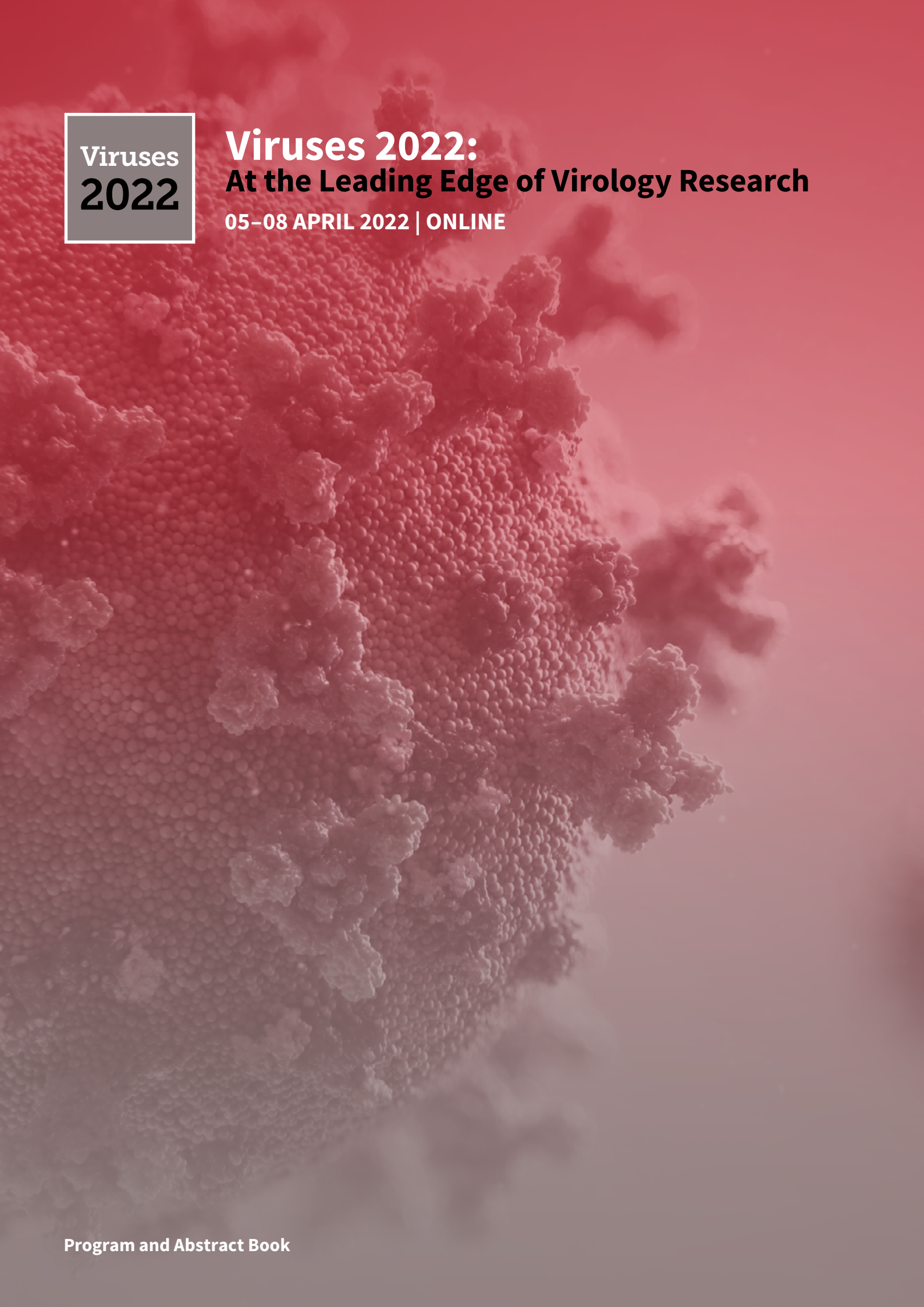

Viruses 2022

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At the Leading Edge of Virology Research

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Viruses 2022 - At the Leading Edge of Virology Research

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Welcome from the Chair

Dear Colleagues,

It is with great pleasure that I announce the conference Viruses 2022—At the Leading Edge of Virology Research, to be held virtually 5-8 April 2022 at 13:30-19:00h CEST/07:30-13:00h EDT.

As we enter the third year of the COVID-19 pandemic, the central importance of virology to all aspects of the human enterprise has never been clearer. The pandemic starkly illustrates the impact that viruses can have on human health and activity, as well as underscoring how advances in virus research can rapidly mitigate the most severe outcomes. While the COVID-19 pandemic has been on everyone's mind, we should not lose sight of the significance of other viral infections or overlook the utility of viruses or viral components that can be harnessed to serve useful and important functions; nor should we ignore the power of viral systems in studies across a wide range of biomedical disciplines, including molecular and cell biology, immunology, chemical biology, evolutionary biology, and structural biology.

This conference is sponsored by MDPI, the publisher of the open-access journal Viruses, and follows the very successful meetings Viruses 2016—At the Forefront of Virus-Host Interactions (January 2016; Basel, Switzerland), Viruses 2018—Breakthroughs in Viral Replication (February 2018; Barcelona, Spain), and Viruses 2020—Novel Concepts in Virology (February 2020; Barcelona, Spain). Like these previous conferences, Viruses 2022 will cover a wide range of topics of interest to participants. In addition to invited talks from world-renowned virologists, the conference will feature oral presentations and posters selected from submitted abstracts. The event will be recorded and will then be made accessible to conference participants on our Sciforum platform

I, and the Viruses 2022 Scientific Committee (Drs. Shan-Lu Liu, Mark Young, Britt Glaunsinger, Kathy Spindler, Veronique Ziegler-Graff), look forward to your participation in this exciting conference.

Eric O. Freed, Ph.D.

Viruses 2022 Chair and

Editor-in-Chief, Viruses

*** Certificate of Attendance**
Upon request, the participants of the event will receive an electronic Certificate of Attendance by email once the event is concluded.

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General Information

Viruses (ISSN: 1999-4915) *i*s a peer-reviewed, open access journal of virology, published monthly online by MDPI. The American Society for Virology (ASV), the Spanish Society for Virology (SEV), the Canadian Society for Virology (CSV), the Italian Society for Virology (SIV-ISV), the Australasian Virology Society (AVS) and more societies are affiliated with Viruses and their members receive a discount on the article processing charges.
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Program

Program Overview

5 April 2022	6 April 2022	7 April 2022	8 April 2022
Welcome from the Chair **Session 1. General Topics in Virology**	**Session 2. Antiviral Therapeutics, Vaccines, and Host Defenses**	**Session 4. Mechanisms of Virus Replication**	**Session 6. Viral Pathogenesis and Evolution**
Break	Break	Break	Break
Poster Session I	**Session 3. Antiviral Innate Immunity**	**Session 5. Virus-Host Interactions - Part I** Break **Poster Session II**	**Session 7. Virus-Host Interactions - Part II** Chair Closing

Conference Program

Day 1 - Tuesday 5 April 2022

Time (EDT)	Time (CEST)	Speaker	Title
08:00-08:10	14:00-14:10	Welcome from the Chair - Eric Freed	
Session 1. General Topics in Virology			
Session Chair: Eric Freed			
08:10-08:50	14:10-14:50	Ralph Baric	Emerging Coronviruses: Mechanisms of Pathogenesis and Control
08:50-09:15	14:50-15:15	Nicholas Heaton	Genetic approaches to understand and prevent influenza disease
09:15-09:30	15:15-15:30	You Zhang	*sciforum-057502* - Human LAMP1 markedly accelerates the kinetics of Lassa virus fusion and promotes fusion pore dilation
09:30-09:45	15:30-15:45	Baldeep Khare	*sciforum-057557* - Usutu virus SAAR-1776: new insights from 2.4 Å resolution cryo-EM structures of a mature flavivirus
09:45-10:00	**15:45-16:00**	**Break**	
Session Chair: Gregory Melikian			
10:00-10:25	16:00-16:25	Katherine Spindler	Evasion of Host Antiviral Responses PKR and GCN2 in an Animal Model of Adenovirus Infection
10:25-10:50	16:25-16:50	Eric Freed	Role of membrane lipids and inositol hexakisphosphate in HIV-1 replication
10:50-11:05	16:50-17:05	Isabelle Reichert	*sciforum-058159* - Lipid droplet biogenesis modulates the formation of the hepatitis C virus replication organelle
11:05-11:20	17:05-17:20	Tamari Chkuaseli	*sciforum-057501* - Determinants of Stop Codon Readthrough Efficiency in an RNA Virus
11:20-11:35	17:20-17:35	Déborah Bernard	*sciforum-057882* - Subcellular relocalization of A. thaliana ALY proteins upon infection with turnip yellows virus (TuYV)
11:35-12:05	**17:35-18:05**	**Break**	
Flash Talks and Poster Session I			
Session Chair: Nathalie Grandvaux			
12:05-12:10	18:05-18:10	Jibin Sadasivan	*sciforum-057203* - A viral protein impairs stress granule formation by modulating Nup358
12:10-12:15	18:10-18:15	Lifeng Liu	*sciforum-057413* - Investigating the effects of apolipoprotein E on Herpes simplex virus infection.

12:15-12:20	18:15-18:20	Frederique Laprise	*sciforum-057493* - SARS-CoV-2 accessory protein ORF8 downregulates the polymeric Ig receptor
12:20-12:25	18:20-18:25	Bernadett Papp	*sciforum-057299* - Rapid virus-driven reprogramming predisposes oral epithelial cells to lytic infections
12:25-12:30	18:25-18:30	Erik Schubert	*sciforum-058291* - Human adenovirus type 5 infection alters mitochondrial DNA and membrane dynamics
12:30-13:20	18:30-19:20	Poster Session I (Break Out Rooms)	

Day 2 - Wednesday 6 April 2022

Time (EDT)	Time (CEST)	Speaker	Title
Session 2. Antiviral Therapeutics, Vaccines, and Host Defenses			
Session Chair: Viktor Müller			
07:30-07:55	13:30-13:55	Yoshihiro Kawaoka	Addressing the Threat of Emerging Viral Infections
07:55-08:10	13:55-14:10	Vera van Vliet	*sciforum-057919* - Ubiquitin variants as antivirals against SARS-CoV-2
08:10-08:25	14:10-14:25	Hong-Su Park	*sciforum-057495* - A single intranasal immunization with avian paramyxovirus type 3 expressing the SARS-CoV-2 spike protein protects hamsters against SARS-CoV-2
08:25-08:40	14:25-14:40	Jitendriya Swain	*sciforum-057473* - Late phases of SARS-COV-2 replication in pulmonary cells triggers reorganization of F-actin nanostructures required for infection
08:40-08:55	**14:40-14:55**	**Break**	
Session Chair: Amanda Hargrove			
08:55-09:10	14:55-15:10	Claudia Claus	*sciforum-058342* - Interferon signalling influences the impact of glycolysis on virus infections
09:10-09:25	15:10-15:25	Emmely Treffers	*sciforum-057337* - Phosphoproteomics analysis of chikungunya virus-infected cells uncovers a novel host shut-off mechanism based on eEF2 phosphorylation induced by an increase in cAMP by the alphavirus nsP2 NTPase
09:25-09:40	15:25-15:40	Felipe Diaz-Griffero	*sciforum-057181* - GS-CA1 and Lenacapavir induce a conformational change that stabilizes the HIV-1 core and blocks viral uncoating
09:40-10:10	**15:40-16:10**	**Break**	
Session 3. Antiviral Innate Immunity			
Session Chair: Andrew Broadbent			

10:10-10:35	16:10-16:35	Ricardo Rajsbaum	Regulation of Antiviral Innate Immunity and Virus replication by Unanchored Ubiquitin Chains and the E3-Ubiquitin Ligase TRIM6
10:35-11:00	16:35-17:00	Sonja Best	Manipulation of mitochondrial dynamics by flaviviruses: how and why
11:00-11:15	17:00-17:15	Ella Sklan	*sciforum-057453* - Sandfly fever viruses attenuate the type I interferon response by targeting the phosphorylation of JAK-STAT components
11:15-11:30	17:15-17:30	Daniel Morales	*sciforum-057899* - The evolutionary dynamics of post-translational modifications in betacoronaviruses as a potential driver of rapid viral divergence
11:30-11:45	**17:30-17:45**	**Break**	
Session Chair: Michelle Flenniken			
11:45-12:10	17:45-18:10	Mark Young	Viral community structure and dynamics in a simplified natural ecosystem
12:10-12:25	18:10-18:25	Ana Rita Ferreira	*sciforum-058357* - HCMV protein vMIA interferes with the peroxisomal and mitochondrial antiviral responses via distinct mechanisms
12:25-12:40	18:25-18:40	Margarida Ribeiro	*sciforum-057471* - Sensing of SARS-CoV-2 infected cells by plasmacytoid dendritic cells promotes a local antiviral response
12:40-12:55	18:40-18:55	Alberto López Muñoz	*sciforum-057494* - Cell Surface SARS-CoV-2 Nucleocapsid Protein Modulates Innate and Adaptive Host Immunity

Day 3- Thursday 7 April 2022

Time (EDT)	Time (CEST)	Speaker	Title
Session 4. Mechanisms of Virus Replication			
Session Chair: Marjolein Kikkert			
07:30-07:55	13:30-13:55	Ke Lan	NDRG1 facilitates both latent and lytic replication of Kaposi's sarcoma-associated herpesvirus
07:55-08:20	13:55-14:20	Montserrat Barcena	Nidovirus Replication Organelles
08:20-08:35	**14:20-14:35**	**Break**	
08:35-09:00	14:35-15:00	Anne Simon	Structure and function of umbravirus cap-independent translation enhancers and novel associated hairpins
09:00-09:15	15:00-15:15	Marc Jamin	*sciforum-057367* - Structural description of the Nipah virus phosphoprotein and of its interactions with viral and cellular partners

09:15-09:30	15:15-15:30	Martin Blackledge	*sciforum-058050* - The intrinsically disordered SARS-CoV-2 nucleoprotein and its dynamic complex with the viral protein nsp3
09:30-10:00	**15:30-16:00**	**Break**	
Session 5. Virus-Host Interactions - Part I			
Session Chair: Martin Blackledge			
10:00-10:25	16:00-16:25	Véronique Ziegler-Graff	Host and vector manipulation by plant viruses to promote transmission
10:25-10:40	16:25-16:40	Konrad Thorsteinsson	*sciforum-058306* - Binding kinetics of human noroviruses to histo-blood group antigens predict susceptibility of human intestinal enteroids to infection
10:40-10:55	16:40-16:55	Marc Talló	*sciforum-057465* - CHIKV uses two different tRNA-related strategies to favor viral protein expression
10:55-11:10	**16:55-17:10**	**Break**	
Session Chair: Vinay Pathak			
11:10-11:35	17:10-17:35	Shan-Lu Liu	Cell-to-cell transmission of Emerging and Re-emerging viruses: studies of HIV, EBOV and SARS-CoV-2
11:35-11:50	17:35-17:50	Alfonso Gomez Gonzalez	*sciforum-057351* - A viral ubiquitination switch attenuates innate immunity and triggers nuclear import of virion DNA and infection
11:50-12:05	17:50-18:05	Pontus Öhlund	*sciforum-057519* - Small RNA sequencing and transcriptomic analysis to investigate the response to acute infection of the insect-specific Lammi Virus in an Aedes albopictus derived cell line
12:05-12:30	**18:05-18:30**	**Break**	
Flash Talks and Poster Session II			
Session Chair: Delphine Muriaux			
12:30-12:35	18:30-18:35	Candace Fox	*sciforum-058277* - Human Coronavirus Infected Lung Cells Recruit Complement Inhibitors Vitronectin and Clusterin and Delay Complement-Mediated Lysis
12:35-12:40	18:35-18:40	Gregory Melikyan	*sciforum-057382* - Interferon-Induced Transmembrane Proteins Inhibit Viral Fusion by Imposing Negative Membrane Curvature and Increasing Lipid Order and Bending Modulus
12:40-12:45	18:40-18:45	Santanu Biswas	*sciforum-057742* - Modulation of HIV Replication in Monocyte-Derived Macrophages (MDM) by Host Antiviral Factors Secretory Leukocyte Protease Inhibitor and Serpin Family C Member 1 Induced by Steroid Hormones
12:45-12:50	18:45-18:50	Alan Xiaodong Zhuang	*sciforum-055438* - The circadian clock component BMAL1 regulates SARS-CoV-2 entry and replication in lung epithelial cells

12:50-12:55	18:50-18:55	Scarlett Guoli Shi	*sciforum-057743* - Rapalogs downmodulate intrinsic immunity and promote cell entry of SARS-CoV-2
12:55-13:45	18:55-19:45	Poster Session II (Break Out Rooms)	

Day 4 - Friday 8 April 2022

Time (EDT)	Time (CEST)	Speaker	Title
Session 6. Viral Pathogenesis and Evolution			
Session Chair: Juana Diez			
07:30-07:55	13:30-13:55	Zhengli Shi	Swine acute diarrhea syndrome coronavirus infection in suckling mice and characterization of a potential drug target
07:55-08:10	13:55-14:10	Ariana Arduini	*sciforum-057485* - Investigating ORF8-mediated immune evasion in SARS-CoV-2 variants of concern
08:10-08:25	14:10-14:25	Judith Olejnik	*sciforum-057551* - Restricted Infection of Macrophages by SARS-COV-2 Induces Pro-Inflammatory Responses
08:25-08:40	14:25-14:40	Kelsie Brooks	*sciforum-058340* - SARS-CoV-2 Infection Induces Modest Microbial Translocation in Rhesus Macaques and Gut-Associated Inflammation in Monkeys and Humans
08:40-08:55	**14:40-14:55**	**Break**	
Session Chair: Marlene Bouvier			
08:55-09:20	14:55-15:20	Paul Turner	Predicting evolutionary genetics of virus-bacteria interactions in patients receiving phage therapy
09:20-09:45	15:20-15:45	Stan Brouns	TBC
09:45-10:00	15:45-16:00	Alan Xiaodong Zhuang	*sciforum-055437* - Circadian control of hepatitis B virus replication
10:00-10:15	16:00-16:15	Huogen Xiao	*sciforum-057482* - Evidence that proteolytic cleavage of plant proteins by potyvirus NIa proteases occurs at various subcellular localizations in infected cells
10:15-10:45	**16:15-16:45**	**Break**	
Session 7. Virus-Host Interactions - Part II			
Session Chair: Elisa Crisci			
10:45-11:10	16:45-17:10	Ileana Cristea	TBC
11:10-11:25	17:10-17:25	Lena Noe	*sciforum-057444* - Innate Immune Sensing of Bovine Foamy Virus primary microRNA?

11:25-11:40	17:25-17:40	Peter Kin Kui Lai	*sciforum-058341* - SERINC5 Restricts Influenza A virus Infection by Modulating Hemagglutinin Conformation
11:40-11:55	17:40-17:55	Nash Rochman	*sciforum-058254* - Epistasis at the SARS-CoV-2 RBD Interface and the Propitiously Boring Implications for Vaccine Escape
11:55-12:10	**17:55-18:10**	**Break**	
Session Chair: Ester Ballana			
12:10-12:35	18:10-18:35	Britt Glaunsinger	Controlling the message: CoV-2 nsp1 targets the ribosome to restrict gene expression at multiple levels
12:35-12:50	18:35-18:50	Kritika Kedarinath	*sciforum-058280* - CD24 Expression in Neuroblastoma Cells Alters Type I Interferon Signaling and Susceptibility to Zika Virus Infection
12:50-13:05	18:50-19:05	Steven Baker	*sciforum-057486* - Alternative splicing liberates a cryptic cytoplasmic isoform of mitochondrial MECR that antagonizes influenza virus
13:05-13:20	19:05-19:20	Maïlys Piau	*sciforum-057631* - Grapevine fanleaf virus (GFLV) protein 1A interacts with Nicotiana benthamiana isoforms ATG8a, ATG8c and ATG8i of ATG8, an autophagy related protein
13:20-13:30	19:20-19:30	Chair Closing - Eric Freed	

Sessions

S1. General Topics in Virology
S2. Antiviral Therapeutics, Vaccines, and Host Defenses
S3. Antiviral Innate Immunity
S4. Mechanisms of Virus Replication
S5. Virus-Host Interactions
S6. Viral Pathogenesis and Evolution

Abstracts

S1. General Topics in Virology

Keynote Speaker

Emerging Coronaviruses: Mechanisms of Pathogenesis and Control

sciforum-060783

Ralph S. Baric

1 Department of Microbiology and Immunology; Department of Epidemiology; Rapidly Emerging Antiviral Drug Discovery Initiative, University of North Carolina, Chapel Hill, NC, USA

Emerging coronavirus infections have caused considerable morbidity and mortality in human populations. The 21st century has seen the emergence of three novel human coronaviruses associated with high mortality rates in humans, culminating in the COVID19 pandemic which has caused over 6 million deaths worldwide, yet the underlying molecular mechanisms that regulate severe disease outcomes remains uncertain. Using a variety of genetic, biochemical, virologic and immunologic approaches, this presentation focuses on the pathogenic mechanisms associated with acute end stage lung disease as well as chronic long-term disease manifestations associated with SARS-CoV2 and other related emerging human coronavirus infections. In addition, a pathway is defined that has resulted in the development of several coronavirus broad-spectrum direct acting antivirals as well as universal vaccine platforms, which are designed to not only help control disease severity associated with the current pandemic but provides countermeasures against future coronavirus disease threats. Current strengths and limitations in acute and chronic disease control are identified as critical high value research campaigns designed to mitigate the severity of chronic disease sequalae.

Invited Speaker

Evasion of Host Antiviral Responses PKR and GCN2 in an Animal Model of Adenovirus Infection

sciforum-060494

Katherine Spindler

1 Department of Microbiology and Immunology, University of Michigan, Ann Arbor, MI, USA

The host antiviral response, activation of protein kinase R (PKR), is well appreciated by virologists. PKR senses dsRNA produced by DNA and RNA virus infections. This results in phosphorylation of eIF2alpha, inhibiting host and viral protein synthesis and thus inhibiting virus replication. Many viral mechanisms are known to overcome this host PKR response. For example, human adenoviruses encode virus-associated (VA) RNAs that inhibit PKR. We have shown that mouse adenovirus type 1 (MAV-1), which does not produce VA RNAs, instead degrades PKR via a proteasomal mechanism. This is the first DNA virus known to counteract PKR by degrading it. We have recently shown that an early viral protein is involved in the PKR degradation by MAV-1. We have produced a new strain of mice that completely lack PKR expression, and we are using these mice and viral mutants to characterize the MAV-1–PKR interaction. A less well appreciated host response to viral infection is activation of GCN2, another eIF2alpha kinase. GCN2 senses amino acid deprivation and oxidative stress, and it is antiviral for HIV-1, Sindbis virus, mouse cytomegalovirus, and MAV-1. Infection of GCN2-knockout mice results in higher viral loads, higher cytokine and chemokine production, and more immunopathology than controls. The MAV-1–mouse model enables us to characterize these PKR and GCN2 antiviral responses in a natural animal host.

Invited Speaker

Role of Membrane Lipids and Inositol Hexakisphosphate (IP6) in HIV-1 Replication

sciforum-060781

Eric Freed

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

HIV-1 particle assembly takes place on the plasma membrane of the infected cell and is driven primarily by the viral Gag polyprotein precursor. Concomitant with release of the nascent virion from the plasma membrane, the viral protease cleaves the Gag precursor into a number of mature domains, including matrix (MA), capsid (CA), nucleocapsid (NC), and p6, and two small spacer peptides, SP1 and SP2. Protease-mediated Gag cleavage triggers virion maturation, during which the newly liberated CA protein assembles into the conical capsid core, which contains the viral genomic RNA and the viral enzymes reverse transcriptase and integrase.
HIV-1 assembly takes place in specialized plasma membrane microdomains, often referred to as lipid rafts, that are highly concentrated in saturated lipids like cholesterol and sphingolipids. As a result of assembly in lipid rafts, the viral membrane is enriched in raft-resident lipids. Our recent work has demonstrated, by using a range of biochemical and structural biology techniques, that disruption of an enzyme involved in ceramide biosynthesis blocks HIV-1 protease-mediated Gag processing, resulting in a severe disruption of particle maturation and infectivity. These findings demonstrate a critical interplay between the lipid composition of the HIV-1 lipid bilayer and Gag processing and particle maturation. We have also been investigating the role of the cellular metabolite inositol hexakisphosphate (IP6) in HIV-1 assembly and maturation. IP6 binds the immature Gag lattice during virus assembly, and also binds the mature capsid lattice during virus maturation. IP6 may also regulate the import of dNTPs into the capsid during the early stages of HIV-1 infection. IP6 thus plays central roles throughout the virus replication cycle. We have used a forced-evolution approach to study the role of IP6 in both immature particle assembly and virion maturation by introducing mutations in IP6 binding sites, propagating the resulting mutants in culture, and characterizing the second-site compensatory mutations that arise to rescue replication and infectivity. These topics will be presented in my talk.

Age-specific mitigation strategies can reduce sex disparity in COVID-19 mortality

sciforum-058261

Levente Zsichla, Viktor Müller

[1] Institute of Biology, Eötvös Loránd University, Budapest, Hungary

Heterogeneities in the susceptibility to infection, severe outcome or death create inequalities of disease burden in an epidemic. In the case of Covid-19 numerous factors influence the clinical outcome of the infection, with age being the most important followed by comorbidities and biological sex.
Mitigation strategies in the current pandemic have included examples that addressed some of these known risk factors by prioritizing access to vaccination, use of antiviral drugs or non-pharmaceutical interventions based on age, comorbidities, or high occupational risk exposure. However, the considerable disparity of COVID-19 mortality risk based on female/male sex has remained unaddressed in practice.
Given the strong age-dependence of COVID-19 fatality, introducing sex-specific age thresholds for targeted interventions offers a simple strategy towards increased equity with respect to biological sex. We used demographic and epidemiological data to estimate the age adjustment (the difference between male and female age thresholds for targeted interventions) that is needed to 'equalize' several measures of mortality between both sexes. For example, we found that to introduce targeted interventions at the same threshold mortality risk, the age threshold for the interventions needs to be about 7 years lower for men compared with women. In addition, we also calculated the age adjustments needed to achieve the same ratio of prevented deaths, the same overall death rate, or an equal number of years of potential life lost for both sexes. We found that while males need to be protected in younger ages than females for all measures of mortality, the age adjustment needed to achieve equity between both sexes varies considerably for the different measures considered.

