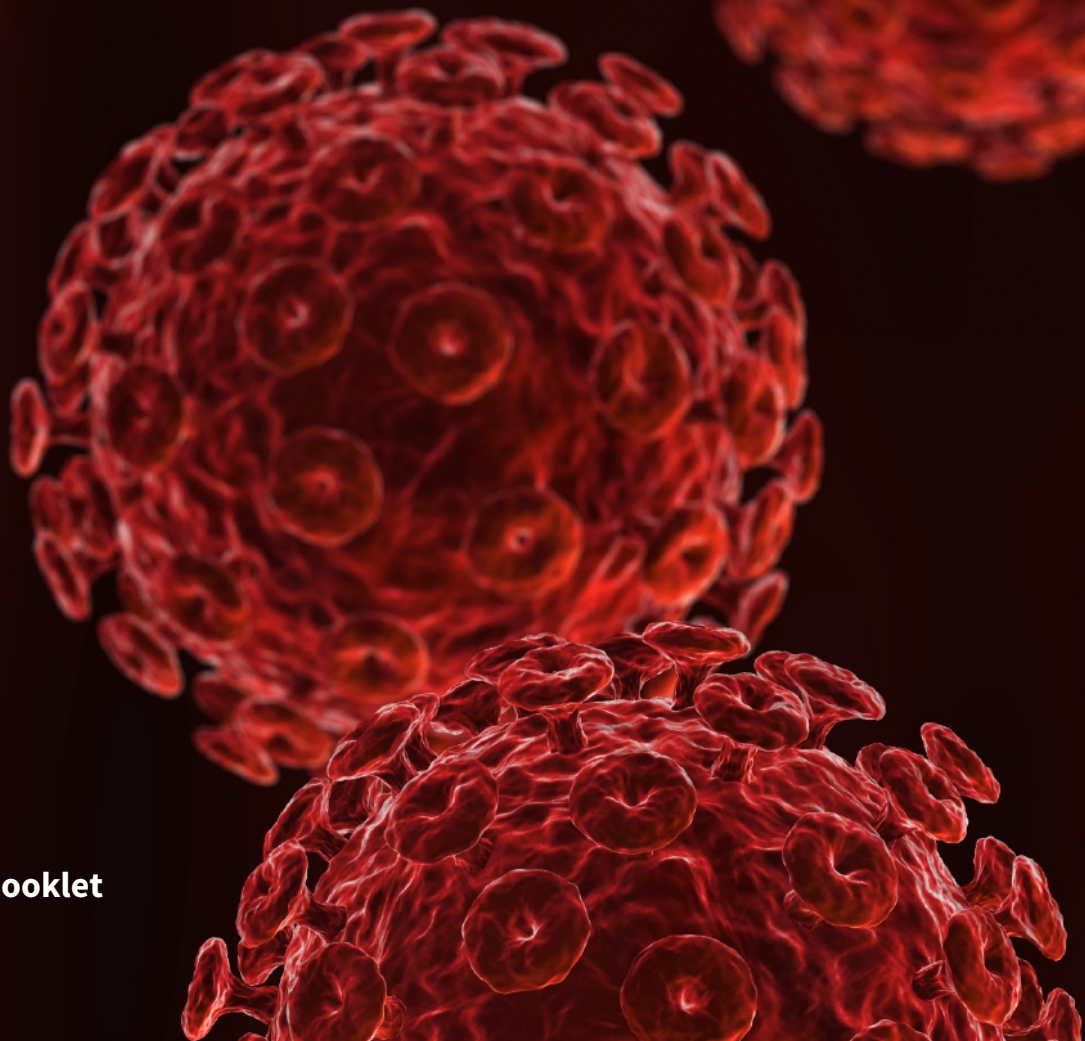


viruses
2020

Novel Concepts in Virology

5-7 FEBRUARY 2020 | BARCELONA, SPAIN



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***Viruses* 2020—Novel Concepts in Virology**

AXA Convention Centre

Barcelona, Spain

5–7 February 2020

MDPI • Basel • Beijing • Wuhan • Barcelona • Belgrade



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Dr. Eric O. Freed

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Dr. Michelle Flenniken

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Conference Secretariat

Dr. Sara Martinez

Mr. Aimar Xiong

Ms. Penny Zhang

Email: viruses2020@mdpi.com

Welcome from the Chairs

Dear Colleagues,

It is with great pleasure that we announce the conference *Viruses 2020—Novel Concepts in Virology* to be held in Barcelona, Spain, 5-7 February 2020.

Because of their global impact on human, animal, and plant health and their utility as tractable model systems, viruses continue to play a central role in all aspects of biomedical research, ranging from molecular and cell biology, structural biology, and immunology to evolution, epidemiology, and bioinformatics. This conference will bring together leading virologists from around the world and across the broad field of virology to share results of their recent studies. Meeting participants will have the opportunity to present posters and short talks on their work and discuss their research in a stimulating and collegial environment.

The 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* held in January 2016 in Basel, Switzerland and *Viruses 2018—Breakthroughs in Viral Replication*, held in February 2018 in Barcelona.

We very much look forward to seeing you at this exciting meeting in Barcelona.



Dr. Eric O. Freed
Conference Chair



Dr. Albert Bosch
Conference Chair

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viruses

an Open Access Journal by MDPI

Viruses (ISSN 1999–4915; CODEN: VIRUBR) is 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.

Among other databases, *Viruses* is indexed by the Science Citation Index Expanded (Web of Science), MEDLINE (PubMed) and other databases. Full-text available in PubMed Central.

Journal Webpage: <https://www.mdpi.com/journal/viruses>

Impact factor: **3.811** (2018)

5-Year Impact Factor: 3.916 (2018)

Conference Venue

Auditorium AXA of the AXA Convention Centre
Avinguda Diagonal, 547, 08029 Barcelona, Spain

Registration Desk

The desk for registration, information and distribution of documents will be open from 08:00 on 5 February 2020.

Certificate of Attendance

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

Disclaimer

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

Insurance

The organizers do not accept liability for personal accident, loss, or damage to private property incurred as a result of participation in the *Viruses 2020—Novels Concepts in Virology*. Delegates are advised to arrange appropriate insurance to cover travel, cancellation costs, medical, and theft or damage of belongings.

Emergency Information

All emergencies in Spain: 112 (no area code needed)
Ambulance (Ambulancia) and health emergencies: 061 or 112
Fire brigade (Cuerpo de bomberos): 080 or 112
Spanish National Police (Policía nacional): 091

Barcelona

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

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



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

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

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

For a complete overview, see [wikitravel.org](https://www.wikitravel.org/wiki/en/Barcelona) or visit [barcelonaturisme.com](https://www.barcelonaturisme.com).



The AXA Convention Centre

The event will be held at the Auditorium AXA of the AXA Convention Centre, which is part of an enormous complex located on the main artery of Barcelona that integrates a shopping centre, two hotels, 48.000 m² of offices, a parking lot, two schools, a sport centre and a public park. City communications are excellent and access from Barcelona's Airport and Sants Station is very quick.

The avant-garde design and construction quality emerge from each and every detail of the building, turning the L'ILLA complex into an emblematic reference of the city.



AXA Convention Centre (Source: www.axa.es)

As a whole, it is more than an auditorium: it is an infrastructure designed to offer quality, flexibility and integral attention through its wide range of services.

How to Reach the Venue

Address: [Avinguda Diagonal, 547, 08029 Barcelona, Spain.](#)



Venue Location (Source: www.axa.es)

Conference Dinner

Thursday 6th February, 20:30

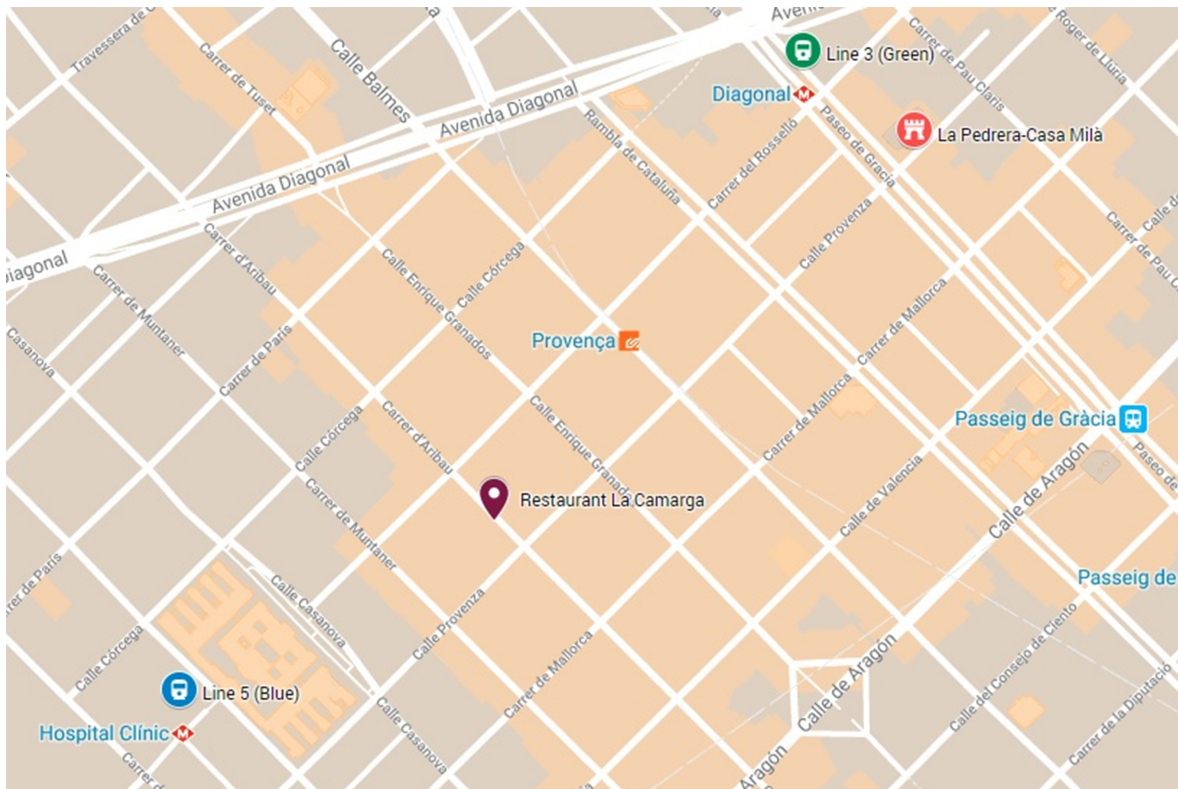
Price: 50€

Sold Out. To join the waiting list please ask at the Information Desk.



The conference dinner will be held at La Camarga, a cutting-edge restaurant specialized in Mediterranean cuisine with deep gastronomic roots that inspires dishes full of contemporary flavours and textures

La Camarga is located at [Carrer d'Aribau, 117](#), only a few minutes away from *Passeig de Gràcia*, one of the major avenues in Barcelona and one of its most important shopping and business areas, containing several of the city's most celebrated pieces of architecture (such as *La Pedrera* or *Casa Batlló*). You can easily reach the restaurant either by taxi, bus or Metro. If you were to choose the second option, the closest metro stations are "Hospital Clínic" on Line 5 (blue), or "Diagonal" on Line 3 (green).



Keynote Speakers



Prof. Peter Palese
Department of Microbiology
Icahn School of Medicine at
Mount Sinai, New York, NY,
USA

Invited Speakers



Dr. Michelle Flenniken
Montana State University,
Bozeman, MT, USA



Dr. Cristina Risco
National Center for
Biotechnology,
Madrid, Spain



Dr. Susan Weiss
University of Pennsylvania,
Philadelphia, PA, USA



Prof. Rotem Sorek
Weizmann Institute of Science,
Rehovot, Israel



Prof. Diane E. Griffin
Johns Hopkins Bloomberg
School of Public Health,
Baltimore, MD, USA



Dr. Stacy M. Horner
Duke University Medical
Center, Durham, NC, USA



Dr. Graham F. Hatfull
University of Pittsburgh,
Pittsburgh, PA, USA



Prof. K. Andrew White
York University,
Toronto, ON, Canada



Dr. Stanley M. Lemon
The University of North
Carolina at Chapel Hill,
Chapel Hill, NC, USA



Dr. Marco Vignuzzi
Institut Pasteur,
Paris, France



Prof. Martin Beer
Institute of Diagnostic
Virology (IVD),
Greifswald, Insel Riems,
Germany



Dr. Jason McLellan
The University of Texas
at Austin,
Austin, TX, USA



Prof. Rosa M. Pintó
University of Barcelona,
Barcelona, Spain

Program at a Glance

| <p style="text-align: center;"><i>Viruses 2020–Novel Concepts in Virology</i> 5–7 February 2020, Barcelona, Spain</p> | | | |
|--|--|--|---|
| | Wednesday 5 February 2020 | Thursday 6 February 2020 | Friday 7 February 2020 |
| Morning | Check-in Opening Ceremony Session 1. <i>General Topics in Virology</i> | Session 3. <i>Antiviral Innate Immunity</i> | Session 5. <i>Mechanisms of Virus Replication</i> |
| | Coffee Break | Coffee Break (Conference Group Photo) | Coffee Break |
| | Session 1. <i>Part II</i> | Session 3. <i>Part II</i> | Session 5. <i>Part II</i> |
| | Lunch | Lunch & Poster Session A | Lunch & Poster Session B |
| Afternoon | Session 1. <i>Part III</i> | Session 4. <i>Viral Pathogenesis</i> | Session 6. <i>Viral Genetics and Evolution</i> |
| | Coffee Break | Coffee Break | Coffee Break |
| | Session 2. <i>Structural Virology</i> | Session 4. <i>Part II</i> | Session 6. <i>Part II</i> |
| | Selected Posters Flash Presentations | | |
| | | Conference Dinner | Closing Remarks & Awards Ceremony |

Conference Program

Day 1: Wednesday 5 February 2020

08:00–09:00 Check-In

09:00–09:15 **Opening Ceremony**

Session 1. *General Topics in Virology (Part I)*

Chair: Shan-Lu Liu

09:15–10:00 **Keynote Opening Lecture**

Peter Palese

The Long Road to a Universal Influenza Virus Vaccine

10:00–10:30 **Jason McLellan (Viruses Young Investigator Award Winner 2019)**

Structure of the RSV Polymerase Complex Reveals a Tentacular Arrangement of the Viral Phosphoprotein

10:30–10:45 **Ariane C. Gomes**

HCMV gB-MF59 vaccine primes the immune system of seronegative individuals to mount greater immunological responses upon challenge with virus at the time of solid organ transplant

10:45–11:00 **Ritu Gaur**

Insights into the activity of second-generation maturation inhibitors against HIV clade C

11:00–11:30 **Coffee Break**

Session 1. *General Topics in Virology (Part II)*

Chair: Peter Nagy

11:30–12:00 **Graham Hatfull—Invited Speaker**

Mycobacteriophages: Diversity, dynamics, and therapy

12:00–12:15 **Cristina Howard-Varona**

Unveiling infection strategies across diverse marine phage-host systems

12:15–12:30 **Hugo Oliveira**

Bacteriophage depolymerases: evolutionary insights and antivirulence strategies against bacterial pathogens

12:30–12:45 **Tae-Jin Choi**

Bacteriophage biocontrol of *Acidovorax citrulli*, the causal agent of bacterial fruit blotch

12:45–13:00 **Victor Latorre**

Valosin-containing protein (VCP/p97) is a potential antiviral target against Mononegavirales

13:00–14:00 **Lunch**

Session 1. *General Topics in Virology (Part III)*

Chair: Anna Cliffe

14:00–14:30 **Michelle Flenniken—Invited Speaker**

Honey Bee Viruses, Colony Health, and Antiviral Defense

14:30–14:45 **Ioly Kotta-Loizou**

A mycovirus mediates the virulence of an insect-killing fungus against the malaria mosquito vector

14:45–15:00 **Lotty Birnberg**

- Small RNAs virome characterization reveals arthropod-associated viruses in Anopheles atroparvus from the Ebro Delta, Spain
- 15:00–15:15 **Maria Vittoria Salvati**
The persistent infection of tick cells by Hazara orthonairovirus is mediated by virus-derived DNA forms
- 15:15–15:30 **Candace Fox**
Histone Deacetylase Inhibitors Enhance Cell Killing and Block Interferon-Beta Synthesis Elicited by Infection with an Oncolytic Parainfluenza Virus
- 15:30–16:00 **Coffee Break**

Session 2: Structural Virology
Chair: Polly Roy

- 16:00–16:30 **Cristina Risco – Invited Speaker**
Imaging Viral Factories
- 16:30–17:00 **K. Andrew White – Invited Speaker**
Regulation of RNA Virus Processes by Viral Genome Structure
- 17:00–17:15 **Reidun Twarock**
Viral genome conformations and contacts across different life-cycle stages
- 17:15–17:30 **Jamil Saad**
Structural basis for Env incorporation into HIV-1 particles
- 17:30–17:45 **Ekaterina Heldwein**
Structural basis for capsid recruitment and coat formation during HSV-1 nuclear egress
- 17:45–18:00 **James Munro**
Conformational dynamics related to membrane fusion observed in single Ebola GP molecules
- 18:00–18:45 **Selected Posters Flash Presentations**
Chair: Jamil Saad
- Alex Compton:** The intrinsic link between metabolic and antiviral states of the cell (Poster No. 130)
 - Ornela Chase:** Deciphering the RNA Silencing Suppressor Function in the Potyvirus SPV2 (Poster No. 52)
 - Matthias Götte:** Independent Inhibition of the Polymerase and Deubiquitinase activities of the Crimean–Congo Hemorrhagic Fever Virus Full-Length L-protein (Poster No. 82)
 - Jiri Hejnar:** CRISPR/Cas9 Editing of Viral Receptors and Biotechnological Approach to Host Resistance (Poster No. 51)
 - Shan-Lu Liu:** Restriction of HIV by TIM and SERINC and Counteraction by Nef (Poster No. 14)
 - Antonio Mas:** Usutu Virus NS5: Characterization of Polymerase Activity, Protein-Protein Interaction and Cellular Localization (Poster No. 138)
 - Craig McCormick:** KSHV activates unfolded protein response sensors but suppresses downstream transcriptional responses to support lytic replication (Poster No. 12)
 - Delphine Muriaux:** Specific lipid recruitment by the retroviral Gag protein upon HIV-1 assembly: from model membranes to infected cells (Poster No. 121)
 - Jack Stapleton:** Yellow Fever Virus Vaccine Reduces T Cell Receptor Signaling and the Levels of Phosphatase PTPRE In Vivo (Poster No. 143)®

Day 2: Thursday 6 February 2020

Session 3: Antiviral Innate Immunity (Part I)

Chair: Gregory Melikyan

- 09:00–09:30 **Susan Weiss—Invited Speaker**
Activation and antagonism of the OAS-RNase L pathway
- 09:30–09:45 **Saguna Verma**
Modeling of Zika virus infection and antiviral immunity in 2D and 3D in vitro systems of human testis
- 09:45–10:00 **Linda Brunotte**
Human IFN-alpha; subtypes display non-redundant antiviral activities against H3N2 influenza A virus in the human lung.
- 10:00–10:15 **Carlos Maluquer de Motes**
Poxin/vSchlafen: a novel viral strategy to subvert cytosolic DNA sensing
- 10:15–10:30 **Gregory Melikyan**
Mechanism of inhibition of viral fusion by interferon-induced transmembrane proteins
- 10:30–11:00 **Coffee Break**
- 10:30–10:35 **Conference Group Photograph**

Session 3: Antiviral Innate Immunity (Part II)

Chair: Susan Weiss

- 11:00–11:30 **Stacy Horner—Invited Speaker**
A role for the RNA modification m6A at the virus-host interface
- 11:30–11:45 **Idoia Busnadiego**
Non-canonical interferon-stimulated pathways in antiviral defense
- 11:45–12:00 **Matthew Murray**
Type I interferon activity promotes a cellular environment that supports the establishment of latency by human cytomegalovirus
- 12:00–12:15 **Daniel Blanco-Melo**
An inability to maintain the ribonucleoprotein genomic structure results in host detection of negative strand RNA viruses
- 12:15–12:30 **Dong-Yan Jin**
Interferon antagonism of Epstein-Barr virus tegument proteins
- 12:30–12:45 **Kevin Groen**
Induction of the type-I IFN response by Human Metanpneumovirus lacking SH, G or M2.2 expression
- 12:45–13:00 **Daniela Ribeiro**
Peroxisomes as platforms for cytomegalovirus' evasion from the cellular antiviral signalling

13:00–15:00 **Lunch**

13:30–15:00 **Poster Session A (1–73)**

Session 4: Viral Pathogenesis (Part I)

Chair: Craig McCormick

- 15:00–15:30 **Rotem Sorek—Invited Speaker**
Beyond CRISPR: The immune system of bacteria

- 15:30–15:45 **Sylvie Alonso**
Relative contribution of non-structural protein 1 in dengue pathogenesis
- 15:45–16:00 **Autumn LaPointe**
Noncapped genomic RNA are critical for Alphaviral infection and pathogenicity
- 16:00–16:15 **Ifeanyi Ezeonwumelu**
SAMHD1 is a modulator of nucleos(t)ide analogue efficacy
- 16:15–16:30 **Jonathan Kinder**
Examining human metapneumovirus infection and spread in a human airway epithelial model

16:30–17:00 **Coffee Break**

Session 4: *Viral Pathogenesis* (Part II)

Chair: Ioly Kotta-Loizou

- 17:00–17:30 **Martin Beer—Invited Speaker**
Lethal encephalitis of unknown origin - elucidation by metagenomics
- 17:30–17:45 **Luca Zaeck**
High-resolution 3D imaging of virus infections in solvent-cleared organs; novel insights into virus replication and tropism in vivo
- 17:45–18:00 **Saveez Saffarian**
Dynamics in HIV Gag lattice detected by time-lapse iPALM
- 18:00–18:15 **Diego Sebastian Ferrero**
From structure to mechanisms of Zika virus induced neurodevelopmental disease
- 18:15–18:30 **Brett Lindenbach**
A sensitive Yellow Fever Virus entry reporter identifies valosin-containing protein (VCP/p97) as an essential host factor for Flavivirus uncoating
- 18:30–18:45 **Lingyan Wang**
Analysis of Programmed Cell Death Induced by HCV Infection
- 18:45–19:00 **Anna Cliffe**
Reactivation of Herpes Simplex Virus (HSV) from Latency in Response to Neuronal Hyperexcitability

20:30 **Conference dinner**

Day 3: Friday 7 February 2020

Special Session. New Coronavirus Outbreak
Chairs: Eric Freed and Albert Bosch

- 09:00–09:10 **Susan Weiss**
Human Coronaviruses as Emerging Pathogens
- 09:10–09:20 **Christine Prat**
Latest Virus Outbreaks: nCov2019 and Zika Virus, Preparedness is the Key. Lessons Learnt from the Perspective of the "European Virus Archive Global (EVAG)" EU Funded Research Infrastructure
- 09:20–09:30 **Q&A/Discussion Round**

Session 5. Mechanisms of Virus Replication (Part I)
Chair: Jack Stapleton

- 09:30–10:00 **Rosa Pintó – Invited Speaker**
Hepatitis A virus replication stimulates the expression of genes coding for proteins involved in the syndecan-ALIX- mediated exosome biogenesis
- 10:00–10:15 **Selena Sagan**
A moonlighting microRNA: mechanism(s) of miR-122-mediated viral RNA accumulation
- 10:15–10:30 **Alexander Rouvinski**
Functional and structural characterization of novel insect restricted Negev viruses and their interaction with the host cells.

