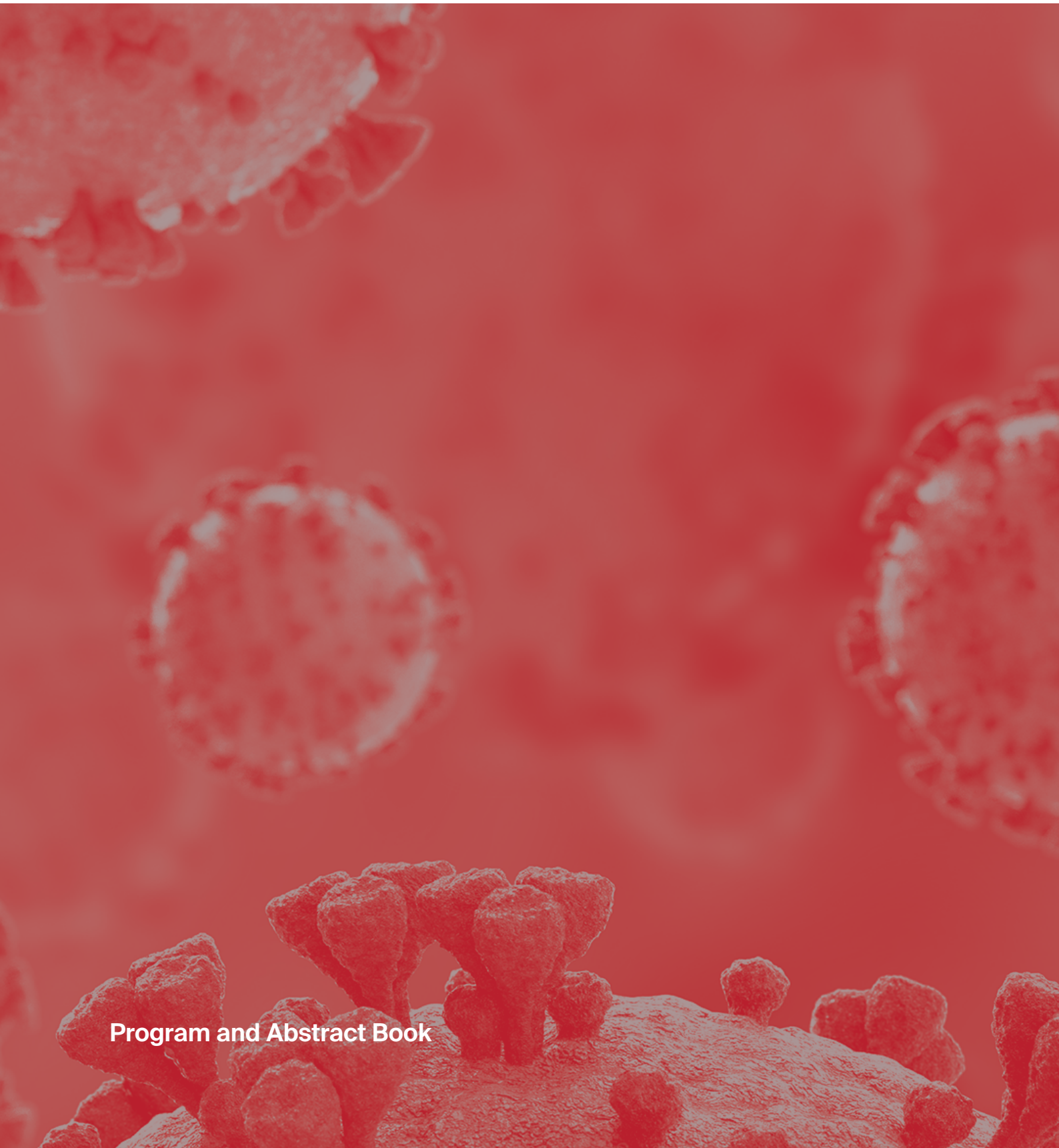
Viruses

2024

Conference

Viruses 2024 – A World of Viruses

14–16 February 2024 | Barcelona, Spain



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Viruses 2024—A World of Viruses

AXA Convention Centre
Barcelona, Spain
14–16 February 2024

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Dr. Albert Bosch

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Prof. Dr. Svetlana Folimonova
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Prof. Dr. Linda Saif
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Conference Secretariat

Mr. Cédric Spinnler
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Dr. Elena González

Contents

Welcome from the Chairs

Dear Colleagues,

We are delighted to announce the upcoming Virology event "Viruses 2024 – A World of Viruses", to be held in Barcelona, Spain, from February 14–16, 2024.

Viruses are a constant threat to global health, and their study remains a critical area of research. This conference aims to bring together top scientists, researchers, and industry experts from around the world to share their findings on the latest developments in viral pathogenesis and immune responses. From molecular and cellular biology to immunology, epidemiology, and public health, the conference will provide a platform for attendees to engage in lively discussions on the most significant issues in virology.

The event is sponsored by MDPI and the open-access journal Viruses and follows the successful meetings held in 2016 in Basel, Switzerland, in 2018 and 2020 in Barcelona, Spain, and in 2022 online. We anticipate that this conference will provide attendees with a unique opportunity to learn about the latest research and to network with colleagues from around the world.

We welcome abstracts for posters or short talks related to the conference theme, which will be considered for presentation at the conference. Meeting participants will have the opportunity to present their work and discuss their research in a stimulating and collegial environment.

We look forward to welcoming you again in Barcelona.

Dr. Eric Freed
Conference Chair

National Cancer Institute, NIH - Frederick, MD, USA

Dr. Albert Bosch
Conference Chair

Enteric Virus Laboratory, School of Biology, University of Barcelona, Spain

Keynote Speakers

Prof. Dr. Luis Enjuanes
National Center of Biotechnology (CNB-CSIC),
Madrid, Spain

Dr. Charles M. Rice
The Rockefeller University,
New York, USA

Invited Speakers

Prof. Dr. Melanie Brinkmann
Institute of Genetics, Technische Universität Braunschweig (TU Braunschweig), Germany

Prof. Dr. Wolfram Brune
Leibniz Institute of Virology (LIV), Hamburg, Germany

Dr. Ulrich Desselberger
Department of Medicine, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK

Prof. Dr. Santiago F. Elena
Institute for Integrative Systems Biology, CSIC, Valencia, Spain

Prof. Dr. Svetlana Folimonova
University of Florida, USA

Prof. Dr. Felicia Goodrum
The University of Arizona, Tucson, USA

Prof. Dr. Margaret Kielian
Albert Einstein College of Medicine, Bronx, NY, USA

Professor Rosa Maria Pintó
Enteric Virus Laboratory, Department of Genetics, Microbiology and Statistics, School of Biology, University of Barcelona, Spain

Prof. Dr. Félix Rey
Virology Department, Institut Pasteur, Paris, France

Prof. Dr. Linda Saif
The Ohio State University, USA

Prof. Dr. Mikael Skurnik
University of Helsinki, Finland

Prof. Dr. Nicola J. Stonehouse
Faculty of Biological Sciences, School of Molecular and Cellular Biology, University of Leeds, UK

Dr. Carolien van de Sandt
Department of Microbiology and Immunology, The University of Melbourne, Peter Doherty Institute for Infection and Immunity, Melbourne, Australia

Abridged Program

Wednesday, 14 February 2024
9:00 am – 6:30 pm CET (Check-In: 8:00 pm CET)

Thursday, 15 February 2024
9:00 am – 6:30 pm CET (Conference Dinner: 8:30 pm CET)

Friday, 16 February 2024
9:00 am – 6:30 pm CET

	Wednesday, 14 February 2023	Thursday, 15 February 2023	Friday, 16 February 2023
Morning	Check-In / Opening Ceremony	S3. Antiviral Innate Immunity	S5. Virus–Host Interactions (Part 2)
	S1. General Topics in Virology (Part 1)		
	Coffee Break	Coffee Break (Conference Group Photo)	Coffee Break
	S1. General Topics in Virology (Part 2)	S4. Structure and Mechanisms of Virus Replication (Part 1)	S5. Virus–Host Interactions (Part 3)
	Lunch Break	Lunch Break & Poster Session A	Lunch Break & Poster Session B
Afternoon	S2. Antiviral Therapeutics, Vaccines, and Host Defenses (Part 1)	S4. Structure and Mechanisms of Virus Replication (Part 2)	S6. Viral Pathogenesis and Evolution (Part 1)
	Coffee Break	Coffee Break & Poster Session A	Coffee Break & Poster Session B
	S2. Antiviral Therapeutics, Vaccines, and Host Defenses (Part 2)	S5. Virus–Host Interactions (Part 1)	S6. Viral Pathogenesis and Evolution (Part 2)
	Flash Poster Presentation Session		Closing Ceremony
		Conference Dinner	

Conference Program

General Information

Viruses 2024–A World of Viruses will be held at the AXA Coventiion Centre, Barcelona, Spain, on 14 – 16 February 2024. This conference This conference aims to bring together top scientists, researchers, and industry experts from around the world to share their findings on the latest developments in viral pathogenesis and immune responses. From molecular and cellular biology to immunology, epidemiology, and public health, the conference will provide a platform for attendees to engage in lively discussions on the most significant issues in virology.

Conference Venue

AXA Coventiion Centre
Avinguda Diagonal, 547, 08029 Barcelona

Registration Desk

The desk for registration, information and distribution of documents will be open from 8.00h on 14 February 2024.

Certificate of Attendance

Participants of the event will be able to downlod an electronic Certificate of Attendance by accessing their dashboards on Sciforum.net once the event is concluded. The certificates will be found under the “My Certificates" category.

Disclaimer

Delegates will receive a name-badge at the Information Desk, upon registration. The badge must be worn prominently in order to gain access to the congress area during the event. Admission will be refused to anyone not in possession of an appropriate badge.

Insurance

The organizers do not accept liability for personal accidents, loss, or damage to private property incurred as a result of participation in *Viruses 2024–A World of Viruses*. Delegates are advised to arrange appropriate insurance to cover travel, cancellation costs, medical, and theft or damage of belongings.

Barcelona and Catalonia

Barcelona is the capital and largest city of Catalonia and is Spain's second largest city, with a population of over one and half million people.

Located on the northeastern Mediterranean coast of Spain, this city has a rich and diverse history, with its roots dating back to Roman times. The fruitful medieval period established Barcelona's position as the economic and political centre of the Western Mediterranean. The city's Gothic Quarter bears witness to the splendour enjoyed by the city from the 13th to the 15th centuries.

The 20th century ushered in widespread urban renewal throughout Barcelona city, culminating in its landmark Eixample district, which showcases some of Barcelona's most distinctive Catalan art-nouveau, or modernista, buildings. The Catalan Antoni Gaudí, one of the most eminent architects, designed buildings such as La Pedrera, the Casa Batlló and the Sagrada Família church, which have become world-famous landmarks.

In 1992, Barcelona gained international recognition by hosting the Olympic games which brought about a massive upturn in its tourism industry. For visitors, this has translated into the very modern, yet incredibly old city you see now in the 21st century, where new elements work to both preserve and celebrate both the city’s heritage and origins.

Barcelona is plenty of outdoor markets, restaurants, shops, museums, and churches. The city is also very walkable, with an extensive and reliable Metro system for more far-flung destinations.

For a complete overview, see wikitravel.org or visit barcelonaturisme.com.

The AXA Convention Centre

Address: Avinguda Diagonal, 547, 08029 Barcelona

The conference will be held at the AXA Convention Centre in Barcelona, Spain. The AXA Convention Centre is located in a vibrant modern zone of the city, next to the main road and with easy access from the airport, as well as the urban and suburban areas.

It is part of "L'Illa Diagonal", a modern complex which include a shopping centre, two 4-stars hotels, several offices, a sports centre, a public park and a parking with 2500 places.

MDPI, the Multidisciplinary Digital Publishing Institute, is a publisher of fully peer-reviewed, Open Access journals with a focus on robust and rapid editorial processes. Our aim is to ensure that high-quality research is verified and made available to the research community as quickly as possible. MDPI stands at the forefront of the Open Access movement, having launched its first online journal Molecules in 1996. Today, MDPI is a leader in Open Access, publishing more than 400 journals across all research disciplines and offering publishing-related initiatives to scholars:

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an Open Access Journal by MDPI

Viruses (ISSN 1999-4915) is an open access journal that provides an advanced forum for studies of viruses. It publishes reviews, regular research papers, communications, conference reports and short notes. Our aim is to publish papers that are of significant impact to the virology community. We encourage scientists to publish their experimental and theoretical results in as much detail as possible. The full experimental details must be provided so that the results can be reproduced. We also encourage the publication of timely reviews and commentaries on topics of interest to the virology community. Papers are either published in the open journal or in Special Issues devoted to specific topics of interest to the field. We accept proposals for review articles that are not part of Special Issues.

Q2
(*Virology*)
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Q1
(*Infectious Diseases*)
submission to first decision provided to authors

15
days
Submission to First Decision

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Special Events:

Viruses 2024 - A World of Viruses
Guest Editor: Dr. Eric O. Freed
Deadline: 30 April 2024

Viruses 2024 Early Career Investigator Award
Nomination Deadline: 29 February 2024

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Emergency Information

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Spanish National Police: 091

Abstracts - Talks

S1. General Topics in Virology

A cure for hepatitis C, now what?

Charles M. Rice
The Rockefeller University, New York, USA

Nearly fifty years after the discovery of non-A, non-B posttransfusion hepatitis and fifteen years later, the elucidation of the causal agent, hepatitis C virus, we now have highly effective treatments with virologic cure rates approaching 100%. There are still challenges to achieving HCV eradication on a global scale, but in this talk I will highlight a few of the non-HCV related research projects that have sprung up in our lab. The first area concerns HBV, with nearly 300 million chronic carriers at risk of developing liver cancer. There is no reliable cure for chronic hepatitis B. I will describe a new approach to studying this virus that has already uncovered new insights into the intricacies of HBV replication and provides a pre-clinical platform for assessing viral resistance to new classes of inhibitors that are being developed to achieve a functional, treatment-free cure. The second theme concerns innate antiviral immunity, a topic we began to study many years ago in the interferon era of HCV treatment. Lately, we have become interested in whether antigen-independent innate immune memory, elicited by virus infection, can influence the outcome of secondary infection by an unrelated virus. I will describe some recent work in this area using a mouse model of SARS-CoV-2 infection.

Design of RNA Replicons as Vaccines for Human Pathogenic Coronaviruses, and of Antivirals to Protect from their Infection

Luis Enjuanes*, Isabel Sola, Sonia Zúñiga, Diego Muñoz-Santos, Marta Villarejo-Torres, Ana Marchena-Pasero, María Rueda-Huélamo, Jesús Hurtado-Tamayo, Ricardo Requena-Platek, José M. Honrubia, María Guzmán, Carlos M. Sánchez, Jorge Ripoll-Gómez, y Mercedes Ruiz

National Center of Biotechnology (CNB-CSIC), Madrid, Spain

An alternative strategy to protect against pathogenic CoVs is the identification of antivirals. CoV E protein has a PDZ-binding motif (PBM) at its carboxy-terminus. PBMs may bind over 400 cellular proteins containing PDZ domains, making them relevant for the control of cell function. Three highly pathogenic human CoVs have been identified to date: SARS-CoV, MERS-CoV and SARS-CoV-2. We investigated the relevance of their E protein PBMs in virulence. Gene expression patterns in the lungs of mice infected with SARS-CoVs encoding different PBMs were analyzed by RNA sequencing. We found that the SARS-CoV-2 E protein PBM dysregulated gene expression related to ion transport and cell homeostasis. Specifically, decreased expression of cystic fibrosis transmembrane conductance regulator (CFTR) mRNA, which is essential for alveolar edema resolution, was observed. Compounds that increased CFTR activity, used in the treatment of cystic fibrosis significantly reduced SARS-CoV-2 growth in cultured cells and were protective for CoVs infected mice. These results indicated that SARS-CoV-2 E protein virulence is mediated in part by the reduction of CFTR expression. Interestingly, the administration of a combination of two drugs that restored CFTR levels protected at least 80% of SARS-CoV-2 infected mice, and helped the identification of antivirals for pathogenic CoVs.

Dynamics of CD8+ T Cell Immunity to Circulating and Candemic Ciruses

Carolien van de Sandt

Department of Microbiology and Immunology, The University of Melbourne, Peter Doherty Institute for Infection and Immunity, Melbourne, Australia

Viruses like influenza and SARS-CoV-2 remain a constant global threat, causing significant morbidity and mortality. A robust CD8+ T-cell response plays an important role in protection against severe disease, particularly in the absence of neutralizing antibodies. We and others demonstrated that CD8+ T-cell receptors recognize conserved viral proteins, providing broad cross-reactivity across viral strains and subtypes. This in combination with the long-lived properties of CD8+ T-cells makes them an attractive target for novel T-cell-based vaccine strategies aiming to protect against severe disease outcomes. However, age is a major factor in determining disease duration and outcome during both seasonal epidemic and pandemic outbreaks. The mechanisms underlying age-related changes in the virus-specific CD8+ T-cell population across the human lifespan and therefore the potential impact of aging on T-cell-based vaccine effectiveness are far from clear. We investigated how virus-specific T-cell receptor composition and functionality develop across immunologically-distinct phases of human life, we identified a linear differentiation-trajectory from newborns to children then adults, followed by a divergence and a clonal reset in the elderly. Elderly gene profiles closely resembled those observed in newborns and children, despite being clonally-different. However, only child- and adult-derived T-cells had the potential to differentiate into highly cytotoxic virus-specific CD8+ T-cells, which was linked to highly functional public (shared) T-cell receptor signatures. Suboptimal private (not shared) T-cell receptor-signatures detected in elderly led to poorer proliferation, polyfunctionality, avidity and recognition of peptide mutants, despite displaying no signs of exhaustion. Our study suggests that priming T-cells at different stages of life might greatly affect CD8+ T-cell responses towards viral infections. Another factor that could affect T-cell-based vaccines are immunomodulatory treatments often prescribed to autoimmune patients. Our data demonstrate that TNF-treated IBD patients benefit from SARS-CoV-2 mRNA vaccination by inducing a robust spike-specific CD8+ T-cell responses.

Decoding RNA Structures for Insights into Viral Life Cycle Dynamics

Silvi Rouskin

Harvard Medical School, Boston, MA, USA

RNA's structure is central to the behavior and life cycle of viruses, acting as a pivotal determinant in their function and interactions with host cells. Previously, we developed tools based on chemical probing for analyzing RNA structures in cellular contexts. These tools allowed us to discover new regulatory mechanisms within multiple viruses. Notably, we found alternative RNA structures that are crucial for the splicing regulation of HIV-1 (Tomezsko et al., Nature 2020) and the frameshifting mechanisms of SARS-CoV-2 (Lan et al., Nature Communications 2022).
Building on this work, our current research is focused on generating a large database of experimentally determined RNA structures of viruses, including multiple coronaviruses, influenza, and herpes viruses such as KSHV and EBV. In parallel, we are developing a machine learning model that aims to predict RNA structures with precision, relying solely on their sequence information. Our current model, trained on a subset of sequences from this database, has a higher accuracy then the current state-of-the-art ML models for structure prediction. By achieving an understanding of RNA structures, we gain deeper insights into their function and mechanistic roles. Moreover, this knowledge will be invaluable in accelerating the design and development of therapeutic agents.

Identification of a Potential Entry Fusion Complex Based on Sequence Homology of African Swine Fever and Vaccinia Virus

Jesús Urquiza López, Covadonga Alonso

National Institute of Agricultural and Food Research and Technology (INIA-CSIC), Madrid, Spain

African swine fever virus (ASFV) belongs to the family of Nucleocytoplasmic Large DNA Viruses (NCLDVs), which share a common phylogeny. Little is known about the internalization of ASFV in the host cell and the fusion membrane events occurring at the early stages of infection. Poxviruses are large, enveloped, double-stranded DNA viruses and are also members of the NCLDV family. Poxviruses, such as vaccinia virus (VACV), are considered unique in having an elaborate entry fusion complex (EFC) comprising 11 highly conserved proteins integrated into the membrane of mature virions. Recent studies have described diverse connections between VACV EFC proteins using new methodological techniques. Here, we describe an approach to elucidate a potential ASFV entry fusion complex which resulted in finding ten candidate proteins with structural similarities with VACV EFC proteins. This could unveil crucial functions of these ASFV proteins due to the similarities between both virus families, which could constitute an ASFV EFC.

Comprehensive Analysis of SARS-CoV-2 Variant Outcompetition Dynamics Through Water-Based Epidemiology

Albert Carcereny Sánchez[1,2], David García Pedemonte[1,2], Maria Isabel Costafreda Salvany[1,2], Cristina Fuentes Pardo[2], Susana Guix Arnau[1,2], Rosa Maria Pintó Solé[1,2], Albert Bosch Navarro[1,2]

[1] *Enteric Virus Laboratory, School of Biology, University of Barcelona, Barcelona, Spain*
[2] *Research Institute of Nutrition and Food Safety (INSA-UB), Barcelona, Spain*

Since the early beginning of the SARS-CoV-2 pandemic, wastewater-based epidemiology has proven to be a useful complementary tool to clinical surveillance. In wastewater, both the amount of circulating virus and the occurrence of variants can be monitored using PCR and NGS techniques. Our study aimed at tracking the dynamics of SARS-CoV-2 variants at the population level through wastewater analysis, to identify factors that may be related with the dominance of a given variant over a previous one.
Variants from Alpha to Omicron BA.5 were weekly monitored using duplex RT-qPCR assays targeting the signature indels of each variant. NGS was performed monthly to validate the RT-qPCR results and, especially from Omicron BA.1 onward, to estimate the proportions of different circulating sublineages. The ability of each emerging variant to replace the previously dominant one was estimated by calculating its outcompetition rate (OR) using duplex RT-qPCR data. The variant entry ratio (ER), the diversity of the receptor variant swarms (Shannon entropy, SE), and herd immunity were also estimated.
Significant differences were found between the OR of the different variants, with Delta and Omicron BA.1 being the fastest ones. A multi-correlation study was performed between the OR, the variant ER, the SE, and herd immunity. The OR showed significant positive correlations with the variant ER and herd immunity, and negative correlation with the SE. The variant ER negatively correlated with the SE and herd immunity, while these two latter parameters showed a strong positive correlation.
Overall, wastewater surveillance not only enables us to monitor SARS-CoV-2 evolution in the population, but also allows for a deeper analysis of different factors that may play a key role in virus behaviour.

HBV-miR-3 Tweaks Hepatocyte Lipid Metabolism: Novel Insights into HBV Pathogenesis

Shruti Chowdhari[1], Auroni Deep[1], Jasmine Samal[2], Ekta Gupta[2], Vivekanandan Perumal[1]

[1] *Kusuma School of Biological Sciences, Indian Institute of Technology, Delhi, India*
[2] *Institute of Liver and Biliary Sciences, Delhi, India*

Chronic HBV (CHBV) infection has been associated with chronic hepatitis, fibrosis, steatosis and cirrhosis; it is also the leading cause of HCC. Few HBV-encoded miRNAs have been reported, but their involvement in HBV pathogenesis remains poorly studied. In this study, we investigate the cellular targets of HBV-miR-3 (an HBV-encoded miRNA) and identify its role in HCC.
Our transcriptome-wide analysis revealed that the ectopic expression of HBV-miR-3 in hepatocytes differentially altered the expression of cellular transcripts. Gene ontology analysis of differentially expressed genes indicated lipid metabolic processes among the top biological processes affected. Interestingly, in silico prediction showed potential HBV-miR-3 binding sites in the 3'UTRs of several host genes including cellular cholesterol exporter ABCA1. Quantitative real time polymerase chain reaction (RT PCR) validation demonstrated that the ABCA1 transcript levels were significantly lower in HBV-miR-3-expressing Huh-7 and HepG2 cells. 3'UTR luciferase reporter assay confirmed that ABCA1 is a direct target of HBV-miR-3. Functional assays further demonstrated that the overexpression of HBV-miR-3 led to increased cellular cholesterol and lipid droplet accumulation in hepatocytes, characteristic of hepatic steatosis. Hepatocytes play a central role in cholesterol and lipid homeostasis. Reduced cholesterol efflux has been associated with lipotoxicity, inflammation and steatosis which are well-known HCC risk factors. Furthermore, we observed that HBV-miR-3 also increased cell proliferation, colony formation and cell migration in hepatocytes. HBV-miR-3-induced cancer hallmark processes were reversed by the lowering of intracellular cholesterol levels with simvastatin, indicating the role for the HBV-miR-3-ABCA1 axis in hepatocarcinogenesis through dysregulation of lipid metabolism. In addition, we found an inverse correlation between HBV-miR-3 levels and ABCA1 transcript levels in liver biopsies (n=20) of patients with CHBV. In sum, our work highlights the etiological role of HBV-encoded miRNA in the dysregulation of cholesterol metabolism in liver that may contribute to HCC.

Human Enterovirus and Poliovirus Diversity Assessment Using Next-Generation Sequencing: A Wastewater-Based Epidemiology Study in Catalonia, Spain (2022-2024)

David Garcia Pedemonte[1], Albert Carcereny[2], Maria Isabel Costafreda[1], Carme Chacón[3], Margarita Palau[4], Belén Galofré[5], Miquel Paraira[5], Andrés Antón[6], Rosa Maria Pintó[1], Albert Bosch[1], Susana Guix[1]

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[2] *Enteric Virus Laboratory, Department of Genetics, Microbiology and Statistics, School of Biology, and Research Institute of Nutrition and Food Safety (INSA-UB), University of Barcelona, Barcelona, Spain*
[3] *Public Health Office, Health Departament, Generalitat de Catalunya, Spain*
[4] *General Directorate of Public Health, Ministry of Health, Madrid, Spain*
[5] *Aigües de Barcelona, Barcelona, Spain*
[6] *Microbiology Department, Vall D'Hebron Institut de Recerca (VHIR), Vall D'Hebron Hospital Campus, Barcelona, Spain*

Wastewater surveillance provides a broader perspective on the pathogens circulating in the community. Due to the reports of poliovirus detection in 2022 in London and New York wastewater, as well as the vaccine-derived poliomyelitis (VDPV) case in New York and many other reports on VDPV cases in other parts of the world, serious public health concern has prompted the development of direct detection methods through NGS sequencing approaches.
The aim of our study was to monitor enterovirus species and poliovirus in samples from two wastewater treatment plants in the urban area of Barcelona in various periods of 2022 and 2023. Two amplicon nanopore NGS direct detection methods were used, a generic approach involving a semi-nested RT-PCR of a VP1 region of 400 bp, allowing for the direct identification of all enterovirus serotypes, and a nested RT-PCR of the entire capsid region, followed by the amplification of full 1000 bp VP1, allowing a more specific enterovirus C, including poliovirus, to be detected. All amplicons were sequenced using nanopore (MinION) technology and a bioinformatic pipeline based on the Vsearch software was designed, enabling the sequences from an in-house enterovirus database to be compared. The surveillance shows a high prevalence of Echovirus 11 throughout the duration of the study, with Echovirus 9, Coxsackievirus B5 and Coxsackievirus B2 being also present with minor frequencies. Four enteroviruses associated to polio-like flaccid paralysis and other severe neural syndromes were detected, although in very low proportions: enterovirus A71, D68, C99 and C109, these latter two belonging to Cluster C enteroviruses. Nevertheless, no poliovirus sequences were detected in the present study.

S2. Antiviral Therapeutics, Vaccines, and Host Defense

Neutral Sphingomyelinase 2 is Required for HIV-1 Maturation

Eric O. Freed

HIV Dynamics and Replication Program, Center for Cancer Research, National Cancer Institute, Frederick, Maryland, USA

HIV-1 assembly occurs at the inner leaflet of the plasma membrane in highly ordered membrane microdomains. The size and stability of membrane microdomains is regulated by activity of the sphingomyelin hydrolase neutral sphingomyelinase 2 (nSMase2) that generates ceramide. nSMase2 is the major sphingomyelinase in mammalian cells that is localized primarily to the inner leaflet of the plasma membrane. Because of its key role in ceramide biosynthesis, we hypothesized that nSMase2 might be required for HIV-1 assembly, release, or maturation. To investigate the role of nSMase2 in HIV-1 replication, we either knocked down nSMase2 with shRNA or treated virus-producing cells with the small-molecule inhibitor PDDC and monitored virus assembly, release, maturation, and infectivity. We found that disruption of nSMase2 impairs HIV-1 Gag and GagPol processing, resulting in a profound impairment in particle maturation and infectivity. Electron microscopy analysis showed that disrupting nSMase2 in virus-producer cells alters the morphology of HIV-1 particles. We found that disruption of nSMase2 also severely inhibits the maturation and infectivity of other primate lentiviruses but has no effect on the gammaretrovirus murine leukemia virus (MLV). Using a sub-optimal dose of PDDC in HIV-1 infected human T cells we were able to select and identify mutations that confer partial resistance to PDDC. These studies demonstrate a previously undescribed role for nSMase2 in the morphogenesis and maturation of primate lentivirus and suggest that specific alterations in the lipid composition of assembling virus particles profoundly impact late stages of the lentiviral replication cycle

Putting a Kink in HIV-1 Particle Assembly: Rocaglamide Inhibits HIV-1 Replication by Altering the Gag–Genomic RNA Interaction

Paul Rosenfeld[1], Gatikrushna Singh[2], Juan Ji[3], Amanda Paz Herrera[3], Bradley Seufzer[2], Xiao Heng[3], Kathleen Boris-Lawrie[2], Alan Cochrane *[,4]

[1] Institute of Medical Sciences, University of Toronto, Toronto, CA
[2] Department of Veterinary and Biomedical Sciences, University of Minnesota Twin Cities, Saint Paul, MN, USA
[3] Department of Biochemistry, University of Missouri, Columbia, Missouri
[4] Dept. of Molecular Genetics, University of Toronto, Toronto

Our examination of RNA helicases for their effects on HIV-1 protein expression and particle assembly identified Rocaglamide (RocA), a known modulator of eIF4A1 function, as an inhibitor of HIV-1 replication in primary CD4+ T cells and three cell systems. HIV-1 attenuation by low nM RocA doses was associated with reduced viral particle formation without a marked decrease in Gag production. Rather, the co-localization of Gag and HIV-1 unspliced RNA assemblies and was impaired by RocA treatment in a reversible fashion. Ribonucleoprotein (RNP) immunoprecipitation studies recapitulated the loss of Gag-unspliced RNA assemblies upon RocA treatment. Parallel biophysical studies determined that neither RocA nor eIF4A1 independently affected the ability of Gag to interact with gRNA, but together, they distorted the structure of the HIV-1 RNP visualized by electron microscopy. Taken together, several lines of evidence indicate that RocA induces the stable binding of eIF4A1 onto the RNA virus genome in a manner that interferes with the ordered assembly of Gag along the viral genomic RNA required to generate infectious virions.

Nucleocapsid-Specific Monoclonal Antibodies with Cross-Reactivity to Diverse Betacoronaviruses as Diagnostic Tools for Future Zoonotic Spillovers

Sneha Sree Mullapudi[1], Yee-Joo Tan[1, 2], Jackie Chu Woei Bin[2]

[1] *Infectious Diseases Translational Research Programme and Department of Microbiology and Immunology, National University of Singapore, Singapore*
[2] *Institute of Molecular and Cell Biology, Agency for Science, Technology and Research (A*STAR), Singapore*

The nucleocapsid (N) protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the most abundant, conserved, and immunogenic protein in the virion and plays a crucial role in viral replication and genome packaging. In this study, we aim to generate new monoclonal antibodies (mAbs) binding to the N-protein of SARS-CoV-2 with broad cross-reactivity to other betacoronaviruses, because such mAbs could be potentially beneficial for the diagnosis of uncharacterized coronaviruses in future disease outbreaks. Thus far, we have generated three anti-N mAbs, namely 7A7, 1H1, and 1G1, by immunizing mice sequentially with MERS-CoV-N and SARS-CoV-2-N recombinant proteins to stimulate broad antibody responses. Their binding to the N-protein expressed in mammalian cells was analyzed using Western blot and immunofluorescence assay. All three mAbs bound to the native and denatured forms of MERS-CoV-N; however, their binding was limited to the native SARS-CoV-2-N protein probably due to the conformational disruption of the binding epitope in the denatured SARS-CoV-2-N. Among these mAbs, 7A7 showed the strongest binding for both MERS-CoV-N and SARS-CoV-2-N (half maximal effective concentration, EC50~10 ng/ml), followed by 1H1 and 1G1.
Our results showed that 7A7 and 1H1 cross-reacted with majority of the human, bat, and pangolin betacoronaviruses tested, but did not bind to alphacoronaviruses. Furthermore, the binding epitopes of these mAbs, determined using overlapping 15mer-peptides, are highly conserved in multiple betacoronaviruses. The mAbs will be further used to set up a sensitive sandwich ELISA for detecting virions.
Since three different betacoronaviruses have crossed the species barrier to infect humans in the past 20 years, a possible spillover of a new betacoronavirus in the future cannot be ruled out. In such an unfortunate event, our panel of new cross-reactive anti-N mAbs binding to multiple betacoronaviruses and their respective binding epitopes could be exploited to develop new strategies for theranostics and vaccines.

AI Predicts High-Confidence Interactions Between Zoonotic Respiratory Virus Proteins and Host Factors, Revealing Antiviral Drug Candidates

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Intra- and extra-virus protein–protein interactions are targets of choice for the development of drugs against viral infections. To facilitate candidate target discovery, we have taken advantage of the AI AlphaFold-Multimer system (Google/DeepMind; Evans et al BioRxiv 2022) to develop a novel algorithm that predicts direct physical protein–protein interactions of human and viral origins. This new software, named AlphaFold-pairs (Poitras et al BioRxiv 2023), was used to define protein–protein interactions for the reassortant Eurasian avian-like (EA) H1N1 swine G4 genotype virus (Sun et al PNAS 2020), a virus under strict surveillance for its elevated risk to cause a pandemic. Our results not only define binary interactions among G4 virus proteins, but also interactions between virus proteins and host factors. Early data reveal high-score interactions with a number of key host proteins, including human molecular chaperones and co-chaperones, which are an indication of a role of the selected viral factors in the modulation of protein folding and/or protein complex assembly. Because some of the identified host factors have previously been found to be targets of therapeutic effectors, sometimes used to treat specific diseases and some having already been approved by the US FDA, we predict that some of these compounds can be successfully repurposed for the discovery of antivirals. Our progress in this direction will be discussed.

Identification of Novel Small-Molecule Inhibitors for Rabies Virus

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Rabies is an overwhelmingly fatal disease with almost no treatment options. The prevention of rabies infection can be achieved through before-exposure or immediate post-exposure prophylactic treatment with antibodies. However, the treatment window is extremely narrow, and failure to prevent virus transmission from the site of infection to the brain could be fatal as antibodies are not effective in protecting across the blood–brain barrier. To identify novel anti-rabies small molecules, we used computational approaches (pharmacophore-based modeling) in combination with a pilot qHTS screen to identify novel hits for rabies. We screened 29,367 compounds using a target-agnostic assay measuring engineered live virus Nano-Luciferase signal in Vero cells. The screening results were then used as input for the in silico modeling approach. The developed models were applied for the virtual screening of a larger NCATS library of 42,533 compounds. Of the 384 compounds proposed for experimental validation, 76 were confirmed as positives, providing an impressive hit rate of 20%. One of these hits showed nanomolar activity in a confirmatory GFP reporter assay in Vero cells, surpassing the activity of previously known actives in this assay. Based on the potency and initial ADME data, we selected one chemotype for our chemistry campaign to develop a probe with improved activity and ADME profile. We primarily used rat, mouse, and hamster ADME data to guide our SAR campaign to perform efficacy studies in a RabV-infected hamster model. These efforts led to the identification of a probe, NCGC00871245, which showed an improved potency below 100 nM in RabV-infected BSR cells and good ADME. To elucidate the mechanism and target of this compound, we synthesized a potent photoaffinity-tagged diazirine analog. Utilizing this diazirine analog, we employed a protein pull-down protocol in conjunction with an integrated proteomics approach. We have identified potential candidates of target proteins that our compounds bind to impede Rabies virus infection within cells.

Epithelial–Mesenchymal Transition Transcriptional Traits After Persistent Hepatitis C Virus Infection Elimination by Direct-Acting Antivirals in Cell Culture

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Chronic hepatitis C virus infection (HCV) causes liver inflammation and fibrosis, leading to the development of severe liver disease, such as cirrhosis or hepatocellular carcinoma (HCC). Approval of direct-acting antiviral (DAA) drug combinations has revolutionized chronic HCV therapy, with virus eradication in >98% of treated patients, regardless of the genotype and liver disease status. However, HCC incidence in formerly HCV-infected patients remains above that of the general population. It has been proposed that the efficacy of these antiviral drugs is such that viral eradication may occur with a reduced contribution of infected cell clearance by the immune system, in contrast to previously used interferon-based therapies. It is thus formally possible that cured patients indeed carry formerly infected cells that display irreversible alterations due to prolonged chronic HCV infection.
To directly test this hypothesis in a defined experimental system, we used cell culture models of persistent HCV infection. Combining differential transcriptomes of infected and non-infected proliferating cell culture populations as well as quiescent, hepatocyte-like cultures, we observed a major reversion of infection-related transcripts after complete infection elimination. However, a small number of transcripts were abnormally expressed in formerly infected cells, both in the proliferating and quiescent cell models. Among these, we observed a permanently elevated expression of epithelial–mesenchymal transition (EMT) markers in quiescent hepatocyte-like cell cultures even after the elimination of infection, including transcriptional traits that were not observed in proliferating, formerly infected cell populations. Interestingly, some of these alterations were also observed in the liver biopsies of virologically cured patients. Overall, our data suggest a direct and permanent impact of persistent HCV infection on the host cell transcriptome even after virus elimination, possibly contributing to development of HCC.

Virus-Like Particles as a Next-Generation Vaccine Against Polio

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The Global Polio Eradication Initiative (GPEI) pledged to eradicate poliovirus (PV) from the planet. However, vaccine-derived viruses remain a major concern, as are individuals who persistently shed PV, sometimes for decades. There is an imperative to develop completely safe polio vaccines to ensure against reintroduction of the virus, following eradication.

We are developing a novel, safe vaccine based on recombinantly expressed virus-like-particles (VLPs). PV, like many other picornaviruses, produces empty capsids during its normal replication cycle. These contain no viral genome, but are antigenically indistinguishable from mature virions and can elicit similar protective immune responses. Empty capsids can be expressed in a variety of systems while retaining characteristics of naturally produced particles. However, they are less stable than virions and readily convert to an alternative conformation, which does not elicit protective immunity. By selection and combination of stabilising mutations, we have generated modified versions without altering their antigenic properties for all three PV serotypes. By co-expression of the modified structural protein precursor proteins together with the viral protease, we have successfully produced stabilised VLPs in yeast, insect cells, plants and mammalian cells and in a cell-free system. High-resolution cryoEM structure determination shows that the VLPs are essentially indistinguishable from naturally produced particles. Partial optimisation of expression in yeast (Pichia pastoris) using bioreactors has resulted in yields comparable with those obtained in current PV vaccine production, but using less expensive infrastructure and culture media.

Finally, these VLPs can be at least as immunogenic than current vaccines in a transgenic mouse protection model and in the rat seroconversion model.

The WHO consortium for the development of a virus-free VLP polio vaccine: University of Leeds, University of Oxford, National Institute of Biological Standards and Control, John Innes Centre, University of Florida, University of Reading, the Pirbright Institute.

Control of CNS-Resident Lyssavirus Infection by Peripherally Administered Human Monoclonal Antibodies F11 and A6

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Lyssaviruses are the causative agent of rabies, a disease characterized by a uniformly fatal encephalitis in humans. Although there is a highly effective therapy for the pre-symptomatic phase of infection, there is no validated therapy for post-symptomatic rabies. Using our recently developed bioluminescence imaging-based system to longitudinally trace lyssavirus infection dynamics and intensity, we demonstrate in mice the efficacy of two neutralizing human monoclonal antibodies (hmAbs), F11 and A6, against both Australian bat lyssavirus and rabies strain CVS-11. When administered through the circulatory system, F11 and A6 reverse disease signs and offer long-term protection against death following infection with lethal lyssavirus strains. Remarkably, this hmAb therapy is effective even after the emergence of disease signs and robust viral replication in the central nervous system (CNS). Importantly, hmAb-mediated virus neutralization is not sufficient to confer a protective effect. Rather, host CD4+ T cells and an intact hmAb Fc region are required for the therapeutic activity of the antibody. Flow cytometry and histology show that therapy results in significant changes in the spectrum of CNS-infiltrating immune cells, with a higher representation of CD4+ T cells and MHC-II-positive myeloid cells in the brains of hmAb-treated infected animals, as compared to controls. Although this hmAb-augmented immune response limits viral replication in the CNS, viral replication does persist, but at a stable and very low level, following antibody treatment. Collectively, our data show that hmAb therapy can facilitate a durable CD4+ T cell-dependent antiviral response against an otherwise fatal CNS-resident lyssavirus infection.