GS-CA1 and Lenacapavir induce a conformational change that stabilizes the HIV-1 core and blocks viral uncoating

sciforum-057181

Anastasia Selyutina[1], Pan Hu[1], Sorin Miller[2], Lacy M Simons[3], Hyun Jae Yu[4], Judd F. Hultquist[3], KyeongEun Lee[2], Vineet N. KewalRamani[2], Felipe Diaz-Griffero[1]

[1] Albert Einstein College of Medicine
[2] National Cancer Institute at Frederick
[3] Northwestern University Feinberg School of Medicine
[4] Frederick National Laboratory

The HIV-1 capsid is an important pharmacological target for antiviral drugs such as PF74, GS-CA1, and Lenacapavir (LEN). Like PF74, GS-CA1 inhibited the interaction of cleavage and polyadenylation specificity factor 6 (CPSF6) and nucleoporin 153 with the HIV-1 core. However, inhibition of HIV-1 infection by GS-CA1 did not require CPSF6 expression in primary $CD4^+$ T cells. One of the hallmark activities of PF74 is its ability to accelerate uncoating of the HIV-1 core during infection, similar to the way that rhesus tripartite motif-containing protein 5 blocks HIV-1 infection. In contrast to PF74, GS-CA1 and LEN stabilized the core during infection, and GS-CA1, unlike PF74, allowed the capsid to enter the nucleus. This nuclear entry is consistent with the fact that GS-CA1 does not inhibit reverse transcription. Unlike PF74, GS-CA1 does not disaggregate preformed CPSF6 complexes in nuclear speckles, suggesting that PF74 and GS-CA1 have different mechanisms of viral inhibition. GS-CA1 stabilized the HIV-1 core, possibly by inducing a conformational change in the capsid. In support of this hypothesis, we demonstrated that HIV-1 capsids bearing the mutation N74D regained the ability to bind CPSF6 when in the presence of GS-CA1. We show here that GS-CA1 binds to the HIV-1 core, changes the conformation of the capsid, stabilizes the core, and thereby prevents viral uncoating and infection.

A simplified multiplex PCR assay for simultaneous detection of six viruses infecting chilli species in India and its application in field diagnosis

sciforum-058249

Oinam Priyoda Devi[1], Susheel Kumar Sharma[2], Keithellakpam Sanatombi[1], Konjengbam Sarda Devi[2], Virendra Kumar Baranwal[3]

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Chilli plants (*Capsicum* sp.) are susceptible to numerous viral infections, which leads to a significant threat to its production. India being one of the leading producer and exporter of chillies has, witnessed predominant outbreaks of multiple viruses infecting chilli cultivars. These demands for the development of a simplified diagnostic procedure capable of detecting all viruses at a time. A simplified and robust diagnosis of such viral infections can be achieved through a multiplex-PCR assay which can simultaneously detect and distinguish the infection of all targeted viruses. The present study reports the development of a multiplex-PCR (mPCR) assay for simultaneous detection of five RNA and one DNA virus, predominantly infecting different *Capsicum* species in India. Five RNA viruses viz, capsicum chlorosis virus (CaCV), chilli veinal mottle virus (ChiVMV), large cardamom chirke virus (LCCV), cucumber mosaic virus (CMV) and pepper mild mottle virus (PPMoV) and a DNA virus, chilli leaf curl virus (ChiLCV), were successfully detected with high specificity and sensitivity using the developed mPCR assay, employing 6 pairs of virus-specific primers designed from their respective conserved genomic regions. The concentration of the primers, annealing temperature, and extension time were optimized to achieve robust detection of targeted viruses in a single reaction. The implementation of mPCR assay in detection of chilli samples collected from chilli growing fields of different parts of North East India showed robustness in the detection of multiple virus infections in naturally infected chilli leaf samples and thus validated the developed assay. The developed mPCR assay could be used as a differential diagnostic tool for simultaneous detection of multiple viruses in chilli, thus aiding in tracking the spread of infection for quarantine and certification programmes.

Analysis of multiple SARS-CoV-2 variants using ddPCR

sciforum-057328

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COVID-19 pandemic has highlighted the need for rapid quantitation of virus from a multitude of different sample types. Diagnostic QRTPCR methods have been developed to diagnose samples for the viral load. QRTPCR remains the gold standard for detecting viral genomes in clinical samples. Recently, there has been interest to study multiple SARS-CoV-2 variants of concern (VOC) in animal studies. Standard QRTPCR methods are not suitable for quantitating multiple closely related sequences in the same sample. The new generation droplet digital PCR has been used to detect rare variants in cancer research, where the targets differ by few nucleotides or by a single nucleotide polymorphism. To date, studies of multiple SARS-CoV-2 variants have been analyzed using next generation sequencing. NGS approach, however, involves labor and is cost prohibitive for many studies. We have therefore developed a ddPCR technique to quantitate multiple SARS-CoV-2 variants in a single sample. We have analyzed two different pairs of SARS-CoV-2 VOCs in a hamster COVID-19 model. We have also obtained NGS data for the same samples for a comparison.
This work was supported by the Division of Intramural Research NIAID, NIH.

Comparison of the proteomes of porcine monocyte-derived macrophages and a stable porcine cell line after infection with African swine fever virus

sciforum-057410

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African swine fever, a highly lethal OIE-notifiable disease of swine (*Sus scrofa*) is caused by African swine fever virus (ASFV). Since its introduction into Georgia in 2007, ASFV has spread over the Eurasian continent and is currently threatening the global pig industry due to a lack of a vaccine or treatment.
To validate the suitability of a stable cell line for *in vitro* infection experiments we use quantitative mass spectrometry to compare the intracellular viral proteomes and the impact of ASFV infection on the cellular proteomes of porcine macrophages, the natural target cell of ASFV, and a susceptible wild boar lung derived cell line, WSL. Porcine monocyte-derived macrophages (moMΦ) were isolated from different donor pigs and infected in parallel with WSL cells with a marker-gene expressing recombinant of ASFV strain Kenya1033.
No major differences in the expression of viral or host proteins were observed after ASFV infection of the two cell populations. However, a number of individual proteins were expressed in a cell type-specific manner.
Since both cell types exhibit very similar responses to ASFV infection, we conclude that WSL cells can be used as a convenient system to study ASFV infections *in vitro*. However, the observed subtle differences concerning virus and host protein expression warrant further studies on cell type-specific expression patterns.

Cytolytic properties and genome analysis of Rigvir® oncolytic virotherapy virus and other echovirus 7 isolates

sciforum-058314

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Human picornaviruses have inherent ability to target, invade, and destroy cancer cells due to natural receptor tropism. For example, CAVATAK (proprietary formulation of cell-adapted coxsackievirus A21), which binds to DAF receptor, is being evaluated in multiple clinical trials against a range of cancers. Viralytics, the company behind CAVATAK, was recently acquired by MSD with the price of $394 million showing the commercial potential of human picornaviruses as oncolytic virotherapy agents. Rigvir® (formulation of cell-adapted echovirus 7 [E7]) was approved in Latvia back in 2014 for melanoma oncolytic therapy and is claimed to be effective against numerous cancer types. While Rigvir®'s license was revoked in 2019 by the State Medicines Agency of Latvia, the drug remains to be approved for use in other countries, such as Uzbekistan, Georgia, and Armenia. We have performed genome and structural analyses of six E7 isolates including Rigvir®, and compared their infection profiles in eight cell lines, which included both cancer and non-cancer cell lines. Phylogenetic analyses placed Rigvir® in its own clade, most distant to other E7 isolates. Genome analyses revealed 9 unique mutations in the Rigvir® capsid region (VP1 n=2, VP2 n=5, VP3 n=2), with 6 mutations exhibiting surface exposure. Mutation E/Q/N232G was found in a region that has been previously characterized as a part of DAF contact surface. Additionally, the E/Q/N232G mutation is structurally close with another Rigvir® mutation, K/N/L208R. However, the exact significance of these mutations is unclear. The replication potential of these viruses in six cancer and two native cell lines was determined by end-point titration, immunofluorescence microscopy and RT-qPCR. No significant differences in replication rate or cytotoxicity were detected, which further brings under question the specificity of Rigvir® as oncolytic virotherapy agent compared to native E7 isolates.

Determinants of Stop Codon Readthrough Efficiency in an RNA Virus

sciforum-057501

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Stop codon readthrough (RT) is a common alternative protein expression strategy utilized by RNA viruses to maximize their coding capacity. Pea enation mosaic virus 1 (PEMV-1) is a coding-sense RNA plant virus in the genus *Enamovirus* (family *Solemoviridae)* that uses RT to express a C-terminally extended minor capsid protein, termed CP-readthrough domain (CP-RTD). Limited incorporation of CP-RTD subunits into virus particles is essential for aphid transmission. The details of how RT is regulated in PEMV-1 have not yet been investigated, however, a potential long-distance base-pairing RNA-RNA interaction between RT-proximal and -distal sequences has been implicated. Through RNA solution structure probing, secondary structure modeling, site-directed mutagenesis, and *in vitro* translation assays, we demonstrate that there are local RNA secondary structures positioned proximal and distal to the RT site in PEMV-1 that are united via at least two distinct long-distance RNA-RNA interactions. These long-distance interactions result in the rearrangement of local RNA structures and the formation of a unique and complex long-range RNA structure that is required for promoting efficient ribosome readthrough. These results provide compelling experimental evidence that CP readthrough in PEMV-1 does indeed involve long-range RNA-RNA interactions and bolsters the concept that CP-RTD production in the genera *Polerovirus* and *Luteovirus* also involve long-range interactions.

Human LAMP1 markedly accelerates the kinetics of Lassa virus fusion and promotes fusion pore dilation

sciforum-057502

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Lassa virus (LASV) is a lethal pathogen endemic in West Africa that causes severe hemorrhagic fever. LASV recognizes a cell surface receptor α-dystroglycan (α-DG) which mediates the internalization of the virus. In acidic endosomal compartments, LASV switches from α-DG to the receptor, lysosomal-associated membrane protein 1 (LAMP1) and undergoes low pH-triggered fusion with the endosomal membrane. However, recent studies showed that, while LAMP1 is enhances LASV infection, apparently by raising the pH threshold for triggering virus fusion, it is dispensable for virus entry into cells lacking human LAMP1. It is currently not known which step(s) of LASV fusion is augmented by LAMP1. Here, we showed that, as expected, human LAMP1 (hLAMP1) overexpression markedly enhanced LASV pseudovirus fusion with human A549 cells and avian DF-1 cells expressing the avian LAMP1 ortholog that doesn't support LASV fusion. Interestingly, LASV fusion with the plasma membrane forced by low pH exposure was more efficient that entry via an endosomal pathway. By tracking double-labeled LASV pseudoviruses in live cells, we show that the kinetic of forced single LASV fusion is markedly faster and fusion pore dilation is greatly enhanced by hLAMP1 expression in DF-1 cells. In addition, forced LASV pseudovirus fusion with parental DF-1 cells in the presence of a soluble hLAMP1 fragment was also markedly accelerated, implying that the hLAMP1 effect on LAV fusion is independent of anchoring to the target membrane. Collectively, our findings provide new insights into the mechanism of LASV entry enhancement by hLAMP1. This work was supported by the NIH R01 AI053668 grant to GBM.

Usutu virus SAAR-1776: new insights from 2.4 Å resolution cryo-EM structures of a mature flavivirus

sciforum-057557

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Usutu virus (USUV) is an emerging arbovirus that belongs to the Japanese Encephalitis serocomplex. USUV was originally identified in South Africa and was introduced to Europe by migratory birds as early as the late nineties, and since then multiple introductions of the virus have occurred. In the last two decades, USUV has spread across Europe and has been responsible for widespread avian outbreaks and fatalities. In humans, USUV infections are characterized typically by asymptomatic presence; in some cases, USUV can lead to severe disease in immunocompromised as well as in healthy human adults. Despite its rapid spread across Europe and pervasive presence in the general human population, USUV pathogenesis and molecular mechanisms of disease are poorly understand. To understand the structural basis of USUV pathogenesis, we determined two structures of the mature USUV SAAR 1776 virus using cryo-electron microscopy at a resolution of 2.4 Å. The molecular structure of mature USUV uncovers a herringbone arrangement of the envelope protein (E) and membrane protein (M) on the icosahedral shell of the virion. High resolution details reveal that a cysteine residue in the fusion loop of one of the three E monomers in the asymmetric subunit of the virus is engaged in a disulfide bond; however, only one of the structures displays this feature. Both structures display three lipid sites, unlike other lower resolution flavivirus structures reported previously. We analyze these details in USUV in context of current knowledge of the mature flavivirus structures described to-date.

Virus Safety of Xenotransplantation

sciforum-057408

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Xenotransplantation using pig islet cells or organs is under development to alleviate the shortage of human donor islet cells or organs for the treatment of diabetes or organ failure. Multiple genetically modified pigs were generated to prevent rejection. Xenotransplantation may be associated with transmission of potentially zoonotic porcine viruses. In order to prevent this, we developed highly sensitive PCR-based and immunological methods for the detection of numerous xenotransplantation-relevant viruses. These methods were used for the screening of donor pigs and xenotransplant recipients. Of special interest are the porcine endogenous retroviruses (PERVs), which are integrated in the genome of all pigs, which are able to infect human cells, and which cannot be eliminated by methods that other viruses can. We showed, using droplet digital PCR, that the number of PERV proviruses is different in different pigs (usually around 60). Furthermore, the copy number is different in different organs of a single pig, indicating that PERVs are active in the living animals. We showed that in the first clinical trials treating diabetic patients with pig islet cells, no porcine viruses were transmitted. However, in preclinical trials transplanting pig hearts orthotopically into baboons, porcine cytomegalovirus (PCMV), a roseolovirus, and porcine circovirus 3 (PCV3), but no PERVs, were transmitted. PCMV transmission resulted in a significant reduction of the survival time of the xenotransplant. But we showed that PCMV can be eliminated from donor pigs by early weaning. PERVs were also not transmitted by inoculation of human cell-adapted PERV into small animals, rhesus monkey, baboons and cynomolgus monkeys, even when pharmaceutical immunosuppression was applied. Since PERVs were not transmitted in clinical, preclinical and infection experiments it remains unclear whether they should be inactivated in the pig genome by CRISPR/Cas. In summary, using our sensitive methods the safety of xenotransplantation can be ensured.

Abstracts

S2. Antiviral Therapeutics, Vaccines, and Host Defenses

In silico evaluation of quinoxaline derivatives as potential inhibitors of the receptor-binding domain (RBD) of the spike protein of SARS-CoV-2

sciforum-057559

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SARS-CoV-2 is the causative agent of pandemic COVID-19. The increase in the number of cases globally is alarming and represents a constant public health challenge. Pharmacological treatments are limited, so the search for new therapeutic options is imperative. The spike protein represents an attractive drug target to prevent infection. The time- and resource-saving strategies such as virtual screening and molecular docking have proven to be powerful tools to identify molecules with the potential to interfere with the interaction between spike (RBD region) and the ACE2 cell receptor. This work was aimed to evaluate *in silico* new compounds as potential blocking agents of the spike (RBD)-ACE2 interaction. All ligands were collected from an "in-house" library called PBL (Pharmaceutical Biotechnology Laboratory). These compounds are quinoxaline derivatives (privileged scaffold in medicinal chemistry), which possess *in vitro* activity against different targets such as trypanocidal, leishmanicidal, anti-*Mycobacterium*, among others. The crystallographic structure with code PDB 6M0J was retrieved from Protein Data Bank and the RBD region selected for molecular docking with Auto Dock Vina 1.1.2 centered on residues of interest: K417, Y449, Y453, A475, N487, T500, N501, and G502. We considered eligible those compounds with the highest number of interactions with residues of interest and the best free energy of binding (FEB). Two hundred derivatives were tested, which **Q-1**, **Q-2**, **Q-3**, **Q-4**, and **Q-5** evidenced a FEB value of -7.4, -7.2, -6.8, -6.5, and -6.5 kcal/mol, respectively. They managed to interact with K417, Y449, Y453, and N501 through hydrogen bonds and hydrophobic contacts that stabilize the complex formed between RDB and each ligand. In conclusion, this study allowed us to detect and predict five molecules as potential RBD blockers of the spike protein by interacting strongly with residues of interest. Further *in vitro* and *in vivo* studies are required to demonstrate their effect.

CDK Inhibitors Impact HIV-1 Susceptibility By Modulating In Vivo Immunologic Response

sciforum-057361

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Cyclin-dependent kinase inhibitors (CDKi) are currently used for the treatment of metastatic breast cancer (BC) with well-described effects on viral replication and immune cell responses in vitro. However, *in vivo* evidence from treated patients is limited. Here, using a longitudinal cohort of CDKi-treated BC patients, we determined treatment impact on HIV-1 susceptibility and immune response to define its potential as anti-HIV agents.
Plasma and PBMCs from BC patients treated with CDKi (palbociclib, ribociclib and abemaciclib) were collected before treatment initiation and 3 months afterwards. Plasma cytokine and inflammation markers were determined by Luminex (n=47). Immune cell subsets and T-cell immune function was characterized by multicolor flow cytometry (n=14). HIV susceptibility was assessed in patients (n=14) by *ex vivo* infection with GFP-expressing HIV-1 and in primary cells from healthy controls *in vitro* treated with CDKi.
Immunophenotypic characterization of PBMCs from patients 3-months after CDKi treatment showed reduced proportion of CD4+ T-central memory cells (p=.0491) and decreased expression of the immune exhaustion marker PD1+ (p=.0059), suggesting improved immune cell function without significant changes in T-cell activation. Susceptibility to *ex vivo* HIV infection was impaired in CDKi-treated, effect that was reversed upon treatment discontinuation, consistently with *in vitro* data, where all CDKi inhibited HIV-1 infection in MDMs and CD4+ T-lymphocytes from healthy controls. A relation between the number of CD4+ T-central memory cells and HIV-1 infection was observed, further suggesting the impact of in vivo CDKi treatment on HIV-1 susceptibility. Plasma cytokine expression showed a modulation of antiviral immune response 3 months after treatment initiation, presenting significantly less EGF (p=.0222) and increased IL-10 (p=.0310).
In vivo treatment with CDKi turns immunologic responses to a phenotype eventually favoring HIV-1 control by decreasing immune exhaustion and cells representing the main source of virus reservoir; demonstrating the potential of CDKi as putative therapeutic strategy against HIV.

Rapid high throughput infectivity assay for screening antiviral drugs and monitoring vaccine effectiveness against emerging variants using AI-based analysis of automated brightfield microscopy

sciforum-057499

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Viral infectivity assays are essential for the development of viral vaccines and antiviral drugs. Plaque and TCID50 assays can take as long as 2-14 days to develop. Alternatives like fluorescent focus assay (FFA) are faster but require antibodies and extensive sample preparation or GFP-labeled viruses.
ViQi, Inc. has developed a quantitative viral infectivity assay using machine learning and automated brightfield microscopy on 96-well plates to identify phenotypic changes within cells associated with viral infection before being humanly visible. As with FFA, these changes can be detected within a few hours after infection depending on the virus, but do not require any sample preparation or fluorescence imaging, and can be done on live cells. Each virus and cell line requires training a new machine learning model which only needs to be done once to establish and validate the assay. Once a model is trained, assays can be processed automatically within hours of infection, producing same-day or next-day results. This technology will reduce turn-around time, increase reliability, lower labor costs, ease automation, and increase throughput. These can be substantial advantages for BSL-3 class viruses.
We have trained machine learning models on seven viruses so far including DNA, RNA, enveloped, and non-enveloped viruses. In each instance, the dilution calibration curve achieved an R^2 of no less than 0.9, and the linear range of the assay was typically equivalent or more broad than that of plaque assays (typically readable over a 10-fold range). Because this technology relies on models sensitized to potentially specific phenotypes of viral production, it may have potential as a rapid, broad-based identification and diagnostic tool.

Substantial enhancement of anti-HIV-1 inhibitory peptide knock-in rates in primary human lymphocytes via the rational design of donor DNA, Cas9 and application of NHEJ inhibitors

sciforum-057469

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We have previously developed CRISPR/Cas9-based gp41 fusion inhibitory peptide knock-in (KI) platform, as an alternative to the currently used lentiviral strategy for delivering anti-HIV therapeutic genes (mBio, 2022). This system allows expressing gp41 peptides MT-C34 and 2P23 on the surface of CD4 lymphocytes from the endogenous CXCR4 promoter. By optimizing CRISPR/Cas9 components, KI efficiency reached 35% in the CEM CD4+ T cell line and 5% in primary CD4 lymphocytes.
In this study, we aimed to improve the level of homology-directed repair (HDR) in primary lymphocytes by enhanced nuclear import of Cas9 and donor DNA. We explored this by testing the type and number of nuclear localization signal (NLS) in Cas9, and examining the role of DNA transport signal (DTS), NFkB tandem binding site, and protospacer (sg) inserted into the donor DNA. The CEM cell line or CD4 lymphocytes were electroporated with modified donors and CRISPR/Cas9 plasmids or ribonucleoprotein complex (RNP). The efficiency of nonhomologous end joining (NHEJ) and HDR were evaluated by measuring the levels of CXCR4 knockout (KO) and MT-C34 expression, respectively. We found that increasing the number of NLS leads to higher editing rates. Adding the 5'-sg and 3'-sg, DTS, or NFkB sites to the flanks of donor DNA maximized KI level, whereas a further increase in the number of these sequences had no effect. Among the NHEJ inhibitors tested, DNA-PK inhibitor M3814 displayed the highest (2 folds) increase in HDR in primary lymphocytes. Interestingly, RNP stabilizing polyglutamic acid greatly enhanced the level of CXCR4 KO but did not improve HDR. Altogether, our results suggest that it is possible to further increase KI rates in human primary cells which is an essential step in the translation of this technique to clinical practice. This work was supported by the grant №22-25-00310 from the Russian Science Foundation.

A mercapto-pyrimidine pharmacophore inhibits the papain-like protease of SARS-CoV-2 and viral replication in cells via a reversible covalent mechanism

sciforum-057381

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The papain-like protease (PLpro) of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an essential for SARS CoV-2 replication in cells and also dampens the host antiviral immune response to the virus. PLpro is therefore an excellent target for developing therapeutics and PLpro inhibitors have the potential to significantly improve the treatment of SARS-CoV-2. However, despite significant efforts, effective inhibitors for PLpro have not been developed yet. In this article, we present a mercapto-pyrimidine fragment that can inhibit PLpro enzymatic activity and also SARS-CoV-2 replication in cells. The mercapto-pyrimidine fragment was identified through high throughput screening of a large chemical library consisting of 115,000 diverse compounds. The mercapto-pyrimidine fragment inhibited PLpro with an IC50 of 3.8μM and biochemical characterization of it revealed that it is a cysteine targeted reversible covalent inhibitor, which undergoes an addition-elimination reaction with thiols. Mercapto-pyrimidine fragments are a new class of reversible inhibitors and have a great potential for inhibiting cysteine proteases, such as PLpro.

A single intranasal immunization with avian paramyxovirus type 3 expressing the SARS-CoV-2 spike protein protects hamsters against SARS-CoV-2

sciforum-057495

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Live-attenuated vaccines for intranasal delivery induce both systemic immunity and mucosal immunity in the respiratory tract and have the ability to potently inhibit or reduce respiratory virus replication at the mucosal sites of infection. We used avian paramyxovirus type 3 (APMV3) as a vaccine vector to express the SARS-CoV-2 spike (S) protein. We selected APMV3 as a vector due to the absence of pre-existing immunity in humans and its attenuation by host range restriction. APMV3 is not a USDA select agent, unlike the closely related avian paramyxovirus type 1 (Newcastle disease virus), which has also been used as an experimental vaccine vector for intranasal administration. APMV3 expressing the SARS-CoV-2 S protein stabilized in the prefusion conformation by six proline substitutions (APMV3/S-6P) replicated to high titers in embryonated chicken eggs. In hamsters, a single intranasal dose of APMV3/S-6P induced strong serum IgG and IgA responses to the S protein and its receptor-binding domain, and strong serum neutralizing antibody responses to the early SARS-CoV-2 isolate USA-WA1/2020. Serum antibodies from APMV3/S-6P-immunized hamsters also efficiently neutralized variants of concern including lineages B.1.1.7 (Alpha variant) and B.1.351 (Beta variant). In hamsters immunized with an empty APMV3 vector, challenge with SARS-CoV-2 USA-WA1/2020 induced weight loss, a surrogate for clinical disease, and a strong inflammatory cytokine response in lungs. After challenge, SARS-CoV-2 replicated to high titers in lungs and nasal turbinates of animals immunized with the empty vector. In contrast, hamsters immunized with APMV3/S-6P had neither detectable weight loss nor lung inflammation after SARS-CoV-2 challenge. In these APMV3/S-6P-immunized animals, SARS-CoV-2 challenge virus was undetectable by viral culture in lungs and nasal turbinates. Thus, a single intranasal dose of APMV3/S-6P was highly immunogenic and protective against SARS-CoV-2 challenge, suggesting that APMV3/S-6P is suitable for clinical development.

Adaptive immune response to the spike protein of SARS-CoV-2 in healthy and oncology donor cohorts

sciforum-057313

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97 peripheral blood samples were collected from donors classified according to three variables: cancer, Covid-19 positivity and vaccination. In this work we performed PBMCs staining after incubation with SARS-CoV-2 S peptivators and ELISA assays.
Anti-S1 antibodies titres were quantified, without detecting significant differences between cancer and no-cancer, whether they were vaccinated with SARS-CoV-2 mRNA vaccines or with previous Covid-19 infection. However, vaccinated cancer donors who previously had Covid-19 showed increased titres of anti-S1 antibodies compared with the same group in no-cancer donors.
According to T cell responses, increased percentage of activated CD4 and CD8 cells stimulated with S peptides were quantified in donors who had Covid-19 compared with vaccinated donors. We also observed that vaccinated donors who previously had Covid-19 presented elevated numbers of T cells compared with the rest of donor groups. No significant differences between cancer and no-cancer donors were observed.
Cytokines profiling in S-specific T cells from donors with and without cancer showed co-expression of pro-inflammatory IL-17A and IFNγ cytokines both in CD4 and CD8 cells in donors who underwent Covid-19 but also in vaccinated donors that also had Covid-19. Interestingly, this profile was not observed in vaccinated donors who did not previously had Covid-19.
Cancer patients generate a similar immune response against covid-19 than donors without cancer. The signature of T cell responses in donors who had Covid-19 was an inflammatory Th17 type.
SARS-CoV-2 mRNA vaccines trigger similar antibody and T cell responses in cancer and no-cancer cohorts.
In addition, vaccinated donors do not generate a T cell response as strong as that observed in donors that had previous Covid-19 disease. However, vaccinated donors show higher titres of anti-S1 antibodies than donors with previous Covid-19. Finally, we can highlight that Covid-19 disease enhances the immune response against S1 protein in donors that were previously vaccinated.