10:30–11:00 **Coffee Break**

Session 5. Mechanisms of Virus Replication (Part II)
Chair: Brett Lindenbach

- 11:00–11:30 **Diane Griffin – Invited Speaker**
Control of alphavirus replication in neurons
- 11:30–11:45 **Oscar Burrone**
Impact of capsid anchor length and sequential processing on the assembly and infectivity of Dengue virus
- 11:45–12:00 **Thejaswi Nagaraju**
4D analyses show that replication compartments are clonal factories in which Epstein-Barr Viral DNA amplification is coordinated
- 12:00–12:15 **Peter Nagy**
Exploitation of host factors and cellular pathways by tombusviruses for the biogenesis of the viral replication organelles
- 12:15–12:30 **John Patton**
Rotaviruses as Neonatal Vaccine Expression Vectors against Other Enteric Pathogen

12:30–14:30 **Lunch**

13:00–14:30 **Poster Session B**

Session 6. Viral Genetics and Evolution (Part I)
Chair: Alex Compton

- 14:30–15:00 **Eric Freed – Invited Speaker**
Mutations in the HIV-1 Envelope Glycoprotein Confer Broad, Multi-Class Drug Resistance

- 15:00-15:15 **Miguel Muñoz-Alía**
Defining Measles virus hemagglutinin antigenic drift by systematic B-cell epitope elimination
- 15:15-15:30 **Nancy Beerens**
Emergence and selection of a highly pathogenic avian influenza H7N3 virus
- 15:30-15:45 **Matthew Evans**
Deep mutational scanning to map how Zika envelope protein mutations affect viral growth and antibody escape
- 15:45-16:00 **Ron Geller**
Globally defining the effects of amino acid mutations across a picornavirus capsid

16:00-16:30 **Coffee Break**

Session 6. *Viral Genetics and Evolution (Part II)*

Chair: Nathalie Arhel

- 16:30-17:00 **Marco Vignuzzi – Invited Speaker**
Good and bad neighbourhoods in viral sequence space: predicting, altering, targeting virus populations
- 17:00-17:15 **Juan Vicente Bou**
Coxsackieviruses undergo intercellular transmission as pools of sibling viral genomes associated to membranes
- 17:15-17:30 **Joanna Kaczorowska**
Revolution in evolution of human “Anellome”
- 17:30-17:45 **Pablo Martinez-Vicente**
Divergent traits and ligand-binding features of the cytomegalovirus CD48 gene family
- 17:45-18:00 **Closing Remarks and Awards Ceremony**

Poster Session A

Thursday 6 February 2020

| | | |
|----|------------------------|--|
| 1 | Richard Brown | Cd302 and Cr11 are novel restrictors of hepatotropic virus cross-species transmission |
| 2 | José Lasalde-Dominicci | A Center for the Rapid Analysis of Clinical-Grade Biologics: The Biophysical Characterization of HIV-1 Env Protein |
| 3 | Oren Kobiler | Abortive Herpes Simplex Virus Infection of Non-Neuronal Cells Results in Quiescent Viral Genomes That Can Be Reactivated |
| 4 | Monique Merchant | An Endogenous Retrovirus from Human Hookworm Encodes an Ancient Phlebovirus-Like Class II Envelope Fusion Protein |
| 5 | Sebastian Dorawa | Characterization of DNA Polymerase from <i>Thermus thermophilus</i> MAT72 Phage Tt72 |
| 6 | Kathakali Das | Characterization of Glycoproteins From Insect-Specific Goutanap and Negev Viruses |
| 7 | Nick De Regge | Comparing Different Larval Food Sources and Temperature Regimes for Rearing of <i>Culicoides obsoletus/scoticus</i> Complex Midges, the Predominant Bluetongue, and Schmallenberg Virus Vectors in Northern Europe |
| 8 | Nicolas Maldonado | Cytopathic BVDV-1 Induces Type I Interferon Expression through IRF-1 and IRF-7 Transcriptional Factors in MDBK Cells |
| 9 | Aliona Špakova | Development of a Tubular Bacteriophage-Based Vaccine Platform that Induces an Immune Response in Mice |
| 10 | Rachel Palinski | Full Genomic Sequencing Of Vesicular Stomatitis Virus Isolates From The 2004-2006 US Outbreaks Reveals Associations of Viral Genetics To Environmental Variables |
| 11 | Hannah Wallace | Knock Out of Cell Death Pathway Components Results in Differential Caspase Expression in Response to HCV Infection |
| 12 | Craig McCormick | KSHV activates unfolded protein response sensors but suppresses downstream transcriptional responses to support lytic replication |
| 13 | Gamaliel López-Leal | Phages Do Encode Antibiotic Resistant Genes in <i>Acinetobacter baumannii</i> |
| 14 | Shan-Lu Liu | Restriction of HIV by TIM and SERINC and Counteraction by Nef |
| 15 | Grace Carter | Resurrecting extinct retroviruses in the chimpanzee genome as a tool to better understand the evolution of simian immunodeficiency virus (SIV) |
| 16 | Xabier Muniz-Trabudua | Siglec-1 Expressed on Dendritic Cells is a New receptor Implicated in Arenavirus Uptake. |
| 17 | Natalia Araujo | The First Complete Genome Sequences of Hepatitis C Virus Subtype 2b from Latin America: Molecular Characterization and Phylogeographic Analysis |
| 18 | Sébastien Nisole | TRIM8 Controls Interferon Response in Plasmacytoid Dendritic Cells by regulating pIRF7 Stability |
| 19 | Maggie Bartlett | Unique Features of Immunity within the Immunoglobulin Heavy Chain Locus of Egyptian Rousette Bats |
| 20 | Mohammad Amin Behzadi | A Cross-Reactive Mouse Monoclonal Antibody against Rhinovirus Mediates Phagocytosis In Vitro |
| 21 | Ella Sklan | A Genome-Wide CRISPR Activation Screen Identifies Genes Involved in Protection from Zika Virus Infection |
| 22 | Berati Cerikan | A novel system to study dengue virus replication organelle formation independent from viral RNA replication |

| | | |
|----|--------------------------|--|
| 23 | Min-Suk Song | A Simple and Rapid Cloning Method for Broad Subtypes of the Influenza A Virus Genome |
| 24 | Ester Ballana | ADAR1 Function Regulates Innate Immune Activation and Susceptibility to Viral Infections |
| 25 | Samuel Connell | African Swine Fever Virus Multigene Family Genes Inhibit the Type-I Interferon Response by Acting on the NF κ B and IRF3 Signalling Pathways at the Transcription Factor Level or Below |
| 26 | Jan Haviernik | An E460D Substitution in the NS5 Protein of Tick-Borne Encephalitis Virus Confers Resistance to the Inhibitor Galidesivir (BCX4430) and Also Attenuates the Virus in Mice |
| 27 | Camila Pereira | An Epitranscriptomic Switch at the 5'-UTR Controls Genome Selection during HIV-1 Genomic RNA Packaging |
| 28 | Han Young Seo | Analysis of cis-Acting RNA Elements Required for Zika Viral RNA Synthesis Initiation by the Viral RNA Polymerase |
| 29 | Ronit Sarid | Lytic Reactivation of the Kaposi's Sarcoma-Associated Herpesvirus (KSHV) Is Accompanied by Major Nucleolar Alterations |
| 30 | Barbara Schnierle | Analysis of Humoral Immune Responses in Chikungunya Virus (CHIKV)-Infected Patients and Individuals Vaccinated with a Candidate CHIKV Vaccine |
| 31 | Haewon Jung | Anti-Norovirus Activity of Orally Administered Neogaroheptaose, a TLR4 Agonist from Red Algae |
| 32 | Fernanda Fredericksen | Antioxidant Effect of <i>A. chilensis</i> on the Production of Infectious Viral Particles of ISAv and Its Consequences on the SUMOylation of NP Protein |
| 33 | Dmitriy Mazurov | Application of SORTS, a Novel Gene-Edited Cell Selection Method for HIV Study and Therapy |
| 34 | Tim Passchier | Architecture of Hendra henipavirus Ribonucleoprotein Complexes Elucidated through Transmission Electron Microscopy |
| 35 | Alba Folgueiras González | Atypical Porcine Pestivirus Molecular Evolution within a Persistently Infected Swine Farm |
| 36 | Sunoh Kwon | bis-Benzylisoquinoline Alkaloids Inhibit Human Coronavirus OC43 Infection in MRC-5 Human Lung Cells |
| 37 | Joan Martí-Carreras | BKTyper—Web Application for VP1 and NCCR Polyoma BK Typing |
| 38 | Aleksandra Maslennikova | Cell Surface-Expressed GPI-Anchored Peptides from the CHR Domain of Gp41 Are Potent Inhibitors of HIV-1 Fusion |
| 39 | Joanne Haney | Characterising Interactions between Influenza A Virus and Respiratory Syncytial Virus During In Vitro Coinfection |
| 40 | Malgorzata Lobočka | Characteristic of a Distant Relative of Teseptimavirus Genus Phages that Acquired the Ability to Lysogenize Its Host |
| 41 | Mirko Cortese | Characterization of Dengue Virus Nonstructural Protein 4A for Its Role in Viral Replication Organelle Biogenesis |
| 42 | Melissa Maginnis | Characterization of JC Polyomavirus Entry by Serotonin Receptors |
| 43 | Florian Bakoa | Characterization of the Neurovirulence of the FNV Vaccine Strain of Yellow Fever Using the BBB-Minibrain Model |
| 44 | Marie Galloux | Characterization of the Respiratory Syncytial Virus N ⁰ -P Complex Paves the Way for Studying the Mechanism of Genome Encapsulation |
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Abstracts

Session 1. General Topics in Virology

Abstract

The Long Road to a Universal Influenza Virus Vaccine

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Seasonal and pandemic influenza virus infections can cause significant disease worldwide. Current vaccines only provide limited, short-lived protection, and antigenic drift/shift in the hemagglutinin (HA) surface glycoprotein necessitates their annual reformulation and re-administration. To overcome these limitations, universal influenza virus vaccine strategies aim at eliciting broadly protective antibodies to conserved epitopes of the HA. We have developed two approaches. (1) The first is based on “chimeric” HA constructs which retain the conserved stalk domain of the HA and have exotic HA heads. Vaccination and boosting with such constructs successfully redirects the immune system in animals and in humans towards the conserved immune sub-dominant domains of the HA stalks; this results in an antigenic silencing of the HA heads and a protective immune response facilitated by the conserved HA stalks. In mice and ferrets such a strategy protects the animals against homo-subtypic and hetero-subtypic challenge with influenza A strains as well as against influenza B variants. It is hoped that vaccine constructs expressing three components (i.e. conserved group 1 HA stalks, conserved group 2 HA stalks, and conserved influenza B HA stalks) will be protective against all future seasonal and pandemic strains. (2) The “mosaic” HA approach is based on antigenic silencing of the major immunodominant antigenic sites of the HA heads by only replacing those epitopes with corresponding sequences of exotic avian HAs, yielding “mosaic” HAs. In mice, a prime-boost vaccination regime with inactivated viruses expressing “mosaic” HAs elicited highly cross-reactive antibodies against the stalk domain of the HAs that were capable of eliciting Fc-mediated effector functions *in vitro*. Extensive trials will be necessary in the future in order to identify the optimal vaccination regime (“chimeric” HA-based versus “mosaic” HA-based) in humans.



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Abstract

Structure of the RSV Polymerase Complex Reveals a Tentacular Arrangement of the Viral Phosphoprotein

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Numerous interventions are currently in the process of clinical development for respiratory syncytial virus (RSV) infection, including the use of small molecules that target viral transcription and replication. These processes are catalyzed by a complex comprising the RNA-dependent RNA polymerase (L) and the tetrameric phosphoprotein (P). RSV P performs many functions, including recruitment of viral proteins to the polymerase complex. Despite their critical roles in RSV transcription and replication, the structures of L and P have remained elusive, though RSV P is thought to be intrinsically disordered in solution, with the exception of its oligomerization domain. Here, we describe the 3.2 Å cryo-EM structure of RSV L bound to tetrameric P. The structure reveals a striking tentacular arrangement of P in which each of the four monomers adopt a distinct conformation. The structure also provides a rationale for the inhibitor-escape mutants and mutations observed in live attenuated vaccine candidates. These results provide a framework for determining the molecular underpinnings of RSV replication and transcription and should facilitate the design of effective RSV inhibitors.



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Abstract

HCMV gB-MF59 Vaccine Primes the Immune System of Seronegative Individuals to Mount Greater Immunological Responses upon Challenge with Virus at the Time of Solid Organ Transplant

Ariane C. Gomes ¹, Ilona Baraniak ¹, Isabela Sodi ¹, Fabien Piras-Douce ², Claire Atkinson ¹, Sylvie Pichon ², Paul Griffiths ¹ and Matthew B. Reeves ¹

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Human cytomegalovirus (HCMV) infection poses a significant threat for prenatals and immunocompromised individuals, including those undergoing bone marrow or solid organ transplant (BMT and SOT, respectively). Although antivirals exist, concerns around toxicity and use in neonates, emergence of drug-resistant strains, and cost drive the need for a vaccine. Arguably, the most successful HCMV vaccine studied to date is the recombinant glycoprotein-B (gB) with MF59 adjuvant (gB/MF59) which, in 3 Phase II trials, demonstrated 43–50% efficacy in preventing HCMV acquisition in seronegative healthy women or adolescents and reduced duration of viremia after SOT. However, the mechanism of vaccine protection in seronegative recipients remains undefined. Pertinently, no evidence for the induction of a neutralising antibody response has been reported in retrospective studies of sera from two of the phase II trials suggesting a more complex mechanism of protection.

The SOT transplant model provides a unique opportunity to study the effects of viral challenge at a known time of infection (i.e. transplant). Thus, we evaluated samples from the high-risk cohort of seronegative SOT patients enrolled in the Phase II gB/MF59 vaccine trial who received organs from seropositive donors. The data show that, recipients of vaccine prior to transplant, mounted a faster gB-specific humoral response post-transplant compared to placebo recipients. Additionally, in contrast to the response induced by the vaccine in the pre-transplant phase, CMV neutralisation by sera post-transplant was readily detected and confirmed to be directed against gB.

Taken together, we hypothesise that the vaccine primed the immune system of seronegative recipients to mount a faster and more robust response following challenge with virus at time of transplant suggesting that a prime-boost response may underpin improved outcome upon HCMV challenge in SOT vaccine recipients.



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Abstract

Insights into the Activity of Second-Generation Maturation Inhibitors against HIV Clade C

Dibya Ghimire, Yuvraj KC and Ritu Gaur

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Maturation inhibitors represent a new underdeveloped class of antiretroviral agents that block virus maturation by binding to the target of protease (PR)–Gag precursor (Pr55^{Gag}). Development of a maturation inhibitor is based on a number of small molecules that are capable of blocking the cleavage event between p24-CA and spacer peptide 1. The bulk of literature on HIV antiretroviral therapy and drug resistance has primarily been derived from HIV-1B, and relatively less is known about the context of HIV-1C, that is responsible for more than 95% of HIV infections of India and half of these infections globally. We and others have shown that presence of maturation inhibitor resistance mutations would make HIV-1B particles either less fit or dependent on these drugs to replicate. By contrast, it is unclear how HIV-1C would naturally acquire these mutations yet remain replication competent in the absence of the selective pressure of maturation inhibitors. Bevirimat, the first-in-class MI, was found to be inactive against HIV-C due to polymorphisms in the SP1 region. We have identified a novel second generation of HIV maturation inhibitors with high potency against HIV clade C (IC₅₀ values in the low nM range). The mutations identified during selection experiments revealed the putative binding pocket of these compounds on HIV-1 subtype C Gag. While working on the role of CA-CTD subdomain in HIV-1 Gag assembly and release, we have found that core glycine-rich residues of the β-turn motif are crucial in Gag-membrane binding, multimerization, and assembly. Furthermore, we have identified a novel mutation in CA-CTD that is dependent on both classes of MIs. In conclusion, our studies provide insights into the mechanistic action of MIs on HIV-1 Gag processing and stabilization.



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Abstract

Mycobacteriophages: Diversity, dynamics, and Therapy

Graham Hatfull

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Bacteriophages—viruses that infect bacteria—are the most numerous biological entities in the biosphere. The phage population is not only vast but dynamic and old, and perhaps not surprisingly, highly diverse genetically. We are exploring and defining this biological diversity by isolating and genomically characterizing individual phages, and comparative genomic analyses. The development of large integrated research-education programs such as the Science Education Alliance Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) program has established a large collection of 15,500 individual phages—all isolated on bacterial hosts within the phylum Actinobacteria—of which over 3000 are fully sequenced. These genomes are highly diverse genetically, even if isolated on the same bacterial strain, and are richly populated in genes of unknown function. We propose that many of these genes are involved in the dynamic relationships between bacteria and their viruses, either promoting or countering viral defense systems, or denying competing phages the opportunity to superinfect infected cells. Examples of these include prophage-mediated defense systems in which integrated prophages of temperate viruses lysogenically express genes that prevent attack by unrelated (heterotypic) phages. These systems are prevalent among temperate phages and can act with exquisite specificity in their defense profiles. Phages can also encode their own counter-defense systems to negate the functions of these prophage-mediated defenses. These contributions to microbial dynamics dictate the phage susceptibility profiles of individual strains of pathogenic bacteria, adding complexity to the prospects for broad therapeutic use of phages against bacterial infections. However, we have shown that administration of a three-phage cocktail engineered and personalized to a specific highly antibiotic resistant strain of *Mycobacterium abscessus* contributes to substantial clinical improvement.



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Abstract

Unveiling Infection Strategies across Diverse Marine Phage–Host Systems

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Bacterial viruses (phages) are amongst the smallest, most powerful biological entities on Earth. Through infection, phages impact host metabolism, bacterial mortality, and evolution. In the oceans, 20%–40% of surface microbes are infected, with 1023 new infections each second. Yet, infections remain virtually uncharacterized, as the available phage isolates underrepresent the diversity of marine phage–host interactions. Additionally, while sequencing efforts reveal ‘who is there?’, a gap between sequence and function prevents answering ‘what are they doing?’ and ‘how?’.

We have developed new Bacteroidetes and Proteobacteria marine phage–host model systems with which to connect genomes, infection strategies, and functions using both traditional and genome-wide ‘omics experiments. We ask: How do infections by genomically divergent phages compare? Are there links between phage–host genomes and infection strategies? Our findings are as follows. In Bacteroidetes, a phage infecting 2 nearly identical strains (host38 and host18) under identical conditions is more fit and efficient on host38. By contrast, on host18, it is less fit and, except for phage transcription, it fails at efficiently mastering all stages of the infection: from adsorption through to cell lysis.

In Proteobacteria, genomically unrelated podovirus and siphovirus phages infecting the same strain reprogram host metabolisms very differently. Namely, siphovirus-infected cells hardly differ from uninfected and mainly repress energy-consuming processes such as motility and translation. By contrast, podovirus-infected cells greatly differ from uninfected cells in transcription and in uniquely shifting central carbon and energy metabolism. Additionally, the siphovirus is more complementary to the host than the podovirus in %GC, amino acids, and codon usage. We found that phage–host genome complementarity may drive the resource demand and fitness of a phage: the phage most complementary to its host easily accesses intracellular resources, infects with little reprogramming, and accomplishes the largest fitness, which has not previously been shown. Together, this work helps to uncover infection efficiency strategies, and connect genomes with metabolisms in marine phage–host systems.



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Abstract

Bacteriophage Depolymerases: Evolutionary Insights and Antivirulence Strategies against Bacterial Pathogens

Hugo Oliveira, Priscila Pires, Luis Melo, Ana Mendes, Alexandra G. Fraga, Jorge Pedrosa and Joana Azeredo

University of Minho, Braga, Portugal

To be able to enter and replicate in exopolysaccharide slime- or capsule-surrounded bacteria, bacteriophages (viruses of bacteria) have evolved the ability to overcome the EPS structure by producing virion-associated proteins with polysaccharide depolymerization activities. We have isolated >20 bacteriophages infecting several species of the *Acinetobacter baumannii*–*Acinetobacter calcoaceticus* complex and demonstrate that they encode depolymerase enzymes to specifically recognize bacterial capsular types as ligands for host adsorption (1). These enzymes are integrated at the C-terminus of bacteriophage tail spikes, genes with high genetic plasticity, to facilitate recognition of a vast variability of polymorphic capsular K antigens of their hosts.

We also show that depolymerases are effective antimicrobial agents against multidrug resistant *A. baumannii*. Heterologously expressed enzymes were shown to be active in several environment conditions, refractory to resistance development, nontoxic to mammalian cells and, most importantly, able to render *A. baumannii* fully susceptible to the host complement effect. Depolymerases can strip bacterial cells from their capsules, which diminishes bacterial virulence and exposes them to the host immune system. This innovative antimicrobial approach has already been applied in (i) *Galleria mellonella* caterpillar, (ii) murine sepsis, and (iii) human serum (3, 4). A single intraperitoneal injection of 50 µg of depolymerase is able to protect 60% of mice from death, with a significant reduction in the pro-inflammatory cytokine profile (4). Overall, this work demonstrates the great diversity of bacteriophage depolymerases, their role in phage infection and evolution, and their therapeutic properties, i.e., as antivirulence drugs against capsulated bacteria.



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Abstract

Bacteriophage Biocontrol of *Acidovorax citrulli*, the Causal Agent of Bacterial Fruit Blotch

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Bacterial fruit blotch caused by *Acidovorax citrulli* is known to be the major threat to cucurbit crop production worldwide. The pathogen can penetrate into seed coat and cause disease symptoms at any stage of plant growth, which results in fruit loss. Two main genotypes (genotype I and II) are reported in *A. citrulli*, in which genotype II is the main cause of BFB in watermelon and group I is known to be causal agent of BFB in melon. To date, there are no commercially available cultivars resistant to BFB, and available strategies could not completely manage the disease. In this study, we aim to isolate bacteriophages to control BFB. Samples collected from watermelon, melon, and pumpkin were used to isolate bacteriophages. All isolated bacteriophages were tested against 42 strains of *A. citrulli*, among which two phages with the ability to lyse a greater number of hosts were selected and characterized. Bacteriophage ACP17 from *Myoviridae* family, with a head size of 100 ± 5 nm and tail of 150 ± 5 nm infected 29 strains of *A. citrulli* mostly belonging to genotype group I, whereas the second isolated bacteriophage, ACPWH from *Siphoviridae*, with a head size of 60 ± 5 nm and tail of 180 ± 5 nm infected 39 *A. citrulli* strains. Genome analysis of both bacteriophages using NGS showed that ACP17 and ACPWH have double-stranded DNA with sizes of 156,972 kb and 424,299 kb, respectively. The presence of bacteriophage as seed coat showed a germination rate of up to 90% in the presence of *A. citrulli* in contrast to untreated seed, which showed no germination, or germinated juveniles with BFB symptoms in the presence of *A. citrulli*. The results of this study show that phage infecting *A. citrulli* can be used as potential biocontrol agent for controlling BFB.