High Activity of Remdesivir and Other Broad-Spectrum Antivirals Against Multidrug-Resistant Hepatitis C Virus in Vitro

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The emergence of multidrug resistance (MDR) can challenge the efficacy of hepatitis C virus (HCV) antiviral therapy, threatening global virus elimination programs. In cell culture experiments, we selected an HCV genotype 3a (HCV-3a) virus variant with resistance to all three classes of direct-acting antivirals: protease and NS5A inhibitors and nucleotide analog (NA) sofosbuvir. The MDR-HCV variant did not respond to treatment with the highly potent glecaprevir/pibrentasvir/sofosbuvir regimen, which efficiently eliminated the original virus. The MDR-HCV variant exhibited multiple mutations across the genome, including known resistance-associated substitutions (RAS) A156L (NS3-protease), Y93H (NS5A), and S282T (NS5B-polymerase). In growth kinetics assays, the MDR-HCV variant propagated slightly slower than the original variant and exhibited decreased replication. The HCV-MDR variant exhibited cross-resistance to 2'C-modified NAs, similar susceptibility to the broad-spectrum NAs remdesivir and galidesivir, and enhanced susceptibility to the mutagenic NAs ribavirin, favipiravir, and molnupiravir, compared to the original variant. Surprisingly, the HCV-MDR variant exhibited hypersusceptibility to interferon alpha-2b, which significantly increased the efficacy of interferon alpha-2b/ribavirin combination treatment. Enhanced ribavirin activity was mediated primarily by sofosbuvir RAS S282T, which boosted the mutagenic effect of ribavirin. Compared to the original variant, infection with the MDR-HCV variant led to lower activation of myxovirus resistance gene 1, an interferon-induced protein, due to inefficient phosphorylation of protein kinase R. Remdesivir activity was enhanced by protease and NS5A inhibitors and by favipiravir but it was reduced by ribavirin. Remdesivir exhibited a remarkably high barrier against HCV-3a resistance, as long-term treatment with increasing drug concentrations did not lead to the emergence of variants with decreased drug susceptibility. In conclusion, we provide a comprehensive characterization of multi-DAA resistance in HCV-3a and show a proof-of-concept of successful treatment of HCV-MDR by existing broad-spectrum antiviral therapies.

Protection Afforded from Two mRNA Vaccine Candidates in a Lethal Mouse Heartland Virus Infection Challenge Model

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Heartland virus (HRTV) is an emerging tick-borne phlebovirus first identified in the United States in 2009 in two farmers presenting with thrombocytopenia and leukopenia. More than 60 HRTV cases have subsequently been reported, including many hospitalizations and several fatal cases associated with older individuals with medical comorbidities. No licensed vaccines or therapies are currently available. We developed two mRNA vaccine candidates based on glycoprotein genes (Gn and Gc) of HRTV formulated in lipid nanoparticles (LNPs), characterized RNA integrity and protein expression in vitro, and conducted a pre-clinical efficacy study in mice. A highly lethal HRTV challenge dose was initially determined in an established interferon receptor knock-out mouse model to develop a challenging model for HRTV vaccine efficacy evaluation. The efficacy study involved immunization of mice with two doses of mRNA vaccine 3 weeks apart with either vaccine candidate or LNPs (naïve control). The immunized and naïve control mice were subsequently challenged with the lethal dose of HRTV four weeks after final vaccination. Three dose levels of each vaccine candidate were evaluated with all candidates eliciting high anti-HRTV neutralizing antibody titers. Complete protection against lethal HRTV challenge and viremia were observed, even in the lowest dose groups. These initial results demonstrate that Gn- and Gc-based mRNA constructs may be promising candidate vaccines for protection against human disease and warrant further development and evaluation.

A High-Throughput Investigation of Genetic Design Constraints in Domesticated Influenza a Virus for Transient Gene Delivery

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Replication-incompetent single cycle infectious Influenza A Virus (sciIAV) has already demonstrated utility as a research and vaccination platform, as protein-based therapeutics are increasingly attractive due to their high selectivity and potent efficacy but still suffer from low bioavailability and high manufacturing costs. Transient RNA-mediated delivery is a safe alternative that allows for the expression of protein-based therapeutics within the target cells or tissues but is limited by delivery efficiency. Here, we develop recombinant sciIAV as a platform for transient gene delivery in vivo and in vitro for therapeutic, research, and manufacturing applications (in vivo antimicrobial production, cell culture contamination clearance, and production of antiviral proteins in vitro). While adapting the system to new proteins, we encountered expression differences dependent on genetic context. To investigate this phenomenon. We applied high-throughput screening to map these within the 3` untranslated and coding regions of the hemagglutinin-encoding segment 4. This screen revealed permissible mutations in the 3` UTR and a depletion of RNA-level motifs in the N-terminal coding region.

S3. Antiviral Innate Immunity

In the Spotlight: HCMV Immune Evasins Reveal an Antiviral Function of Bone Morphogenetic Protein 9

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Herpesviruses are renowned for their ability to stay in the organism lifelong in a latent state, intermitted with episodes of reactivation. This form of infection is possible due to a high degree of adaptation of host and virus. While primary infection with human cytomegalovirus (HCMV) usually elicits only mild symptoms in healthy individuals, it can cause life-threatening symptoms up to end-organ failure in immunocompromised patients. Moreover, HCMV can cross the placental barrier in a pregnant woman and establish infection in the foetus, which can lead to various dysfunctions, malformations or even the death of the unborn child and represents the worldwide leading viral cause of congenital birth defects. In my talk, I will give an overview about our recent and ongoing activities regarding immune modulation by this complex virus, which is a prerequisite to avoid elimination by the host's immune response. In particular, I will talk about our recent finding that the cytokine bone morphogenetic protein 9 (BMP9) has antiviral activity against HCMV, that BMP9 is secreted upon viral infection, and that the virus encodes a specific defense mechanism to circumvent BMP9 signaling and thus evade repression, providing ample evidence that this pathway is physiologically relevant during HCMV infection.

Discovery of an Interferon-Independent Antiviral Pathway and Its Antagonism by Poxviruses

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The facilitates chromatin transcription (FACT) complex is an ancient chromatin remodeling factor that regulates eukaryotic gene transcription. However, whether FACT regulates host immune responses to infection is unclear. Here, we identify a novel antiviral pathway mediated by FACT that is distinct from the interferon (IFN) response, which restricts poxvirus replication at the step of virion assembly. We show that early viral gene expression triggers this pathway by promoting nuclear accumulation of SUMOylated Spt16 subunits of FACT. These SUMOylated Spt16 subunits are required for FACT-mediated expression of ETS-1, a downstream transcription factor that we show to invoke a virus restriction program. However, poxvirus-encoded A51R proteins block ETS-1 expression by using a conserved hydrophobic motif to directly bind SUMOylated Spt16 subunits in the cytosol and tether them to microtubules, thereby inhibiting their nuclear import. Moreover, we show that A51R antagonism of FACT enhances poxvirus virulence in mice. Notably, we show that ETS-1 expression is neither induced by, or required for, IFN response signaling, indicating that FACT-ETS-1-dependent antiviral responses are distinct from the IFN pathway. Finally, we demonstrate that FACT also restricts several unrelated RNA viruses, suggesting a broader role for FACT in antiviral immunity. Our study reveals the “FACT-ETS-1 Antiviral Response (FEAR)” pathway to be critical for eukaryotic antiviral immunity and describes a unique mechanism of viral immune evasion.

What Makes a Common Cold Virus? Respiratory Viruses Differentially Interface with Host Interferon Responses in the Nasal Epithelium

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Respiratory viruses initially infect the nasal epithelium, where induction of antiviral responses may result in local control of viral replication and limited spread to the lower airway. Using primary nasal epithelial-derived air-liquid-interface (ALI) cultures, we compared a panel of respiratory viruses to identify features differentiating highly pathogenic from common cold-associated viruses. Four human coronaviruses (HCoVs)—two lethal (SARS-CoV-2, MERS-CoV) and two common cold-associated (HCoV-229E, -NL63)—and human rhinovirus-16 (HRV-16) replicate in nasal cultures. Common cold-associated viruses (HCoV-229E, HCoV-NL63, HRV-16) replicate more efficiently at 33°C (nasal temperature) than 37°C (lung temperature). Nasal epithelial cells efficiently clear common cold viruses, whereas productive infection with SARS-CoV-2 and MERS-CoV persists for >20 days. ulk RNA sequencing of infected cultures revealed divergent induction of interferon (IFN) signaling. While pathogenic HCoVs result in near complete shutdown (MERS-CoV) or significant delay (SARS-CoV-2) in IFN signaling, the common cold-associated viruses robustly induce IFN. Given this gradient of IFN induction, we conducted infections in the presence of an IFN signaling inhibitor, ruxolitinib. Strikingly, we found that ruxolitinib treatment rendered nasal cells unable to clear common cold-associated viruses, with minimal impact on SARS-CoV-2 and MERS-CoV replication. Furthermore, MERS-CoV recombinant viruses with mutations in IFN antagonist proteins promoted a phenotype mirroring common cold-associated viruses—with robust IFN signatures and subsequent clearance. Finally, we hypothesized that SARS-CoV-2 has evolved toward a common cold phenotype however, omicron infections showed increased IFN induction but failed to exhibit temperature-sensitivity or clearance due to relative IFN insensitivity compared to wild-type SARS-CoV-2. These findings illustrate unique features of common cold-associated viruses in the nasal epithelium—preferred replication at nasal temperature (33°C), robust IFN induction, and IFN-mediated clearance. Given the pivotal role of IFN signaling in controlling respiratory viral infections, this pathway should be leveraged as an antiviral strategy.

Differential Innate Antiviral Signaling in Human and Bat Cells

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Background
Emerging viruses pose substantial threats to health, biodiversity, and economic prosperity. Understanding the mechanisms of antiviral signaling in taxa that act as viral reservoirs, such as bats, can identify the mechanisms for viral suppression in non-human species. Bats are a diverse order containing over 1,400 species, several of which are implicated as reservoir hosts in virus spillovers. Experimental viral infections demonstrate that bats exhibit effective antiviral responses, including strong interferon responses, and dampening of inflammatory responses that is correlated with negative outcomes in humans.

Methods
We performed a computational analysis of mammalian interferon regulatory factor (IRF) family members, including multiple species within the two suborders of bats. We subsequently produced in-depth sequence alignments and performed functional analyses on residues found to be positively selected in multiple bat IRF species. Additional RNA-Seq analyses were performed in wild-type and IRF3 knockout human and bat cells under resting or poly(I:C)-treated conditions.

Results
Our studies revealed strongly conserved immune modulators, signaling pathways, and antiviral responses between humans and bats. However, we also identified positively selected residues in key IRFs and identified unique regulation of signaling pathways, including the regulation of IL-6.

Conclusion
Our study provides genetic and functional evidence for enhanced IRF-mediated antiviral responses in bats and adds further support to speculation that bats have positively selected for multiple adaptations in their antiviral immune responses. A full understanding of the strategies used by bats to augment innate antiviral immunity while dampening proinflammatory sequela will inform the strategic development of novel pan-antiviral therapeutic strategies, such as small molecules and mRNA delivery, to effectively skew the human immune response to a “bat-like” immune response.

A New Job for an Old Friend: Hsc70-4 Localizes at the Cell Surface to Mediate the Internalization of dsRNA in Drosophila Melanogaster

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The small interfering RNA pathway constitutes a pivotal antiviral defense against RNA viruses in insects, functioning through RNA interference mediated by the Ago2-guided cleavage of viral genomes. This systemic mechanism requires the recognition and transport of double-stranded RNA (dsRNA) of viral origin. Despite the known cellular uptake of dsRNA through endocytosis, the specific protein(s) responsible for this internalization remain elusive. Here, we investigate the role of Hsc70-4, a cytosolic protein known for its chaperone activity, as a potential cell surface receptor or co-receptor for dsRNA internalization in the insect model *Drosophila melanogaster*. Immunofluorescence assays were conducted on permeabilized and non-permeabilized S2 cells using a specific anti-Hsc70-4 antibody to determine its subcellular localization. Permeabilized cells exhibited cytoplasmic and plasma membrane staining, whereas non-permeabilized cells showed punctate staining on the outer surface of the plasma membrane, indicating the presence of Hsc70-4 on the cell surface. To assess the role of Hsc70-4 as a receptor/co-receptor for dsRNA uptake, we employed immunofluorescence and luciferase-based silencing assays. The pretreatment of S2 cells with anti-Hsc70-4 antibody significantly reduced the internalization of Cy3-labeled dsRNA, suggesting Hsc70-4's involvement in the uptake process. Luciferase assays revealed a direct correlation between antibody concentration during pretreatment and decreased silencing efficiency, further supporting Hsc70-4's role as a dsRNA receptor. Finally, we evaluated the ability of Hsc70-4's to bind dsRNA in vitro using electrophoretic mobility shift assays and found that Hsc70-4 binds dsRNA specifically in a sequence-independent manner. Altogether, our experiments provide evidence that Hsc70-4 is expressed on the cell surface of drosophila cells where it may act as a receptor for extracellular dsRNA. This finding constitutes a previously undescribed function for Hsc70-4, and sheds light on the molecular mechanisms underlying insect antiviral defense.

A Cytomegalovirus Inflammasome Inhibitor Reduces Proinflammatory Cytokine Release and Pyroptosis

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In response to viral infection, cells can initiate programmed cell death (PCD), leading to a reduction in the release of viral progeny. Viruses have therefore evolved specific mechanisms to curb PCD. Cytomegaloviruses (CMVs) are sophisticated manipulators of cellular defenses and encode potent inhibitors of apoptosis and necroptosis. However, a CMV inhibitor of pyroptosis has not been identified and characterized. Here we identify the mouse cytomegalovirus M84 protein as an inhibitor of pyroptosis and proinflammatory cytokine release. M84 interacts with the pyrin domain of AIM2 and ASC to inhibit inflammasome assembly. It thereby prevents Caspase-1-mediated activation of interleukin 1β (IL-1β), IL-18, and Gasdermin D. Growth attenuation of an M84-deficientMCMV in macrophages is rescued by knockout of either Aim2 or Asc or by treatment with a Caspase-1 inhibitor, and its attenuation in infected mice is partially rescued in Asc knockout mice. Thus, viral inhibition of the inflammasome-pyroptosis pathway is important to promote viral replication in vivo.

S4. Structure and Mechanisms of Virus Replication

The Enigmatic Yersinia Jumbo Bacteriophage YerA41

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Yersinia phage YerA41 is morphologically similar to jumbo bacteriophages. The isolated genomic material of YerA41 could not be digested by restriction enzymes and used as a template by conventional DNA polymerases. Nucleoside analysis of the YerA41 genomic material, carried out to find out whether this was due to modified nucleotides, revealed the presence of a ca 1 kDa substitution of thymidine with apparent oligosaccharide character. We identified and purified the phage DNA polymerase (DNAP) that could replicate the YerA41 genomic DNA even without added primers. Cryo-electron microscopy (EM) was used to characterize structural details of the phage particle. The storage capacity of the 131 nm diameter head was calculated to accommodate a significantly longer genome than that of the 145,577 bp genomic DNA of YerA41. Indeed, cryo-EM revealed, in contrast to the 25 Å in other phages, spacings of 33–36 Å between shells of the genomic material inside YerA41 heads suggesting that the heavily substituted thymidine increases significantly the spacing of the DNA packaged inside the capsid. In conclusion, YerA41 appears to be an unconventional phage that packages thymidine-modified genomic DNA into its capsids along with its own DNAP that has the ability to replicate the genome.

Structural Basis for Gag Assembly and Env Incorporation into HIV-1 Particles

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During the late phase of the HIV-1 infection cycle, Gag polyproteins are targeted to the plasma membrane (PM) for assembly and virus release. Gag–PM binding is mediated by interactions of the matrix (MA) domain with phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$). Concurrent to Gag assembly, the envelope (Env) protein is recruited to the PM for incorporation into virus particles. Several lines of evidence suggest that Env incorporation is mediated by the cytoplasmic tail of gp41 (gp41CT) and the MA domain of Gag. Both gp41CT and a well-formed MA lattice are essential for incorporation and infectivity. It appears that it is not sufficient to only embed gp41CT in the MA layer, but it is necessary for the MA layer to undergo a cleavage-induced maturation step for gp41 to become fully active. Recent studies proposed a model in which the MA domain undergoes a structural transformation to form distinct MA lattices during assembly and upon maturation. The structural details of the MA lattice (immature and mature) bound to the membrane, factors that govern the MA conformational switch, factors that (de)stabilize the MA lattice, and the structural basis for MA–gp41CT interaction during assembly and upon maturation are still lacking. We show that MA forms a lattice even in the absence of a membrane, that $PI(4,5)P_2$ is capable of binding to alternate sites on MA, consistent with novel and perhaps distinct MA–membrane binding mechanisms during assembly of the immature particle and upon maturation. We also devised “controlled assembly” approaches, coupled with single particle cryo-electron microscopy, to generate biologically authentic substructures of immature and mature gp41CT–MA complexes. Our findings may present an opportunity to develop new antiviral therapeutic agents that inhibit assembly, Env incorporation, and ultimately virus production.

SARS-CoV-2 Virus-Like Particles Reveal Chaperones of Viral Assembly

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Coronaviruses are the largest positive-strand RNA viruses known to infect humans. Their large genome size necessitates a similarly large viral particle to encapsulate the helical nucleocapsid–RNA complex. The construction of this large viral particle, complete with the iconic spike protein to enable entry into a naive cell, is not a trivial task. To characterize the molecular mechanisms that enable such a complex and reproducible feat, we examined the biochemical and cell biological features of viral assembly and developed an improved system to generate virus-like particles (VLPs) for SARS-CoV-2. Our platform consists of the four major SARS-CoV-2 structural proteins, where the membrane (M) protein orchestrates interactions between spike (S), envelope (E) and nucleocapsid (N), which, together with a cellular lipid membrane, form a secreted VLP. Interestingly, we observed that secreted VLPs are heterogeneous and many of these aberrant particles lack S, limiting their application to the study of viral assembly, immunogenicity or entry. Therefore, we screened the SARS-CoV2 viral accessory proteins for their ability to stabilize spike in viral particles and identified orf3A as a putative chaperone of spike. We found that orf3A binds directly to spike through its cytoplasmic domain to re-localize and stabilize spike in the cell, enabling particle assembly. Therefore, our findings demonstrate that orf3A is a prime antiviral target in the viral assembly process and showcase the importance of chaperones in viral assembly.

Alphavirus Cell-to-Cell Transmission

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Alphaviruses such as chikungunya virus (CHIKV) are important human pathogens that cause millions of human infections worldwide. Alphavirus infection or expression of the alphavirus structural proteins can induce the formation of intercellular long extensions (ILE) that project from infected cells and form stable non-continuous membrane bridges with neighboring cells. The mechanistic role of ILE in alphavirus infection was unclear. Using a co-culture system and flow cytometry methods we quantitatively evaluated transmission of CHIKV from infected to uninfected cells in the presence of neutralizing antibody. Endocytosis and endosomal acidification were critical for virus cell-to-cell transmission, while the CHIKV receptor MXRA8 was not. By using distinct antibodies to block formation of extensions and by evaluation of transmission in HeLa cells that did not form extensions, we showed that ILE mediate CHIKV cell-to-cell transmission. In vivo, pretreatment of mice with a neutralizing antibody blocked infection by direct virus inoculation, while adoptive transfer of infected cells produced antibody-resistant host infection. Studies with mapped monoclonal antibodies defined the domains of viral envelope proteins involved in ILE formation. Sera from a panel of human CHIKV patients but not control sera were found to block ILE formation. Our data suggest that ILE can shield CHIKV from neutralizing antibodies and promote efficient intercellular virus transmission, while the antibody response during human infection can counteract ILE to limit intercellular transmission.

Significance of Cellular Lipid Metabolism for the Replication of Rotaviruses and other RNA Viruses

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Species A rotaviruses (RVA) are a major cause of severe acute gastroenteritis in infants and young children worldwide and in the young of many mammalian and avian host species. RVA vaccination programs have significantly decreased RVA-associated disease in young children, although to different degrees in different parts of the world. Thus, more work on the molecular mechanisms of RVA replication is required.

During their replication RVAs form cytoplasmic inclusion bodies termed viroplasms in which early morphogenesis and viral RNA replication take place. Viroplasms form complexes with lipid droplets (LDs), cellular organelles serving as storage site for neutral lipids and cholesterol and being involved in intracellular transport chains. Compounds disturbing LD homeostasis, e.g. by blocking fatty acid (FA) biogenesis or by fragmentation of LDs, were found to decrease RVA replication (Cheung W et al, *J Virol* 2010; **84**: 6782-98; Crawford SE, Desselberger U, *Curr Opin Virol* 2016; **19**: 11-15). Lipid-disturbing compounds could be explored as RVA and possibly more broadly active candidate antivirals.

Disturbance of the cellular lipid metabolism also affects the replication of other RNA viruses, such as HCV (Vieyres G, Pietschmann T, *Cells* 2019; **8**: 233), Dengue virus (Samsa MM et al, *PLoS Pathog* 2019; **15**: e1000632), SARS-CoV-2 (Roingeard R et al, *Cell Mol Life Sci* 2022;**79**: 425); HIV-1 (Waheed AA et al, *Proc Natl Acad Sci USA* 2023; **120**: e2219475120); picornaviruses (Laufman O et al, *Cell* 2019; **178**: 275-89), and noroviruses (Doerflinger SY et al, *PLoS Pathog* 2017; **13**: e1006705).

For HIV-1 it was found that inhibitors of enzymes converting sphingomyelin to ceramide and phosphorylcholine block Gag processing and impair the infectivity of HIV-1 progeny in cell culture (Waheed AA et al, 2023) and also in a humanized BLT mouse model (Yoo SW et al, *Proc Natl Acad Sci USA* 2023; **120**: e2219543120). Thus, drugs interfering with ceramide formation may be suitable for the development of broadly active HIV-1 antivirals.

High-Speed Atomic Force Microscopy Reveals Real-Time Dynamics of Influenza a Virus Transcription

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The Influenza A virus genome is composed of eight different single-stranded viral RNA (vRNA) segments assembled into viral ribonucleoprotein complexes (vRNP) in which the RNA polymerase (RNApol) binds to the 5' and 3' ends of the vRNA, while nucleoproteins (NPs) bind the remaining vRNA. The vRNP represents the minimal transcriptional machinery of influenza viruses, although its mechanism is not fully understood. Recent studies have proposed the so-called Processive Helical Track model, which suggests that vRNP undergoes conformational changes that allow RNApol to progressively access the entire template vRNA for copying, without disassembling the overall vRNP structure. This mechanism involves a significant local restructuring of the vRNP, but the highly dynamic interactions between its components allow it to regain its initial structure after each transcription cycle, enabling it to perform several consecutive rounds of transcription. Monitoring these conformational changes is crucial for a better understanding of the mechanistic processes that take place during vRNA synthesis. Here, we aimed to address this challenge by following with High-Speed Atomic Force Microscopy (HS-AFM) the conformational dynamics of individual mini-recombinant vRNP (rRNP) during transcription. Our results support the Processive Helical Track model, showing reversible conformational changes in the rRNP and events of multiple consecutive rounds of such changes. In addition, we provide the first estimations of the average transcription rates of the RNApol within the rRNP complex and its modulation by NTP analogs that affect the structure of the nascent RNA.

Dynamics of Type I and III Interferon Responses Against Viral Infections at Single-Cell Resolution

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Type I and III interferon (IFN-I/III) responses act as pivotal first lines of innate immunity, promptly counteracting viral replication and alerting immune cells. Recent reports have suggested that the kinetics of IFN-I/III –response is a critical determinant of disease severity. For example, in the context of SARS-CoV-2, delayed IFN-I signalling is associated with elevated virus replication and promotes the severity of the disease, including a high level of inflammation and hypercytokinaemia, referred to as *'cytokine storm'*. Nonetheless, little is known about the dynamics of IFN-I/III responses at the single-cell level and their intersection with different host signalling pathways. Here, we designed a genetic tool to enable real-time tracking of those responses and modelled the dynamics of IFN-I/III responses in infected cells. This was achieved by novel tandemfluorescent reporters designed to temporally and spatially define cells that are presently responding versus cells that were previously activated. This was combined with tracking infected cells thanks to the insertion of a fluorescent reporter into the viral genome and itsanalysis by live imaging with single-cell resolution. Moreover, we developed and optimised a bioinformatics pipeline to track the intensity of the response at the single-cell level and used a machine learning method to generate a model of spatio-temporal response within the cell network. Our study, focussed on SARS-CoV-2, points out key features of IFN-I/III responses. In particular CRISPR-Cas9-mediated inhibition of specific signalling cascades revealed how paracrine signalling contributes to the spatial regulation of viral spread. We thus validated our novel system as an instrument to define the impact of host cellular regulators on the activation and shut-off of the response, and the potential change in behaviour of the cells concomitantly associated with the innate immune response.

Trans-Complementation of Functions of Non-Structural Protein 1 of Alphaviruses

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Alphaviruses are mosquito-transmitted enveloped positive-strand RNA viruses. Chikungunya virus (CHIKV), Ross River virus (RRV), Semliki Forest virus (SFV), and Eastern equine encephalitis virus (EEEV) are re-emerging pathogens causing outbreaks and large epidemics. For example, CHIKV has caused millions of cases of disease in over 50 countries. However, there are no approved vaccines or drugs to prevent and treat alphavirus infection. Developing antivirals requires understanding its viral replication. Non-structural protein 1 (nsP1) is the sole membrane anchor of the alphavirus replication complex, forming a dodecameric ring that interacts with other nsPs. Its guanine-7-methyltransferase (MTase) and guanylyltransferase (GTase) activities are essential for the capping of viral RNA genomes.
In this study, tetracycline-inducible stable cell lines expressing nsP1 or its mutants from different alphaviruses were generated. Using a *trans*-complementation assay, it was found that the activity of CHIKV replicase, lacking enzymatic activities associated with the nsP1 region, can be rescued by co-expression of wild-type nsP1 of matching or closely related alphaviruses (SFV, RRV). The compensation was less effective, or not observed at all, for replicase mutants where the defect was due to its compromised membrane attachments. Adding the defective nsP1 to the CHIKV replicase which harbored the same type of mutation further reduced the activity of replicase; however, the activities of mutant replicase were restored when it was supplemented with nsP1 harboring different types of functional defects. Expression of wild-type nsP1 of CHIKV or closely related viruses (SFV, RRV) allowed for the rescue, replication and propagation of CHIKV mutants lacking RNA-capping activities. Thus, stable tetracycline-inducible cell lines can be used as tools to *trans*-complement alphaviruses with lethal defects in nsP1, allowing us to obtain conditionally infectious virions which can be used at low-biosafety-level laboratories for high-throughput neutralization assays and antiviral testing.

MARCHF E3 Ubiquitin Ligases Block Furin Proprotein Convertase Activity by a Non-Degradative Mechanism via K33-Linked Polyubiquitination

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The membrane-associated RING-CH-type finger (MARCHF) proteins are a subfamily of the RING-type E3 ubiquitin ligase family, which was originally discovered in KSHV. MARCHF proteins broadly ubiquitinate immunoreceptors and target them to lysosomes for degradation, leading to immune evasion or suppression. Previously, we reported that MARCHF8 blocks the furin cleavage of class I fusion proteins to inhibit HIV-1, Ebola virus, and influenza virus. To understand this antiviral mechanism, we investigated MARCHF8's interaction with furin. We found that like MARCHF8, MARCHF2 and MARCH9 also inhibited the class I fusion protein cleavage by furin, and the inhibition was all dependent on the E3 ligase activity. Furin is a 794-aa type I transmembrane (TM) protein with a large lumenal region and a 56-aa cytoplasmic tail (CT). Although the furin cleavage activity was independent of its CT, this domain was targeted by MARCHF2/8/9. MARCHF2/8/9 bound the CT and triggered K33-linked polyubiquitination on the K748, K760, and K789 residues. Notably, this polyubiquitination did not cause furin degradation, but inhibited furin self-processing. In addition, furin intracellular trafficking was altered, resulting in the shedding of the outside cells. These results provide into new insights into the broad MARCHF antiviral activity by targeting furin.

S5. Virus-Host Interactions

Understanding Superinfection Exclusion by an RNA Virus

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Citrus tristeza virus (CTV), a member of the family Closteroviridae, is the most destructive viral pathogen of citrus, which continues to threaten citrus industries in many different regions worldwide. CTV possesses the largest among plant viruses nonsegmented positive-sense RNA genome (ca. 19.3 kb) and has numerous variants that are classified into at least seven different genotypes or strains based on the analysis of the nucleotide sequences of their genomes. One of the major focuses of our research is understanding the mechanism of CTV superinfection exclusion (SIE), a phenomenon in which a primary viral infection prevents a secondary infection with the same or closely related virus. SIE has been observed commonly for many important viruses of humans, animals, and plants and impacts the pathogenicity and evolution of viral populations. Previously, we discovered that SIE by CTV functions in the virus strain-dependent manner: it occurs only between variants of the same strain but not between variants of different strains. Furthermore, with CTV, SIE operates at two different levels - the whole organism and the cellular levels - and is mediated by multiple viral factors. One of these factors is the viral membrane-associated protein, p33. The other factor is encoded in the 5'-proximal region of the virus genome, which shows highest divergency between the genomes of different strains. This region drives the production of a 5'-co-terminal non-coding subgenomic RNA, LMT1, that is produced in copious amounts early in virus infection. Our latest work shows that LMT1 could be implicated in SIE. This talk will present our current knowledge on the involvement of the viral factors in SIE and outline possible implications in the development of novel virus-based therapeutics to reduce the effect of CTV-induced diseases.

A Segment of Human Papillomavirus L2 Capsid Protein Supports Virus Entry in a Sequence-Independent Manner

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The human papillomaviruses (HPV) are small non-enveloped DNA tumor viruses whose capsid consists of the L1 major capsid protein, which forms the outer shell of the capsid and binds to the cell surface, and the L2 minor capsid protein, which directs the viral DNA to the nucleus during virus entry. After HPV is internalized into the endosome lumen, a cell-penetrating peptide (CPP) near the C terminus of the L2 protein mediates the protrusion of much of L2 through the endosome membrane into the cytoplasm. Once protruded, L2 can bind cellular factors, such as retromer and COPI, required for the intracellular retrograde trafficking of HPV during entry. The L2 proteins of all examined papillomaviruses are predicted to contain an ~110-residue disordered segment upstream of the CPP and the adjacent retromer binding site. Large deletions in this segment inhibit the cytoplasmic protrusion of HPV16 L2, thereby inhibiting association with retromer, exit from the endosome, and infectivity. Surprisingly, the ability of these deletion mutants to infect cells can be restored by inserting protein segments with diverse compositions and chemical properties into this region, including randomly scrambled sequences, a tandem array of a short sequence, and segments of unrelated human, protozoan, and procaryotic proteins, including the intrinsically disordered region of a cellular protein. These results indicate that this segment of L2 supports HPV entry in a manner independent of its amino acid sequence. Further analysis indicates that the length of the disordered segment determines its virus entry activity. We propose that this segment of L2 has to be long enough to allow it to protrude through the endosome membrane to bind retromer. This work reveals a novel and unexpected aspect of protein function.

Interferon-λ Induces a Neuron-Specific Antiviral Response Against Alpha Herpesvirus Infection

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Alpha herpesviruses (α-HV) infect host mucosal epithelial cells prior to establishing a life-long latent infection in the peripheral nervous system. The initial spread of viral particles from the mucosa to the nervous system and the role of intrinsic immune responses at this barrier is not well understood. Using primary neurons cultured in compartmentalized chambers, prior studies performed on Pseudorabies virus (PRV) have demonstrated that type I and type II interferons (IFNs) induce a local antiviral response in axons via distinct mechanisms leading to a reduction in viral particle transport to the neuronal nucleus. A new class of interferons known as type III IFNs (IFN-λ) has been shown to play an immediate role against viral infection in mucosal epithelial cells. However, the antiviral effects of type III IFNs within neurons during α-HV infection are largely unknown. In this study, we focused on elucidating the antiviral activity of type III IFN against PRV neuronal infection, and we compared the STAT signaling and interferon-stimulated gene (ISGs) induction pattern in neurons to non-neuronal cells. We found that IFN pre-exposure of both primary neurons and fibroblasts significantly reduces PRV virus yield, albeit by differential STAT activation and ISG induction patterns. Notably, we observed that IFN-λ triggers the expression of a subset of ISGs, mainly through STAT1 activation, to induce an antiviral state in primary peripheral neurons. Overall, our studies suggest that type III IFNs may play a major role in coordinating the intrinsic and innate immune responses against alpha herpesvirus spread from the mucosal epithelial barrier to the nervous system.

Interferon-Inducible KLK13 Inhibits Sars-Cov-2 Cell Entry and Syncytia Formation via Proteolytic Cleavage of Spike Protein

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused the COVID-19 pandemic, which has had a devastating impact on global public health. Host cells initiate the innate immune response and express restriction factors upon viral infection. Here, we showed that kallikrein-related peptidase 13 (KLK13), a serine protease expressed in airway ciliated epithelial cells with high specificity, efficiently cleaved the spike protein of SARS-CoV-2 and SARS-CoV, resulting in the inhibition of SARS-CoV-2 cell entry and cell–cell fusion. Mechanistically, we found that KLK13 collocated and interacted with the SARS-CoV-2 spike protein on the cell membrane, enabling proteolytic cleavage. We also confirmed that the enzymatic activity of KLK13 is responsible for the specific cleavage. The entry of SARS-CoV-2 spike-pseudotyped lentiviruses into host cells with *KLK13* overexpression was reduced. Furthermore, we found that the mRNA level of *KLK13* was significantly upregulated upon stimulation by poly(I:C), suggesting that *KLK13* is an interferon-stimulated gene (ISG). Consistently, the *KLK13* expression level was increased in the airway epithelia of COVID-19 patients. Furthermore, KLK13 was found to be secreted into several biological fluids, including the nasal mucus of COVID-19 patients. Collectively, our study reveals that KLK13 is an ISG that antagonizes SARS-CoV-2 infection by cleaving its spike protein. The identification of KLK13 as a novel restriction factor being secreted into nasal mucus paves the way for the development of prophylactic antivirals.

Discrepancy Between Immune Response of Green Monkey Survivors and Those That Succumbed After Large-Particle Aerosol Exposure to Nipah Virus Malaysia Strain (NiV_M)

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The objectives of this study were to identify critical host response mechanisms and to determine a role for specific populations of immune cells in the development and progression of disease by comparing the immunopathogenesis of green monkeys that succumbed to NiV_M infection with those that survived (with or without seroconversion). The long-term goal is to identify potential host targets for the development of therapeutics to treat NiV infection.

This study included a cohort of six green monkeys that were exposed to around 500 PFU NiV_M via large-particle aerosol. Of the two survivors, one animal was exposed but uninfected, and one animal survived and seroconverted. Three animals succumbed to NiV disease. One animal succumbed to a non-NiV-related infection and was excluded from analyses. Abnormalities were identified in brain magnetic resonance imaging (MRI) and histopathology of the three animals that succumbed and the seroconverted survivor.

Cytokine levels and immune cell populations were measured over time in plasma and selected tissue. The surviving seroconverted animal had little systemic inflammation with early activation of epithelial cells and platelets. The adaptive immune response appears to be activated, as evidenced by the increase in interleukin 15 (IL15), which triggers natural killer (NK) cells, T cells, and B cells. This animal had a productive T-cell response through the increase in IL2, interferon gamma (IFNG), and IL4. Two separate peaks of IL4 occurred, one around Day 12 and another around Day 41, corresponding with peaks in immunoglobulin M (IgM) and IgG, respectively. The three animals that succumbed showed similar trends in IL15, IL2, and IFNG a few days later than the survivor. Delayed IL4 production, corresponding to delays in antibody production, was also observed in these animals.

Overall, the cytokine and immune cell population data may elucidate critical differences in animals surviving versus succumbing to Nipah virus disease.

Wobble Uridine Transfer RNA Modifications Shape Influenza A Virus Infection in a Codon-Dependent Manner

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Influenza A viruses (IAV) rely completely on the host's translation machinery, including transfer RNAs (tRNAs), to decode its viral RNA genome. tRNA pools typically reflect cellular codon preferences and hence are optimized to translate host mRNAs, which are biased towards guanosine (G)- or cytosine (C)-ending codons. In contrast, IAV is skewed towards adenosine (A)- or uridine (U)-ending codons, for which cognate host tRNAs are scarce. Still, the translation of the IAV genome is highly efficient, indicating that the virus adapted to remodel host tRNA pools to its codon usage.
tRNAs undergo a plethora of chemical modifications, known as the tRNA epitranscriptome, that are critical for translation accuracy in the anticodon stem. These modifications are dynamic and can quickly change in response to external cues to rewire gene expression under specific conditions. As viruses are sources of cellular stress, the reprogramming of host tRNA modifications is likely to occur as a strategy to enhance the decoding of codon-biased virus transcripts.
We set to explore if and how the host tRNA epitranscriptome is reshaped during a single IAV infection cycle. Our results revealed that the levels of several wobble uridine modifications were significantly reduced later in infection. Silencing the catalysts of these modifications markedly reduced viral translation in a codon-dependent manner. Furthermore, mechanisms related to the unfolded protein response (UPR) pivotal for IAV were disrupted. Following the buildup of insoluble proteins in the endoplasmic reticulum lumen, the UPR-PERK arm was activated and enhanced host antiviral responses.
Taken together, our data highlight the relevance of the host tRNA epitranscriptome for proper viral RNA translation and host surveillance escape. Our findings reveal, for the first time, additional mechanisms by which the host attempts to counteract IAV infection and uncover new potential host-based antiviral targets, paving the way for developing innovative therapies.

The Roles of Severe Acute Respiratory Syndrome Coronavirus 2 Accessory Protein orf3a in Nf-κB Activation and Tumor Necrosis Factor Receptor-Associated Factor Binding

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Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) caused a global pandemic in 2019 (COVID-19). Excessive inflammation is a hallmark of severe COVID-19, and several proteins encoded in the SARS-CoV-2 genome are capable of stimulating the inflammatory pathways. Among these, the accessory protein open reading frame 3a (ORF3a) has been implicated in COVID-19 pathology through multiple mechanisms, such as inducing cell death through apoptosis or inflammasome activation, leading to pyroptosis, disrupting the cellular membrane structures, and impairing autophagy. Here, we investigated the roles of ORF3a in binding to the TNF receptor-associated factor (TRAF) proteins and inducing nuclear factor kappa B (NF-κB) activation. X-ray crystallography and an fluorescence polarization assay revealed low-level affinity binding between an ORF3a N-terminal peptide and the TRAFs, and a dual-luciferase assay demonstrated NF-κB activation by ORF3a. Nonetheless, the mutation of the N-terminal TRAF-binding sequence Pro-ILe-GIn-Ala-Ser in ORF3a did not significantly diminish NF-κB activation in our assay. Thus, our results suggest that the SARS-CoV-2 protein may activate NF-κB through alternative mechanisms.

The Interplay Between HCMV and Cellular Pathways Regulating Cholesterol

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The human cytomegalovirus virus (HCMV) persistently infects 40-99% of the global population in a dormant state called latency. Reactivation from latency is associated with a poor prognosis for the immunocompromised. The *UL133-UL138* locus in the HCMV genome encodes repressors and activators of replication to establish and maintain latency or to reactivate from latency, respectively. Identifying host pathways targeting or regulated by these viral determinants is key to defining the mechanistic basis by which HCMV senses and responds to changes in host biology to maintain latency or to reactivate. *UL136* has emerged as a key HCMV modulator of the switch between latency and reactivation. *UL136* encodes 5 protein isoforms (p33, p26, p25, p23, and p19, based on molecular weight) that differ only in their N-terminal sequences. Intriguingly, the full-length, membrane-bound p33 isoform is unstable and targeted for rapid proteosomal turnover ($t_{1/2} \leq 1h$). Stabilization of p33 results in a replicative virus that cannot establish latency, suggesting that the short half-life of UL136p33 is necessary to establish latency. We have defined IDOL (inducible degrader of LDLR) as the host E3 ligase targeting UL136p33 for rapid turnover. IDOL is differentially expressed in response to changes in cholesterol homeostasis and hematopoietic cell differentiation. Induction of IDOL results in a failure to reactivate from latency whereas loss IDOL is associated with reactivation, indicating that rapid turnover of p33 is critical for fine-tuning its concentrations to levels required for maintenance of latency. Further, we find that HCMV targets LDLR for turnover independent of canonical mechanisms, including IDOL. Our findings suggest a model whereby the virus highjacks IDOL regulate the levels of p33 in response to host cues to modulate the decision to maintain the latent state or exit latency for reactivation and encodes yet undefined mechanisms for regulating some IDOL targets. Understanding how HCMV regulates and is regulated by host pathways important for cholesterol homeostasis will expand the current paradigms underlying the HCMV latency-reactivation switch.