Antiviral effect of polysaccharide-coated silver nanoparticles against Epstein-Barr virus

sciforum-058304

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Among herpesviruses, the Epstein-Barr virus (EBV) distinguished by its lymphotropism is able to persist in B-lymphocytes, can lead to the development of lymphoproliferative diseases, formation of tumors, immunodeficiencies. Nanosilver attracts attention as an antibacterial and antiviral agent. The aim of our work was to determine the antiviral effect of polysaccharide-coated silver nanoparticles (PCAgNPs) in chronic and lytic EBV infections *in vitro*.

The cytotoxicity of PCAgNPs relative P3HR1 and B95-8 cells was investigated using the MTT, neutral red (NR) and trypan blue (TB) dyes. The inhibiting effect of PCAgNPs on EBV at chronic and lytic 2.5 mM sodium butyrate-induced infections was evaluated by using Rt-PCR.

PCAgNPs showed greater toxicity to B95-8 cells (characterized by high cancer capacities) than to P3HR1 cells. CC_{50} values were 55.5, 27.8 and 15.2 μg/mL for B95-8 cells and 67.2, 59.8, and 73.8 μg/mL for P3HR1 cells, obtained by NR, TS and MTT, respectively.

The antiviral effect of nanosilver in the concentrations of 0.2-20 μg/ml in B95-8 cells was studied according to 3 schemes: 1) simultaneous application; 2) prophylactic and 3) therapeutic. The greatest inhibitory effect was shown by PCAgNPs in the first scheme: 34-67.5% inhibition. 2 μg/ml of nanosilver inhibited EBV by 55-75% in all schemes. The concentrations of 20 and 0.2 μg/ml of PCAgNPs were ineffective in prophylactic and therapeutic schemes.

PCAgNP in the concentrations of 0.1–10 μg/mL inhibited EBV in P3HR1 cells under conditions of chronic reproduction by 9.5–65%. The inhibition of replication was observed by 52-63% at all concentrations with the combined action of PCAgNP at the same concentrations with Ganciclovir 10 μg/ml.

Thus, PCAgNP at non-toxic concentrations effectively inhibited EBV replication under conditions of chronic and lytic EBV-infections in lymphoblastoid cells. A synergistic enhancement of the antiviral effect was observed when combined with Ganciclovir.

Broad spectrum, host-directed compounds block cellular entry of influenza A virus and SARS-CoV-2

sciforum-057573

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In the fight against the ongoing COVID-19 pandemic, whereas vaccination remains a cornerstone in prophylaxis, antivirals constitute an essential element in treating the affected patients. However, majority of the potential antivirals currently being tested are targeted against SARS-CoV-2-encoded proteins. Therefore, the concern regarding the development of drug-induced resistance due to the rapid emergence of mutant viral strains remains. While screening a library of small molecules, we identified a group of urea-derivatives that blocked SARS-CoV-2 infection in tissue culture cells by >99%. Investigating the mechanism of action of those molecules, we found that they targeted cellular endocytosis and endocytic maturation processes as evident by a significant reduction in EGF and transferrin uptake, and a block in intra-vesicular acidification. Further, we found that the molecules are capable of transporting chloride ions across the cell membrane, possibly interfering with the cellular endocytic machinery. Since the molecules showed a general effect on endocytosis, we hypothesized that they could be capable of blocking infection by other viruses that enter host cells via endocytosis. Interestingly, we found that all the molecules that inhibited SARS-CoV-2 infection, also blocked influenza A virus (IAV) infection by >99% by impeding its endocytic uptake. We observed a similar extent of inhibition of infection by the molecules against different IAV strains. Finally, we tested the efficacy of the molecules against IAV infection in mice and observed significant recovery and reduction in lung viral titer compared to the control mice. Taken together, our results demonstrate that the urea-derivate compounds identified through our study efficiently block entry of IAV and SARS-CoV-2, which could be developed as a new class of broad-spectrum antiviral agents and could be an important addition to the armamentarium in the combat against the prevailing COVID-19 pandemic.

Cross-protection as a biocontrol strategy against fanleaf degeneration: a vineyard case study

sciforum-057957

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Grapevine fanleaf virus (GFLV) is one of the main agents of fanleaf, an endemic disease distributed worldwide. In most vineyards, fanleaf causes severe decline and serious economic losses. However, vines GFLV infections are not always symptomatic and induce a complex patterns of symptoms types and severities. GFLV populations are characterized by high genetic diversity, high rate of mixed infection and frequent recombination events identified all along its bipartite RNA genome.
No effective measures are available to control the disease. Thus, our research team explores mild strain cross-protection (MSCP) as a strategy to mitigate fanleaf. In MSCP, primary infection with mild isolate(s) is used to protect plants from subsequent infection by closely related severe variants. One essential requirement for efficient and long-term cross-protection is the ability of the mild isolates to protect against a broad range of challenger variants. Moreover, MSCP programs conducted against citrus tristeza suggested that keys of its success rely on the selection of mild isolates adapted to each cultivar and cultivation environment.
Therefore, we started the evaluation of different GFLV mild isolates at mitigating fanleaf and we characterized the genetic variability of the virus populations in different French winegrowing areas. In this study, the efficacy of cross-protection was assessed by analyzing vines primo-infected with GFLV isolate VAC-Bα. These vines were planted in a commercial highly fanleaf diseased vineyard ten years ago. Following RNA-Seq analyzes, 36 molecular variants of GFLV RNA-1 and 27 molecular variants of GFLV RNA-2 were recovered from 20 vines. Half of the vines were only infected with the primary isolate, showing a great resilience of the primary infection. Our findings will be discussed with the perspective of implementing the MSCP as a sustainable approach against fanleaf and constitute essential data for the proof of concept of this biocontrol strategy in viticultural agrosystems.

Developing high-throughput screening method for RNase III inhibitors based on FRET-labelled-siRNA

sciforum-058339

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The most severe virus disease in Sweetpotato is caused by the synergic infection of *Sweet potato chlorotic stunt virus* (SPCSV) with several plant viruses from different genera, which are responsible for severe yield loss all over the world. However, there are no approved treatments against the most severe disease currently. As its infection mechanism, SPCSV encodes an RNase III (CSR3) that suppresses antiviral RNA silencing defense of plants. Thus, CSR3 may be an ideal inhibitor identification target. Here we have developed a novel high-throughput screening (HTS) assay based on fluorescence resonance energy transfer (FRET) for inhibitor identification of CSR3. Compatible with FRET dyes, a FRET-labelled siRNA substrate was generated for the HTS that was suitable to monitor changes in the enzymatic activity of CSR3. After assay optimization, to demonstrate the usefulness of the assay, we carried out HTS for CSR3 inhibitors allowing selection of 109 potential candidates out of thousands of compounds from different small molecule libraries. In addition, parallel screening tests of the selected candidates were also carried out for a similar protein RNase III from *E.coli*. Our results show that the two RNase III proteins are inhibited by different molecules, indicating specificity. Hopefully, the HTS methods can be widely applied in drug discovery for RNase III like proteins.

Diphyllin a broad-spectrum antiviral compound

sciforum-057262

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Diphyllin is a natural arylnaphtalide lignan extracted from tropical plants, which are of particular importance in traditional Chinese medicine and also have antiviral activity against SARS-CoV-2. This compound has been described as a potent inhibitor of vacuolar (H+)ATPases and thus of the endosomal acidification process required by numerous enveloped viruses to initiate their respective viral infection cascades after entering host cells by receptor-mediated endocytosis. The (H+)ATPase is a proton pump that acidifies the interior of endosomes. Compounds such as diphyllin can inhibit this mechanism of action and act as broad-spectrum antiviral compound. We demonstrate the antiviral activity of diphyllin against a number of enveloped viruses belonging to the families Flaviviridae, Phenuiviridae, Rhabdoviridae, and Herpesviridae. Diphyllin is not cytotoxic to Vero and BHK-21 cells and exerts submicromolar or low-micromolar antiviral activity against tick-borne encephalitis virus, West Nile virus, Zika virus, Rift Valley fever virus, rabies virus, and herpes simplex virus type 1. Our study shows that diphyllin is a broad-spectrum antiviral agent that targets the host cell and blocks replication of several phylogenetically unrelated enveloped RNA and DNA viruses.

Electron microscopy to study the mechanism of action of antiviral drugs

sciforum-058172

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Emerging and reemerging viruses are a major health concern. To combat present and future pandemics caused by pathogenic viruses, the validation of broad-spectrum antivirals that can be quickly applied to the clinic is a priority. There is a long list of drugs, already approved for the treatment of different diseases, which have shown promising results as potential candidates for repurposing as broad-spectrum antivirals. With a combination of methods that includes high-throughput screening of clinically tested compounds, virology methods, biochemistry and light and electron microscopy, in this work we have tested the antiviral capacity of 5 repurposed drugs to treat Bunyamwera virus (BUNV) infection in Vero cells. BUNV is the prototype of the *Bunyavirales* order, a large group of RNA viruses that includes important pathogens for humans, animals and plants. Two members of the order *Bunyavirales*, Rift Valley Fever virus (RVFV) and Crimean-Congo Hemorrhagic Fever virus (CCHFV), have been included in the WHO list of prioritized infectious diseases and reports of novel bunyaviruses that cause severe disease are frequent. Our study showed that one of the selected compounds efficiently inhibits BUNV infection. Electron microscopy showed that this compound inhibits the assembly of BUNV spherules that are the intracellular replication organelles of bunyaviruses. As a consequence of this blockade, the production of new infectious viruses is blocked. The treatment inhibits also the characteristic BUNV-induced fragmentation of the Golgi complex previously reported in infected Vero cells. Our results show that electron microscopy morphological analysis helps to understand how antivirals work, their side effects, and why they fail.

Favipiravir Induced Reductions in Zika Virus Infectivity Contribute to Antiviral Activity in HeLa Cells

sciforum-057496

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Zika virus (ZIKV) is a mosquito-borne flavivirus associated with an increased risk for congenital abnormalities in neonates and neurologic complications in children and adults. Although ZIKV infection can substantially impact patient development and quality of life, there are currently no approved antiviral therapies or vaccines to treat or prevent infection. This study aims to evaluate the antiviral potential of the nucleoside analogue favipiravir (FAV) against ZIKV in two clinically relevant human cell lines. HeLa (cervical) and SK-N-MC (neuronal) cells were infected with ZIKV and treated with increasing concentrations of FAV. Viral supernatant was sampled daily and infectious viral burden was quantified by plaque assay on Vero cells. Changes in ZIKV infectivity were measured by calculating viral specific infectivity. FAV potently inhibited ZIKV in HeLa cells and the calculated EC_{50} value in this cell line (247.3 uM) is therapeutically achievable. Robust activity in HeLa cells is likely linked to drug induced changes in viral infectivity since treatment caused infectious titers and viral infectivity to decrease in a time and concentration dependent manner. In contrast, viral suppression achieved in SK-N-MC cells was modest (EC_{50} 388.8 uM) and FAV induced alterations in specific infectivity were slight in this cell line. These results demonstrate FAV ability to substantially alter viral infectivity is cell line dependent and suggest FAV's robust anti-ZIKV activity in HeLa cells is related to the strong effect on viral infectivity observed in this cell line. We are actively working on characterizing host cell factors that explain differences in this infectivity phenomenon.

High-level resistance to integrase strand transfer inhibitors via accumulation of HIV-1 Env mutations

sciforum-058209

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Integrase strand transfer inhibitors (INSTIs) are highly potent antiretrovirals. Several regions of the HIV-1 genome have been reported to affect DTG sensitivity, however, the basis for high-level resistance to INSTIs is poorly understood. We recently reported that mutations in the HIV-1 envelope glycoprotein (Env) can broadly reduce viral susceptibility to antiretrovirals. We also identified multiple Env mutations in participants failing an INSTI-containing regimen (ACTG study A5273). The aim of the current study was to examine whether HIV-1 can develop high-level resistance to the INSTI dolutegravir (DTG). We propagated the lab-adapted NL4-3 strain with increasing concentrations of DTG (0.1 – 2,000 nM). Longitudinal sequence analysis of IN- and Env-coding regions revealed the sequential accumulation of multiple Env mutations. By contrast, no INSTI-resistance mutations in the IN-coding region were identified. The *env* amplicons from virus replicating at 256 nM DTG were cloned into NL4-3 and the replication kinetics, cell-free infectivity, and cell-cell transfer efficiency were examined. A clone with heavily mutated Env (4XEnv) exhibited faster-than-WT replication. Replication of WT NL4-3 was inhibited at 3 nM DTG, while 4XEnv could replicate at DTG concentrations up to 3 μM, indicating that accumulation of Env mutations can lead to high-level resistance to DTG in the context of spreading infection. Interestingly, 4XEnv impairs cell-free viral infectivity, but can efficiently transfer via cell-cell contact, relative to WT NL4-3, indicating that the selected Env mutations increase the efficiency of cell-cell transfer, resulting in high-level resistance to DTG. Long-term passaging of clinically relevant HIV-1 isolates [subtype B NL(AD8) and subtype C transmitted founder K3016] also revealed that the Env-dependent pathway to high-level DTG resistance is independent of virus strain. Our findings advance the understanding of how HIV-1 can evolve resistance to antiretrovirals, including the potent INSTI DTG, in the absence of mutations in genes targeted by the drug.

In silico molecular docking analysis of bioactive compounds from Nigerian medicinal plants against SARS-CoV-2 proteins

sciforum-057820

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The COVID-19 pandemic has become a threat to the world at large ever since it has been declared a pandemic since March 11 by WHO. Nigeria has recorded low infection, mortality and morbidity due to COVID-19 compared to the global average. In Nigeria ethnomedicine, it is believed that the consumption of herbal remedies may in part explain the low burden of disease in Nigeria and most of Sub-Sahara Africa. As a first step in investigating the antiviral activity of medicinal plants against SARS-Cov-2, this study screened bioactive substances from Nigeria medicinal plants for binding affinities against COVID-19 virus proteins. Thirty-five phytochemicals (out of 80 tested phytochemicals) satisfied Lipinski's rule of five and verber's rule. Ten out of those 35 phytochemicals were docked against SARS-CoV-2 Mpro, SARS-CoV-2 spike protein, TMPRSS2, and SARS-CoV-2 spike protein-ACE-2 complex. The results showed that Apigenin has the best binding affinity against SARS-CoV-2 Mpro and SARS-CoV-2 spike protein with -7.8kcal/mol and -7.2kcal/mol respectively. Corydine, with a binding affinity of -5.9kcal/mol, has the best binding affinity against SARS-CoV-2 spike protein-ACE-2 complex. Catechin was the topmost docked phytochemical against TMPRSS2 with a binding affinity of -7.3kcal/mol. Future experiments will explore the therapeutic potential of Apigenin, Corydine and Catechin singly and in combination against SARS-CoV-2.

Neutralization Landscape of influenza hemagglutinin stem to characterize antibody behavior and decompose antibody mixtures

sciforum-057425

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Current influenza vaccines protect against influenza strains antigenically matched to those used for vaccine preparation, but vaccine efficacy becomes minimal against antigenically mismatched influenza variants circulating among humans or emerging viruses from the animal reservoir. Discovery of broadly neutralizing antibodies (bnAbs) targeting conserved epitopes on influenza hemagglutinin (HA) set the blueprint for universal influenza vaccines that can elicit broader protective immunity. However, due to the lack of standardized, high-throughput influenza neutralization assay, deep characterization of the breadth and potency of bnAbs was not performed until recently. Here, we used the neutralization profiles of 27 bnAbs against a panel of 51 representative replication-restricted reporter influenza viruses to develop a new form of antigenic cartography called Neutralization Landscape that captures the full scope of antibody-virus interactions for those targeting the HA stem. Furthermore, using the Neutralization Landscape analysis, we decompose the collective neutralization of 14 antibody mixtures to characterize the composition and functional properties of the stem antibodies within. Our framework offers deeper understanding of the antibody-virus interactions and represents a new tool for in-depth analysis of polyclonal sera to study the antibody repertoire elicited by influenza vaccination or infection, which will provide insights towards developing a universal influenza vaccine strategy.

Optimized production and description of non-fluorescent and fluorescent SARS-CoV-2 Virus-Like Particles

sciforum-057724

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SARS-CoV-2 is an RNA enveloped virus from the *betacoronaviridae* family. Since 2019, this virus is responsible of the COVID19 and millions of deaths worldwide. Developing non-infectious SARS-CoV-2 derived virus-like-particles (VLP) are indispensable tools to facilitate vaccinal as well as basic research out of BSL3 laboratory. Here, we focus on setting up an optimal assembly and production of SARS-CoV-2 derived-VLP. Based on previous productive assays [*Swann et al 2020*], we propose an optimal system required for VLP formation composed of the main SARS-CoV-2 structural proteins, membrane M, nucleocapsid N, and enveloppe E and able to incorporate the Spike S. Based on transcriptomic data analysis of infected cells, we identify the optimal viral mRNA ratio allowing SARS-CoV-2 particle formation. Respecting that ratio, the structural M, N, E and S proteins were expressed by DNA transfection in HEK293T productive cells. Along this line, we implemented the incorporation of S, and fluorescent tagged on M and N proteins. We efficiently produced VLP incorporating S, and fluorescent and photoswitchable VLP. Thanks to super-resolution microscopy, we assessed VLP size and concentration showing a size of 100-120nm diameter for MNE VLP and 140nm for VLP-S. The VLP concentration was stimated at 1.10e12 particle/ml. SARS-CoV-2 VLP can also tolerate the integration of several fluorescent protein tags on M and N without impairing particle assembly then offering a good tool to visualize particles. We then show the incorporation of the Spike onto the VLP using immuno-spotting technique and revealing ~35% Spike incorporation on VLP, like for wild-type SARS-CoV-2 particles. Finally, the functionality of the Spike was shown by monitoring fluorescent VLP-S entry in human hACE2-A549 cells. Our condition of VLP production reveal VLP that resemble SARS-CoV-2 particles, albeit non-infectious and usable out of a BSL3, for studying virus assembly, virus entry, or VLP-based vaccins.

Pharmacological inhibition of IKK induces reactivation of latent HIV-1

sciforum-057447

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Background. Over 160 latency-reversing agents (LRAs) have been identified for the human immunodeficiency virus (HIV). However, none of them has yet led to functional cure. We evaluated the use of IKK kinase inhibitors (IKKi) targeting to different IKK subunits and IKK related kinases (TBK-1 and IKKε) as an alternative pathway to reverse HIV-1 latency.
Methods. HIV reactivation by IKKis was evaluated in latently infected lymphoid Jurkat (J-HIG) and myeloid HL-60 (HL-HIG) cells, by quantifying the percentage GFP+ cells by flow cytometry. Production of HIV CA p24 antigen induced by IKKis was measured in ACH-2 cells. Anti-HIV activity on acute infection was monitored in Jurkat and HL-60 cells infected with VSV-G-pseudotyped NL4-3-EGFP. For HIV-1 reactivation *ex vivo*, CD4+T cells from HIV+ individuals were treated for 72h with IKKis (4μM). HIV RNA in culture supernatants was quantified using an ultrasensitive semi-nested RT-qPCR. Cell activation markers (CD25, CD69 and HL-DR) and T-cell subsets were analyzed in CD4+ T cells from HIV-/HIV+ volunteers. Innate immune signalling pathways were assessed by western blot.
Results. Regardless of combination or not with anti-HIV drugs, IKKi triggered up to 1.8 fold increase in HIV reactivation in J-HIG cells. In HL-HIG, HIV expression was 1.5 fold-higher for TBK-1 inhibitors. HIV CA p24 antigen induced by IKKis was higher compared to HDACi in ACH-2 cells. IKKis targeting TBK-1 (MRT67307) and IKKβ (TCPA-1) induced viral transcription in HIV+ CD4+T cells *ex vivo*. IKKis did not upregulate HLA-DR, CD25, CD69 markers nor modify T-cell subsets. Innate immune signaling was partially blocked by decreased phosphorylation of STAT1 and subsequent signaling pathway.
Conclusion. IKKis targeting TBK-1 and IKKε present significant HIV-1 reactivation capacity in lymphoid and myeloid compartments. Low toxicity and the absence of cell activation, suggest their use as alternative LRA in new HIV therapeutic approaches.

Polyphenols and Polyphenol coated nanoparticles, promising future antivirals

sciforum-058330

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Abstract: To efficiently lower the virus infectivity and combat virus epidemic or pandemic, it is important to discover broadly acting antivirals. Here, we investigated two naturally occurring polyphenols, epigallocatechin gallate (EGCG) and resveratrol (RES), and polyphenol functionalized nanoparticles for their antiviral efficacy. Concentrations in the low micromolar range permanently inhibited infectivity of high doses of enteroviruses (107 PFU/mL). Sucrose gradient separation of radiolabelled viruses, dynamic light scattering, transmission electron microscopic imaging, and an in-house developed real-time fluorescence assay revealed that polyphenols prevented infection mainly through clustering of the virions into very stable assemblies. Clustering and stabilization were not compromised even in dilute virus solutions or after diluting the polyphenols-clustered virions by 50-fold. In addition, the polyphenols lowered virus binding on cells. In silico docking experiments of these molecules against 2-fold and 3-fold symmetry axes of the capsid, using an algorithm, developed for this study, discovered five binding sites for polyphenols out of which three were novel binding sites. Our results altogether suggest that polyphenols exert their antiviral effect through binding to multiple sites on the virion surface leading to aggregation of the virions and preventing RNA release and reducing cell surface binding.

Rufinamide-Induced Autophagy Cell Death in HIV-1-Infected Macrophages

sciforum-058343

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Background: The major barrier to HIV cure is the HIV-1 reservoir. Macrophage reservoirs contribute to HIV-1 persistence, disease progression, and comorbidities in people living with HIV, including HIV-associated neurocognitive disorders and cardiovascular disease. Unlike dividing CD4+ T cells, which experience rapid cell-death due to accelerated viral replication, macrophages experience slow viral replication kinetics, and do not die upon HIV-1 infection. We hypothesized that accelerating HIV-1 replication in macrophages would result in cell-death of only infected macrophages, decaying the reservoir.
Methods: We launched a screening campaign of > 2700 agents (FDA approved/Phase 3) to identify agents that increase viral replication and confer cell-death only to infected macrophages (flow-cytometry). Data were confirmed using a replication competent HIV-1 (89.6). Toxicity across relevant human uninfected cells were measured (flow-cytometry). Measure of effect of identified agents on SAMHD1 and dNTP were measured (western blots). Death markers were measured (flow-cytometry). Autophagosomes were visualized (electron microscopy;EM).
Results: We identified rufinamide, a blood-brain-barrier penetrant, FDA approved agent that specifically kills only infected macrophages, including newly established infection and already-existing infection, without toxicity to uninfected macrophages or other relevant human cells. Rufinamide does not increase dNTP levels or SAMHD1/pSAMHD1, implying the phenotype is not related to increase in dNTP pools. Impact of HIV-1 accessory protein *Vpr* was evaluated due to known role in toxicity/cell-death. The Vpr deletant replication kinetics were not affected by rufinamide, indicating Vpr plays a role in the cell-death phenotype induced by rufinamide. Autophagosomes were observed via EM in rufinamide-treated HIV-vector treated macrophages, demonstrating autophagy as a contributing cell-death mechanism.
Conclusions: Rufinamide induces cell-death specific to HIV-1 infected macrophages, independent of SAMHD1 or dNTP levels. Autophagy is a mechanistic contributor. These data are promising towards eventual human trials to purge the myeloid reservoir, given its repurposed status and existing body of safety in humans.

Small molecule screening identifies Syk kinase inhibition and Rutaecarpine as modulators of macrophage training and SARS CoV-2 infection

sciforum-058196

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Biologically active small molecules can impart modulatory effects in immune cells, in some cases, providing extended long-term memory. In a screen of biologically active small molecules for regulators of TNF induction, we identified several compounds with the ability to induce training effects on human macrophages. Rutaecarpine was identified both as an acute and long-term modulator, enhancing LPS-induced pro-inflammatory cytokine secretion and relieving LPS tolerance in human macrophages. Rutaecarpine inhibited b-glucan induced H3K4Me3 marks at the promoters of several proinflammatory cytokines, highlighting the potential of this molecule to modulate epigenetic topology. In further work, a Syk kinase inhibitor (SYKi IV) screen hit promoted an enhanced response to LPS, similar to that previously reported for b-glucan-induced training. Macrophages trained with SYKi IV showed a high degree of resistance to influenza A, SARS CoV-2 and OC43 coronavirus infections, highlighting a potential application of this molecule and other Syk kinase inhibitors as a prophylactic treatment for viral susceptibility.

Unraveling the antiviral activity of plitidepsin by subcellular and morphological analysis

sciforum-057818

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To treat infection caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) effective antiviral treatments are urgently needed. To this purpose, an interesting approach relies on the use of compounds against cellular host factors used by the virus to complete its replication cycle. Plitidepsin is an antitumor agent of marine origin that has also shown a potent pre-clinical efficacy against SARS-CoV-2, with similar potency against several variants including omicron. Plitidepsin targets the host protein eEF1A (eukaryotic translation factor 1 alpha 1) interfering with viral infection at an early, post-entry step. Because electron microscopy is a valuable tool to study the mechanism of action of antiviral drugs, in this work we have used transmission electron microscopy (TEM) to evaluate the effects of plitidepsin in cultured Vero E6 cells infected with SARS-CoV-2. In the absence of plitidepsin, TEM morphological analysis of infected cells showed double-membrane vesicles (DMVs) that are the genome replication organelles of coronaviruses, and numerous intracellular and extracellular viral particles. In fact, in the absence of plitidepsin 100% infected cells contained viral structures. However, when treated with plitidepsin, neither DMVs nor viral particles were found in Vero E6 cells treated with SARS-CoV-2. Immunogold detection of SARS-CoV-2 nucleocapsid (N) protein and double-stranded RNA that is an intermediate of viral replication, provided clear signals in cells infected in the absence of plitidepsin, but complete absence in cells infected and treated with plitidepsin. The present study shows that plitidepsin blocks the biogenesis of viral replication organelles and the morphogenesis of virus progeny. Electron microscopy morphological analysis coupled to immunogold labeling of viral products offers a unique approach to understand how antivirals such as plitidepsin work. Furthermore, this work shows that targeting host factors can offer a pan-antiviral strategy to combat present and future pathogenic viruses.