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Abstract

Valosin-Containing Protein (VCP/p97) is a Potential Antiviral Target against Mononegavirales

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The viral order Mononegavirales consist of eight virus families. Members of these families include some of the most infectious (MeV), lethal (EBOV and RABV), and most common viruses (RSV). Despite their medical importance, few vaccines and no antiviral treatments are available for treating infections with these viruses. Being obligate cellular parasites, viruses must rely on the cellular machinery for their replication. One example of this is the widespread use of molecular chaperones, which assist the correct folding of newly synthesized proteins, refold misfolded or aggregated proteins, and play key roles in maintaining proteostasis in cells. Targeting chaperones required for viral replication may, therefore, provide an antiviral approach. In this work, we set out to identify all members of the cytoplasmic chaperone network that are involved in the replication of RSV using an RNA interference screen. Among our hits is valosin-containing protein (VCP; also known as p97), a chaperone involved in ubiquitin-mediated protein degradation, which has been shown to play a role in the life cycle of several viruses. We have investigated the role of VCP during RSV and vesicular stomatitis virus (VSV) infections using specific VCP inhibitors. Our results suggest that VCP activity is necessary for RSV and VSV replication and may constitute a promising antiviral approach for the Mononegavirales.



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Abstract

Honey Bee Viruses, Colony Health, and Antiviral Defense

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Honey bee colony losses are influenced by multiple abiotic and biotic factors, including viruses. To investigate the effects of RNA viruses on honey bees, we infected bees with a model virus (Sindbis-GFP) in the presence or absence of dsRNA. In honey bees, dsRNA is the substrate for sequence-specific RNAi-mediated antiviral defense and is a trigger of sequence-independent/antiviral responses. Transcriptome sequencing identified more than 200 differentially expressed genes, including genes in the RNAi, Toll, Imd, JAK-STAT, and heat shock response pathways, and many uncharacterized genes. To confirm the virus limiting role of two genes (i.e., *dicer* and *MF116383*) in honey bees, we utilized RNAi to reduce their expression *in vivo* and determined that virus abundance increased. To evaluate the role of the heat shock stress response in antiviral defense, bees were heat stressed post-virus infection and virus abundance and gene expression were assessed. Heat stressed bees had reduced virus levels and greater expression of several heat shock genes compared to controls. To determine if these genes are universally associated with antiviral defense, bees were infected with another model virus (flock house virus) or deformed wing virus and gene expression was assessed. *Dicer* expression was greater in bees infected with either FHV or Sindbis-GFP compared to mock infected bees, but not in deformed wing virus infected bees. To further investigate honey bee antiviral defense mechanisms and elucidate the function of key genes (*dicer*, *ago-2*, *MF116383*, and Hsps) at the cellular level, primary honey bee larval hemocytes were transfected with dsRNA or infected with Lake Sinai virus 2 (LSV2). These studies indicate that *MF116383* and Hsps mediate dsRNA detection and that *MF116383* is involved in limiting LSV2 infection. Together, these results further our understanding of honey bee antiviral defense, particularly dsRNA-mediated antiviral responses, at both the individual bee and cellular levels.



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Abstract

A Mycovirus Mediates the Virulence of an Insect-Killing Fungus against the Malaria Mosquito Vector

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The cosmopolitan insect-pathogenic fungus and popular biocontrol agent *Beauveria bassiana* can be used to control *Anopheles* mosquito populations and restrict the spread of malaria, the deadliest vector-borne infectious disease worldwide caused by the protozoan parasite *Plasmodium*. Here, we establish that infection with a double-stranded (ds)RNA mycovirus, Beauveria bassiana polycovirus (BbPmV)-1, significantly reduces *B. bassiana* virulence against *A. coluzzii*, the main vector of malaria. The BbPmV-1-mediated hypovirulence can be at least partially attributed to slow fungal growth on the mosquitos. Analysis of dual next-generation sequencing of the *B. bassiana* and *A. coluzzii* transcriptomes provided insight into the molecular mechanisms of the BbPmV-1-mediated effects. BbPmV-1-free *B. bassiana* has a wide impact on the *A. coluzzii* transcriptome, affecting immunity and metabolism, and led to identification of novel immune response proteins. BbPmV-1 regulates the gene expression profile of its fungal host, directing the use of available resources towards sporulation and suppressing the mosquito immune system. Additionally, BbPmV-1-infected and -free *B. bassiana* strains differentially modulate mosquito gut microbiota; the former reducing the bacterial genus *Elizabethkingia* and the latter *Serratia*. Co-transfection of mosquitos with *B. bassiana* and *P. berghei* revealed a reduction of ookinetes in the presence of BbPmV-1, potentially due to upregulation of a mycotoxin. Finally, BbPmV-1-mediated hypovirulence is at least partially dependent on the *A. coluzzii* RNAi pathway, and silencing of the *dicer-2* gene restores virulence. Taken together, our data clearly demonstrate the crucial role of mycovirus infection in mediating *B. bassiana* virulence against *A. coluzzii* and suggest that BbPmV-1 protects *A. coluzzii* from *B. bassiana*, the mosquito's own immune system, potentially harmful gut microbiota, and *Plasmodium* parasites.



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Abstract

Small RNAs Virome Characterization Reveals Arthropod-Associated Viruses in *Anopheles atroparvus* from the Ebro Delta, Spain

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Even though malaria was eradicated from Europe after the mid-20th century, in 2017, more than 8000 imported cases were reported in the continent. Due to travel routes to endemic areas, climate change, and the presence of native vector mosquitoes (genus *Anopheles*), the re-establishment of autochthonous malaria transmission is a current concern. *Anopheles atroparvus* (Van Thiel, 1972) is one of the nine sibling species within the Palearctic *Anopheles maculipennis* complex, which formerly were considered the main vectors of the disease in the European continent. The microbiota (bacteria and viruses) of vector species has been demonstrated to play a significant role in the biology of these organisms, including their infection susceptibility and their capacity to transmit disease-causing agents. Recently, with the improvement of metagenomics techniques, several viruses that naturally infect vector mosquitoes have been identified. The purpose of the present study was to characterize, for the first time, the virome present in *An. atroparvus* from the Ebro Delta and assess its evolution after ten generations in the laboratory.

Small RNA sequencing was used to characterize the virome from wild-caught *An. atroparvus* females and from the tenth generation produced under controlled laboratory conditions. Through this approach, we were able to identify viral lineages previously reported in other invertebrates, such as Chac virus and several Partiti-like viruses. A reduction in the viral composition was observed during the colonization process.

The present study contributes to the understanding of the viral diversity of a medically relevant vector species in its natural setting and under confinement, and sets a baseline for further studies to assess the potential implications of these viruses in the transmission of pathogens.



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Abstract

The Persistent Infection of Tick Cells by *Hazara orthonairovirus* is Mediated by Virus-Derived DNA Forms

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Crimean-Congo haemorrhagic fever (CCHF) is a severe disease for humans caused by *CCHF orthonairovirus* (CCHFV), a class 4 pathogen. *Hyalomma* ticks are the viral reservoir, and they represent the main vector. CCHFV can be transmitted to its hosts during the tick blood feeding.

We have previously shown that CCHFV can persistently infect *Hyalomma*-derived tick cell lines without any cytopathic effect. However, the mechanism allowing the establishment of a persistent viral infection in ticks is still unknown. It has been recently reported that *Hazara orthonairovirus* (HAZV) can be used as a BSL-2 viral model instead of CCHFV to study viral/vector interaction. The aim of our study is to elucidate the mechanism allowing the establishment of the CCHFV persistent infection in ticks using HAZV as a model. We used classical and molecular methods applied to virology to characterize the establishment of the HAZV persistent infection in two *Hyalomma anatolicum*-derived cell lines, Hae/CTVM8 and Hae/CTVM9.

As for CCHFV, we showed that HAZV persistently infects tick cells without any sign of cytopathic effect, and that cells can be cultured for more than one year. The persistent infection is established in 7–9 days post-infection and viral titer is maintained at a lower level with respect to the earlier time points. Interestingly, short viral derived DNA forms (vDNAs) start to be detected in parallel with the beginning of viral replication and are maintained in persistently infected cells. Experiments with the antiretroviral drug AZT suggest that vDNAs are produced by a retrotranscriptase activity; furthermore, we collected evidence that vDNAs are not integrated and seem to be involved in downregulation of viral replication by promoting cell survival. In conclusion, vDNA synthesis might represent a strategy to control the replication of RNA viruses in ticks, as recently demonstrated in insects, allowing the persistent infection in viral vectors.



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Abstract

Histone Deacetylase Inhibitors Enhance Cell Killing and Block Interferon-Beta Synthesis Elicited by Infection with an Oncolytic Parainfluenza Virus

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Previous results have shown that infection with the cytoplasmic-replicating parainfluenza virus 5 mutant P/V-CPI⁻ sensitizes cells to DNA damaging agents, resulting in the enhanced killing of airway cancer cells. Here, we have tested the hypothesis that histone deacetylase (HDAC) inhibitors can also act during P/V-CPI⁻ infection to enhance cancer cell killing. Using human non-small cell lung cancer and laryngeal cancer cell lines, 10 HDAC inhibitors were tested for their effect on viability of P/V-CPI⁻ infected cells. HDAC inhibitors, such as scriptaid, enhanced caspase-3/7, -8, and -9 activity induced by P/V-CPI⁻ and overall cell toxicity. Scriptaid-mediated enhanced killing was eliminated in lung cancer cells that were engineered to express a protein which sequesters double-stranded RNA. Scriptaid also enhanced cancer cell killing by two other negative strand RNA viruses—the La Crosse virus and vesicular stomatitis virus. Scriptaid treatment enhanced the spread of the P/V-CPI⁻ virus through a population of cancer cells and suppressed interferon-beta induction through blocking phosphorylation and nuclear translocation of interferon regulatory factor 3 (IRF-3). Taken together, these data support a role for combinations of a cytoplasmic-replicating RNA virus, such as the P/V-CPI⁻ mutant, with chemotherapeutic agents.



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Abstracts

Session 2. Structural Virology

Imaging Viral Factories

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Viruses remodel cellular compartments to build their replication factories. Remarkably, viruses are also able to induce new membranes and new organelles. As a result of recent advances in light and electron microscopy, we are starting to be aware of the variety of structures that viruses assemble inside cells. Viral factories are intracellular compartments harboring replication organelles that contain viral replication complexes and the sites of virus particle assembly. This lecture will revise the most relevant imaging technologies for studying the biogenesis of viral replication factories. Live cell microscopy, correlative light and electron microscopy, cryo-TEM, and three-dimensional imaging methods are unveiling how viruses manipulate cell organization. In particular, methods for molecular mapping in situ, in two and three dimensions, are revealing how macromolecular complexes build functional replication complexes inside infected cells. The combination of all these imaging approaches is uncovering the viral lifecycle events with a detail never seen before.



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Abstract

Regulation of RNA Virus Processes by Viral Genome Structure

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The genomes of RNA viruses contain a variety of RNA sequences and structures that regulate different steps in virus reproduction. Events that are controlled by RNA elements include (i) translation of viral proteins, (ii) replication of viral RNA genomes, and (iii) transcription of viral subgenomic mRNAs. Studies of members of the family *Tombusviridae*, which possess plus-strand RNA genomes, have revealed novel ways in which RNA genome structure is utilized to control different viral processes. Recent advances in our understanding of RNA-based viral regulation in select tombusvirids will be presented.



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Abstract

Viral Genome Conformations and Contacts across Different Lifecycle Stages

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Single-stranded RNA viral genomes (gRNA) are dynamic molecules that permit packaging into virions and their subsequent extrusion during infection. For viruses with such genomes, we have discovered a previously unsuspected mechanism that regulates their assembly. This regulation is the result of multiple cognate coat protein (CP)–gRNA contacts distributed across the RNA. Collectively, these interactions make assembly highly efficient and specific. The regions of the gRNA packaging signals (PSs), driving this assembly are potential drug targets, whilst manipulation of PS–CP contacts with nonviral RNA cargos is a route towards bespoke virus-like particles.

Infectivity depends on virions being able to transfer their gRNAs into host cells. The starting point for this transfer appears to be an encapsidated RNA with a defined three-dimensional structure, especially around the PSs. A combination of asymmetric cryo-EM structure determination and X-ray synchrotron footprinting were used to define these contacts and structures in a number of viral examples, including hepatitis B virus and enteroviruses. These tools allow us to look beyond the outer CP layer of the virion shell and to see the functional, asymmetric components that regulate viral infectivity. This revealed yet more unexpected aspects of critical infection mechanisms, such as the RNA conformational changes required for encapsidation, the details of PS–CP contacts regulating assembly, and the conformational “memory” imposed by encapsidation.



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Abstract

Structural Basis for Env Incorporation into HIV-1 Particles

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During the late phase of the HIV-1 replication cycle, the Gag polyproteins are transported to the plasma membrane (PM) for assembly. Gag targeting and assembly on the PM is dependent on interactions between its matrix (MA) domain and phosphatidylinositol 4,5-bisphosphate (PI(4,5)P₂). Subsequent to Gag assembly, the envelope (Env) protein is recruited to the PM for incorporation into virus particles. Evidence suggests that incorporation of Env is mediated by interactions between the MA domain of Gag and the cytoplasmic tail of gp41 (gp41CT), a mechanism that remains to be elucidated. MA trimerization appears to be an obligatory step for this interaction. The interplay between gp41CT, MA trimer, and membrane has yet to be determined. Our lab has pioneered methods and approaches to investigate, at the molecular level, how retroviral MA domains of Gag interact with membranes, a key requirement for understanding Gag assembly and Env incorporation. Herein, we devised innovative approaches that will enable structural characterization of the gp41CT–MA–membrane interactions. We employed structural biology (NMR and cryo-EM), biophysical, and biochemical tools to generate a macromolecular picture of how the MA domain of Gag binds to membrane and how it interacts with gp41CT. Towards this end, we: (i) determined the three-dimensional structure of HIV-1 gp41CT and characterized its interaction with membrane, (ii) engineered trimeric constructs of gp41CT and MA to recapitulate the native and functional states of the proteins, and (iii) utilized membrane nanodisc technology to anchor the MA and gp41CT proteins. Our studies will allow for a detailed structural characterization of the gp41CT–MA–membrane interactions, which will advance our knowledge of HIV-1 Gag assembly and Env incorporation.



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Abstract

Structural Basis for Capsid Recruitment and Coat Formation during HSV-1 Nuclear Egress

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During herpesvirus infection, nascent viral capsids egress the nucleus into the cytoplasm by an unusual mechanism whereby capsids bud at the inner nuclear membrane. This process is mediated by the conserved heterodimeric nuclear egress complex (NEC), anchored to the inner nuclear membrane, that deforms the membrane around the capsid by forming a hexagonal array. However, how the NEC coat interacts with the capsid and how proper curvature of the coat is achieved to enable budding are yet unclear. Here, we show that binding of a capsid protein, UL25, promotes the formation of a pentagonal rather than hexagonal NEC arrangement. Our results suggest that during nuclear budding, interactions between the UL25 bound to the pentagonal capsid vertices and the NEC introduce pentagonal insertions into the hexagonal NEC array to yield an NEC coat of the appropriate size and curvature, leading to productive budding and egress of UL25-decorated capsids.



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Abstract

Conformational Dynamics Related to Membrane Fusion Observed in Single Ebola GP Molecules

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The Ebola virus (EBOV) envelope glycoprotein (GP) is a membrane fusion machine required for virus entry into cells. Following endocytosis of EBOV, the GP1 domain is cleaved by cellular cathepsins in acidic endosomes, exposing a binding site for the Niemann-Pick C1 (NPC1) receptor. NPC1 binding to cleaved GP1 is required for entry; but how this interaction translates to GP2 domain-mediated fusion of viral and endosomal membranes is not known. Here, using a virus-liposome hemifusion assay, and single-molecule Förster resonance energy transfer (smFRET)- imaging, we found that acidic pH, Ca²⁺, and NPC1 binding act synergistically to induce conformational changes in GP2 that drive lipid mixing. Acidic pH and Ca²⁺ shifted the GP2 conformational equilibrium in favor of an intermediate state primed for NPC1 binding. GP1 cleavage and NPC1 binding enabled GP2 to transition from a reversible intermediate to an irreversible conformation, suggestive of the post-fusion 6-helix bundle. Thus, GP senses the cellular environment to protect against triggering prior to arrival of EBOV in a permissive cellular compartment.



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Abstracts

Session 3. Antiviral Innate Immunity

Abstract

Activation and Antagonism of the OAS–RNase L Pathway

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The oligoadenylate synthetase–ribonuclease L (OAS–RNase L) system is a potent antiviral pathway that severely limits the pathogenesis of many viruses. Upon sensing dsRNA, OASs produce 2',5'-oligoadenylates (2-5A) that activate RNase L to cleave both host and viral single-stranded RNA, thereby limiting protein production and virus replication and spread, leading to apoptotic cell death. Endogenous host dsRNA, which accumulates in the absence of adenosine deaminase acting on RNA (ADAR)1, can also activate RNase L and lead to apoptotic cell death. RNase L activation and antiviral activity during infections with several types of viruses in human and bat cells is dependent on OAS3 but independent of virus-induced IFN and, thus, RNase L can be activated even in the presence of IFN antagonists. Differently from other human viruses examined, Zika virus is resistant to the antiviral activity of RNase L and instead utilizes RNase L to enhance its replication factories to produce more infectious virus. Some betacoronaviruses antagonize RNase L activation by expressing 2',5'-phosphodiesterases that cleave 2-5A and thereby antagonize activation of RNase L. The best characterized of these PDEs is the murine coronavirus (MHV) NS2 accessory protein. Enzymatically active NS2 is required for replication in myeloid cells and in the liver. Interestingly, while WT mice clear MHV from the liver by 7–10 days post-infection, RNase L knockout mice fail to effectively clear MHV, probably due to diminished apoptotic death of infected cells. We suggest that RNase L antiviral activity stems from direct cleavage of viral genomes and cessation of protein synthesis as well as through promoting death of infected cells, limiting the spread of virus. Importantly, OASs are pattern recognition receptors and the OAS–RNase L pathway is a primary innate response pathway to viruses and capable of early response, coming into play before IFN is induced or when the virus shuts down IFN signaling.



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Abstract

Modeling of Zika Virus Infection and Antiviral Immunity in 2D and 3D In Vitro Systems of Human Testis

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The spectrum of pathogens that can be sexually transmitted is broad and now includes Zika (ZIKV), Ebola and Lassa viruses as the recently recognized pathogens that can establish persistence in the testes. ZIKV can be detected in human seminal fluid for more than six months after infection and has been associated with low sperm count. Recent data collectively suggest the ability of these viruses to hide in the immune privilege compartment of the seminiferous tubules to escape peripheral immunity. The immunosuppressive environment of testes is tightly governed by an elaborate communication network between different resident cell types, including Sertoli cells (SC) and Spermatogonia stem cells (SSC), that produce cytokines and hormones to maintain local immune homeostasis. Our research focuses on understanding the immune mechanisms underlying ZIKV persistence in the human testis. We demonstrated that human SC were highly susceptible to ZIKV as compared to other testicular cell types. Our *in vitro* human blood-testis barrier model showed that ZIKV could transmigrate across the barrier without affecting the barrier integrity. Subsequent RNA-seq analysis of infected SC revealed modulation of pathways associated with antiviral defense and SC-SSC network. We also found that AXL receptor tyrosine kinase plays a dual role in ZIKV infection of SC by enhancing ZIKV entry and negatively modulating antiviral state. Our recent data also revealed that multicellular 3D human testicular organoids can be used to study long-term infection of ZIKV and its effect on SSC. The ability of ZIKV to establish persistent infection in the testes clearly suggests involvement of unique immune suppressive pathways, characterization of which will provide much-needed insights into the new complications observed in recent RNA virus outbreaks. These newly developed 2D and 3D *in vitro* tools will be valuable in testing efficacy of antiviral drugs to clear testicular infection of ZIKV and other emerging pathogens.



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Abstract

Human IFN- α Subtypes Display Non-Redundant Antiviral Activities against H3N2 Influenza A Virus in the Human Lung

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Influenza viruses (IV) cause recurring outbreaks of respiratory infections. Annual vaccines and antiviral drugs are available, but vaccine strain mismatches and drug resistance require the development of new treatments. Interferon alpha (IFN- α) is a potent inhibitor of IV replication by inducing antiviral restriction factors. Although humans encode 12 subtypes, only IFN- α 2 has been studied for IV treatments, with limited success. Here, the antiviral activities of all 12 human IFN- α subtypes against seasonal H3N2 IV was compared in a translational human study model to identify the best candidate for new antiviral treatments.

Our results demonstrate that human IFN- α subtypes display individual antiviral activities against H3N2 in the human lung. While subtypes α 1 and α 21 did not reduce viral replication, IFN- α 2 displayed intermediate antiviral activity. However, the most potent subtypes α 4, α 5, and α 16 inhibited H3N2 replication up to 230-fold more potently than α 2 in pre- and post-infection settings. Intriguingly, analysis of the IFN- α subtype signature before and after H3N2 infection revealed a preference for the induction of weak subtypes, while potent subtypes α 4, α 5, and α 16 were not induced. Strong antiviral activity was characterized by increased induction of restriction factors MxA, ISG15, OAS1, and PKR. The importance of MxA for subtype-specific antiviral activities was further investigated in Δ MxA cells. In conclusion, this study identified human IFN- α subtypes α 4, α 5, and α 16 as the most promising candidates for the treatment of H3N2 infections. Furthermore, we provide novel insights into the immune regulatory properties of IFN- α subtypes and their dependency on MxA in human lung tissue.