A Mouse Parvovirus Disrupting Glioblastoma Stem Cell-Based Tumours Through Patient-Specific p53 Deregulations

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Glioblastoma multiforme (GBM) remains a major type of cancer with no effective therapy currently available. In attempting to develop a GBM therapy, two strains of the Minute Virus of Mice (MVMp and MVMi), a non-pathogenic mouse parvovirus with previously evidenced oncolytic capacity against GBM cell lines, were tested infecting primary glioblastoma stem cells (GSCs) isolated from GBM patients. GSC neurospheres accumulated assembled capsids, but restricted viral NS1 cytotoxic protein expression by an innate PKR/eIF2α-P response. Inter- and intra-patient GSC heterogeneity was dissected by their diverse innate responses and the ratio between structural and non-structural viral gene expression. Viral infection triggered a comprehensive DNA damage response involving cell cycle arrest, neurosphere disorganisation, and bystander disruption of GSC-derived brain tumour architecture in rodent models. Notably, MVM infection preferably targeted GSC subpopulations within patients showing weak innate responses and harbouring p53 gain-of-function mutants and/or p53-Ser15 phosphorylation. Further studies showed the possibility to enhance the anti-GBM cytotoxic effects of MVM by combination with chemotherapy. This study provides a molecular foundation for personalised biosafe viral therapies against devastating glioblastoma, using oncolytic virus-targeting stem cells with weakened innate responses and/or deregulated p53 signalling.

The Interactive Effects of *Wolbachia* and *Nop60B* on Sindbis Virus Replication

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Arboviruses pose a threat to more than half of the world's population. The intracellular bacterium, *Wolbachia,* is found in 40-60% of insect species, and mosquitoes can host it alongside other human pathogens. *Wolbachia* colonization protects invertebrate hosts against infection from single-stranded, positive-sense RNA viruses, a phenomenon known as pathogen blocking. Despite a lack of understanding of the mechanism, *Wolbachia*-infected mosquitoes are being released in the field to reduce the transmission of mosquito-borne viruses. We performed screening to identify the host factors involved in the *Wolbachia*-mediated pathogen blocking of Sindbis virus (SINV) in *Drosophila melanogaster*. We identified a host pseudouridine synthase gene, *Nop60B,* which has previously been shown to display significant differential isoform usage in flies colonized with *Wolbachia* compared to the *Wolbachia*-free counterparts. Thus, we hypothesized that the *Wolbachia*-induced changes in *Nop60B* may affect SINV replication. We induced the variation in *Nop60B* expression in transgenic RNAi flies with and without *Wolbachia* to measure SINV replication. There is a significant positive correlation between the SINV RNA and *Nop60B* expression in *Wolbachia*-free flies. *The Wolbachia*-colonized flies show a weak negative correlation between the SINV RNA and *Nop60B* expression. We identified *Nop60B* isoform differences in these samples, which may explain the opposite trends. Furthermore, we used a *D. melanogaster* cell line cleared of *Wolbachia* to overexpress the Nop60B protein. Overexpression leads to an increase in SINV infectivity and the intracellular RNA levels. Overall, these experiments suggest that the pseudouridine synthase, Nop60B, is proviral and *Wolbachia* colonization dismantles the proviral effect. Pseudouridine is highly abundant in the SINV RNA; we hypothesize that Nop60B may directly target the SINV RNA. Future experiments will focus on overexpressing Nop60B in *Wolbachia*-colonized cells and mapping pseudouridine residues in the SINV and cellular RNA. The results of these experiments will contribute to our understanding of *Wolbachia* virus–host interactions and antiviral mechanisms in general.

HIV-1 Alters Late Endosome/Lysosome Motility to Assemble at Virus-Containing Compartments in Macrophages

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Macrophages are one of the main targets of HIV-1 and are thought to represent an important viral reservoir in infected individuals. Differing from T cells, HIV-1 assembly in macrophages takes place in intracellular compartments termed virus-containing compartments (VCCs) which play a role in HIV-1 transmission and antibody evasion. Our previous research in HeLa cells (where HIV assembly is as in T cells) suggested that late endosomes/lysosomes (LELs) play a role in HIV-1 Gag trafficking towards assembly sites; however, their role during assembly at VCCs is not fully explored. Here, using an HIV-1-inducible pro-monocytic cell line (THP-1 GagZip), we studied the role of LELs during viral assembly in macrophages using high-resolution microscopy techniques in fixed and live-cell settings. Additionally, the HIV-1 association with endosomes was evaluated using flow cytometry, as well as the effect on HIV-1 assembly caused by LEL repositioning or by the inhibition of HIV-1 myristoylation. We determined that the THP-1 GagZip cell line resembles the VCC phenotype observed in human macrophages 72 hrs post HIV-1 induction. Furthermore, we provide evidence that supports the presence of LELs at VCCs, as well as the presence of HIV-1 Gag on endosomal membranes. Live-cell imaging revealed that HIV-1 repositions LELs towards the plasma membrane and modulates their motility when colocalizing with Gag. Finally, we found that Arl8b-mediated LEL repositioning is indispensable for VCC formation, while membrane binding impairment inhibited VCC formation by inhibiting myristoylation. Here, we provide a model to study VCCs in a robust way, as well as extensive evidence to support that HIV-1 hijacks and modulates LEL motility to promote assembly in infected macrophages. Future investigations will focus on uncovering HIV-1 Gag interacting partners that modulate LEL trafficking, aiming to inform on the design of new strategies in order to be one step closer to the cure of HIV-1.

Establishment of Chronic Chikungunya Infection in Mosquito Cells Requires Dynamic Changes of the Host and Virus Translation Landscapes

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Mosquito-borne viruses, such as chikungunya (CHIKV), cause acute pathogenic infections in human cells and chronic infections in mosquito cells with minimal consequences to mosquito fitness. How these viruses efficiently expand in both host cells, causing two different infection outcomes, is unknown. Combining polysome profiles and ribosome footprinting, we comprehensively analyzed temporal translational changes in C6/36 mosquito cells upon CHIKV infection. Our findings reveal that CHIKV infection triggers a transient repression of host mRNA translation, contrasting with infections in human cells. Furthermore, CHIKV infection activates translation of some host-specific mRNAs related to ribosome biogenesis and tRNA metabolism, augmenting the host's translation capacity, and that of odorant biding proteins. Viral RNAs maintain high translation rates throughout infection and experience mild repression at later time points when host mRNA translation is recovered. Collectively, our results elucidate the establishment of a virus–host equilibrium, wherein host translation is restored while CHIKV RNA translation remains at sufficient levels to sustain productive virus production. These findings also offer mechanistic insights into the heightened olfactory capacities observed in arbovirus-infected mosquitoes. Ongoing experiments in CHIKV-infected *Aedes aegypti* aim to validate and extend these findings in vivo.

Host Factor Senataxin Restricts DNA Virus Gene Expression at the Transcriptional Level

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Viruses are intracellular parasites that hijack many cellular proteins and functions to complete their replicative cycle, including cellular transcription machinery. In response, cells have evolved mechanisms to limit viral replication by different means. Here, we describe and characterize a new host restriction factor, Senataxin (SETX), which targets the viral episomal DNA transcription process to limit viral gene expression in different viral model systems. Silencing of SETX increased hepatitis B virus gene expression upon infection, as well as reporter gene expression in integration-deficient lentiviral and retroviral vectors and adeno-associated viral (AAV) vector transductions. Moreover, SETX silencing also increased transfected plasmid-derived reporter expression, while ectopic SETX overexpression reduced reporter accumulation, demonstrating the restrictive role of SETX on incoming DNA expression. Analysis of retroviral DNA integration in SETX-silenced cells ruled out any effect of SETX on the integration of episomal DNA. In situ DNA and RNA labeling experiments performed in SETX-silenced cells showed a reduction in cellular DNA replication and global RNA synthesis, confirming previously reported functions of SETX in DNA repair and cellular mRNA metabolism. In fact, gene ontology and pathway enrichment analyses of RNA-seq data from SETX-silenced cells revealed expression changes in DNA replication and nucleosome assembly pathways. Importantly, a ChIP-seq analysis demonstrated an increased binding of cellular RNA polymerase II (RNAPII) to the AAV genome in SETX-silenced cells, which in fact produced an increase in AAV genome-derived transcription, as determined by TT-seq analysis. Collectively, these results suggest that SETX restricts episome-derived gene expression by preventing RNAPII binding to episomal DNA. These results provide basic knowledge that could be useful for the development of new antivirals as well as improved gene therapy strategies in the future.

Interferon Lambda mRNA Synthesis Is Induced by Double-Stranded RNA Produced During Hepatitis Delta Virus Replication

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Hepatitis delta virus (HDV) is a significant human pathogen that causes acute and chronic liver disease. As the type member of Kolmioviridae, HDV replicates via an unusual double rolling-circle mechanism that uses host RNA pol II as an RNA-dependent RNA polymerase. The pathogen recognition receptor MDA5, which is activated by long double-stranded RNAs, has been shown to play a key role in sensing HDV replication in infected cells, leading to the production of interferons, including IFN-λ. However, it is not clear which RNA species generated during HDV replication are detected by MDA5. Perhaps MDA5 recognizes the HDV genome and/or antigenome RNAs, which form quasi-double-stranded structures in which about 70% of the 1680 bases are paired. The mechanisms of HDV RNA replication are poorly understood, and it is not clear whether dsRNA is produced in HDV-infected cells. To address this question, IFN-λ mRNA production was analyzed following transfection of hepatoma cells with various non-replicating HDV RNAs. Only cells transfected with double-stranded HDV RNAs exhibited significant increases in IFN-λ mRNA levels. Neither the HDV genome nor antigenome alone induced production of IFN-λ mRNA. This result suggested that dsRNA might be produced during HDV replication and could be responsible for IFN-λ mRNA production. We used immunofluorescence analysis of cells replicating HDV to determine whether dsRNA is produced. Our results indicated that dsRNA is detectable within nuclei early during HDV replication, and later moves to foci in the cytoplasm adjacent to the nucleus. Notably, the appearance of these cytoplasmic, nuclear-adjacent dsRNA foci is temporally associated with peak levels of IFN-λ mRNA. We conclude that dsRNA is produced during HDV replication and suggest that this dsRNA leads to the induction of IFN-λ synthesis.

The Class II Enveloped Viruses “Accompanying” Glycoprotein

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Class II viral membrane fusion proteins (MFPs) are special as they are not autonomous and require an accompanying protein (AP) to be functional as an AP/MFP heterodimer. The AP exerts a chaperone function for folding of the MFP into a metastable state that can be triggered to induce membrane fusion for entry. The AP/MFP heterodimers interact laterally at the membrane surface to make a regular glycoprotein lattice that drives virus budding by imposing high curvature to make a closed particle.

The trigger for membrane fusion is heterodimer dissociation upon binding of protons in the acidic environment of the endosome. Upon dissociation from the AP, the MFP undergoes a conformational change that encompasses insertion of the fusion loop into the endosomal membrane and oligomerization into the characteristic post-fusion trimer of hairpins that drives the fusion of the viral envelope with the endosomal membrane.

Our structural studies have revealed that the AP from viruses as different as alphaviruses and hantaviruses are evolutionary related, indicating that their fusion machinery is derived from an common ancestor in which the AP has evolved more rapidly than the MFP, which is more conserved in structure. In my presentation, I will discuss the implications of these relations for other viruses with class II fusion proteins, as well as for the eukaryotic counterparts driving gamete fusion for zygote formation across eukaryotes.

Promotion of SARS-CoV-2 Replication by NSP1 Mediated Through Activation of Calcineurin-NFAT Signaling

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Various agents have been extensively tested for the suppression of SARS-CoV-2 replication. Among them, the immunosuppressant cyclosporine A (CsA) serves as a pan-coronavirus inhibitor. However, the mechanism by which CsA suppresses coronavirus replication remains elusive. In this study, we report on the hijacking of CsA-inhibited NFAT signaling by SARS-CoV-2 NSP1 protein. We further demonstrate that this leads to the upregulation of the expression of a cellular RNA helicase named DDX5, which promotes SARS-CoV-2 replication. Through its association with calcineurin A (CnA), NSP1 impedes the binding of the regulatory factor RCAN3 to CnA to induce the activation of NFAT. An NSP1-deficient recombinant SARS-CoV-2 was constructed. The role of NFAT in SARS-CoV-2 replication and its activation by NSP1 were validated by using the NSP1-deficient virus. SARS-CoV-2 replication was effectively inhibited by calcineurin inhibitors including CsA and VIVIT. They also synergized with nirmatrelvir to exert antiviral activity against SARS-CoV-2. Our findings shed light on the molecular mechanisms by which CsA and calcineurin inhibitors inhibit SARS-CoV-2 replication. The work was supported by HKRGC (C7142-20GF and T11-709/21-N).

Bax Inhibitor 1 (BI-1) and bZIP60 Transcription Factor Regulate Virus Replication and Cell-To-Cell Movement Independently While Integrating IRE1-led ER Stress Signaling

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Potexviruses, such as Plantago asiatica mosaic virus (PlAMV), depend upon the endoplasmic reticulum (ER) to promote replication and cell-to-cell movement. The TGB3 protein is a key viral protein that embeds in the ER and influences ER stress signaling via the inositol-requiring enzymes IRE1a and IRE1b. Bax inhibitor 1 is an ER-localized protein with six transmembranes that control the IRE1alpha branch of the unfolded protein response (UPR) in humans. The role of BI-1 in the regulation of the Arabidopsis IRE1a and IRE1b is unknown. IRE1 signaling triggers the plant unfolded UPR machinery which serves to manage virus spread in host plants, but the mechanism of how it acts on the virus is poorly understood. UPR in plants involves an inositol-requiring enzyme (IRE1A) and basic leucine zipper60(BZIP60). IRE1 is an ER resident type-1 transmembrane protein with a sensor domain in its luminal side and kinase and endonuclease domain in its cytoplasmic sides. The endonuclease domain is responsible for the unconventional splicing of a short intron from the bZIP60 mRNA, producing a truncated transcription factor that activates gene expression to maintain cellular homeostasis during environmental assaults. This study showed that BI-1 and bZIP60 regulate virus replication and cell-to-cell movement independently. BI-1 appears to be involved in regulating the IRE1a and IRE1b sensors and ER stress responses affecting virus accumulation.

Sarbecovirus Membrane Proteins Inhibit Activating Transcription Factor 6 Activation

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Coronavirus (CoV) spillover events have caused three recent outbreaks, including the COVID-19 pandemic. In addition to these highly pathogenic CoVs, there are four seasonal 'common cold' CoVs that circulate among humans. It is not well understood how the differences between CoVs contribute to pathogenesis or why SARS-CoV-2 is a more highly transmissible and pathogenic virus compared to other CoVs. The non-structural and accessory proteins of CoVs have been investigated for the disruption of host cell signalling pathways, while the CoV structural proteins such as Membrane (M) have been overlooked. M is the most abundant viral glycoprotein in the virus envelope, and it coordinates the other CoV structural proteins at the ER-Golgi intermediate compartment (ERGIC) for assembly. The ERGIC is an organelle that facilitates the bidirectional trafficking of cargo between the ER and Golgi, and it is rarely a site of virus assembly. Activating Transcription Factor 6 (ATF6) is an ER stress-sensing protein part of the unfolded protein response that is activated by proteotoxic stress. Upon activation, ATF6 translocates to the Golgi and is cleaved by the resident proteases, liberating a transcription factor to upregulate genes to restore ER homeostasis. We have discovered that the SARS-CoV-2 Spike protein activates ATF6, while the SARS-CoV-2 M proteins inhibit ATF6 activation. Additionally, the SARS-CoV-2 and SARS M proteins are superior at inhibiting ATF6 activation compared to the common cold coronavirus M proteins. Preliminary functional domain mapping has determined that the cytoplasmic tail of the SARS-CoV-2 M protein is dispensable for ATF6 inhibition. Our current objectives aim to determine how M inhibits ATF6 and characterize the significance of ATF6 inhibition for viral replication. The findings from this research could identify new roles for the SARS-CoV-2 M protein interfering with the ATF6 pathway and reveal how this could be exploited to elicit antiviral effects during SARS-CoV-2 replication.

Host-Encoded CTCF Regulates HCMV Latency via Chromatin Looping

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Human cytomegalovirus (HCMV) is a prevalent pathogen that establishes life-long latent infection in hematopoietic cells. While this infection is usually asymptomatic, immune dysregulation leads to viral reactivation, which can cause significant morbidity and mortality. However, the mechanisms underpinning reactivation remain incompletely understood. The HCMV major immediate early promoter (MIEP)/enhancer is a key factor in this process, as its transactivation from a repressed to a active state helps drive viral gene transcription necessary for reactivation from latency. Numerous host transcription factors bind the MIE locus and recruit repressive chromatin modifiers, thus impeding virus reactivation. One such factor is CCCTC-binding protein (CTCF), a highly conserved host zinc finger protein that mediates chromatin conformation and nuclear architecture. However, the mechanisms by which CTCF contributes to HCMV latency were previously unexplored. Here, we confirm CTCF binds two convergent sites within the MIE locus during latency in primary $CD14^+$ monocytes, and following cellular differentiation, CTCF association is lost as the virus reactivates. While mutation of the MIE enhancer CTCF binding site does not impact viral lytic growth in fibroblasts, this mutant virus fails to maintain latency in myeloid cells. Furthermore, we show the two convergent CTCF binding sites allow looping to occur across the MIEP, supporting transcriptional repression during latency. Indeed, looping between the two sites diminishes during virus reactivation, concurrent with activation of MIE transcription. Taken together, our data reveal that three-dimensional chromatin looping aids in the regulation of HCMV latency and provides insight into promoter/enhancer regulation that may prove broadly applicable across biological systems.

S6. Viral Pathogenesis and Evolution

Accumulation Dynamics of Defective Genomes During Experimental Evolution of Betacoronaviruses

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Virus-encoded RdRps often generate aberrant RNA genomes, known as defective viral genomes (DVGs). When coinfected with a helper virus providing necessary proteins, DVGs can multiply and spread. While DVGs depend on the helper virus for propagation, they can interfere with the helper virus replication, impact immune responses, and affect viral persistence and evolution. Understanding the dynamics of DVGs alongside standard viral genomes during infection remains elusive. To address this, we conducted long-term evolution experiments with human coronavirus OC43 (HCoV-OC43) and murine hepatitis virus (MHV) in susceptible cell cultures at either a high or low multiplicity of infection (MOI). We then performed HTS at regular time intervals, reconstructed DVGs, and analyzed their accumulation and evolution. Our findings indicate that the DVG fraction of the viral populations evolved to exhibit greater diversity and abundance, with deletions and insertions being the most common types and the size of DVGs depending on MOI conditions. Notably, some deletions showed limited temporal existence, while others became more prevalent over time at high MOI. We identified hotspot regions for deletions, primarily within regions of structural and accessory proteins and associated with the existence of short sequence repeats. In conclusion, our study illustrates the widespread formation of DVGs during betacoronavirus evolution, influenced by MOI and virus-specific factors.

Immune Checkpoint Activation with Metabolism and Immune Disfunctions During Lethal Classical Swine Fever Virus Infection

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Classical swine fever (CSF) is one of the most important transboundary viral diseases affecting domestic and wild pigs. Infection with CSF virus (CSFV) results in a strong immunosuppression accompanied by aberrant pro-inflammatory responses, which is related to disease severity and viral replication. To gain a better understanding of CSF pathogenesis, transcriptomic analysis was performed using porcine bone-marrow-derived dendritic cells (poBMDCs) infected ex vivo with two different cDNA-derived CSFV, the low-virulence strain Pinar de Rio (vPdR-36U) carrying a poly-U insertion in the 3' untranslated region (UTR) or the lethal double-mutant vPdR-H30K-5U. These two viruses were previously characterized in vivo. The transcriptomic profile of vPdR-36U- or vPdR-H30K-5U-infected poBMDCs versus uninfected poBMDCs revealed 946 and 2643 differentially expressed genes (DEGs), respectively. In total, 191 DEGs were observed when the two virus-infected cells were compared. Importantly, poBMDCs infected with the lethal CSFV strain overexpressed immune checkpoint molecules, limiting the adaptive immune responses and involved in immune exhaustion and immunosuppression mechanisms, in particular PD-L1, CD276, and LAG3, while the CTLA-4 and CD154 genes were down-regulated. In addition, the transcription of tristetraprolin (TTP) that plays a role in binding mRNA 3'-UTR AU-rich regions in mRNA decay was turned off in the presence of the lethal virus. KEGG pathways analyses showed that innate immunity-related pathways were overexpressed, while adaptive immunity-related pathways were underexpressed in cells infected with the lethal CSFV. The DNA replication pathways and the cellular senescence pathways were overexpressed, and the metabolic pathways and the biosynthesis of amino acid pathways, among others, were underexpressed similarly by the lethal and the non-lethal viruses. Taken together, these findings shed new light on the mechanisms of viral disease immunopathogenesis and thus represent a basis for the development of tools for the detection of early immune system dysfunction markers due to viral infections.

Discordant Genetic and Antigenic Diversification of Norovirus GII.4 Sydney_2012 After a Decade of Circulation in Humans

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Noroviruses are a major cause of severe gastroenteritis in humans worldwide, and these viruses are characterized by their large genetic diversity. The most predominant genotype, GII.4, presents the continued emergence of phylogenetically discrete variants that escape the immune responses to previous infections. In most cases, these variants predominate for 2-3 years until they are replaced by a newly emerging variant. The last variant to emerge, Sydney_2012, has been circulating at a high incidence level globally for over a decade, raising the question of whether this variant is undergoing antigenic diversification. We retrieved >1400 capsid sequences from the GII.4 Sydney_2012 variant from public databases and performed sequence analyses to determine the genetic changes indicative of antigenic diversification. Phylogenetic analyses show two major Sydney_2012 lineages, with viruses scattered within the tree topology and no single cluster dominating during a given year or geographical location. Fourteen residues presented high variability, seven of which mapped to four major antigenic sites. Notably, >50% of the viruses presented mutations at two or more antigenic sites. The patterns of residue composition confirmed that two Sydney_2012 virus populations (phylogenetic clusters) co-circulated globally, and that residues 297 and 372 from antigenic site A are genetically linked. Despite mutations in the antigenic sites, minimal changes in reactivity were detected in the Sydney_2012-specific polyclonal sera, and only four (out of thirty-two) Sydney_2012-specific mAbs lost binding, which were all mapped to antigenic site A. Notably, modern Sydney_2012 viruses with mutations at residues 297, 372, and 373 reconstituted binding with the Farmington_Hills_2002-specific polyclonal sera and mAbs, suggesting constraints in the evolvability of antigenic site A. Together, this study demonstrates that despite the extensive genetic diversification on antigenic sites, Sydney_2012 viruses present minimal antigenic diversification, raising questions about the determinants for the predominance of GII.4 variants in the human population.

Evolving Coronaviruses: Spike Recombinants and Mutants Influence Intra/Interspecies Transmission and Virulence

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The rapid global spread of the betacoronavirus (CoV), SARS-CoV-2 ignited an overwhelming and ongoing pandemic. It was preceded by the deadly SARS (2003) and MERS (2012) betaCoV zoonoses. Most mammalian CoVs, including the endemic human CoVs, likely originated from ancestral CoVs in bat reservoirs, with some infecting intermediate animal hosts prior to spillover into humans or other animals (see https://doi.org/10.1073/pnas.2202871119). Notably betaCoVs from wild ungulates (cervids) experimentally infect cattle; historically cattle likely transmitted them to other species (humans, pigs, dogs, poultry). Like other RNA viruses, CoVs frequently mutate and recombine to generate new CoV species or variants that escape existing immunity and acquire new tissue or host tropisms that increase their transmissibility and broaden their host range and interspecies/zoonotic transmission or alter their virulence. Especially notable are recombination and mutations in the Spike gene. To highlight these concepts, swine CoVs and the alphaCoV-1 host reservoir community in pigs, cats and dogs provide precedents for understanding CoV emergence and evolution in a host or in multispecies hosts, with lessons applicable to SARS-CoV-2. At least 34 species, many of them wildlife, are susceptible to SARS-CoV-2 natural or experimental infection. Ominously, if SARS-CoV-2 becomes established in an animal reservoir(s), the virus can persist, mutate and continue to evolve in the new host species, extending its host range and its potential to reinfect humans or other species. In addition, novel CoVs will continue to emerge in animals and humans that may pose future pandemic threats (WHO “Disease X”). My talk will address comparative aspects of CoV evolution, infections and interspecies transmission and underscore the disease threats that CoVs pose to animals and humans.

The Hepatocyte Traffic Network in the Hepatitis A Virus Infectious Cycle: An Evolutionary-Driven Use?

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Eleven years ago, it was described the occurrence of a double "phenotype" of the hepatitis A virus: the quasi-enveloped virions (eHAV) which circulate in the blood and are responsible for the cell-to cell transmission, and the naked virions (nHAV) which are shed in feces and are responsible of the inter-host transmission. This discovery has led to a new paradigm of the cell cycle of HAV, from entry to egress.

While it is well established that eHAV particles correspond to exosome-containing immature capsids, their biogenesis is still under debate. We will discuss the use of the syndecan-ALIX–mediated exosome biogenesis, and the role of the RAB GTPases on the vectorial egress of HAV from polarized hepatocytes.

Finally, the different pattern of interaction with ALIX of a "wild-type-like" strain and a strain with an amino acid replacement at position 134 of VP2, closely located to one of the late domains present in the HAV capsid, will be discussed from an evolutionary perspective.

Persistent SARS-CoV-2 Infection in Primary Human Small Intestinal Tissues as an in Vitro Model for Characterizing Long COVID

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SARS-CoV-2 remains a major public health burden due in part to its continued spread, the emergence of new variants, and the encumbering symptoms of post-acute sequalae of SARS-CoV-2 (PASC or long COVID). While long COVID remains poorly understood, self-identified gastrointestinal symptoms in patients, in addition to the demonstrated expression of viral protein in the intestines or viral RNA in stool, suggest that the gastrointestinal tract plays an important role in SARS-CoV-2 persistence during long COVID. We hypothesize that the human gut may serve as a reservoir to support long-term infection with SARS-CoV-2. As no small animal models have demonstrated long COVID in the gut, we utilized primary human tissue models to study the biology of prolonged Sars-CoV-2 infection and evaluate potential therapeutics. To achieve this goal, we infected primary human transwell-matured small intestine tissues (EpiIntestine-FT, MatTek) with several SARS-CoV-2 variants, including Wash-1, Beta, Delta, and Omicron subvariants, BA.2, and EG5.1 (Eris). We monitored infection for up to 32 days and measured temporal and tissue-specific variant infectivity/replication, transcriptomic changes, and secreted cytokine responses. Transcriptomic profiling demonstrated sustained up-regulation of interferon-stimulated genes throughout the infection. Furthermore, the cytokine profiles of the tissues displayed continued secretion of inflammatory cytokine CXCL10. Despite the sustained innate immune response, high levels of viral RNA and infectious virions were detected as far as 32 days post-infection. We also compared Sars-CoV-2 infections in the gut with infections in primary human air–liquid interface matured large airway (EpiAirway, MatTek) and alveolar (EpiAlveolar, MatTek) tissues to demonstrate that the responses were gut-specific.

Investigating the Role of Accessory Protein ORF8 Secretion in the Pathogenesis of SARS-CoV-2 and Its Variants

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As COVID-19 continues to claim lives worldwide, the ongoing emergence of SARS-CoV-2 variants compromises efforts to limit the pandemic's spread. These variants, among which Omicron remains dominant, are often defined by mutations that enhance viral pathogenicity. Along with the viral spike, Open Reading Frame (ORF) 8 stands out as one of the fastest evolving viral sites. As the only secreted SARS-CoV-2 protein identified in COVID-19 patient sera, it elicits a strong humoral response and has been correlated with disease severity. Notably, ORF8 mediates viral immune evasion; bearing a unique Ig-like fold, ORF8 has been suggested to induce a cytokine storm by mimicking proinflammatory cytokines like IL-17. It was also proposed to antagonize cell-mediated immunity by suppressing Major Histocompatibility Complex (MHC) I and Fcg receptor 3A (FcgR3A; CD16a), altogether promoting SARS-CoV-2 immune evasion and pathogenesis. It is unknown whether these ORF8 abilities are enriched in SARS-CoV-2 variants and how ORF8 is secreted to exert its effects. We characterized polymorphisms in the ORF8-coding region of past and present SARS-CoV-2 variants, determining their differential impact on ORF8 release into cell culture supernatants. Additionally, our comparison of ORF8 proteins among closely related CoVs found that SARS-CoV ORF8 was secreted at considerably higher levels than SARS-CoV-2 and similar CoVs. Through extensive mutagenesis, we identified the involvement of arginine residues in the initial β-sheet of SARS-CoV ORF8, which are absent in SARS-CoV-2 ORF8. This could be a contributing factor to the higher fatality rate of the 2003 SARS epidemic compared to COVID-19. We believe that studying rapidly evolving regions like ORF8 will provide insights into crucial SARS-CoV-2 pathogenic and evolutionary strategies, as well as inspire the development of targeted antivirals aimed at mitigating immune impairment to improve the outcome of severe COVID-19 cases.

Structure and Dynamics of Enterovirus Genotype Networks

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Non-polio enteroviruses (NPEVs), such as Enterovirus (EV)-D68, EV-A71, and Coxsackievirus B3 (CV-B3), are members of the *Picornaviridae* family, which cause around 10 to 15 million infections and tens of thousands of hospitalizations annually in the United States, especially in young children. Despite causing mostly mild or asymptomatic disease, these viruses are associated with diverse clinical manifestations, including respiratory disease, acute flaccid myelitis, and myocarditis. Rapid viral evolution is required for efficient within-host spread and pathogenesis of the related poliovirus, type 1. To address the within-host evolution of NPEVs, we developed a high-resolution, virus-inclusive sequencing pipeline to quantify the transcriptional responses and viral diversity in individual cells using the 10X single-cell sequencing platform combined with PacBio long-read sequencing. Using our method, we captured infected Rhabdomyosarcoma (RD) cells at 6 hours post-infection, and reconstructed hundreds of viral haplotypes from individual CV-B3-, EV-A71-, and EV-D68-infected cells with 10 to 1000x coverage across the viral genome. We validated the approach by describing the population diversity of two viral populations derived from long-term passage (EV-A71 and EV-D68) and one recently derived from a plasmid template (CV-B3), revealing complex genotypic networks that underly ongoing adaptation in these populations. To further explore these dynamics, we performed five serial passages of EV-A71 in vitro. The results reveal how EV-A71 populations traverse the mutational network to alter the engagement of its receptor, SCARB2. Within these five passages, two dominant genotypes emerged, both with multiple mutations in the capsid proteins. We mapped the identified genotype back onto the individual infected cell transcriptional data to correlate the observed mutations with the transcriptional outcomes of infection. In the future, we will use this technique to link the heterogeneity of cell and virus populations during infection in both in vitro and in vivo models, providing insight into how these pathogens evolve, spread, and cause disease.

Evolutionary Pathways to Resistance to Flavivirus STAT2 Antagonism

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All Flaviviruses need to suppress the host interferon response for efficient infection and pathogenesis. Many of these viruses, including the Zika (ZIKV), dengue (DENV), and yellow fever viruses (YFV), do so by using their nonstructural 5 (NS5) proteins to antagonize the Signal Transducer and Activator of the Transcription 2 (STAT2) protein. Analyses conducted by us and others indicated that the STAT2 evolved under positive selection within multiple mammalian lineages. Furthermore, STAT2 antagonism by these Flaviviruses is species-specific and impacts virus tropism, as mice are only infectible with ZIKV if interferon signaling is artificially impaired or with a mouse bearing human STAT2 in place of the mouse gene. To more broadly examine how STAT2 evolution has impacted antagonism susceptibility, we evaluate the capacity of various Flaviviruses' NS5 proteins to antagonize the STAT2 from 38 mammalian species. We found that the resistance to such antagonism evolved numerous times during STAT2 evolution and that the susceptibility varies between the different Flavivirus NS5 proteins despite their sequence and structural conservation. By mapping the resistance determinants of rodent STAT2 proteins, we found that resistance was acquired at least twice in rodent evolution, as evident by those determinants differing between the rodent lineages. Most strikingly, we identified that the resistance to DENV NS5 was acquired early in the evolution of lemurs, but was then later lost by some species. This suggests that there was a cost to developing resistance to STAT2 antagonism, which can promote the selection of susceptibility under the right circumstances. These results suggest the possibility that flavivirus-like antagonism has impacted STAT2 evolution. The observed resistance to STAT2 antagonism in some mammalian lineages likely limits the host tropism of Flavivirus infection and pathogenesis, and illustrates the hurdles and possible routes of adaptation for the creation of small animal models.

Neurovirulence of the Australian Outbreak Japanese Encephalitis Virus Genotype 4 Is Lower Compared to Genotypes 2 and 3 in Mice and Human Cortical Brain Organoids

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Human infections with Japanese encephalitis virus (JEV) are a leading cause of viral encephalitis, with ≈70,000 symptomatic cases (≈40% with long-term neurological sequelae) and ≈20,000 deaths reported annually. An outbreak of JEV genotype 4 was recently reported in Australia, with an isolate ($JEV_{NSW/22}$) obtained from a stillborn piglet brain. Herein, we characterize the neuropathology of $JEV_{NSW/22}$, JEV_{FU} (genotype 2) and $JEV_{Nakayama}$ (genotype 3) in adult C57BL/6J wild-type mice, mice deficient in interferon regulatory factor 7 (*Irf7*$^{-/-}$), and mice deficient in type I interferon receptor (*Ifnar*$^{-/-}$). In C57BL/6J and *Irf7*$^{-/-}$ mice with lethal outcomes, viral antigen was detected, inter alia, in the cortex, thalamus, and hippocampus, and histopathological lesions recapitulated those seen in humans and primates. JEV was universally lethal in *Ifnar*$^{-/-}$ mice by day 3 with histological signs of brain hemorrhage, but produced no other detectable brain infection or lesions, with viral protein detected in blood vessels but not neurons. We thus describe a new *Irf7*$^{-/-}$ mouse model for $JEV_{NSW/22}$, which had increased viremia compared to C57BL/6J mice, allowing for lethal neuroinvasive infection in one mouse. Overall, $JEV_{NSW/22}$ was less neurovirulent than other JEV isolates in C57BL/6J and *Irf7*$^{-/-}$ mice and was more sensitive to type I interferon. All JEV isolates showed robust cytopathic infection of human cortical brain organoids, albeit lower for $JEV_{NSW/22}$. Our study establishes $JEV_{NSW/22}$ mouse models of infection, allowing for lethal neuroinvasive infection that was rarer than for other JEV genotypes. These models can be deployed in pre-clinical studies to further understand JEV disease pathogenesis.

Unraveling the Measles Puzzle: Genotypes Shifting Landscapes, Selection, and Recombinants

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The measles virus, belonging to the Paramyxoviridae family, is highly contagious (estimated R_0 of 18) and primarily affects children. Despite mandatory vaccination in France for children born after January 1st, 2018, two major epidemic resurgences occurred between 2008 and 2012 and in the period ranging from 2017 to 2019. This research aims to better understand the evolutionary dynamics and circulation of the virus.
Retrospective random sampling was conducted among the 12,604 samples received at the French Measles National Reference Center between January 1st, 2010, and December 31st, 2022, resulting in the selection of 1,253 samples. These samples were deep-sequenced using Illumina® MiSeq technology with an in-house protocol, and the generated genomes were analyzed using Bayesian statistical methods, as well as in-house R and BASH scripts for diversity and evolutionary analyses. Temporal phylogenetic analysis was performed using the BEAST software. Temporal variability was estimated by calculating root-to-tip, and selection phenomena were assessed by determining the dN/dS ratios and site substitution rates. The results revealed a high genotypic diversity over the 13-year period, identifying eight different genotypes. D4 was the predominant genotype from 2010 to 2012, followed by a shift in favor of D8 in 2013. The phylogenetic analyses demonstrate a gradual lineage evolution, with D8 showing significant diversification and the apparent extinction of D4 in 2015. Two major D8 subclades co-circulated between 2013 and 2022. Genes involved in innate immune response or cellular receptor binding were under purifying or diversifying selection. Additionally, potential recombinants were detected.
Selective pressure directly fuels the two recent major epidemic outbreaks in France, providing insights into the implication of genotype evolutionary differences and natural selection on the epidemiological success of different lineages. This research illuminates the evolutionary dynamics of measles and the factors contributing to its resurgence. It underscores the importance of both long-term and continuous surveillance to unravel measles' evolutionary puzzle and pave the way for better outbreak control and preventio

Abstracts - Poster Exhibition

Poster Session A—Thursday 14 February

A1. New Insights into the Antiviral Activity and Mode of Action of Pateamines

Francesca Magari[1], Henri Messner[1], Florian Salisch[2], Stella Maria Schmelzle[3], Ger van Zandbergen[3], Alois Fürstner[4], John Ziebuhr[2], Andreas Heine[1], Christin Müller[2], Arnold Grünweller[1]

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The SARS-CoV-2 pandemic brought us to a closer realization of how critical it is to develop pan-antivirals. Especially when viruses mutate fastly, direct-acting antivirals are often not the preferred choice. On the contrary, through the inhibition of host factors which are essential for the viral life cycle it is rather unlikely that viruses can escape, thus preventing drug resistance. In this context, the human RNA helicase eIF4A, which is part of the translation initiation complex eIF4F, unwinds RNA secondary structures in 5`-UTRs of selected mRNAs. Since the protein synthesis of a large number of RNA viruses requires eIF4A, the latter is a promising target for the design and development of broad-spectrum antivirals.

It is already known that potent eIF4A inhibitors like the rocaglate silvestrol are able to inhibit RNA virus replication at low nanomolar concentrations via a mechanism called RNA-clamping. Interestingly, pateamines, which are chemically different from rocaglates, can functionally mimic this clamping mechanism. Here, we analyzed pateamines for their mode of action in 23 eIF4A variants and compared their potential drug sensitivity and resistance with those of rocaglates. Our findings reveal mechanistical differences in the binding behavior of pateamines compared to rocaglates when interacting with an eIF4A-RNA complex, especially in respect to Phe163. Furthermore, we also observed that unlike rocaglates, pateamines do not depend on the presence of polypurine tracts in RNA substrates for efficient RNA clamping. Importantly, pateamines demonstrate remarkable anti-coronaviral activity at non-toxic concentrations, achieving potent effects in the low nanomolar range.

Taken together, our results show that pateamines broaden our toolbox for combating viruses that rely on the host enzyme eIF4A for conducting their viral protein synthesis. In addition, we demonstrated that pateamines can serve as effective eIF4A inhibitors in rocaglate-resistant pathogens.

A2. SARS-CoV-2 Endoribonuclease Antagonizes Antiviral Signaling

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Coronaviruses (CoVs) produce dsRNA during genome replication and mRNA synthesis. Upon sensing dsRNA, antiviral pathways such as interferon (IFN) signaling, OAS-RNase L, and PKR are activated. CoVs encode numerous proteins that antagonize the activation of these antiviral host responses. Specifically, the conserved nsp15 contains an endoribonuclease (EndoU) domain that cleaves viral RNA to limit dsRNA accumulation. The inactivation of nsp15 enzymatic activity of murine hepatitis virus or Middle East respiratory syndrome coronavirus results in increased IFN signaling during infection and attenuation of viral replication. We wanted to determine if nsp15 contributed similarly to the innate immune evasion of severe acute respiratory syndrome (SARS)-CoV-2. Studies that have attempted to characterize SARS-CoV-2 nsp15 have solely utilized overexpression; such systems lack the complexity of a replicating CoV and are therefore limited in their analysis. Thus, we constructed a recombinant (r)SARS-CoV-2 with an enzymatically inactivated EndoU (nsp15^{mut}). Following rSARS-CoV-2 nsp15^{mut} infection of the primary nasal air-liquid interface (ALI) cultures, we observed increased IFN induction and expression of IFN-stimulated genes (ISGs), along with a significant reduction in viral titers. To confirm that nsp15 antagonizes IFN induction, infected nasal ALI cultures were treated with ruxolitinib, a Janus kinase inhibitor that prevents STAT signaling. In the presence of ruxolitinib, rSARS-CoV-2 nsp15^{mut} showed decreased ISG expression with viral titers equivalent to the wild-type virus. To characterize this phenotype further, we generated a rSARS-CoV-2 double mutant expressing the catalytically inactive nsp15^{mut} and an ablation of sarbeco-specific accessory protein ORF6 (ORF6^{stop}). The ORF6-encoded protein has been shown to block STAT1 translocation and nuclear export by interacting with nucleoporin 98 and the mRNA export factor RAE1, respectively. Future studies will focus on characterizing the impact of rSARS-CoV-2 nsp15^{mut}/ORF6^{stop} on viral replication, dsRNA production, IFN/ISG signaling, and transcription factor localization.