Unravelling the mode of action against the SARS-CoV-2 of an EMA/FDA approved anti-cancer drug

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In 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged and spread quickly globally putting immense strain on all healthcare systems. Despite the available vaccines, there is still a need for effective therapeutic agents against coronavirus disease 19 (COVID-19) and its sequelae. One of the approaches to seek effective antiviral agents is drug repurposing. PARP inhibitors, a set of antineoplastic drugs, have emerged as possible remedies for COVID-19. Three PARP inhibitors were selected in our screen: rucaparib, olaparib and talazoparib. Rucaparib exhibited anti- SARS-CoV-2 activity in our *in vitro* screen. We excluded the possibility that Rucaparib could inhibit the SARS-CoV-2 Nsp3 macrodomain that reverses the ADP- ribosylation of key viral proteins. Next, we investigated whether rucaparib blocks viral entry. Both an *in vitro* neutralization assay and a reporter viral uptake assay showed reduced viral replication/uptake after rucaparib treatment. Our results suggest, that rucaparib blocks the entry of the virus into the host cells through an unknown target. Further studies might be necessary to fully understand the mechanism of action and its clinical relevance.

Abstracts

S3. Antiviral Innate Immunity

Invited Speaker

Manipulation of mitochondrial dynamics by flaviviruses: how and why

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In cells infected with dengue virus or Zika virus (ZIKV), mitochondria accumulate near sites of replication and are thought to support expansion of membranes that the viruses uses as replicative platforms. However, the mechanisms and consequences of this phenomenon to viral pathogenesis are not clear. By examining the interactions of ZIKV with innate immune responses, we have uncovered an ability of these viruses to suppress mitophagy, or the selective removal of damaged mitochondria. The major pathway of mitophagy is controlled by two proteins, PINK1 (kinase) and Parkin (E3 ubiquitin ligase), often mutated in familial Parkinson's disease (PD). Increasing evidence suggests that defects in mitophagy are associated with increased inflammation that may be pathogenic. Here, we demonstrate that ZIKV nonstructural protein 5 (NS5) antagonizes mitophagy by binding to the host protein Ajuba and preventing its translocation to depolarized mitochondria where it is required for PINK1 activation and downstream signaling. Consequent mitophagy suppression amplifies the production of pro-inflammatory chemokines through protein kinase R (PKR)-dependent sensing of mitochondrial RNA. In Ajuba-/- mice, ZIKV induces early expression of pro-inflammatory chemokines associated with significantly enhanced dissemination to tissues. This work identifies Ajuba as a critical regulator of mitophagy and demonstrates a role for mitophagy in limiting systemic inflammation following infection by globally important human viruses.

Invited Speaker

Viral community structure and dynamics in a simplified natural ecosystem

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It is widely suspected that viruses play a major role in the structure, function, and evolution of microbial communities, however a detailed understanding of their role in natural ecosystems is lacking. One factor limiting our understanding of the role of viruses in ecosystems is our poor understanding of the total virus diversity and dynamics in any single ecosystem. High temperature (>75C) acidic (pH 4) hot springs found in Yellowstone National Park, USA (YNP) provide an opportunity to define the near complete virus community due the highly simplified microbial communities present in these hot springs. Here we combine temporal viral community sequence analysis of several different hot springs (viral metagenomics), viral network analysis, and cellular DNA and RNA sequencing to develop a more complete understanding of the virus community structure and their activity. We define 198 virus groups that define the majority of virus types present in these hot springs of which 179 have no characterized representatives. Most of these virus groups are likely new archaeal viruses that are widely distributed among the sampled hot springs. A surprising high percentage of sequence reads in both the cellular DNA and RNA datasets map to the virus network, indicating that most if not all cells are interacting with and replicating viruses. Overall, this work provides a roadmap for directing the isolation and characterization of viruses and examining their role in these hot spring ecosystems.

Invited Speaker

Regulation of Antiviral Innate Immunity and Virus replication by Unanchored Ubiquitin Chains and the E3-Ubiquitin Ligase TRIM6

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The host antiviral innate immune response requires activation of multiple signaling factors leading to the production of type I interferons (IFN-I) and inflammatory cytokines. To avoid hyperinflammation, these signaling pathways are regulated by post-translational modifications, including protein ubiquitination. The E3-ubiquitin ligase TRIM6 catalyzes the synthesis of unanchored K48-linked polyubiquitin chains, which are not covalently attached to any protein, and activate the IKKε kinase for induction of IFN-I mediated antiviral responses. To identify other host factors regulated by unanchored ubiquitin in a physiological setting, we developed a method to isolate unanchored Ubiquitin-interacting factors from lung tissue. We identified DHX16 as a potential pattern recognition receptor (PRR), which we found has antiviral effects against different viruses, including Influenza, Zika and SARS-CoV-2. DHX16 recognizes a motif in Influenza virus RNA segments that undergo splicing as its pathogen-associated molecular pattern (PAMP). In addition, newly generated *Trim6*$^{-/-}$ mice shows that TRIM6 can play both protective and detrimental roles depending on the virus infection and the inflammatory environment. For example, Ebola virus replication is decreased in the lungs of *Trim6*$^{-/-}$ upon intranasal administration. In contrast, TRIM6 plays a protective role against Influenza, via the IFN-I pathway, but can exacerbate disease via induction of chemokines and infiltration of inflammatory cells. Although *Trim6*$^{-/-}$ mice have increased Influenza virus titers, they also show reduced signs of pathology and reduced neutrophil infiltration in the lungs. Overall, TRIM6 and unanchored ubiquitin are emerging as important factors in balancing protective and detrimental inflammatory responses and the importance of TRIM6 is highlighted by the fact that it can be hijacked by viruses leading to enhanced pathology.

The circadian clock component BMAL1 regulates SARS-CoV-2 entry and replication in lung epithelial cells

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The coronavirus disease 2019 pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) coronavirus, is a global health issue with unprecedented challenges for public health. SARS-CoV-2 primarily infects cells of the respiratory tract via spike glycoprotein binding to angiotensin-converting enzyme (ACE2). Circadian rhythms coordinate an organism's response to its environment and can regulate host susceptibility to virus infection. We demonstrate that silencing the circadian regulator *Bmal1* or treating lung epithelial cells with the REV-ERB agonist SR9009 reduces ACE2 expression and inhibits SARS-CoV-2 entry and replication. Importantly, treating infected cells with SR9009 limits SARS-CoV-2 replication and secretion of infectious particles, showing that post-entry steps in the viral life cycle are influenced by the circadian system. Transcriptome analysis revealed that *Bmal1* silencing induced interferon-stimulated gene transcripts in Calu-3 lung epithelial cells, providing a mechanism for the circadian pathway to limit SARS-CoV-2 infection. Our study highlights alternative approaches to understand and improve therapeutic targeting of SARS-CoV-2.

Assessing the functional impact of IFITM heteromultimers during restriction of virus entry

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Human interferon-induced transmembrane (IFITM) proteins play an important role in innate immunity by preventing virus entry into host cells. IFITM1, IFITM2 and IFITM3 exhibit different antiviral specificities. IFITM3 restricts influenza A virus and Zika virus while IFITM1 is a more potent inhibitor of SARS-CoV, SARS-CoV-2 and Ebola virus. However, little is known about how these related proteins regulate one another or whether they collaborate during virus restriction. We previously identified a ^{91}GxxxG^{95} motif that is critical for IFITM3 homomultimerization and its antiviral function. Here, we found that IFITM3 interacts with IFITM1 in cells using immunoprecipitation coupled to mass spectrometry, and that ^{91}GxxxG^{95} of IFITM3 is required for this interaction. We confirmed this finding by showing that ectopic IFITM3 and IFITM1, as well as IFITM3 and IFITM1 induced by type-I interferon, co-immunoprecipitate from cell lysates. The ^{91}GxxxG^{95} in IFITM3 is conserved in IFITM1 (^{71}GxxxG^{74}). When this motif was mutated in IFITM3, IFITM1, or both, co-immunoprecipitation between the two proteins was significantly reduced. These results suggest that the same determinants govern both homo- and heteromultimerization among the human IFITM family. We are now determining where the IFITM3 and IFITM1 interaction takes place on the subcellular level using live-cell fluorescence microscopy. To determine whether the interaction between IFITM proteins is important for their antiviral activities, we co-transfected cells with IFITM3 and IFITM1 variants that are competent for heteromultimerization or not and challenged them with Influenza A virus or SARS-CoV-2. We found that co-expression of heteromultimerization-defective IFITM mutants led to loss of virus restriction. Our findings suggest that a IFITM3-IFITM1 complex regulates the antiviral potential of both proteins, providing a potential explanation for the evolutionary expansion of the IFITM locus in primate ancestors.

CD24 Expression in Neuroblastoma Cells Alters Type I Interferon Signaling and Susceptibility to Zika Virus Infection

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Zika virus (ZKV), a member of the *Flaviviridae* family, has recently re-emerged and caused a global health emergency. While ZKV infection can result in varying degrees of tropism for a variety of cells and tissues (such as neural or reproductive tissues), the question of which host cellular factors modulate susceptibility and infectivity by ZKV remain largely unknown. Our prior work demonstrated that ZKV replication was dramatically reduced in a neuroblastoma SK-N-AS cell line, and this was relieved by ectopic expression of a cellular protein CD24. Here, we have tested the hypothesis that CD24 suppressed the cellular anti-viral Type I Interferon (IFN-I) response to ZKV infection, thereby allowing a previously non-permissive SK-N-AS cell line to be susceptible to ZKV infection. Flow cytometry data showed that the parental SK-N-AS cells (with undetectable CD24 levels) were highly restricted for ZKV low multiplicity of infection (MOI) spread through the population of cells, whereas this was enhanced in SK-N-AS cells with ectopic CD24 expression. Upon treatment with exogenous IFN-I, robust anti-viral gene responses were observed in the parental CD24-negative cells, compared to the CD24-expressing cells which showed a low response. Parental CD24-negative cells showed constitutive basal levels of phosphorylated STAT1, a key factor in the IFN-I signaling pathway. By contrast, CD24-expressing cells had low basal phosphorylated STAT1 levels. Parental CD24-negative cells also showed an upregulation in other key players of IFN-I pathway, IRF-1, IKKE, and NF-kB, while CD24-expressing cells displayed a downregulation of these proteins. These studies support the importance of basal and induced IFN-I as a key factor in restricting ZKV infection of neuroblastoma cells and lay the foundation for determining how CD24 expression represses the IFN-I signaling pathway

HCMV protein vMIA interferes with the peroxisomal and mitochondrial antiviral responses via distinct mechanisms

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Peroxisomes have recently emerged as important organelles during viral infections. Numerous reports show that different viruses modulate peroxisome dynamics and metabolism to promote proper virus particle formation and propagation. Peroxisomes are also central signalling platforms for the cellular antiviral response. Upon recognition of viral RNA at the cytoplasm of infected cells, RIG-I-like proteins interact with MAVS at peroxisomes and mitochondria, inducing its oligomerization and, consequently, the downstream production of direct antiviral effectors. Different studies demonstrate that several viruses have evolved specific mechanisms to counteract the peroxisome-dependent antiviral signalling. We have shown that the immediate early human cytomegalovirus (HCMV) protein vMIA, known for its anti-apoptotic functions, is also able to inhibit the peroxisome-dependent antiviral response. Here, we further unravel the mechanisms involved and demonstrate that, contrarily to mitochondria, this signalling inhibition is independent of the organelle's morphology and does not involve the impairment the interaction between MAVS and the endoplasmic reticulum protein STING. Importantly, we demonstrate that vMIA interacts with MAVS and impedes its oligomerization and the consequent activation of the downstream signalling at both peroxisomes and mitochondria. We furthermore demonstrate that MFF is essential for vMIA's inhibition of peroxisomal MAVS oligomerization but plays no essential role on the mitochondrial MAVS signalling inhibition.
Our results reveal that HCMV has developed distinct mechanisms to interfere with the peroxisomal and mitochondrial antiviral responses, likely adapting to their intrinsic differences and role throughout the viral infection. These results highlight the relevance of peroxisomes as platforms for antiviral signalling against HCMV and unravel specific molecular mechanisms that may be further explored as targets for antiviral therapy.

Human Coronavirus Infected Lung Cells Recruit Complement Inhibitors Vitronectin and Clusterin and Delay Complement-Mediated Lysis

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The complement (C') system is a significant pathway of the innate immune response which viruses and infected cells must evade for survival. We sought out to better understand the innate immune response to a highly prevalent and reoccurring RNA human coronavirus (CoV)-OC43. CoV-OC43 is a major public health concern that imposes a huge burden on the economy and health care industry. Using real-time cell viability assays and flow cytometry, we have shown the kinetics of C'-mediated lysis of CoV-OC43 infected cells was substantially delayed as compared to the rapid and potent C'-mediated killing of cells infected with parainfluenza virus type 5 (PIV5). In the case of CoV-OC43 infected cells that were co-infected with PIV5, the C'-mediated lysis was delayed to the same extent as CoV-OC43 infected cells alone, suggesting that CoV-OC43 infection can overcome highly activating signals induced by PIV5 infection. Our prior work has shown respiratory virus infection can upregulate expression of C' inhibitors such as CD59. Vitronectin (VN) and clusterin (CLU) are key regulators of the terminal C' pathway found in normal human serum (NHS) that can prevent membrane attack complex formation and cell death. Here we investigated the hypothesis that human respiratory virus infected cells can utilize the C' inhibitors VN and CLU. VN and CLU could be recruited from exogenous serum to the surface of CoV infected cells. VN wasn't bound to OC43-infected cells after treatment with antibody-depleted serum. Reconstitution experiments with purified IgG and VN showed that human antibodies are both necessary and sufficient for VN recruitment to OC43-infected cells. Taken together, we propose a novel role for VN and CLU recruitment by coronavirus infected cells to delay C'-mediated death – findings that have implications for viral pathogenesis and tissue tropism.

Innate Immune Sensing of Bovine Foamy Virus primary microRNA?

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As the innate immune system with its recognition receptors for double-stranded RNA plays a key role in the defense mechanisms against viruses, the question arises whether the approximately 125 nt-long and mostly double-stranded pri-miRNA of Bovine Foamy Virus (BFV) is detected via innate sensing. This dumbbell-shaped pri-miRNA, which is transcribed by RNA-Polymerase III, has been described by Whisnant et al. and has no known counterpart in other living organisms. Due to a lack of knowledge regarding the possible role of this unique structure as an agonist of innate cytosolic dsRNA sensing, we conducted experiments to analyse its effect. Different methods were established to gain insight into innate immune sensing of the in-vitro T7 RNA polymerase-transcribed BFV pri-miRNA. Either one of the stem-loops of the pri-miRNA or the exact sequence of the pri-miRNA as a scrambled sequence without predicted secondary structures were used as intrinsic controls. In addition, the correct synthesis and therefore the correct sizes of the different in-vitro-transcribed pri-miRNAs were validated using Bioanalyzer RNA 6000 Pico assay analyses. To minimize possible side effects of reaction intermediates, the pri-miRNA was treated with DNase I or RNase H to exclude RNA-DNA hybrids and different purification systems were used. After varying pre-treatments, the pri-miRNA was transfected into human A549 cells which constitutively express IRF-3 tagged with eGFF. The system established allows live-cell-imaging and analyses of the effects of pri-miRNA with different pre-treatments in comparison to the diverse controls. Initial analyses yielded pri-miRNA-specific IRF-3 aggregation and nuclear accumulation.
The significance of these studies lies not only in the context of BFV research but in the establishment of these methods regarding the further applicability in other viral systems.

Interferon signalling influences the impact of glycolysis on virus infections

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Cellular metabolism and especially a fast response through glycolysis provide energy and metabolic intermediates not only for a viral replication cycle, but also for the antiviral innate immune response. We hypothesized that the response to interferon (IFN) influences virus-associated metabolic alterations, especially glycolysis. We addressed this in a loss-of-function approach on human A549 cells with knockout of the type I IFN receptor, either solely (IFNAR KO) or together with the receptor of type III IFN (IFNAR/IFNLR1 KO). Usutu virus (USUV) and rubella virus (RuV) were used as representative virus infections based on their impact on cellular metabolism. Extracellular flux analysis was performed to determine the cellular metabolic phenotype together with the application of metabolic stressors. Virus replication efficiency was assessed besides analysis of the IFNs generated during infection. For both viruses an increase in replication was detected on A549 with IFNAR/IFNLR1 KO in comparison to both, IFNAR KO cells and control cells. Moreover, during infection an increase in glycolysis was noted on IFNAR/IFNLR1 KO A549. As shown for RuV, IFN-associated signalling influenced the impact of the glucose analogue 2-deoxy-glucose as an inhibitor of glycolysis on virus infection: on control cells an altered infection rate, but only a slight influence in the absence of IFN signalling (IFNAR/IFNLR1 KO A549) was detected. Moreover, the generation of IFN β during RuV infection was influenced by type III IFN-associated signalling. In conclusion, virus infection-associated alterations in glycolysis and as such dependency on glycolysis can be influenced by type I and type III IFN signalling.

Interferon-Induced Transmembrane Proteins Inhibit Viral Fusion by Imposing Negative Membrane Curvature and Increasing Lipid Order and Bending Modulus

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Interferon-induced transmembrane proteins (IFITMs) are small transmembrane proteins that potently inhibit entry/fusion of diverse enveloped viruses. We have previously demonstrated that IFITM3 accumulates in endosomes carrying single influenza virus and captures viral fusion at a hemifusion stage by blocking the formation of a fusion pore. To elucidate the mechanism by which these proteins interfere with virus entry, we examined the effects of a purified recombinant IFITM3 reconstituted into giant unilamellar vesicles (GUVs). IFITM3 incorporation into GUVs induced inward vesicle budding implying that this protein promotes negative membrane curvature. We mapped this membrane bending activity of IFITM3 to a short amphipathic helix (AH) corresponding to the residues 59-68 of the N-terminal cytoplasmic region, which has been previously shown to be critical for the IFITM3's antiviral activity. Addition of the AH peptide to GUVs induced inward vesicle budding. Substitutions in the AH peptides that have been shown to disrupt the amphipathic helix and compromise the IFITM3's antiviral activity greatly diminished the ability of AH to promote GUV vesiculation. In addition to imposing negative membrane curvature, AH increased lipid order, as well as the membrane bending modulus. To determine if AH is necessary and sufficient for antiviral activity, we incorporated AH into the inner or outer leaflets of the plasma membrane and observed significant inhibition of virus-cell fusion or exocytosis, respectively. Collectively, our results imply that induction of negative curvature and stiffening of the cytoplasmic leaflet of endosomal membranes by the IFITM3 AH stabilizes the hemifusion diaphragm and thereby disfavors viral fusion. This work was supported in part by NIH R01 AI135806 grant to GBM.

Modulation of HIV Replication in Monocyte-Derived Macrophages (MDM) by Host Antiviral Factors Secretory Leukocyte Protease Inhibitor and Serpin Family C Member 1 Induced by Steroid Hormones

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The impact of steroid hormones estrogen and progesterone on human immunodeficiency virus type 1 (HIV-1) replication is well documented. However, the exact mechanism involved in the regulation of HIV-1 replication by estrogen and progesterone is still unclear. In the present study, we wanted to elucidate the molecular mechanisms underlying the modulation of HIV-1 replication by estrogen and progesterone. To achieve this goal, we used real-time quantitative PCR arrays (PCR arrays) to identify differentially expressed host genes in response to hormone treatments that are involved in antiviral responses. Our in vitro results suggest that treatment with high doses of estrogen and progesterone promotes the expression of host antiviral factors Secretory leukocyte protease inhibitor (SLPI) and Serpin family C member 1 (SERPIN C1) among others produced in response to HIV-1 infection. SLPI is an enzyme that inhibits human leukocyte elastase, human cathepsin G, human trypsin, neutrophil elastase, and mast cell chymase. SERPIN C1 is a plasma protease inhibitor that regulates the blood coagulation cascade by the inhibition of thrombin and other activated serine proteases of the coagulation system. A dose dependent downmodulation of HIV-1 replication was observed in monocyte-derived macrophages (MDMs) pre-treated with the two proteins SLPI and SERPIN C1. Further investigations suggests that the host antiviral factors, SLPI and SERPIN C1 act at the pre-integration stage, inhibiting HIV-1 viral entry and leading to the observed downmodulation of HIV-1 replication. Our studies would help identify molecular mechanisms and pathways involved in HIV-1 pathogenesis.

Rapalogs downmodulate intrinsic immunity and promote cell entry of SARS-CoV-2

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SARS-CoV-2 infection in immunocompromised individuals is associated with prolonged virus shedding and the evolution of viral variants. Rapamycin and its analogs (rapalogs, including everolimus, temsirolimus, and ridaforolimus) are FDA-approved as mTOR inhibitors in clinical settings such as cancer and autoimmunity. Rapalog use is commonly associated with increased susceptibility to infection, which has been traditionally explained by impaired adaptive immunity. Here, we show that exposure to rapalogs increases susceptibility to SARS-CoV-2 infection in tissue culture and in immunologically naïve rodents by antagonizing the cell-intrinsic immune response. By identifying one rapalog (ridaforolimus) lacking this function, we demonstrate that rapalogs promote Spike-mediated entry into cells by triggering the lysosomal degradation of IFITM2 and IFITM3. Rapalogs that promote virus entry inhibit the mTOR-mediated phosphorylation of TFEB, a transcription factor controlling lysosome biogenesis and degradative capacity. In the hamster model of infection, injection of rapamycin four hours prior to virus exposure resulted in elevated virus titers in lungs and accelerated weight loss, while ridaforolimus had milder effects. Furthermore, rapamycin significantly elevated mouse-adapted SARS-CoV-2 titers in lungs of mice. Overall, our findings indicate that preexisting use of certain rapalogs may elevate host susceptibility to SARS-CoV-2 infection and disease by activating a lysosome-mediated suppression of intrinsic immunity.

Role of the RNA modification N^6-methyladenosine (m^6A) during HIV-1 replication in monocyte-derived human macrophages and microglias

sciforum-058284

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Chronic inflammation is a hallmark of people living with HIV despite efficient viral suppression by ART, being the main cause of mortality and development of non-AIDS comorbidities. Growing evidence suggests that active transcription of the integrated HIV-1 provirus and the presence of the viral RNA in the cytoplasm of infected macrophages significantly contributes to the establishment of this pro-inflammatory state. Moreover, reports have shown that the post-transcriptional RNA modification m^6A would play a crucial role regulating viral RNA sensing and activation mechanisms in infected cells. The present work aims to determine whether the presence of m^6A in HIV-1 RNA is involved in viral replication and cellular activation mediated by innate immune sensors. In this context, during the characterization of HIV-1 infection in Thp-1 macrophages and the microglia cell line C20, we observed a peak of viral RNA at 48 hpi with a subsequent decrease in RNA levels until 5 days of infection, with is in concordance with the expression of the viral p24 protein. We also observed induction of the mRNA of some pro-inflammatory cytokines like IFN-a, IFN-b, CXCL10 and IL-8 that could have a relation with the expression of the viral RNA during the infection. We have observed a variations in the expression of the m^6A methyltransferase METTL3 during the 5 days of infection in C20 microglias. Some experiments of overexpression of METTL3 and the demthylase FTO in Thp-1 macrophages have shown an impact in the replication of the virus and the expression of the pro-inflammatory cytokines IFN-b and CXCL10.
We are currently investigating the role of the m^6A modification in the post-transcriptional control of the HIV-1 RNA and the immune activation triggered by this viral transcript.

Sandfly fever viruses attenuate the type I interferon response by targeting the phosphorylation of JAK-STAT components

sciforum-057453

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Sandfly fever viruses are emerging *Phleboviruses* typically causing mild febrile illness. Some strains, however, can cause severe and occasionally fatal neuro-invasive disease. Like most viruses, *Phleboviruses* have devised various strategies to inhibit the type I interferon (IFN) response to support a productive infection. Still, most of the strategies identified so far focus on inhibiting the sensing arm of the IFN response. While the effect of sandfly virus infection on signaling from the IFN receptor is less characterized. Therefore, we tested the effect of sandfly fever virus Naples (SFNV) and Sicily (SFSV) infection on IFN signaling. We found that infection with either of these viruses inhibits signaling from the IFN receptor by inhibiting STAT1 phosphorylation and nuclear translocation. We show that the viral nonstructural protein NSs mediates these effects, but only NSs from SFNV was found to interact with STAT1 directly. Thus, we tested the upstream IFN signaling components and found that Janus kinase 1 (Jak1) phosphorylation is also impaired by infection. Furthermore, the NSs proteins from both viruses directly interacted with Jak1. Last, we show that IFN inhibition by SFNV and SFSV is most likely downstream of the IFN receptor at the Jak1 level. Overall, our results reveal the multiple strategies used by these related viruses to overcome host defenses.

Sensing of SARS-CoV-2 infected cells by plasmacytoid dendritic cells promotes a local antiviral response

sciforum-057471

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The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is responsible for the current COVID-19 pandemic. Type I and III interferons (IFN-I/ λ) are pivotal antiviral molecules rapidly produced following virus infection that can directly inhibit viral spread and potentiate the adaptive immunity. In the course of SARS-CoV-2 infection, a delayed and/or weak production of IFN-I is thought to lead to an increased viral load, and hereby, an exacerbated release of proinflammatory cytokines. This hypercytokinemia contributes to COVID-19 severity. Here, we demonstrate that the plasmacytoid dendritic cells (pDCs) are predominant IFN-I/λ source by sensing SARS-CoV-2-infected cells. We show that sensing of viral RNA by pDCs requires sustained cell adhesion with infected cells, via cell adhesion established by the αLβ2-integrin/ICAM-1 complex. Moreover, using a recombinant infectious SARS-CoV-2 expressing a Neon-Green reporter, live-imaging of pDC-antiviral response reveals that the sustained contact of pDC with infected cell decreases the replication in the infected cells when in direct physical contact with the activated pDC. This specialized function enables pDCs to efficiently turn-off viral replication, likely by a concentrated flux of antiviral effectors at the contact site with infected cells. Therefore, we propose that pDC activation is essential to locally control SARS-CoV-2-infection. By exploring the pDC response in patients, we further demonstrate that pDC responsiveness correlates with the severity of the disease and in particular, this response is impaired in severe COVID-19 patients. Thus, the ability of pDCs to respond to SARS-CoV-2-infected cells could be a key to understand severe cases of COVID-19.

Ubiquitin variants as antivirals against SARS-CoV-2

sciforum-057919

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Almost two years into the pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), there is still a dire need for potent antivirals to fight the effects of this virus on the human body. Similar to SARS-CoV and MERS-CoV, SARS-CoV-2 encodes a viral papain-like protease, named PLpro. This protease cleaves part of the viral polyprotein into non-structural protein subunits, which is crucial for the virus life cycle. Additionally, the protease is able to bind and cleave both (poly-) ubiquitin and the ubiquitin-like protein ISG15 from host cellular substrates, which provides the virus with a way to evade the innate immune response during infection. Because of the many activities of PLpro, it is an attractive target for potent antiviral agents. In an innovative approach, sequence variants of ubiquitin (UbVs) were developed to specifically bind to SARS-CoV-2 PLpro with high affinity, thereby acting as antiviral agents. A crystal structure of SARS-CoV-2 PLpro in complex with the UbVs shows that the UbV molecules bind to PLpro away from the catalytic core of the protease. Yet, the binding is able to inhibit multiple activities of the protease *in vitro* and in cell culture. Moreover, SARS-CoV-2 specific UbVs greatly reduce viral replication in cell culture. Delivery options, development of resistance against this antiviral strategy, and broadness of activity against coronavirus variants of concern will be further investigated and/or discussed.

Abstracts

S4. Mechanisms of Virus Replication

Invited Speaker

NDRG1 facilitates both latent and lytic replication of Kaposi's sarcoma-associated herpesvirus

sciforum-060694

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Kaposi's sarcoma-associated herpesvirus (KSHV) latently infects host cells and establishes lifelong persistence as an extra-chromosomal episome in the nucleus. To persist in proliferating cells during viral latency, the viral genome typically replicates once per cell cycle and is distributed into daughter cells. This process involves host machinery utilized by KSHV, however the underlying mechanisms are not fully elucidated. We found that N-Myc downstream regulated gene 1 (NDRG1), a cellular gene known to be non-detectable in primary B cells and endothelial cells which are the major cell types for KSHV infection *in vivo*, was highly upregulated by KSHV in these cells. We demonstrated that NDRG1 facilitates viral genome replication by interacting with proliferating cell nuclear antigen (PCNA) and the latent viral protein latency-associated nuclear antigen (LANA) during viral latency. Interestingly, we further demonstrated that NDRG1 can interact with KSHV ORF44, the viral helicase expressed during lytic replication. We found that this interaction promotes viral lytic replication by maintaining the stability of ORF44. These findings suggest that NDRG1 plays an important role in both KSHV latent and lytic replication, and provide new clues for understanding of KSHV persistence.

Invited Speaker

Nidovirus replication organelles

sciforum-060646

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The remodeling of intracellular membranes is a hallmark of positive-strand RNA viruses infecting eukaryotes. These virus-induced membrane structures support the viral RNA synthesis and are often referred to as viral replication organelles. They are thought to provide optimal microenvironments for viral replication and a shield from innate immune sensors. A fascinating example are the replication organelles induced by coronaviruses and other members of the *Nidovirale*s order. Nidoviruses generate typical double-membrane vesicles (DMVs), inside which viral RNA synthesis is presumed to take place. Strikingly, in conventional electron microscopy, these DMVs appear as closed compartments with no openings to the cytosol. Therefore, it has been long unclear how newly made viral RNA could be exported to the cytosol for packaging into new virions and translation into viral proteins.

Using cellular cryotomography to study coronavirus-induced DMVs, we unveiled a crown-shaped molecular pore spanning the two membranes of the DMVs. Six copies of the largest viral transmembrane non-structural protein constitute the core of this pore complex. We have expanded our investigations to arteriviruses, a distantly related nidovirus family, and found similar DMV pores. Moreover, DMV pore complexes were also formed when expressing only subsets of the arterivirus transmembrane nonstructural proteins. This indicated that the formation of a basic pore structure is independent of the presence of viral RNA, the replication machinery or the structural proteins. In infected cells, viral nucleocapsids were often observed in close association with the DMV pores.

Collectively, our results suggest that DMV-spanning pore complexes is a conserved feature in nidovirus replication, which highlights their important role in the viral replication cycle. These DMV pore complexes are likely RNA export channels that coordinate viral replication and encapsidation. They represent a novel class of viral complexes that offer new targets for future antiviral strategies.

Invited Speaker

Structure and function of umbravirus cap-independent translation enhancers and novel associated hairpins

sciforum-060485

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Umbraviruses have a positive sense genomic RNA with four ORFs and a lengthy 600 to 700 nt 3' UTR. Multiple different 3'cap-independent translation enhancers (3'CITEs) have been identified in umbravirus 3'UTRs, including (i) the pea enation mosaic virus 2 (PEMV2) PTE and Kl-TSS, located in the central portion of the 3'UTR, and the 3'end proximal T-shaped structure (TSS); and (ii) the centrally located BTE and 3' proximal TSS for opium poppy mosaic virus (OPMV). For PEMV2, translation of the gRNA requires only two of the 3'CITEs whereas the subgenomic RNA uses all three. Recently, we found additional, critical translation elements 7 to 11 nt upstream from all umbravirus centrally located 3'CITEs. These "CITE-associated structures" or CAS have a similar hairpin with conserved residues at the base and apical loop, and some have two hairpins just upstream that connect to each other by kissing-loop interactions. We made multiple mutations in the CAS and 3'CITEs to determine their relationship and analyzed the effects of the mutations on virus accumulation *in vivo*, translation *in vitro*, and SHAPE (Selective 2' Hydroxyl Acylation analyzed by Primer Extension) structure probing. We showed that: (i) CAS mutations have differential effects on translation in *in vivo* assays using luciferase reporter constructs containing genomic RNA or subgenomic RNA 5' sequences, with some mutations enhancing translation and others negatively impacting translation; (ii) addition of CAS elements *in trans* reduced translation efficiency of full-length genomic RNA *in vitro*; (iii) all CAS mutations significantly reduced virus accumulation in infected cells; and (iv) all mutations in the OPMV CAS led to the formation and stabilization of the downstream 3'TSS 3'CITE, suggesting that the CAS suppresses the structure of the 3'TSS. Overall, our data indicate that for umbraviruses, a complex role exists between the CAS and very different 3'CITEs, and suggest that CAS elements may also be associated with 3'CITEs of other viruses.

Characterization of Zika virus replication in fluctuating environments

sciforum-058204

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Mosquitoes are the main vectors for numerous human pathogens including Dengue virus and Zika virus (ZIKV). ZIKV is an RNA virus that gained worldwide attention when it emerged in the Americas in 2015 and associated with birth defects. Although significant research efforts have been devoted to understanding ZIKV infection, we still lack an effective vaccine and antiviral treatments. Currently, the only mitigation strategy to stop outbreaks is to target the mosquito vector population. ZIKV replication and transmission in the mosquito are highly dependent on temperature. Mosquitoes are ectotherms, meaning their body temperature reflects their environment. Previous studies have characterized Zika transmission across constant temperature conditions to produce thermal suitability risk maps. In nature, Zika infected mosquitoes would not experience constant temperature conditions, but night-to-day temperature fluctuations. To explore the efficiency of ZIKV replication under fluctuating conditions, we monitored viral replication in mosquito C6/36 cells in incubators capable of recapitulating day/night cycles. Incubators fluctuated a total of 10°C over the course of the day. While constant incubation at cool temperatures (20°C) prevents the production of high ZIKV titers, incubation in a fluctuating environment that only reaches temperatures >20°C for part of the day enables ZIKV to replicate to high titers. We serially passaged ZIKV under constant or fluctuation conditions and examined the genetic and phenotypic changes. Virus adapted to replicate in both cool constant and fluctuating conditions replicated faster and to higher titers than Parental virus at 20°C. However, adapted viruses replicated similarly to Parental virus at 28°C. Sequence analysis of the adapted ZIKV is underway. Further study of ZIKV's ability to replicate and transmit in natural temperature environments will enable us to update the maps of temperature suitability and enable us to explore the genetic signatures associated an expanded temperature range.

Generation of a reverse genetic system and recombinant virus to study Lloviu virus, a bat-borne European filovirus

sciforum-058331

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The genomes of many previously unknown RNA viruses closely related to known human pathogens have been discovered in animal reservoir hosts in recent years via next generation sequencing. Despite the possibility of zoonosis and potential pathogenesis in humans, most of these viruses remain unstudied or understudied in part since they have not yet been cultured. The creation of recombinant versions of these viruses via reverse genetics would overcome this obstacle but is unfortunately hindered by missing viral genome ends in most cases. Lloviu virus (LLOV), an uncultured filovirus that is closely related to the highly pathogenic Ebola virus, is a perfect example of such a virus. We used minigenome systems to complement the missing LLOV genomic ends and identified cis-acting elements required for LLOV replication that were lacking in the published sequence. Leveraging these data, we generated recombinant full-length LLOV clones and rescued infectious virus. Recombinant LLOV (rLLOV) forms filamentous virions, typical of filoviruses, and induces the formation of characteristic inclusions in the cytoplasm of the infected cells, as observed by electron microscopy. Primary human target cells of Ebola virus, including macrophages and hepatocytes, are permissive to rLLOV infection, suggesting that humans could potentially serve as hosts. However, hallmark inflammatory responses in human macrophages observed upon Ebola virus infection are not induced by rLLOV. Additional tropism studies identified pneumocytes as supportive of robust rLLOV and Ebola virus infection. We also utilized rLLOV for antiviral testing, targeting multiple facets of the replication cycle. The wealth of information already obtained using rLLOV as well as its future potential highlights the usefulness of generating recombinant versions of uncultured viruses of pathogenic concern as we seek to build our arsenal for pandemic preparedness.

Integrative structural approaches reveal dynamic interactions of NS2B-NS3 protease from DENV4 during the post cleavage stage

sciforum-058839

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Flavivirus such as dengue virus (DENV), West Nile Virus (WNV), are a serious threat to public health. The flavivirus single stranded RNA genome is translated into a polyprotein cleaved by viral and cellular proteases into different structural proteins and non-structural proteins. Non-structural (NS) protein 3 is a multifunctional protein with N-terminal protease and C-terminal helicase. The NS3 protease responsible for cleavage of viral polyprotein requires co-factor NS2B for enzymatic activity and folding. Due to its essential role in replication, NS2B-NS3 protease is an attractive antiviral target. Despite availability of crystal structures, dynamic interactions of the N- and C-termini of NS2B co-factor have been elusive due to their flexible fold. Although structurally similar, NS2B-NS3 protease activity is affected differently by the presence of NS2B/3 cleavage at NS2B C-terminus in DENV and ZIKV. In this study, we employ integrative structural approaches, a combination of X-ray crystallography and crosslinking mass spectrometry along with biochemical assays to elucidate the interactions of flexible NS2B/3 N- and C-termini during the post-cleaved stage. We identified a novel second cis-cleavage site between NS2B/NS3. We report a novel crystal structure of DENV4 NS2B-NS3 protease. In contrast to ZIKV, the C-terminus of DENV NS2B exits the active site and occupies the active site of the neighboring NS2B-NS3 molecule with NS2B cofactor folding in a semi-closed conformation. The presence of second cleavage between NS2B/NS3 with semi-closed NS2B co-factor explain why the NS2B-NS3 cleavage site at NS2B C-terminus do not inhibit the protease activity for DENV as it is the case for ZIKV NS2B-NS3. Mapping of NS2B N- and C-termini with NS3 indicates the inter-domain interactions occur mainly on the solvent exposed beta-barrel of NS3 protease. Our integrative approach enables a comprehensive understanding of the folding and dynamic interactions of DENV NS3 protease and its cofactor NS2B at the post cleavage state.

Structural description of the Nipah virus phosphoprotein and of its interactions with viral and cellular partners

sciforum-057367

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Nipah virus (NiV) is one of the most pathogenic and deadly viruses infecting humans. We have combined several biophysical methods, including X-ray crystallography, NMR spectroscopy, SEC-MALLS, and small-angle X-ray scattering, to characterize the structure of the entire phosphoprotein and its interactions with the nucleocapsid and with a cellular partner (Jensen 2020; Bourhis 2022). (1) We showed that the long intrinsically disordered N-terminal region contains short regions of residual secondary structure that represent known or putative binding sites for partners. (2) We mapped a region, common to both V and W proteins that binds with low affinity to STAT1 and confirmed the involvement of key P amino acids in this interaction. (3) We have shown that NiV P is tetrameric. (4) We solved the crystallographic structures of the tetramerization domain (4gjw.pdb) and of the C-terminal X-domain (P_{XD}) (7pno.pdb) alone and in complex with the intrinsically disordered C-terminal tail of the N protein (N_{TAIL}) (7pon.pdb). In the crystal, isolated NiV P_{XD} adopts a dimeric conformation incompetent to bind its partner, but in complex with N_{TAIL}, it folds into a functional 3-helix conformation. (5) We have shown that in solution, P_{XD} exchanges between several conformations: a three-helix bundle form and partially unfolded monomeric and dimeric conformations. We propose a model where the thermodynamic stability of P_{XD} controls the attachment of P to the nucleocapsid. (6) By combining the data, we obtained an atomistic representation of the complete protein as an ensemble of conformations. The protein appears as a nanometer-size octopus comprising a central tetrameric core and displaying binding sites on its flexible arms to recruit multiple viral and cellular partners and providing new insights into how this protein fulfills its multiple functions.
Jensen et al. 2020 Biophys. J. 118, 2470-2488.
Bourhis et al. 2022 Submitted

The intrinsically disordered SARS-CoV-2 nucleoprotein and its dynamic complex with the viral protein nsp3

sciforum-058050

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The processes of genome replication and transcription of SARS-Cov-2 represent important targets for viral inhibition. Beta-coronaviral nucleoprotein (N) is a highly dynamic cofactor of the replication-transcription complex (RTC), whose function depends on an essential interaction with the N-terminal Ubiquitin-like domain of nsp3 (Ubl1). Here we describe the intrinsic conformational behaviour of N alone and in complex with nsp3 (affinity 30-200nM) at atomic resolution using nuclear magnetic resonance spectroscopy. The interaction implicates two linear motifs in the intrinsically disordered linker domain (N3), a hydrophobic helix (^{219}LALLLLDRLNQL^{230}) and a disordered polar strand (^{243}GQTVTKKSAAEAS^{255}), that mutually engage to form a bipartite interaction, folding N3 around Ubl1. This results in substantial collapse in the dimensions of dimeric N, forming a highly compact molecular chaperone, that regulates binding to RNA, suggesting a key role of nsp3 in the association of N to the replication-transcription complex. This interaction may contribute to localization of N to the exit pores of double membrane vesicles where viral RNA is replicated. The identification of distinct linear motifs that mediate an important interaction between essential viral factors provides future targets for development of novel strategies against Covid-19.
Bessa et al., Sci. Adv.8, eabm4034 (2022) https://www.science.org/doi/full/10.1126/sciadv.abm4034

Abstracts

S5. Virus-Host Interactions

Invited Speaker

Host and vector manipulation by plant viruses to promote transmission

sciforum-060391

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The majority of plant viruses that represent a major threat in agriculture causing significant yield losses worldwide rely on insect vectors. During infection viruses modify the physiology of their host plant in ways that can influence the interactions with vectors. For example, virus infection can induce leaf yellowing, promote emission of volatile compounds and modify phloem sap composition that can have an indirect effect on vector behavior. These modifications can affect vector preference for infected plants and change their feeding behavior and/or performance which can as a consequence, alter viral acquisition and propagation.

Many examples of plant and vector manipulations have been described in the last few years but the molecular mechanisms underlying these effects are largely unknown. To investigate these tripartite interactions we used a pathosystem composed of the model plant *Arabidopsis thaliana* and *turnip yellows virus* (TuYV), a polerovirus which is restricted to vascular tissues and transmitted by the aphid *Myzus persicae* in a circulative non replicative mode.

Global transcriptomic and metabolic approaches were implemented to explore the changes that poleroviruses produce on their host plant over time in presence or absence of aphids. We observed important modifications in gene expression and metabolomic profiles in plants that were both aphid-infested and TuYV-infected compared to plants only infested with aphids. In particular 58% of the plant genes deregulated by aphids alone were relieved in viral infection conditions suggesting that TuYV has a significant influence on the transcription status of genes normally deregulated by aphid infestation. Among them transcription factors and other signaling and stress response related genes were predominant. These results showing that TuYV induces significant changes in favor of transmission by aphids likely will be discussed in the light of specific aphid transmission experiments.

Invited Speaker

Controlling the message: CoV-2 nsp1 targets the ribosome to restrict gene expression at multiple levels

sciforum-060484

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Non-structural protein 1 (nsp1) has been identified as a pathogenicity factor in betacoronaviruses due to its ability to dampen host innate immune responses. CoV-2 nsp1 inserts its C-terminal domain into the mRNA entry tunnel of the 40S ribosome, thereby blocking cellular mRNA translation and leading to subsequent mRNA decay, yet a 5′ leader sequence present on viral transcripts confers escape from this repression. It remains unclear how these activities are coordinated and the extent to which other regions of nsp1 are involved host shutoff. We addressed these questions by performing a structure-function analysis of CoV-2 nsp1, demonstrating that regions outside of the defined 40S interaction domain contribute to interactions with the 40S ribosome and other translation factors as well as enable viral transcript escape from repression. Furthermore, we show that nsp1-induced mRNA degradation activates a novel cellular pathway that represses nascent cellular mRNA synthesis by RNA polymerase II. Collectively, our results suggest that multiple nsp1 domains coordinate access to the ribosome and that this mechanism of translational inhibition broadly impacts both upstream and downstream stages of mRNA biogenesis and fate.

Examining the functional relevance of APOBEC3D and APOBEC3F hetero-oligomerization to HIV-1 restriction

sciforum-057507

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APOBEC3 (A3) enzymes are one of the intrinsic viral restriction factors, most well studied in HIV-1 infection. Out of the seven members of this enzyme family, A3C, A3D, A3G, A3F, and A3H have retroviral restriction ability, although they are targeted for degradation in infected cells by HIV-1 Vif protein. APOBEC3 enzymes have cytosine deaminase activity that converts cytosine to uracil in single-stranded DNA. Most APOBEC3 enzymes form homo-oligomers and A3F and A3G have been found to also form hetero-oligomers. Hetero-oligomerization of A3F and A3G has given higher retroviral restriction ability and imparted a greater resistance of A3F to Vif mediated degradation. In this work, we show that A3D can also form hetero-oligomers with A3F. We have characterized the restriction of HIV-1 by A3D and A3F alone and in combination and found that there is no increase in capacity to restrict HIV-1 or resist Vif-mediated degradation by hetero-oligomerization. It instead appears that A3D can antagonize A3F restriction of HIV-1 replication. Previous data has shown low levels of mRNA of A3D in primary CD4+ cells. Our data suggest that this has evolved to avoid A3D-mediated antagonism of other A3 enzymes. Altogether our data suggest a unique function of A3F and A3G hetero-oligomerization and provide new observations about co-expression of A3D with other A3 enzymes.

Binding kinetics of human noroviruses to histo-blood group antigens predict susceptibility of human intestinal enteroids to infection

sciforum-058306

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Human norovirus (hNoV) infections are one of the leading causes of gastroenteritis worldwide. However, the study of hNoVs has long been hampered due to lack of adequate *in vitro* culture systems, and most knowledge limited to binding studies revealing that histo-blood group antigens (HBGAs)-carrying glycosphingolipids on intestinal cells, are involved in the infectious cycle. Recently, the development of a hNoV culture system based on human intestinal enteroids (HIEs) produced from patients' intestinal biopsies, has facilitated the study of norovirus infections *ex vivo*. This new HIE culture system allows us to test how differences in HBGA expression correlate to the infectious behavior.

We isolated and characterized lipid extracts by mass spectrometry from seven different HIE cultures, from patients with varying HBGA expression and thus varying susceptibility to hNoV infection. We then used these extracts to study the binding kinetics of norovirus virus-like particles to membranes made from these lipids using total internal reflection fluorescence microscopy. This allows us to study the dynamics of virus-membrane interactions on a single particle scale and to gain knowledge on the attachment and detachment behavior of the virus. (1)

We see that the virus has higher affinity for membranes of susceptible cells, but even membranes from non-susceptible cells show intermediate binding. This behavior correlates nicely with histo-blood group antigen expression. We also observe that disassociation behavior correlates better with susceptibility to infection than association. (2)

This project demonstrates the importance of host-membrane interactions in the norovirus infectious process and the potential of HIEs in studying hNoVs.

(1) Bally et al. (2011) Interaction of Single Viruslike Particles with Vesicles Containing Glycosphingolipids. *Phys. Rev. Lett.* **107**, 188103.
(2) Rimkute, Thorsteinsson et al. (2020) Histo-blood group antigens of glycosphingolipids predict susceptibility of human intestinal enteroids to norovirus infection. *J. Biol. Chem.* **295**, 15974–15987.

Investigating the effects of apolipoprotein E on Herpes simplex virus infection

sciforum-057413

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For decades human polymorphic apolipoprotein (apoE, isoform 4) or human herpes simplex virus type 1 (HSV1) has been suggested as an important risk factor for the development of Alzheimer disease (AD), a serious and increasing issue among elder populations worldwide. Recent epidemiological studies have revealed that interactions between apoE4 and HSV1 associates with higher risk of AD. However, mechanistic studies of apoE-HSV1 interactions at molecular levels are scarce. Here, we investigate the effects of apoE on the HSV1 infectious life cycle in *in vitro* cell experiments. Analysis of HSV1 growth curves show that HSV1 production is promoted in presence of any of the 3 apoE isoforms, while demonstrating varied isoform-dependent pro-viral effects. Such a pro-viral role of apoE4 is abolished when the lipid binding and self-association regions are removed. Following the life cycle of virus infection, HSV1 binding and entry were quantified and the results show that apoE inhibits viral attachment to cell membrane but does not affect virus entry kinetics/efficiencies. Further studies by qPCR-based quantification reveal that harbouring apoE2, 3, or 4 leads to an increase of HSV1 extracellular release but unchanged levels of either intracellular or cell plasma associated viral genome copies, strongly indicating that that apoE promotes virus release from the plasma membrane without affecting virus replication or intracellular trafficking. Our data reveal two opposing effects of apoEs on HSV1 infection both targeting interactions at the plasma membrane where the apoE's role in facilitating virus release dominates over the protein's ability to inhibit HSV1 binding, resulting in an overall pro-viral effect of apoE.

Visualization Of Single Viral Rna Translation Of The Murine Leukemia Virus By Suntag Tagging

sciforum-057476

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The genomic RNA (gRNA) of the murine leukemia virus (MLV) encodes structural (Gag), enzymatic (Pol), and envelope (Env) proteins. The gRNA also serves as the genome that is bound by Gag to form new viral particles. The translation of the gRNA is a crucial step that is poorly understood at the level of single-molecule.
We developed a system to image and quantify the translation of single MLV gRNAs in real-time combining the SunTag labeling for visualizing nascent polypeptides and MS2 labeling to visualize single RNA molecules. Colocalization of SunTag and MS2 signals will provide information about where, when, and how fast the translation is taking place in the cytoplasm as well as the number of ribosomes per RNA. We introduced 24-SunTags inside the ORF of gag in a plasmid that contains the whole genome of MLV and 24-MS2 hairpins in pol gene. Murine cells stably expressing a GFP antibody against SunTag and a Red MS2-Coat-Protein were transfected with MLV-SunTag-MS2 and 24- or 48-hours individual cells were imaged in a fluorescence microscope.
The results show that only ~20% of cytoplasmic MLV gRNA colocalizes with SunTag signals, meaning that a small fraction of MLV gRNA is undergoing translation. This level of colocalization decreases to 7 % when cells are treated with the translation inhibitor puromycin. This result reveals the two functions of gRNA: puro-sensitive translation (13%) and puro-insensitive assembly (7%). To discriminate these two functions, cells were co-transfected with two MLV plasmids, each carrying MS2 or ST tags. In this context, colocalization will correspond only to Gag-RNA complexes leading to the assembly of new viruses. Further analysis will be performed with Gag mutants defective for assembly. We hypothesize that the localization of the gRNA could influence its function, so we are currently looking for a specific subcellular compartment of the gRNA translation.

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

sciforum-058334

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Glioblastoma multiforme (GBM) remains a major type of cancer with no current effective therapy. On the other hand, multiple studies support that the efficacy of cancer therapies must be primarily shown against the cancer stem cells driving tumourigenesis. We have therefore attempted to develop a GBM therapy using two strains of the Minute Virus of Mice (MVMp and MVMi), a non-pathogenic parvovirus with evidenced oncolytic capacity and well characterized tropism determinants. To this aim, primary glioblastoma stem cells (GSCs) isolated from GBM patients were challenged with the MVM strains and the system was comprehensively studied in neurospheres. GSC neurospheres accumulated assembled capsids, but restrict viral NS1 cytotoxic protein expression by an innate PKR/eIF2α-P response. Inter- and intra-patient GSC heterogeneity was dissected by their diverse innate responses and the ratio between structural and non-structural viral gene expression. Further, viral infection triggered a comprehensive DNA-damage response involving cell cycle arrest, neurosphere disorganization, and bystander disruption of GSC-derived brain tumour architecture in rodent models. Notably, the MVM infection preferably targeted those GSC subpopulations within patients showing weak innate responses, and harbouring p53 gain-of-function mutants and/or Pp53Ser15 phosphorylation. This study provides a molecular foundation for personalized biosafe viral therapies against devastating glioblastoma and other cancers with weakened innate responses and /or deregulated p53 signalling.

A viral protein impairs stress granule formation by modulating Nup358

sciforum-057203

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Stress granules (SG) are ribonucleoprotein complexes that assemble during translation inhibition following cellular stress. Inhibition of SG assembly has been observed under virus infection across species, suggesting a conserved fundamental viral strategy. However, the significance of SG modulation during virus infection is not fully understood. The 1A protein encoded by the model dicistrovirus, cricket paralysis virus (CrPV) can inhibit SG formation and bind to and degrade Ago-2 in an E3 ubiquitin ligase-dependent manner to block the antiviral RNA interference pathway. A specific R146 residue is important for SG inhibition and virus infection in both Drosophila S2 cells and adult flies. Here, we uncouple CrPV-1A's functions and provide insights in its underlying mechanism for stress granule inhibition. In this study, we show that expression of wild-type but not mutant R146A CrPV-1A in S2 and HeLa cells inhibits SG formation when challenged with potent stress granule inducers arsenite or DTT, indicating that 1A-mediated SG inhibition is stress-independent. CrPV-1A's ability to inhibit SG formation does not require the Ago-2 binding domain but does require the E3 ubiquitin-ligase binding domain. Overexpression and infection studies showed that wild-type CrPV-1A but not mutant R146A CrPV-1A localizes to the nuclear membrane which correlates with nuclear enrichment of poly(A)+RNA. Transcriptome analysis demonstrated that R146A mutation dramatically dampens host transcriptomic landscape in CrPV-infected cells. Finally, inhibition of stress granule formation by CrPV-1A requires NUP358 in an R146 dependent manner. We propose that CrPV utilizes a multiprong strategy for productive virus infection whereby the CrPV-1A protein interferes with a nuclear event that contributes to the inhibition of SG assembly.