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Abstract

Poxin/vSchlafen: A Novel Viral Strategy to Subvert Cytosolic DNA Sensing

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Cells contain numerous immune sensors for detecting virus infection. A critical sensor is the 2',3'-cyclic GMP-AMP (cGAMP) synthase (cGAS), which recognises cytosolic DNA and mounts strong innate immune responses via cGAMP and stimulator of interferon genes (STING). How activation of the cGAS/STING DNA sensing pathway impacts on host protective responses during viral infection, however, remains ill-defined. Poxviruses are large DNA viruses that, contrary to most, carry out their lifecycle in the cytosol of infected cells. Poxviruses must thus have evolved strategies to subvert cellular cytosolic DNA sensing. Here, we demonstrate that virulent poxviruses, but not attenuated vaccine strains, prevent STING activation and that this antagonism is essential for virulence. We identified viral Schlafen (vSlfn) as the main viral STING inhibitor and showed that ectromelia virus, the causative agent of mousepox and a model for human smallpox and monkeypox, was severely attenuated in the absence of vSlfn. Both vSlfn-mediated virulence and STING inhibitory activity mapped to the recently discovered poxin cGAMP nuclease domain. RNA-sequencing transcriptomics from virus-infected tissues revealed a strong interferon response in the absence of vSlfn that reduced viral titres in target organs and protected animals from subcutaneous, respiratory, and intravenous infection. Interestingly, vSlfn is conserved in all poxviruses transmitted to rodents, but is partially or totally inactivated in poxviruses infecting humans, suggesting an important function in transmission and host range. Our findings demonstrate the importance of unleashing DNA sensing during viral infection and support the manipulation of DNA sensing as an efficient therapeutic strategy in diseases triggered by infectious agents or tissue damage-mediated release of self-DNA.



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Abstract

Mechanism of Inhibition of Viral Fusion by Interferon-Induced Transmembrane Proteins

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Interferon-induced transmembrane protein 3 (IFITM3) inhibits infection of diverse enveloped viruses by blocking virus–cell membrane fusion through a mechanism that is poorly understood. At the same time, arenaviruses, such as the Lassa Fever virus (LASV), are not affected by IFITM3 expression. To elucidate the basis for virus restriction by IFITM3, we visualized co-trafficking and fusion of sensitive Influenza A virus (IAV) and resistant Lassa Fever Virus (LASV) in living cells expressing a fluorescently tagged IFITM3 construct. Single virus and endosome tracking showed that IAV, but not LASV, entered IFITM3-enriched endosomes where the virus remained trapped at a hemifusion stage. In contrast, LASV entered the cells and fused with endosomes lacking detectable levels of fluorescent IFITM3, implying that this virus escapes restriction by utilizing endocytic pathways that are distinct from those transporting IAV. Accordingly, forcing the IFITM3 proximity to LASV glycoprotein through incorporation of the restriction factor into LASV pseudoviruses diminished their ability to undergo fusion. Using an IFITM3-derived membrane-active peptide and giant unilamellar vesicle (GUVs), we found that this peptide inserts into the lipid bilayers and changes membrane curvature. Our results thus demonstrate the need for IFITM3 accumulation at the sites of virus entry and suggest that this factor inhibits the transition from hemifusion to full fusion by imposing an unfavorable curvature to the cytoplasmic leaflet of a cell membrane. This work was partially supported by the NIH R01 grant AI053668.



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Abstract

A Role for the RNA Modification m⁶A at the Virus–Host Interface

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The RNA modification N⁶-methyladenosine (m⁶A) plays an important role in the post-transcriptional control of eukaryotic mRNA fate and dynamically modulates important biological processes. We have previously found that m⁶A modification of viral RNA genomes regulates infection by RNA viruses in the *Flaviviridae* family. However, the function of epitranscriptomic m⁶A modification of cellular mRNA during viral infection is still unclear. Our work now reveals that infection by the *Flaviviridae* members dengue virus (DENV), Zika virus (ZIKV), West Nile virus (WNV), and hepatitis C virus (HCV) similarly alters the m⁶A modification of a set of specific cellular transcripts without affecting the overall distribution of m⁶A across the epitranscriptome. We find that some of the observed changes in m⁶A modification are a result of innate immune sensing and ER stress, cellular pathways that are activated by *Flaviviridae* infection, suggesting that signal transduction from these pathways influences the specificity of the m⁶A methyltransferase complex. We then further elucidate how m⁶A regulates expression of these specific transcripts during *Flaviviridae* infection and how post-transcriptional control of these transcripts by m⁶A impacts viral infection. Taken together, these observations provide insights into how m⁶A modification of cellular transcripts is altered during viral infection and how this influences the outcome of *Flaviviridae* infection.



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Abstract

Noncanonical Interferon-Stimulated Pathways in Antiviral Defense

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The host interferon (IFN) response is one of the first barriers that viruses have to circumvent to establish productive infection. Upon binding of IFN to its receptor, phosphorylation and activation of the JAK/STAT pathway is a well studied event. However, little is known about the activation of other pathways and their role in modulating this response. This led us to investigate how changes in the phosphorylation status of proteins induced by IFN stimulation can activate novel pathways with an impact on the IFN response.

To this end, we conducted label-free quantitative phosphoproteomic analysis and identified several hundreds of proteins with significant changes in serine/threonine phosphorylation upon stimulation with type I, II, or III IFNs. Interestingly, we found many modified proteins with a previously unidentified role in IFN signaling.

In order to address whether they represent novel factors acting at different levels of the IFN response, we first analyzed 68 proteins significantly modified upon stimulation with type I IFN and performed two siRNA screening assays. To study their contribution to IFN signaling, we used reporter A549 cells expressing GFP under an IFN-stimulated promoter; to assess their effect on antiviral activity we used influenza A and vesicular stomatitis reporter viruses (IAV-Renilla and VSVGFP, respectively). Depletion of 17 of these genes showed an impact on IFN-stimulated promoter as assessed by a reduction in reporter GFP expression. Additionally, depletion of 8 of these genes in the presence of IFN significantly rescued IAV-Renilla and VSVGFP replication.

Our current work aims to analyze the impact of modified residues on the function of the factors of interest through the generation of phospho-mutant versions; identify the enzymes responsible for phosphorylation changes; and assign the identified factors to specific signaling pathways. The knowledge gained provides new insights into the molecular mechanisms underlying the IFN response.



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Abstract

Type I Interferon Activity Promotes a Cellular Environment That Supports the Establishment of Latency by Human Cytomegalovirus

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Type I interferons (IFN) are potent inducers of an antiviral state in response to infection and have been demonstrated to inhibit cytomegalovirus (CMV) replication both *in vitro* and *in vivo*. CMV, like all herpes viruses, has the capacity to establish lifelong infections of host through the establishment of latency. As the very early stages of viral entry can trigger IFN responses, we investigated the impact of IFN on the establishment of latent human CMV (HCMV) in myeloid progenitor cells.

Here, we show that priming of myeloid THP1 cells with type I IFN prior to infection skews infection towards a more efficient establishment of latency. This is evidenced by the detection of reduced lytic gene expression, increased latent gene expression, and increased levels of reactivation following differentiation. Blockade of IFN signalling with neutralising antibodies antagonised the latent phenotype, suggesting that endogenous IFN production upon infection contributed to the effect observed. Intriguingly, whilst both IFN α 2 and IFN β can drive latent infection individually, their effects were dose-dependent and demonstrated a biphasic impact on the establishment of latency, with the highest doses of IFN preventing both lytic and latent infection.

These data demonstrate that HCMV derives an unexpected benefit from IFN production. They support a hypothesis that although antiviral in nature, concentration-specific effects of IFN may be evident in the cells, which can modulate different outcomes post-infection in persistent viruses such as HCMV. Work is ongoing to identify the IFN concentration-specific effects responsible for a cellular environment that favours the establishment of latency.



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Abstract

An Inability to Maintain the Ribonucleoprotein Genomic Structure Results in Host Detection of Negative Strand RNA Viruses

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Cellular biology has a uniformity that is not shared amongst viruses. One of the most unique biological strategies for genomic replication derives from negative strand RNA viruses that package their genetic material in a reverse complement orientation. The genomes of negative strand RNA viruses are wrapped around a nucleoprotein (NP) scaffold and associated with a viral RNA-dependent RNA polymerase (RdRp), forming a ribonucleoprotein (RNP) complex readable only by their own RdRp. While this replication strategy provides genomic stability and enables precise stoichiometric balance of each viral component, it poses an inherent dependency on maintaining RNP structure. Here, we demonstrate that inadequate levels of NP culminate in a replicative catastrophe for this viral order as it promotes the production of defective viral genomes and the subsequent induction of an antiviral host response. Moreover, we show that difficulties in maintaining high NP levels must be buffered by a viral antagonist in order to sustain replicative fitness. Given that this dynamic is universal amongst negative strand RNA human pathogens, we propose that NP represents an optimal target for antiviral-based therapeutics.



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Abstract

Interferon Antagonism of Epstein–Barr Virus Tegument Proteins

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The Epstein–Barr virus (EBV) successfully infects 95% of all adults but causes Burkitt’s lymphoma, Hodgkin’s lymphoma, gastric carcinoma, nasopharyngeal carcinoma or other malignancies in only a small subset of infected individuals. The virus must have developed effective viral countermeasures to evade host innate immunity. In this study, we performed functional screens to identify EBV-encoded interferon (IFN) antagonists. Several tegument proteins were found to be potent suppressors of IFN production and/or signaling. The large tegument protein and deubiquitinase BPLF1 antagonized type I IFN production induced by DNA sensors cGAS and STING or RNA sensors RIG-I and MAVS. BPLF1’s ability to suppress innate immune signaling required its deubiquitinase activity. BPLF1 functioned as a catalytically active deubiquitinase for both K63- and K48-linked ubiquitin chains on STING and TBK1, with no ubiquitin linkage specificity. Induced expression of BPLF1 in EBV-infected cells through CRISPRa led to an effective suppression of innate DNA and RNA sensing. Another EBV tegument protein BGLF2 was found to suppress JAK-STAT signaling. This suppression was ascribed to a more pronounced K48-linked polyubiquitination and proteasomal degradation of BGLF2-associated STAT2. In addition, BGLF2 also recruited tyrosine phosphatase SHP1 to inhibit tyrosine phosphorylation of JAK1 and STAT1. A BGLF2-deficient EBV activated type I IFN signaling more robustly. Taken together, we characterized the IFN antagonism of EBV tegument proteins BPLF1 and BGLF2, which modulate ubiquitination of key transducer proteins to counteract type I IFN production and signaling in host cells. Supported by HMRF 17160822, HMRF 18170942, and RGC C7027-16G.



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Abstract

Induction of the Type I IFN Response by Human Metanpneumovirus Lacking SH, G, or M2.2 Expression

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The human metapneumovirus (HMPV), a member of the *Pneumoviridae* family, is a major cause of respiratory illness primarily in young children, the elderly, and immunocompromised individuals. Fundamental understanding of the viral evasion of innate immune responses is crucial for the rational design of antiviral therapies. Several studies have reported on how HMPV subverts innate immune responses, with roles for SH, G, and M2.2 proteins. However, reports are often conflicting. It has also been reported that eliminating the M2.2 ORF results in insertions and deletions around the M2.2 ORF, which could result in an M2.2-independent interaction with the immune system. We aimed to investigate how HMPV interacts with the innate immune response. Therefore, recombinant viruses lacking M2.2, SH, or G protein expression were generated either by deletion or by ablation of protein expression through mutations. Phenotypic analysis revealed that viruses lacking M2.2 expression are attenuated on interferon-competent A549 cells, but not on interferon-deficient cells. Deletion of ORFs compared to ablation of expression through mutations did not result in differences in replication kinetics. Viruses lacking M2.2 expression induced interferon- β protein production, indicating interferon-antagonistic functions of the M2.2 protein, as previously reported. Phenotypic analysis of A549 cells knocked out for RIG-I, MAVS, and PKR revealed the role of RIG-I in the immune response towards HMPV. Next-generation sequencing analysis of viruses lacking M2.2 expression, but not G or SH expression, showed hypermutation throughout the virus genome. The hypermutation patterns suggest a role for ADAR editing. We addressed the question of whether RIG-I activation by viruses lacking M2.2 expression is due to hypermutated genomes or the absence of M2.2 as interferon antagonist. Additionally, we investigated the role of ADAR in HMPV infection. We present our data on the possible influence of ADAR in HMPV infection by next-generation sequencing of viral stocks in cell knockdowns of ADAR generated by CRISPR-interference.



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Abstract

Peroxisomes as Platforms for Cytomegalovirus' Evasion from Cellular Antiviral Signaling

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Peroxisomes, in concert with mitochondria, have been established as platforms for the establishment of a rapid and stable antiviral immune response, due to the presence of MAVS (mitochondrial antiviral signaling protein) at their membranes. Upon intracellular recognition of viral RNA, RIG-I (retinoic acid inducible gene-I)-like proteins interact with MAVS, inducing its oligomerization and the establishment of a signaling cascade that culminates with the production of direct antiviral effectors, preventing important steps in viral propagation.

We and others have demonstrated that different viruses have developed specific mechanisms to counteract peroxisome-dependent antiviral signaling. We have shown that the human cytomegalovirus (HCMV) protein vMia hijacks the peroxisome transport machinery to travel to the organelle, interact with MAVS, and inhibit the immune response.

Here, we further unravel the mechanisms by which HCMV is able to evade peroxisome-dependent antiviral signaling. We demonstrate that vMIA localizes at the peroxisomes in a complex with MAVS and STING (stimulator of interferon genes). Furthermore, vMia interacts with MFF (mitochondrial fission factor) at the peroxisomal membrane, which we show to be essential for vMia's-dependent inhibition of the antiviral immune response. Importantly, we demonstrate that vMia's interaction with MAVS impedes its oligomerization and the consequent activation of the downstream signaling cascade. Interestingly, our results underline important differences between vMIA's mechanisms of action at peroxisomes and mitochondria.

Our results unravel novel mechanisms involving the interplay between the human cytomegalovirus and peroxisomes that may ultimately contribute to the discovery of novel targets for antiviral combat.



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Abstracts

Session 4. Viral Pathogenesis

Abstract

Beyond CRISPR: The Immune System of Bacteria

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The arms race between bacteria and phages led to the evolutionary development of sophisticated anti-phage defense systems, among which is the CRISPR-Cas system. As CRISPR-Cas is present in less than 50% of all bacteria, it is conceivable that additional bacterial anti-phage defense systems are yet to be discovered. The talk will discuss a systematic effort for the discovery of new defense systems that are located in “defense islands” in microbial genomes, and progress in understanding their abundance and mechanisms of action. Specifically, we will report surprising parallels between the human and the bacterial innate immune systems.



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Abstract

Relative Contribution of Nonstructural Protein 1 to Dengue Pathogenesis

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Dengue is an arthropod-borne viral disease that is endemic in the subtropical and tropical world, and ranges from mild to life-threatening conditions, including hemorrhage and shock, with the latter resulting from increased vascular permeability. There are no effective treatments against dengue and the only vaccine available on the market (Dengvaxia), a live attenuated vaccine, has been facing a serious setback due to the recent discovery that it could increase the risk of severe dengue in serologically dengue naïve vaccinees. As such, subunit dengue vaccine approaches have regained some traction, and the nonstructural 1 (NS1) protein of dengue virus (DENV) has surfaced as a potential candidate. Recent studies have reported that NS1 plays a significant role in dengue pathogenesis, whereby secreted NS1 (sNS1) was shown to interact with the endothelium and induce vascular leakage both in vivo and in vitro. Consistently, NS1 immunity was found to protect against dengue in symptomatic mouse models. Those studies were conducted using either mouse-adapted DENV strains or DENV strains of poor in vivo fitness that necessitates a very high infectious dose to establish infection. However, we found that NS1 immunization failed to confer protection in symptomatic dengue mouse models using two non-mouse-adapted DENV2 strains that are highly virulent. Furthermore, although purified NS1 increased vascular permeability in vitro, exogenously administered NS1 did not enhance vascular leakage and disease severity in sublethally infected mice. Virus chimerization approaches supported that the prME structural region, but not NS1, plays a critical role in driving in vivo fitness and virulence of the virus through induction of key pro-inflammatory cytokines. This work highlights that the role of NS1 in dengue pathogenesis is DENV strain-dependent and warrants re-evaluation of NS1 as a dengue vaccine candidate.



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Abstract

Noncapped Genomic RNA Are Critical for Alphaviral Infection and Pathogenicity

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Alphaviruses are positive-sense RNA arthropod-borne viruses that represent a significant threat to public health. During alphaviral replication, significant quantities of viral genomic RNAs that lack a canonical 5' cap structure are produced and packaged into viral particles, despite the fact that the noncapped genomes cannot be translated and are essentially noninfectious. Previously, we have reported that the capping efficiency of nsP1, the alphaviral capping enzyme, of Sindbis virus (SINV) could be modulated via point mutation. It was found that increasing RNA capping efficiency led to decreased viral growth kinetics via decreased particle production, despite increased innate immune evasion; whereas decreasing capping efficiency had wild type growth kinetics and particle production. This led to the conclusion that the noncapped viral RNAs meaningfully contribute to the biology of alphaviral infection at the molecular level. To determine the importance of the noncapped viral RNAs *in vivo*, we characterized the impact of altered capping efficiency in a murine model of infection utilizing a neurovirulent strain of SINV. Mice infected with the nsP1 mutant with decreased capping exhibited wild type rates of mortality, weight loss, and neurological symptoms. Conversely, the mice infected with the increased capping nsP1 mutant showed significantly reduced mortality and morbidity compared to mice infected with wild type virus. Interestingly, viral titers in the ankle, serum, and brain were equivalent between the wild type virus and the two mutant viruses. Importantly, examination of the brain tissue revealed that mice infected with the increased capping mutant had significantly reduced immune cell infiltration and expression of proinflammatory cytokines compared to the decreased capping mutant and wild type virus. Collectively, these data indicate that the noncapped viral RNAs have important roles during the early and late stages of alphaviral infection and suggest a novel mechanism by which noncapped viral RNA aids in viral pathogenesis.



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Abstract

SAMHD1 is a Modulator of Nucleos(t)ide Analogues' Efficacy

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Nucleos(t)ide analogues are commonly used in the treatment of infectious disease and cancer. SAMHD1 is a dNTP triphosphohydrolase involved in the regulation of intracellular dNTP pool, whose function has been linked to viral restriction, cancer development, and autoimmune disorders. Here, we evaluate SAMHD1 function on antiviral and antiproliferative efficacy of a wide range of nucleos(t)ide analogues currently used to treat infections and cancer. Anti-HIV-1 and cytotoxic activity of compounds was assessed in primary and established cell lines in the presence or absence of SAMHD1. SAMHD1 effectively modified the anti-HIV-1 activity of all nucleos(t)ide analogues tested, whereas sensitivity to a non-nucleoside inhibitor (nevirapine) or nucleoside phosphonates (cidofovir and tenofovir) was not affected. Interestingly, SAMHD1 could either enhance (gemcitabine, capecitabine, fluorouracil, and floxuridine) or inhibit (Ara-C, fludarabine, cladribine, clofarabine, and nelarabine) the antiviral potency of anticancer analogues, an effect that was not dependent on the specific nucleotide targeted. When cytotoxicity was evaluated, SAMHD1-dependent changes were less evident and restricted to the increased efficacy of fluorouracil and floxuridine and reduced efficacy of nelarabine and ara-C in the presence of SAMHD1. In summary, our results demonstrate that SAMHD1 modifies the efficacy of a wide variety of nucleoside analogues used to treat infections, cancer, and other diseases. In addition, the anti-HIV activity of nucleos(t)ide analogues may represent a more sensitive measure of SAMHD1 impact on drug efficacy. Thus, modulation of SAMHD1 function may constitute a promising target for the improvement of multiple therapies.



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Abstract

Examining Human Metapneumovirus Infection and Spread in a Human Airway Epithelial Model

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Human metapneumovirus (HMPV) is a leading cause of respiratory illness, infecting most people by the age of 5, with reinfection common throughout life, and children, the elderly and immunocompromised individuals are most likely to be seriously impacted by HMPV infection. While one of the most common causes for hospitalizations for respiratory infections, there are still no therapeutics or vaccinations against HMPV. To better understand HMPV infection, analysis of viral entry and spread in physiologically relevant systems is needed. Here, we utilized human airway epithelium (HAE) tissues as a 3-D model system to analyze critical aspects of viral lifecycle including infection, replication body formation, cellular morphology modification and viral spread. These tissues more accurately recapitulate the lung environment including cellular polarization, functional cilia and mucous production. HMPV is able to infect the apical surface and demonstrates efficient spread up to 48hpi but decreases from 72hpi up to 144hpi. Apical release of HMPV was poor, supporting findings in standard tissue culture that HMPV exists primarily in a cell associated form. Similar to what was seen in non-polarized monolayers, HMPV forms inclusion bodies in infected cells in the HAE cultures, and these inclusion bodies contain N, P and vRNA localized to punctate cytosolic structures. Pre-treatment of virus with a monoclonal antibody (54G10) targeting the fusion protein (F), completely inhibited viral entry. Conversely, cells infected and subsequently treated with 54G10 modestly, but significantly inhibited spread, supporting that spread of HMPV is primarily cell-to-cell mediated as opposed to release and reentry. Thus, this work identifies key aspects of the HMPV lifecycle using a physiologically relevant model system of viral infection.



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Abstract

Lethal Encephalitis of Unknown Origin-Elucidation by Metagenomics

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Novel and (re)emerging viruses cause frequent threats to both human and animal health. Diagnostic metagenomics using unbiased next-generation sequencing (NGS) is the key method for the identification of new pathogens. With the today available state-of-the-art platforms, NGS can be broadly used to identify also novel and unknown pathogens in different sample materials (even as point-of-care diagnostics) or to characterize the complete genomes of all types of pathogens. Nevertheless, these extreme numbers of sequence fragments resulting from NGS-analyses requires not only novel diagnostic pipelines including powerful software tools for big data analysis, but also a new dimension of knowledge and resources. We developed and validated therefore a universal workflow for metagenome diagnostics for the analysis of disease syndromes in both animals and humans. The metagenomics-pipeline will be presented and several examples with the detection and characterization of novel viruses will be shown. The power of diagnostic metagenomics will be presented with different examples focussing on lethal encephalitis cases in both animals and humans where we were able to identify a series of novel or unexpected viral pathogens. Furthermore, the detection of zoonotic pathogens was only possible by a “one-health” approach and the close relationship between veterinary and human medicine. The major aim of the presentation is to get an idea about the capabilities of modern NGS-based metagenomics, and to learn more about the newly detected viral pathogens. Since a large proportion of severe encephalitis cases still remains unexplained, a main conclusion is the recommendation that those cases should be analysed by using a modern and powerful metagenomics workflow.