A3. Dynamics of Type I and III Interferon Responses Against Viral Infections at Single-Cell Resolution

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Type I and III interferon (IFN-I/III) responses act as pivotal first lines of innate immunity, promptly counteracting viral replication and alerting immune cells. Recent reports have suggested that the kinetics of IFN-I/III –response is a critical determinant of disease severity. For example, in the context of SARS-CoV-2, delayed IFN-I signalling is associated with elevated virus replication and promotes the severity of the disease, including a high level of inflammation and hypercytokinaemia, referred to as '*cytokine storm*'. Nonetheless, little is known about the dynamics of IFN-I/III responses at the single-cell level and their intersection with different host signalling pathways. Here, we designed a genetic tool to enable real-time tracking of those responses and modelled the dynamics of IFN-I/III responses in infected cells. This was achieved by novel tandemfluorescent reporters designed to temporally and spatially define cells that are presently responding versus cells that were previously activated. This was combined with tracking infected cells thanks to the insertion of a fluorescent reporter into the viral genome and itsanalysis by live imaging with single-cell resolution. Moreover, we developed and optimised a bioinformatics pipeline to track the intensity of the response at the single-cell level and used a machine learning method to generate a model of spatio-temporal response within the cell network. Our study, focussed on SARS-CoV-2, points out key features of IFN-I/III responses. In particular CRISPR-Cas9-mediated inhibition of specific signalling cascades revealed how paracrine signalling contributes to the spatial regulation of viral spread. We thus validated our novel system as an instrument to define the impact of host cellular regulators on the activation and shut-off of the response, and the potential change in behaviour of the cells concomitantly associated with the innate immune response.

A4. Chikungunya Virus Release Rate is Decreased by Phosphatidylserine Receptors

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Chikungunya virus (CHIKV) is an enveloped alphavirus transmitted through the bites of infectious *Aedes* mosquitoes. According to the World Health Organization, vector-borne diseases account for more than 17% of all infectious diseases, causing over 700,000 deaths every year. The prevention of this disease is mostly characterized by vector control strategies and the prevention of mosquito bites. Phosphatidylserine (PS) receptors, such as T-cell immunoglobulin (Ig) and mucin domain proteins (TIM), have been shown to interact with the enveloped viruses, mediating multiple stages of their replication cycle. The previous studies have shown that Chikungunya virus utilizes TIM receptors for mediating the entry into host cells in a process known as apoptotic mimicry. However, the PS receptors can also impede the release of other enveloped viruses, such as HIV and the Japanese Encephalitis Virus. With this study, we aimed to determine if the PS receptors affect the release of CHIKV viral particles. We utilized a Chikungunya virus tagged with a luminescence reporter gene, nano-luciferase, attached directly to the envelope protein E2. This tag allows us to produce luminescence viral particles that can be quantified in supernatants and cell lysates using a luminometer. We found that the presence of PS receptors, particularly TIM proteins on the surface of the host cells, reduced the release efficiency of CHIKV, while the cells knocked out for TIM and AXL showed an increase in the number of viral particles in the supernatant. This study highlights the importance of phosphatidylserine in mediating not only the entry of CHIKV, but also its release.

A5. Development and Characterization of a Neurological Sequelae Mouse Model for EEEV Infection

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Eastern equine encephalitis virus (EEEV) is an arthropod-borne virus that causes encephalitic disease in both humans and equines. Infection can result in death or neurological sequelae in surviving patients. The significant mortality and morbidity associated with EEEV underscores the need for useful medical interventions. Well-characterized animal models reflective of human disease are critical to the development of prospective therapeutics and vaccines because drug and vaccine candidates will need to be approved via the FDA's Animal Rule. To date, few studies have described EEEV pathogenesis in lethal mouse models and there is no neurological sequelae model currently available. We are addressing this deficiency by developing and characterizing a novel sublethal–sequelae model of EEEV infection and characterizing the host responses associated with cognitive deficits. We hypothesized that animals that appear to have recovered from EEEV infection in fact suffer from substantial cognitive deficits resulting from neurodegeneration associated with viral infection. To verify this, mice were challenged with various doses of EEEV (FL93-939) or attenuated strains of EEEV. Symptomatic mice all succumbed to the viral challenge, with higher-dose groups averaging a quicker time to death. However, mice challenged with attenuated strains of EEEV had reduced or no clinical symptoms, and the majority recovered from the infection. Ongoing studies will determine the potential lasting cognitive impairments in mice that have recovered from EEEV infection through a battery of behavioral testing followed by pathological analysis of formalin-fixed brains of the surviving mice. Developing a model of this nature is critical for understanding the consequential neuropathologies associated with EEEV infection.

A6. Urine Sampling as a Prognostic Tool for COVID-19: A Prospective Interventional Study

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Introduction: Urine samples may prove useful beyond the detection of viral load itself. Negative results of samples of upper respiratory tracts via RT-PCR do not rule out COVID-19. Additional detection methods may prove useful for prognosticating disease. Research on urine sampling for COVID-19 is promising but has limitations. This study aims to explore urine sampling as a novel diagnostic and prognostic tool.
Methods: 148 urine samples were collected from 37 patients at regular intervals from admission. Symptoms onset was documented. Urine samples were collected periodically; PCR tests were performed upon admission and every 3 days for 14 days or until recovery.
Results: Among the 37 patients, 21 had mild symptoms, and 16 had moderate to severe. All had pneumonia; 4 (11%) had Acute Respiratory Distress Syndrome, 3 (8%) bacteraemia, 2 (5%) anaemia and cardiac complexities. 6 (16.2%) had positive COVID-19 viral RNA in urine from day 11 to 23. Patients with PCR-positive urine required Intensive Care Unit in 50% of cases, correlated with symptom severity.
Conclusion: Urine sampling has diagnostic, prognostic, and safety implications for COVID-19, including potential occupational and health risks from viral shedding in toilets. This first extensive study of regular interval urine sampling emphasizes its role in understanding COVID-19 prognosis and potential implications for community infection recognition and prevention. The findings suggest the role of urine testing could be extended to other epidemic viral infections for potential prognosis. Novel urine tests could enable broader infection recognition and prevention. Further research is needed to validate these findings and determine the optimal use of urine sampling as a prognostic tool for COVID-19. This study provides valuable insights into improving the assessment and management of suspected COVID-19 cases.

A7. Expression and Purification of Crimean Congo Haemorrhagic Fever Antigens for the Development of Serodiagnosis

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Introduction: Crimean—Congo hemorrhagic fever virus (CCHFV) is a zoonotic disease, and has caused a endemic in many regions (including Africa, Asia, the Middle East, and south-eastern Europe). However, the prevalence of CCHF is not monitored in most of the endemic countries due to the limited availability of diagnostic assays.

Methods: In this study, we designed expression vectors and established a protocol to purify recombinant CCHFV glycoproteins (Gps) and nucleoproteins (NPs). We designed an antigen bait to isolate human monoclonal antibodies and investigated the utility of these proteins for serodiagnosis in an enzyme-linked immunosorbent assay (ELISA) to detect CCHFV-specific antibodies. The CCHFV genes from the South African isolate SPU187/90 were codon-optimized and cloned into pCDNA3.1+ and human embryonic kidney cells (293F) were transfected with the plasmid. The expressed proteins were purified from the supernatant using nickel resins and concentrated using centrifugal filters.

Results: The antigens purified were used to develop an in-house CCHFV ELISA to detect IgG from CCHF survivors and we efficiently detected CCHFV-specific IgG in human samples. We compared our data with a commercially available CCHFV immunofluorescence kit and our ELISA showed similar results.

Conclusion: The results obtained demonstrate that CCHFV antigens can be produced to a reasonable yield from 293 F and these antigens can be used as reagents for serodiagnosis.

A8. Prevalence of Rotavirus Infec-Tion Among Children Under 5 Years of Age with Acute Diarrhea Admitted to Banadir Hospital, Mogadishu, Somalia: A Retrospective Cross-Sectional Study

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Background:
Diarrhoea is a major cause of morbidity and mortality in children under 5, responsible for approximately 4 billion cases and 1.1 million deaths annually. In developing countries, it causes two million deaths each year. The major causative organism responsible is rotavirus, which is responsible for one-third of hospitalisations with approximately 40% mortality. Globally, rotavirus is the most frequent cause of severe acute gastroenteritis (AGE)/acute watery diarrhoea among young children.

Problem Statement:
There is still a high burden of rotavirus-related diarrhoeal disease in Somalia. Over half of all countries in the world have introduced rotavirus vaccines into their national immunisation programmes, but many of the countries with the highest burden of rotavirus disease have not, including Somalia.

Methods:
A total of 4,239 children presenting with acute watery diarrhoea to the Cholera Treatment Center (CTC) were recruited from June 2022 to July 2023. A stool sample specimen was collected from each enrolled child and tested for rotavirus antigen using enzyme-linked immunosorbent assay (ELISA ProSpecT® Rotavirus Microplate Assay) for the detection of rotavirus in fecal specimens.

Results:
A total of 213,984 patients were admitted to the hospital for various diseases. Of these, 4,239 were admitted due to acute diarrhoea (2%) during the 1-year period of surveillance. Out of these, 1,240 cases were sampled for laboratory confirmation and 453 cases (36.5%) were positive for rotavirus antigen. Rotavirus detection rates were the highest in children aged 3–24 months (76.3%); the detection rate was 14.6% in children aged 6 months and 11% in children aged 25–59 months.

Conclusion and Recommendations:
There is a high burden of rotavirus gastroenteritis among children below 5 years of age hospitalized for acute diarrhoea due to the lack of a rotavirus vaccine. This highlights the need for the introduction of the rotavirus vaccine into the Somali national immunization program.

A9. Presence of Epstein–Barr Virus in Patients with Myalgic Encephalomyelitis/Chronic Fatigue Syndrome

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Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a complex multifactorial disease that causes increasing morbidity worldwide. Many individuals with ME/CFS symptoms remain undiagnosed due to the lack of diagnostic biomarkers. Its etiology is still unknown, but since some studies have demonstrated that specific proteins encoded by Epstein–Barr virus (EBV) could contribute to the immune and neurological abnormalities exhibited by a subgroup of patients with ME/CFS, the aim of this study was to analyze the presence of EBV in patients and healthy controls. In total, 86 patients with clinically diagnosed ME/CFS corresponding to the 1994 Fukuda criteria and 44 matched healthy controls were analyzed for the presence of EBV using the digital PCR method (26000 partition plate) according to QIAcuity EG PCR protocol from Qiagen. Results showed that 10 out of 86 (12%) patients and 5 out of 44 (11%) controls were EBV-positive. Although no significant difference in the frequency of EBV was found between the groups, more positive partitions were detected in the ME/CFS patient group (up to 109 positive partitions; median concentration 38.70 copies/µl) in comparison to the control group (up to 51 positive partitions; median concentration 12.36 copies/µl). This study's findings suggest that further research should be conducted to explore the potential role of EBV in ME/CFS, paving the way for investigations into the virus's contribution to the pathophysiology of the disease and potential therapeutic implications.

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A10. Mayaro, an Emerging Arbovirus in Brazil

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Mayaro (MAYV) is an Alphavirus (Togaviridae) isolated in Trinidad, Caribbean, in 1954. Like yellow fever virus, MAYV is maintained in nature as a zoonosis of primates transmitted by Haemagogus mosquitoes. MAYV epizootics in primates spread through river border forests. MAYV can also be transmitted by Aedes aegypti, an anthropophilic mosquito common in Brazilian cities. MAYV is a causative of acute febrile illnesses and, like Chikungunya virus (CHIKV is a phylogenetically related virus), produces arthritides that can last for months. We also found that the processing and storage of blood bags used in banks did not prevent MAYV from remaining viable in concentrates of red blood cells and platelets. Outbreaks of Mayaro fever are well known in the Amazon Region of Brazil. In recent decades, outbreaks of MAYV have been increasingly reported in the Central Plateau of Brazil. Large outbreaks of Chikungunya have also been reported in the same areas where MAYV has been found. Due to this, MAYV is mostly misdiagnosed because cases are clinically confused with those caused by CHIKV: patients have a similar course of disease and also, in laboratory diagnosis, both viruses show cross-reactions in serological tests. Recently, we found that mice immunized against CHIKV have partial cross-protection against a secondary MAYV infection. MAYV is spreading southward and we have found evidence that this virus has already infected people in the highly populated State of São Paulo, Southeastern Brazil. Therefore, epidemiological surveillance systems in Brazil and other Latin American countries should expand their usual epidemiological surveillance of dengue, Zika, and Chikungunya and implement surveillance for Mayaro as well as for other native arboviruses.

A11. Loop-Mediated Isothermal Amplification and Lateral Flow Immunochromatography Technology Used for the Rapid Diagnosis of Influenza AB

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Influenza viruses cause highly contagious respiratory diseases that cause millions of deaths worldwide. The rapid detection of influenza virus is essential for an accurate diagnosis and the initiation of appropriate treatment. We developed a multi-loop-mediated isothermal amplification (LAMP) and lateral flow immunochromatographic assay capable of simultaneously detecting influenza A/B. Primer sets for influenza A and influenza B were designed to target conserved regions of segment 7 and the nucleoprotein gene, respectively. The internal control primer set was designed within a conserved region of human actin beta mRNA. The kit was optimized by testing various ratios of primer sets for influenza A/B and internal control (IC). Test tubes containing the reagent mixtures were placed on a heat block at 62oC for 30 min. For the LAMP LFA assay, a detection device with a compact size and plastic housingcomprising a sample pad, gold-conjugated pad, nitrocellulose membrane, and absorbent pad was developed. The indirect detection of the LAMP products involves the use of monoclonal antibodies (mAbs) against sets of tags, digoxigenin, fluorescein isothiocyanate (FITC), and biotin. A total of 267 (82 influenza-A-positive, 70 influenza-B-positive, and 115 influenza-B-negative) nasopharyngeal swab samples were collected, all of which were confirmed by influenza AB real-time PCR. The performance of the influenza A/B multiplex LAMP–LFA assay was compared with that of the commercial Allplex SARS-CoV-2/FluA/FluB/RSV assay (Seegene, Seoul, Korea). The influenza A/B multiplex LAMP-LFA assay showed 98.2% specificity for uninfected clinical samples (113/115) and sensitivities of 91.4% (75/82) and 85.5% (62/70) against influenza A and B clinical samples, respectively. The concordance rate between the influenza A/B multiplex LAMP–LFA assay and RT-PCR was 93.6%. The performance of the influenza A/B multiplex LAMP - LFA assay show high concordance with RT-PCR, indicating that it is reliable for use in a low-resource environment.

A12. X-Ray Irradiation for Inactivation of Acutely Infected Cellular Samples, for Downstream Immunological Processing

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Since the SARS-CoV-2 pandemic, the need to process high-containment samples in low-containment labs has highlighted the pursuit for a high-fidelity inactivation method, benefiting less-equipped facilities. Among various inactivation methods, gamma and x-ray irradiation have emerged as gold-standard methods for inactivation of cultured virus, for use as LFD and ELISA controls This is due to their effectiveness, preservation of viral structures, and avoidance of hazardous chemicals. X-rays provide finer dose control and enhanced safety and security relative radiological sources. However, it remains uncertain how high-dose x-ray irradiation impacts biological elements, such as cells, within a sample. This study aims to explore the feasibility of x-ray inactivation of acutely infected patient cellular samples and effect of high dose irradiation on cell viability and surface marker detection.

Using patient sample surrogates with Zika virus +/- high density VERO cells, the minimum dose required for a Log_{10} reduction of the viral titre (D-value) was established. The presence of VERO cells in Zika samples had no measurable effect on the radiation dose required to inactive said samples, supported by $TCID_{50}$ testing, under the same condition. Inactivation was confirmed with 3 week-long continuous passages that showed no cytopathic effects when infected with irradiated samples, which was further corroborated by qRT-PCR on samples collected throughout the from the cell culture during the same time frame, showing no increase in genome copies.

Incremental irradiation of PBMCs up to 8kGy and subsequent analysis using flow cytometry detected an increasing loss in cell viability with increasing dose. Immunological markers including CD3, CD4 and CD8 were still reliably detected at 8kGy however, showing a degree of resilience of these cell types to irradiation.

Overall, this work suggests the potential utility of x-ray irradiation to inactivate patient blood and tissue samples whilst maintaining immunological marker integrity, with further patient sample testing underway.

A13. Deciphering Structural Elements of Viral RNAs

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Viruses have the ability to efficiently encompass large amounts of information in relatively small genomes, allowing such miniscule particles to effectively infect cells and replicate. The viral repertoire of tools for that matter includes frameshifting stimulating elements (FSEs) and particular splicing site structures, both of which grant certain viruses the possibility to produce more proteins out of a single original RNA molecule. Other structural elements, like hairpins located at the very 3' end of some viral RNAs, provide stability to the transcript, without the need for polyadenylation (poly-A).
In this study, we aimed to thoroughly characterize several of these structures, such as the FSE of SARS-CoV, SARS-CoV-2 and HIV, the 3' splice site of Influenza A, or the 3' Stem loop from *Herpesvirus saimiri*'s HSUR transcript, in an in vitro system where we could effectively change different conditions in a rigorously controlled context. As viral RNAs are subjected to changing environments during their life cycle, it is critical to understand how structural elements react to shed light on viral mechanisms and guide therapeutical efforts, while also allowing other previously unknown structural behaviors to be predicted.
With that in mind, we generated a library of viral sequences and studied their structures in varying conditions (such as Mg concentration and temperature), using in vitro transcription followed by DMS-MaPseq, a widely used technique used for structural studies. We then assessed the obtained information by means of a newly developed web app, with a user-friendly interface aimed at facilitating this type of analysis to the general community.

A14. Unveiling Viral RNA Structures Using DMS-MaPseq

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RNA is a highly dynamic molecule capable of carrying out various functions throughout life. Its versatility is due to its structure and ability to adopt alternative conformations. This is especially relevant in the case of viruses; because of their limited coding capabilities, they take advantage of their secondary structures for multiple mechanisms regarding replication, transcription, translation, and other vital processes. This project makes use of dimethyl sulfate mutational profiling and sequencing (DMS-MaPseq), a high-throughput chemical probing technique used to determine the secondary structures of RNA molecules. DMS-MaPseq was used to determine the structure of in vitro-derived herpesvirus transcripts and the human coronavirus OC43 genome. As a starting point, the structures for five Epstein—Barr virus (EBV) and five Kaposi's Sarcoma-associated herpesvirus (KSHV) RNA transcripts were generated. One such structure generated from KSHV is the polyadenylated nuclear (PAN) RNA, which agrees with a previously published structure generated through an alternative structure-probing method. The ultimate purpose of this project is to generate a database containing the RNA secondary structures of as many viruses as possible. This will encompass both DNA and RNA viruses, and samples will be obtained from in vitro-generated RNA and live virus-infected cells. This viral RNA structural database can be used by any researcher interested in elucidating the roles of viral RNAs.

A15. Efficient Benchtop Purification of SARS-CoV-2 Whole Virions for Immunological Studies

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Background: Research on COVID-19 pathogenesis and immunology requires efficient in vitro model systems involving purified SARS-CoV-2 virions. This study aimed to develop a rapid and benchtop purification protocol for viruses in a model of SARS-CoV-2, eliminating cytopathic agents and non-viral substances to facilitate immunological investigations.
Methods: The SARS-CoV-2 delta variant was propagated in Vero E6 cells within a BSL-4 facility. The virus was completely inactivated via UV-inactivation, and then confirmed by multiple assays. The purification process included filtration through a 0.22 μm filter, anion exchange chromatography, and optional desalting. Purity was assessed via BCA assay, and viral recovery via RT-PCR and rapid antigen tests. Immunological interactions were evaluated using PBMCs from healthy donors, co-incubated with purified and unpurified virus suspensions, and analyzed via flow cytometry.
Results: The purification process removed 99% of total proteins, leaving predominantly viral proteins. The PCR and rapid tests showed minimal reduction in virus content, recovering approximately 70% of viral RNA. Unpurified virus caused significant PBMC destruction, while purified virus did not.
Discussion: This 30-minute benchtop purification method efficiently removes cellular proteins, making it suitable for immunological research. Testing with active viruses is needed to assess infectivity. Anion exchange magnetic beads proved highly effective. The discrepancies in PCR and rapid test results after desalting warrant further investigation.
Acknowledgment: This research was conducted in collaboration with the National Laboratory of Virology, University of Pécs, and supported by the National Research, Development, and Innovation Office of Hungary (project numbers: 2020-2.1.1-ED-2020-00100 and RRF-2.3.1-21-2022-00010).

A16. Employing Flock House Virus-like Particles (VLPs) as Versatile Drug Delivery Agents

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Chemotherapy against cancer comes with a lot of complications, including a lack of selectivity for tumor cells, poor effectiveness and biodistribution, and a short circulation time. Several nanotechnology-based platforms such as liposomes, synthetic polymers, and protein-based nanocarriers have been developed to circumvent these issues. The structural proteins of many viruses can self-assemble spontaneously into virus-like particles (VLPs) under appropriate conditions, which makes them potentially effective nanocarriers for drug encapsulation and delivery. We have exploited the inherent advantages of an insect nodavirus, Flock House Virus (FHV), to engineer a targeted nano-vehicle. Since FHV lacks mammalian cell tropism, making use of click chemistry, we devised a quick chemical conjugation method to attach targeting peptides to FHV virus-like particles (VLPs). Further, the natural affinity of FHV for hydrophobic molecules has been utilized to encapsulate hydrophobic chemotherapeutic drugs, like doxorubicin and camptothecin, by the transient destabilization and re-stabilization of the capsid via the modulation of Ca^{2+} concentration. These modified, drug-encapsulated VLPs have demonstrated high anticancer efficacy in murine colorectal and melanoma models. Their effectiveness as targeted drug delivery vehicles is being investigated in a murine lung cancer model as well. The cellular internalization and trafficking of these VLPs will be tested in addition to their apoptotic efficiency in the respective cancer cell lines. Effective targeted delivery of chemotherapeutic drugs via VLP-based nano-vehicles is expected to address some of the primary challenges associated with cancer therapy.

A17. Determination of the in Vitro Isolation Conditions of Dobrava, Puumala and Tula Hantaviruses

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Hantaviruses are negative single- stranded RNA viruses which found worldwide and known to cause severe human diseases, such as HCPS and HFRS. Natural hosts are rodents, soricomorphs and bats. In Hungary, three types of hantaviruses are circulating: Dobrava (DOBV), Puumala (PUUV) and Tula (TULV), which are carried by mice and voles. DOBV and PUUV viruses cause human disease, while the human pathogenicity of the TULV is still questionable. Since hantaviruses are difficult to reproduce on cell lines, we found it necessary to develop a virus infection protocol that would make experiments with hantaviruses more efficient and faster. In the protocol, we defined the most important parameters for the preparation of virus isolates, such as the type of cell line, the age of the cells, the number of cells, and the type of cytopathic effect for all Hungarian Hantavirus species. The results of infections in 96-well cell culture plates were analyzed with Cytosmart Omni. We determined that the cytopathic effect shows a different picture in the case of DOBV, PUUV, TULV. The age of the cells has a minimal influence, while the confluence of the cells greatly affects the success of the infection.

A18. Oncolytic Parainfluenza Virus Enhances the NK Cell-Mediated Killing of Pediatric Cancer Cells

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Pediatric cancer is one of the leading causes of childhood death, and current treatment options are commonly associated with chronic health conditions that persist into adulthood. Natural killer (NK) cell adoptive immunotherapy is a promising treatment for tumor therapy. However, pediatric cancers are known to exhibit resistance to NK cell killing, potentially due to an increase in NK cell inhibitory ligands on their surface. We hypothesized that the resistance of pediatric cancers to NK cell killing can be overcome using tumor-targeting viruses called oncolytic viruses. Our oncolytic parainfluenza virus 5 (O-PIV5) provides strong activating signals for the NK-cell mediated killing of infected tumor cells. We have demonstrated, through real-time cell viability assays, that O-PIV5 is effective at enhancing the NK cell-mediated killing of pediatric cancer cells, but the mechanism of enhancement is currently unknown. Cell surface analysis showed that interferon-gamma (IFN-y) treatment caused NK cell inhibitory ligands to be upregulated on the surface of pediatric cancer cells, a response that is blocked by infection with O-PIV5. The PIV5 V protein is known to block IFN signaling within infected cells. Consistent with a role for V protein in enhancing the NK cell killing of O-PIV5 infected cells, we demonstrated that the lentiviral delivery of V protein to pediatric cancer cells prevented the IFN-y induced upregulation of NK cell inhibitory ligands. Our oncolytic virus and NK cell combination therapy provides a promising therapeutic option to effectively target and eliminate pediatric tumors.

A19. Exploring the Diversity of Mosquito-Derived Viruses in Serbia With in Vitro and Molecular Biological Methods

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Technological advances in the field of sequencing and bioinformatic analyses have led to the discovery of a diverse group of viruses, namely insect-specific viruses (ISVs). The hallmark characteristics of ISVs are that they exclusively infect mosquitoes and phlebotomine sandflies and cannot replicate in vertebrate hosts and their cell lines. Based on the currently available data, ISVs may have a function in the suppression of arbovirus replication, providing a biological tool for arbovirus control. Furthermore, ISVs can also be reliable agents for vaccine and diagnostic platform developments. For this study, we used the remaining mosquito samples collected in Serbia in 2014. During the in vitro surveillance, we screened a total of 283 mosquito pools for viral cytopathic effect on the C6/36 (*Aedes albopictus*) cell line. From the inoculated samples, 72 showed cytopathic effects after the second passage. Metagenomic analyses of 72 samples proved the presence of diverse mosquito-specific viruses in 68 samples belonging to several mosquito species from different collection sites in Serbia. We demonstrated the possibility of establishing local mosquito-derived virus collections to facilitate future co-infection and interference studies. Our results provide pillars for more detailed genetic and evolutionary analyses of our insect-specific virus isolates to support further evolutionary, vector competence, vaccine, and diagnostic platform developments in the future. Regarding the complexity of the mosquito–virus interactome, such works will represent the first step for applied arbovirus surveillance studies.

A20. High-Throughput Thermodynamic Measurements Using Dimethyl Sulfate Chemical Probing of Viral RNAs

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Viral RNA performs some of its functions by adopting specific structural elements, which, in turn, influence the virus's functionality. While understanding the complete structure of viral RNA is essential, measuring the thermodynamics of base pairing at the single nucleotide resolution could significantly impact the way we predict RNA structures. The traditional experiments that measure the melting temperature of base-paired RNA are cumbersome and can only analyze one sequence at a time. However, the recent advancements in RNA structure determination have shifted from analyzing individual molecules to employing high-throughput methods such as DMS-MaPseq. This approach offers insights into the base-pairing patterns of thousands of RNAs simultaneously with an unparalleled single-nucleotide precision.
We have leveraged a specially designed library, comprising thousands of structured RNAs, to introduce a method that measures their thermodynamic stability using DMS-MaPseq. This library encompasses multiple primary "parent" structures sourced from viral RNA. By progressively destabilizing these structures one base pair at a time and assessing their reactivity with DMS, we can pinpoint the exact moment a structure begins to unfold, giving us a direct measure of its thermodynamic stability. Consequently, we can recreate a detailed melting curve at the single-nucleotide resolution both in vitro and within cells. This method does not just offer invaluable thermodynamic insights into viral RNAs, but also sheds light on RNA folding more broadly.

A21. The Use of a Genetic Screen for the Identification of Mild Beet Yellowing Virus Resistance

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A recent study reported that polerovirus can be effectively used as a virus-induced gene silencing (VIGS) vector to induce a prominent yellow-vein-clearing phenotype in *Arabidopsis thaliana*, without compromising the virus's infectivity (Bortolamiol-Bécet et al., 2018). This capacity to induce visible silencing effects has previously proven to be a powerful tool in discriminating infected plants from non-infected ones in high-throughput plant phenotyping screenings (Beyene et al., 2017; Sheat et al., 2022).
Based on these findings, my project aims to employ this technology for the rapid screening of a collection of sugar beet varieties to identify those resistant to viral yellows. To achieve this, a recombinant virus was engineered by adding a fragment of a gene involved in chlorophyll synthesis in sugar beet to the 3' end of the cDNA clone of the *Beet mild yellowing virus* (BMYV).
After two weeks' post-agroinfection of *Beta vulgaris* plants, we observed a characteristic vein yellowing in newly developed leaves, indicating the ability of the recombinant virus to trigger visible symptoms in sugar beets. Interestingly, only symptomatic plants were found to be infected by the BMYV, while non-infected plants remained asymptomatic.
Similar to the findings of Bortolamiol-Bécet et al., 2018, our preliminary results also revealed no significant difference in infectivity between the wild-type and the recombinant viral infection, ensuring that the screening process was unbiased.
During our phenotypic screening, we observed varying levels of intensity of VIGS symptoms across the different sugar beet genotypes tested. Some plants exhibited only mild yellowing vein clearance on a few leaves, while others displayed pronounced yellowing-vein-clearing symptoms on all their leaves. However, no correlation between the intensity of VIGS symptoms and the viral load was found.
In summary, we developed a rapid high-throughput visual diagnostic tool for evaluating sugar beets resistant to BMYV infection.

A22. Long-Term Infection and Pathogenesis in a Novel Mouse Model of Human Respiratory Syncytial Virus

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Intensive efforts have been made to develop models of hRSV infection or disease using various animals. However, the limitations such as semi-permissiveness and short duration of infection have impeded their applications in both the pathogenesis of hRSV and therapeutics development. Here, we present a mouse model based on a Rag2 gene knockout using CRISPR/Cas9 technology. Rag2-/- mice sustained high viral loads upon intranasal inoculation with hRSV. The average peak titer rapidly reached1×109.8 copies/g and 1×106TCID50 in nasal cavity, as well as 1×108 copies/g and 1×105 TCID50 in the lungs up to 5 weeks. Mild interstitial pneumonia, severe bronchopneumonia, elevated cytokines and NK cells were seen in Rag2-/- mice. A humanized monoclonal antibody showed strong antiviral activity in this animal model, implying that Rag2-/- mice that support long-term stable infection are a useful tool for studying the transmission and pathogenesis of human RSV, as well as evaluating therapeutics.

A23. Localization of RNA-Dependent RNA Polymerase from Picornaviruses in Cells Using Fluorescent NTPs and Synthetic Transporters

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Positive-sense single-stranded RNA viruses are a large family that includes many dangerous human pathogens such as yellow fever and poliovirus. The key enzyme for viral replication is the RNA-dependent RNA polymerase (RdRp). The structure of this enzyme is rather well understood. However, in infected cells, RdRp always synthesizes new RNA strands on the surface of membranes that form the so-called viral replication organelles [1]. Fluorescent nucleotide analogs could be used to visualize the synthesis of new RNA strands.
In this study, we investigate several fluorescent nucleotide analogs, and whether they are incorporated by polioviral or coxackieviral RdRp into a nascent RNA strand. We choose the most promising of them and, using synthetic nucleoside triphosphate transporters, deliver these NTPs into cells [2]. Using a confocal microscope, we observe a colocalization of fluorescently labelled NTPs with fluorescently labelled replicons in cells. After replication and fixation, we mark incurred nascent RNA strands with a dsRNA antibody.
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A24. Dynamics of SARS-CoV-2 Variants of Concern and Co-circulation of Viral Respiratory Pathogens: Insights from a Brazilian Study

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Respiratory viruses have a strong impact on public health, often leading to widespread epidemics and pandemics with significant mortality. The recent SARS-CoV-2 pandemic, for instance, resulted in about 7 million deaths. Emerging SARS-CoV-2 variants, known as Variants of Concern (VOCs), have shown increased viral virulence, and Brazil has reported five circulating VOCs. In our study, we conducted RT-qPCR analyses on 1601 clinical samples collected from patients with respiratory symptoms between December 2020 and March 2022. Among these, 850 samples tested positive for SARS-CoV-2, leading to further sequencing of the spike genomic region. For the 751 negative samples for SARS-CoV-2, a multiplex RT-qPCR protocol was used to detect three further respiratory viruses: Influenza A (IAV), Influenza B (IBV), and Respiratory Syncytial Virus (RSV). For SARS-CoV-2, our analysis revealed shifts in the VOCs' prevalence over time. Initially, VOC Alpha was predominant, but with the emergence of the Gamma variant in January, 90% of samples were classified as Gamma by March. From August to December 2021, all sequences were identified as Delta. However, by the end of December 2021, the Omicron variant was detected, and all subsequent samples belonged to this variant. Analysis of the spike region confirmed the VOC RT-qPCR findings and identified amino acid substitutions, including Y351C, R355M, V367G, R403K, S477F, V510I, V539A, N542H, N544H, and insertion 1612A. Among the SARS-CoV-2-negative samples, our analysis revealed 4% as IAV, 0.1% as IBV, and 3% as RSV. Our findings suggest a shift in VOC predominance over time. Additionally, the substantial number of negative samples for the screened viruses indicates the potential presence of other respiratory pathogens, emphasizing the need for surveillance. This underscores the importance of epidemiological studies on various respiratory viruses, not only SARS-CoV-2, as these viruses continuously circulate.

A25. Distribution of SARS-CoV-2 Variants Through Pandemic Waves in Tourist Split-Dalmatia County

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Background:
At the Teaching Public Health Institute of Split-Dalmatia County (SDC), SARS-CoV-2 was first detected at the very beginning of the pandemic (end of March 2020). In this analysis, we wanted to summarize data on the frequency of positive results for SARS-CoV-2 and variant distribution in South Croatia for the 3-year pandemic period.
Materials and methods:
During a three-year period (April 1, 2020 to March 31, 2023), we tested 473,467 patients for SARS-CoV-2 by real-time PCR. Furthermore, 2,095 SARS-CoV-2 PCR-positive respiratory specimens tested between June 2021 and February 2023 were selected and sent to the Croatian Institute of Public Health in Zagreb in order to monitor the appearance of different variants during the pandemic period in South Croatia.
Results:
Out of the total number of 473,467 samples tested for SARS-CoV-2, 33.7% were positive. The positivity rates ranged from 2.2% to 75.4% each month in the 3-year period. The total of tested patients and the corresponding positivity rate were significantly different depending on the age of the tested patients. However, the highest positivity rates were detected with the appearance of variants in SDC, especially the Omicron variant, due to which the positivity rate reached 75.4% of tested patients.
Conclusions:
SARS-CoV-2 positivity rates during the 3-year pandemic period in Split-Dalmatia County were most related to the appearance of new variants, with the Omicron variant being the most frequently detected. Positivity rates did not indicate a connection with the tourist season.

A26. An Accord Analysis of Biochemical and Immune-Inflammatory Markers Predicting the Clinical Outcome of COVID-19 Patients in the Indigenous Race and Ethnic Population of Arunachal Pradesh to Aid in Designing Discriminatory and Risk Stratification Tools

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Introduction: The COVID-19 pandemic has affected diverse populations worldwide, with varying degrees of disease severity. Indigenous communities often face unique challenges in managing infectious diseases. This study aims to investigate the predictive value of biochemical and immune-inflammatory markers in determining the severity of COVID-19 among the indigenous race and ethnic population of Arunachal Pradesh, India, and also to examine how it agrees with the findings from the non-indigenous population of India.
Methods: A prospective hospital-based study was conducted in the Dedicated COVID hospital (DCH), Arunachal Pradesh, over the period from August 2020 to January 2022. A total of 420 patients diagnosed with COVID-19 were analysed. The neutrophil to lymphocyte ratio (NLR), lymphocyte to monocyte ratio (LMR), platelet to lymphocyte ratio (PLR), C-reactive protein (CRP), D-Dimer, and liver enzymes were estimated. Serum ferritin and interleukin-6 (IL-6) were measured and compared. Three models were used in logistic regression to check for the predictive potential of biochemical parameters, and the disease severity score was calculated using a non-linear regression algorithm.
Results: CRP, Ferritin, IL6, and D-Dimer reported the highest odds of predicting severity for COVID-19 patients among the indigenous and ethnic population. The mean of NLR, LMR, and PLR for the severe group was found to be significantly elevated, predicting their association with the disease severity. For validation, 30% of the total dataset was used as testing data (n=126) with a sensitivity of 4.1% and a specificity of 4.5%. Severe cases exhibited significant deviations from mild cases, with elevated levels of inflammatory markers, altered lymphocyte counts, and abnormal platelet profiles, which is in accordance with the findings in the non-indigenous population of India. These markers are reported to be potential predictive tools for disease severity.
Conclusions: The study of these factors can aid in implementing targeted interventions, and in improving patient outcomes, in this specific and unique demographic group.

A27. Eco-Geography Variables of Dengue Fever and Aedes in Latin America and New Autochthonous Transmission Localities: What to Expect in the USA and Europe?

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Dengue fever is the arboviral disease transmitted by *Aedes aegypti* and *Ae albopictus* with the highest prevalence and incidence worldwide. In Ecuador, a geographic model that can be extrapolated to many locations in Latin America and new areas of local transmission, we have determined that there are eight interrelated variables that limit or trigger its introduction and the transition from endemic to epidemic transmission in the three continental regions (Coast, Sierra, and Amazon). These variables range from the vector invasive capacity and/or its persistence to physical factors such as orography, slopes, and altitude; diversity and water availability; climatic factors such as minimum temperatures, daily temperature intervals, annual patterns of precipitation/accumulated precipitation; socioeconomic variables (storage patterns due to deficiencies in the supply of drinking water through pipes, deficiencies in solid waste collection and disposal services); and demographics such as population density and the type of human mobility, which are all related to the ecology (larvae and adults) and physiology (eggs) of the vector. The interrelation and synergy of these variables determine the incidence and prevalence of dengue in a differential way in the three regions of Ecuador, with a west–east geographical order from highest to lowest in the following sequence: Coast Pacific > Sierra Pacific slope > Low Amazon > Sierra Amazonian slope > High Andean Sierra (without transmission, greater than 1,700 m), and northern latitudinal geographic > southern Ecuador for epidemic expansion. This is evident in a regional pattern segmented by quadrants of the ecology of the disease. The expansion to subtropical areas in the United States, southern South America, and the Mediterranean part of Europe demonstrates that climate change, migration, and socio-economic changes in public services can be associated with the introduction of vectors, their establishment and persistence, and a possible transition to endemicity.

A28. Design of a Modified Respiratory Syncytial Virus Viral Entry Reporter for the Study of Antiviral Activity of New Compounds

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Respiratory syncytial virus (RSV) is the most common cause of lower respiratory tract infections in children, causing severe complications (such as bronchiolitis and, in more severe cases, pneumonia and death). However, to date, there is no effective antiviral or vaccine against RSV. In this work, we produced a novel recombinant RSV with a betalactamase enzymatic reporter that allowed us to determine the moment in which the virions deposit the viral content into the cytoplasm. Using this strategy, we evaluated the antiviral effect of Baicalin (BE), Baicalein (BLE), Hesperidin (H), Kaempferol (K), Myricetin (M), Naringin (NG), Naringenin (NGN), Tangeretin (T), and Quercetin (Q) on virus entry. We also evaluated the hemolytic effect and cytotoxicity effect of these flavonoids on NHBE cells. The results showed that these flavonoids do not possess hemolytic activity, nor do they induce apoptosis and/or necrosis in NHBE cells. Our findings show that only Myricetin and Quercetin affected RSV viral entry events in NHBE cells. This new platform can also be used to evaluate new compounds.

A29. Discovery and Characterization of Potent and Selective SARS-CoV-2 Antivirals with Pan-Coronavirus Potential

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The latest events caused by the SARS-CoV-2 pandemic highlight the need to develop means to stop the expansion of these pathogens. The use of prophylactic vaccines has considerably reduced the impact of these outbreaks. However, viruses continue to circulate among the population and therapeutic tools are needed to treat infected individuals in the form of effective antivirals. Using a phenotypic screening approach, a new family of antiviral molecules has been identified and a medicinal chemistry programme for the generation of preclinical candidates has been initiated. Structure—activity and structure—property relationship studies allowed or the optimization of the chemical family and the selection of lead compounds with activity and optimal therapeutic window in cell culture. The identified family showed selective activity against human coronaviruses, such as SARS-CoV-2, hCoV-229E, and MERS-CoV, without any measurable activity against other RNA viruses, suggesting pancoronavirus antiviral activity. The time of additional experiments indicates that these compounds strongly inhibit infection at a post-entry step and that they reduce the accumulation of subgenomic viral RNAs in cells inoculated at a high multiplicity of infection. Moreover, compounds interfere with replication of a subgenomic SARS-CoV2 replicon, reinforcing the notion that they inhibit a step that affects the replication of viral RNA. In vitro enzymatic studies have ruled out that the main viral protease (Mpro) is targeted by these compounds. Finally, the genetic resistance profiles of lead compounds have been determined by directed evolution in cell culture. These studies revealed that selective pressure imposed by the compounds that cluster together in a non-structural region of the SARS-CoV-2 genome is responsible for the formation of double membrane vesicles within the putative RNA replication compartment. Reverse genetics studies are underway to formally demonstrate the identity of the resistance-associated mutations.

A30. Evaluation of Pfizer Vaccine Efficiency Against a New VOC of SARS- CoV-2: XBB 1.1

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The wide diversity of SARS-CoV-2 variants which have emerged in recent months from the different Omicron sub-lineages has been of great concern to the EMERGEN consortium coordinated by Santé Publique France and ANRS-MIE. These new variants of concern (VOCs) harbor a large set of mutations in the viral surface protein Spike, giving rise to immune escape and raising concerns about the efficacy of Pfizer vaccines in immunized populations. To this end, we investigated the efficacy of monovalent and bivalent booster (Omicron BA.4/BA.5-containing) mRNA COVID-19 vaccines against the VOC Omicron XBB 1.1 in a Syrian hamster model.
Strikingly, we observed a 1.8- and 4.7-fold decrease in virus load in the lungs and a 1.7- and 17.7-fold marked decrease in nasal turbinates after the monovalent vaccine and bivalent booster, respectively. Consistently, we observed that a complete vaccine regimen was required to successfully maintain an appropriate balance between the inflammatory response and robust immune protection. These data were also in agreement with the histopathological evaluation since the vaccinated animals exhibit fewer lesions and less lung damage as compared to unvaccinated ones.
Taken together, these data strongly support the notion that the Pfizer bivalent vaccine displays a significant immune protective effect against the new XBB 1.1 Omicron variant.

A31. Structural and Functional Characterization by Cryo-EM of a Novel Monoclonal Antibody with Pan-Neutralizing SARS-CoV-2 Activity

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During previous COVID-19 waves, therapeutic administration of monoclonal antibodies (mAbs) was highly effective in preventing hospitalizations and deaths related to this disease. In this work, we characterize, from a structural point of view, an mAb (17T2) with high and broad neutralizing activity against pre-Omicron and Omicron SARS-CoV-2 variants, isolated from a convalescent COVID-19 individual infected during the first wave of the COVID-19 pandemic.
Cryo-electron microscopy (cryo-EM) analysis showed that 17T2 binds to the Omicron BA.1 spike protein with all three receptor-binding domains (RBD) in the "up" position and recognizes a large epitope that overlaps with the receptor-binding motif. A comparison with a structurally similar neutralizing mAb (S2E12) was carried out to understand the differences in neutralizing activity, which led to the detection of point mutations in key residues that may lead to increased interaction with the RBD in the case of 17T2.
These results highlight the impact of small structural antibody changes on neutralizing performance and identify 17T2 mAb as a potential candidate for future medical interventions.

A32. Discovery and Characterization of Broad-Spectrum Antivirals Against RNA Viruses with Dual Mode of Action Against SARS-CoV-2

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The SARS-CoV-2 pandemic has highlighted the need to be prepared for emerging viral infections. Despite vaccine availability, effective antiviral drugs may still be needed to improve the clinical outcomes of infected individuals. With the aim of identifying new antivirals, a collection of experimental compounds was analyzed using a phenotypic screening approach. A family of compounds with antiviral activity in the low micromolar range was identified and a hit-to-lead medicinal chemistry program was started to carry out the lead identification. Structure–activity relationship studies led to the selection of lead compounds with improved activity. Time-of-addition experiments revealed that these leads display antiviral activity against SARS-CoV-2 infection at a post-entry step. Antiviral activity against distant RNA viruses such as vesicular stomatitis, West Nile and dengue viruses was maintained, suggesting broad-spectrum activity. Target deconvolution was initially approached using morphological profiling by means of a Cell Painting Assay (CPA). The obtained profiles of compound-perturbed cells were compared with the profiles of reference compounds with known activity. Profile similarity hinted towards the same target and mode of action. CPA prioritized the hypothesis of a previously known cellular target involved in a well-defined cellular metabolic pathway. Antiviral activity was abolished by adding the metabolic product of the putative molecular target enzymatic activity. Only SARS-CoV-2 was still susceptible to antiviral treatment in the presence of the rescue metabolite, suggesting a secondary, selective mechanism against SARS-CoV-2. A chemical proteomics approach confirmed that our lead compounds interfere with the hypothesized cellular pathway, similarly to a well-characterized clinical candidate drug of the same target. However, cells treated with some of the new compounds displayed an additional cluster of host factors altered by the treatment, underscoring a potential dual mode of action against SARS-CoV-2, as suggested by the metabolite rescue experiments.

A33. Identification of Antiviral Compounds of Marine Origin Using a High-Throughput Screening Process

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Each virus has its own characteristics, but many of them share some characteristics that provide the basis for the development of broad-spectrum antivirals, a strategy that may offer a solution against many viruses (including the emerging ones). For example, broad-spectrum kinase inhibitors approved for antitumor therapy have demonstrated the potential to disrupt intracellular viral trafficking, and therefore, inhibit a wide range of viruses. PharmaMar is a company dedicated to the exploration of the seabed, with the aim of finding and isolating new compounds with antitumor activity. As a result, it has a large collection of pure compounds with potential broad-spectrum antiviral activity. To address this study, a primary high-throughput screening (HTS) was designed with a panel of five representative viruses from different families (*Herpesviridae, Flaviviridae, Picornaviridae, Paramyxoviridae* and *Coronaviridae*), in which the percentage of cytotoxicity (CC) and the viral percent inhibition (PI) of each compound at three different concentrations (5, 0.5 and 0.05 μM) for 72 hours were measured. The compounds were classified based on their CC and PI, and those with a lower CC and higher antiviral activity level (PI) were selected. Through bioinformatic analysis, the selected compounds were clustered using different filters to identify those that were virus-specific, broad-spectrum, or at a low cytotoxic level. Those screened compounds underwent a secondary dose–response assay to confirm their PI, CC, and selectivity index (SI). Of the nearly 2,000 compounds analyzed, 60 are active in three or more viruses, and there are at least 2 families of compounds that are active in four or five of the viruses. The results obtained have been contrasted with the known compounds from the same families that also present antiviral activity and validated in a secondary assay, demonstrating the power of screening and its ability to identify compounds with broad-spectrum antiviral activity in a short period of time.

A34. Lethal Infection of Dengue Virus Clinical Strains in IFN-α/β Receptor-Deficient Mice

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Background
Suitable animal models are essential for the pre-clinical evaluation of antivirals/vaccines for dengue virus (DENV) infection. Since immunocompetent mice are naturally resistant to DENV, IFN-α/β and γ receptor-deficient mice have conventionally been used for dengue studies. However, a lack of an intact IFN-γ signaling pathway limits the adaptive immune function in response to DENV infection in these mice. Here, we show that clinical strains of DENV1 and DENV2 are capable of causing lethal infection in adult IFN-α/β receptor-deficient mice (A129 mice).
Methods
A129 mice were infected with clinical DENV1 and DENV2 isolates in the presence of enhancing antibodies. IFN-α/β and γ receptor-deficient mice (AG 129 mice) were used for comparison.
Results
Infection with 5 x 10^7 pfu of DENV2 in A129 mice caused 75–100% mortality on days 4–6 post infection, which was similarly observed in AG129 mice. Surprisingly, infection with 1 x 10^7 pfu of DENV1 was 80% lethal in A129 mice, while the same virus dose did not cause mortality in AG129 mice, indicating that this clinical DENV1 isolate is more virulent against A129 mice than AG129 mice. Treatment with anti-TNF-α antibody was 100% protective in A129 mice lethally infected with DENV1 or DENV2, suggesting that TNF-α is responsible for disease severity, as was demonstrated in AG129 mice lethally infected with DENV in past studies.
Conclusion
Our results demonstrate that clinical DENV strains are capable of inducing severe diseases in A129 mice, which are prevented by anti-TNF-α antibody treatment. Since the important role of IFN-γ signaling in adaptive immune responses, either the cellular and humoral arms, has been well recognized, lethal A129 mouse models would contribute to the development of dengue vaccines in which functional immunity is required for pre-clinical assessment.

A35. Cell Culture Studies of the Barrier to Resistance of the Broad-Spectrum Antiviral Remdesivir Against Hepatitis C Virus

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The failure of hepatitis C virus (HCV) treatment due to direct acting antiviral (DAA) resistance can compromise the efficacy of highly active HCV therapy. The repurposing of existing antivirals such as the broad-spectrum nucleotide analog (NA) remdesivir (RDV) could be of interest for treating DAA-resistant HCV infections. We investigated the efficacy and barrier to resistance of RDV against HCV genotype 2 (J6/JFH1) in Huh7.5 cells.
The drug efficacy was assessed through dose–response assays. A long-term treatment of original HCV-2a with increasing concentrations of RDV was used to select drug escape viral variants. Resistance-associated substitutions (RASs) were identified using next-generation sequencing and their effect was assessed via reverse genetics. The fitness of escape and mutant viruses was assessed in growth kinetics assays based on the infectivity titer, and replication was investigated using subgenomic replicons expressing luciferase.
RDV was highly effective against HCV-2a, with an effective concentration 50% (EC_{50}) value of 43.5 nanomolar. An RDV escape variant exhibited a 4.7-fold increase in EC_{50} compared to the original virus. Cross-resistance was observed to GS-6620 (6.3-fold increase in EC_{50}), but not to sofosbuvir (2.1-fold increase in EC_{50}). An RDV escape showed faster growth kinetics compared to the original variant. Escape correlated with the emergence of substitutions throughout the viral genome including E143Q, T179A, and M289V/L in the polymerase. All substitutions increased RDV EC_{50}, particularly in combination (6.6-fold increase in EC_{50}), with E143Q identified as a major contributor to resistance. Except for M289L, all other substitutions decreased fitness, particularly when combined. The E143Q/T179A/M289L mutant virus was genetically unstable both in vitro and in vivo, losing the E143Q mutation gradually in Huh7.5 cells and human liver chimeric uPA/SCID mice.
RDV showed remarkable efficacy and high barrier to resistance in cell cultures. Clinical trials are needed to assess remdesivir's therapeutic relevance.

A36. Antiviral Activity of Glycopeptide Antibiotic Derivatives Against Chikungunya, Zika and SARS-CoV-2

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Viruses cause mild to severe health complications each day around the world. In the case of most infections, specific antiviral agents are not available, and only the symptoms are being treated. Chikungunya, Zika and SARS-CoV-2 are phylogenetically distant viruses. All three viruses have caused major epidemics throughout history and are still present in various parts of the world. Chikungunya (*Alphaviridae*) can cause fever, rashes, arthralgia and myalgia. The joint problems and pain can persist for several months or even years. A Zika virus (*Flaviviridae*) infection during pregnancy can result in severe complications, as it can cause microcephaly or other brain abnormalities to the unborn fetus. SARS-CoV-2 (*Coronaviridae*) can cause life-threatening conditions, like acute respiratory distress syndrome. The aim of our studies was to target these three distant viruses when testing potential antiviral agents. Derivatives of active substances already used in clinic practice, such as glycopeptide antibiotics, can be repurposed and their antiviral activity can be tested. We examined the antiviral effect of twenty-four derivatives against Zika MR766, Chikungunya (Guatemala isolate) and SARS-CoV-2 B.1.5. The in vitro testing of a newly synthesized theicoplanin-based drug was evaluated for its ability to inhibit infection using microscopic observations, MTT assay and RT-qPCR to measure viral particle number variation. Six derivatives showed strong antiviral efficacy against all three viruses, and one had a moderate effect. All six derivatives are conjugated with perfluoralkylated chains. Our study indicates that perfluoralkylated glycopeptide antibiotic derivatives can be used as broad-spectrum antivirals, but more studies are needed to determine their full potential in antiviral therapies. In the future, we would like to further investigate the effect of these drugs, both by expanding them to new viruses, and in terms of their mechanism of action.

A37. Human HERC5 Plays a Vital Role in the Type I Interferon Response Against Sars-Cov-2 and Is Antagonized by PLpro

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The virulence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in humans largely relies on its ability to counteract the type I interferon (IFN-I) response. Since the emergence of SARS-CoV-2, variants of concern have evolved that demonstrate enhanced infectivity and evasion of the IFN-I response. Human 'homologous to the E6-AP carboxyl terminus and regulator of chromosome condensation 1-like domain-containing protein 5' (HERC5) is a multifunctional antiviral protein that is vital for the IFN-I response towards evolutionarily diverse viruses and is best known as the main cellular E3 ligase for ISG15. HERC5 is significantly upregulated in SARS-CoV-2-infected individuals; however, the antiviral effectiveness of HERC5 against SARS-CoV-2 and the mechanisms of HERC5 evasion were previously unexplored. Our data show that HERC5 is a key mediator of the IFN-I response towards SARS-CoV-2 and the alpha, beta, gamma, and omicron variants by inhibiting viral replication and protecting cells from virus-induced cytopathic effects (CPEs) in vitro. Although HERC5 protected cells from delta variant virus-induced CPE and inhibited the production of the virus from cells, it exhibited a reduced ability to inhibit intracellular viral replication, suggesting that HERC5 predominantly acts at a later stage in viral particle production. Through HERC5 protein mutagenesis, we discovered that the amino-terminal RCC1-like domain and carboxyl-terminal HECT domain can be omitted without the loss of activity. Conversely, the 322 amino acid spacer region connecting these domains is indispensable for HERC5 antiviral activity. Further, our data suggest that SARS-CoV-2 variant papain-like protease (PLpro) exhibits differential activity against HERC5-mediated ISGylation, with delta PLpro being the most potent antagonist. In summary, we provide evidence that HERC5 is an important contributor to the IFN-I response towards SARS-CoV-2 infection and inhibits viral replication with E3 ligase-dependent and -independent mechanisms. Prospective antiviral treatments should target the restoration of HERC5 activity during infections, possibly by inhibiting PLpro.

A38. Structural Basis for mpox Virus Inhibition by Targeting Its Methyltransferase VP39

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Mpox is a zoonotic disease with pandemic potential. It is caused by the mpox virus (MPXV), a double-stranded DNA virus from the Poxviridae family. MPXV replicates in cytoplasm and thus it must encode for the DNA/RNA replication machinery. Besides the DNA-dependent DNA and RNA polymerases, it also encodes for the RNA capping enzymes, crucial for the translation and stability of viral RNA and for immune evasion [1]. One of the key components of the mpox virus' RNA capping machinery is the RNA methyltransferase (MTase) VP39.
To gain insight into the molecular details of the enzymatic function of MPXV VP39, we co-crystalized it with the pan-MTase inhibitor sinefungin or with the side product of the reaction, S-adenosylhomocysteine (SAH) [2]. Inspection of the SAH-binding site of MPXV VP39 revealed an unoccupied cavity adjacent to the adenine base of SAH, filled with a network of coordinated solvent molecules. We screened a small in-house library of SAH analogs bearing substituents at the 7-deaza position of the adenine base and identified several compounds that inhibited the VP39 enzyme significantly better than sinefungin. The identified VP39 inhibitors were co-crystalized with the VP39 protein to reveal the binding mode of the inhibitors. Finally, the selected VP39 inhibitors were successfully tested for their ability to inhibit MPXV replication in cells [3].
The discovery of this group of SAH-derived compounds can be the first step in the development of a completely new group of antivirals, aimed at blocking viral methyltransferases.
This work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) , Funded by the European Union—Next Generation EU.
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A39. Development of SARS-CoV-2 and mpox Virus Methyltransferase Inhibitors Has Revealed Compounds with Dual Specificity

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RNA methyltransferases (MTases) play a crucial role in synthesizing the 5' RNA cap, which is essential for RNA stability and efficient translation (Nencka et al., 2022). Over the past couple of years, both our team and others have developed numerous inhibitors targeting coronaviral MTases (Bobileva et al., 2021; Inniss et al., 2023; Otava et al., 2021). We have also successfully determined the crystal structures of these inhibitors bound to coronaviral MTases like nsp14 and nsp16/nsp10. More recently, we have extended our research to include the monkeypox virus MTase VP39. Our crystallographic studies, including VP39 in complex with the broad-spectrum MTase inhibitor sinefungin and various other inhibitors, have unveiled distinctions between coronaviral and poxviral MTases (Silhan et al., 2023; Skvara et al., 2023). However, they have also highlighted the potential for these enzymes to be targeted by the same or highly similar compounds (Silhan et al., 2023).

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A40. Bleomycin and Zeocin Preferentially Induce EBV-Positive Gastric Cancer Cell Death

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The Epstein—Barr virus (EBV) infects more than 90% of people worldwide and causes EBV-related cancers, such as EBV-associated gastric cancer. However, there has been no specific drug for EBV-related cancers. We have recently noticed that both Bleomycin and Zeocin induce cell death in response to DNA damage response (DDR), damaging EBV-positive cells than EBV-negative cells more specifically. Therefore, we investigated the molecular background of Zeocin-induced cell death in the EBV-positive gastric cancer cell line, AGS. Zeocin treatment more strongly induced DDR and phosphorylated γH2AX expression in EBV-positive cells than EBV-negative cells. Zeocin treatment also increased the number of annexin V-expressing apoptotic cells and cleaved caspase 3-positive cells in EBV-positive cells compared to EBV-negative cells. Furthermore, the Zeocin treatment of EBV-positive cells induced the expression of BZLF1, which induced EBV lytic replication and increased the production of infectious virus particles. Almost similar results were obtained with the use of Bleomycin. However, these phenomena were not recognized by MKN28 cells with mutations in P53. In conclusion, Bleomycin and Zeocin may induce DDR in an EBV-positive cell-specific manner, which cause host cell death via the activation of EBV lytic replication and subsequent caspase-3-dependent apoptosis.

A41. Immuno-Informatics-Driven Multi-Epitope-Based Vaccine Design Against SARS-CoV-2 Variants of Concern: Insights into Molecular Interactions and Immunological Responses

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The SARS-CoV-2 virus, responsible for the COVID-19 pandemic, poses a significant health threat to humans. Although SARS-CoV-2 is susceptible to mutations, specifically in the spike protein, the substantial conservation in the nucleocapsid protein can be exploited for a degree of cross-protective effectiveness. Hence, to achieve effective cross-protection against existing and future strains, a nucleocapsid (N) protein-based multi-epitope vaccine may have the ability to reduce the severity of the illness. To address this issue, we employed an immuno-informatics approach to formulate a novel vaccine candidate against SARS-CoV-2 variants of concern (VOCs). In this study, we predicted 07 CD4+ and 06 B-cell linear epitopes to design a multi-epitope-based vaccine (MEV) with various peptide linkers and an adjuvant. The designed MEV shows a suitable physicochemical landscape against SARS-CoV-2 VOCs. The 3D structure of the MEV was also developed and evaluated using different web servers, which resulted in a stable protein structure. We then assessed the interaction between MEV and immune receptors HLA-B*08:01 and TLR-4 using docking analysis. Additionally, our immune simulation results suggested that the MEV can trigger innate and adaptive immune responses. Therefore, we anticipate that the designed MEV will offer protection against the existing VOCs of SARS-CoV-2 and any emerging strains.

A42. SARS-CoV-2 nsp14 Methyltransferase Activity Assay for Inhibitor Screening

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Since the outbreak of the COVID-19 pandemic, efforts have been made to find ways to halt the replication of the causative agent, SARS-CoV-2. In addition to targeting the viral RNA polymerase, the capping machinery could serve as a potential drug target [1]. This is because the cap is essential for the stability of viral RNA and, most importantly, for evading the innate immune system. Coronaviruses encode unique proteins to cap their mRNAs. First, they attach GTP to the newly formed RNA, likely through a sophisticated cooperation between nsp12 and nsp9. Then, they methylate the GTP nucleobase at position 7 using the nsp14 N7-methyltransferase. Finally, they install a methyl group on the 2′-hydroxyl group of the first following nucleoside using the nsp16 2′-O-methyltransferase.
We have developed a method coupled with the ECHO-MS system for measuring methyltransferase activity and used it to screen for inhibitors of SARS-CoV-2 nsp14 N7-methyltransferase [2]. Using this method, we have tested and selected compounds that inhibit nsp14 with an IC_{50} of around 20-50 nM. These inhibitors are analogs of S-adenosyl-L-homocysteine, which have been rationally designed to improve specificity and activity.
This work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID project no. LX22NPO5103) and funded by the European Union's Next-Generation EU.
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A43. Development and Validation of a New In-House Phenotypic Assay for HIV-1 Drug Resistance Testing in Clinical Samples

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The global efforts to combat the HIV/AIDS pandemic are being hampered by the increasing prevalence of resistance to antiretroviral drugs (ARVs). The testing of HIV drug resistance is crucial in determining the best treatment regimen especially for the patients with a history of treatment failure. Genotypic assays that detect resistance mutations in viral genes are the mainstay resistance tests. However, the interpretation of the results is sometimes difficult, especially in cases of new or complex patterns of mutations. Therefore, phenotypic assays are recommended in cases of uncommon mutations or a complex pattern of mutations, like the case of resistance to multiple classes of drugs. In our study, we established a new in-house phenotypic assay that enabled the culturing of plasma-derived infectious HIV in the presence of ARVs. Our assay is based on the use of specially prepared polycationic amyloid fibrils of synthetic prostatic acid phosphatase that are able to capture and efficiently isolate the infectious HIV from the plasma. We compared drug resistance testing using the new assay and a genotypic assay and found that the new assay could detect the resistance that was missed by the genotypic assay. This could be due to the fact that the amyloid fibrils can bind and isolate all the virus particles from the plasma, including the minor HIV variants, which also contribute to drug resistance and are usually missed during genotypic testing. The studies are ongoing using post COVID-19 clinical samples to determine whether the disruption of treatments during the pandemic could have introduced new drug resistance patterns.

A44. Pan-Pox-Specific T Cell Responses in HIV-1-Infected Individuals After JYNNEOS Vaccination

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People living with HIV (PLWH) are the individuals most affected by the current Monkeypox virus (MPXV) outbreak that was first announced in May 2022. Here, we describe pan-pox-specific antibody and T cell responses in a cohort of HIV-1-infected individuals after receiving the non-replicative, attenuated smallpox vaccine JYNNEOS from Bavarian Nordic (BN). Intradermal (i.d.) and subcutaneous (s.c.) vaccination was safe without major side effects. Low antibody but efficient T cell responses were induced. Dose-sparing i.d. vaccination was superior to s.c. vaccination and promoted T cell polyfunctionality and the expression of the gut-homing marker α4β7 integrin on lymphocytes. HIV-1-infected individuals with CD4 T cell counts 500/mm 3 blood required at least a booster vaccination to exhibit efficient virus-specific T cell responses. The magnitude of the Th1 response after this booster directly correlated with the CD4 T cell count of the vaccinees. In summary, we provide an early indication of how to best proceed with preventive vaccination against mpox in a group of individuals with high infection risks.

A45. Accelerating Drug Discovery Aagainst SARS-CoV-2: Utilizing High-Throughput Screening and Nano-Luciferase Reporter Viruses

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The World Health Organization reported that the pandemic caused by SARS-CoV-2 in 2019 resulted in 771 million cases and nearly 7 million deaths worldwide. Research has shown that many of the proposed drugs have limited or no clinical usefulness for treating SARS-CoV-2 and the discovery of new drugs against SARS-CoV-2 has become a matter of utmost importance to prepare for future pandemics.
The most restrictive requirement for drug discovery targeting live SARS-CoV-2 is the BLS-3 specification, followed by the selection of an appropriate cell line for virus infection, and lastly, the multifaceted and time-consuming process that involves virus inactivation, immunostaining, and imaging.
Our group has installed a High-Throughput Screening (HTS) platform that utilizes the recombinant reporter virus icSARS-CoV-2-nLuc in conjunction with an engineered A549 cell line expressing ACE2 + TMPRSS2 receptors, as well as a semi-automated process relying on different automatic liquid handlers. First, we correlated the luminescence signal with the virus titer and confirmed the sensitivity of icSARS-CoV-2-nLuc to various reference drugs. Then, we validated the signal obtained at 24 h post infection with the nLuc in vitro using various microplate formats following the NIH guidelines, and we confirmed the reliability of the automated dispensing systems, which consistently produced robust results. Finally, we screened the MMV Global Health Priority box and discovered several promising hits against SARS-CoV-2 that will be subjected to further characterization.
By bypassing the classical method of virus detection and relying on the nLuc signal, we obtained a screening capacity of 10K compounds a week and the possibility to switch the screening against any recombinant virus expressing the nLuc gene. Furthermore, we are implementing other reporter viruses, like icMERS-CoV-nLuc, to the HTS workflow as well as an IVIS protocol to accelerate the in vivo drug screening.

A46. Development and Validation of Mayaro Virus Nanoluciferase as a Potent Tool for Drug Discovery

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Mayaro (MAYV) virus has drawn the attention for its ability to cause febrile illnesses, clinical complications, and chronic manifestations. Furthermore, there is no licensed antiviral therapy to treat Mayaro fever. In this context, novel tools to the development and validation of antiviral against MAYV represent an important approach for drug discovery. Additionally, adamantane derivatives exhibit interesting pharmacological properties and present previously reported inhibitory activity against some viruses. Here we assessed the antiviral activity of an adamantane derivative (A1), against MAYV *in vitro*, and also validated its antiviral action against Zika virus (ZIKV) in order to assess its potential broad-spectrum activity. A stable infectious clones of MAYV harboring the nanoluciferase reporter under the CMV promoter (MAYV-*nanoluc*) was developed. For antiviral assays, Vero-E6 cells were treated with A1 in a two-fold serial dilution ranging from 2 to 400µM and infected with MAYV-*nanoluc* at a multiplicity of infection (MOI) of 0.1 for 24h, or with $ZIKV_{PE243}$ at an MOI of 0.01 for 72 hours. MAYV replication levels were quantified by measuring *nanoluciferase* activity, and ZIKV titers were evaluated by focus formation units using immunofluorescence assay. The results demonstrated that A1 presented a dose-dependent antiviral activity, with an EC_{50} of 80.3 µM, CC_{50} of 194.2 µM, and selectivity index (SI) of 2.42 for ZIKV. For MAYV, EC_{50} and CC_{50} values were 88.5 µM and 291.9 µM, respectively, presenting a SI of 2.3. Our findings demonstrate the applicability of the MAYV infectious clone as a powerful tool for high-throughput antiviral screening aiming to identify drug candidates against MAYV. Additionally, we employed this system we identified aminoadamantane (1) as an interesting derivative to be repurposed for the treatment of Mayaro and Zika fever, which may further stimulate its potential as a broad-spectrum antiviral drug.

A47. New Synthetic Pd(II) Aminoadamantine Complexes Strongly Impair SARS-CoV-2 Infection

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Introduction: Despite the vaccination and the use of emergency repurposing drugs, COVID-19 still poses as a threat to health system. It is essential to develop new antiviral molecules that can abrogate SARS-CoV-2 infection in infected patients. Here we evaluated activity of new and unique Pd(II) complexes with aminoadamantanes Adamantine (Pd-atd), Rimantadine (Pd-rtd), and Memantine (Pd-mtn) against SARS-CoV-2 infection. **Methods:** Dose-response assay was performed with Pd-atd, Pd-rtd, and Pd-mtn in A549-AT cells in the presence or absence of SARS-CoV-2-Wuhan infectious clone expressing NeonGreen (SARS-CoV-2-NeonGreen). Time of drug-addition assay was performed with each compound. Broad-spectrum activity was evaluated using SARS-CoV-2-MCherry expressing Delta or Omicron spikes as well as both wild-type variants. Cell-cell transmission and resistance-inducing assays were also performed for each compound. **Results:** Pd-atd, Pd-rtd, and Pd-mtn had a selectivity index (SI) of 14.3, 36.2, and 23.5, respectively. Pd-atd inhibited all stages of viral replication (higher inhibition in entry steps), while Pd-rtd and Pd-mtn impaired attachment and uncoating. The complexes did not decrease Delta titters, however decreased Omicron's in two-folds. Additionally, the molecules also did not inhibit SARS-CoV-2-MCherry-S-Delta and showed low inhibition of SARS-CoV-2-MCherry-S-Omicron. Cell-cell transmission decreased in all treatments. Pd-rtd induced viral resistance after 1 passage in cell culture recovering higher titters compared to the control, however lost resistance with the continued passaging. Pd-atd and Pd-mtn did not induce resistance until the 5th passage. **Conclusion:** Pd(II) complexes were able to inhibit SARS-CoV-2, mainly on entry steps with high SI, and further studies are necessary to identify the exact mechanism of action.

A48. An Atlas of Structural and Dynamic Variation in the HIV-1 Env Glycoprotein

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The extraordinary sequence diversity across HIV-1 isolates is exemplified by the virally encoded surface glycoprotein, Env. At present, we have a limited understanding of the extent of structural variation and the impact on Env's antigenic profiles that results from the underlying sequence differences among HIV isolates. In order to understand how broadly neutralizing antibodies achieve breadth and potency, it is essential not only to identify the conserved aspects of their target epitopes, but also to identify how they cope with and manage the extremely variable contexts in which the conserved features are embedded. Here, we use hydrogen/deuterium exchange mass spectrometry (HDX-MS) to compare the structural dynamics in native-like Env trimers across a panel of HIV-1 isolates that is representative of the global diversity of the circulating virus. The impact of epitope dynamics and stability on antibody binding and neutralization are examined. These results provide a structural dynamic atlas of variation embodied in diverse Env trimers and offer new insights into the nature of neutralization-sensitive versus resistant isolates of HIV-1. Understanding the structural link between the neutralization phenotype, the activity of an antibody, and a given Env is anticipated to be pivotal for the development of engineered vaccines against HIV-1.

A49. Humoral and Cellular Immune Responses Induced by DNA Expressing Bivalent Immunogen-Fusing Capsid Proteins from Porcine Circovirus Genotypes 2a and 2b

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Porcine circovirus type 2 (PCV2) is the main causative agent of porcine circovirus-associated disease (PCVAD), one of the crucial diseases that has a profound impact on the swine industry worldwide. While most commercial PCV vaccines are developed based on PCV genotype 2a (PCV2a), PCV2b has become a predominant genotype since 2003. In this study, we developed and evaluated a bivalent vaccine capable of inducing immune responses against both PCV2b and PCV2a. We designed a new bivalent PCV2 immunogen combining PCV2a and PCV2b capsid proteins in the form of fusion protein (PCV2b-2a), and then further modified with signal sequence (SS) and a fusion protein linking granulocyte macrophage-colony stimulating factor (GM-CSF) with interleukine-4 (IL-4) (GMCSF/IL4 or GI). Delivered by DNA vector pVAX1, four immunogen constructs were efficiently expressed in a mammalian cell line. An immunogenicity study in BALB/c mice revealed that the DNA co-expressing the PCV2b-2a immunogen and GMCSF/IL4 (pVAX1.PCV2b-2a-GI) was most potent at inducing PCV2-specific immune responses, both antibody and T cell responses. Interestingly, DNAs bearing PCV2b-2a and PCV2b-2a-GI both skewed immune response towards Th1 phenotype (IgG2a > IgG1). By testing predicted peptides in immunoassays, the most immunoreactive B-cell and T-cell epitopes were identified on 3 and 5 regions of the PCV2 Cap protein, respectively. Sera from mice immunized with DNA expressing PCV2b-2a and PCV2b-2a-GI significantly inhibited PCV2a cell entry, both at serum dilution 1:8. Importantly, the vaccines induced immune responses that recognized both genotype-specific and PCV2-conserved epitopes, suggesting broad immune responses across PCV2 genotypes. These results suggest the great potential of our PCV2b-2a-based vaccine, which can be further developed with other vaccine platforms to achieve both vaccine efficacy and production cost.

A50. Development of Antiviral Agents and Structural Analysis of the Lambda-2 Pentameric Turret mRNA Capping Complex from Mammalian Orthoreovirus

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Mammalian Orthoreovirus (MRV), a non-enveloped icosahedral virus (60 to 100 nm in diameter), harbors a linear, segmented double-stranded RNA genome (~23.5 kb). MRV infections are prevalent in mammalian hosts, causing mostly mild symptoms in humans. Taxonomically, MRV belongs to the *Orthoreovirus* genus within the *Reoviridae* family and *Spinareovirinae* subfamily. It is further classified into four distinct serotypes: Type 1 Lang (T1L), Type 2 Jones (T2J), Type 3 Dearing (T3D), and Type 4 Ndelle (T4Nd). The MRV genome comprises ten segments, categorized as three large (L1-3), three medium (M1-3), and four small (S1-4) segments, encoding λ (lambda), μ (mu), and σ (sigma) proteins, respectively. Notably, the outer capsid Lambda-2 (λ2) protein demonstrates multifunctionality, catalyzing RNA guanylyltransferase, RNA-7N-, and RNA-2'O-methyltransferase activities crucial for mRNA capping of the viral plus-strand transcripts, culminating in the formation of the 5' cap structure. The λ2 protein forms a pentameric structure characterized by a hollow cylindrical configuration with projecting turrets, facilitating the extrusion of capped transcripts into the cellular cytosol. This research study involves several steps. First, MRV-L2 genes from serotypes T1L and T3D are cloned into the pSUMO vector. Then, bacterial expression systems are used to overexpress the λ2 protein, followed by purification through fast protein liquid chromatography (FPLC) techniques. High-throughput screening methodologies encompass the utilization of a pan-MTase inhibitor, sinefungin, in addition to an in-house library of SAH analogs, featuring compounds bearing diverse aliphatic, aromatic, or heterocyclic substituents. Also, computational molecular screening and docking studies will be performed to identify potential inhibitors against the λ2 protein. Finally, structural analyses of the λ2 pentameric turret are conducted using cryogenic electron microscopy (cryo-EM) techniques in the presence of the developed inhibitors. The development of novel inhibitors targeting the MRV λ2 methyltransferase domain holds promise in mitigating potential future Reoviral infections.

A51. The Amazon Rainforest as a Source of Antiviral Molecules Against Mayaro Virus

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Introduction: The Mayaro virus (MAYV) is the etiological agent of Mayaro fever, a disease characterized by fever, rashes, myalgia, and arthralgia that can progress to a chronic condition, resulting in long-term polyarthritis and polyarthralgia. Currently, there are no antiviral drugs for the management of Mayaro fever. In this context, the Amazon rainforest presents a large and untapped source of molecules to be investigated as antiviral candidates to combat MAYV. **Methods:** Vero E6 cells were treated with 23 compounds isolated from the Amazon rainforest at the highest non-cytotoxic concentrations previously determined by MTT viability assays, and were infected with MAYV expressing nanoluciferase (MAYV-*nanoluc*) at a multiplicity of infection (MOI) of 0.1. After 24h, the cells were harvested using *Renilla*-luciferase lysis buffer, and virus replication levels were quantified by measuring *nanoluciferase* activity through the *Renilla*-luciferase assay system. The effective concentration of 50% (EC_{50}) and cytotoxic concentration of 50 % (CC_{50}) were calculated by plotting a dose–response curve for compound Am7, with concentrations ranging from 3.1 to 400µM in a two-fold serial dilution with the absence or presence of MAYV-*nanoluc* infection. A time-of-drug-addition assay was also performed to investigate the effects of the compound on different stages of the MAYV cycle. **Results:** Among the tested molecules, compounds Am7, Am20, and Am21 inhibited MAYV infection by 49%, 43%, and 36%, respectively. Since Am7 presents the best antiviral ratio, the EC_{50} and CC_{50} were determined, with values of 87 and 219.1 µM, respectively, resulting in a selectivity index (SI) of 2.52. This molecule exhibited a virucidal activity, inhibiting entry and reducing MAYV infection by over 79%. **Conclusion:** Am7 demonstrates potential as a template for the design of novel antiviral drugs against Mayaro fever, requiring further studies to investigate its mode of action.

A52. Potent Immune Induction by Epitope-Based DNA Vaccine Against Porcine Epidemic Diarrhea Virus

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Porcine epidemic diarrhea virus (PEDV), the causative agent of porcine epidemic diarrhea (PED), continues to cause economic losses in the swine industry worldwide. A spike (S) protein is the main target for PEDV vaccine development. However, it is a large and complex glycoprotein composed of around 1383 amino acids, thus liming protein production yield in host cells. To minimize the size of the immunogen but maintain the immunogenic regions important for immune induction, here, we developed a new DNA-based PEDV vaccine expressing a newly designed immunogen, namely, PEDVSME, a chimeric protein that combines the core of epitopes (COE) domain with three known B-cell epitopes: peptide M, S1D6 (bearing epitopes SS2 and SS6), and 2C10. Vectored by DNA vector pTH, the PEDVSME protein was produced at a high level in mammalian cells. The DNA vaccine pTH.PEDVSME was more potent at inducing a PEDV-specific immune response than the pTH-expressing COE in BALB/c mice. The vaccine induced a balanced Th1/Th2 immune response (comparable level of IgG2a and IgG1). ELISA testing with synthetic epitope peptides revealed epitope 2C10 as the most immunodominant B-cell epitope, followed by epitope S1B-17 in the COE domain. Sera from immunized mice could neutralize PEDV, and a competitive neutralization assay with peptides S1B-17 and 2C10 (S2B-33 in our study) indicated that antibodies recognizing these two epitopes have a neutralizing activity. Interestingly, B-cell epitopes S1D6 and 2C10 were also shown to have the ability to induce T cell response as shown by the ELISpot and IFN-g cytokine ELISA results, suggesting their dual functions. Taken together, the PEDVSME-based DNA vaccine showed its potency to stimulate both antibody and cellular immune responses in mice; thus, it is worth further development with other vaccine platforms and investigation in pigs, the real targets of this vaccine.

A53. Immunodominant Linear B-Cell Epitope Profiles of Proteins p30, p54, p72, and CD2v From African Swine Fever Virus and Development of Peptide-Based ELISA

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African swine fever (ASF) is a dangerous disease currently affecting the swine industry worldwide. Both detection kits and vaccines are important for ASF monitoring and control. Although African swine fever virus (ASFV) is a complex virus and its genome encodes > 150 proteins, four proteins, namely p30, p54, p72, and CD2v, are among the proteins that are most studied and frequently used for the development of detection kits as well as vaccines. To provide more insights into B-cell epitope profiles of these four proteins, we tested B-cell epitope peptides obtained from immunoinformatics prediction with pig sera collected from pigs in three different stages of ASFV infection: (i) survivor pigs, (ii) healthy pigs from low-incidence farms, and (iii) sick (infected) pigs. An antibody ELISA test conducted using a commercial kit showed a positive result only with sera from survivor pigs, while testing with recombinant p30, p54, and p72 proteins showed high OD450 in sera from survivor pigs and healthy pigs from low-incidence farms, while OD450 in sera from sick pigs was lower. We were able to identify the immunodominant epitopes of each protein using ELISA and synthetic peptides. Interestingly, immunodominant epitope profiles were different in pigs with different stages of infection. We then applied this information to the development of a peptide-based antibody ELISA test. By using the mixtures of the chosen peptides as antigens, our peptide-based ELISA test offered a higher sensitivity compared to the commercial ASF ELISA kit. Our ELISA test could detect broad ranges of samples including serum, saliva (oral fluid), and colostrum/milk. In addition to the ELISA test, profiles of B-cell epitopes may be useful and applicable for ASFV vaccine development.

A54. Exploring the Antiviral Potential of Steroid–Styryl Compounds Against Influenza A Virus Infection

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The influenza A virus (IAV) poses a significant threat to global public health as a causative agent of the flu. So far, vaccines are the best way to prevent and control IAV infections. However, their effectiveness is compromised by the virus' high mutation rate, demanding frequent modifications in vaccine composition for every flu season. Antivirals serve as a crucial line of defense in combating IAV infections. Unfortunately, the persistent emergence of resistant strains has drastically reduced their efficiency. There is hence an urgent need to develop potent new antivirals against IAV.
Our group has previously demonstrated the antiviral potential of the steroid compound HASQ-6Cl, which induced a significant decrease in the formation of new infectious viral particles by inhibiting protein aggregation and virus particle assembly. In this work, we analyzed the antiviral capabilities of modified versions of HASQ-6Cl, specifically eight different hybrid compounds incorporating a steroid moiety and/or styryl group with a specific physicochemical with a predicted antiaggregation capacity.
Several of these compounds presented antiviral activity, with HASC-4F standing out as the most promising candidate. From a structure–activity relationship perspective, it was concluded that the styryl moiety has a notable impact on viral propagation inhibition, with its effectiveness further enhanced by the presence of a fluorine atom at carbon-4 of the aromatic ring of the styryl group. Furthermore, we have demonstrated that HASC-4F's antiviral effect is IAV-specific, and it does not seem to impact virus protein translation.
Our results suggest that steroid- and/or styryl-based compounds hold substantial promise as candidates for the development of novel and effective antivirals against IAV infections.

A55. Lithium: A Double-Edged Sword in HIV-1 Cure Strategies

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Background: The major barrier to an HIV-1 cure is the rapid establishment of latently infected cells. Targeting the latent reservoir can be achieved by promoting deep latency, thereby containing the virus expression and spread. Lithium has been shown to intersect with the NF-κB pathway and autophagy, the latter being implicated in HIV-1 cure strategies.
Hypothesis: Lithium intersects with TNF-α pathway and attenuates the virus expression.
Methodology: Latently-infected J-lat 10.6 cells were used to analyze how LiCl influences HIV-1 reactivation. In parallel, we infected SupT1 cells with the dual reporter HIV Nef_CRMZY at 14 days post infection, to analyze how LiCl affects latent and productive infections (productive infection: ZsGreen^{+}Crimson^{+}; latent infection: ZsGreen^{+}Crimson^{-}). In both cases, the cells were treated with LiCl for 24 hours ahead of HIV-1 reactivation using PMA or TNF-α. Reactivation was measured by flow cytometry and Western blotting, and autophagy induction was measured by immunofluorescence.
Results: LiCl induced autophagy in J-lat cells. LiCl markedly decreased virus reactivation by TNF-α, and to a lesser extent by PMA, suggesting that LiCl antagonizes the TNF-α signalling pathway independent of LiCl-induced autophagy. In the ATG5 knock-down cells, the effect of LiCl regarding virus expression was even greater, suggesting that autophagy induction may favour HIV-1 reactivation. Consistently, the LiCl treatment of the SupT1-HIV Nef_CRMZY-infected cells increased the basal level of HIV-1 expression, but the addition of TNF-α did not reactivate HIV-1 to the same extent, suggesting that, although LiCl alone induces virus expression, it attenuates TNF-α-induced reactivation.
Conclusion: Lithium acts as a latency-reversal or latency-promoting agent in different contexts. First, autophagy induction may favour reactivation, while in the second, autophagy is dispensable, and the intersection of LiCl with TNF-α plays a major role. These results demonstrate the complexity of host–pathogen interactions and how drugs introduced in different contexts during the infection can change the outcome of virus replication.

A56. Harnessing the Innate Immune Response Through NOD1 Agonists Prevents SARS-CoV-2 Infection in Human Lung Epithelial Cells

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The difficulty of exerting a direct antiviral activity in the lungs is one of the major roadblocks to managing Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). Boosting the innate immune response of the respiratory mucosa at early stages of the infection is a potential intervention for treating respiratory infections.
We evaluated IL8 production upon 24h incubation with immunomodulators targeting the main pattern recognition receptors (PPRs) in lung epithelial A549 or myeloid THP-1 cells. NOD-like receptor (NLR) agonists were characterized through their proinflammatory profile in A549 and the activation of the NF-κB and interferon-sensitive response element (ISRE) pathways in A549-Dual-hACE2-TMPRSS2 cells. The specificity of NOD1 and NOD1/2 agonists in lung cells was assessed by transient downregulation of NOD1gene expression (siRNA) and selective NOD1 and NOD1/2 inhibitors. Systemic activation by NOD agonists was evaluated in PBMCs. The antiviral activity of NOD agonists was determined in A549-Dual cells in vitro-challenged with SARS-CoV-2-GFP as measured by flow cytometry at 48h infection.
NLR agonists TriDAP (NOD1) and M-TriDAP (dual NOD1/2) showed up to a 3.3-fold increase in IL8+ cells in a dose-dependent manner, without impairing cell viability. Their activity was 2-fold-higher compared to the LPS control. NOD1 and dual NOD1/2 agonists induced the NF-κB and ISRE pathways. NOD1 downregulation resulted in a 93% decrease in IL-8+ cells cocultured with TriDAP or M-TriDAP. Selective NOD1 and NOD1/2 inhibitors impaired the NOD1-induced activation of NF-κB/ISRE. PBMCs were unresponsive to NOD1 agonists, suggesting tissue specificity of NOD1 in lung epithelial cells, without a global systemic activation. Finally, Tri-DAP and M-TriDAP promoted an antiviral environment that prevented SARS-CoV-2 replication in lung epithelial cells.

A57. Bunyavirus Induction of Host Cell Complement Responses

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Complement (C') is a powerful component of the innate immune system that recognizes and lyses virus-infected cells. The negative-stranded RNA virus La Crosse Virus (LACV) is transmitted by mosquito bites, with skin cells being the first to be infected before the virus disseminates to the central nervous system (CNS). Here, we have examined the interactions of C' with LACV-infected human skin cells. LACV productively infects human dermal fibroblasts and their infection results in the production of progeny virions. We have found that in contrast to infection with parainfluenza virus, LACV-infected dermal fibroblasts are resistant to C'-mediated lysis. We hypothesized that this resistance is due to the LACV-induced upregulation of host cell inhibitors or regulators of C' activation (RCAs). RT-qPCR analysis of dermal fibroblasts infected with LACV or UV-inactivated LACV showed an induction of the host gene C' Factor H (CFH) by ~10- and ~22-fold, respectively. In contrast, other RCAs, such as CD46, CD55, and CD59, showed no induced expression. We hypothesize that induced endogenous CFH plays a role in the resistance of LACV-infected skin cells to C'-mediated lysis. Elucidating the mechanisms by which LACV evades C' enables future research in developing therapeutics that can counter this evasion, preventing severe disease.

A58. 7D Antagonizes CXCL4-Dependent Enhancement of Dengue Virus Infection by Augmenting Antiviral Immune Response in Mice

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Dengue is a major arboviral infection, causing significant morbidity and mortality in more than 100 countries. Effective antivirals and vaccines against dengue virus (DENV) are not available to date and there has been significant focus on the development of antivirals against the disease. Our recent study described that CXCL4, or PF4, is pro-viral for DENV. Its plasma level directly correlated with viral load in dengue-infected patients. This platelet chemokine inhibits interferon (IFN)-α/β synthesis by inhibiting the CXCR3: p38 pathway. AMG487, a CXCR3 antagonist, reverses this process, but it was withdrawn from clinical trials due to non-linear pharmacokinetics. In this study, we developed compound 7D as a promising candidate for inhibiting the replication of DENV2 in vitro and in vivo. 7D inhibited the replication of both DENV2 and DENV4 by improving IFN-α/β synthesis in U937-DC-SIGN cells, antagonizing the CXCL4:CXCR3: p38 pathway. 7D supplementation (8 mg/kg body weight) to DENV2-infected IFNα/β/γR$^{-/-}$ AG129 or C57BL6 WT mice significantly improved disease symptoms including thrombocytopenia and leukopenia, decreased vascular leakage, and increased survival. Further, 7D treatment induced IFNα/β levels in plasma and reduced the frequency of TNFα^{+ve} CD4 CD8 double-positive T lymphocytes. 7D also increased the activation of STAT3 and the proliferation of antigen-specific B lymphocytes and neutralizing antibody levels in DENV2-infected mice. Together, our studies identify compound 7D as an immuno-modulator, increasing IFN-α/β synthesis by suppressing the CXCL4:CXCR3: p38 pathway and augmenting neutralizing antibodies by promoting STAT3 activation in plasma cells.

A59. Alphaviruses Triggering the Type I IFN Response in the Absence of Viral RNA Templates

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Alphavirus infection in vertebrate hosts leads to the activation of the type I IFN response which has been commonly associated with the production of viral RNA species. More recent evidence suggests that some alphaviruses also activate such a response in the absence of viral RNA templates, as has been shown for Semliki Forest virus (SFV) and Sindbis virus (SINV). In our current study, we aim to characterize the genus further in this respect by analysing a panel of 10 alphaviruses and their replicases, including a selection of their mutant variants. We expressed solely the viral replicases in HEK293T cells, and the replicase activity in producing PAMP RNAs using cellular templates was then evaluated by isolating the subjected cellular RNA, followed by the transfection of the latter into COP-5 cells for IFN-b detection using ELISA. Although the observed levels of induced IFN-β vary significantly between different alphavirus replicases, the ten assayed replicases all induced detectable IFN-β levels via non-viral PAMP RNAs and more prominent IFN-β induction was observed for SFV, SINV, Barmah Forest virus (BFV), and chikungunya virus (CHIKV). Furthermore, in the case of CHIKV and BFV replicases, mutations that slowed down the non-structural polyprotein processing led to a significant boost in the induced IFN-β levels via non-viral PAMP RNAs. It is also noteworthy that some alphavirus replicases produce non-viral PAMP RNAs when expressed in C6/36 cells, a property that needs to be studied further perhaps in the context of the invertebrate innate immune system. Our results suggest that the activation of the type I IFN response using cellular RNAs as templates to produce PAMP RNAs might be a common feature of the alphavirus replicase, yet the extent of this property needs further investigation.

A60. Antiviral Strategies of Interferons to Control Intestinal Adenoviral Infection

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HAdV can cause infections of the gastrointestinal (HAdV-C5, HAdV-F41) and the respiratory (HAdV-C5) tracts. Most infections in immunocompetent patients are clinically inapparent. However, immunocompromised patients, especially children after allogenic human stem cell transplantation (HSCT), frequently suffer from severe infections. In these cases, infections can lead to disseminated disease, which is associated with increased morbidity and mortality. HAdV can establish persistent infections, especially in the adenoids, tonsils and the gastrointestinal tract. In immunocompetent hosts, interferon type I and II signaling (IFN type I/II) represses HAdV E1A protein expression, which reduces viral DNA replication and might lead to HAdV persistence in lymphocytes. Our aim is to understand intestinal adenoviral reactivation in immunosuppressed patients. We hypothesize that immunosuppression triggers the reactivation of persistent infections due to inhibition of the IFN signaling pathways. Consequently, the virus begins to replicate in lymphocytes, spreads to the intestinal epithelium, and causes disseminated disease. Our experiments showed that IFNα treatment during HAdV-C5 and -F41 infections repressed viral DNA replication, protein expression and progeny production in primary-like colon (HCEC-1CT) and lung carcinoma (A549) cells. Although IFNα repressed HAdV-C5 DNA replication in HCEC-1CTs for three weeks, low levels of viral DNA were still detectable, which indicates viral persistence in this cell line.

A61. Mechanism of the ERK Pathway Regulation of Enterovirus 2A Protein in Viral Replication

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Enterovirus A71 (EV-A71) is the most common pathogen causing severe hand, foot and mouth disease (HFMD) in children, which can lead to neurological or cardiorespiratory complications and even death. Enteroviruses can utilize various signaling cascades in their life cycle, and studies performed by us and others have demonstrated that the extracellular-signal-regulated kinase (ERK) pathway is essential for EV-71 through its involvement in the internal ribosome entry site (IRES) of EV-A71 by shutting down host cap-dependent translation mechanisms. However, the interacting viral proteins and mechanisms remain unclear.

Here, we found that ① $2A^{pro}$ of enterovirus EV-A71 is an important factor in up-regulating the efficiency of the viral IRES, which depends on the proteolytic activity of $2A^{pro}$. ERK pathway activity is essential for $2A^{pro}$ enzyme activity to mediate the translation process of the IRES and the key mechanism of $2A^{pro}$ cleavage of eukaryotic initiation factor 4GI (eIF4GI) in host cells. ② Ser/Thr125 residues of enterovirus $2A^{pro}$ are phosphorylated by ERK1/2, which favors enteroviral $2A^{pro}$ proteolysis, therefore enhancing virus replication and pathogenicity toward susceptible mice, and this phosphorylation site and its function are highly conserved in enteroviruses.

In summary, we report for the first time that host ERK-mediated phosphorylation of Ser/Thr125 in enterovirus $2A^{pro}$ increases viral host fitness to ensure its proliferation and pathogenicity. In addition, weakened viruses containing the S/T125A mutation may be a general strategy for developing attenuated vaccines against enteroviruses.

A62. Stabilization of a Single-Stranded DNA of Adeno-Associated Virus by Inverted Terminal Repeats

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Parvoviruses have evolved to possess a linear, single-stranded DNA (ssDNA) genome ranging from 4 to 6.3 kb. A member of the Parvoviridae, adeno-associated virus (AAV), contains an approximately 5 kb linear ssDNA within its capsid. This ssDNA features two 145 base inverted terminal repeats (ITRs) positioned at each end, and the ITRs have a T-shaped hairpin secondary structure that plays a crucial role in viral replication. To investigate the impact of ITRs on ssDNA stability, we conducted a DNA denaturation–reannealing assay in 10 mM magnesium acetate, 50 mM potassium acetate, and 20 mM tris-acetate buffer at pH 7.9. While conventional double-stranded DNA (dsDNA) fragments retain a reannealing capability of over 50% for sizes under 9.31 kb, gradually losing this capability as the sizes increase, the dsDNA fragments ranging from 2.5 to 6.3 kb in rAAV did not exhibit a reannealing profile. This suggests that the presence of ITRs at both ends hinders the annealing process between the complementary strands. These results indicate that ITR structures preferentially induce an ssDNA conformation under 6.3 kb in size, and that the stability of AAV ssDNA contributes to the viral life cycle, including processes such as infection, replication, and packaging. Considering the size of parvovirus genomes, it appears that their genomes require flexibility in the complementary DNA strands, and simultaneously, this adaptability needs to be regulated by specific palindromic inverted terminal repeats at both ends.

A63. Late Reactivation of Herpes B Virus After a Monkey Bite: A Case of Severe Meningoencephalitis

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Background

Macacine alphaherpesvirus 1 (McHV-1), also known as herpes B virus (BV), is an alphaherpesvirus endemic to several macaque species capable of causing zoonotic infections in humans with high mortality rates (~80%).[2]
BV is an occupational hazard for people working with macaques and a concern for tourism, zoos and the growing illegal pet trade.[3,4]
Evidence of reactivation in humans has rarely been reported.[5,6]

Case presentation

A 66-year-old woman, with no history of chronic diseases, was admitted to the hospital presenting with fever and drowsiness followed by an altered level of consciousness requiring intubation in an intensive care unit. Lumbar puncture revealed lymphocytic pleocytosis with mild hyperproteinorrachia.
Herpes simplex virus 1 DNA, herpes simplex virus 2 DNA, varicella zoster virus DNA, and enterovirus RNA remained undetectable by polymerase chain reaction (PCR) on repeated cerebrospinal fluid (CSF) samples.
The patient's relatives revealed an episode of lymphocytic meningitis 54 years prior following a bite from an infected monkey.
Medical information was ascertained from the patient's original medical files at the hospital where she was admitted and recovered at in 1965. She did not have any other exposure to monkeys during her life.
Following this information, her CSF and blood were tested for BV (McHV-1) DNA using a targeted PCR, which detected the McHV-1 genome in both samples.
Initial treatment with acyclovir was switched to ganciclovir.[7]
The patient exhibited complete recovery. We considered lifelong antiviral prophylaxis with acyclovir.[7]

Conclusion

We report for the first time that BV can reactivate in humans after 54 years of latency, causing severe meningoencephalitis.
Clinicians should consider BV as part of the differential diagnoses when a patient's medical history reveals exposure, even if it was a long time ago, to monkeys. The optimal duration of antiviral prophylaxis after BV central nervous system infection remains controversial.[7] However, this case provides support for lifelong antiviral prophylaxis in these patients.

A64. Fate of KSHV Tegument Proteins ORF45 and ORF52 During de Novo Infection

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Kaposi's sarcoma-associated herpesvirus (KSHV) is a human oncogenic virus primarily linked with the development of Kaposi's sarcoma. Like other herpesviruses, mature KSHV particles comprise a double-stranded DNA enclosed within an icosahedral capsid which is coated with a tegument layer, predominantly composed of proteins, and wrapped within a phospholipid envelope.
In this study, we investigated the fate of the KSHV tegument proteins open reading frame 45 (ORF45) and ORF52 during de novo infection of SLK cells in which KSHV establishes latency. ORF45, an immediate early lytic protein, is known to prevent virus-mediated type I interferon induction, while ORF52, a late lytic protein, inhibits the enzymatic activity of cGAS.
Confocal immunofluorescence microscopy revealed that docked capsids at the nuclear pores lacked both ORF45 and ORF52, indicating their prior dissociation from the capsids before nuclear docking. A time-dependent nuclear accumulation of ORF45 was noticed, whereas ORF52 surrounded the nucleus at early time points and its intensity diminished over time. Cycloheximide treatment abolished the nuclear accumulation of ORF45, confirming its de novo expression post infection. In line with these findings, Western blot analysis revealed increasing levels of ORF45 at early stages post infection, followed by its decline. ORF45 exhibited multiple bands indicative of likely post-translational modifications. No expression of the late lytic viral protein ORF65 or production of infectious virions was detected. Of note, cells treated with endosome acidification inhibitors demonstrated no dissociation of either ORF45 or ORF52 from the capsids, which failed to dock at the nuclear pores. This hindered the expression of ORF45, thereby preventing the establishment of latent infection.
Taken together, our findings reveal that capsids at nuclear pores lack the tegument proteins ORF45 and ORF52, with their dissociation contingent on endosome acidification. Furthermore, we revealed a transient de novo expression of ORF45 post infection, occurring prior to the establishment of KSHV latency.

A65. The KSHV ORF20 Short Isoform Participates in Coordinating Lytic Reactivation for Enhanced Infectious Particle Production

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Kaposi's sarcoma-associated herpesvirus (KSHV) is a cancer-causing agent that establishes a long-lasting infection. This study focuses on the conserved herpesviral protein ORF20 encoded by KSHV, which is a member of the herpesvirus UL24 protein family and encodes three co-linear isoforms (long, intermediate and short). Like the other members of the UL24 family, all the ORF20 isoforms contain five conserved homology domains and a predicted conserved PD-(D/E)XK endonuclease motif, whose potential enzymatic function has not been established yet. In an effort to determine the role of ORF20 during KSHV infection, a recombinant ORF20-Null KSHV genome, which fails to express all three ORF20 isoforms, was prepared. This genome was reconstituted in the iSLK cells to establish a latent infection, which resulted in the earlier accumulation of viral lytic transcripts and proteins and a significant decrease in the viral yield upon lytic reactivation. This was coupled with an increase in cell death at the early time points following lytic reactivation. The functional complementation of the ORF20-Null mutant with the ORF20 isoforms rescued KSHV production upon the expression of the short isoform, whereas its endonuclease mutant form failed to complement lytic reactivation. When assessing the localization of the short ORF20 isoform to its mutant form using immune fluorescent microscopy, we found they both localized predominantly to the nucleolus. RT-qPCR analysis confirmed that ORF20 exhibits late transcription kinetics. These findings suggest that the ORF20 protein participates in coordinating lytic reactivation, while the short isoform, with its putative endonuclease motif, is essential for this activity.

A66. Non-nucleotide RNA-Dependent RNA Polymerase Inhibitor That Blocks SARS-CoV-2 and Flaviviral Replication

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Coronaviruses (family Coronaviridae) and flaviviruses (family Flaviviridae) are positive-sense RNA viruses that cause numerous important human and animal diseases, such as acute diarrhea, vomiting, encephalitis, acute flaccid paralysis, hemorrhagic fevers or congenital abnormalities and fetal death. Unfortunately, both families have been proven to hold pandemic potential, as demonstrated recently by the ZIKV, MERS-CoV and SARS-CoV outbreaks. Therefore, effective treatment strategies are urgently needed to treat patients infected with flaviviruses. RNA-dependent RNA polymerase (RdRp) is a key enzyme in the replication of RNA viruses and therefore is the one of the most important targets for the treatment of viral infection [1, 2, 3].
In this study, we tested the ability of helquat-like ligand PR-673 against coronaviral and flavivirus RdRps. We used in vitro polymerase assays to show that these compounds interfere with RNA syntheses performed by the RdRps.
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Poster Session B—Friday 15 February

B1. Utilising Engineered Filamentous Bacteriophages as an Innovative Antigen Delivery Platform Combined with Adjuvant Gene Electrotransfer of IL-12 Plasmid Effectively Eradicated Aggressive Murine Melanoma

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Bacteriophages (prokaryotic viruses) possess many intriguing properties that make them valuable bioengineering tools. Their genome can be genetically modified, while bacteriophages per se induce an immunogenic response. Our study aimed to establish non-lytic M13 filamentous bacteriophages as antigen-delivering nanoparticles in combination with IL-12 plasmid with adjuvant activity as a vaccine for cancer immunotherapy. Genetically engineered bacteriophages expressing the tumour peptides MAGE-$A1_{161-169}$, $gp100_{25-33}$, or MELAN-A_{26-36} were produced using phage display nanotechnology. C57BL/6 mice were vaccinated intraperitoneally with 3×10^{12} pfu of bacteriophage cocktail five days after 5×10^{4} B16.F10 cell injection, and the vaccination was repeated three times at four-day intervals. When combining bacteriophages with gene electrotransfer (GET) of IL-12, plasmid DNA (0.5 mg/mL) was injected intratumourally when tumours reached 50 mm^3. The therapy's effect was monitored by measuring tumour volumes. Mice that were tumour-free at 90 days post treatment were considered cured, re-injected with tumour cells, and monitored for 90 more days. Tumours were collected on the third and sixth day of therapy and processed for HE staining and staining of CD4+ and CD8+ lymphocytes and macrophages. T cell cytotoxicity was assessed using the EliSpot assay. A significant overall enhancement in survival rate was observed in groups vaccinated with genetically engineered bacteriophages in combination with IL-12 GET, with some mice experiencing complete tumour regression. Mice treated solely with genetically engineered bacteriophages also exhibited a noteworthy improvement in survival rates; however, these mice did develop slowly growing tumours. Control animals exhibited no protection against the tumour challenge. Immunohistochemistry confirmed a strong infiltration of immune cells into the tumour and the surrounding skin. Additionally, we demonstrated the presence of activated antigen-specific T cells secreting IFN-γ and granzyme B. In summary, immunotherapy using an innovative bacteriophage-based vaccine combined with adjuvant IL-12 GET effectively triggered immune responses and successfully inhibited tumour growth, showing promise as a novel approach for melanoma treatment.

B2. The Double Life of Lipid Droplets: New Functions in Antiviral Innate Immune Defense

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As multifunctional organelles ubiquitously present in eukaryotic cells, lipid droplets (LDs) play a critical role for multiple pathogens. Among others, several members of the *Flaviviridae* family evolved to exploit LDs as a lipid source or a platform for the biogenesis of their replication organelle. In addition to their supporting function during viral infections, a recent study reported systemic changes of the LD proteome upon bacterial detection and a key defensive role for LDs in the host's antibacterial immune response.
While this study suggests that LDs might serve as molecular switches for innate immune signaling in the context of bacterial infection, the implication of LDs in antiviral immune responses is still elusive. In order to shed light on the antiviral functions of LDs, we systematically profiled changes in the LD proteome of several cell lines upon different innate immune stimuli, utilizing label-free mass spectrometry.
Collectively, this unbiased approach identified more than 4,000 different proteins in the LD fractions, of which 80–300 proteins were differentially enriched upon immune elicitation. In addition to well-known LD markers, previously reported LD-associated proteins with antiviral functions, as well as a number of novel innate immunity effectors, were also detected. The overexpression of a selection of the newly identified LD-associated proteins revealed a systemic impact on the replication of HCoV-229E and HCV reporter viruses. Follow-up mechanistic studies to assess the mechanisms of action and the relevance of lipid droplet localization for a selection of the newly identified LD-associated proteins are currently underway.

B3. Identification of a Secreted Form of the Rotavirus Outer Capsid Protein VP7

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Manipulation of the host immune system facilitates enhanced transmission of viruses during host infection. Different viruses employ a wide array of strategies to subvert the host immune system. One such strategy is the secretion of virus-encoded immunomodulatory proteins to regulate the innate and adaptive immune response. During an investigation of rotavirus infection within polarized intestinal epithelial cells, we identified a secreted form of the structural glycoprotein VP7. Antibodies against VP7 are neutralising and anti-VP7 serology is a correlate of immune protection against rotavirus infection. The secretion of VP7 was inhibited by brefeldin A and monensin, suggesting transit through the Golgi, and this is supported by the observation of a complex glycosylation signature not observed in the intracellular and virion-associated forms of VP7. Additionally, secreted VP7 was bound by confirmation-dependent neutralising antibodies, indicating that the secreted protein can act as an antigen decoy to inhibit the neutralisation of the infectious virus in the gut and thus improve transmission of the virus. This represents a novel mechanism of rotavirus antagonism of host immunity, but mimics a similar strategy observed across other viruses.

B4. Host Hsp70 Chaperone Network Participates in the Particle Formation of SFTSV

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Severe fever with the thrombocytopenia syndrome virus (SFTSV), a causative agent of a tick-borne hemorrhagic disease, hijacks various host biological machineries for its lifecycle, as is the case with other viruses. The stability of viral proteins is entirely dependent on the machinery of the infected cells, highlighting the interplay between cellular proteostasis components and their specific substrates. In this study, we investigated the role of the host Hsp70 chaperone network in SFTSV propagation. Our immunofluorescence analyses showed that certain Hsp70 isoforms and the cochaperone DnaJ formed puncta upon SFTSV infection. This puncta formation was also observed upon the overexpression of a viral protein. Treatment with allosteric inhibitors of Hsp70 action significantly reduced SFTSV propagation in Huh7 cells and in primary human umbilical vein endothelial cells (HUVECs). Time-of-drug addition experiments showed that Hsp70 function is crucial for virus particle production, but not for viral RNA replication. These data indicate that SFTSV recruits the host Hsp70 chaperone complex through the expression of the viral protein and promotes virus particle production, suggesting that specific Hsp70 recruits specific DnaJ and functions in a directional manner.

B5. Long-Range RNA:RNA Base Pairing Regulates Active Conformations of Frameshift Stimulating Elements in Coronaviruses

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All RNA viruses fold their genomes into structures that mediate essential processes in their life cycles. One ubiquitous task, regulating protein stoichiometry, is accomplished by many viruses using an RNA structure called a frameshift stimulating element (FSE). An FSE causes a fraction of ribosomes to shift into the -1 or +1 reading frame, thereby bypassing a stop codon in frame 0. The ratio of proteins upstream to downstream of the FSE is thus controlled by the fraction of ribosomes that the FSE causes to frameshift.
Every coronavirus requires frameshifting to express RNA processing enzymes, including its replicase complex. Reporter assays using the SARS-CoV-2 FSE have indicated that it folds into an RNA pseudoknot that causes 20-40% of ribosomes to frameshift. However, ribosome profiling studies have suggested double the frameshifting rate, around 50-70%. Moreover, we found that in live SARS-CoV-2, the FSE folds into an ensemble of structural states, none of which is consistent with the pseudoknot. These findings suggest that the structure and function of the SARS-CoV-2 FSE depend on external factors.
We hypothesize that one factor is RNA:RNA base pairing between the FSE and another genomic region. To investigate, we develop Structure Ensemble Ablation by Reverse Complement Hybridization and Mutational Profiling (SEARCH-MaP) and discover that the FSE base pairs with an RNA element one kilobase downstream in nearly 50% of the molecules. Using Structure Ensemble Inference by Sequencing, Mutation Identification, and Clustering (SEISMIC), we model and extensively interrogate the secondary structure of this one-kilobase RNA:RNA interaction. This structure overlaps with and would thus preclude the pseudoknot, hinting that it provides a mechanism to regulate frameshifting in SARS-CoV-2. Finally, we find similar long-range RNA:RNA base pairing in other SARS-related coronaviruses and in transmissible gastroenteritis coronavirus (TGEV), suggesting that such RNA structures are a general strategy among coronaviruses.

B6. Quantifying Genetic Interactions Between Mutant Genomes Using Single-Cell RNA Sequencing Approaches

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Detecting genetic interactions within viral populations typically relies on bulk methods to quantify interactions between mutants, usually based on gross measures of virus production to quantify the effects of interactions. However, recently developed single-cell sequencing approaches provide new avenues to quantify the interactions of viral mutants in individual cells and, significantly, to correlate these interactions with richer viral and cellular phenotypes associated with infection with specific mutants. Here, we use a novel method to examine genetic interactions between enterovirus mutants during virus infection and replicon transfection experiments. We quantify positive-to-negative strand ratios across thousands of individual infected cells to identify and quantify actively replicating cells and to observe cell phenotypes associated with infection with specific mutants. The ability to map genetic interactions to viral and cellular phenotypes simultaneously will enable high throughput screens of viral mutants and host factors and enable more precise dissection of the interactions between mutants within viral populations, including complementation and dominant negative effects.

B7. Structural Basis for RNA-Cap Recognition and Methylation by the Mpox Methyltransferase VP39

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Mpox, formerly known as monkeypox, is a zoonotic disease caused by the mpox virus (MPXV), which has gained attention due to its rapid and widespread transmission, with reports from more than 100 countries. The virus belongs to the Orthopoxvirus genus, which also includes variola virus and vaccinia virus. In poxviruses, the RNA cap is crucial for the translation and stability of viral mRNAs and also for immune evasion [1]. This study presents the crystal structure of the mpox 2′-O-methyltransfarase VP39 in a complex with a short cap-0 RNA. The RNA substrate binds to the protein without causing any significant changes to its overall fold and is held in place by a combination of electrostatic interactions, π-π stacking and hydrogen bonding. The structure also explains the mpox VP39 preference for a guanine base at the first position; it reveals that guanine forms a hydrogen bond that an adenine would not be able to form [2].

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B8. Interaction of SCoV-2 NSP7 or NSP8 Alone with NSP12 Causes Constriction of the RNA Entry Channel: Implications for Novel RdRp Inhibitor Drug Discovery

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RNA-dependent RNA polymerase (RdRP) is a critical component of the RNA virus life cycle, including SCoV-2. Among the Coronavirus-encoded proteins, non-structural protein 12 (NSP12) exhibits polymerase activity in collaboration with one unit of NSP7 and two units of NSP8, constituting the RdRp holoenzyme. While there is abundant information on SCoV-2 RdRp-mediated RNA replication, the influence of interplay among NSP12, NSP7, and NSP8 on template RNA binding and primer extension activity remains relatively unexplored and poorly understood. Here, we recreated a functional RdRp holoenzyme in vitro using recombinant SCoV-2 NSP12, NSP7, and NSP8, and established its functional activity. Subsequently, molecular interactions among the NSPs in the presence of a variety of templates and their effects on polymerase activity were studied, wherein we found that NSP12 alone exhibited notable polymerase activity that increased significantly in the presence of NSP7 and NSP8. However, this activity was completely shut down, and the template RNAprimer complex was detached from NSP12 when one of the two cofactors was present. Through computational analysis, we found that the template RNA entry channel was more constricted in the presence of one of the two cofactors, which was relatively more constricted in the presence of NSP8 compared to that in the presence of NSP7. In conclusion, we report that NSP7 and NSP8 together synergise to enhance the activity of NSP12, but antagonise when present alone. Our findings have implications for novel drug development, and compounds that obstruct the binding of NSP7 or NSP8 to NSP12 can have lethal effects on viral RNA replication.

B9. Structural Alterations During the Disassembly of Non-enveloped Viruses

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Capsid of non-enveloped viruses exhibit extreme structural stability, however factors like receptor interaction, low endosomal pH etc. trigger capsid disassembly allowing their entry in the host cells. This disassembly process represents an important aspect in viral infectivity and any disturbance in this can significantly impede infectivity. Despite its importance, due to difficulty in isolating the disassembly intermediates from infected cells in sufficient quantities for detailed structural analysis it has been difficult to study the disassembly process in depth. We aim to address this by generating in vitro disassembly intermediates of two non-enveloped viruses: *Feline Calicivirus* and *Flock House Virus* in a sequential fashion, using a judicious combination of viral uncoating conditions, mimicking the milieu encountered during cellular entry, and subsequent characterization using Cryo-electron microscopy. These structural analyses of disassembly intermediates by Cryo-EM based methods, including single particle reconstruction and tomographic reconstructions, is expected to provide a temporal map of virus disassembly and genome release. This knowledge is of paramount importance for designing of therapeutic molecules to block this critical early stage of infection and prevent virus propagation. We posit that understanding the underlying chemistry of virus disassembly can lead to design of inhibitors to prevent this initial infection step effectively and offer new avenues for antiviral intervention strategies.

B10. Insights Into Molecular Interactions Between nsp14 and nsp10 in SARS-CoV-2 Replication

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Coronaviruses are large RNA viruses, with genomes of over 30 kb in some species. They cause a range of diseases in birds, mammals, and humans. While most human coronaviruses lead to mild respiratory infections, in the last two decades, we saw two major epidemics and a global pandemic, mainly caused by the virus SARS-CoV-2, causing severe respiratory illnesses and fatalities. Within the coronavirus genome, there is a gene encoding a non-structural protein 14 (nsp14), which possesses 3'-5'exonuclease and methyltransferase enzymatic activities. The exonuclease nsp14 participates in the repair of misincorporated nucleotides during viral genome replication, and its presence is exceptionally rare among RNA viruses. The exonuclease activity of nsp14 is significantly enhanced by the binding of another viral protein, nsp10, which lacks enzymatic activity, but acts as a critical cofactor for several enzymatically active coronavirus nsps. Both nsp14 and nsp10 are highly conserved and have similar sequences among the various coronavirus species.
To characterize the exonuclease activity of nsp14 and its interactions with nsp10, mutant versions of the nsp14 and nsp10 proteins were created using PCR mutagenesis. Both the wild-type and mutant versions of nsp14 and nsp10, as well as a truncated version of nsp10, were produced in a bacterial expression system utilizing *E. coli* bacteria. The exonuclease activity of each nsp14 variant was observed *in vitro* using activity assays with ssRNA, dsRNA, and dsRNA, with mismatched base pairs as the substrates in the presence or absence of nsp10 variants. The results of this study provide insights into the functioning of nsp14 exonuclease and its interactions with nsp10, which may serve as a basis for more detailed characterization in future research.

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B11. Structural Basis of Broad Protection Against Phylogroup 1 Lyssavirus by Antibodies A6 and F11

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Lyssaviruses, a group of deadly neurotropic viruses, pose a severe threat to both humans and animals. Infections with rabies and related lyssaviruses are often fatal once they infiltrate the central nervous system, leaving a limited window for effective intervention. However, a beacon of hope emerges in the form of a human monoclonal antibody, F11, which has demonstrated remarkable potential in treating established infections, even after the virus has breached the CNS. F11 exhibits the capacity to reduce viral load within the brain and effectively reverse symptoms through a CD4 T cell-dependent mechanism. In our study, we delve into the structural and epitope characteristics of F11 and its closely related antibody, A6, which shares over 90% sequence identity. Utilizing electron microscopy single particle analysis, we uncovered that these two antibodies target domain III of the lyssavirus glycoprotein G. Notably, our findings indicate that this recognition is exclusively compatible with the prefusion conformation of G. Our results offer valuable insights into the therapeutic efficacy of these antibodies, and sets the stage for further enhancement of these antibodies' efficacy, holding significant promise for the treatment and prevention of lyssavirus infections.

B12. Human Rhinoviruses: An Eminent Contributor to Pediatric Acute Respiratory Distress Syndrome

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The primary objective of this research was to evaluate the incidence of Pediatric Acute Respiratory Distress Syndrome (PARDS) among children hospitalized with Acute Respiratory Infection (ARI) and to determine the causative agents when viral in origin. Over a span of four years (2017-2021), our study observed 736 patients under the age of 18 who were admitted to the Children's Hospital Zagreb exhibiting ARI symptoms with suspected viral involvement. In addition to adhering to established clinical and laboratory protocols, we collected nasopharyngeal and pharyngeal swabs and conducted multiplex PCR analysis to detect 15 respiratory viruses.
Among the cohort of 736 patients, 22 children developed PARDS, accounting for a prevalence of 2.99%. This group comprised 15 males and 7 females, with a median age of 1.75 years. All PARDS patients received oxygen therapy, with five necessitating mechanical ventilation. Fourteen out of the twenty-two children were prescribed antibiotics, and fourteen of them had pre-existing medical conditions. Clinically, ARDS onset was associated with preceding conditions such as pneumonia (eight cases), acute bronchitis (five cases), asthma exacerbation (four cases), bronchiolitis (three cases), suspected bronchodysplasia in one child, and aspiration during an extended stay in the PICU in another child.
In 18 of the cases, a viral pathogen was identified, with 14 cases attributed to a single virus and 4 cases indicating the presence of two or more viruses. No respiratory virus was detected in four children with PARDS. Notably, human rhinovirus was identified in 10 children, of whom 6 exhibited rhinovirus as the sole confirmed pathogen. Our findings suggest that rhinovirus stands out as a prevalent cause of viral-induced PARDS.

B13. Disruption of Host Cell Calcium Signaling Pathways Reduces Polyomavirus Infection

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JC polyomavirus (JCPyV) infects 50–80% of the human population. In healthy individuals, JCPyV causes a persistent, asymptomatic infection in the kidneys. In severely immunocompromised individuals, JCPyV can cause a fatal brain infection called progressive multifocal leukoencephalopathy (PML). PML causes a lytic infection of myelin-producing glial cells and becomes progressively debilitating, sometimes resulting in death within one year of symptom onset. There are currently no targeted, approved treatments for PML, underscoring the importance of continued research on JCPyV and PML. In an effort to identify antiviral therapeutics, the Maginnis laboratory performed a large-scale drug screen using the National Institutes of Health Clinical Collection (NIH-CC), which contains FDA-approved drugs and therapeutics in various stages of clinical trials. Over 700 drugs were screened for their capacity to reduce JCPyV infection. Results demonstrated that multiple FDA-approved drugs from several drug classes reduce JCPyV infection and that multiple “hits” identified were drugs that target cellular calcium signaling pathways. Calcium channel blockers and related calmodulin inhibitors have been further characterized by viral infectivity assays, with results supporting a role of calcium signaling during JCPyV infection. Our data also demonstrate that calmodulin inhibitors reduce simian virus 40 (SV40), BK polyomavirus (BKPyV), and SARS-CoV-2 infections, indicating that calmodulin may be an antiviral target for a broad range of viral infections. Importantly, trifluoperazine (TFP), a calmodulin inhibitor, significantly impaired JCPyV infectivity in primary human kidney cells as well. Further investigation into the impact of TFP on the JCPyV infectious cycle showed that TFP is blocking JCPyV infection at the point of viral entry. Exploring pre-approved drugs from the NIH-CC is a promising and demonstrated method for repurposing therapeutics to identify potential treatments for PML and other viral diseases.

B14. SARS-CoV-2 Accessory Protein ORF8 Intercepts the dIgA-pIgR Mucosal Immune Pathway

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Since its emergence in 2019, SARS-CoV-2 has posed a significant threat to global health and resulted in over 6.6 million deaths. Despite the deployment of safe and effective vaccines, SARS-CoV-2 transmission persists and is closely monitored due to its high mutation rate. Although current intramuscular immunization strategies mitigate COVID-19 disease severity, their capacity to prevent breakthrough SARS-CoV-2 spread and infection is limited due to poor stimulation of mucosal immunity. As such, mucosal vaccines are actively pursued to generate a potent immune response in the lung mucosa to restrict SARS-CoV-2 infection and establishment. Consequently, it is essential to understand both the mucosal immune response to SARS-CoV-2 and the antagonization mechanisms employed by the virus, which are unfortunately not well understood. Recent findings have highlighted a marked reduction in the expression of crucial immune receptors in COVID-19 patients, including the polymeric Ig receptor (pIgR). This receptor is a key mediator of mucosal immunity as it maintains homeostasis by conferring the transcytosis and secretion of dimeric IgA (dIgA) across epithelial cells and into the lung mucosa, allowing the neutralization of infectious pathogens such as SARS-CoV-2. In this study, we report that while the SARS-CoV-2 accessory protein Open Reading Frame 8 (ORF8) decreases pIgR expression in the infected cells, the secreted ORF8 can bind to cell-surface pIgR and enter neighboring cells to exert antagonization of transcytosis alongside its immunomodulatory functions, which together may dampen mucosal immunity and exacerbate SARS-CoV-2 infection in the lungs. Our results not only suggest a function of ORF8 in the exploitation of dIgA-mediated mucosal immunity but also supports ORF8's function as a polyvalent immunomodulator, moreover highlighting ORF8 as a potential mucosal vaccine target.

B15. Functional Characterization of the Interplay Between the APOBEC Host Deaminases and the SARS -CoV-2 Proteins, and Their Role in SARS -CoV-2 Evolution

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The SARS-CoV-2 is a 30kb-long single-stranded positive sense RNA virus. It evolves due to host antiviral defenses, mainly driven by APOBEC deaminase activity. APOBEC3 enzymes play a crucial role in restricting viruses by introducing mutations into their genome. These mutations occur in specific contexts, specifically when cytidine is preceded by thymine or uracil, known as the 5'TC or 5'UC motif. The selective pressure exerted by APOBEC3 enzymes results in a depletion of these motifs in exposed viruses, a phenomenon termed the "APOBEC3 footprint."
The goal of this project is to test whether one or several members of the APOBEC3 family can indeed restrict SARS-CoV-2 replication. Preliminary data lead us to focus on three candidates: APOBEC3A, APOBEC3H and APOBEC3G. By overexpressing these proteins in A549-ACE2 and VeroE6 cells, which support SARS-CoV-2 replication, we observed a significant reduction in viral replication in cells expressing APOBEC3H and a mutant form of APOBEC3G (APOBEC3G T218A) that cannot be enzymatically inactivated through phosphorylation. This result suggests that APOBEC3H and the non-phosphorylatable APOBEC3G mutant have a restriction effect on SARS-CoV-2. Further research is needed to elucidate the precise antagonistic mechanism of APOBEC3G, potentially involving phosphorylation of its T-218 residue.
Viruses evolved different mechanisms to antagonize or even use the APOBEC3 response to favor the genetic diversification of the viral genome. The antagonization relies on their use of viral proteins that recruit APOBEC proteins and target them for degradation or delocalization away from the viral replication center. We therefore proposed creating an interactome map of SARS-CoV-2 proteins with various APOBEC proteins using high-throughput GPCA assays. Initial results indicated an interaction between APOBEC3G (A3G) and the N protein of SARS-CoV-2, prompting ongoing investigations into the impact of this interaction on A3G's antiviral activity.

B16. SARS-CoV-2 Infection of Multiple Cell Types and Molecular and Immune Signatures of COVID-19 in Lungs Using Spatial Single-Cell Transcriptome Analysis

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The pathology and underlying molecular mechanism of COVID-19 remain poorly defined. We previously detected SARS-CoV-2 infection in diverse cell types in lung tissues, and confirmed pseudovirus infection of these cells from 5 organ systems. Here, we investigated the spatial single-cell molecular and cellular features of COVID-19 lung tissues. We detected 10,414,863 transcripts of 221 genes and segmented them into 1,719,459 cells that were mapped to parenchymal and immune cells, all infected by SARS-CoV-2. Compared to controls, COVID-19 lungs exhibited reduced alveolar cells (ACs) and increased innate and adaptive immune cells. Spatial analysis revealed regions with high infection rates correlated with high cell densities (HIHD). HIHD regions expressed high levels of virus entry-related factors ACE2, FURIN, TMPRSS2 and NRP1, and co-localized with organizing pneumonia (OP) and lymphocytic and immune infiltration, which exhibited increased ACs and fibroblasts but decreased vascular endothelial cells and epithelial cells, mirroring the tissue damage and wound healing processes. Sparse non-negative matrix factorization (SNMF) analysis of niche features identified 7 signatures that captured structure and immune niches. Trajectory inference based on immune niche signatures defined two pathological routes. Trajectory A progressed with increased NK cells and granulocytes, likely reflecting the complication of microbial infections. Trajectory B was marked by increased HIHD and OP, possibly accounting for the increased immune infiltration. The OP regions were marked by high numbers of fibroblasts expressing extremely high COL1A1 and COL1A2 levels. Single-cell RNA-seq analyses of COVID-19 and idiopathic pulmonary fibrosis lung tissues identified similar cell populations consisting mainly of myofibroblasts. Immunofluorescence staining revealed activated IL6-STAT3 and TGF-b-SMAD2/3 pathways in these cells, likely mediating COL1A1 and COL1A2 upregulation, and excessive fibrosis. This study provides a spatial single-cell atlas of cellular and molecular signatures of fatal COVID-19 lungs, revealing the complex spatial cellular heterogeneity, organization, and interactions that characterized the COVID-19 lung pathology.

B17. Human Herpesvirus-6 Encoded G-Protein-Coupled Receptors U12 and U51 and Thyroid Autoimmunity

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Background and aims. Studies have linked HHV-6 with thyroid autoimmunity, yet the exact mechanisms of HHV-6 involvement remain unclear. We propose that two poorly studied HHV-6 proteins, U12 and U51, may contribute to autoimmunity. They are homologous to chemokine receptors (G-protein-coupled receptors), can be expressed on cell surfaces, and can influence viral replication. By conducting molecular and immunological investigations, we aim to elucidate the potential role of U12/U51 in thyroid autoimmunity.
Methods. Thyroid tissue and plasma samples from 81 AIT patients (harboring HHV-6 in the thyroid) were investigated. RNA was isolated from thyroid tissues, used for cDNA synthesis, and nPCR to detect U12/U51 mRNA. FFPE thyroid tissues were microscoped to visualize U12/U51. U12 and U51 antibodies were detected with the Luminex system.
Results. A total of 46% of thyroid tissue samples harbored U12/U51 mRNA. Thyroid tissues with mRNA harbored significantly higher viral loads (1390 vs 120 viral copies/10^6 cells, $p = 0.01$). Nearly all AIT patients had U12/U51 antibodies, and in those with an active HHV-6 infection, antibody levels were significantly higher. Microscopy revealed the colocalization of HHV-6 and U12/U51 in the thyroid.
Conclusions. The combination of the presence of U12/U51 mRNA and viral protein-specific antibodies with the visualization of both HHV-6 and its GPCRs in the thyroid suggests that both could be involved in autoimmunity development/exacerbation. HHV-6 and U12/U51 could enhance thyroid cell destruction, autoantigen release, and inflammation through viral replication enhancement or through direct antibody binding and the subsequent immune response against U12/U51.

B18. Evaluation of Haematological and Immunological Parameters of the ASFV Lv17/WB/Rie1 Strain and Its Derived Mutant Lv17/WB/Rie1/d110-11L in Domestic Pigs

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African swine fever virus (ASFV) is the etiological agent of a haemorrhagic disease that threatens the global pig industry. There is an urgency to develop a safe and efficient vaccine, but the knowledge of the immune-pathogenetic mechanisms behind ASFV infection is still very limited. In this work, we evaluated the haematological and immunological parameters of domestic pigs vaccinated with the ASFV Lv17/WB/Rie1 strain or its derived mutant Lv17/WB/Rie1/d110-11L, and then challenged with virulent Armenia/07 ASFV. Circulating levels of C-reactive protein (CRP), 13 key cytokines, and 11 haematological parameters were evaluated throughout the study. Lv17/WB/Rie1 triggered an inflammatory response, with increased levels of CRP and pro-inflammatory cytokines (IL-1α, IL-1β, IL-2, IL-6), and induced lymphopenia, thrombocytopenia, and a decline in red blood cell (RBC) parameters, although this was transitory. Lv17/WB/Rie1/d110-11L triggered only transitory thrombocytopenia and a mild inflammatory reaction, with no increase in serum levels of pro-inflammatory cytokines, but it raised IL-1Ra levels. Both strains counteracted several adverse reactions elicited by virulent challenge, like thrombocytopenia, a decline in RBC parameters, and inflammation. Within this paper, we provided a deep portrayal of the impact of diverse ASFV strains on the domestic pig's immune system. A better understanding of these immune-pathological mechanisms would help in designing suitable vaccines against this disease.
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B19. Investigating the Role of WNK Signaling in Viral Replication

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The WNK-SPAK/OSR1 signaling pathway responds to changes in osmolarity to regulate ion transport. Upon the activation of this pathway, WNK kinases phosphorylate downstream effector kinases SPAK and OSR1, which in turn phosphorylate ion cotransporters to modulate their activity and restore ion balance. While this pathway has been well studied in the context of osmolarity, little is known about its immunological role. Stress proteins, such as WNK, have been found to have a dual role in response to viral infection. They can assist in formulating an antiviral response against the infection or their functions can be hijacked by the invading virus to promote various steps of its infection cycle. The role of WNK signaling in responding to viral infection has not been investigated and should be elucidated to uncover a potential novel role. I hypothesize that WNK signaling promotes the replication of multiple virus families through the regulation of the intracellular ion environment. I further hypothesize that viral entry activates WNK signaling by altering the intracellular concentration of chloride ions. Thus far, the effect of WNK and SPAK inhibition on viral replication was tested in human fibroblast cells. Cells were infected with vesicular stomatitis virus (VSV) or herpes simplex virus 1 (HSV-1) followed by treatment with either WNK or SPAK inhibitors. Treatment of cells with either inhibitor reduced the replication of both VSV and HSV-1 when compared to DMSO-treated controls. These results suggest the involvement of the WNK signaling pathway in promoting viral replication. Further experimentation will expand on these findings by testing the inhibitors on additional virus families and elucidating the mechanism by which WNK signaling promotes viral replication. Additionally, the interaction between viral entry and activation of WNK signaling will be investigated.

B20. Cell-To-Cell Transmission of SARS-CoV-2: Roles of the ACE2 Receptor, Membrane Fusion and Endosomal Entry

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Enveloped viruses can spread through cell-free infection and/or cell-to-cell transmission. Cell-to-cell transmission is known to evade antibody neutralization, thus contributing to efficient virus infection and pathogenesis. We have shown that a SARS-CoV-2 spike is more efficient in mediating cell-to-cell transmission compared to that of SARS-CoV, which in part is due to the high fusogenicity of the SARS-CoV-2 spike. However, although cell–cell fusion enhances the cell-to-cell transmission of both SARS-CoV and SARS-CoV-2, the treatment of cocultured cells with endosomal entry inhibitors also impairs the cell-to-cell transmission of both viruses, suggesting endosomal membrane fusion as an underlying mechanism. Interestingly, ACE2 enhances the efficiency of cell-to-cell transmission, but it is not absolutely required for some cell types. Compared with cell-free infection, the cell-to-cell transmission of SARS-CoV-2 is much more refractory to inhibition by neutralizing antibodies induced by vaccines or convalescent sera of COVID-19 patients. Although Alpha and Beta variants have similar cell-to-cell transmission capability, Delta and Omicron variants exhibit distinct patterns of cell-to-cell transmission. We also discovered that, compared to prototype Omicron, newly emerged Omicron subvariants possess enhanced cell–cell fusion capability, correlating with increased cell-to-cell transmission. We identified several amino acid mutations, including H655Y and T547K, that govern the low fusogenicity and endosomal entry of Omicron variants. Overall, our study reveals some unique features of the cell-to-cell transmission of SARS-CoV-2 and its variants, which could have implications for viral tropism, persistence, and pathogenesis.

B21. SIGMAR1 Silencing Interferes with the Formation of HCV-Induced Double-Membrane Vesicles

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Sigma-1 receptor (SIGMAR1), an ER membrane protein concentrated in mitochondria-associated ER membranes (MAMs) and lipid droplets, is linked to different cellular functions including stress survival and development. SIGMAR1 functions have been associated with neurodegenerative diseases, cancer, and viral infections. Despite its potential clinical significance as molecular target for therapy, the precise mechanisms underlying these diverse functions remain elusive. This research delves into SIGMAR1 pivotal role as a proviral factor, shedding light on its intricate biological functions in the host cell.

Prior research found that SIGMAR1 plays a critical role in forming functional hepatitis C virus (HCV) replicase complexes, limiting HCV infection by constraining viral RNA replication without affecting primary translation. Single-cycle infection experiments conducted with a panel of plus-strand RNA viruses indicated a strong reduction in infection efficiency exclusively for HCV, implying a selective role in early stages of HCV infection.

Next, we determined whether SIGMAR1 silencing interfered with the formation of double membrane vesicles (DMVs), the putative replicase compartment for HCV, using replication-independent viral protein expression systems and transmission electron microscopy (TEM). Remarkably, DMVs were formed upon overexpression of viral non-structural proteins both in controls and SIGMAR1-deficient cells. However, detailed structural analysis suggests structural aberrancies of HCV-induced DMVs in SIGMAR1-deficient cells, reinforcing the critical role of SIGMAR1 in HCV replicase compartment formation.

In light of these findings, current research aims at defining molecular and functional interactions between SIGMAR1 and HCV non-structural proteins as well as with other host factors potentially intervening in the formation of DMVs in a SIGMAR1-dependent manner. An unbiased approach to identify SIGMAR1 interaction networks during HCV infection promises to provide a comprehensive view of SIGMAR1 function in the viral realm.

B22. The Identification of Novel Cellular Targets for a HIV-1 Cure Through Non-clonal Latency Models From Myeloid and Lymphoid Origin

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Strategies targeting the HIV latent reservoir have been unsuccessful so far. The most used preclinical models, including latently infected clonal T cell lines, fail to mimic the heterogeneity of the latent reservoir. Here, we develop and functionally characterize novel in vitro non-clonal HIV-1 latency models from myeloid and lymphoid origin, finally leading to the identification of new druggable cellular targets.
Viral latency was established in Jurkat, MOLT, U937, and HL60 cells through long-term infection with an Env-deficient GFP+ NL4-3 virus. Latency-reversing (LRAs) and -promoting agents (LPAs) were used to assess the HIV-1 latency modulation capacity of models using flow cytometry. The quantification of integrated proviruses was determined by qPCR. The whole-transcriptome profiling and identification of differentially expressed genes (DEGs) were performed by RNA-seq, R-studio, GSEA, and Cytoscape.
In our models, up to 62% of reported LRAs/LPAs modulated latent HIV with robust, comparable, and reproducible results (3.3±0.4 fold change for vorinostat 5μM). HIV-integrated provirus quantification resulted in 1-3 copies/cell and was correlated with reactivation capacity induced by vorinostat (r^2=0.6), panobinostat (r^2=0.8), or PMA (r^2=0.8) LRAs. Whole-transcriptome profiling differentially clustered lymphoid and myeloid cells, and infected and non-infected cells. DEG analysis in the latent models identified TRIM2 and HSPA6 (p0.05), both previously linked to HIV pathogenesis. Further characterization by gene set enrichment analysis pointed out the downregulation of cell cycle and metabolism pathways and the upregulation of TNFα signaling (q0.001) in the latent models. A screening with more than 500 compounds was performed to functionally validate the transcriptomic data, which identified novel putative latency modulators targeting HSP, JAK/STAT, or Aurora kinase proteins. Those findings were confirmed ex vivo in primary CD4+ T cells from PLWH.
Non-clonal models represent a reliable alternative that allows for the identification of novel cellular targets for a HIV cure.

B23. The Functions of CCR5 in SARS-CoV-2 Infection

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The binding of spike protein of SARS-CoV-2 with human angiotensin converting enzyme II (ACE2) that triggers viral membrane fusion with cellular fusion is critical for viral entry. Recently, clinical studies showed that a specific antibody, Leronlimab, against CCR5, an entry co-receptor of HIV, reduced SARS-CoV-2 plasma viremia in severe COVID-19 patients and the CCR5-Δ32 deletion was found to be associated with susceptibility to COVID-19 and disease severity. Therefore, we hypothesized that CCR5 might act as a co-receptor for SARS-CoV-2 to interact with spikes for promoting viral-cell fusion. To investigate this possibility, we conducted a fluorescence-based cell-cell fusion assay by which the binding of the spike with ACE2 and the spike-mediated membrane fusion could be examined. The interactions between spike and CCR5 were also validated by co-immunoprecipitation assay. According to the results of the cell-cell fusion assay, the treatment of either **Maraviroc**, a pharmacological inhibitor of CCR5, or **Met-RANTES**, a specific antibody against CCR5, depicted less effect on the spike-mediated cell binding, but significantly reduced the spike-mediated cell fusion. Among variants of spike protein, CCR5 inhibitors could block wild-type and Delta variant of spikes-mediated cell fusion but not Omicron spike variant-mediated cell fusion. These observations suggested that CCR5 was mainly involved in specific type of spike variants-mediated membrane fusion. Furthermore, the results of co-immunoprecipitation revealed that spike, ACE2 and CCR5 form a complex during or after the spike-mediated cell fusion in a ACE-2 dependent manner. This study contributes to the understanding of the roles of CCR5 in SARS-CoV-2 infection and may also provide a novel target for the development of anti-SARS-CoV-2 drugs.

B24. KU Heterodimer Restricts Viral Episomal DNA Gene Expression

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The genome of DNA viruses undergoes transcription and replication processes, reminiscent of cellular DNA metabolism, in order to complete their replicative cycles. Some viruses contain either partially double-stranded (i.e., hepatitis B virus (HBV)) or single-stranded DNA genomes (i.e., adeno-associated virus (AAV)) that need to be repaired by cellular factors to produce episomal DNA molecules that serve as transcriptional templates for viral gene expression. The production of these episomes is a rate-limiting step in the replicative cycle of these viruses, but once formed they are hallmarks of HBV persistence and successful AAV-based gene therapy strategies. In this study, we analyzed the role of the cellular heterodimer Ku70/Ku80 in the context of these viral models. Silencing of these proteins produced an increase in the accumulation of HBV proteins (i.e.: HBeAg and core), and in the AAV-derived reporter gene expression, suggesting that they negatively regulate viral gene expression. Moreover, ectopic expression of wild-type Ku70 protein reduced AAV-derived reporter expression, confirming that the phenotype is not related to off-target effects. To further characterize the step targeted by the Ku heterodimer, we used AAV vectors that contain double-stranded DNA genomes (scAAV). These scAAV vectors are more efficient in episome formation and gene expression than their single-stranded genome-containing counterparts. Silencing of Ku proteins also produced an increase in scAAV-derived reporter expression, suggesting that Ku proteins do not target the second-strand DNA synthesis step. Indeed, reporter expression was also enhanced in plasmid transfection experiments performed in parallel. Collectively, these results suggest that Ku70/Ku80 heterodimers regulate episomal gene expression by targeting a step downstream of episomal DNA formation. Further studies are underway to define the steps and mechanisms by which these cellular proteins perform their restrictive functions.

B25. Regulation of IRF7 Expression: A Novel Approach to Control HIV-1 Transcription and Latency

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Emerging evidence suggests that the modulation of innate immunity could impact viral latency and contribute to HIV reservoir clearing. Our data indicated that IRF7 expression correlates with HIV latency reversal. Here, we demonstrate the key role of IRF7 in HIV-1 transcription and latency, identifying novel therapeutic agents useful in HIV eradication.
Latency was evaluated by non-clonal models. Gene and protein expression were assessed by qPCR and WB. IRF7 loss-/gain-of-function models were developed by siRNA or plasmid overexpression. The role of IRF7 in HIV-1 transcription was evaluated by measuring LTR-driven transactivation in TZM-bl cells and co-immunoprecipitation. The immunophenotyping of ex vivo-treated CD4+ T-cells from ART-suppressed PLWH was performed by flow cytometry and latency reactivation/promotion activity was measured by qPCR in cell supernatants. IRF7 expression correlates with the latency reversal/promoting capacity of LRAs/LPAs, including PMA, PNB, and JQ1, as well as the JAK2 inhibitors fedratinib and pacritinib, among others (r^2= 0.8; p-value=0.0012). IRF7 downregulation impaired the latency reversal capacity of LRAs (p=0.005) but its overexpression enhances the Tat-mediated transactivation of integrated HIV in the presence of LRAs (p=0.05). Coimmunoprecipitation studies showed physiological interaction between Tat protein and IRF7, relating IRF7 to HIV-1 transcription control. As pacritinib dowregulated IRF7, it was used to further explore the role of IRF7 in HIV latency. Pacritinib significantly blocked HIV-1 reactivation both alone and in combination with PMA or VOR in non-clonal models of HIV-1 latency (50% reduction, p=0.05) and ex vivo in CD4+T cells from ART-suppressed PLWH (1log reduction). Immunophenotypic characterization of pacritinib-treated CD4+T cells from PLWH showed no major changes in CD4+T cell subsets oractivation markers. Moreover, pacritinib significantly reduced the presence of multispliced HIV-1 transcripts in primary CD4+T cells.
IRF7 controls latent HIV-1 transcription and plays a role in HIV-1 reactivation/blockade. The modulation of IRF7 expression through pharmacological agents might represent an asset to current HIV-1 cure strategies.

B26. Three-Dimensional Remodeling of SARS-CoV-2-Infected Cells Revealed by Cryo-SXT

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Plus-strand RNA viruses are proficient at remodeling host cell membranes for optimal viral genome replication and the production of infectious progeny. These ultrastructural alterations result in the formation of viral membranous organelles and may be observed using different imaging techniques providing nanometric resolution. Guided by confocal and electron microscopy, this study describes the generation of wide-field volumes using cryogenic soft X-ray microscopy on SARS-CoV-2-infected human lung adenocarcinoma cells at ALBA synchrotron. Confocal microscopy showed an accumulation of double-stranded RNA and nucleocapsid protein in compact perinuclear structures, preferentially found around centrioles at late stages of the infection. Transmission electron microscopy (TEM) showed an accumulation of membranous structures in the vicinity of the infected cell nucleus, forming a viral replication organelle containing characteristic double-membrane vesicles and virus-like particles within larger vesicular structures. Cryogenic soft X-ray tomography (cryo-SXT) revealed very similar viral replication organelles to those observed by TEM, but indicated that the vesicular organelle observed in TEM sections is indeed a vesiculo-tubular network that is enlarged and elongated at late stages of the infection. Overall, our data provide additional insight into the molecular architecture of SARS-CoV-2 replication organelles.

B27. Rapid Hepatitis C Virus Replication Machinery Removal After Antiviral Treatment with Direct-Acting Antiviral Drugs Monitored by Multimodal Imaging

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Hepatitis C virus (HCV) infection in a cell culture constitutes an excellent model of persistent infection, whereby the virus takes control of the infected cell without killing it. This strong interference with host cell homeostasis is manifested by the profound remodeling of the host endomembrane system, as well as the strong induction of virtually all the stress response pathways in the cells. Specific direct-acting antiviral (DAA) drugs against HCV can revert this process, and the ultrastructural events that follow a viral replication blockade shortly after antiviral treatment can be defined using confocal immunofluorescence, transmission electron microscopy (TEM) as well as correlative cryogenic light-soft X-ray tomography (CLXT), with the use of a table-top device for the latter.

The analysis of DAA-treated HCV replicons indicate that most viral antigens and RNA are eliminated within the first 48 hours of treatment, concomitant with the reversion to the baseline expression of HCV-induced stress markers, such as ATF3. A general survey of the control cells and HCV replicons using CLXT indicates that the HCV-induced membranous alterations are no longer visible after 24 hours of treatment and that a substantial fraction of NS5A, a viral component of the replicase, is located in pleomorphic, high-absorption contrast organelles in the DAA-treated cells. The three-dimensional reconstruction of these cells suggest that these organelles are spatially organized in layers proximal to the cell nuclei in the areas with reduced mitochondrial content. The TEM studies confirmed the rapid elimination of the viral machinery and the concurrent appearance of large endo-lysosomes and multivesicular bodies, suggesting a major role for this recycling machinery in the elimination of HCV-induced membranous compartments. These and previously obtained results suggest that the HCV replication compartment is constantly produced and recycled by the endo-lysosomal system and that this equilibrium is unbalanced by DAA treatment, resulting in the rapid removal of the viral machinery.

B28. IFITM Proteins Diminish SARS-CoV-2 Infectivity by Targeting Spike Protein

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Interferon-induced transmembrane proteins, also known as IFITMs, are an important product of Interferon-Stimulated Genes (ISGs). They have been extensively characterized to play a vital role in antiviral immunity by inhibiting the entry of various viruses, such as HIV-1, influenza A viruses, flaviviruses (like Dengue and West Nile Virus), and coronaviruses (SARS-CoV, SARS-CoV-2). They primarily act as entry restriction factors but have also been shown to negatively affect infectivity by interacting with the envelope proteins of viruses like HIV-1. While much is known about their impact on viral entry, their influence on the later replication stages, like assembly, of coronaviruses remains unclear. In our study, we uncovered a novel effect of IFITMs on the assembly of SARS-CoV-2 using a virus-like particle (VLP) system. The VLP system uses SARS-CoV-2 structural proteins along with a luciferase-linked packaging gene to measure the infectivity of the produced particles. Remarkably, we found that VLPs produced in the presence of IFITMs exhibited significantly reduced infectivity. Further investigation revealed that this reduction was due to a modification in the processing of the spike protein. After eliminating the possibility of protease cleavage, we found that this is an alteration in the glycosylation of spike. This modification may impact spike-mediated viral entry, antibody binding, and neutralization. Our study not only enhances the understanding of SARS-CoV-2 assembly and release from host cells, but will also aid the development of potential therapeutics targeting these later stages of viral replication.

B29. Dissecting the Structural Regulation of Pro- and Anti-Viral Host microRNAs by ADAR Editing

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Extensive research has shown that microRNAs (miRNAs) are key players in the dense virus–host interactome. Across diverse viral families, both host and virally encoded miRNAs regulate viral replication, infectivity, and latency. As of 2018, an unbiased review of the literature found 46 unique host miRNAs known to possess pro- or anti-viral activity. Because their presence or absence can ultimately determine host fate, it is crucial to understand how these miRNAs are regulated. One such mechanism of regulation is the A-to-I editing of primary miRNA (pri-miRNA) hairpins by the Adenosine Deaminase Acting on RNA (ADAR) family of enzymes. These editing events are thought to stabilize or destabilize the highly conserved secondary structure of pri-miRNA hairpins, either enhancing or attenuating their recognition through downstream structure-dependent maturation machinery. Remarkably, 30 of the 46 miRNAs are known ADAR targets, but the effect of editing on their maturation remains unknown. Further complicating the interplay of ADAR and their virus-relevant pri-miRNA substrates, ADAR expression is frequently upregulated by the host innate immune response, and evolutionary pressure has driven the development of viral mechanisms for suppressing or evading ADAR editing. In combination, these competing effects can dramatically change editing profiles during infection. To better understand how ADAR editing alters the maturation of pro- and anti-viral miRNAs, we generated a library containing all known edited pri-miRNA stems with an effect on viral fitness. We then interrogated the structure/function relationship of editing across 730 unique positions on these pri-miRNAs. By deconvolving ADAR editing and chemical mapping data at single-nucleotide resolution, we identified structural perturbations induced by editing on pri-miRNA stems and linked these perturbations to differential miRNA biogenesis in vitro. These experiments provide insight into an underexplored mechanism by which cells and their viral invaders engage in a molecular “tug-of-war” to dynamically shape the mature miRNA landscape to their benefit.

B30. Cell-type Dependent Differences in JC Polyomavirus Signaling Pathway Activation

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JC polyomavirus (JCPyV) infects the majority of the population and causes an incurable persistent infection in the kidneys. In immunocompromised individuals, JCPyV can become reactivated in the central nervous system and infect glial cells, oligodendrocytes and astrocytes, which are critical for myelin production. The molecular mechanisms of JCPyV infection of astrocytes are poorly understood, in part due to most studies being limited to an immortalized cell model. To better understand the cellular and molecular basis of JCPyV infection in astrocytes, we established an innovative infection model using normal human astrocytes (NHAs). We determined that viral infection was regulated differently in primary and immortalized cell types, due to viral factors, such as T antigen expression, and cellular factors, including activation of cellular signaling pathways. Using RNA Sequencing analysis and complementary molecular approaches, we defined cell-type specific differences in the regulation of the MAPK pathway through dual-specificity phosphatases (DUSPs) and also determined the importance of the PI3K/AKT signaling pathway in NHAs. Through this comparative genomic analysis, we elucidated how JCPyV orchestrates differential gene expression and regulation of cellular signaling pathways to mediate infection in primary astrocytes. Outcomes of this research provide an enhanced understanding of cell-type dependent differences in viral infection and can be applied to our broader understanding of cell signaling pathways and future development of antiviral therapies.

B31. Neurovirulence of the Australian Outbreak Japanese Encephalitis Virus Genotype 4 Is Lower Compared to Genotypes 2 and 3 in Mice and Human Cortical Brain Organoids

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Human infections with Japanese encephalitis virus (JEV) are a leading cause of viral encephalitis, with ≈70,000 symptomatic cases (≈40% with long-term neurological sequelae) and ≈20,000 deaths reported annually. An outbreak of JEV genotype 4 was recently reported in Australia, with an isolate ($JEV_{NSW/22}$) obtained from a stillborn piglet brain. Herein, we characterize the neuropathology of $JEV_{NSW/22}$, JEV_{FU} (genotype 2) and $JEV_{Nakayama}$ (genotype 3) in adult C57BL/6J wild-type mice, mice deficient in interferon regulatory factor 7 (*Irf7*$^{-/-}$), and mice deficient in type I interferon receptor (*Ifnar*$^{-/-}$). In C57BL/6J and *Irf7*$^{-/-}$ mice with lethal outcomes, viral antigen was detected, inter alia, in the cortex, thalamus, and hippocampus, and histopathological lesions recapitulated those seen in humans and primates. JEV was universally lethal in *Ifnar*$^{-/-}$ mice by day 3 with histological signs of brain hemorrhage, but produced no other detectable brain infection or lesions, with viral protein detected in blood vessels but not neurons. We thus describe a new *Irf7*$^{-/-}$ mouse model for $JEV_{NSW/22}$, which had increased viremia compared to C57BL/6J mice, allowing for lethal neuroinvasive infection in one mouse. Overall, $JEV_{NSW/22}$ was less neurovirulent than other JEV isolates in C57BL/6J and *Irf7*$^{-/-}$ mice and was more sensitive to type I interferon. All JEV isolates showed robust cytopathic infection of human cortical brain organoids, albeit lower for $JEV_{NSW/22}$. Our study establishes $JEV_{NSW/22}$ mouse models of infection, allowing for lethal neuroinvasive infection that was rarer than for other JEV genotypes. These models can be deployed in pre-clinical studies to further understand JEV disease pathogenesis.

B32. Novel Binding Partners Related to Lipid Metabolism and Endocytosis Found in the African Swine Fever Virus Interactome

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The ultimate mechanisms of pathogenesis underlying viral diseases are often unknown. Hence, the development of therapies to prevent and to treat infections caused by highly pathogenic viruses is rarely achieved and requires a deep knowledge of the viral genes' functionality and viral protein interactions with the hosts. The proteomic analysis of African swine fever virus (ASFV) potential fusion proteins has yielded the identification of cellular systems that are relevant for infections, including functional validations using inhibitors.
ASFV is a highly complex virus with a double-stranded DNA genome, affecting domestic and wild pigs, and is the only member of the *Asfarviridae* family. ASFV causes African swine fever, a devastating disease that is produces a panzootic, significantly impacting the swine industry worldwide due to its rapid distribution and the absence of a commercial vaccine or therapy.
Nowadays, the development of countermeasures is limited by insufficient knowledge regarding the function of the majority of its more than 150 viral genes, which interact with the host proteins to reprogram the cellular environment for efficient viral replication and immune evasion.
Understanding the functions of viral proteins and their cellular interactors is crucial for the rational development of new therapeutics. Hence, our laboratory tried to decipher the relevant interactions of the virus with cellular proteins, thus understanding how the virus exploits the host cell.
Our group identified several new druggable targets for antivirals in the endocytic pathway, endoplasmic reticulum–membrane contact sites, and lipid metabolism. Some of those are the enzymes carnitine acyl transferase, or carnitine palmitoyltransferase, and long-chain acyl-CoA synthetase.
Here, we have revealed a number of cellular proteins interacting with their viral counterparts that could be involved in fusion events between viral and cellular membranes and other events throughout the infectious cycle, providing a number of useful targets for the development of specific antivirals.

B33. Effect of Pistachio Extracts on Herpes Simplex Virus-1 (HSV-1)-Induced Inflammation

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Background: Great efforts are currently being devoted to the search for alternative clinical treatments against HSV infections to reduce side effects, overcome drug resistance, and fight the inflammatory response. Polyphenols are active compounds with both immunomodulatory and antiviral activity and are present in large quantities in pistachios (Pistacia vera L.). **Aims:** We have investigated the immunomodulatory effect of polyphenols-rich extracts from natural raw (NRRE) and roasted unsalted (RURE) pistachio kernels on human monocytic cells (THP-1 cells), exploring the role played by NF-κB, as well as key genes and pathways of the inflammasome through the combined approach of RT2 profiler PCR arrays and bioinformatics tools. The expression levels of cytokines and chemokine transcripts related to NF-κB signaling were evaluated to understand whether pistachios abrogated the inflammatory response mediated by HSV-1 replication. **Materials and Methods:** THP-1 cells were infected with HSV-1 and treated with pistachio extracts (NRRE or RURE) at 0.6 mg/mL for 24h. Real-time PCR using RT2 Profiler PCR Arrays in combination with RT2 SYBR® Green Master mixes was employed. **Results and Conclusion:** The potent activation of inflammatory cytokines and chemokines induced by HSV-1 is completely abrogated by in vitro treatment with pistachio extracts. Based on these results, we encourage the formulation of adjuvant drugs using pistachio extracts against acyclovir-resistant HSV-1 strains. **Funding Sources:** This work was funded by American Pistachio Growers (APG, CA, US).

B34. Enveloped Viruses Take Advantage of Massive Endocytosis Mechanisms in CD169-Mediated Uptake in Dendritic Cells

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Dendritic Cells (DCs) are key in the immune response against viruses. However, some enveloped viruses, such as HIV-1 and SARS-CoV-2, enter DCs in a structure named the Viral-Containing Compartment (VCC), where viral particles remain infectious, facilitating their dissemination. Upon viral sensing, DCs upregulate CD169/Siglec-1 expression, a cellular receptor that recognizes GM-1 and GM-3 on the viral envelope, promoting viral internalization. Although the VCC formation process is unknown, previous data showed the involvement of cholesterol dependency, actin dispensability and transient deregulation of cortical actin. We hypothesized that enveloped viruses exploit Massive Endocytosis (MEND) machinery to form the VCC.

Live cell confocal imaging of DCs was conducted to obtain a 3D reconstruction of VCC formation and measure its dimensions and dynamics. Actin and cholesterol staining was performed in fixed and live cells to assess their roles during this process. PI3K inhibitors and protein palmytoilation inhibitors were used to evaluate their effect in the uptake of Viral-Like Particles (VLPs) via confocal microscopy. Images were processed with ImageJ and data analysis with GraphPad.

The HIV-1 VLP uptake dynamics and the VCC size (2.9 ± 0.7 μm diameter and 20 ± 9.9 μm^3 volume) indicate massive plasma membrane invagination. Actin was not observed in the early steps of VCC formation, but is essential for VCC maintenance. PI3K and protein palmitoylation inhibition arrested VLP entry, reducing the rate of VCC formation in DCs from 70% to 40% and 20%, respectively. Cholesterol coalescence determined VCC formation, as the cholesterol probe signal doubled in the VLP uptake regions.

These data suggest that MEND mechanisms participate in the formation of the VCC in DCs. This work provides new insights into virus–host interactions concerning myeloid cells, revealing new therapeutical targets. Blocking VCC formation offers potential cross-protection against enveloped viral infections that use CD169 to disseminate throughout the body.

B35. Investigating the Interplay Between Host and Viral Factors in SARS-CoV-2 Infection

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The impact of SARS-CoV-2 virus infections on global health is still huge, despite the availability of safe and effective vaccines. The emergence of novel viral variants of concern that escape the vaccine-triggered immune response is a challenge to halting its spread. A better understanding of the physio-pathogenetic mechanisms of SARS-CoV-2 and identification of the host factors involved are absolute priorities in the search for effective countermeasures to stop the circulation of this virus. SARS-CoV-2 infection results in a profound remodeling of cellular organelles, including an extensive fragmentation of the Golgi apparatus, an alteration of the mitochondrial network, and the translocation of peroxisomes to the perinuclear region close to double membrane vesicles (DMVs), the replication organelles where replication of the viral genome occurs. The molecular mechanisms underlying these cellular alterations and their biological significance are still poorly understood. Here, through an integrated approach that combines high-throughput screening with virological assays, we identified host factors involved in SARS-CoV-2 replication and cellular organelle remodeling during SARS-CoV-2 infection.

B36. The Presence of Common Herpesviruses (EBV, HHV-6, and HHV-7) in the Thyroid Gland—Implications for Disease Development and Exacerbation

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Background
Several human herpesviruses have long been associated with autoimmunity. Strong evidence has been presented for the association between HHV-6 and autoimmune thyroiditis (AIT). Incidental results have also linked other herpesviruses, such as HHV-7 and EBV. This study aims to investigate the prevalence of common herpesviruses in thyroid tissue samples and to elucidate the potential of their involvement in AIT development either on their own or in concert with one another.
Methods
Post-operative thyroid tissue samples and whole blood from 58 AIT patients were used for nucleic acid isolation and the subsequent detection of HHV-6, HHV-7, and EBV, and HHV-6 active infection markers using nested PCR. Additionally, the HHV-6 load was determined using qPCR with a commercially available kit by Saccace.
Results
All thyroid tissue samples harbored the DNA of at least one of the herpesviruses, and only 1 of the 58 blood samples was herpesvirus DNA-negative. HHV-6 was most commonly found in thyroid samples (58/58), followed by EBV (45/58), and HHV-7 (23/58). HHV-6 was found in only 8 of the 58 blood samples. Conversely, EBV and HHV-7 were commonly found in the blood—50/58 and 43/58, respectively. A statistical analysis revealed that thyroid tissue samples were more frequently HHV-6+ (p0.01) as compared to blood samples, which were more likely to be HHV-7+ (p0.01). Active HHV-6 infection was determined in 34 of the 58 thyroid tissue samples. Those thyroid tissue samples that were not actively infected were more frequently EBV+. Thyroid tissue samples harboring HHV-6 and EBV harbored higher median HHV-6 loads than those harboring only HHV-6.
Conclusions
Although HHV-6 remains the most commonly detected virus in autoimmunity-affected thyroid tissues, our results demonstrate that other commonly autoimmunity-associated herpesviruses, especially EBV, may also be linked with autoimmune thyroiditis. Our preliminary results warrant a deeper look into the potential synergistic role of EBV and HHV-6 in AIT.

B37. An Integrated Approach Reveals Oncolytic Activity and Novel Receptor Requirements of Bioselected Echovirus Variants

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Oncolytic viruses have emerged as a promising class of antitumor agents, designed to selectively target and lyse tumor cells while minimizing the damage to surrounding healthy tissue. Among these viruses, Echovirus 1 (Faruk strain) and Echovirus 12 (Travis strain) have exhibited notable oncolytic activity against model cancer cell lines. However, the identification of critical cell receptors for viral internalization remains a central challenge in oncolytic virotherapy.
In this study, we aimed to unravel the intricacies of viral entry and assess the receptor requirements for Echovirus variants. We employed a multifaceted approach that involved the use of CRISPR/Cas9-mediated knock-out of the FCGRT gene in HEK293T cells to confirm the necessity of the FcRn receptor for viral internalization. We then applied a bioselection approach to generate a number of virus strains capable of efficient cell entry in the absence of the canonical receptor. The bioselected viruses were subsequently assessed for their oncolytic activity across a diverse panel of tumor cell lines with varying levels of FcRn expression.
Our subsequent analysis revealed that certain amino acid substitutions were responsible for changes in the viral entry pathway of the bioselected strains. Moreover, we observed a decrease in the inactivating activity of rat antisera raised against canonical virus strains compared to the new ones, indicating differences in antigenic determinants between these variants. Finally, to reveal host factors crucial for viral oncolysis, we used a CRISPR screen of FCGRT KO cells infected with the bioselected virus strains and identified novel cellular determinants of viral oncolytic activity. Our data highlight the potential of a bioselection approach in antitumor therapy.

B38. Using Machine Learning to Identify Predictive Correlates in Data From an in Vivo Ferret Model for Influenza a Risk Assessment

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The ability of influenza A viruses to infect humans across species boundaries necessitates the continual assessment of novel and emerging strains using a variety of viral, host, environmental, and ecological factors. To guide pandemic preparedness efforts, in vivo assessments of viral pathogenicity and transmissibility in the ferret model are a crucial part of many risk assessment rubrics. However, there is no clear understanding of which biological factors and/or data points from the ferret risk assessment efforts are most useful for predicting pathogenesis and transmission outcomes. Here, we compiled information from 20+ years of risk assessment operations using the ferret model, including information on 125 different influenza A viruses. Our dataset includes viruses collected during avian surveillance programs, zoonotic viruses that have jumped species barriers to cause human infection, and well-adapted human seasonal circulating strains, spanning the H1, H2, H3, H5, H7, and H9 subtypes. Several experimental results, including mortality, weight loss (as a marker of disease severity), and respiratory droplet transmission, were predicted using machine learning (ML) techniques. We identified key empirically determined characteristics that are most consistently linked to high balanced accuracy, specificity, and sensitivity, among other metrics. Gradient boosting machines, random forest, and neural networks performed consistently better, with a balanced accuracy over 0.93 for lethality, 0.78 for weight loss, and 0.97 for transmission. Our findings show that ML algorithms can be used to summarize complex in vivo experimental work into succinct summaries, determine the best methods for gathering important experimental metrics, and inform and enhance risk assessment criteria for pandemic preparedness that take in vivo data into account. Collectively, this study highlights the need for future investment in ML approaches for a better analysis, and contributes to a more sophisticated integration of statistical and ML methods into in vivo research for more data and biological insights.

B39. RING Finger Proteins from Herpesviruses of Aquatic Vertebrates

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Aquaculture provides important resources for human food and high-nutrition proteins over the world. However, the aquaculture industry often suffers from various diseases, especially viral diseases including those caused by herpesviruses. Carp including crucial carp (*Carassius auratus* gibelio) and common carp (*Cyprinus carpio*) are important economic fishes. *Carassius auratus* herpesvirus (CaHV) is a member of the genus *Cyprinivirus* (family *Alloherpesviridae*), which causes acute gill hemorrhages and high mortality in cultured crucial carp. In recent years, we focused on CaHV, including virus identification and purification, pathologic analysis, genome sequencing, and functional gene characterization.

The architecture of the CaHV genome shows insertions, deletions, and the absence of a terminal direct repeat compared to other *cyprinid* herpesviruses (CyHVs). Five viral proteins (39L, 52L, 131R, 136L, and 143R) were found to contain a RING domain. Proteins containing the domain have been proved to participate in various cellular processes. A further sequence analysis based on genomes from members of the order Herpesvirales showed that the number of viral RING finger proteins decreased with the elevation of host evolutionary status. For example, most herpesviruses isolated from fishes possess five RING finger proteins, while the herpesviruses infecting humans (such as HSV-1 and KSHV) only encode two RING finger proteins. Ubiquitination assays with prokaryotic and eukaryotic expressed proteins showed that the five CaHV proteins all have E3 ubiquitin ligase activity, which relied on the RING domains, indicating that the RING finger proteins may have important functions in virus infection. A furtherfunctional analysis revealed that the CaHV RING finger proteins could function individually or cooperatively to resist the host innate immune response through different pathways, thus facilitating virus replication.

B40. Comprehensive Transcriptome Analysis Reveals the Distinct Immunity-Related Gene Expression Patterns of Tumor Microenvironment in HPV-Associated and HPV-Non Associated Tonsillar Squamous Cell Carcinoma

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Tonsillar squamous cell carcinoma (TSCC) has an aggressive clinical course with poor disease outcomes and is the most prevalent type of oropharyngeal squamous cell carcinoma (OPSCC) cancer. The tumor microenvironment (TME) is heterogeneous and complex and is a crucial factor in achieving an effective cancer therapy. This study aimed to gain a more comprehensive understanding of the transcriptomes of the TME in HPV-associated and HPV-non-associated TSCC by studying the gene expression profiles of 168 genes linked to various cellular mediators and factors involved in inflammation, immunity crosstalk, transcription, signal transduction, oncogenesis, tumor suppression, angiogenesis, and apoptosis. We also detected and genotyped HPV in TSCC. We identified 40 genes related to communication between the tumor cells, and the cellular mediators of inflammation and immunity crosstalk were differentially expressed. In the HPV-positive TSCC patients, 33 genes were overexpressed; of those, 27 genes were unique in this group, while the HPV-negative TSCC patients had 10 up-regulated genes. The results further showed that 48 gene transcripts of oncogenesis, tumor suppression, angiogenesis, and apoptosis were up-regulated in the HPV-positive and the HPV-negative TSCC patients. In the HPV-positive TSCC patients, 37 genes were overexpressed, while the HPV-negative TSCC patients had 11 up-regulated genes. The TME of the HPV-associated and HPV-non-associated TSCC patients were found to be distinct, showing the dysregulation of various genes associated with cellular mediators and factors involved in inflammation, immunity crosstalk, transcription factors, immune signalling pathways, signal transduction, oncogenesis, tumor suppression, angiogenesis, and apoptosis. It is quite a surprising finding that in one patient, we observed a rather remarkable down-regulation of 88 genes. Furthermore, we detected six hr-HPV genotypes in 81% of the TSCC patients. The most common types were HPV-16 and HPV-35, followed by HPV-45, HPV-18, HPV-39, and HPV-31. In conclusion, we observed distinct heterogeneity in the HPV-associated and non-associated TSCC microenvironment transcriptomes.

B41. Mutations at the Conserved N-Terminal of the Human Rhinovirus Capsid Gene VP4 and Their Impact on the Immune Response

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Rhinoviruses (RVs) are the main cause of chronic obstructive pulmonary disease and are associated with exacerbation development, community-acquired pneumonia in children, and significant morbidity, mortality, and hospitalization. This study investigates the impact of changes in the amino terminal of the conserved VP4 epitope of different RV serotypes on pulmonary cytokine and chemokine responses and disease severity. Respiratory samples were collected from pediatric pneumonia patients who were either attending the outpatient department (OPD) or admitted to the Intensive Care Unit (ICU). Rhinovirus-positive samples were genetically characterized, and the gene expression of pulmonary Th1 and Th2 cytokines/chemokines was profiled using RT-PCR arrays. Genetic sequencing and 3D modeling revealed changes in the amino terminal of the conserved viral protein 4 (VP4) epitope in the RV-A101 serotype, specifically serine, at several crucial positions for binding with host immune cells. This finding led us to further investigate the pulmonary immunity of gene expression related to Th1 and Th2 cytokines and chemokines in pneumonia patients infected with RV-A101 and RV-C8. Dysregulation of pulmonary gene expression related to Th1 and Th2 immunity was observed in pneumonia patients with RV-A101 and RV-C8 infections. These findings contribute to a better understanding of RV immunity and the potential mechanisms involved in the pathogenesis of severe RV infections. However, further comprehensive functional studies are required to confirm the causal relationship.

B42. Exploring the Intriguing Role of the Turnip Yellows Virus's P3a Protein

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Turnip yellows virus (TuYV) is a plant virus that contributes to a significant yield losses in rapeseed crops worldwide. Like any other member of the *Polerovirus* genus, it possesses atypical features, such as a phloem-restricted tropism, and a plethora of translation mechanisms to express its proteins. Despite the fact that it was identified and studied for fifty years, researchers still struggle to unravel the molecular mechanisms related to viral replication and movement. Ten years ago, we discovered P3a, a 45-amino-acid-long protein expressed via a small and non-canonical ORF, which turned out to be mandatory for the long-distance movement of the virus (Smirnova *et al.*, 2015).
Based on this work, my thesis project aims to decipher to what extent the P3a is involved in the movement process of the TuYV, with a major focus on the molecular level. My research encompasses various aspects, including the assessment of the subcellular localization of recombinant P3a proteins with different cell markers in confocal microscopy and the search for cellular partners using an IP-MS/MS approach performed in two models (*N. benthamiana* and *A. thaliana*). These main experiments are being followed by others to confirm the hypothetical interactions previously detected in order to test the impact of knock-out backgrounds on the viral movement within the plants upon infection. A description of the workflow and interesting results will be presented in this poster, as well as new working hypotheses and future leads to explore.

B43. New Insights Into the Nuclear Export of Plant Pararetrovirus mRNAs

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Unlike most phytoviruses, the first stages of the CaMV life cycle take place in the nucleus of infected cells. From the viral genome, a circular dsDNA, two RNAs are synthesized, 35S and 19S RNAs. While the 19S RNA encodes only the P6 protein, the 35S RNA, which can be spliced, encodes all seven viral proteins; it is also the pre-genomic RNA that is retro-transcribed to form new viral genomes and possesses a highly structured 5'NTR region. After their synthesis and maturation, the RNAs are exported to the cytoplasm to be translated or retrotranscribed and encapsidated. The export of viral RNAs had not been studied until very recently. In 2021, we demonstrated that the 5'NTR region, which binds the viral proteins P4 and P5, also allows the recruitment of the adaptor proteins ALY and MOS11 involved in the TREX-1 nuclear export pathway of mRNAs (Kubina et al., 2021).
To identify the nuclear protein interactome of the 5'NTR region of CaMV 35S RNA, we adapted and implemented an in vitro capture approach based on the use of a 5'NTR-DNA-biotin chimera immobilized on magnetic streptavidin-coated beads. The results obtained via nLC-MS/MS allowed the identification of several potentially interesting proteins, including the nucleoporins NUA, Nup35S, Nup88, HOS1 and Ran-GAP2, as well as the TREX-1 components THO2 and THO7B. Another very interesting candidate is the MED18 subunit of the Mediator complex. Mediator is involved in transcription regulation but also interacts with the TREX-2 complex to couple transcription to mRNA export in yeast (Schneider et al., 2015). Moreover, *med18* KO Arabidopsis plants are resistant to CaMV infection (Hussein et al., 2020). We have demonstrated that MED18 also physically interacts with the viral export factor P4 in vitro and in vivo, and are currently evaluating the inhibition of CaMV mRNA nuclear export in *med18* KO plants.

B44. SARS-CoV-2 Nucleoprotein Is a Viral Regulatory Interactor of Protein Phosphatase 1 (PP1)

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Several studies have explored the importance of Protein Phosphatase 1 (PP1) in the context of viral infections, demonstrating that different viruses encode regulatory interactors of PP1 (RIPPOs), while others encode proteins that recruit host RIPPOs. In both scenarios, the PP1 activity is modulated to promote the viral life cycle and to evade the host immune response. Most RIPPOs have intrinsically disordered short linear motifs (SLiMs), such as the RVxF motif, that mediate binding to PP1. Here, we aimed to investigate whether specific proteins from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) might function as viral RIPPOs. A search was conducted to identify PP1-binding SLiM consensus sequences in SARS-CoV-2 proteins. The protein regions containing such SLiMs were evaluated to predict their disorder propensity and structural conformation. To validate the interaction between PP1 and SARS-CoV-2 nucleoprotein, *in vitro* binding assays and pull-down experiments were performed. The PP1 activity was also assessed in the presence of nucleoprotein. The *in-silico* analysis revealed that SARS-CoV-2 nucleoprotein possesses a RVxF motif that meets the requirements for PP1 docking, suggesting a potential interaction with PP1. The *in vitro* binding assay demonstrated that PP1 binds to the nucleoprotein, confirming a direct interaction between these proteins. Additionally, in cells transfected with SARS-CoV-2 nucleoprotein, the interaction with PP1 was also observed. Notably, the presence of nucleoprotein did not affect the PP1 activity either *in vitro* or in transfected cells. This suggests that PP1 activity remains unaltered but is directed towards certain substrates. Altogether, our preliminary findings indicate that SARS-CoV-2 nucleoprotein possesses a functional RVxF motif, enabling direct binding to PP1. These results support the hypothesis that PP1 has a putative role in SARS-CoV-2 viral infection. Further experimental research is required to unveil the molecular functions of PP1:nucleoprotein holoenzyme.

B45. The Activation of the Mitochondrial Unfolded Protein Response Regulated the Formation of Stress Granules

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How cells cope with harmful changes to their environment is critical to a healthy life. The integrated stress response (ISR) is a signalling network activated in response to physiological and pathophysiological conditions. The phosphorylation of the eukaryotic translation initiation factor eIF2α is a key ISR trigger leading to both a global attenuation of translation to preserve cellular resources, and the condensation of mRNAs and proteins into cytoplasmic biomolecular condensates called stress granules (SGs), thereby remodelling cytoplasmic contents and rewiring cellular functions. In addition to this first line of defence, the mitochondrial unfolded protein response (UPR^{mt}) protects mitochondria from harmful conditions through the induction of mitochondrial chaperones and proteases. Growing evidence implicates the ISR as a central element of UPR^{mt} regulation in mammalian systems.
Here, we present evidence that UPR^{mt} induction results in eIF2a phosphorylation and initial SG formation. SGs are then disassembled in mid/late UPR^{mt}, potentially due to an upregulation of SG disassembly chaperones. Moreover, GADD34 is induced in a later phase, protecting cells from prolonged stress by impairing the further assembly of SGs in response to eIF2a-dependent pathways. In addition, impairing the ability of cells to form SGs improved mitochondrial respiration during UPR^{mt} activation, indicating that the presence of SGs negatively affects mitochondrial homeostasis. These findings suggest a novel crosstalk between SGs and the UPR^{mt} that could contribute to restoring mitochondrial functions under stress. Furthermore, our research uncovers the impact of this crosstalk during viral infections, particularly with flaviviruses and coronaviruses, which manipulate both the SG response and mitochondrial dynamics to establish infection within host cells.

B46. Characterization of Structural Remodelling of Cellular Organelles Upon Coronaviridae Infections

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The COVID-19 pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), highlights the importance of elucidating the molecular mechanisms governing viral replication and its interactions with the host.
It was previously shown that SARS-CoV-2 genome replication occurs within double-membrane vesicles (DMVs), and viral infection remodels the cellular organelles both at the molecular and the structural level. In this study, we investigate how these alterations observed during SARS-CoV-2 infection are conserved among different Coronaviridae. To this aim, we combined imaging techniques, such as live-cell imaging and electron microscopy, to characterize how infection from a common-cold human coronavirus (OC43) alters the cellular organelles. We show that, similar to SARS-CoV-2, the formation of HCoV-OC43 DMVs is accompanied by a general remodelling of the cellular endomembrane system, such as the induction of Golgi fragmentation and the altered organization of peroxisomes.
By comparing observations from HCoV-OC43 and SARS-CoV-2 infections, the aim is to identify both shared features and differences that characterize Coronavirus interaction with the host cell.

B47. A role for SR Proteins in the Maintenance of HIV-1 Latency

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Reversal of HIV-1 latency remains a significant barrier to achieving a cure for this infection, and recent studies have highlighted issues in viral RNA processing as one component of the latency barrier. Key to regulating RNA processing are the host SR (serine–arginine-rich splicing factor) proteins. As part of our effort to understand the role that individual SR proteins play in regulating HIV-1 replication, we examined their interaction with HIV-1 RNA and the effect of their depletion on viral gene expression. eCLIP analysis determined that these factors had extensive overlap in their binding sites on the viral RNA. Despite similar binding profiles, of the eight SR proteins (SRSF1-7, 9) tested, depletion of only SRSF3, 5, and 9 yielded marked increases in HIV-1 protein expression and viral RNA accumulation in two T cell lines examined (JLat 10.6, CEM-T4). While SRSF3 and 5 depletion increased HIV-1 promoter function, loss of SRSF9 affected post-initiation events. In the context of CEMT4 cells, increased HIV-1 gene expression was predominately due to a >14-20 fold increase in the percentage of cells expressing Gag upon SRSF3 or SRSF9 depletion, an effect that could be further augmented by the addition of various latency-reversing agents. Effects on promoter function were not unique to HIV-1, as subsequent analyses determined that SRSF3 or 9 depletion also modulated the promoter function of multiple host genes. Together, these observations highlight an unexpected role for SRSF3, 5, and 9 in the regulation of HIV-1 latency through effects on viral promoter function.

B48. Peroxisomes and Viruses: A Dynamic Duo

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During viral infections, host cell organelles are explored by both the virus and the cellular immune system. Peroxisomes, subcellular organelles that fulfil an essential role in multiple metabolic pathways, have emerged as important antiviral and pro-viral platforms in the context of viral infections. Several viruses have been shown to modulate the morphology and/or metabolism of peroxisomes to either evade the cellular antiviral response or promote virus particle formation and propagation. Nevertheless, research in this field is still relatively incomplete as mechanistical details are scarce and, for most viruses, only specific infection stages have been analyzed. Furthermore, peroxisomes are highly dynamic organelles that can change their number and morphology upon diverse stimuli, and these parameters have been shown to be manipulated by different viruses. However, there is still a need to investigate how these changes occur during the virus lifecycle and in the context of antiviral signaling. Hence, our work aims to disclose the flow in peroxisome dynamics during viral infection and upon activation of the cellular antiviral response. For this purpose, we conducted a spatiotemporal imaging analysis of peroxisome dynamics during influenza A virus (IAV) infection and upon stimulation of the peroxisome-dependent antiviral signaling with an RNA molecule that mimics viral RNA. Furthermore, we interfered with the peroxisomal fission machinery and evaluated its importance for antiviral signaling. Our results indicate that stimulation of the peroxisomal MAVS induces a certain level of peroxisome elongation and spatial redistribution of these organelles and suggest that peroxisome proliferation may play an important role in antiviral signaling. In addition, in the context of viral infection, peroxisome dynamics were shown to be altered by IAV in a lifecycle-dependent manner.

B49. Deciphering the Transfer Ribonucleic Acid-Modifying Enzyme Landscape in Non-severe Human Coronavirus Infection

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Respiratory viruses extensively manipulate the host cell, including its protein synthesis and processing machinery. Our research focuses on how respiratory RNA viruses alter the host cell transfer RNA (tRNA) pool and its associated components during infection. tRNAs are essential for protein synthesis and are also heavily modified by the tRNA-modifying enzymes (TMEs) to ensure efficient translation. Through studying the interplay between the viruses, tRNAs and their associated components, we aim to uncover the specific mechanisms by which respiratory viruses advantageously manipulate the host cell.
Our recent observations have shed light on the intriguing interaction between influenza A virus (IAV) and the host cell tRNA TME population, resulting in significant alterations in viral protein translation. To advance our comprehensive understanding of this phenomenon, we determine whether this manipulation strategy extends to other respiratory viruses, particularly human coronaviruses, with an emphasis on human coronavirus 229E (hCoV-229E).
When the abundance of tRNAs and the mRNA codon requirements is balanced within a cell, the hCoV-229E genome exhibits a preference for adenosine (A)- and uridine (U)-ending codons, which usually does not correlate with the host's preferences. Despite this discrepancy in codon usage, the hCoV-229E infection persists in mammalian cells, suggesting that the virus may dynamically adapt cellular tRNA pools to match its RNA codon requirements.
Our investigation of the interplay between hCoV-229E and the TMEs sheds some light on the intricate molecular mechanisms governing viral replication. Utilizing siRNAs that target the TMEs, less viral particle production is observed, accompanied by an increase in protein aggregation, suggesting its possible role in maintaining proper protein folding dynamics and proteostasis. These initial observations hint at the elaborate influence of the TMEs on viral particle production and proteostasis, contributing to the discovery of new targets for host-directed antiviral therapy.

B50. Genetic Characterization of Influenza A(H1N1) pdm09 and A(H3N2) Viruses Circulating in Mauritius from 2017 to 2019

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Background: Mauritius, a tropical island to the West of Africa, experiences circulation of influenza with no distinct pattern, and data on circulating strains are limited. Here, we describe the genetic and evolutionary characteristics of Influenza A(H1N1) pdm09 and A(H3N2) strains that circulated in Mauritius from 2017 to 2019.
Methods: Influenza A virus isolates (N=118) from National Influenza Surveillance Programs between 2017 and 2019 were used. Influenza A(H1N1)pdm09 accounted for 77.1% (91/118) and A(H3N2) for 22.7% (27/118) of isolates. We PCR-amplified all eight genome segments in a single reaction followed by Oxford Nanopore sequencing. Gene assembly and mutational analysis were performed and a phylogenetic tree was constructed using the Genome Detective Virus tool, Nextclade, flusurver and figtree.
Results: Among 51.6 % A(H1N1)pdm09 (47/91) 6B subclades with varying annual dominance were 6B.1(13/16, 2017), 6B.1A.5a (5/12, 2018) and 6B.1A.5a (14/19, 2019). An HA mutational analysis identified two amino acid substitutions, A232E and D239G, involved in host cell receptor binding. Influenza A(H3N2) viruses (70.4%, 19/27) belonged to 3C.2 and subclades with varying dominance: 3C.2(4/6, 2017), 3C.2a1b.1 (7/10,2018) and 3C.2a1b.2b (2/3, 2019). Over the 3 years, the number of N-linked glycosylation sites (PNGs) in the HA of A(H1N1)pdm09 was mostly seven (44/47) and was more variable among A(H3N2) sequences. Four A(H1N1)pdm09 strains had the D151E mutation and two A(H3N2) viruses had the D151N mutation in NA, which is associated with a reduced sensitivity to neuraminidase inhibitors. The number of PNGs across NA protein sequences of A(H1N1)pdm09 (46/47) and A(H3N2) (14/19) remained fairly constant at 8.
Discussion and Conclusions: In Mauritius, A(H1N1)pdm09 6B subclades vary, with 6B.1A.5a emerging and dominating in 2018 and 2019. From 2017 to 2019, A(H3N2) 3C.2 dominance changed in the following way: 3C.2 > 3C.2a1b.1 > 3C.2a1b.2b. Mutations affecting sensitivity to neuraminidase inhibitors were limited.

B51. Genomic Insights Into SARS-CoV-2 Epidemiological Waves and Seasonal Fluctuations: A Study of the Epidemic in Cyprus

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The persistent global challenge presented by the COVID-19 pandemic, instigated by the emergence of SARS-CoV-2 in December 2019, has compelled a profound examination of its evolution. This ongoing transformation of SARS-CoV-2 has driven heightened transmission rates, immune resistance, and recurrent waves of infection worldwide. Our extensive molecular epidemiology research into SARS-CoV-2 infection in Cyprus, spanning the period from April 2020 to October 2022, has been dedicated to unraveling the complex interplay between SARS-CoV-2 variants and the seasonal dynamics driving infection waves within a specific region. Employing comprehensive genomic analyses, we methodically analyzed 7,648 viral sequences, delineating 231 distinct variants and lineages. Notably, a subset of these variants played a pivotal role in catalyzing waves of infection that subsided and flowed with the changing seasons. In Cyprus, our investigation unveiled a compelling sequence of six distinct infection waves that unfolded against the backdrop of seven key lineages and their sublineages. The inaugural wave, witnessed in the spring of 2020, bore the genetic signature of B.1.1.29, a member of the B.1.1 parental lineage. Subsequent waves were steered by B.1.258 in autumn 2020 through to winter 2021, followed by Alpha in spring 2021, Delta in summer 2021, and the Omicron 1 and 2 variants during the winter and spring of 2022. The sequence of epidemiological waves finished in the sixth wave, steered by Omicron 5, during the summer of 2022. Beyond the meticulous scrutiny of genetic variant dynamics, our research illuminated the intricate patterns of SARS-CoV-2 migration, with Cyprus serving as a focal point. These findings underscore the prevailing paradigm, suggesting that SARS-CoV-2's propagation and the concomitant waves of infection were precipitated by the introduction of distinct, highly transmissible lineages. Notably, our study dispelled earlier assumptions regarding the virus's seasonality, as the influx of more infectious variants and subsequent surges in infection occurred across the entire spectrum of seasons.

B52. HERV Expression in SARS-CoV-2-Positive Placentas

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Introduction: HERV activation is a well-known phenomenon associated to several inflammatory pathologies including COVID-19 disease. COVID-19 has been related with a higher risk of suffering pregnancy disorders with placental damage. Since SARS-CoV-2 has been detected in placental samples, this study aimed to investigate the possible relationship between placental HERV expression, pregnancy complications and placental barrier function in COVID-19 disease.
Methods: We quantitively analyzed mRNA expression of a set of endogenous retrovirus elements in placental samples taken from women diagnosed with COVID-19 at some point during pregnancy, using qPCR. We also analyzed mRNA expression of interferon (IFN) genes.
Results: Placental samples from SARS-CoV-2 positive mothers, either with pregnancy disorders or not, exhibited significantly lower levels of *ERVW1*, *HERVW*, *HERVK* and *HEMO* expression. Relative expression levels of *HERVK ENV*, *LINE-1* and *HERVH* were similar to controls when analyzing the whole subset of placentas from pregnant women with COVID-19. However, our data showed a few cases in which *HERVH* was upregulated. In mothers with confirmed COVID-19 disease, higher levels of IFN I and III expression were detected regardless of the presence of SARS-CoV-2 transcripts in the placenta.
Conclusions and discussion: While HERV expression is reportedly induced in blood lymphocytes and other tissues as a response to SARS-CoV-2 infection, we detect lower levels when analyzing the expression of retroviral elements from different families in the placenta of COVID-19 patients. Careful study of cases with placental SARS-CoV-2 transcripts have confirmed the absence of HERV induction even in these cases, with the exception a pair of high positive placentas in which COVID-19 has activated the expression of the human endogenous retrovirus *HERVH*. We also have found indications of an IFN-based anti-viral protection mechanism that is active in human placentas in SARS-CoV-2 infected mothers, regardless of the presence of viral transcripts in the placenta.

B53. Large-Scale Genomics and Deep Mutational Scanning Reveal the Limited Role of Immune Pressure on the Genetic Diversification of the Outer Capsid (VP7) of G3 Rotaviruses

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Rotavirus is a major global cause of acute gastroenteritis in children under five years old. Although the implementation of oral vaccines has greatly reduced rotavirus infection in high-income countries, their efficacy is variable in other regions. The cause is likely multifactorial, but antigenic diversification is suspected to play a role in diminishing vaccine efficacy. Here, we collected all (N=17,973) sequences of VP7, an immunodominant structural protein, from human and animal rotaviruses available in GenBank and performed sequence analyses with 2255 G3 rotavirus sequences detected during the period 1973-2022. The generic analysis suggested no significant accumulation of mutations over time, with ~6 (range 1-15) amino acid differences in any given year. A Shannon entropy analysis identified 25 highly variable residues, 10 mapped to three known antigenic sites, and 5 directly contiguous to antigenic sites. To assess the importance of these sites against neutralizing antibodies, we incorporated all possible mutations to 51 residues (all 29 antigenic site residues and 22 high-entropy, surface-facing residues) of the VP7 (VP7m) from SA11 (G3, prototype rotavirus). Two VP7m lineages with distinct mutation profiles were developed via reverse genetics and successfully rescued across five passages. These lineages were tested against neutralizing concentrations of anti-G3 mouse monoclonal and anti-SA11 rabbit sera. Breakthrough mutations were identified against the sera and monoclonal. High-resolution sequencing showed that a single codon mutation at residue 94 (antigenic site 7-1a) is sufficient for escape from monoclonal neutralization. Similarly, a mutation at residue 212 (antigenic site 7-1b) enabled rotavirus escape against anti-SA11 sera. Notably, residue 94 is conserved in rotavirus at a global level, while residues from antigenic site 7-1b, including 212, are highly variable. The latter suggests that only a subset of the variable sites respond to anti-viral activity from polyclonal sera. Together, despite transient and extensive genetic diversification, antigenic pressure seems to have a minimal effect on rotavirus genetic drift.

B54. Might a Greater Variability of Porcine Rotavirus A Hamper Vaccine Efficacy in Pigs?

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Rotaviruses (RVs) belong to the *Reoviridae* family and are a major cause of acute viral gastroenteritis in young animals, including piglets.
During our study in 2016–2019, 167 samples of domestic pig faeces were screened for the presence of RVA and 44.9% tested positive (n=75). The most common porcine G-genotypes were G9 (36% of all RVA-positive samples), G4 (26%), G5 (17%), and G11 (12%) in combination with different P-types (mostly with P[6] and P[13]). Most commercial farms in the Czech Republic use rotavirus vaccines intended for the active immunisation of pregnant sows and gilts to induce colostral and lactogenic immunity in newborn piglets. However, those vaccines only include the OSU RVA strain, which is of G5P[7] genotype.
Ongoing surveillance of porcine RVA genotypes (2020–2023) showed another increase in the representation of non-G5 genotypes (50% of G9, 28% of G4, and 7% of G11). Only 5% of successfully genotyped samples were of G5 genotype.
Similar changes in the proportions of human RVA genotypes were demonstrated in several large-scale molecular epidemiological studies after the introduction of vaccination in different countries, suggesting the possibility of RVA's escape from vaccine-induced immune pressure.
Therefore, continuing surveillance studies of porcine rotavirus genotypes are needed to monitor the dynamics of RVA and to be able to responsibly evaluate the effectiveness of RVA vaccines used in swine production.

B55. A Sex-Specific CD4+ T Cell Response Limits Coxsackievirus B Pathogenesis in Mice

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Sex is a significant contributor to the outcome of human infections. Males are frequently more susceptible to viral, bacterial, and fungal infections, often attributed to weaker immune responses. In contrast, a heightened immune response in females enables better pathogen elimination but leaves females more predisposed to autoimmune diseases. Unfortunately, the underlying basis for sex-specific immune responses remains poorly understood. Here, we show a sex difference in the $CD4^+$ T cell response to an enteric virus, Coxsackievirus B3 (CVB3). We found that CVB3 induced CD4+ and CD8+ T cell expansion in female mice but not in male mice. CVB3 infection also led to the expansion of $CD62L^{lo}$ $CD4^+$ T cells in the mesenteric lymph node and spleen of female but not male mice as early as five days post inoculation, indicative of activation. Using a recombinant CVB3 virus expressing a model $CD4^+$ T cell epitope, we found that the expansion of $CD4^+$ T cells in females is viral-specific and not due to bystander activation. Finally, the depletion of $CD4^+$ T cells before infection led to enhanced mortality, indicating that CD4+ T cells protect against CVB3 in female mice. These data demonstrate that CVB3 induces a $CD4^+$ T cell response in female mice, and highlight the importance of sex-specific immune responses to viral pathogens.

B56. Impact of Direct Acting Antivirals (DAAs) on Morphogenesis and Plasticity of Hepatic Cells

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Hepatitis C virus (HCV) infection is a major public health concern, often resulting in severe liver diseases such as steatosis, fibrosis, and hepatocellular carcinoma (HCC). While direct-acting antivirals (DAA) have revolutionized HCV treatment by ensuring viral elimination, the risk of disease progression and cancer development persists.
The epithelial-mesenchymal transition (EMT) is the most studied form of plasticity and represents a hallmark of cancer. The role of EMT in HCC is well established, however, the precise impact of DAA on this process remains poorly understood. To address this gap, we conducted research using a 3D-cell culture model based on organoids using Huh7 cells and those expressing the full-length HCV genomic replicon (Huh7-R).
Our investigations by immunofluorescence and confocal microscopy, revealed that Huh7 cells form organoid with canaliculi in 3D culture, while the Huh7-R cells presented multi-lumen phenotype and exhibited visible membrane protrusions, loss of polarity, and EMT characteristics. Treatment of these cells with DAA restored polarity, including the formation of bile canaliculi. Additionally, both immunofluorescence and immunoblot analyses demonstrated that DAA strongly decreased expression of the EMT markers induced by HCV. Transcriptomic analysis, including an EMT score assessment, supported these findings.
However, it's worth noting that while DAA treatment significantly rescued polarity and down-regulated the expression of EMT markers in Huh7-R cells, it never fully restored them to the levels observed in control. Consequently, residual EMT conditions following HCV cure may serve as a foundation for the development and progression of chronic liver diseases.
In conclusion, our research highlights the significance of EMT as a critical process to investigate when developing prognostic and therapeutic strategies for HCV infection and its associated liver diseases. By better understanding the impact of DAA on EMT, we can potentially enhance our ability to address the ongoing health challenges posed by HCV infection.

B57. Extracellular Vesicle Spreading of CVB3: Genetic Basis and Evolutionary Implications

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In recent years, scientific researchers have unveiled groundbreaking insights into the collective transmission of viruses, fundamentally challenging the traditional virology paradigm of "one infectious unit; one infectious particle." One prominent avenue for collective viral propagation is via the extracellular vesicles (EVs), raising intriguing questions about the underlying genetic mechanisms and their evolutionary implications. This study aimed to shed light on extracellular vesicle transmission. CVB3, an enterovirus, is renowned for its release of virion clusters within the EVs.
Through experimentation, we have discerned that CVB3 adapts to selective pressures by modifying its capacity for extracellular vesicle transmission. Notably, the N63H mutation within the VP3 protein dramatically reduced the group transmission of CVB3 by over 100-fold, providing compelling evidence for a genetic cause of group dissemination in the EVs. Our in-depth analysis of the genetic diversity within CVB3 virion clusters, employing fluorescence microscopy and flow cytometry to study the eGFP and mCherry-encoded CVB3 variants, reveals that these clusters primarily consist of closely related viral particles, thus discouraging the co-transmission of distinct viral genetic variants. Intriguingly, when we pitted the wild-type CVB3 against the N63H mutant, we found that extracellular vesicle transmission incurred substantial costs in the culture conditions due to the dispersal of the groups. However, it notably accelerated the infection and the release of viral progeny in the cells infected as a group. Consequently, while vesicle co-transmission is infrequent and primarily fosters short-lived cooperation events based on diversity, en bloc transmission could play a pivotal role, especially during the initial stages of the infection cycle and host-to-host transmission.
These discoveries offer valuable insights into the genetic mechanism and evolutionary consequences of extracellular vesicle transmission. This knowledge may hold the key to the development of enhanced health and epidemiology protocols, as well as the creation of more effective antiviral drugs.

B58. Uncovering Enterovirus Structural Plasticity Through Deep Indel Screening

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Indels are important sources of novelty and innovation in protein evolution. In RNA viruses, high mutation rates allow them to explore a broad range of evolutionary pathways leading to significant phenotypic changes through altered engagement with receptors or other host factors. In this work, we expand the mutational landscape explored in RNA viruses to indels focusing on Enterovirus A71 (EV-A71) as a prototype for this emerging viral family. We engineered ~50,000 indel variants across the complete viral proteome and quantified their fitness effects using a novel experimental-computational sequencing pipeline. As a general trend, most indels were lethal to the virus. The rare, rescued hits were identified reproducibly confirming the ability of our screen to fully explore the evolutionary potential of indels in viral proteins. The identified hotspots correlate with sites of indels within the Enterovirus family, mapping to regions that do not interfere with receptor binding and enzymatic activity. Our work reveals the tolerance for indels across the Enterovirus proteome and identifies regions of evolutionary capacity that require further monitoring.

B59. Dissecting Mechanisms of Coronavirus Recombination

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As exemplified by the ongoing SARS-CoV-2 pandemic, coronaviruses represent a significant and understudied public health threat. A key factor in the vast genetic and phenotypic diversity of coronaviruses is their propensity for recombination. Recombination events have been implicated in the emergence of all three pandemic coronaviruses. Additionally, coronaviruses play host to molecular characteristics unique among RNA viruses. Coronaviruses utilize discontinuous transcription to produce subgenomic template RNAs. This process is regulated by RNA hairpin structures known as transcription regulatory sequences (TRSs), which recruit the viral replicase, resulting in a joining of the 5' leader sequence with each of the structural and accessory body genes. While thought to be a result of template switching during discontinuous transcription, the mechanism underlying high-frequency recombination in coronaviruses has not been elucidated. Additionally, viral protein nsp14, a proofreading exonuclease and methyltransferase, has been shown to regulate recombination location and frequency, but the mechanism underlying this phenomenon remains uninvestigated. Here, we utilize a diverse library of viral sequences and an in cellulo SARS-CoV-2 replicon to identify the structure and sequence motifs associated with high-frequency recombination. A library of dually barcoded constructs containing coronaviral sequences flanking various TRS sites is introduced to cells expressing the SARS-CoV-2 replicase, and chimeric molecules resulting from recombination are identified by long-read sequencing. Elucidating the mechanism of recombination in coronaviruses is essential to our understanding of pathogenic virus emergence and tracking the evolutionary trajectory of these important pathogens.

B60. Genetic Diversity Among Rose Rosette Virus Isolates: A Roadmap Towards Studies of Gene Function and Pathogenicity

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Rose rosette virus (RRV) is an enveloped virus with a negative-strand RNA genome and is a member of the genus *Emaravirus* within the family *Fimoviridae.* RRV has seven genome segments, and all are monocistronic, except RNA6, which is bicistronic. The mutation spectrum within a virus species impacts virus fitness, adaptive responses, antigenic drift, and natural selection. Mutations can offer biological advantages or disadvantages, influence the behavior of a virus population, and can also lead to the accumulation of defective interfering (DI) RNAs. This study was undertaken to gain insights into the genetic diversity and the nature of the genetic changes among the full genomes of sequenced RRV isolates. An analysis of nucleotide diversity across the concatenated isolates showed lower genetic differences among RNAs encoding the core proteins required for encapsidation than the latter genome segments. Recombination breakpoints were identified near the junctions of several genome segments, suggesting that the genetic exchange of segments contributes to differences among isolates. The maximum likelihood (ML) analysis of individual RNA segments revealed different relationship patterns among isolates, which supports the notion of genome reassortment. We tracked the branch positions of two newly sequenced isolates to highlight how genome segments relate to segments of other isolates. These results suggest greater diversity among RRV isolates than previously recognized.

B61. Viral Non-coding RNA Resistant to Exoribonuclease: A Synergic Actor of RNA Silencing Suppression and Promoter of Viral Long-Distance Movement

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Beet necrotic yellow vein virus (BNYVV) provokes sugar beet rhizomania. This rod-shaped multipartite virus possesses four to five positive strand RNAs. Its viral long-distance movement in beet requires movement proteins, the viral suppression of RNA silencing (VSR) and the accumulation of a viral non-coding RNA (ncRNA) acting synergistically on RNA silencing suppression. The ncRNA is produced through a partial degradation of BNYVV genomic RNA3 by the cellular 5'-3' exoribonuclease (XRN). The XRN enzyme is blocked by a structure involving the 20 nts long "coremin" pattern and a 20 nts long downstream sequence. The published folding of this ncRNA3 does not fit with an exoribonuclease-resistant RNA (xrRNA). An alternative fold is proposed which is reminiscent of the Flavivirus sfRNAs, an xrRNA involved in viral pathogenicity.
Our goal consists in the validation of the model by introducing mutations disrupting base pairings and reintroducing compensatory base changes in "coremin". The accumulation of ncRNA has been tested in the viral context. We will present whether ncRNA is still able to act in synergy with the VSR and promote systemic movement.

B62. Drivers and Dynamics of a SARS-CoV-2 Sister Clade Sarbecovirus During a Long-Term Follow-up of a Rhinolophus Bats' Colony

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SARS-CoV-2, a Betacoronavirus Sarbecovirus responsible for the COVID-19 pandemic, probably originated in Rhinolophus spp. bats. With more than 1400 species worldwide, bats are known to harbour a great diversity of alpha- and betacoronaviruses.
Our study aims to unravel the origin of viral diversity in wildlife, the dynamics of new linages appearance and the drivers and mecanisms of emergences. Thus, we conducted an extensive longitudinal survey of a Rhinolophus ferrumequinum maternity colony that harbours BBCoV, a european SARSr-CoV.
More than 2500 non-invasive faecal samples were collected at high frequency rate and screened by Sarbecovirus qRT-PCR to investigate BBCoV prevalence and individual excretion dynamics overtime. Consensus genomes were deep-sequenced and analysed using a time-resolved Bayesian method. We also investigated the selection pressures at different scales and on intra-host diversity through the study of SNPs (single nucleotide polymorphism) frequencies.
Between 2014 and 2020, 1168 BBCoV-positive individuals were detected. The variations in BBCoV circulation across the year are closely related to the seasonal behaviour of the colony (gathering of individuals, births, weaning, infection-reinfection dynamics). The phylogenetic reconstruction shows a gradual emergence of BBCoV, though punctuated by the rooting of most recent genomes to 2016 lineages, suggesting the existence of recombination events, confirmed by recombinant detection analyses. Intra-host diversity dynamics, heterogeneous across individuals and time, is subjected to differential selection pressures across the genome. Indeed, positive selection pressures on specific sites in the ORF1a seem to rule BBCoV posterior circulation.
In conclusion, this in-depth study of a SARS-like Sarbecovirus species in Europe provides a robust natural model to explain how natural circulation of coronaviruses can opportunistically lead to the birth of better-fitting variants and new lineages, that may spill-over in other species as a first step toward emergence.

B63. Characterization of SARS-CoV-2 Substitutions Causing Remdesivir Resistance

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Remdesivir is a broad-spectrum antiviral that efficiently inhibits severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Here, we performed head-to-head comparisons of the levels of remdesivir resistance conferred by novel and previously described substitutions using a subgenomic SARS-CoV-2 replicon system in Vero E6 cells.
Two novel substitutions, E796D (nsp12) and A225S (nsp14), were found after treatment with increasing concentrations of remdesivir. Compared to the wild-type, the E796D substitution (previously observed in an immunocompromised individual after treatment), conferred an 11-fold replication increase in the presence of remdesivir. Addition of Q225S increased replication 16-fold under the same conditions. Neither substitution negatively affected baseline replication or viral propagation.
Nsp12 substitutions S759A or E802D have similarly been previously described in vitro and in treated individuals. S759A conferred the highest levels of replication in the presence of remdesivir (189-fold increase). However, it greatly impacted baseline replication (38-fold decrease). Nsp12 substitutions V166A, N198S, V792I, or C799F co-selected with S759A in vitro, but when combined with S759A, none of them restored baseline replication. However, under remdesivir treatment, V166A, V792I or C799F increased replication (38-, 15-, and 33-fold, respectively), particularly when combined with S759A: 822- and 240-fold increases for S759A+V792I and S759A+V166A+N198S+C799F, respectively. E802D only increased replication 3-fold in the presence of remdesivir, and it did not affect baseline replication.
In conclusion, we found two novel substitutions (nsp12-E796D and nsp14-A225S) that significantly decrease susceptibility to remdesivir. Compared to previously reported nsp12-substitutions, E796D+A225S causes intermediate resistance. Changes at the catalytic S759 alone or combined with additional substitutions in/or adjacent to motif D conferred the highest drug resistance levels.

B64. Double Trouble and Diagnostic Dilemma—Dengue and Scrub Typhus Co-infection Resulting in the Mimicry of Similar Clinical Implications in the Hosts: A Study Based on the Sub-Tropical Region of Arunachal Pradesh, India

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INTRODUCTION: With all the explosive studies of co-infection cases where multiple pathogens can circulate simultaneously, this study focuses on viral and bacterial co-infections such as dengue (a mosquito-borne disease caused by a Flavivirus) and scrub typhus (caused by the *Orientia tsutsugamushi* bacterium) co-infection. Both are common and often debilitating tropical and sub-tropical diseases. Demographically, this study was a preliminary study, to investigate the broader aspect of dengue and scrub typhus co-infection in the sub-tropical region of Arunachal Pradesh, India, and their detailed pathogenesis, viral sequences paramount to the co-infection cases, and the molecular characterisation of the pathogen.
Rationale: This study was based on the previously reported case of septic shock syndrome (SSS), which resulted in fatal cases in patients co-infected with dengue and scrub typhus in other parts of India, yet no case report was available from the sub-tropical region of Arunachal Pradesh.
Methodology: A seasonal study was conducted during which patients presenting with acute febrile illnesses and related symptoms were tested for dengue and scrub typhus infections using serological and molecular diagnostic methods. The patients' demographic and clinical data were collected and analysed to identify co-infected cases.
Results: This study revealed a notable prevalence of co-infection cases, with a substantial percentage of the studied population exhibiting simultaneous infections of dengue and scrub typhus. Most of the co-infected individuals presented a range of similar clinical symptoms, including high fever, headache, rash, and myalgia. The clinical presentation in co-infected patients was more severe and prolonged compared to single/mono infections. Furthermore, co-infected individuals experienced a delay in diagnosis and treatment due to overlapping symptomatology.
Conclusions: Dengue and scrub typhus co-infection results in a complex clinical presentation, making timely diagnosis and treatment difficult. This study highlights the necessity of a comprehensive approach to tackle the complications of co-infection cases.

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