A viral ubiquitination switch attenuates innate immunity and triggers nuclear import of virion DNA and infection

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Antiviral defense and virus exclusion from the cell nucleus restrict foreign nucleic acid influx and infection. How the genomes of DNA viruses evade cytosolic pattern recognition and cross the nuclear envelope is incompletely understood. Here, we show that the virion protein V of adenovirus functions as a linchpin between the genome and the capsid, thereby securing the particle integrity. Absence of protein V destabilizes cytoplasmic particles and promotes premature genome release, raising cytokine levels through the DNA sensor cGAS. Non-ubiquitinable V yields stable virions, genome misdelivery to the cytoplasm, and increased cytokine levels. In contrast, normal protein V is ubiquitinated at the nuclear pore complex, dissociates from the virion depending on the E3 ubiquitin ligase Mib1 and the proteasome, and allows genome delivery into the nucleus for infection. Our data uncover previously unknown cellular and viral mechanisms of viral DNA nuclear import in pathogenesis, vaccination, gene therapy, and synthetic biology.

Alternative splicing liberates a cryptic cytoplasmic isoform of mitochondrial MECR that antagonizes influenza virus

sciforum-057486

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Viruses must balance their reliance on host cell machinery for replication while avoiding host defense. They often exploit conserved essential host genes whose critical role for the cell limits mutational escape. Conversely, host antiviral genes are often nonessential and can undergo mutation or regulated expression to thwart infection and limit self-damage. Influenza A viruses are zoonotic agents that frequently switch hosts, causing localized outbreaks with the potential for larger pandemics. The host range of influenza virus is limited by the need for successful interactions between the virus and cellular partners. We used immuno-competitive capture-mass spectrometry to identify cellular proteins that interact with human- and avian-adapted viral polymerases. Here, we dissect the antiviral activity executed by the gene encoding mitochondrial enoyl CoA-reductase (MECR). MECR protein is localized to mitochondria where it functions in mitochondrial fatty acid synthesis (mtFAS). While a small fraction of the polymerase subunit PB2 localizes to the mitochondria, PB2 did not interact with full-length MECR. By contrast, RNA-seq revealed a minor splice variant that creates cytoplasmic MECR (cMECR), which interacts with PB2. By binding the viral polymerase, cMECR suppresses viral replication by blocking assembly of viral ribonucleoprotein complexes (RNPs). MECR ablation through genome editing or drug treatment is detrimental for cell health, creating a generic block to virus replication. Using the yeast homolog Etr1 to supply the metabolic functions of MECR, we showed that specific antiviral activity is independent of mtFAS and lies solely within cMECR. Thus, a cryptic antiviral activity is embedded within a key metabolic enzyme, possibly protecting it from viral countermeasures.

Association between Epstein-Barr virus infection activity status and autoantibodies profile in patients with Systemic Lupus Erythematosus

sciforum-057959

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Systemic lupus erythematosus (SLE) is an autoimmune disease. Among many known and still elusive underlying triggers, Epstein-Barr virus (EBV) causes lifelong infection which is involved in development of SLE. Viral dual nature of switching between latent and active expression in B cells fits nicely within periods of flares and remission in SLE patients. The aim was to investigate the association between EBV infection status and autoantibodies during flares in SLE patients. A total of 48 patients with active SLE were enrolled in this study. Anti-EBV-CA (capsid antigen) IgM and IgG, anti-EBV-EA (early antigen) IgM and IgG and anti-EBNA1 (nuclear antigen) IgG were detected by ELISA, while EBV-DNA was detected by nested-PCR. Values of autoantibodies were taken from the hospital database. The association was evaluated using Spearman`s rank correlation coefficient in IBM SPSS ver. 21. EBV DNA replication was detected in 16.6% and EBV antibodies profile of active infection in 58.3% of SLE patients. Seropositivity for anti-EBNA1-IgG and anti-EBV-CA-IgG were 97.9% and 100%, respectively. EBV-CA-IgM, EBV-EA-IgG and EBV-EA-IgM seropositivity was 31.2%, 37.5% and 18.7%, respectively. Statistically significant positive moderate association between EBV-EA-IgG and anti-cardiolipin-IgG antibodies (p=0.003), negative moderate association between EBNA1-IgG and antiβ-IgG and antiβ-IgM (for both p0.001), and negative mild association between EBV-CA-IgG and antiβ-IgG and antiβ-IgM (p=0.041 and p=0.044, respectively) was detected in SLE patients with active EBV infection. In SLE patients with non-active EBV infection statisticaly significant positive association was detected only between EBV-CA-IgG and total amount of IgG autoantibodies (p=0.018).These findings indicate the importance of determining EBV antibodies profile in diagnosis of EBV reactivation in SLE patients due to its potential impact on further course of SLE. Also, demonstrated linkages between some autoantibodies and anti-EBV antibodies once again showed a role of EBV as a possible trigger in pathogenesis of SLE.

Cell Surface SARS-CoV-2 Nucleocapsid Protein Modulates Innate and Adaptive Host Immunity

sciforum-057494

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Despite the unprecedented global response to SARS-CoV-2, which has generated over 200,000 publications in 2 years, critical aspects of SARS-CoV-2 biology, pathogenesis and immunomodulation remain uncertain, including mechanisms underlying the cytokine storm and coagulation dyscrasias. Nucleocapsid (N) protein, the most abundant viral protein expressed during infection, induces strong antibody and T cell responses. N is considered to be strictly localized intracellularly. However, cell surface expression of viral nucleoproteins is the rule, not the exception, among RNA viruses including influenza A, vesicular stomatitis, measles and respiratory syncytial viruses. Cell surface viral nucleoproteins have been reported to induce immunosuppression, but also to serve as antibody targets. We find that N is expressed on the cell surface of live cells in high copy numbers. It binds to infected and non-infected neighboring cells by electrostatically associating with glycosaminoglycans. Using biolayer interferometry (BLI), we found that N binds specifically to heparan sulfate/heparin with high affinity, but not to other glycosaminoglycans on the cell surface. BLI screening between SARS-CoV-2 structural and accessory proteins and 64 human cytokines, revealed high-affinity interaction between N and a set of 11 human chemokines, including CXCL12b. Importantly, N inhibited CXCL12b-mediated leukocyte migration in chemotaxis assays, as did SARS-CoV-1 and MERS-CoV nucleoproteins. We also found that anti-N antibodies bound to the surface of infected cells can activate Fc receptor-expressing cells.
These data indicate that cell surface N may play an important role in host adaptive immunity to SARS-CoV-2 and in manipulating innate immunity at the early stages of infection.

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

sciforum-057465

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Viruses are obligatory intracellular parasites that intimately adapt to the host they infect. How mosquito-borne viruses such as chikungunya virus (CHIKV) expand so efficiently in humans and mosquitoes, two organisms one million years apart in evolution, and why they kill the human cells but chronically infect the mosquito ones remain unsolved fundamental questions. Previously, we showed that CHIKV alters the human tRNA epitranscriptome to adapt the host translational machinery to the viral RNA genome enriched in sub-optimal codons in such a way that they become optimal for the viral gene expression. Here, we address whether this mechanism is conserved in mosquito-infected cells. By using RNA-seq and ribosome profiling we obtained a high-resolution time course analyses of the transcriptome and translatome in CHIKV-infected mosquito cells. Interestingly, CHIKV-infection did not induce changes in codon optimality but favoured translation of host mRNAs mainly expressing aminoacyl tRNA synthetases and proteins related to odorant response. The aminoacyl tRNA synthetases are enzymes that attach the appropriate amino acid onto its corresponding tRNA. Thus, their overexpression would favour viral RNA translation without major alterations in the host expression programme, reaching the virus-host equilibrium required to establish chronic infection. The overexpression of odorant-related proteins strongly suggests that virus-induced changes in the host translatome might ultimately affect the mosquito behaviour to favour viral expansion.

Circadian control of hepatitis B virus replication

sciforum-055437

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Chronic hepatitis B virus (HBV) infection is a major cause of liver disease and cancer worldwide for which there are no curative therapies. The major challenge in curing infection is eradicating or silencing the covalent closed circular DNA (cccDNA) form of the viral genome. The circadian factors BMAL1/CLOCK and REV-ERB are master regulators of the liver transcriptome and yet their role in HBV replication is unknown. We establish a circadian cycling liver cell-model and demonstrate that REV-ERB directly regulates NTCP-dependent hepatitis B and delta virus particle entry. Importantly, we show that pharmacological activation of REV-ERB inhibits HBV infection in vitro and in human liver chimeric mice. We uncover a role for BMAL1 to bind HBV genomes and increase viral promoter activity. Pharmacological inhibition of BMAL1 through REV-ERB ligands reduces pre-genomic RNA and de novo particle secretion. The presence of conserved E-box motifs among members of the Hepadnaviridae family highlight an evolutionarily conserved role for BMAL1 in regulating this family of small DNA viruses.

Dynamics of LD in JUNV infected cells: just a product of lipophagy?

sciforum-058348

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Junín virus (JUNV) is one of the members of the *Arenaviridae* family that causes a hemorrhagic fever, the Argentine hemorrhagic fever. In previous studies, we have described that JUNV's replication cycle is closely linked to the lipid metabolism of the cells it infects.
Lipid droplets (LD) are a central hub for lipid storage, handling and trafficking that have been involved both in the replication cycles of viruses from diverse families and in the cell's immune response. As such, we set to study the biology of lipid droplets in the context of JUNV infection.
We have previously shown that the pharmacological modulation of LD availability leads to changes in viral yield, demonstrating that these organelles are important for the proper replication of JUNV. Conversely, we found that cells infected with JUNV contain lower amounts of LD than non-infected cells. LD can be subjected to both lipolysis and lipophagy, mechanisms we studied in order to determine if they were responsible for the observed phenotype. Lipophagy is a specific type of autophagy, which is known to be induced by JUNV. Through confocal microscopy we have found colocalization events between LD and lysosomes. This suggests that the infection with JUNV could be triggering LD degradation through lipophagy, but this might not be the only intervening factor.
We are currently also analyzing the expression of genes involved both in the synthesis and degradation of lipids at different time-points post infection. Preliminary results suggest that the LD dynamic is complex, with the expression of genes, such as DGAT 1 and 2 and PNPLA2, being up or downregulated in a transitory manner at short and long times post infection.

Engineering functional domains of the parvovirus MVM capsid with VEGF blocking peptides for anti-cancer purposes.

sciforum-058332

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We have attempted to direct the capsid of the oncolytic parvovirus Minute Virus of Mice (MVM) against the tumor vasculature by two strategies, (i) as platform to induce α-VEGF antibodies, and (ii) re-targeting the infection to cells expressing VEGF-R1. For this, peptides from three functional domains of MVM capsid were replaced by peptides that block the binding of VEGF to its receptors (VEbp). (i) In the first approach, although most substitutions caused alterations in the assembly or maturation of the virus, some virus chimeras with VEbp substituting residues at the 3-axis efficiently propagated in culture, exposed the heterologous peptides on the surface of the capsid, and induced native anti-VEGF antibodies in mice. (ii) In re-targeting studies, some virus chimeras bearing VEbp in the 2X-axis where the sialic acid receptor binding site localizes, showed greater infectivity than the wt towards human glioblastoma cells, although it did not infect through VEGF-R1. Instead, chimeras bound, as wt, to sialic acid residues, but their infection capacity was determined by the extent of a cell type dependent intracellular capsid structural transition leading to VP2 cleavage, which could be further enhanced by controlled removal of sialic acid from cell surface. Overall, this study provides valuable information to provide anti-neovascularizing immune capacity to oncolytic viruses, and to manipulate parvovirus tropism by altering intracellular capsid structural transitions triggered by sialic acid binding.

Epistasis at the SARS-CoV-2 RBD Interface and the Propitiously Boring Implications for Vaccine Escape

sciforum-058254

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At the time of this writing, December 2021, potential emergence of vaccine escape variants of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a grave global concern. The interface between the receptor-binding domain (RBD) of SARS-CoV-2 spike (S) protein and the host receptor (ACE2) overlap with the binding site of principal neutralizing antibodies (NAb), limiting the repertoire of viable mutations. Nonetheless, variants with multiple mutations in the RBD have rose to dominance. Non-additive, epistatic relationships among RBD mutations are apparent, and assessing the impact of such epistasis on the mutational landscape is crucial. Epistasis can substantially increase the risk of vaccine escape and cannot be completely characterized through the study of the wild type (WT) alone. We employed protein structure modeling using Rosetta to compare the effects of all single mutants at the RBD-NAb and RBD-ACE2 interfaces for the WT, Delta, Gamma, and Omicron variants. Overall, epistasis at the RBD interface appears to be limited and the effects of most multiple mutations are additive. Epistasis at the Delta variant interface weakly stabilizes NAb interaction relative to ACE2 interaction, whereas in the Gamma variant, epistasis more substantially destabilizes NAb interaction. Although a small, systematic trend towards NAb destabilization not observed for Delta or Gamma was detected for Omicron, and despite bearing significantly more RBD mutations, the epistatic landscape of the Omicron variant closely resembles that of Gamma. These results suggest that, although Omicron poses new risks not observed with Delta, structural constraints on the RBD hamper continued evolution towards more complete vaccine escape. The modest ensemble of mutations relative to the WT that are currently known to reduce vaccine efficacy is likely to comprise the majority of all possible escape mutations for future variants, predicting continued efficacy of the existing vaccines.

Evidence that proteolytic cleavage of plant proteins by potyvirus NIa proteases occurs at various subcellular localizations in infected cells

sciforum-057482

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Potyviruses constitute a large and economically important group of plant positive-strand RNA viruses. The potyvirus NIa protease (NIa-Pro) is a chymotrypsin-like cysteine protease closely related to the picornavirus 3C protease. We have shown that potyvirus NIa-Pros can cleave host proteins *in vitro* and in infected cells (Xiao et al, 2022, J Virol, JVI0144421). To examine cleavage in infected cells, we transiently expressed epitope-tagged versions of candidate plant proteins and simultaneously launched virus infection from a turnip mosaic virus (TuMV) infectious clone. We previously confirmed efficient cleavage of AtEML2 (At5g06780), an Emsy-like protein belonging to a family of histone readers known to be involved in pathogen resistance. We now report partial cleavage of AtUFD1 (At4g15420), a ubiquitin fusion degradation-like protein probably involved in the endoplasmic reticulum (ER)-associated protein degradation (ERAD) pathway. AtUFD1 also includes a TRAF-like domain, a domain family which has been associated with the regulation of plant immune responses. Cleavage of AtEML2 and AtUFD1 was also observed in plant cells that co-expressed the plum pox virus (PPV) or TuMV NIa-Pros. Using a differential centrifugation fractionation method, we investigated the subcellular localization of the uncleaved proteins and of the cleaved fragments. Uncleaved AtEML2 protein was found predominantly in nuclear-enriched fractions while the N- and C-terminal cleaved fragments accumulated mostly in cytoplasmic-enriched fractions. The cleavage site is located within a weakly predicted bipartite nuclear localization signal (NLS), suggesting that processing of AtEML2 by NIa-Pro disrupted this NLS. Full-length AtUFD1 and the cleaved AtUFD1 fragments were detected predominantly in cytoplasmic-enriched fractions in spite of a strongly predicted NLS in the N-terminal region of the protein. These results suggest that NIa-Pro is able to cleave plant proteins at diverse subcellular localization in infected cells, consistent with the known association of mature or precursor NIa-Pro with the nucleus, cytoplasm and ER membranes.

Grapevine fanleaf virus (GFLV) protein 1A interacts with *Nicotiana benthamiana* isoforms ATG8a, ATG8c and ATG8i of ATG8, an autophagy related protein

sciforum-057631

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Grapevine fanleaf virus (GFLV) causes fanleaf disease in grapevine and is one of the most detrimental viruses to vineyards worldwide. It belongs to the genus *Nepovirus*, family *Secoviridae*, order *Picornavirales*.
GFLV protein 1A of unknown function was used as a bait in a yeast two hybrid assay. Isoforms ATG8a, ATG8c and ATG8i of the autophagy-related protein ATG8 were found among the most represented candidates. Autophagy is a catabolic pathway conserved among eukaryotes that ensures degradation and recycling of intracellular material. It is also involved in antiviral defense and some viruses have developed ways to escape or hijack it.
We study the role of the interaction between GFLV protein 1A and *Nicotiana benthamiana* ATG8 isoforms ATG8a, ATG8c and ATG8i in the life cycle of the virus. First, we randomly generated 1A mutants and selected nine clones for their loss of interaction with ATG8a in yeast two hybrid. A second test confirmed their loss of interaction also with ATG8c and ATG8i. In the viral context, five of those 1A mutants were impaired in systemic infection of *N. benthamiana* whereas the remaining mutants were comparable to the WT virus. Interestingly, two defective mutants harbored substitution mutations in predicted ATG8-interacting motifs. In parallel, we down-regulated *nbatg6*, another autophagy-related gene, or *nbatg8* isoforms in *N. benthamiana* plants prior to virus inoculation to better understand the role of ATG8 and/or autophagy in GFLV multiplication. Results will be discussed considering pro- and antiviral effects of ATG8 on other viruses such as turnip mosaic virus, a *Potyvirus*, or mouse hepatitis virus, a *Coronavirus*.

Human adenovirus type 5 infection alters mitochondrial DNA and membrane dynamics

sciforum-058291

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Mitochondria are double-membrane organelles that orchestrate essential cellular functions including ATP synthesis and apoptosis. Furthermore, mitochondria are effective stimulators of the innate immune system through the release of mitochondrial components into the cytosol. One such stimulus is mitochondrial DNA (mtDNA) which is recognized by receptors of the innate immune system, such as cGAS, due to its resemblance to bacterial DNA. Several viruses, such as influenza virus and dengue virus, have been shown to induce the release of mtDNA. In this study we utilize biochemical and microscopical methods to study how human adenovirus type 5 (HAdV-C5) infection alters mitochondrial DNA dynamics. We show that HAdV-C5 induces the release of mtDNA into the cytosol of infected cells and that this release correlates with the expression of the viral structural protein pVI during the late phase of infection. Notably, ectopic expression of the pVI protein is sufficient to release mtDNA into the cytosol. Further, the pVI protein harbors an amphipathic helix which targets the protein into the mitochondria and alters the mitochondrial membrane integrity. Elimination of the mitochondrial Bax and Bak proteins reduces the pVI-mediated mtDNA release. In addition, accumulation of the pVI protein in mitochondria leads to the hyperpolarization of the inner mitochondrial membrane. Collectively, our study reveals a novel interplay between the HAdV-C5 infection and mitochondrial homeostasis.

Identifying host ZDHHC palmitoyl acyltransferases responsible for the palmitoylation of hepatitis E virus (HEV) ORF3 protein.

sciforum-058215

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Hepatitis E Virus (HEV) is responsible for a large percentage of acute hepatitis worldwide, causing an estimated 40,000 - 70,000 annual deaths. Despite HEV's impact, no HEV-specific therapies exist. Therefore, there's key interest in HEV's essential proteins, as well as their interactions with host proteins, in order to elucidate potential therapeutic targets. One such therapeutic candidate is HEV's ORF3, a protein necessary for HEV's apical release and shedding of virus into the feces. Essential for ORF3 functioning, is a post-translational modification called palmitoylation, which is understood to localize ORF3 to cellular membranes for viral budding. The specific ZDHHC palmitoyl acyltransferase(s) (PATs) responsible for this modification, however, are unknown. Identifying the host acyltransferase(s) could offer a tempting drug target to treat HEV infection. In order to identify ORF3-palmitoylating enzyme(s), separate DHHCs (murine) were over-expressed in ORF3-expressing HEK293T cells. Successive rounds of co-immunoprecipitation identified 7 PATs with relatively stronger interactions with ORF3, revealing high-interest PATs. Immunofluorescence assay (IFA) in HEK293T identified that DHHC 2 and DHHC 12 colocalized with ORF3. Further IFA in HEK293T cells revealed colocalization of DHHC2 with ORF3 and Rab11a on recycling endosomes - a key organelle believed to be hijacked by HEV prior to viral egress. These results suggest that ORF3 may be predominately palmitoylated by ZDHHC2 at recycling endosomes prior to apical viral release, though Co-IP results still suggest a degree of redundancy among multiple PATs when palmitoylating ORF3. Further testing of high-interest PATs' interactions with ORF3 cysteine-mutants could help explain redundancy, such as sequential palmitoylation of ORF3's eight N-terminal cysteines. Other ongoing experiments include IFA to assess DHHC/ORF3 colocalization in relevant hepatic cell lines and CRISPR KO of PATs to observe changes in ORF3 palmitoylation after PAT KO.

Influenza A virus induces the accumulation of insoluble protein aggregates to support efficient virus particle formation

sciforum-058317

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Influenza A virus (IAV) is the main causative agent of seasonal respiratory epidemics in humans. Its ability for genetic reassortments and rapid evolution hinder vaccine development and lead to occasional lethal pandemics. IAV has evolved several strategies to manipulate and control host cellular mechanisms in order to support efficient virus particle formation and viral propagation, among which, the exploitation of host factors involved in protein homeostasis.
In general, stress-induced disruption in protein homeostasis results in the accumulation of insoluble proteins and toxic aggregates, a transversal hallmark of several pathological conditions. The dynamic virus-host interplay can also elicit alterations in the host proteome and protein distribution within the cell to benefit viral replication.
In this study, we performed a detailed analysis of the variations in proteostasis-related mechanisms throughout the course of an IAV infection cycle. Our results demonstrate that IAV infection disturbs the host cell protein homeostasis by inducing the accumulation of detergent-insoluble proteins, mostly coinciding with the viral ribonucleoproteins' egress from the nucleus and viral protein translation. Our results also suggest that this accumulation is associated with a virus-induced deregulation of the host cell RNA metabolism. Disturbance of protein aggregation by the presence of a specific chemical compound strongly impacts the formation of proper infectious virus particles, highlighting the relevance of the observed insoluble protein aggregates for IAV propagation.
Our results reveal a novel mechanism by which IAV interferes with the host cell proteostasis-related mechanisms and may uncover specific targets that can be explored for the development of novel antiviral therapies.

Inhibition of the STING-mediated interferon response by novel oxidative posttranslational modification

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Accumulating evidence supports a role of reactive oxygen species (ROS) in the regulation of several pathways involved in virus-host interactions, particularly those leading to the induction of antiviral type I and III Interferons. Dysregulation of these pathways lead to autoimmune and inflammatory diseases. The redox regulation of virus-host interactions is well documented, but the underlying mechanisms of the redox regulation remain ill-defined. ROS are known to modify signaling protein structure and activity through reversible oxidative post-translational modifications of Cysteines (Cys ox-PTM). To unveil the Cys ox-PTMs that affect signaling proteins involved in the innate antiviral response, we performed a proteome wide identification of the Cys ox-PTMs induced by ROS in vivo using maleimide-based bioswitch labeling coupled to mass spectrometry. We identified 2720 unique Cys ox-PTM sites encompassing 1473 proteins with distinct abundance, location and functions. Enrichment of Cys-ox-PTMs was found in proteins involved in numerous signaling pathways, many relevant to virus-host interactions. Among these, we uncovered the oxidation of STING, the central adaptor of the innate immune type I interferon pathway induced upon detection of DNA viruses. We provide demonstration of reversible oxidation of Cys^{148} and Cys^{206} of STING in cells. Proteomic analyses led us to establish a new model in which Cys^{148} oxidation is constitutive, while Cys^{206} oxidation is inducible by oxidative stress or by the natural ligand 2'3'-cGAMP. Using in silico molecular modeling and mutational analyses, we found that STING oxidation on Cys^{206} plays an inhibitory role to prevent STING hyperactivation through the constraint of a conformational change associated with the formation of inactive polymers containing intermolecular disulfide bonds. This study establishes a direct mechanism by which ROS control the cGAS/STING-dependent innate immune response. It provides new ground for the design of therapies targeting STING relevant to viral infections, such as vaccination, but also to autoinflammatory disorders.

IRE1-dependent unfolded protein response is reprogrammed to support influenza A virus propagation

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Influenza A virus (IAV) is a zoonotic pathogen, and one of the main causes of annual respiratory epidemics and occasional pandemics. Thoughout its infection cycle, IAV takes advantage of a combination of cellular and viral mechanisms to ensure proper virion formation and propagation. Some of these strategies encompass the manipulation of cellular pathways involved in protein synthesis, folding, and degradation.

To cope with misfolded proteins and proteotoxicity, the unfolded protein response (UPR) comprises three complementing signaling pathways. Upon stimulation of at least one of the key UPR activators (IRE1, PERK, and ATF6), a programmed signaling cascade leads to altered rates of protein synthesis, translocation and quality control, preferential degradation of mRNA encoding for ER-localized proteins, and the controlled synthesis of stress-attenuating proteins.

Current evidence on IAV interaction with the host cells' UPR, as well as its effects on viral propagation, are still unclear. In this study we analyzed the activation of the three different branches of UPR signaling at different time points within a single cycle of infection. Our results demonstrate a deregulation of both PERK and IRE1 UPR branches upon infection, while the ATF6 branch remains unaffected. Importantly, while PERK inhibition did not affect the production of infectious IAV particles, virus production is attenuated in the presence of an IRE1 inhibitor. Our results indicate that IRE1 pathway activation is relevant for proper IAV particle formation and infection propagation, offering new insights into the interplay between IAV and the host cell protein homestasis and unraveling novel mechanisms that may be explored as targets for the development of novel antiviral strategies.

IRF7 Regulates HIV-1 Latency Reversal Independent Of Cytokine Signalling Blockade

sciforum-057423

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The mechanisms governing HIV persistence remain to be fully elucidated. Understanding these mechanisms is key for developing successful strategies for eliminating the viral reservoir, and ultimately curing HIV infection. Increasing interest has focused on targeting the immune response to HIV latent infection, including modulation of the JAK/STAT pathway. Here, we show that IRF7 drives HIV latency reversal by a subclass of Janus kinase inhibitors (JAKinibs).
Non-clonal GFP-expressing *in vitro* models of HIV-1 latency were used to evaluate the reactivation capacity of JAKinibs by flow cytometry (FC). Immunophenotyping of *ex vivo* treated CD4+T cells from uninfected donors and ART-suppressed HIV+ individuals were performed by multicolor FC and latency reactivation measured by qPCR amplification of supernatant HIV-1 RNA. RNAseq of *in vitro* JAKinib-treated cells was performed for whole transcriptome profiling. qPCR and western blot were used to validate gene and protein expression.
Selective-JAK2inibs (2/8), including fedratinib, reversed HIV-1 latency in all tested *in vitro* models (p0.001). Fedratinib *ex vivo* treatment of HIV+ CD4+T cells resulted also in significant HIV reactivation, without major changes in immune cell activation, but presenting an increased effector memory CD4+T populations. Whole transcriptomic profiling supported reactivation data, showing common gene expression signatures between LRA-treated cells (fedratinib and PMA) in contrast to other JAKinibs, albeit fedratinib presented significantly fewer affected genesets in the pathway analysis. Interestingly, we observed a significant induction of IRF7 expression upon fedratinib treatment (6-fold, p0.001), despite blockade of the JAK/STAT pathway and downregulation of proinflammatory cytokines. IRF7 expression correlated with reactivation capacity by JAK2inibs (rho=0.86, p=0.006). siRNA knockdown of IRF7, limited HIV reactivation (p=0.005), further demonstrating the role of IRF7 in HIV latency.
Our findings indicate that IRF7 could be an important modulator of HIV-1 latency and might play a crucial role in HIV-1 eradication strategies.

Late phases of SARS-COV-2 replication in pulmonary cells triggers reorganization of F-actin nanostructures required for infection

sciforum-057473

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The severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) is worldwide the main cause of the still ongoing COVID-19 pandemic. After SARS-COV-2 infection of human pulmonary cells, intracellular viral replication take place during spatio-temporal regulated processes in different cellular compartments resulting in the destruction of the host cells and causing severe respiratory diseases. Although mechanisms of trafficking in cellular compartments implicated in SARS-COV-2 infection have been explored, little is known about the role of the cytoskeleton during SARS-COV-2 replication in human pulmonary cells. Here we show that SARS-COV-2 infection induces dramatic changes of cytoskeletal F-actin nanostructures in human pulmonary cells, which are required for late phases of SARS-COV-2 replication. We found that SARS-COV-2 infection induces ring-like F-actin nanostructures surrounding viral intracellular organelles, suggesting a spatial and functional interplay between F-actin nanostructures and viral M cluster formation during assembly of SARS-COV-2 particles. Further, we found that SARS-COV-2 infection promote filopodia-like structures loaded with viruses to neighbour cells, suggesting these structures as mechanism for cell-to-cell SARS-COV-2 infection spread. Strikingly, gene expression profil analysis using RNAseq reveal a major role of alpha-actinins superfamily proteins in the late phases of SARS-COV-2 replication, and related inhibitor treatments of SARS-COV-2-infected pulmonary cells reduce viral replication. Thus our results demonstrate an important role of F-actin nanostructures in SARS-CoV-2 replication.

Lipid droplet biogenesis modulates the formation of the hepatitis C virus replication organelle

sciforum-058159

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A hallmark of *plus-strand RNA virus* infection is the reshuffling of host cell membranes to form viral replication organelles. In the case of hepatitis C virus (HCV) infection, this replication organelle is called "membranous web" and appears as accumulation of double membrane vesicles (DMVs) that are derived from the ER membrane.
In our lab, we found that excess of DGAT2, a key enzyme in the lipid droplet (LD) biosynthesis, strongly inhibits HCV infection. In this project, we aim to identify the viral and cellular determinants behind this inhibitory phenotype.
We found that DGAT2 specifically inhibits the replication step of the HCV viral life cycle, while it had minimal effect on the replication of other RNA viruses of the *Flaviviridae, Hepeviridae* and *Coronaviridae* families. Using correlated light electron microscopy, we could correlate this phenotype to a defect in DMV formation. Analysis of a series of DGAT2 mutants also indicates that reticular rather than LD association of DGAT2 is crucial to its antiviral activity. Since both DMVs and LDs are derived from the ER membrane, we hypothesize that the DGAT2-induced LD biosynthesis alters the ER lipid landscape and thereby impairs the HCV replication organelle formation. We used a combination of lipidomics, fluorescent lipid biosensor imaging and lipid supplementation assays to identify key lipid species involved in the DGAT2 sensitivity of HCV. Finally, we serially passaged HCV in DGAT2-overexpressing cells and are currently examining the adaptation phenotype in order to identify the viral determinants and mechanism of DGAT2 replication inhibition.
In conclusion, the tight regulation of HCV RNA replication by DGAT2 expression allowed us to better understand the lipid requirements for HCV to form a functional membranous web. It also highlights the singularity of the biogenesis of this replication organelle among the *Flaviviridae* and the other plus-strand RNA viruses.

Phosphoproteomics analysis of chikungunya virus-infected cells uncovers a novel host shut-off mechanism based on eEF2 phosphorylation induced by an increase in cAMP by the alphavirus nsP2 NTPase

sciforum-057337

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Chikungunya virus (CHIKV) is a reemerging alphavirus. Since 2005, it has infected millions of people during outbreaks in Africa, Asia, and South/Central America. CHIKV replication depends on host cell factors at many levels and is expected to have a profound effect on cell physiology. To obtain more insight into cellular responses to infection, stable isotope labeling with amino acids in cell culture and liquid chromatography-tandem mass spectrometry were used to assess temporal changes in the cellular phosphoproteome during CHIKV infection. Among the ~3,000 unique phosphorylation sites analyzed, the largest change in phosphorylation status was measured on residue T56 of eukaryotic elongation factor 2 (eEF2), which showed a >50-fold increase at 8 and 12 h p.i. Infection with other alphaviruses (Semliki Forest, Sindbis and Venezuelan equine encephalitis virus) and a picornavirus (CVB3) triggered a similarly strong eEF2 phosphorylation. Expression of a truncated form of CHIKV nsP2, containing only its N-terminal and NTPase/helicase domains (nsP2-NTD-Hel), sufficed to induce eEF2 phosphorylation, which could be prevented by mutating the Walker A and B motifs of the NTPase domain. Alphavirus infection or expression of nsP2-NTD-Hel caused a decrease in cellular ATP levels and an increase in cAMP levels. This did not occur when catalytically inactive NTPase mutants were expressed. The wild-type nsP2-NTD-Hel inhibited cellular translation independent of the C-terminal nsP2 domain, which was previously implicated in directing the virus-induced host shut-off. We hypothesize that the alphavirus NTPase activates a cellular adenylate cyclase that increases cAMP levels, thus activating eukaryotic elongation factor 2 kinase. This in turn triggers eEF2 phosphorylation and translational inhibition which contributes to the alphavirus-induced shut-off of cellular protein synthesis.

Rapid virus-driven reprogramming predisposes oral epithelial cells to lytic infections

sciforum-057299

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Kaposi's sarcoma-associated herpesvirus (KSHV) is an oncogenic virus that infects oral epithelial cells, where it can replicate whereby promoting viral transmission. Our global transcriptome analysis of KSHV-infected primary human gingival epithelial cells (HGEP) revealed that the host transcription factor FOXQ1 is one of the most induced human genes within hours of KSHV infection. While FOXQ1 is known to regulate cell proliferation and epithelial differentiation, its role in viral infection is unknown. Here, we found that siRNA-mediated depletion of FOXQ1 after KSHV infection abrogated both KSHV lytic gene expression and viral replication in telomerase-immortalized gingival epithelial cells (TIGK), revealing FOXQ1 as a lytic cycle promoting host factor. We detected bivalent chromatin with both activating and repressive histone modifications on FOXQ1 promoter, granting FOXQ1 to be poised for rapid induction in oral epithelial cells. By screening KSHV ORFs, we identified the tegument protein ORF45 and the viral transcription factor RTA to be sufficient for inducing FOXQ1 in HGEP, highlighting the role of incoming and rapidly expressed viral factors in triggering FOXQ1 induction. We found that ORF45 is nuclear at four hours of infection in TIGK and triggers the nuclear accumulation of the ERK substrate p90 ribosomal s6 kinase (RSK) and its phosphorylation. In contrast, a point mutant of ORF45, which lacks its known RSK activity-sustaining function, neither triggers RSK activity nor induced FOXQ1 expression. Importantly, while deficient lytic gene expression was observed in RTA knockout KSHV-infected TIGK, yet only ORF45/RTA double-knockout KSHV infection or pathway inhibition failed to induce FOXQ1 in infected cells, indicating that incoming tegument protein ORF45 is prerequisite for host reprogramming. Taken together, we described a novel positive feedback loop in which incoming and rapidly expressed KSHV immediate-early proteins drive rapid reprogramming of oral epithelial cells, which promotes productive viral life cycle in the oral epithelium.

Restricted Infection Of Macrophages By SARS-COV-2 Induces Pro-Inflammatory Responses

sciforum-057551

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been associated with immune hyperactivation and high levels of pro-inflammatory cytokines. Lung infiltration by CD169+ inflammatory monocytes and presence of activated CD169+ alveolar macrophages suggest that monocyte/macrophages are key drivers of severe disease. While most macrophages do not express ACE2, the entry receptor for SARS-CoV-2, a subset of CD169+ macrophages have been reported to express ACE2 and are viral antigen positive in post-mortem tissues.
To determine the mechanisms by which macrophages contribute to inflammation in SARS-CoV-2 infection, we infected monocyte-derived macrophages (MDMs) and PMA-differentiated THP-1 macrophages engineered to constitutively express CD169, ACE2, or CD169 and ACE2 with SARS-CoV-2 or lentiviruses pseudotyped with SARS-CoV-2 spike protein (S). Expression of ACE2 in THP-1/PMA and MDMs restored productive virus infection, which was not observed in naïve cells, suggesting that macrophages are permissive to SARS-CoV-2 replication when entry is facilitated in an ACE2 dependent manner. Interestingly, expression of both CD169 and ACE2 in macrophages led to enhanced kinetics and magnitude of infection, suggesting an entry-enhancing role of CD169. However, while SARS-CoV-2 S-mediated entry and fusion was observed in both CD169+ THP-I/PMA cells and MDMs in the absence of ACE2, these cells were not productively infected. SmRNA-FISH and qRT-PCR analysis revealed low amounts of various SARS-CoV-2 RNA species in infected CD169+ cells without viral particle production indicative of an abortive infection. Importantly, CD169-mediated SARS-CoV-2 infection of macrophages accompanied by restricted expression of viral RNAs led to induction of pro-inflammatory cytokines, which could be inhibited by blocking the viral polymerase using remdesivir. Furthermore, knockdown of cytosolic RNA sensors (RIG-I and MDA-5) or MAVS significantly attenuated inflammatory cytokine expression in CD169+ macrophages, confirming that restricted expression of cytosolic viral RNAs in macrophages is sensed by cellular nucleic acid sensors and triggers innate immune activation.

Role of the RNA helicase DDX3 on Zika virus replication

sciforum-058283

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Zika virus (ZIKV) is a mosquito-borne virus with pandemic potential considered a global threat to public health by the WHO. This pantropic virus infects different cell types from neurons to immune cells including microglia, monocytes, macrophages, and dendritic cells. During ZIKV infection, host cells activate the innate immune response and previous reports suggest that ZIKV uses host proteins to avoid/abrogate cellular defenses to favor its own replication. Indeed, different RNA viruses including HIV-1, dengue virus and Hepatitis C virus usurp immune response-related cellular factors, such as the RNA helicase DDX3, to promote viral replication.

In this work, we explored the involvement of DDX3 during ZIKV replication in a human microglia cell line. We observed that viral RNA and proteins reach a peak at 24 hours post-infection (hpi) decreasing at 48 hpi. On the other hand, activation of innate immune response in infected microglia followed a different kinetic in which the levels of the IFN-b and CXCL10 mRNAs reach a peak at 36 hpi.

Interestingly, our data show that DDX3 protein levels increase from 12 to 24 hpi and the pharmacological inhibition of DDX3 enzymatic activity results in a decrease of viral protein levels and viral titers.

Since DDX3 possess a role in the MAVS-dependent immune signaling, we are currently investigating the involvement of DDX3 in innate immune response triggered by ZIKV infection.

SARS-CoV-2 accessory protein ORF8 downregulates the polymeric Ig receptor

sciforum-057493

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SARS-CoV-2 is the causative agent of the COVID-19 pandemic, which has claimed over 5.5 million lives. The rapid mutation and evolution of SARS-CoV-2 has led to the emergence of multiple variants of concern, such as Delta and Omicron, which continue to strain and threaten global health. Furthering our understanding of SARS-CoV-2 viral pathogenesis is expected to inform the development of new therapeutics which are crucial to the effective management of COVID-19 spread and clinical severity. COVID-19 patients suffer from a range of mild to severe symptoms, where the latter is characterized by lung tissue injury and the consequent severe respiratory distress syndrome. The mucosal immunity of the airways and lungs are expected to play a crucial role in defending against SARS-CoV-2 infection. A key player in mucosal immunity is the secreted dimeric IgA (dIgA) antibody which can bind and neutralize SARS-CoV-2 particles. It is unknown whether SARS-CoV-2 possesses a mechanism to evade dIgA-mediated immune protection. In this study, we report that SARS-CoV-2 accessory protein, Open Reading Frame 8 (ORF8), is able to profoundly downregulate the polymeric Ig Receptor (pIgR). pIgR is expressed in the airway and gastrointestinal epithelial cells. Its main function is to transport dIgA from the basolateral side of epithelial cells to the apical (luminal) side, so that dIgA can protect the mucosal surface from pathogen infections. Our results suggest a function of ORF8 in evading dIgA-mediated mucosal antiviral immunity, thus exacerbating SARS-CoV-2 infection and pathology in the lungs.

Sequence and structural determinants of membrane insertion of fusion loops from class II viral fusion proteins - a molecular dynamics simulation study on Chikungunya virus E1 protein

sciforum-057547

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Enveloped viruses enter cells through a membrane fusion event. In Chikungunya virus (CHIKV) cell entry, its envelope protein, E1, performs membrane fusion. E1 is a class II viral fusion protein and has three β-sheet domains, and two hydrophobic loops at the tip of the domain II insert into the host membrane to start membrane fusion. Contrary to the internal fusion loops in class II viral fusion proteins, in class I viral fusion proteins, fusion peptides (N-terminal hydrophobic region of class I fusion proteins) are unordered, and adapt helical, boomerang conformation when inserted into the host membrane, and insert only into one leaflet, and in an oblique orientation to the membrane. The fusion peptides from HIV gP41 and influenza HA (class I fusion proteins) are thoroughly characterized. The sequence pattern and structural positioning of residues critical for membrane fusion function are conserved and are important determinants for the function. A conserved glycine residue in the N-terminal end, hydrophobic residues interspersed with polar residues, and position of aromatic residues in the fusion peptide determine the depth to which the fusion peptide would insert into the membrane and the boomerang conformation required for fusion. Interestingly, our analysis of fusion loop sequences from different class II viral fusion proteins shows sequence pattern conservation similar to that of class I fusion peptides. In order to understand the fusion loop insertion into the membrane and conformation of the fusion loops in the membrane, we performed a molecular dynamics simulation study with the CHIKV E1 fusion loops in POPC: Cholesterol: POPE (1:2:1 molar ratios) membrane models. Analysis of the MD simulation trajectory revealed that fusion loops partition into one leaflet of the lipid bilayer about 2Å. The fusion loop shows 5Å fluctuation from its pre-fusion structure and has a hook-like conformation upon insertion into the lipid bilayer.

SERINC5 Restricts Influenza A virus Infection by Modulating Hemagglutinin Conformation

sciforum-058341

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Serine incorporator 5 (Ser5), a transmembrane protein, has recently been identified as a host antiviral factor against human immunodeficiency virus (HIV)-1 and gammaretroviruses like murine leukemia viruses (MLVs). It is counteracted by HIV-1 Nef and MLV glycogag. Due to its antiviral activity against diverse retroviruses, we aimed to determine whether its activity extends to influenza A virus (IAV). Here, we demonstrated that Ser5 inhibited HIV-1-based pseudovirions bearing IAV hemagglutinin (HA); as expected, the Ser5 effect on this glycoprotein was antagonized by HIV-1 Nef protein. With the study of the incorporation-defective Ser5 mutant (Ser5K130A) and the co-immunoprecipitation assay, we discovered that the antiviral effect of Ser5 was not only associated with its incorporation, but also depended on its interaction with HA. In addition, Ser5 inhibited the virus-cell and cell-cell fusion induced by HA. Most importantly, we found that Ser5 inhibited the infectivity of full-length intact IAV. Single-molecule FRET (smFRET) analysis further revealed that Ser5 destabilized HA on virions and blocked the coiled-coil formation of HA in the IAV fusion process. In summary, Ser5 is a potential antiviral factor against IAV which inhibits the membrane fusion of IAV.

Small RNA sequencing and transcriptomic analysis to investigate the response to acute infection of the insect-specific Lammi Virus in an *Aedes albopictus* derived cell line

sciforum-057519

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Mosquitoes serve as vectors for a large number of viral pathogens, and their associated diseases cause a major health burden across the globe. In addition, mosquitoes are also infected with a group of viruses known as insect-specific viruses (ISVs) that are unable to infect vertebrates. ISVs are thought to be ancestors to the pathogenic arboviruses and are discussed as tools in different control strategies. However, little is known about how these viruses interact with the mosquito and its immune system. Therefore, we have analyzed the innate immune response to acute infection of the insect-specific Lammi virus (LamV) in the *Aedes albopictus* derived cell line U4.4.
Small RNA sequencing was conducted to study the RNA interference (RNAi) pathway, which is believed to be the primary defense against viral infection in mosquitoes. Results, revealed that LamV elicited a strong virus-derived small interfering RNA (vsiRNA) response that increased over time and that targeted the whole viral genome. Additionally, LamV-infected cells produced virus-derived Piwi-like RNAs (vpiRNAs); however, they were mainly derived from the antisense genome and did not show the typical ping-pong signatures. Furthermore, a transcriptomic analysis was performed to investigate the altered gene expression upon LamV-infection. The transcriptome profiles showed that LamV seem to evade the immune system with few altered immune related genes. however, the active viral infection induced a strong stress response in the cell with an increased expression of different heat-shock proteins. These proteins are known to be induced during stress, such as a during a pathogen infection or a heat shock, and act as chaperones to guide misfolded proteins. The induction of a strong RNAi response but no altered gene expression of immune related genes, including those involved in the RNAi pathways suggest that the ISV interact with the host differently than the medically important flaviviruses.

Sneaky SUO: plant protein promoting potyviral translation

sciforum-058296

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SUO is required for miRNA mediated translation repression and contains two GW repeats that interact with Argonaute (AGO) proteins. It inhibits translation independently of miRNA biogenesis and stability. *suo* is a loss of function mutant which reduces the activity of miR156/miR157 and AGO1 causing early expression of adult vegetative traits in *Arabidopsis thaliana (A.t.)*. To study if *suo* has an effect on potyvirus infections, we infected *suo- A.t.* and *suo* knock-down *Nicotiana benthamiana (N.b.)* with turnip mosaic virus (TuMV:GFP) and potato virus A (PVA:Rluc), respectively. Col0 plants and empty hairpin-infiltrated *N.b*. were used as controls, respectively. Viral gene expression was quantified by GFP-ELISA for TuMV, while Dual luciferease assay was performed to quantify Rluc levels from PVA:Rluc. Viral RNA transcript levels were assessed by measuring relative gene expression using RT-qPCR. We observed a two-fold and three-fold reduction in viral protein levels when SUO was downregulated in TuMV-*A.t*. and PVA-*N.b*. pathosystems, respectively. Viral transcript levels in both pathosystems did not change compared to the controls. In the absence of SUO, viral translation was inhibited. This result suggests that the virus hijacks SUO to assist the infection by promoting viral translation. Interestingly, when we further investigated if any of the three SUO homologs and AGO1 transcripts are affected by PVA infection in *N.b*., we did not observe any significant change in their transcript levels. Based on our results, we put forward a conjecture that SUO likely contributes to translation associated control of potyviral infection in an transcription independent manner.

Subcellular relocalization of *A. thaliana* ALY proteins upon infection with turnip yellows virus (TuYV)

sciforum-057882

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Turnip yellows virus (TuYV) belongs to the genus *Polerovirus* and newly to the family *Solemoviridae* infects a large number of plant species of agronomical interest and causes important losses for rapeseed crops.
As for all other viral pathogens, TuYV infectious cycle requires the expression of viral proteins whose function depends on their dynamic interactions with host proteins. Yeast two-hybrid screen identified ALY proteins as potential interactants of two TuYV proteins. In *Arabidopsis thaliana*, four proteins of the AtALY family (ALY1 to 4) have been described. They participate redundantly in the nuclear export of cellular mRNAs mediated by the THO-TREX multiprotein complex. One of the viral proteins found to interact with AtAly is the multifunctional protein RT involved in aphid transmission and viral movement in the plant. This protein is found either as a truncated form integrated in the viral capsid or as a complete form in the cytoplasm. We confirmed the interaction of the RT protein with the four AtALYs *via* co-immunoprecipitation experiments.
Confocal microscopy analysis of agro-infiltrated *Nicotiana benthamiana* leaves shows a relocalization of the four AtALY-eGFP into perinuclear vesicles upon co-expression with the TuYV expressing the RFP-tagged RT protein. The use of a nuclear pore marker (Nup36-eGFP) and a double-stranded RNA marker (B2-eGFP), a signature of viral replication, allowed us to associate these vesicles with replication complexes whose membranes are potentially derived from the nuclear envelope. In the future, we will investigate whether the relocalization of the AtALYs proteins is induced by the viral infection or by the expression of the RT protein alone.
Finally, we have shown that the inhibition of expression of the four AtALY genes in a quadruple mutant of *A. thaliana* induced an overaccumulation of TuYV, which suggests that AtALY proteins could be used as novel targets to fight against its dissemination.

Syncytia formation in fibroblasts and epithelial cells infected with human Cytomegalovirus

sciforum-058271

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Cell-cell fusion is used by many viruses as a means to transfer progeny in a manner protected from detection and neutralization by host immune defenses. Although the presence of syncytia has been observed in human cytomegalovirus (CMV) infected cultures and in the organs of severely infected individuals since the 1970s, the mechanics and significance of their formation for CMV replication and spread have never been investigated. We thus tested a set of cell types highly relevant to CMV pathogenesis *in vivo* for their ability to undergo syncytia formation after infection with different CMV strains. We show that: 1) foreskin fibroblasts, MRC-5 lung fibroblasts, labial fibroblasts, ARPE-19 retinal pigment epithelial cells, human renal epithelial cells and human nasal epithelial cells do not spontaneously fuse; 2) labial and lung fibroblasts are more prone to fusion than foreskin fibroblasts; 3) retinal and renal, but not nasal epithelial cells readily undergo syncytia formation; 4) fusion is enhanced by infection with strains that can efficiently assemble the gH/gL/UL128-131A pentameric complex and 5) fusion is blocked when viral DNA replication is inhibited and is impaired when nucleocapsid egress from the nucleus is halted. Together, these data suggest that infection-induced syncytia formation requires the synthesis of late viral proteins, is stimulated by the presence of maturing viral progeny and of a well-formed virion assembly compartment and is likely modulated by the expression of cell type-specific proteins with roles in in membrane manipulation and/or cell shaping, and whose identity is currently under intense scrutiny.

Taking a Closer Look at Fluorescent Filovirus Glycoprotein Pseudotypes

sciforum-057368

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A pseudotyped virus particle is a particle displaying the structural core of one virus and the functional envelope glycoprotein (GP) of a heterologous virus of interest. Pseudotypes are a common tool in the context of studying virus binding and entry of BSL3 and BSL4 pathogens within a BSL2 setting using infection assays. Fluorescent pseudotypes have been developed and used for microscopy applications but are much less established. In addition, not much is known about their physico-chemical properties and heterogeneity, presenting a potential hurdle for single-particle applications.
In this work, we produce fluorescent pseudotypes of the Filoviruses Ebola and Marburg using a lentiviral pseudotyping system. Both Filoviruses are amongst the deadliest human pathogens. For this reason and because they carry a single envelope GP which determines viral tropism and mediates virus entry, filovirus pseudotypes have been proposed as attractive models to study filovirus attachment and entry. First, we characterize their physico-chemical properties. Specifically, we focus on the following questions: do all pseudotypes show the same binding behavior; do all particles carry the same amount of GPs; does the fluorescent tag interfere with the interactions we would like to study with these pseudotypes? This leads to the development of improved production and purification protocols. Second, we implement the improved pseudotypes in binding assays based on single-particle tracking. Here, we focus on unraveling the influence of the GP's mucin-like domain in modulating virus attachment to and detachment from cell-surface glycosaminoglycans.
A better understanding of the properties of the pseudotypes in general will allow for improved experiments with more homogenous samples and an improved understanding of pseudotype production will equip us better for thorough interpretations of experimental results, as exemplified by our own binding data.

The aryl hydrocarbon receptor and the promyelocytic leukemia protein: Interplay of pro-viral and anti-viral factors during *in vitro* dengue virus infection

sciforum-058279

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The dengue virus (DENV) is considered the most important human virus transmitted by arthropods worldwide. Environmental impacts are known as a shaping factor of the host susceptibility and the pathogen itself. The analyses of environmental cues on the infection became crucial to understand the pathogenesis and develop new antiviral strategies against viruses.
The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor classically associated to xenobiotics clearance mechanisms. It is known that AHR modulates the immune system and it has emerged as pro-viral factor during viral infections.
The promyelocytic leukemia protein (PML) is the major component of a multi-component nuclear body (NB) in human cells: PML-NBs. PML-NBs exert a variety of cellular functions including antiviral intrinsic immune responses.
Previous results from our group indicated that PML exerts anti-viral activity whilst AHR has a pro-viral role during DENV infection. The aim of our work was to evaluate the potential interplay between AHR and PML during DENV infection *in vitro*.
DENV increases AHR expression and promotes its nucleo-cytoplasmic shuttling in early infection stages. Additionally, cells infected with DENV exhibit differential nucleus/cytoplasmic localization of both, PML and AHR. Poly I:C (20μg/ml) treatment triggers changes in the expression and localization of AHR and PML, differentially and in a time-dependent manner. This suggest that AHR and PML might be counter-regulated within the nucleus. Moreover, PML over-expression induced an accumulation of AHR and its co-localization with specific PML isoforms at NBs. Furthermore, PML over-expression promoted an accumulation of the viral polymerase (NS5) at PML-NBs and NS5 over-expression impacted on endogenous PML and AHR expression levels, suggesting a potential interaction network between AHR, PML and NS5.
In sum, AHR and PML might have antagonistic roles during DENV infection and constitute a novel potential antiviral target which has to be further explored.

The function of APOBEC3F and APOBEC3H hetero-oligomers in HIV-1 restriction

sciforum-057385

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APOBEC3 (A3) deoxycytidine deaminases have a role in primate intrinsic immunity against retroviruses, such as HIV-1. Restriction of HIV-1 occurs after deamination of cytidine to form uracil in single-stranded (-)DNA during reverse transcription. In humans, five of the seven A3 enzymes show HIV-1 restriction capability (A3G, A3F, A3H, A3D, A3C). HIV-1 suppresses A3 restriction though the viral infectivity factor (Vif) protein that promotes ubiquitination and proteasomal degradation of A3s. A3H is the most polymorphic A3s with seven Haplotypes and four mRNA splicing variants. The A3H Haplotype (Hap) I allele is the most common and is resistant to Vif-mediated degradation. However, it is unstable in cells and does not restrict HIV-1 replication efficiently. Here, we assessed the ability of co-encapsidation of A3H Hap I with other A3s in HIV-1 restriction and Vif-mediated resistance. We found out that co-encapsidation of A3H Hap I with A3F led to partial resistance of A3F to Vif-mediated degradation. We found that A3H Hap I and A3F interact and form a hetero-oligomer that reduced the viral load two-fold more than each A3 expressing alone. These data suggest previously unrecognized role for A3H Hap I in HIV-1 restriction, if it interacts with other A3 enzymes.

The US21 Viroporin Of Human Cytomegalovirus Regulates Cell Adhesion And Migration

sciforum-057290

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Human cytomegalovirus (HCMV) with its viral proteins disrupts most cellular homeostatic circuits. Among the proteins involved, we find those encoded by the US12 gene family, a set of 10 contiguous genes tandemly arranged (from US12 to US21) Only few functions have been associated with the family to date. Previously, we identified US21 protein as a HCMV homolog of cellular TMBIM proteins and an ER-resident virus-encoded calcium-permeable channel able to dysregulate intracellular Ca2+ homeostasis and that inhibit apoptosis. Given the role of Ca2+ in controlling cell adhesion and motility, we investigated whether the pUS21-mediated Ca2+ release might influence these important responses. Using engineered human cell lines to express pUS21, we observed a significant increase in the migration rate of cells expressing pUS21wt, while its mutation in the critical D201 residue that define the pUS21's Ca2+ channel leaking function, affected the ability to stimulate cell motility, thus suggesting an involvement of US21 ability to reduce the Ca2+ content in intracellular stores. Moreover, we performed migration assays in the presence of an inhibitor of calpain 2, a Ca2+-protease that promotes cell motility. The addition of the calpain-2 inhibitor abrogated the pUS21-mediated increase of migration in cells expressing this HCMV protein, on contrary an increase of calpain-2 activity was observed in extracts from cells expressing pUS21wt, thus confirming an involvement of pUS21 in the control of calpain 2 activation. Moreover, the functional relationship between pUS21 and calpain 2 was further suggested by the observation that talin 1, a calpain substrate, interact with pUS21 as observed by mass spectrometry and co-immunoprecipitation. Together, these findings highlight a novel role of the pUS21 viroporin as a cellular regulator as a consequence of the dysregulation that pUS21 exerts on the localized Ca2+-dependent activation of calpain2 and the subsequent talin activity at newly forming protrusions.

Abstracts

S6. Viral Pathogenesis and Evolution

Invited Speaker

Swine acute diarrhea syndrome coronavirus infection in suckling mice and characterization of a potential drug target

sciforum-060613

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Swine acute diarrhea syndrome coronavirus (SADS-CoV) recently emerged as a bat-borne coronavirus that causes high mortality rates in piglets. *In vitro* studies indicate that SADS-CoV has a wide tissue tropism derived from different hosts, including humans. However, whether this virus can potentially threaten other animals remains unclear. Here, we report the experimental infection of suckling mice with SADS-CoV and find that the virus is susceptible to mice under 7 days old. The mortality rate was highest in 2-day-old mice and decreased in the elders with noted multi-tissue infections and damage. A preliminary inflammatory response was observed in the brains of SADS-CoV-infected mice of 7-day-old. Our results indicate that this virus has potential pathogenicity to other young hosts. To explore host dependency factors for SADS-CoV as therapeutic targets, we employed genome-wide CRISPR knockout library screen in HeLa cells and identified the zinc finger DHHC-type palmitoyltransferase 17 (ZDHHC17, ZD17) as an important host factor for SADS-CoV infection. Through truncation mutagenesis, we demonstrated that the DHHC domain of ZD17 is required for the SADS-CoV genomic RNA replication. Treatment of infected cells with palmitoylation inhibitor, 2-bromopalmitate (2-BP), significantly suppressed SADS-CoV infection. Our findings provide insight on SADS-CoV-host interactions and a potential therapeutic application.

Invited Speaker

Predicting evolutionary genetics of virus-bacteria interactions in patients receiving phage therapy.

sciforum-060861

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One possible strategy to combat the antibiotic resistance crisis is a renewed approach to 'phage therapy,' where these administered viruses not only kill the target bacteria, but also predictably select for phage resistance that reduces virulence and/or increases antibiotic sensitivity (evolutionary trade-offs). By utilizing virulence factors as receptor binding sites, the phages exert selection for bacteria to evolve phage resistance by modifying (or losing) the virulence factor, potentially reducing bacterial pathogenicity. We present examples of phages that kill target bacteria while selecting for phage resistance that coincides with useful clinical traits, and compare in vitro data to phenotypic, genetic and metagenomics analyses of microbes isolated longitudinally from patient samples before, during and after emergency phage therapy treatments.

Chapulin: a leap forward on mobile element and structural variant identification.

sciforum-057500

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Transposable elements, including endogenous retroviruses and other endogenous viral elements, represent a substantial proportion of eukaryotic genomes. These elements represent viruses that were active at the time of infection and thus present a remarkable record of historical virus–host associations. Moreover, they can affect gene expression by either inhibition or enhancement. Current approaches rely on parsing sequence alignment files looking for anomalies on read length, read orientation and read depth, however, their performance is often slow and the installation process is troublesome. Here, we present Chapulin, a cross-platform, open-sourced, Rust application for transposable element identification and characterization. By using concurrent computing and native execution, Chapulin identifies a large fraction of mobile element insertions while maintaining a small computational footprint and good performance. As such, Chapulin is potentially expandable to fulfill the identification needs of different classes of mobile element and structural variants in different settings, for instance, clinical samples, population genetic studies. In this fashion, Chapulin facilities the study of viral evolution and host-virus interactions at a deep time scale.

Human pathogenic RNA viruses establish non-competing lineages by occupying independent niches

sciforum-057071

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Many pathogenic viruses are endemic among human populations and can cause a broad variety of diseases, some potentially leading to devastating pandemics. How virus populations maintain diversity and what selective pressures drive population turnover, is not thoroughly understood. We conducted a large-scale phylodynamic analysis of 27 human pathogenic RNA viruses spanning diverse life history traits in search of unifying trends that shape virus evolution. For most virus species, we identify multiple, co-circulating lineages with low turnover rates. These lineages appear to be largely noncompeting and likely occupy semi-independent epidemiological niches that are not regionally or seasonally defined. Typically, intra-lineage mutational signatures are similar to inter-lineage signatures. The principal exception are members of the family Picornaviridae, for which mutations in capsid protein genes are primarily lineage-defining. The persistence of virus lineages appears to stem from limited outbreaks within small communities so that only a minor fraction of the global susceptible population is infected at any time. As disparate communities become increasingly connected through globalization, interaction and competition between lineages might increase as well, which could result in changing selective pressures and increased diversification and/or pathogenicity. Thus, in addition to zoonotic events, ongoing surveillance of familiar, endemic viruses appears to merit global attention with respect to the prevention or mitigation of future pandemics.

Identification of mutational signatures affecting the VP1 gene of BK polyomavirus in vivo

sciforum-057703

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Mutations in the BK polyomavirus (BKPyV) capsid accumulate in kidney transplant (KTx) recipients with persistent virus replication. They are associated with neutralization escape, and it has been reported that they arise as result of cytosine deamination by host cell APOBEC3 enzymes. However, it is not known whether these mutations occur in all, or only a sub-group of KTx recipients, nor whether APOBEC3 is the only source of BKPyV mutations. To address these questions, we amplified the typing region of the VP1 gene, sequenced the amplicons to a depth of 5000-10000x, and identified rare mutations using lofreq, which were fitted to COSMIC mutational signatures with the Mutational Patterns package in R. Background mutations due to Taq polymerase errors were identified in amplicons from plasmids carrying the MM strain BKPyV genome and compared to mutations observed in 88 samples from 23 KTx recipients in France and in Vietnam. Three mutational signatures were consistently observed in urine, serum and kidney biopsy samples, two of which, SBS2 and SBS13, corresponded to APOBEC3 activity. In addition, a third signature with no known etiology, SBS89, was detected both in patient samples, and in extracts from cells infected with the MM strain.
Although these results confirm that APOBEC3 is a major source of BKPyV genome mutations in vivo, they also show that cytosine deamination by APOBEC enzymes is not the only mutagenic mechanism acting on the BKPyV viral population in patients.

SARS-CoV-2 Infection Induces Modest Microbial Translocation in Rhesus Macaques and Gut-Associated Inflammation in Monkeys and Humans

sciforum-058340

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SARS-CoV-2 infections exhibit a broad range of severity, from asymptomatic to fatal. Symptoms are not limited to respiratory tract-associated sneezing, coughing, or rhinitis and may include gastrointestinal (GI) tract involvement with symptoms such as nausea and diarrhea. To further explore the relationship between the GI tract and SARS-CoV-2 infection, we investigated GI pathology longitudinally in rhesus macaques and cross-sectionally in humans during symptomatic infection and at symptom resolution.

Fourteen rhesus macaques were infected intranasally and intratracheally with US/WA-1/2020 SARS-CoV-2 and monitored through acute infection to euthanasia at day 10. Animals experienced mild infection with weight loss less than 5% of baseline mass and no fever. Nasal viral RNA (vRNA) peaked at day 2 post-infection, while vRNA in the throat peaked earlier at day 1 post-infection and at approximately 1.5 logs higher. vRNA was additionally detected in the feces of infected animals, including low levels of subgenomic RNA that indicates viral replication. Furthermore, immunohistochemistry for *E. coli* in fixed colon sections demonstrated modest microbial translocation across the gut epithelium, with slightly elevated translocation scores in infected animals over uninfected controls. Zonulin, indicative of gut damage, was significantly elevated at day 10 over baseline ($p=0.0234$) in a subset of older animals. Soluble CD14 in plasma, suggestive of an inflammatory response to microbial products, was significantly elevated at day 10 post-infection over baseline in all macaques ($p=0.0006$), while humans with moderate COVID-19 experienced significant reduction in soluble CD14 at recovery from infection over earlier symptomatic time points ($p=0.0295$). Additionally, individuals experienced significant reductions in IL-6 at recovery time points ($p=0.0171$).

Despite experiencing mild SARS-CoV-2 infections, rhesus macaques nonetheless demonstrated GI tract involvement and corresponding inflammation, while humans with moderate infection likewise demonstrated systemic inflammation connected to gut pathology. The contributions of SARS-CoV-2 GI tract pathology to systemic disease merit additional exploration.

A global understanding of how Picornavirus capsids escape antibody neutralization

sciforum-058185

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Picornavirus capsids constitute the key target of host humoral responses and therefore play an important role in viral evolution and pathogenesis. While numerous studies have defined monoclonal antibody neutralization sites across picornavirus capsids, a global understanding of the interaction between viral capsids and polyclonal sera is currently lacking. To address this gap, we have used Coxsackievirus B3 (CVB3) populations harboring extreme diversity in the capsid coding region to map all mutations that confer resistance to neutralization by human and mouse polyclonal sera. Using this approach, we have obtained the antigenic profiles of the different sera and analyzed the breadth of escape of the identified epitopes in a structural and evolutionary context. This study presents the first comprehensive picture of the interaction between the CVB3 capsid and polyclonal sera, advancing our understanding of a key aspect of host-pathogen interaction and shedding light on how natural immunity can shape viral antigenic evolution.

First report on detection and molecular characterization of adenoviruses and circo/cycloviruses in the mongoose: Identification of novel viruses

sciforum-057798

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Mongooses, members of the family *Herpestidae*, are small terrestrial carnivores. Due to sharing their habitat with humans and other animals, and their invasive and scavenging behavior, mongooses can serve as potential carriers of viral pathogens, such as rabies. However, to date, limited virological studies have been performed in mongoose populations. We report here the first-time detection and molecular characterization of novel adenoviruses, circoviruses and cycloviruses in the small Indian mongoose (*Urva auropunctata*). Using broad-range nested PCR assays, we detected adenoviruses and circo/cycloviruses in 17 (20.48%, n=83) and 76 (91.56%, n=83) fecal samples, respectively, from the small Indian mongoose on the Caribbean Island of St. Kitts. Based on analysis of partial DNA-dependent DNA polymerase gene sequences (~300 bp), (i) *Bovine atadenovirus E*-like adenoviruses, suggestive of interspecies transmission from ruminants, (ii) novel atadenoviruses that appeared to share a common origin with reptilian atadenoviruses, and (iii) a novel mastadenovirus that was genetically distinct from other mastadenoviruses were identified. Using inverse nested PCRs, the complete genomes of select mongoose associated circoviruses and cyclovirus strains were amplified. The full-length genome sequences of the mongoose associated circo/cycloviruses shared 80% maximum genome-wide pairwise nucleotide sequence identities with those of circo/cycloviruses from other animals/sources, and were assigned to novel circovirus, or cyclovirus species. Despite high genetic diversity, the mongoose associated circo/cycloviruses retained the various features that are conserved among members of the family *Circoviridae*. Our findings provide important insights into virus evolution in wildlife and warrant large-scale surveillance studies on viruses circulating in mongoose populations that would help elucidate potential implications on animal and human health.

Frailty-based analysis of COVID-19 mortality predicts fewer life years lost

sciforum-058270

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Beyond the number of deaths associated with the infection, the mortality of COVID-19 epidemics can be characterized with the years of life lost (YLL) due to the disease. Previous calculations have yielded estimates in the first wave of the epidemic indicating that the infection causes death typically more than a decade earlier than it would occur from other causes of mortality. However, these analyses attributed YLL to each deceased individual according to the mean life expectancy at the individual's age, which entails the assumption that COVID-19 mortality strikes 'unbiased' within a given age group.
We challenge this assumption, arguing that variation in health status within each age group is likely to affect the severity of COVID-19 infections, with a higher risk of death in those who have poorer health. We introduce a frailty-based analysis for the calculation of the YLL in a COVID-19 epidemic, assuming that the fatality rate is linked to the potential remaining life years (an indicator of health condition) in the infected individuals.
Using detailed demographic data from several countries, we decomposed age strata into subgroups according to potential remaining lifespan, and then re-fitted estimated consensus rates of age-dependent COVID-19 fatality as a function of remaining life span (alone, or in combination with age). We demonstrate that statistical models with a dominant effect of remaining life span can yield comparably good fits as earlier age-based models, and in these models the median YLL can be as low as two years. We conclude that the data are equally compatible with a range of alternative scenarios in which the risk of death with COVID-19 might be dominantly determined by either the general health status ('biological' age) or the 'chronological' age of the individual, or by a combination of the two.

Investigating ORF8-mediated immune evasion in SARS-CoV-2 variants of concern

sciforum-057485

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The COVID-19 pandemic continues to have devastating effects on public health. The global spread of SARS-CoV-2 has resulted in significant viral evolution, raising concern for the emergence of variants with increased pathogenicity. A number of these, coined variants of concern (VOCs) have already been identified, among which Omicron now dominates the COVID-19 landscape in many countries. VOCs are characterized by key mutations which were naturally selected to provide a fitness advantage for SARS-CoV-2 transmission and replication. In addition to the viral spike protein, the Open Reading Frame (ORF) 8 is the other fast evolving viral protein, which is secreted as a dimer, bears the immunoglobulin fold, and is present in COVID-19 patient sera. It has been reported that ORF8 downregulates Major Histocompatibility Complex I (MHC-I), suggesting its function in immune evasion. We have further discovered that ORF8 downregulates Fcg receptor 1A (FcgR1A). FcgR1A is the high affinity Fcg receptor, primarily expressed on the surface of myeloid immune cells including monocytes, macrophages, and dendritic cells. The function of FcgR1A is to engage with the IgG/antigen immune complexes and mediate protective effector cell responses. We will report the ability of ORF8 from SARS-CoV-2 VOCs and animal coronaviruses in downregulating MHC-I and FcgR1A, and their impact on antigen presentation and other functions of immune cells. Our data are expected to demonstrate that ORF8 contributes to SARS-CoV-2 immune evasion by downregulating MHC-I and FcgR1A, and that its polymorphisms may contribute to the increased transmission and pathogenesis of SARS-CoV-2 VOCs.

Natural History Study of Aerosol Induced Ebola Virus Disease in Non-Human Primates

sciforum-058219

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Ebola virus disease (EVD) is a serious global health concern because case fatality rates are approximately 50% due to recent widespread outbreaks in Africa. Well-defined nonhuman primate (NHP) models for different routes of Ebola virus exposure are needed to test the efficacy of candidate countermeasures. In this natural history study, four rhesus macaques were challenged via aerosol with a target titer of 1000 plaque-forming units per milliliter of Ebola virus. The course of disease was split into the following stages for descriptive purposes: subclinical, clinical, and decompensated. During the subclinical stage, high levels of venous partial pressure of carbon dioxide led to respiratory acidemia in three of four of the NHPs, and all developed lymphopenia. During the clinical stage, all animals had fever, viremia, and respiratory alkalosis. The decompensatory stage involved coagulopathy, cytokine storm, and liver and renal injury. These events were followed by hypotension, elevated lactate, metabolic acidemia, shock and mortality similar to historic intramuscular challenge studies. Viral loads in the lungs of aerosol-exposed animals were not distinctly different compared to previous intramuscularly challenged studies. Differences in the aerosol model, compared to intramuscular model, include an extended subclinical stage, shortened clinical stage, and general decompensated stage. Therefore, the shortened timeframe for clinical detection of the aerosol induced disease can impair timely therapeutic administration. In summary, this nonhuman primate model of aerosol-induced EVD characterizes early disease markers and additional details to enable countermeasure development.

SARS-CoV-2, Placental Histopathology, Gravity of Infection and Immunopathology: Is there an association?

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(1) Background: As the pandemic months progress, more and more evidence shows that the placenta acts as a "barrier" to SARS-CoV-2, although rare cases of vertical transmission havebeen described.; (2) Methods: In an attempt to investigate the mechanisms of potential transmission and to investigate whether the symptoms of pregnant SARS-CoV-2 positive patients impacted the placental parenchyma, we divided the patients into two groups, depending on the symptomatology (moderate / severe vs asymptomatic / mild) and we analyzed the data. (3) Results: The symptoms (likely to be related to viremia) have a statistically significant impact (p 0.05) on maternal malperfusion, decidual arteriopathy, blood vessel thrombus of fetal relevance. Furthermore, ACE2 does not appear to be statistically different in the two populations analyzed. (4) Conclusions: There is still much to study and discover regarding the mechanisms of the etiopathogenesis of SARS-CoV-2; there is even more to understand about immunopathology and the relationship between placental parenchyma, virus and host response. It seems quite certain that the low inci-dence rate of maternal-fetal transmission is due to a "barrier" effect, so that the placenta acts as the ultimate bulwark against SARS-CoV-2. Similarly, it does not seem that ACE-2, at least in the third trimester of pregnancy, is able to act as a "gateway" to virions, but rather some other recep-tor still to be well characterized. In any case, only a careful and greater analysis of large case studies can throw even clearer light on one of the most complex mechanisms of infection.

Study of microRNA and anti-inflammatory genes in tissues of rabbits infected with Lagovirus europaeus GI.1/RHDV (Rabbit Hemorrhagic Disease Virus)

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Objectives: MicroRNA-155 exhibit both anti-and pro-inflammatory functions, depending on the simulant involved. *Lagovirus europaeus*/RHDV is an etiological factor of rabbit haemorrhagic disease. The disease usually leads to the death of animals due to MOF resulting from the development of DIC. Our previously functional analysis showed that ocu-miR-155-5p can regulate the expression of genes involved in anti-and pro-inflammatory processes correlated with ALF and MOF in rabbits infected with *Lagovirus europaeus*. Therefore the present study aimed to quantify ocu-miR-155-5p and TGF-β1, HGF, c-Met, and IL-10 expression patterns in four different tissue of rabbits infected with *Lagovirus europaeus.*
Material and methods: The specimen of liver, lungs, spleen, and kidneys tissue were collected from rabbits infected with *Lagovirus europaeus*, variant GI.1a/Rossi (n=10) and healthy controls (n=10). The expression of ocu-miR-155-5p was determined by the qPCR reactions. TaqMan®Real-Time PCR Assays has been used to analyze the expressions of TGF-β1, HGF, c-Met, and IL-10.
Results: Ocu-miR-155 expression was significantly increased in liver and kidney tissue; while it was lowered in the lungs and spleen. In the liver, an increase the expression of the TGF-β1 and IL-10 gene was observed; in the kidney, an increase c-Met and IL-10; in the lung an increase c-Met and IL-10; in the spleen, a decrease HGF and an increase c-Met and IL-10.
Conclusion: We have demonstrated that in the liver and kidneys, as a result of infection and inflammation, there is an increase the expression of ocu-miR-155, which increases the expression of the TGF-β1 (liver) and c-Met (kidneys) genes. In addition, the IL-10 produced by cells infiltrating these organs may affect the concentration of c-Met. In the lungs and spleen, miR-155 expression was decreased, accompanied by an increase in c-Met and IL-10 in both tissues.

The evolutionary dynamics of post-translational modifications in betacoronaviruses as a potential driver of rapid viral divergence

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Viruses accumulate amino acid substitutions enabling evolutionary rewiring that may benefit viral fitness by altering host-microbe interactions involved in immune evasion and infectivity. These substitutions may generate mimics of host post-translational modification (PTM) motifs leading to post-translationally modified viral proteins mediated by host enzymes. These PTMs on viral proteins may alter viral fitness. We investigated the evolutionary landscape of PTMs across betacoronaviruses to elucidate if PTMs are potential drivers of adaptive evolutionary rewiring.
Protein families were constructed for all shared proteins in three betacoronavirus clades (11 Sarbecoviruses, 10 Merbecoviruses, and 9 Embecoviruses). PTMs for all sequences were predicted, mapped, and encoded to their corresponding multiple sequence alignment (MSA). Sequence conservation was examined for predicted PTM sites. Evolutionary rates were estimated across the proteome using Rate4Site to determine rate shifts in protein sequence and PTM-encoded MSAs between the clades and against the whole tree.
The most abundant PTMs were N-linked glycosylation, Ser-phosphorylation, and Lys-N6-acetylation. Spike, NSP3, and Nucleocapsid have the most PTMs. Average sequence conservation surrounding PTM sites varied by PTM type across the proteome. S-palmitoylation, Lys-methylation, and Tyr-phosphorylation showed the highest mean conservation surrounding PTM sites, while Pyrrolidone Carboxylic Acid and SUMOylation showed the lowest across the proteome. Evolutionary rate shift analysis of the PTM-encoded MSAs revealed several clade-specific hotspots. Spike, NSP3, NSP12, and Nucleocapsid experienced multiple PTM shifts among clades and the whole tree. While few PTMs are conserved across all clades, multiple clade-specific conserved PTM sites are evident.
The rapid evolutionary dynamics of PTMs, the presence of clade-specific PTMome signatures, and the lack of PTMs conserved across the entire phylogeny support a role for PTMs in driving adaptive evolutionary processes across betacoronaviruses. Through an improved understanding of the evolutionary processes that benefit viral fitness, we can better combat emergent viral pathogens.

Using Genetic Surveillance to Understand the Disrupted Seasonality of Paediatric Respiratory Viruses Despite Physical Distancing

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Background-Rationale: Non-pharmaceutical interventions (NPIs) (i.e., social distancing, mask-wearing, hand sanitisers, border closures, lockdowns, working and schooling from home) effectively reduced SARS-CoV-2 transmission in Australia but also disrupted the seasonal transmission of other respiratory viruses during 2020. Globally, there have been changes in non-SARS-CoV-2 respiratory virus transmission patterns such as reduced overall frequency, off-season transmission or resurgence. In Queensland, Australia, rhinoviruses (RVs) and respiratory syncytial viruses (RSVs) have surged off-season to become predominant causes of respiratory illnesses amongst children. In this study, we seek to better understand the genetic characteristics of circulating RV trends following transmission bottlenecks in the community.

Methodology-Results: Molecular diagnostic assays have been utilised to test for RV and RSV (subtypes A and B) across >2000 nasopharyngeal swabs collected for COVID-19 screening from March to December 2020. Preliminary screening shows RV detection in 659/2219 (30%) children's swabs, exceeding full-year averages ranging from 17-23% since 2016 over typical winter months, and high proportions in children 4 years and under. Sanger sequencing was performed to determine RV genotypes detected throughout 2020 – at the implementation of NPIs in March, 40 RV genotypes were circulating, compared with 48 genotypes collectively from August to December. This is a 40% reduction in predicted genotypes for matched sample numbers.

Outcome-Significance: Despite seeing more RV infections, predicted circulating RVs genotypes reduced by > 40% in 2020. Such non-SARS-CoV-2 infections have resulted in additional economic burden to the individual (eg. lost productivity of parent while caring for a child in self-isolation, frontline worker parents, non-frontline parents, other) and increased testing costs for respiratory viruses causing indistinguishable acute respiratory tract infections (ARIs). Once State and International borders reopen, resurgence of influenza virus (IFV) transmission is likely. This project aims to monitor and report non-SARS-CoV-2 respiratory virus clusters in 2022 to alert Health Workers (HWs).


MDPI