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Abstract

High-Resolution 3D Imaging of Virus Infections in Solvent-Cleared Organs: Novel Insights into Virus Replication and Tropism In Vivo

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The visualization of infection processes in relevant tissues and organs using microscopy methods reveals a unique link between the distribution, tropism, and abundance of pathogens and the physiological structure of the respective organ. To dissect virus replication and the host reaction in vivo at both a global and a single-cell level, conventional 2D imaging approaches can only provide limited insight. However, pathological studies of infected organ material are still mostly restricted to immunostaining of thin sections from paraffin-embedded or frozen samples. While 3D analysis of large tissue volumes is possible via laborious serial sectioning, a variety of problems and artifacts remain. Modern immunostaining-compatible tissue clearing techniques allow for seamless 3D visualization of infection sites in optically cleared thick tissues sections or even entire organs. Benefiting from pure optical slicing, this approach enables the acquisition of multicolor high-volume 3D image stacks for coherent qualitative and quantitative analyses of the infection and its surrounding cellular environment. Here, we demonstrate the utility and power of this methodology by visualizing virus infections in different target tissues. For instance, we reconstructed the cellular context of rabies virus infection sites in mouse brain tissue, allowing thorough investigation and quantitative analysis of rabies virus cell tropism. Systematic comparison of different rabies viruses with varying pathogenicity revealed a remarkable difference for highly virulent street rabies viruses and attenuated lab strains. While virus protein expression was readily detectable at a comparable level in both neurons and non-neuronal glial cells from brains of mice infected with street rabies viruses, it was virtually absent in glial cells of lab strain-infected mice. These data provide novel and detailed insights into the pathogenesis of virus infections and substantially contribute to an improved understanding of virus–host interactions in vivo.



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Abstract

Dynamics in HIV Gag Lattice Detected by Time-Lapse iPALM

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Immature HIV virions have a lattice of Gag and Gag-Pol proteins anchored to the lumen of their envelope. This lattice has significant void spaces that were previously characterized by fluorescence correlation spectroscopy, cryoelectron tomography, and iPALM imaging. In the current study, we demonstrate that HIV virus-like particles (VLPs) assembled by the viral protein Gag tagged at its C terminus with the photoactivable fluorescent protein Dendra, are slightly enlarged (185 ± 20 nm) compared to virus-like particles assembled using only HIV Gag (140 ± 15 nm). We show that the Gag-Dendra lattice observed within these VLPs has similar gaps as observed in Gag-only VLPs. We further used time-lapse iPALM microscopy to image the Gag-Dendra lattice within the lumen of VLPs at two time points separated by 30 minutes. Reconstruction of these time-lapse images shows significant lattice dynamics within the lumen of purified VLPs. Addition of disuccinimidyl suberate (DSS) to the purified VLPs completely abrogated these dynamics. A method to quantify the dynamics of the Gag-Dendra lattice using correlation function analysis is further presented. The HIV Gag lattice, along with the structural lattices of many other viruses, have been mostly considered static. Our study provides an important tool to investigate the dynamics within these lattices and also highlights the effects of fluorescent tags on virion structures.



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Abstract

From Structure to Mechanisms of Zika Virus-Induced Neurodevelopmental Disease

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Zika virus (ZIKV) has explosively emerged over recent years, causing a series of epidemics across the Western world. Neonatal microcephaly associated with ZIKV infection has already caused a public health emergency of international concern. As with other members in the *Flaviviridae* family, ZIKV relies on its nonstructural protein 5 (NS5) for RNA genome capping (by the methyltransferase N-terminal domain) and replication (by the RNA-dependent RNA polymerase (RdRP) domain), representing an attractive crystallisable and antiviral target.

The crystal structures of the ZIKV NS5 protein in two different space groups revealed conserved protein self-interactions to form dimers and higher-order fibrillar oligomers that serve as a platform for the coordination of the different enzymatic activities across neighboring molecules. The presence of dimers in solution was further verified by small angle X-ray scattering (SAXS), analytical ultracentrifugation (AU), and mass spectrometry, and ZIKV/NS5 helicoidal fibers were also observed by negative staining transmission electron microscopy (TEM) and atomic force microscopy (AFM). In addition, our preliminary data indicate that NS5 oligomerization might act as scaffold to interact with host proteins.

In order to extend our findings, we have studied the *in vivo* effects of ZIKV NS5, both wild type and mutants in which NS5 oligomerization was disrupted, and these revealed an unexpected role of this protein in the exhaustion of neural progenitor cell (NPC) pool that may contribute to ZIKV-induced microcephaly. We have also identified a cluster of cilia/centrosome and nuclear envelope proteins of host cells as NS5 interactors. Work is currently ongoing to determine how NS5 interferes with the molecular machinery and behavior of NPCs to provide a better understanding of ZIKV–host interactions, highlighting new potential targets for therapeutic intervention.



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Abstract

A Sensitive Yellow Fever Virus Entry Reporter Identifies Valosin-Containing Protein (VCP/p97) as an Essential Host Factor for Flavivirus Uncoating

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Flaviviruses are enveloped, arthropod-borne, positive-strand RNA viruses that cause significant human disease. While the basic mechanisms of flavivirus entry and fusion are understood, little is known about the post-fusion events that precede RNA replication, such as nucleocapsid disassembly. We recently developed a sensitive, conditionally replication-defective yellow fever virus (YFV) entry reporter to quantitatively monitor the translation of incoming, virus particle-delivered genomes. We validated that viral gene expression can be neutralized by YFV-specific antisera and requires known pathways of flavivirus entry; however, as expected, gene expression from the defective reporter virus was insensitive to a small molecule inhibitor of YFV RNA replication. The initial round of viral gene expression was also shown to require: (i) cellular ubiquitylation, consistent with recent findings that dengue virus capsid protein must be ubiquitylated in order for nucleocapsid uncoating to occur; (ii) valosin-containing protein (VCP)/p97, a cellular ATPase that unfolds and extracts ubiquitylated client proteins from large macromolecular complexes. RNA transfection and washout experiments showed that VCP/p97 functions at a post-fusion, pre-translation step in YFV entry. Together, these data support a critical role for VCP/p97 in the disassembly of incoming flavivirus nucleocapsids during a post-fusion step in virus entry.



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Abstract

Analysis of Programmed Cell Death Induced by HCV Infection

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BACKGROUND:

Virus infection results in host cell damage through various types of programmed cell death. Pyroptosis is a caspase-1-dependent, pro-inflammatory form of programmed cell death that occurs following inflammasome activation. Therefore, pyroptosis may play roles in the development of liver pathogenesis and hepatocellular carcinoma in HCV-infected individuals. The objective of this study was to identify cellular mechanisms by which HCV infection induces programmed cell death.

METHODS:

Infection and transfection experiments were performed in Huh-7.5 and Huh-7.5 CRISPR knockouts for NLRP3, caspase-3, or Gasdermin D. The expression of pro-caspase-1, cleaved (c)-caspase-1, pro-caspase-3, c-caspase-3, HCV core protein, and GAPDH were determined by Western blotting. Virus titers were determined by limiting dilution focus-forming assays.

RESULTS:

When Huh-7.5 cells were infected with HCV, we observed activation of caspase-1 (pyroptosis) at 2 days p.i. and activation of caspase-3 (apoptosis) on day 3. Caspase-1 activation was not observed on day 2 when HCV infection was performed in NLRP3 knockout cells. We also observed lower levels of virus titer in caspase-3 and NLRP3 knockout cells compared to parental Huh-7.5 cells.

CONCLUSION:

Pyroptosis is induced earlier than apoptosis during HCV infection. Moreover, HCV-induced pyroptosis is mediated by the NLRP3 inflammasome. Finally, lytic program cell death promotes HCV replication most likely through enhancement of virus spread. The findings from this study have the potential to identify mechanisms underlying chronic liver inflammation and viral spread in HCV patients.



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Abstract

Reactivation of Herpes Simplex Virus (HSV) from Latency in Response to Neuronal Hyperexcitability

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HSV establishes a latent infection in neurons, in which viral transcription is restricted and viral promoters are associated with heterochromatin. In response to certain stimuli, the virus reactivates to permit transmission. The exact physiological triggers of reactivation, the cell signaling pathways involved, and how signals act on heterochromatin-associated lytic promoters is not understood. Previously, we identified a role for a neural stress pathway involving DLK and JNK activity in HSV reactivation triggered by nerve growth factor (NGF) deprivation. Reactivation was associated with a JNK-dependent histone phospho/methyl switch on lytic gene promoters. Because the same histone phospho/methyl switch occurs in cortical neurons following hyperexcitability (triggered by forskolin) we examined whether HSV reactivation was linked to hyperexcitability and the contribution of JNK activity and histone phosphorylation. Using our primary neuronal model of HSV reactivation, we found that forskolin triggered DLK/JNK-dependent reactivation via a pathway that was distinct from NGF deprivation. The initial burst of HSV lytic gene expression in response to forskolin occurred independently of histone demethylase activity and was accompanied by a histone phospho/methyl switch. To determine whether forskolin-mediated reactivation was linked to neuronal activity, we investigated the contribution of ion channel activity. Inhibition of voltage-gated potassium and sodium channels, or hyperpolarization-activated cyclic nucleotide-gated channels, prevented forskolin-mediated reactivation. In addition, hyperexcitability resulting from removal of a tetrodotoxin block triggered HSV reactivation in a DLK/JNK-dependent manner. We next investigated whether physiological triggers induce HSV reactivation via hyperexcitability. IL-1 induced DNA damage associated with hyperexcitability in adult neurons. IL-1 also triggered DLK/JNK-dependent HSV reactivation that was dependent on ion channel activity. Therefore, these data indicate that neuronal hyperexcitability in response to physiological stimuli, such as inflammation, trigger HSV reactivation and place activation of DLK/JNK and a histone phospho/methyl switch as key events in the hyperexcitability-mediated reactivation.



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Abstracts

Session 5. Mechanisms of Virus Replication

Abstract

Cell Entry by Quasi-Enveloped and Naked Hepatoviruses

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Hepatoviruses are unusual picornaviruses, distinct genetically and structurally from other members of the *Picornaviridae*, exclusively hepatotropic, and released from infected cells without lysis in small membranous vesicles resembling exosomes. These quasi-enveloped virions (eHAV) are infectious and the only form of virus found circulating in blood during acute infection. By contrast, naked virions are shed in feces, having been stripped of membranes by bile salts during passage from the liver through the biliary system. Naked virions are exceptionally stable, promoting efficient inter-host transmission through the environment, whereas the membranes cloaking quasi-enveloped virions protect the virus from neutralizing antibodies, facilitating stealthy spread of infection in newly infected hosts. Since quasi-enveloped eHAV lacks virus-encoded surface proteins, its mechanism of cell entry has been enigmatic. Previous studies in our laboratory have shown that both virion types are internalized primarily by clathrin- and dynamin-dependent endocytosis, facilitated by integrin β_1 , followed by trafficking through early Rab-5A⁺ and late Rab-7a⁺ endosomes. eHAV undergoes further ALIX-dependent trafficking to LAMP1⁺ lysosomes where the quasi-envelope is enzymatically degraded. Although TIM1 (HAVCR) was reported many years ago to be a receptor for HAV, it is not essential for infection with either virion type and acts only to facilitate eHAV entry by binding phosphatidylserine on its surface. Late steps in entry, after degradation of the eHAV membrane, remain uncertain. Remarkably, neither virion type requires PLA2G16 for infection, although this phospholipase is essential for successful transfer of the RNA genome of many -other picornaviruses to the cytoplasm. This, and other unusual features of HAV, including the fact that the assembly of capsid pentamers is driven by the C-terminal pX domain of VP1 rather than VP4, and the exceptional stability of the capsid, greatest at the low pH of endolysosomes, suggest an atypical mechanism for HAV uncoating and genome release.



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Abstract

Hepatitis A Virus Replication Stimulates the Expression of Genes Coding for Proteins Involved in Syndecan–ALIX-Mediated Exosome Biogenesis

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HAV egresses from infected cells cloaked in exosomes, forming quasi-enveloped particles (eHAV). These exosomes contain, among others, ALIX, syntenin and Rab7 proteins, which play a key role in syndecan–ALIX-mediated exosome biogenesis and release. However, while ALIX is critical for eHAV biogenesis, the role of the other two proteins is unclear.

Using two HAV strains (L0 and HP) which differ in their replication capacity, we have analyzed the expression pattern of several genes coding for proteins potentially involved in syndecan–ALIX-mediated exosome biogenesis (syndecan, syntenin, ALIX, TSG101) and release (Rab7, Rab11, Rab27) in the hepatocyte-derived Huh7-AI cell line.

The expression pattern did not significantly differ between non-polarized and polarized hepatocytes, with syntenin, *Rab11*, and *ALIX* showing the highest expression levels. Infection of non-polarized cells with the L0 strain was associated with a moderate increase in the expression of ALIX and syntenin. By contrast, infection with the HP fast-growing strain was associated with a more significant increase in the expression of *ALIX*, syntenin, and *Rab7*. Syntenin and *Rab7* expression also increased during the infection of polarized cells, but only for the HP strain.

Additionally, we analyzed the infection of polarized Huh7-AI cells, grown in Transwell plates, through the basolateral and apical membranes. Surprisingly, infection was more efficient through the apical than through the basolateral membrane for both strains. Virus release was also more efficient through the apical membrane, independently of the entry route for both strains. For infection through the apical membrane, no differences were observed between the release patterns of both strains. However, in infections through the basolateral membrane, no differences were observed regarding the apical release, but the basolateral release was more efficient for the fast-growing strain.

Altogether, these results suggest the involvement of Rab7 in the eHAV basolateral release and of Rab11 in the apical release.



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Abstract

A Moonlighting microRNA: Mechanism(s) of miR-122-Mediated Viral RNA Accumulation

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Hepatitis C virus (HCV) is a positive-sense RNA virus that interacts with a human-liver-specific microRNA, termed miR-122. miR-122 binds to two sites in the 5' untranslated region (UTR) of the viral genome, and this interaction *promotes* HCV RNA accumulation. This interaction is important for viral RNA accumulation in cell culture, and miR-122 inhibitors have been demonstrated to be efficacious in reducing HCV titers in chronic HCV-infected patients. However, the precise mechanism(s) of miR-122-mediated viral RNA accumulation have remained elusive. We have used biophysical analysis and assays for viral replication in cell culture to understand interactions between the human Argonaute 2 (hAgo2):miR-122 complex and the HCV genome. In addition, we have analyzed several resistance-associated variants isolated from patients who underwent miR-122 inhibitor-based therapy to shed light onto novel mechanisms of antiviral resistance. Our results provide a new model for miR-122:HCV RNA interactions and demonstrate that miR-122 plays at least three roles in the HCV life cycle: (1) miR-122 acts as an RNA chaperone to suppress an energetically favorable secondary structure and allows the viral internal ribosomal entry site (IRES) to form; (2) miR-122 binding to the 5' terminus protects the genome from the activity of cellular pyrophosphatases (DOM3Z and DUSP11) and subsequent exonuclease-mediated decay; and (3) the Argonaute (Ago) protein at Site 2 makes direct contact with the HCV IRES, enhancing viral translation. In addition, analyses of several resistance-associated variants isolated from patients that underwent miR-122 inhibitor-based therapy suggests that mutations in the 5' terminus alter the structure of the 5' UTR in a manner that promotes RNA chaperone activity or viral genome stability, even in the absence of miR-122. Taken together, these findings provide insight into the mechanism(s) of miR-122-mediated viral RNA accumulation and suggest new mechanisms of antiviral resistance mediated by changes in RNA structure.



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Abstract

Functional and Structural Characterization of Novel Insect-Restricted Negev Viruses and Their Interaction with Host Cells

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Many viruses transmitted by blood-sucking arthropods have emerged worldwide and cause serious human and animal diseases. Arthropod-borne (arbo-)viruses possess a peculiar capacity to replicate in very different hosts, like mosquitoes and mammals (e.g., dengue and chikungunya). However, the molecular mechanisms that enable arboviruses to enter and replicate in very different hosts, such as arthropods, mammals, and birds, remain highly enigmatic. Insect-restricted viruses provide a reference point regarding this issue, since they lack the strategies of infecting vertebrate hosts and are viewed as evolutionary precursors of arboviruses. Negev viruses comprise a recently discovered insect-restricted taxon exhibiting worldwide distribution among blood sucking arthropods. Negev viruses are enveloped, positive sense, nonsegmented RNA viruses. The virus genome is ca. 10 kb long and contains three ORFs, with the longest *ORF1* (7 kb) encoding putative replicative enzymes. *ORF2* (1.2 kb) and *ORF3* (0.7 kb) do not have any clear homologs and are predicted to encode membrane glycoproteins. We explored the functional and structural aspects of Negev viruses and the role of their membrane glycoproteins during Negev virus entry into the mosquito cells. We employ biochemistry, structural biology, and microscopy to reveal mechanisms of Negev virion organization, and its entry into host cells followed by fusion with the host membranes to allow subsequent genome delivery and replication.



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Abstract

Control of alphavirus replication in neurons

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Sindbis virus causes age-dependent encephalomyelitis in mice. Young mice and immature neurons replicate virus to high titers and die from infection while older mice and mature neurons restrict replication and survive infection. Studies to identify factors that affect maturation-dependent virus replication in neurons have used AP-7 rat olfactory neuronal cells that can be differentiated in vitro, including host transcription factors interferon regulatory protein 7 and NF- κ B and viral proteins E2 and nsP3. IRF7 is required for neuronal survival and deficiency leads to increased susceptibility to TNF α in vitro and paralysis and death of 4-6 week old C57BL/6 mice. Activation of NF- κ B works in concert with PKR to facilitate replication in mature neurons by promoting shut off of host protein synthesis and increasing translation of the viral structural proteins from subgenomic RNA. The macro domain of nsP3 binds and hydrolyses ADP-ribose from ADP-ribosylated proteins and is important for initiation of neuronal infection and for synthesis of viral structural proteins. Thus, neurons regulate translation of the structural proteins from subgenomic RNA required for production of infectious virus.



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Abstract

Impact of Capsid Anchor Length and Sequential Processing on the Assembly and Infectivity of Dengue Virus

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The assembly and secretion of flaviviruses are part of an elegantly regulated process. During maturation, the viral polyprotein undergoes several co- and post-translational cleavage events mediated by both viral and host proteases. Among these, sequential cleavage at the N- and C-termini of the hydrophobic capsid anchor (Ca) at the junction of C-PrM has been considered essential for the production of flaviviruses. Here, using a refined dengue pseudovirus production system, we show that Ca plays a key role in the processing efficiency of dengue virus type 2 (DENV2) structural proteins and the assembly of viral particles. Replacement of the relatively short DENV2 Ca with the homologous regions from West Nile or Zika viruses or, alternatively, increasing its length improved cleavage and hence particle assembly. Furthermore, we show that substitution of the Ca conserved proline residue (Pro-110) to alanine abolishes pseudovirus production, regardless of the Ca sequence length. Using two experimental approaches, we investigated the need for sequential cleavage (first on the cytosolic side, then on the luminal side) and found that while cleavage at the Ca-Pr boundary is essential for the assembly of infective particles, the same is not true for cleavage at the C-Ca boundary. We show that both mature (C) and unprocessed capsid (C-Ca) of DENV2 were equally efficient in packaging the viral RNA and in the assembly of infective particles. This was further confirmed with mutants in which cleavage at the luminal side by the signal peptidase occurred independently of cleavage at the cytosolic side by the viral NS2B/NS3 protease. We thus demonstrate that unlike other flaviviruses, DENV2 capsid does not require a cleavable Ca sequence and that sequential cleavage is not an obligatory requirement for the morphogenesis of infective particles.



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Abstract

4D Analyses Show That Replication Compartments Are Clonal Factories in Which Epstein–Barr Viral DNA Amplification Is Coordinated

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Most DNA viruses must amplify their DNA to form new viral particles. To kickstart their DNA amplification, herpesviruses alter the host cell cycle dynamics by halting G1/S progression. Soon after, the viruses begin amplifying their DNA and halt any detectable cellular DNA synthesis. Viral DNA amplification takes place in specialized regions in the cell known as replication compartments. The genesis and maturation of replication compartments are not well understood. While replication compartments can only be visualized via microscopy, examining DNA synthetic events requires ensemble approaches. We have therefore exploited single-cell assays, including live-cell imaging, fluorescence in situ hybridisation (FISH), and EdU-pulse labeling, in combination with computational simulations and ensemble approaches, to study the role of replication compartments in DNA amplification of Epstein–Barr virus. FISH revealed that each replication compartment initially contains a single DNA molecule which does not travel between compartments. DNA amplification lasts for 13–14 in single cells, as shown by live cell imaging. Replication compartments eventually grow to occupy 30% of the nucleus, which itself grows by 50%. We find that early in the lytic phase, the availability of DNA templates limits DNA synthesis, while late in the lytic phase, the majority of viral DNA molecules no longer serve as templates, which correlates with a drop in levels of replication protein. The eventual decline in DNA synthesis does not result from encapsidation; only 1%–2% of the viral DNA is encapsidated.

The levels of viral DNA synthesis in each compartment were similar. Therefore, the number of compartments determined the total amount of DNA synthesized and, consequently, the levels of amplified DNA. This finding was predicted by computational simulations of the amplification of the two distinct EBV-derived replicons we studied. Our results establish that replication compartments represent clonal factories for DNA amplification that are regulated coordinately during the lytic phase.



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Abstract

Exploitation of Host Factors and Cellular Pathways by Tombusviruses for the Biogenesis of the Viral Replication Organelles

Peter Nagy

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Plus-stranded RNA viruses recruit cellular vesicles and co-opt cellular proteins involved in cellular metabolism and lipid biosynthesis to build viral replicase complexes (VRCs) within the large viral replication compartments. We use tombusviruses (TBSV), which are small (+)RNA viruses, as model plant viruses to study virus replication, recombination, and virus-host interactions using yeast (*Saccharomyces cerevisiae*) surrogate host. Several systematic genome-wide screens and global proteomic and lipidomic approaches have led to the identification of ~500 host proteins/genes that are implicated in TBSV replication. We characterized the role of two-dozen co-opted host proteins, sterols and phosphatidylethanolamine in tombusvirus VRC assembly and viral RNA synthesis. We provide evidence on the critical roles of phosphoinositides and co-opted membrane-shaping proteins in VRC formation. We also present data that tombusviruses hijack the glycolytic and fermentation pathways to obtain ATP, which is required for the biogenesis of the replication compartment. Finally, we show evidence that TBSV usurps COPII and endosomal vesicles to form a unique microenvironment involving peroxisomes and ER to support viral replication. These new insights highlight the amazingly complex nature of virus-host interactions.



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Abstract

Rotaviruses as Neonatal Vaccine Expression Vectors against Other Enteric Pathogens

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Although the incidence of rotavirus diarrheal disease has been reduced by the introduction of neonatal rotavirus vaccines, other enteric viruses, including norovirus, hepatitis E virus (HEV), and astrovirus, remain significant causes of illness. In this study, we investigated the possibility of generating recombinant rotaviruses that express the capsid proteins of other enteric viruses as an approach for creating neonatal multitarget vaccines. As a first step, we examined whether the segmented dsRNA genome of rotavirus could be engineered to express a separate foreign protein through the use of a 2A translational ‘self-cleavage’ element. These attempts were successful, allowing recovery of recombinant rotaviruses with modified-segment-7 RNAs that contained a single ORF encoding a NSP3-2A-fluorescent protein (FP) cassette. By varying the FP introduced into the cassette, genetically-stable rotaviruses were generated that grew efficiently and directed the robust expression of FP as an independent product (e.g., UnaG (green), mRuby (red), mKate (orange), TagBFP (blue), and (YFP) yellow). Subsequently, attempts were made to recover recombinant rotaviruses with modified-segment-7 RNAs that contained a single ORF encoding NSP3-2A fused to the capsid-protein gene of norovirus (VP1, P, or P2), HEV (VP1), or astrovirus (VP34, VP70, or VP90). These attempts resulted in the generation of recombinant viruses that efficiently expressed capsid proteins of other enteric viruses, despite the required addition of up to 2.5 kB of foreign sequence to the 18.5 kB rotavirus genome. Our findings support the idea that rotaviruses can be engineered as plug-and-play expression vectors to create next-generation neonatal vaccines that can induce immunological protection against not only rotavirus but also other enteric pathogens.



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Abstracts

Session 6. Viral Genetics and Evolution

Abstract

Mutations in the HIV-1 Envelope Glycoprotein Confer Broad, Multi-Class Drug Resistance

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Despite the effectiveness of antiretroviral therapy, virological failure can occur in HIV-1-infected individuals, in some cases in the absence of recognized drug resistance mutations (DRMs). This is particularly the case with the highly potent integrase strand transfer inhibitor Dolutegravir (DTG). Here, long-term passage of wild-type virus in the presence of antiretrovirals led to the identification of mutations within the HIV-1 envelope (Env) glycoprotein that broadly increase viral fitness by overcoming blocks to virus replication, in the absence of resistance mutations in the drug-target gene. We have identified a panel of drug-resistant Env mutants that arose in the presence of protease, reverse transcriptase, and integrase inhibitors. Using a GFP-reporter virus, we determined that the selected Env mutations greatly enhance the efficiency of cell-to-cell transfer and increase the effective MOI of the transmitted virus. The Env mutations that confer drug resistance are clustered at the interface between the surface Env glycoprotein gp120 and the transmembrane glycoprotein gp41; biochemical analyses demonstrate that these mutations stabilize the interaction between gp120 and gp41. The study of Env mutants that result in a decreased sensitivity to DTG is of particular interest, as resistance mutations in integrase have been challenging to characterize to date. We are currently investigating the implications of these findings for HIV drug resistance in humanized mouse and nonhuman primate models and in infected humans.



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Abstract

Defining Measles Virus Hemagglutinin Antigenic Drift by Systematic B-Cell Epitope Elimination

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Immunization with live attenuated measles viruses (MeVs) derived from the 1954 David Edmonston isolate still provokes an adaptive immune response that protects against all known circulating MeV genotypes. To understand how the antigenic evolution of MeV is constrained, we used murine monoclonal antibodies to systematically eliminate by reverse genetics all thirty known B-cell epitopes on the MeV hemagglutinin (MeV-H). We found that a minimum elimination of seven MeV-H antigenic sites was required to achieve a variant that could escape polyclonal antibody neutralization. The virus expressing the multiple mutated MeV-H could no longer bind the pathogenicity-determining receptors SLAMF1 and NECTIN4 but entered cells exclusively via the vaccine-specific receptor CD46. Virus transmissibility was associated with full susceptibility to neutralization by human serum. Since escape from serum neutralization required at least seven simultaneous mutations in co-dominant antigenic sites in the MeV-H protein, there is no basis for new measles serotypes to arise by stepwise antigenic drift.



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Abstract

Emergence and Selection of a Highly Pathogenic Avian Influenza H7N3 Virus

Nancy Beerens, Rene Heutink, Ben Peeters

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Low pathogenic avian influenza (LPAI) viruses of subtypes H5 and H7 have the ability to spontaneously mutate to highly pathogenic (HPAI) variants, causing high mortality in poultry. The switch to high pathogenicity is poorly understood, and evidence from the field is scarce. This study provides direct evidence for LPAI to HPAI mutation at a turkey farm during an H7N3 outbreak in the Netherlands. At the farm, only mild clinical symptoms were reported, but the intravenous pathogenicity index measured for the virus isolated from the infected turkeys was consistent with a highly pathogenic virus. Using deep-sequencing, we showed that a minority of HPAI virus (0.06%) was present in the virus preparation. Analysis of different organs of the infected turkeys showed the highest percentage of HPAI virus was present in the lung (4.4%). The HPAI virus contained a 12-nucleotide insertion in the hemagglutinin (HA) cleavage site that was introduced by a single event, as no intermediates with shorter inserts were identified. The HPAI virus was rapidly selected in chickens, after both intravenous and intranasal/intratracheal inoculation with the mixed virus preparation. Full-genome sequencing revealed that both pathotypes contained a deletion in the stalk region of the neuraminidase protein. We identified mutations in HA and polymerase basic protein 1 (PB1) in the HPAI virus, which were already present as minority variants in the LPAI virus. Our findings provide more insight into the molecular changes and mechanisms involved in the emergence of HPAI viruses. This knowledge may be used for timely identification of LPAI viruses that pose a risk of becoming highly pathogenic in the field.



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Abstract

Deep Mutational Scanning to Map How Zika Envelope Protein Mutations Affect Viral Growth and Antibody Escape

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The Zika virus has recently been shown to be associated with severe birth defects. The virus' envelope (E) protein mediates its ability to infect cells and is also the primary target of the antibodies that are elicited by natural infection and vaccines that are being developed against the virus. Therefore, determining the effects of mutations to this protein is important for understanding its function, its susceptibility to vaccine-mediated immunity, and its potential for future evolution. Functional constraints on viral proteins are often assessed by examining sequence conservation among natural strains, but this approach is relatively ineffective for the Zika virus because all known sequences are highly similar. Here, we take an alternative approach to mapping functional constraints on Zika virus' E protein by using deep mutational scanning to measure how all amino-acid mutations to the protein affect viral growth in cell culture. The resulting sequence-function map is consistent with existing knowledge about E protein structure and function but also provides insight into mutation-level constraints in many regions of the protein that have not been well characterized in prior functional work. In addition, we extend our approach to completely map how mutations affect viral neutralization by two monoclonal antibodies, thereby precisely defining their functional epitopes. Overall, our study provides a valuable resource for understanding the effects of mutations to this important viral protein and also offers a roadmap for future work to map functional and antigenic selection to the Zika virus at high resolution.



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Abstract

Globally Defining the Effects of Amino Acid Mutations across a Picornavirus Capsid

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RNA viruses are characterized by extreme mutation rates, which play key roles in their biology and confer them the ability to rapidly adapt to new environments. However, non-synonymous mutations tend to be largely deleterious to protein function, raising the question of how the proteins of RNA viruses maintain functionality in the face of high mutation rates. This is of particular relevance to the capsids of non-enveloped RNA viruses, which form highly complex protein structures that assemble from numerous subunits, interact with cellular host factors to mediate entry and uncoating, and are under strong immune selection. To better understand how viral capsids accommodate mutations, we generated viral populations harboring a large fraction of all possible single amino acid mutations in a picornavirus capsid. We then used high-fidelity next-generation sequencing to derive the relative fitness of these mutations compared to the wildtype sequence. Combining our results with available structural, genetic, and phenotypic data, we are able to provide a comprehensive understanding of the ability of a viral capsid to accommodate mutations.



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Abstract

Good and Bad Neighbourhoods in Viral Sequence Space: Predicting, Altering, Targeting Virus Populations

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Good and Bad Neighbourhoods in Viral Sequence Space: Predicting, Altering, Targeting Virus Populations

All viruses, but especially RNA viruses, generate tremendous diversity in genome composition, including point mutations, duplications, deletions, and insertions. We use *in vitro* and *in vivo* models to perform natural and directed experimental evolution and combine these data with mathematical modelling to determine how virus populations occupy sequence space—a multidimensional hypercube that describes all combinations of nucleotide, codon, or amino acid sequences. We show how these experimental and computational approaches can help monitor, predict, alter, and even target virus evolution and population dynamics, creating new ways to study virus–host interactions and to innovate antiviral approaches. Using arboviruses, enteroviruses, and influenza, we recreate and predict host jumps and emergence events in the lab, redirect evolution towards the ‘bad’ neighbourhoods of sequence space that represent attenuation, and poison the viral population by disturbing the balance between good and bad genomes.



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Abstract

Coxsackieviruses Undergo Intercellular Transmission as Pools of Sibling Viral Genomes Associated to Membranes

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Some viruses are released from cells as pools of membrane-associated virions. By increasing the multiplicity of infection, this type of collective dispersal could favor viral cooperation, but also the emergence of cheater-like viruses such as defective interfering particles. To better understand this process, we examined the genetic diversity of membrane-associated coxsackievirus infectious units. We found that infected cells released large membranous structures containing 8–21 infectious particles on average, including vesicles. However, in most cases (62–93%) these structures did not promote the co-transmission of different viral genetic variants present in a cell. Furthermore, collective dispersal had no effect on viral population sequence diversity. Our results indicate that membrane-associated collective infectious units typically contain viral particles derived from the same parental genome. Hence, if cooperation occurred, it should probably involve sibling viral particles rather than different variants. As shown by social evolution theory, cooperation among siblings should be robust against cheater invasion.



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Abstract

Revolution in Evolution of Human “Anellome”

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Torque teno viruses (TTVs), the most well-known members of the family *Anelloviridae*, are not associated with any disease. They are one of the most abundant and divergent entities in the viral world; however, the cause of their variability is not currently known. In this study, a set of longitudinally collected serum samples from two HIV-1 infected and two non-infected persons was analyzed for the presence of TTVs and other *Anelloviridae* using a genera-specific qPCR. The samples positive for TTVs were selected for the quantitative heteroduplex tracking assay (QHTA), which showed repeating patterns of TTV genotypes. Sanger sequencing of the partial viral sequences revealed that the same strains of TTVs are most probably disappearing and returning in different stages of life, with scarcely any new introductions. The partial sequences were grouped into phylogenetic genogroups, and samples representing each of these genogroups were selected for full-length genome sequencing using an in-house optimized rolling circle amplification(RCA)-Illumina protocol.



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Abstract

Divergent Traits and Ligand-Binding Features of the Cytomegalovirus CD48 Gene Family

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The genesis of gene families through the capture of host genes and their subsequent duplication is a crucial process in the evolution of large DNA viruses. CD48 is a cell surface protein with an ectodomain composed of two immunoglobulin (Ig) domains. Via its N-terminal Ig domain, CD48 interacts with the cell surface receptor 2B4, triggering signal transduction events that regulate leukocyte cytotoxicity. We previously reported the presence of five CD48 homologs (vCD48s) in two related cytomegaloviruses, derived from a common host CD48 ancestor gene acquired by retrotranscription. Recently, we examined one member of this family, A43, showing that it acts as a functional viral decoy receptor, binding with high affinity and stability to 2B4 and impairing NK-cell cytotoxicity. Here, we have characterized the rest of the vCD48s. We show that they are highly glycosylated type I transmembrane proteins which display remarkably distinct features: dissimilar structures (e.g., different size stalks and cytoplasmic tails), biochemical properties, locations (cell surface/soluble), and temporal kinetic classes. We found that, in contrast to A43, none of them interacts with 2B4. Consistent with this, molecular modeling of the N-terminal Ig domains of these vCD48s evidences significant changes in the number and length of their β -strands and inter-sheet loops that participate in the interaction of CD48 with 2B4. This suggests that these vCD48s have diverged to perform new 2B4-independent functions. Interestingly, we determined that one of them, S30, tightly binds CD2, a T- and NK-cell adhesion and costimulatory molecule whose primary ligand is CD58. Thus, altogether, our results show how a key host immune receptor captured by a virus can be subsequently remodeled during viral evolution to yield new molecules with improved affinities to their cognate receptors or with shifted binding specificities to additional immune targets, expanding the repertoire of viral immunevasins.



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Abstracts

Poster Exhibition

1 Cd302 and Cr11 Are Novel Restrictors of Hepatotropic Virus Cross-Species Transmission

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Virus species- and tissue-tropism is governed by host dependency and restriction factors. Hepatitis C virus (HCV) exhibits a narrow species-tropism and murine hepatocytes are refractory to infection. Using murine liver cDNA library screening we identified Cd302, a lectin, and Cr11, a complement receptor, as pan-genotypic restrictors of HCV infection. Cd302 and Cr11 proteins interact directly with each other and virions to impede uptake. In addition, ectopic expression of both factors in permissive human cells co-operatively induces a non-canonical transcriptional program which contributes to the inhibition HCV and hepatitis B virus (HBV) infection *in vitro*. We demonstrate Cd302 and Cr11 expression is constitutive in murine hepatocytes and not inducible by interferon or virus infection: these factors mediate protection independently of the interferon system. Conditional Cas9 disruption of murine hepatocyte Cd302 expression increased HCV replication *in-vivo* and *ex-vivo*, and modulated the intrinsic hepatocyte transcriptome, dysregulating expression of metabolic process and host defense genes. Liver specific ablation of Cd302 expression modulated cellular pathways involved in IL-1R mediated inhibition of RXR function, lipid/xenobiotic metabolism and fatty acid synthesis. In contrast to mice, CR1L expression was absent in human hepatocytes and HCV restriction concomitantly reduced. Therefore, the Cd302/Cr11 axis contributes to limiting hepatotropic virus cross-species transmission to mice via a novel mechanism. Together these data open new avenues for step-wise development of mouse models for these important human pathogens which cause substantial disease burden globally.



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Abstract

2 A Center for the Rapid Analysis of Clinical-Grade Biologics: The Biophysical Characterization of HIV-1 Env Protein

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The development of a prophylactic vaccine against the human immunodeficiency virus (HIV) is of paramount importance in the global drive to halt the spread of the virus. Even after the successful discovery and initial testing of a vaccine candidate, there are hurdles associated with production yield, purification strategy, and in vitro stability that may hinder its development as a biological product. The goal of the Clinical Bioreagent Center (CBC) is to streamline the vaccine development pipeline from a promising lead to the clinic, in part by developing state-of-the-art analytical tools to characterize and to quickly monitor the quality of HIV-1 Env protein, a new vaccine candidate. A method was developed to determine the purity of HIV-1 Env glycoprotein by capillary electrophoresis that provides higher sensitivity of detection of impurities and better resolution as compared to regular gel electrophoresis. Using an Octet QKe system, host cell protein content was confirmed using a kit that has greater precision and linear range than available kits based on ELISA. Imaged capillary isoelectric focusing results highlight the charge heterogeneity of the recombinant HIV-1 Env protein. The binding affinity of the broadly neutralizing antibody, 4E10, to the HIV-1 Env protein was determined by biolayer interferometry. The glycan profile obtained by MALDI spectrometry showed that the recombinant HIV-1 Env protein glycans are distinct from SF162 gp140. These analytical tools can be implemented to ensure that the protein expression and purification conditions do not change the integrity, bioactivity, and therapeutic properties of the vaccine. The methods developed here can be qualified per current good manufacturing practices to facilitate their transfer to a biomanufacturing facility. Our experimental tools have been developed to monitor the quality of the HIV-1 Env protein, with the goal of boosting production yields to expedite its success onto clinical trials. Supported by NIH-NIAID R01AI22935.



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3 Abortive Herpes Simplex Virus Infection of Non-Neuronal Cells Results in Quiescent Viral Genomes That Can Be Reactivated

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Abortive viral infections are defined as cells that have been infected with a virus but did not produce any progeny virus as a result of the infection. Recent single-cell studies have shown that abortive infection is observed frequently, even during the infection of susceptible and permissive cell types. We recovered populations of susceptible and permissive cells that survived infection with herpes simplex virus 1 (HSV-1) at high multiplicity of infection. We found that these abortive cells maintain viral genomes in a quiescent state for at least five weeks. Our results indicate that these viral genomes are maintained inside the nucleus, bound to cellular histones, and are occasionally reactivated to produce new progeny viruses. These findings suggest that abortive HSV-1 infection leads to spontaneous latency-like infection in non-neuronal cells, challenging the current paradigm of herpesvirus latency.



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Abstract

4 An Endogenous Retrovirus from Human Hookworm Encodes an Ancient Phlebovirus-Like Class II Envelope Fusion Protein

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Within the parasitic nematode *Ancylostoma ceylanicum*, a ~20 million year old Bel/Pao LTR retrotransposon encodes an ancient viral class II envelope fusion protein termed Atlas Gc. Typically, retroviruses and related degenerate retrotransposons encode a hemagglutinin-like class I envelope fusion protein. A subset of Bel/Pao LTR-retrotransposons within the phylum Nematoda have acquired a phlebovirus-like env and utilized the encoded fusion machinery to escape the genome as intact exogenous retroviruses. This includes *C. elegans* retroelement 7 virus which was recently reclassified as a member of the genus *Semotivirus*. A 3.76 Å cryoEM reconstruction confirms Atlas Gc as a closely related phleboviral homologue and class II fusion protein in a novel case of gene exaptation. Preliminary biophysical and biochemical characterization indicate Atlas Gc functions under specific physiological conditions targeting late-endosomal membranes, much like modern viral class II envelope fusion proteins. Phylogenetic analyses support the reclassification of the Atlas endogenous retrovirus and five other *A. ceylanicum* ERVs as novel semotiviruses of *Belpaoviridae* of the new viral order of reverse-transcribing viruses *Ortervirales*.



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Abstract

5 Characterization of DNA Polymerase from *Thermus thermophilus* MAT72 Phage Tt72

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Thermophilic phages are recognized as an untapped source of thermostable enzymes relevant in biotechnology; however, their biology is poorly explored. This has led us to start a project aimed at investigating thermophilic phages isolated from geothermal areas of Iceland. Here, we present a structural and functional analysis of DNA polymerase of phage Tt72 that infects thermophilic bacterium *Thermus thermophilus* MAT72.

In silico analysis of the Tt72 phage genome revealed the presence of a 2112-bp ORF encoding protein homologous to the members of the A family of DNA polymerases. It contains a conserved nucleotidyltransferase domain and a 3'→5' exonuclease domain but lacks the 5'→3' exonuclease domain. The amino acid sequence of Tt72 DNA polymerase shows a high identity to two yet uncharacterized DNA polymerases of *T. thermophilus* phages: fYS40 (91%) and fTMA (90%).

The gene coding for Tt72 DNA polymerase was cloned and overexpressed in *E. coli*. The Tt72 *polA* gene is composed of 2112 nucleotides. The overall G+C content of this gene is 31,58%, which is lower than the G+C content of *T. thermophilus* genomic DNA (69.49%). The Tt72 *polA* gene codes for a 703-aa protein with a predicted molecular weight of 80,477. Enzyme was overproduced in *E. coli*, purified by heat treatment, followed by HiTrap TALON column and HiTrap Heparin HP column chromatography, and biochemically characterized. The optimum activity was found at 55 °C, pH 8.5, 25 mM KCl, and 0.5 mM Mg²⁺. Further, the Tt72 DNA polymerase shows strong 3' → 5' exonucleolytic activity.



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6 Characterization of Glycoproteins From Insect-Specific Goutanap and Negev Viruses

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Negeviruses (NVs) are a recently discovered taxon of enveloped, positive sense, single-stranded RNA viruses, infecting blood sucking insects. While classical arthropod-borne (arbo)viruses like dengue and Chikungunya infect both insects and vertebrates, NVs are restricted to insects and do not have any known vertebrate host and are thus classified as insect-restricted viruses. Previous works have predicted a structure consisting of three ORFs, the first with homologous regions to RNA-dependent RNA polymerase, helicase, and methyl transferases in plant viruses. On the contrary, *ORF2* and *ORF3* do not have homologs and are predicted to encode membrane glycoproteins. Their structures, functions, and significance remain vague. We focus on characterization of the viral proteins, structural organization of the virion, and the principles of their interaction with the host cell. We purified the virion particles of Negev virus produced in mosquito cells and identified its structural components. In addition, we have cloned and overexpressed *ORF2* and *ORF3* of *Negeviruses*. Furthermore, we have defined and successfully produced and purified recombinant *ORF2*. Subsequent characterization using gel filtration, ion exchange, and MALS techniques reveal that *ORF2* ectodomains of *Negeviruses* exhibit different higher order assembly patterns: dimerization and multimerization in a concentration- and pH-dependent manner that correspond to their biological role. We combine biochemical, structural, and cell biology techniques to unravel mechanisms of Negev virus interaction with the host cell.



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7 Comparing Different Larval Food Sources and Temperature Regimes for Rearing of *Culicoides obsoletus/scoticus* Complex Midges, the Predominant Bluetongue, and Schmallenberg Virus Vectors in Northern Europe

Charlotte Sohier, Severine Matthijs and Nick De Regge

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During the last decade, Europe was confronted with the emergence of two *Culicoides*-borne viruses, bluetongue virus and Schmallenberg virus. Both diseases caused economic losses in cattle, sheep, and goats. Despite their importance, only limited knowledge is available on the developmental stages of *Culicoides obsoletus* complex midges and a lab-reared colony has not yet been established.

Therefore, this experimental study aims to compare the emergence patterns from field collected *C. obsoletus* complex from egg to adult that were exposed to different combinations of temperature and larval substrates (T1: 1% liquid broth (LB) agar at 24 °C; T2: 1% LB agar with dung patches at 24 °C; T3: 1% LB agar at 28 °C). The rearing dishes were kept in an environmental chamber under a 8 h/16 h dark–light regime and 80%–85% humidity. Algae and nematodes were provided as a food source.

The average period required to develop from egg to adult was 28 days and varied strongly in each of the conditions (T1: 15–47 days; T2: 14–35 days; T3: 20–37 days). A bias of sex ratio was observed towards production of males in all three treatments with only 23.9% (T1), 23.5% (T2), and 0% (T3) females. Larval mortality was highest in T3 (25.9%), followed by T2 (8.6%), and T1 (1.8%). All pupae produced adults within 1 to 10 days, and emergence rates for pupae varied strongly between the treatments: 49.5% (T1), 71.6% (T2), and 38.5% (T3).

This study shows that the *C. obsoletus* complex can be reared under laboratory conditions from blood-fed wild caught females to emerged progeny. More larval substrates and food sources should be tested with the aim of obtaining a 1:1 sex ratio to bring us one step closer to a viable lab-reared colony.



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8 Cytopathic BVDV-1 Induces Type I Interferon Expression through IRF-1 and IRF-7 Transcriptional Factors in MDBK Cells

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Bovine viral diarrhea virus (BVDV-1) is responsible for the Bovine Viral Diarrhea/Mucosal Disease complex, endemic pathology of cattle, as well as for heavy losses for the livestock and dairy industry in the world. Several investigations have shown that BVDV-1 is capable of altering the host animal's immune system, but there is little information available on the molecular and cellular mechanisms involved. The production of interferon (IFN- α/β) is considered a potent and rapid response of the innate immune system against the presence of a virus. In the case of BVDV-1, the antecedents that show whether IFN-expression is triggered during an infection in bovine models are contradictory, and the transcription factors that regulate the expression of this key cytokine to trigger antiviral status have not been established. To investigate the effects of BVDV-1 on the activation of the immune response, Madin–Dardé bovine kidney (MDBK) cells were infected with the cytopathic biotype cpBVDV-1, and the expression of IFN- β , interferon regulatory factors (IRF) and immunity markers was analyzed. Additionally, a transient silencing of the IRF-7 factor was performed. The results obtained show that BVDV-1 is capable of inducing the production of IFN- β , IRF-1, and IRF-7 in a manner similar to polycytidylic acid, evaluated transcript, and protein level. The use of pharmacological inhibitors against IRF-1 and IRF-7 decreases the production of IFN- β , a phenomenon observed by mainly interfering with the activation pathway of IRF-7. These results propose that in an infection with cpBVDV-1, the activation of the IRF-7 factor is required and indispensable for the regulation of the transcription of the IFN- β gene in an *in vitro* model of infection.



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9 Development of a Tubular Bacteriophage-Based Vaccine Platform that Induces an Immune Response in Mice

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Current vaccines against infectious diseases have primarily relied on attenuated or inactivated pathogens. However, self-assembled virus-based nanoparticles (VNPs) are noninfectious multiprotein structures regarded as safe vaccine platforms for an efficient foreign antigen display within a host immune system. Currently, there is a low diversity of self-assembled rod-shaped VNPs. Additionally, there is no information regarding the generation of tailed-bacteriophage nanotubes in yeast and their immunogenicity in mice. Here, we developed a novel tubular VNP-based vaccine platform utilizing a yeast-synthesized recombinant tail tube gp39 protein from bacteriophage vB_EcoS_NBD2 (NBD2). The diameter of these extremely flexible polytubes was ~12 nm, while the length varied from 0.1 to >3.95 μm. In this study, the immunogenicity of polytubes formed by the recombinant gp39 protein and antibody response were tested. The tubular structures formed by the recombinant gp39 protein were immunogenic in mice, while the addition of Freund's adjuvant enhanced anti-gp39 antibody response compared to the use of tubular structures alone. To further examine the applicability of novel polytubes as a vaccine platform, one carboxy-terminal region within the gp39 protein was identified, allowing insertion of six foreign histidine epitopes with no effect on the recombinant protein synthesis or structure self-assembly. This genetic insertion of a foreign epitope within the surface-exposed domains results in repetitive display of antigens on the surface of NBD2 tail tube-originated polytubes. The combination of a repetitive, highly-ordered display of foreign epitopes as well as tubular structure of a vaccine platform can greatly enhance the immune response. Although more studies are needed, our unique flexible and extremely long tubular platform formed by the recombinant tail tube gp39 protein holds the promise of serving as a foreign epitope platform for the development of more effective vaccines.



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Abstract

10 Full Genomic Sequencing of Vesicular Stomatitis Virus Isolates from the 2004-2006 US Outbreaks Reveals Associations of Viral Genetics to Environmental Variables

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Vesicular stomatitis (VS) outbreaks in the western USA occur cyclically approximately every 8-10 years. Phylogenetic evidence based on a 450nt region of the P coding sequences suggests the initial introduction is a single viral lineage closely related to those circulating in endemic areas of Mexico. In 2004, a VS outbreak initiated in southern NM and TX and spread as far north as northern CO. Subsequently, in 2005, VS cases appeared in 9 states (AZ, CO, ID, MT, NE, NM, TX, UT and WY) and in 2006 VS reappeared only in WY. Phylogenetic suggested that a single VS virus New Jersey (VSNJV) lineage caused the 2004 outbreak, and re-emerged in 2005 and 2006. The mechanism of VS emergence and re-emergence remain unclear. Here, we use near full-length genomic sequences of 60 viral strains isolated from 2004–2006 in the US and Mexico to determine their phylogeographic relationships and environmental variables associated with outbreak dynamics. The results confirmed that a single VSNJV lineage caused the 2004–2006 US outbreaks and its closest ancestor was a virus circulating in Colima, Mexico in 2004. We also present evidence that the virus lineage overwintered in 2005 and 2006. Furthermore, rather than a simple geographic relationship, specific viral sublineages or patristic groups were associated to environmental variables, particularly precipitation and temperature. The results confirm the role of environmental factors in the evolution and spread of VSNJV in the USA.



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Abstract

11 Knock Out of Cell Death Pathway Components Results in Differential Caspase Expression in Response to HCV Infection

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INTRODUCTION

Pyroptosis (inflammatory programmed cell death), is induced after activation of an inflammasome, ultimately resulting in pore formation and cell lysis. One factor in the pathology associated with chronic hepatitis C virus (HCV) infection is non-inflammatory caspase-3-mediated apoptosis. Our lab has found both apoptosis and pyroptosis occurring in HCV-infected Huh-7.5 cells. In the context of some viral infections, pyroptosis is beneficial to the virus; for others, pyroptosis is believed to represent an innate antiviral response. This study aimed to test the effects of knocking out components of the inflammasome pathway on caspase activation in HCV-infected cells.

METHODS

FAM-FLICA probes or antibodies were used to visualize active caspase-1 and active caspase-3 *in vitro*. Huh-7.5 cells with components of the pyroptotic or apoptotic pathways knocked out (NLRP3, GSDM-D or caspase-3) were used to determine the effect of their absence on the virus and caspase activation using confocal microscopy and flow cytometry.

RESULTS

Increased levels of caspase-1 were consistently observed in HCV-infected cells compared to uninfected cells and these levels increased with subsequent days post-infection. Inhibition of inflammasome activation using knockout cell lines showed differential activation of caspase-1 and caspase-3, with the inhibition of pyroptosis resulting in a trend towards greater expression of caspase-3, indicative of apoptosis. Inhibition of NLRP3 did not fully omit caspase-1 activation, but it was decreased. Flow cytometry results revealed a small sub-set of cells positive for both caspase-1 and caspase-3.

CONCLUSION

These data confirm the occurrence of pyroptosis in HCV-infected cells and demonstrate involvement of the NLRP3 inflammasome, although other inflammasome sensors might be involved. Since inhibition of one cell death pathway resulted in increased activation of the other, along with the presence of double-positive cells, there may be cross-talk between apoptotic and pyroptotic pathways; the role of this cross-talk during infection remains to be elucidated.



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12 KSHV Activates Unfolded Protein Response Sensors But Suppresses Downstream Transcriptional Responses to Support Lytic Replication

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Herpesviruses usurp host cell protein synthesis machinery to convert viral mRNAs into proteins, and the endoplasmic reticulum (ER) to ensure proper folding, post-translational modification and trafficking of secreted and transmembrane viral proteins. Overloading ER folding capacity activates the unfolded protein response (UPR), whereby sensor proteins ATF6, PERK and IRE1 initiate a stress-mitigating transcription program that accelerates catabolism of misfolded proteins while increasing ER folding capacity. Kaposi's sarcoma-associated herpesvirus (KSHV) can be reactivated from latency by chemical induction of ER stress, which causes accumulation of the XBP1s transcription factor that transactivates the viral RTA lytic switch gene. The presence of XBP1s-responsive elements in the RTA promoter suggests that KSHV evolved a mechanism to respond to ER stress. Here, we report that ATF6, PERK and IRE1 were activated upon reactivation from latency and required for efficient KSHV lytic replication; genetic or pharmacologic inhibition of each UPR sensor diminished virion production. Despite UPR sensor activation during KSHV lytic replication, downstream UPR transcriptional responses were restricted; (1) ATF6 was cleaved to activate the ATF6(N) transcription factor but ATF6(N)-responsive genes were not transcribed; (2) PERK phosphorylated eIF2 but ATF4 did not accumulate; (3) IRE1 caused XBP1 mRNA splicing, but XBP1s protein did not accumulate and XBP1s-responsive genes were not transcribed. Complementation of XBP1s deficiency during KSHV lytic replication inhibited virion production in a dose-dependent manner in epithelial cells. Taken together, these findings indicate that while XBP1s plays an important role in reactivation from latency, it can inhibit virus replication at a later step, which the virus overcomes by preventing its synthesis. These findings suggest that KSHV hijacks UPR sensors to promote efficient viral replication while sustaining ER stress.



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13 Phages Do Encode Antibiotic Resistant Genes in *Acinetobacter baumannii*

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A. baumannii is one of the most important hospital-associated human pathogens nowadays, very well known for having many antibiotic resistance genes (ARGs). The dispersion of these ARGs has been mainly associated with plasmids and transformation; however, the role of phages as spreaders of ARGs has not been explored in this species. Here, we explored the diversity of prophages using 133 *A. baumannii* genomes that represent the breadth of diversity within this species. This species has a considerable quantity of phages that are not closely related to previously described phages. Notably, we determined that many of these phages encode a diverse array of ARGs. The three major matches were the multidrug efflux RND transporter family, the small multidrug resistance efflux pump AbeS, and oxacillinases (OXA) from the OXA-23-like and OXA-51-like gene families. This analysis clearly shows that phage-encoded ARGs are commonplace in *A. baumannii*. Furthermore, we demonstrate that some of them show lytic capacity.

Our findings point to the fact that considerably more attention has to be paid to phages as vehicles of ARGs in this species, and that specialized transduction is not as uncommon as previously suggested.



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14 Restriction of HIV by TIM and SERINC and Counteraction by Nef

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T cell immunoglobulin and mucin domain (TIM) proteins inhibit release of HIV-1 and other enveloped viruses by interacting with cell- and virion-associated phosphatidylserine (PS). We recently provided evidence that the Nef proteins of HIV-1 and other lentiviruses antagonize TIM-mediated restriction. HIV-1 Nef does not appear to downregulate TIM-1 expression but promote its internalization from the plasma membrane and also sequesters TIM-1 into intracellular compartments. Intriguingly, we found that depletion of SERINC proteins attenuates TIM-mediated restriction of HIV-1 release in human CD4⁺ T cells and monocyte-derived macrophages, especially that of Nef-deficient viruses. Consistent with this model, MLV glycoGag and EIAV S2 proteins also counteract TIM-mediated inhibition of HIV-1 release. Pulse-chase metabolic labeling assay shows that the half-life of TIM-1 is greatly extended in the presence of SERINC5, indicating that SERINC5 can stabilize TIM-1. Collectively, our work reveals a new role of Nef in antagonizing TIM-1, which is in part through SERINC5, and highlights a novel yet complex interplay between Nef and HIV-1 restriction by TIMs and SERINC5. Roles of SERINC5 in antiviral innate immunity, which is independent of HIV-1 Nef, will also be discussed.



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Abstract

15 Resurrecting Extinct Retroviruses in the Chimpanzee Genome as a Tool to Better Understand the Evolution of Simian Immunodeficiency Virus (SIV)

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Endogenous retroviruses make up a large component of primate genomes and can serve as sources of medically significant new infections. The most deadly retrovirus in human history, the Human immunodeficiency virus (HIV), made the jump to humans from its 32,000-year-old chimpanzee precursor, the Simian immunodeficiency virus (SIV), just a century ago. Our research aims to uncover novel retroviruses which have become “fossilized” in the chimpanzee genome and compare their sequences to that of SIV to determine how they differ genetically from one another. Using two largely orthogonal pre-existing programs (LTRharvest and LtrDetector), we identified regions in the chimpanzee genome which might represent novel retroviral DNA. We then cross-compared the results from these two programs to find sequences which were identified by both programs, and performed a BLAT search between these sequences and those contained in the RepeatMasker database. Using this method, we identified 4857 potential novel endogenous retroviral sequences in the chimpanzee genome, 370 of which represent regions in which the original LTRharvest and LtrDetector hits overlapped by 90% or higher. We will validate many of these sequences as bonafide endogenous retroviral sequences by reconstructing consensus sequences and manually annotating sequences with viral motifs. These promising findings indicate that there is still much to discover about the origin and evolution of SIV, which will in turn lead to new insights into HIV and how we may be able to treat it in human populations.



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Abstract

16 Siglec-1 Expressed on Dendritic Cells Is a New Receptor Implicated in Arenavirus Uptake

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Arenaviruses are enveloped viruses that cause hemorrhagic fever outbreaks in humans and still lack an effective antiviral treatment. Upon early infection, these viruses target dendritic cells (DCs), which can promote systemic viral dissemination, contributing to pathogenesis. We have previously described that Siglec-1, a sialic acid Ig-like binding lectin-1 expressed on DCs interacts with different enveloped viruses and promotes their capture within a virus-containing compartment. Such is the case of HIV-1 or Ebola virus, which display sialylated gangliosides on their viral envelope that are effectively recognized by Siglec-1. Here, we aimed to study if Siglec-1 on DCs also interacts with arenaviruses such as Junin.

We produced non-infectious Junin viral-like particles (Junin-VLPs) tagged with fluorescent eGFP by transfecting a plasmid encoding the structural Junin Z protein on HEK-293T cells. Junin-VLPs were added to a Raji cell line stably transfected with Siglec-1 or to monocyte-derived DCs activated or not with either Interferon- α or lipopolysaccharide. Viral uptake was analyzed by FACS or confocal microscopy in the presence of an anti-Siglec-1 monoclonal antibody (mAb) or an isotype control. Statistical differences were assessed with the indicated tests.

Raji Siglec-1 cells captured a higher number of Junin-VLPs than Raji cells, and this was blocked with an anti-Siglec-1 mAb ($P = 0.0159$; Mann-Whitney). On primary DCs, activation enhanced Junin-VLP capture ($P = 0.0024$; paired t -test) and Siglec-1 expression. Furthermore, pre-incubation with an anti-Siglec-1 mAb on activated DCs blocked Junin-VLP uptake ($P \leq 0.0002$; one sample t -test), while an isotype control did not. Forty-nine percent of the activated DCs analyzed by confocal microscopy captured Junin-VLPs within a Siglec-1+ virus-containing compartment. Moreover, when HIV-1 was also added, 97% of those compartments retained both viruses.

Thus, we conclude that Siglec-1 is a new receptor involved in arenavirus uptake in DCs and could represent a novel target for an anti-arenavirus treatment.



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17 The First Complete Genome Sequences of Hepatitis C Virus Subtype 2b from Latin America: Molecular Characterization and Phylogeographic Analysis

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The hepatitis C virus (HCV) has remarkable genetic diversity and exists as eight genotypes (1 to 8) with distinct geographic distributions. No complete genome sequence of HCV subtype 2b (HCV-2b) is available from Latin American countries, and the factors underlying its emergence and spread within the continent remain unknown. The present study was conducted to determine the first full-length genomic sequences of HCV-2b isolates from Latin America and reconstruct the spatial and temporal diversification of this subtype in Brazil. Nearly complete HCV-2b genomes isolated from two Brazilian patients were obtained by direct sequencing of long PCR fragments and analyzed together with reference sequences using the Bayesian coalescent and phylogeographic framework approaches. The two HCV-2b genomes were 9318 nucleotides (nt) in length (nt 37–9354). Interestingly, the long RT-PCR technique was able to detect co-circulation of viral variants that contained an in-frame deletion of 2022 nt encompassing E1, E2, and p7 proteins. Spatiotemporal reconstruction analyses suggest that HCV-2b had a single introduction in Brazil during the early 1980s, displaying an epidemic history characterized by a low and virtually constant population size until the present time. These results coincide with epidemiological data in Brazil and may explain the low national prevalence of this subtype.



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18 TRIM8 Controls Interferon Response in Plasmacytoid Dendritic Cells by Regulating pIRF7 Stability

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Proteins of the tripartite motif (TRIM) family have recently emerged as potentially important regulators of innate immune pathways. However, most studies are either based on the overexpression of TRIM proteins in irrelevant cell lines or focus on single specific TRIM proteins, thus failing to provide a comprehensive view of the TRIM protein family in physiologically relevant cells. The rationale of our project was to determine which TRIM proteins are essential for immune cells in mounting an efficient innate response towards viral infections.

In order to evaluate whether some TRIM proteins are key regulators of the interferon (IFN) response, we investigated the consequences of their knockdown in the most relevant cell type, namely, plasmacytoid dendritic cells (pDCs), which are professional type I IFN producer cells. For this purpose, we performed a siRNA screen of TRIM proteins in human primary pDCs in order to identify those that are critical for the IFN response upon viral activation.

Among the candidates that we found to regulate signaling downstream of TLR7, TRIM8 emerged as an essential regulator of IFN production by pDCs upon activation by HIV-1 or influenza virus. We report that TRIM8, which is expressed in the nucleus, protects phosphorylated IRF7 (pIRF7) from proteasomal degradation in an E3-ubiquitin ligase-independent manner. Mechanistically, we found that TRIM8 protects pIRF7 from the peptidyl-prolyl *cis/trans*-isomerase Pin1, which induces its proteasome-dependent degradation, as previously described for pIRF3. The implication of TRIM8 in the context of antiviral defense *in vivo* was validated in a zebrafish model of Chikungunya virus infection. In this model, we showed that TRIM8 positively regulates type I IFN responses and contributes to resistance against viral infection.

Our findings uncover a new regulatory mechanism of type I IFN production in pDCs by which TRIM8 and Pin1 oppositely regulate the stability of pIRF7. Beyond pDCs, our results show that TRIM8 is equally important for IRF7 function in other cell types, including dendritic cells or even cell lines, thus demonstrating that TRIM8 is a universal regulator of IRF7 and therefore of IFN- α expression.



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19 Unique Features of Immunity within the Immunoglobulin Heavy Chain Locus of Egyptian Rousette Bats

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Marburg virus (MARV) presents with a hemorrhagic fever in primates but asymptotically in its known reservoir, the Egyptian rousette bat (*Rousettus aegyptiacus*, ERB). Understanding the biological mechanisms that explain these differential outcomes could be used to develop efficient therapeutics against MARV disease in humans. Since one of the antiviral mechanisms to control viruses is the humoral response, we hypothesize that the B cell repertoire is unique to primates and contributes to the ERB's ability to overcome MARV infection. Immunoglobulin (Ig) heavy and light chains undergo DNA rearrangement to generate a diverse repertoire. To be able to study B cell rearrangement, accurate annotation of the Ig heavy chain (*IGH*) locus is needed. We implemented three complementary strategies to describe and annotate the *IGH* locus of ERBs. First, we identified and annotated genes at the *IGH* locus utilizing the previously described genome and transcriptome of the ERB our group created in collaboration with the CDC and the University of Boston. Second, we sequenced the specific IgM transcriptome of B cells from ERB PBMCs, to confirm or identify new *IGH* germline genes. Third, we generated bacterial artificial chromosomes (BAC) libraries to confirm and improve the layout of the *IGH* locus. We were able to resolve misassemblies of these regions and identify multiple gene expansions unique to ERBs that may contribute their ability to generate B cell diversity and control infections. We found an expansion of genes associated with protection from various viruses in humans, differential expression of ERB isotypes across tissues, and two functional *IgE* genes.



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20 A Cross-Reactive Mouse Monoclonal Antibody against Rhinovirus Mediates Phagocytosis In Vitro

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Human rhinoviruses (HRVs) are the main cause of common cold worldwide. To date, more than 160 serotypes of the virus have been recognized. These viruses are categorized into three major groups: A, B, and C. There are currently no approved vaccines available to prevent infection with HRVs. We designed a mouse immunization strategy that aimed to elicit a humoral response against conserved regions of capsid proteins of HRV-A viruses. To this end, recombinant DNA plasmids expressing the capsid proteins (VP1-4) and two proteases (2A and 3C) of HRV 1A, 16, 49, 68, and 71 were engineered. Mice were sequentially vaccinated with these DNA plasmids at three-week intervals. After a final boost with purified whole virus using the HRV 15 strain, mice spleens were extracted and cells expressing monoclonal antibodies (mAbs) were generated by hybridoma fusion. A total of 98 mAbs with reactivity to different strains of HRV-A were isolated. After isotyping, 22 mAbs expressing an IgG Fc-domain were selected for further expansion and purification. Three mAbs showed broad cross-reactivity against multiple strains of HRV-A viruses by ELISA, including 1A, 1B, 15, 16, and 49. Additional mAbs had strain-specific binding patterns, with a surprising number of mAbs showing reactivity to HRV 15, the strain used for the final vaccination. Using a microneutralization assay, we found that the HRV15-specific mAbs, but not the broadly cross-reactive mAbs, were highly neutralizing. Additional testing in a flow cytometry-based antibody-dependent cellular phagocytosis (ADCP) assay revealed a high degree of ADCP activity for one of the broadly cross-reactive mAbs. Epitope mapping of the neutralizing mAbs via escape mutant viruses revealed binding sites with a shared epitope on VP1 of HRV 15. The epitope of the ADCP-active, non-neutralizing mAb was determined by microarray analysis of cyclic constrained peptides generated from the VP1 capsid protein. This study identified a broadly cross-reactive mAb that mediates phagocytosis. These findings could be used toward the development of vaccines against HRV.



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Abstract

21 A Genome-Wide CRISPR Activation Screen Identifies Genes Involved in Protection from Zika Virus Infection

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Zika virus (ZIKV) is an arthropod-borne emerging pathogen causing febrile illness. ZIKV is associated with Guillain–Barré syndrome and other neurological complications. Vertical transmission of ZIKV can cause fetus demise, stillbirths, or severe congenital abnormalities and neurological complications. There is still no vaccine or specific treatment for ZIKV infection. To identify host factors that can rescue cells from ZIKV infection, we used a genome-scale CRISPR activation screen. Our highly ranking hits included a short list of interferon-stimulated genes (ISGs) previously reported to have antiviral activity. Validation of the screen results highlighted interferon lambda 2 (*IFN-lambda2*) and interferon alpha-inducible protein 6 (*IFI6*) as genes providing high levels of protection from ZIKV infection. Activation of these genes had an effect on an early stage in viral infection. In addition, infected cells expressing single guide RNAs (sgRNAs) for both of these genes displayed lower levels of cell death than did the controls. Furthermore, the identified genes were significantly induced in ZIKV-infected placenta explants. These results highlight a set of ISGs directly relevant for rescuing cells from ZIKV infection or its associated cell death, thus substantiating CRISPR activation screens as a valid tool for identifying host factors impeding pathogen infection.



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22 A Novel System to Study Dengue Virus Replication Organelle Formation Independent from Viral RNA Replication

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Positive-strand RNA viruses, such as dengue virus (DENV), induce extensive rearrangement of intracellular membranes that serve as a scaffold for the assembly of the viral replication machinery. In the case of DENV, the main endomembrane ultrastructure produced in infected cells consists of invaginations of the endoplasmic reticulum, designated as vesicle packets (VPs), which are the assumed sites of viral RNA replication. VPs are observed as arrays of vesicles surrounded by an outer membrane, the formation of which is induced by the viral nonstructural proteins, presumably in conjunction with specific host factors. However, little is known about the mechanisms governing VP formation, which is mainly due to the lack of a replication-independent system supporting the biogenesis of these membranous structures. Here we describe an expression based, viral RNA replication-independent, DENV polyprotein system, designated as pIRO (plasmid-induced replication organelle), which is sufficient to induce VP formation. We show that VPs induced by pIRO expression are morphologically indistinguishable from those found in infected cells, suggesting that DENV replication organelle formation does not require RNA replication. We conclude that the pIRO system is a novel and valuable tool that can be used to dissect the mechanisms underlying DENV replication organelle formation.



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23 A Simple and Rapid Cloning Method for Broad Subtypes of the Influenza A Virus Genome

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Performing reverse genetics (RG) systems on RNA viruses could largely contribute to the understanding of their molecular characteristics. Although the RG system of influenza A virus is well established, the conventional sequence-dependent method for cloning influenza genome segments is time-consuming and requires multiple processes (e.g., enzyme digestion and ligation) and exhibits low cloning efficiency compared to the sequence-independent cloning method. We improved influenza genome cloning into the pHW2000 vector for an RG system by incorporating a sequence-independent circular polymerase extension cloning (CPEC) approach which requires only two steps (reverse transcription and one-pot CPEC-PCR) and takes about 4 hours before the transformation. The specifically designed viral gene and vector primers used for CPEC-PCR have improved cloning efficiency, ranging from 63.6% to 100% based on the results of gene-specific colony PCR, which were additionally confirmed by enzyme digestion. We successfully cloned all genes from broad subtypes of influenza A viruses (H1–H12, N1–N9) and performed rescue using the RG system. Our results demonstrate that this one-pot cloning method for influenza A virus was efficient in terms of required time and cloning rate. In conclusion, this novel cloning method for influenza A virus will contribute to a significant reduction in the time required for genetic studies of emerging influenza viruses.



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Abstract

24 ADAR1 Function Regulates Innate Immune Activation and Susceptibility to Viral Infections

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Viral infection induces innate intracellular antiviral defenses, aimed at restricting virus replication and spread. Therefore, understanding the role and function of innate immune modulators can help to establish novel strategies for viral control. Here, we explore the role of ADAR1 as a regulator of HIV, HCV, and HPV infections, both in vitro and in vivo, in a genetic association study. Depletion of *ADAR1* induces innate immune activation, observed by a significant increase in *IFNB1* mRNA and *CXCL10* expression. Further characterization of *ADAR1* knockdown also showed upregulation of the RNA sensors MDA5 and RIG-I, increased IRF7 expression, and phosphorylation of STAT1. ADAR1 deficiency had differential effects depending on the virus tested: siADAR1 cells showed a significant reduction in HIV-1 infection, whereas *ADAR1* knockdown suggested a proviral role in HCV and HPV infection. In addition, genetic association studies were performed in a cohort of 155 HCV/HIV individuals with chronic coinfection and a cohort of 173 HPV/HIV-infected individuals followed for a median of 6 years (range 0.1–24). Polymorphisms within the *ADAR1* gene were found to be significantly associated with poor clinical outcome to HCV therapy and advanced liver fibrosis in a cohort of HCV/HIV-1 coinfecting patients. Moreover, we identified the low frequency haplotype AACCAT to be significantly associated with recurrent HPV dysplasia, suggesting a role for ADAR1 in the outcome of HPV infection in HIV+ individuals. In conclusion, we show that ADAR1 regulates innate immune activation and plays a key role in susceptibility to viral infections by either limiting or enhancing viral replication. Overall, ADAR1 could be a potential target for designing immune-modulating therapeutic strategies.



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Abstract

25 African Swine Fever Virus Multigene Family Genes Inhibit the Type-I Interferon Response by Acting on the NF κ B and IRF3 Signalling Pathways at the Transcription Factor Level or Below

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African Swine Fever Virus (ASFV) is a haemorrhagic infection of swine, which routinely displays 100% lethality. ASFV is rapidly spreading through East Asia and the EU, posing an existential threat to the global pork industry. No vaccine presently exists.

ASFV is a large dsDNA virus with a 190 kb genome, encoding for over 160 ORF. The terminal genome ends encode numerous paralogous genes divided into 5 clusters, known as multigene family (MGF) genes. The composition of MGFs present varies largely between isolates, and are thought to be responsible for isolate virulence. Previous research has unveiled a role in the modulation of the innate immune system, although the functional mechanism of which remains elusive. Inoculation with naturally attenuated OURT88/3 and the genetically modified attenuated Benin Δ MGF both lacking 6 MGF360 and 4 MGF505 genes induces protection upon challenge with parental virus, but has also been demonstrated to induce IFN production. Furthermore increased sensitivity of viral replication to pre-treatment of cells with type-I IFN is also observed. This previous work has hinted towards the MGF genes ability to subvert the interferon pathway.

We aim to elucidate the functional mechanism of the MGF genes responsible for attenuation, in order to rationally generate a live attenuated vaccine. We have demonstrated that the MGF genes of interest display a strong inhibition of both the NF κ B and IRF3 signalling pathways, seemingly by acting at the transcription factor level or below. Furthermore, the interaction of MGFs with host proteins, and the impact on host interferon responses is under investigation.



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Abstract

26 An E460D Substitution in the NS5 Protein of Tick-Borne Encephalitis Virus Confers Resistance to the Inhibitor Galidesivir (BCX4430) and Also Attenuates the Virus in Mice

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Tick-borne encephalitis virus (TBEV) is a pathogen that causes severe human neuroinfections in Europe and Asia for which there is currently no specific therapy. The adenosine analogue galidesivir (BCX4430), a broad-spectrum RNA virus inhibitor, has entered a phase 1 clinical safety and pharmacokinetics study in healthy subjects and is under clinical development for treatment of Ebola and yellow fever virus infections. Moreover, galidesivir also inhibits the reproduction of TBEV and numerous other medically important flaviviruses. Until now, studies of this antiviral agent have not yielded resistant viruses. In our study, we performed serial in vitro passaging of TBEV in the presence of increasing concentrations of galidesivir (up to 50 μ M) which resulted in the generation of two drug-resistant TBEV mutants. The first TBEV mutant was characterized by a single amino acid change, E460D. The other carried two amino acid changes, E460D and Y453H. Both mutations mapped to the active site of the viral RNA-dependent RNA polymerase (RdRp). Galidesivir-resistant TBEV exhibited no cross-resistance to structurally different antiviral nucleoside analogues, such as 7-deaza-2'-C-methyladenosine, 2'-C-methyladenosine, and 4'-azido-aracytidine. Although the E460D substitution led to only a subtle decrease in viral fitness in cell culture, galidesivir-resistant TBEV was highly attenuated in vivo, with a 100% survival rate and no clinical signs observed in infected mice. Furthermore, no virus was detected in the sera, spleen, or brain of mice inoculated with the galidesivir-resistant TBEV. By contrast, infection with wild-type virus resulted in fatal infections for all animals. Our results contribute to understanding the molecular basis of galidesivir antiviral activity, flavivirus resistance to nucleoside inhibitors, and the potential contribution of viral RdRp to flavivirus neurovirulence.



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Abstract

27 An Epitranscriptomic Switch at the 5'-UTR Controls Genome Selection during HIV-1 Genomic RNA Packaging

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During the late stages of viral replication, the *HIV-1* full-length RNA plays two major roles in the cytoplasm of infected cells by acting as an mRNA encoding the structural proteins Gag and Gag-Pol and as the genome packaged into newly produced viral particles. It was proposed several years ago that the full-length RNA exists as a single population used indistinctly as mRNA or genome. However, the molecular mechanisms involved in the selection of the RNA molecules that will be packaged into nascent virions are still poorly understood. It was recently reported that the *HIV-1* full-length RNA contains N⁶-methyladenosine (m⁶A) residues located at both the 5'- and 3'-UTR as well as at internal positions. Full-length RNA methylation has shown to play important roles during the replication cycle, inducing early degradation upon entry but allowing the accumulation of viral RNA during active viral gene expression. These observations prompted us to study whether m⁶A could serve as a mark that defines the functions of the *HIV-1* full-length RNA as either mRNA or genome for packaging. Here, we show that the presence of m⁶A on the full-length RNA favors Gag synthesis but strongly inhibits RNA packaging. Interestingly, m⁶A-seq analyses revealed that the 5'-UTR of the viral RNA is methylated within the cell, but not in viral particles, and we identified A₁₉₈ and A₂₄₂ as the critical residues responsible for the epitranscriptomic regulation of *HIV-1* full-length RNA packaging. We also observed that FTO-mediated demethylation was required for incorporation of the full-length RNA into viral particles and, interestingly, HIV-1 Gag associates with FTO in the nucleus and drives RNA demethylation. Finally, we demonstrate that pharmacological inhibition of the RNA demethylase activity of FTO suppressed full-length RNA packaging. Together, our data propose a novel epitranscriptomic mechanism whereby the selection of *HIV-1* full-length RNA for packaging is regulated through an epitranscriptomic switch involving the demethylation of two conserved adenosine residues present within the 5'-UTR. This novel epitranscriptomic regulation could also be exploited as a target for pharmacological intervention.



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28 Analysis of *cis*-Acting RNA Elements Required for Zika Viral RNA Synthesis Initiation by the Viral RNA Polymerase

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ABSTRACT

Zika virus (ZIKV) infection causes not only microcephaly in infants but also various neurological disorders including Guillain–Barré syndrome, meningoencephalitis, and myelitis in adults. Currently, neither vaccines nor specific antiviral drugs are available to prevent or treat ZIKV infection. ZIKV plus-strand RNA genome is replicated by a virally encoded RNA-dependent RNA polymerase (RdRp). Thus, the RdRp domain in the C-terminal part of nonstructural (NS) protein 5 is an attractive target for direct-acting antiviral agents (DAAs). Furthermore, identification of the *cis*-acting RNA elements (CREs) recognized by the RdRp would help in understanding their roles in viral RNA replication and, thus, to design a safe attenuated live ZIKV vaccine. In the present study, we cloned and expressed a full-length recombinant ZIKV NS5 to establish an in vitro RdRp assay system. Using the purified NS5 protein capable of copying viral RNA templates, we mapped the CRE required for minus-strand viral RNA synthesis by the Zika viral RNA polymerase. The purified NS5 showed primer-dependent RNA synthesis activity on a homopolymeric RNA template. It was also able to initiate de novo RNA synthesis from viral RNA templates representing the 3'-end region of the plus- and minus-strand RNA of ZIKV. We found that an 83 nt long 3'-UTR is the minimal ZIKV RdRp template from which minus-strand viral RNA synthesis occurs. The in vitro RdRp assay system, established with a full-length NS5, will be useful for understanding the mechanisms of ZIKV RNA genome replication and for the development of anti-ZIKV DAAs.



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29 Lytic Reactivation of the Kaposi's Sarcoma-Associated Herpesvirus (KSHV) Is Accompanied by Major Nucleolar Alterations

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The nucleolus is a subnuclear compartment whose primary function is in the biogenesis of ribosomal subunits. Certain viral infections affect the morphology and composition of the nucleolar compartment and influence rRNA transcription and maturation. However, no description of the nucleolar morphology and function during KSHV infection is available to date.

By using immunofluorescence microscopy, we have documented extensive destruction of the nuclear and nucleolar architecture during lytic reactivation of KSHV. Redistribution of key nucleolar proteins was documented, including the rRNA transcription factor UBF, the RNA polymerase I subunit RPA194, the pre-rRNA processing factor fibrillarin, and the nucleolar phosphoproteins nucleophosmin and nucleolin. Certain nucleolar proteins remained associated whereas others dissociated, implying that nucleolar proteins undergo nonrandom programmed dispersion. Of note, both fibrillarin and UBF did not colocalize with PML nuclear bodies, and no colocalization was detected between fibrillarin and KSHV ORF59 protein, which specifies viral replication compartments. No significant changes in pre-rRNA levels and no accumulation of pre-rRNA intermediates were found by RT-qPCR and Northern blot analysis, respectively. Furthermore, a complete overlap between ITS-1, which represents the primary pre-rRNA product, and fibrillarin was documented, suggesting that the transcription and processing of rRNA proceed. Finally, small changes in the levels of pseudouridylation were documented across rRNA.

Taken together, our results suggest that rRNA transcription and processing persist during lytic reactivation of KSHV, yet they may become uncoupled. It remains to be determined whether the observed nucleolar changes favor productive infection or signify cellular antiviral responses.



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Abstract

30 Analysis of Humoral Immune Responses in Chikungunya Virus (CHIKV)-Infected Patients and Individuals Vaccinated with a Candidate CHIKV Vaccine

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Chikungunya virus (CHIKV) is a mosquito-transmitted alphavirus that causes severe flu-like symptoms. The acute symptoms disappear after one week, but chronic arthralgia can persist for years. Here, humoral immune responses in CHIKV-infected patients and vaccinees were analyzed. Alphavirus neutralization activity was analyzed with pseudotyped lentiviral vectors and antibody epitope mapping was performed with a peptide array. Greatest CHIKV neutralization activity was observed 60–92 days after onset of symptoms. The amount of CHIKV-specific antibodies, their binding avidity, and cross-reactivity with other alphaviruses increased over time. CHIKV and o'nyong-nyong virus (ONNV) were both neutralized to a similar extent. Linear antibody binding epitopes were mainly found in E2 domain B and the acid-sensitive regions (ASRs). In addition, serum samples from healthy volunteers vaccinated with a measles-vectored Chikungunya vaccine candidate, MV-CHIK, were analyzed. Neutralization activity in the samples from the vaccine cohort was lower than in samples from CHIKV-infected patients. In contrast to infection, vaccination induced cross-neutralization with ONNV and the E2 ASR1 was the major antibody target. These data could assist vaccine design and enable the identification of correlates of protection necessary for vaccine efficacy.



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31 Anti-Norovirus Activity of Orally Administered Neoagarohexaose, a TLR4 Agonist from Red Algae

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ABSTRACT

Norovirus infection is the primary cause of non-bacterial acute outbreaks of gastroenteritis worldwide affecting people in a wide range of ages. In immunocompromised patients, it is known to establish chronic infection. In the absence of vaccines approved for clinical uses, there is an urgent unmet need for antiviral drugs that can be used without adverse effects on the elderly and infants. In the present study, we evaluated the antiviral activity of neoagarohexaose (NA6) derived from red algae, in virus-infected cells and mice. We show that NA6 is a nonconventional toll-like receptor 4 (TLR4) agonist with antiviral activity against norovirus. It inhibited murine norovirus (MNV) replication with an EC₅₀ of 1.5 μM in the mouse macrophage cell line RAW264.7. Activation of the TLR4 signaling pathway by NA6 was dependent on myeloid differentiation factor 2 (MD2) and CD14, an accessory protein also known to be required for sensing of the prototype TLR4 ligand lipopolysaccharide. NA6 induced interferon-β and TNF-α production in mouse and human macrophages. Oral administration of NA6 in mice also induced expression of these antiviral cytokines in the ileal Peyer's patches and decreased MNV loads in the distal ileum. Our results disclose an NA6-mediated TLR4 signaling activation in the ileum, highlighting its potential therapeutic application for the treatment of norovirus infection.



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32 Antioxidant Effect of *A. chilensis* on the Production of Infectious Viral Particles of ISAv and Its Consequences on the SUMOylation of NP Protein

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Infectious salmon anemia virus (ISAv) is a pathogen of high economic importance worldwide; it produces a highly fatal clinical symptomatology called Salmon Infectious Anemia (ISA), which is one of the causes of greatest economic losses in Chilean aquaculture Chilean salmon, being responsible for causing a mortality greater than 80% when outbreaks of this pathogen occur in fish farms. ISAv dramatically increases levels of reactive oxygen species (ROS) by increasing the activity of the p38MAPK protein, which activates p47phox, by phosphorylation, allowing the binding of this to the membrane subunits of the NOX2 complex, which is an important positive regulator of ROS levels in cells. Further, it is known that oxidative stress is able to regulate the SUMOylation machinery, producing an increase in SUMOylated proteins. Together with this background and various bioinformatic analyses, it was found that ISAv nucleoprotein (NP) has a highly conserved motive for SUMOylation, and this protein alone is capable of causing strong oxidative stress in transfected cells and is therefore able to regulate the SUMOylation machinery. Immunoprecipitation assays confirmed the bioinformatic analyses, where NP was seen to be SUMOylated, and this signal decreases considerably when cells are treated with a p38MAPK inhibitor. Together with this, the number of copies of NP and the viability in cells infected with ISAv were also evaluated, where it is observed that there is a strong increase in the number of copies of NP and a marked decrease in cell viability, this being contrasted with when, in addition to the infection, the cells are treated with a natural product “maqui” (*A. chilensis*), which, due to its high content of polyphenolic compounds, has been shown to have a high antioxidant capacity, greatly reducing the number of copies of NP and the percentage of mortality compared to cells that are only infected with ISAv.



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Abstract

33 Application of SORTS, a Novel Gene-Edited Cell Selection Method for HIV Study and Therapy

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We have recently developed SORTS (surface oligopeptide knock-in for rapid target selection), a novel method to isolate mammalian cells with gene modifications using FACS-sorting. It relies on CRISPR/Cas9-mediated targeted knock-in of a very short promoterless expression construct (250 bp) comprising a Flag or HA epitope embedded into the smallest GPI-protein CD52, and a polyA signal from the beta-globin. CD52 efficiently delivers the epitope to the cell surface, where it serves as a marker for selection, while polyA terminates transcription and silences target gene expression. Primarily, SORTS was developed to knock out genes encoding intracellular or secreted proteins, which cannot be used as markers for selection of live cells. Using in-frame modification of SORTS, we demonstrated the possibility of HIV-1 provirus inactivation via sorting of GPI-tag positive cells. In order to make the “cured” cells resistant to a subsequent HIV-1 infection, the epitope tag in the CD52 molecule was substituted by one of the fusion inhibitor peptides from the CHR-domain of gp41. We selected a series of cell-surface-expressed, GPI-anchored, C34-based peptides that confer a strong cellular resistance to HIV-1 infection mediated by NL4-3, JRFL or ZM153 Env. These findings together with a monoclonal antibody raised against the C34 peptide provide an opportunity to generate and select HIV-resistant lymphocytes for a therapeutic goal. SORTS was also adapted to engineer transgenic HIV-1 effector T cells and to study cell-to-cell transmission. To facilitate transgenesis, we developed a knock-in strategy to express GPI-tag from the intronic region of the human PPP1R12C gene (AAVS1 locus) and delivered FRT sites of recombination into both alleles. In summary, SORTS is a novel instrument to isolate rare cells with precise genomic modifications with broad applications, including HIV biology.

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34 Architecture of *Hendra henipavirus* Ribonucleoprotein Complexes Elucidated through Transmission Electron Microscopy

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Hendra henipavirus (HeV; *Paramyxoviridae*) is an emerging zoonotic non-segmented negative-sense RNA virus (nsNSV) and the type species of the genus *Henipavirus*. HeV is disseminated by pteropid fruit bats and lethally infects horses and humans with mortality rates of up to 60%–75%. The close relative of HeV, *Nipah henipavirus* (NiV), has caused hundreds of deaths across Asia and is also spread by pteropid bats with a similarly high mortality rate. As an nsNSV, HeV expresses a nucleocapsid protein (N) which, as a multimer, encapsidates the viral RNA (vRNA) genome, forming a structure called a ribonucleoprotein (RNP). Since N/N and N/vRNA interactions are essential for virus replication, they represent potential targets for the design of antiviral therapies. Currently, no high-resolution structural information of either the HeV N protein or HeV RNP architecture is available, and no effective antiviral strategies exist to prevent or treat HeV infections.

We recombinantly expressed and purified HeV RNP-like complexes in a bacterial expression system. Negative stain and cryogenic transmission electron microscopy were employed to elucidate the HeV RNP architecture and ultimately resolve the HeV N protein structure, revealing a helical architecture of approximately 13 monomers per turn.

In addition, the generation of a HeV replicon system allows for functional examination of critical residues involved in the observed N/vRNA and N/N interactions. These residues and contacts may constitute attractive therapeutic targets for the prevention of RNP assembly, and the data improve upon our understanding of nsNSV genome encapsidation and replication mechanics.

Our work adds to the limited availability of structural information on HeV and henipaviruses as a whole. Importantly, these data could facilitate the development of new diagnostic tools and antiviral therapies, such as small molecule inhibitors, to combat HeV and, by extension, potentially NiV.



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Abstract

35 Atypical Porcine Pestivirus Molecular Evolution within a Persistently Infected Swine Farm

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Atypical porcine pestivirus (APPV) is a single stranded RNA virus from the family Flaviviridae. It has been identified as the possible causative agent of congenital tremor (CT) type A-II in newborn piglets. To date, APPV has been reported in several countries in North and South America, Europe, and Asia, showing a worldwide distribution. Although clinical signs are only present in piglets born from gilts and occasionally in piglets born from high parity sows, adult pigs can also be carriers and persistently shed the virus.

In this study, we retrospectively investigated the molecular evolution of APPV in one endemically infected farm between the first outbreak in 2013 and 2019. Monitoring was done consistently over time in a controlled environment with no viral introduction from the outside. During the monitoring period, clinical outbreaks were observed in 2013, 2015, and 2016. Samples from clinically trembling piglets at the time of these outbreaks, as well as from persistent carriers detected during regular monitoring, were used for viral sequence analysis.

This field case study provides new insights into the genetic evolution of this novel pathogen circulating within a farm. With this study, we aimed to provide new information regarding nucleotide and amino acid variability of APPV over time, phylogenetic analysis on complete genome sequences and partial regions, and to evaluate their potential role into the escape from the host-immune system. These findings are essential for future vaccine design strategies against APPV as well as for understanding the genetic variation patterns of other (novel) pestiviruses.



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Abstract

36 bis-Benzylisoquinoline Alkaloids Inhibit Human Coronavirus OC43 Infection in MRC-5 Human Lung Cells

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Although the pharmacological bioactivities of bis-benzylisoquinoline alkaloids mostly isolated from *Stephania tetrandra* and other related species of Menispermaceae have been studied, their antiviral effects against human coronavirus (HCoV) remain poorly characterized. In the current study, we investigated the antiviral activities of bis-benzylisoquinoline alkaloids and their underlying action mechanism in HCoV-OC43-infected MRC-5 human lung cells. An effective dose considerably reduced virus-induced cytopathic effects without cytotoxicity in a time- and dose-dependent manner. Additionally, the antiviral activity was effective at the early stage of HCoV-OC43 infection and the virus-induced interferon (IFN)-related gene expression and production of inflammatory cytokines were decreased after treatment. Taken together, our study indicates that bis-benzylisoquinoline alkaloids may play pivotal roles as natural antiviral components and be potentially preventive and therapeutic against HCoV-OC43 infection.



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37 BKTyper—Web Application for VP1 and NCCR Polyoma BK Typing

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Human polyoma BK virus (BKV) prevalence has been increasing due to the introduction of more potent immunosuppressive agents, mostly in immunocompromised patients. BKV has been linked mostly to polyomavirus-associated hemorrhagic cystitis, and polyomavirus-associated nephropathy. BKV is a circular double stranded DNA virus (circular dsDNA) with an average genome size of 5100 bp and an average GC content of 40%. Its genome codifies for 5 proteins: VP1, VP2, VP3, