron subvariants. These results highlight the use of MVA as a potent and adaptable vaccine vector against new emerging SARS-CoV-2 variants, as well as the promising feature of combining multivalent MVA vaccine candidates.

N° 32

Women-led homes coping with energy poverty: A case study from a vulnerable neighbourhood in Madrid, Spain

Teresa Cuerdo[1]; Miguel Ángel Navas Martín[2]

[1] Instituto de Ciencias de la Construcción Eduardo Torroja (IETcc-CSIC)

[2] Escuela Nacional de Sanidad-ISCIII

People with lower incomes often dwell in homes that lack proper thermal insulation, which hamper their ability to maintain a comfortable indoor climate. This vulnerability is intricately linked to the quality and upkeep of housing, adversely affecting indoor environmental comfort, particularly concerning energy consumption patterns to reach an adequate temperature, and has repercussions on health and well-being. Exploring energy poverty from a qualitative approach allows for a deeper understanding of these individuals' experiences. A qualitative research study was conducted focusing on women facing housing vulnerability and energy poverty, involving semistructured interviews with five households led by women and two key informants, both before and after the COVID-19 confinement, in one of Madrid's most at-risk neighborhoods. The investigation revealed the complexity surrounding this issue and identified three main categories: 1) household structure and financial resources, 2) perceptions and recommendations for home improvements, and 3) the overall health and well-being of households. Additionally, the findings indicate that the dependence on ineffective heating methods, such as electric radiators or butane heaters during winter (which may seem cheaper initially but are unsustainable long-term), reflects energy instability. This, combined with adverse housing conditions and residents' behaviors, significantly affects health and can exacerbate chronic illnesses. Research focusing on vulnerable populations requires urgent actions that go beyond mere awareness, supported by key informant insights. Social workers and educators play a crucial role in enhancing the living conditions of the most deprived individuals; however, they must be supported by the backing of social policies and well-crafted intervention plans and strategies to make their efforts successful.

N° 33

Mapping mutational fitness effects across the coxsackievirus B3 proteome reveals distinct profiles of mutation tolerability

Ron Geller[1]; Cristina Toft[1]; Sebastian Velandia-Álvarez[1]; **Beatriz Álvarez-Rodríguez**[1]

[1] Instituto de Biología Integrativa de Sistemas (I2SysBio) (UV-CSIC)

RNA viruses have notoriously high mutation rates due to error-prone replication by their RNA polymerase. However, natural selection concentrates variability in a few key viral proteins. To test whether this stems from different mutation tolerance profiles among viral proteins, we measured the effect of >40,000 non-synonymous mutations across the full proteome of coxsackievirus B3 as well as >97% of all possible codon deletions in the nonstructural proteins. We find significant variation in mutational tolerance within and between individual viral proteins, which correlated with both general and protein-specific structural and functional attributes. Furthermore, mutational fitness effects remained stable across cell lines, suggesting selection pressures are mostly conserved across environments. In addition to providing a rich dataset for understanding virus biology and evolution, our results illustrate that incorporation of mutational tolerance data into druggable pocket discovery can aid in selecting targets with high barriers to drug resistance.

N° 34

Duplex sequencing to assess the impact of putative exonuclease inhibitors on the MHV-A59 coronavirus

Marianoel Perreira Gómez[1,2]; Sebastián Velandia Álvarez[3]; Ron Geller[3]; Gonzalo Moratorio[2,4]

[1] Facultad de Veterinaria-Universidad de la República

[2] Institut Pasteur de Montevideo

[3] Instituto de Biología Integrativa de Sistemas (I2SysBio) (UV-CSIC)

[4] Facultad de Ciencias-Universidad de la República

Abstract. RNA viruses are highly susceptible to treatment with chemical mutagens, which can lead to a drastic reduction in their biological efficacy and, in some cases, result in the extinction of viral populations through a process known as lethal mutagenesis. This antiviral strategy is based on overloading viral populations with mutations, which reduces the production of viable progeny. Targeting viral replication fidelity by inhibiting error-correcting mechanisms could further increase the mutational load, offering a potential therapeutic approach against coronaviruses. This study aims to evaluate the antiviral effects of two candidate compounds that inhibit the error-correcting activity of coronaviruses, using the murine hepatitis virus (MHV-A59) as a model, which is phylogenetically related to SARS-CoV-2. We investigated viral growth inhibition, specific infectivity and mutational frequency to determine the potential inhibition of the viral exonuclease, a key enzyme involved in replication fidelity.

MHV-A59 viral populations were propagated in murine fibroblast cells under standard culture conditions. Candidate compounds concentrations were selected based on preliminary cytotoxicity studies. Viral growth was quantified using plaque assays, RNA was quantified by RT-qPCR, and specific infectivity was calculated. Sustained treatment at sublethal doses with candidate compounds resulted in decreased viral growth compared to control populations. The reduction in viral growth was accompanied by a sharp decrease in specific infectivity. Preliminary duplex sequencing results for viral populations treated with compound B revealed a higher frequency of mutations compared to untreated populations. To determine the mutational load, viral RNA was extracted from infected cells PCR amplified and subjected to duplex sequencing. Libraries were prepared, sequenced, and analyzed for mutation frequency in viral populations treated with compound B compared to untreated controls.

Analysis of populations treated with compound A and estimation of the viral mutation rate in both treated and control conditions are ongoing to confirm whether these compounds play a role in inhibiting viral exonuclease function. The viral mutation rate under experimental and control conditions will be calculated to determine whether the compounds inhibit viral exonuclease activity, responsible for correcting replication errors. Our findings suggest that inhibiting the error-correcting mechanisms of coronaviruses may reduce viral fitness and serve as an effective antiviral strategy. The observed increase in mutation frequency and corresponding decrease in viral infectivity indicate that compounds targeting the viral exonuclease could enhance lethal mutagenesis, providing a novel approach for controlling coronavirus infections. Further analysis of the viral mutation rate will clarify the role of these compounds in exonuclease inhibition and their potential for therapeutic development.

N° 35

Web portal for Health and Population in Spain

Dariya Ordanovich[1]; Diego Ramiro Fariñas[1]; Pilar Aceituno Nieto[1]; Ana Belén Castillo Belmonte[1]

[1] Instituto de Economía, Geografía y Demografía (IEGD-CSIC)

Abstract. Here we present a dynamic and continuously updated platform that offers a comprehensive overview of social and healthcare resources for the elderly across Spain. At its core is an interactive map that allows users to explore various regions, instantly revealing relevant information about medical and social care facilities available within the selected area. By focusing on data specific to services for the elderly, the platform provides a unique, user-friendly tool for researchers, healthcare professionals, and policymakers to access valuable insights. It not only serves as a powerful visual tool but also offers access to a wealth of scientific publications based on this data, supporting academic contributions and evidence-based decision-making. This platform is an invaluable resource for analyzing spatial patterns of healthcare services for aging populations, making it an essential tool for improving service accessibility and shaping future policies tailored to the needs of Spain's ageing population.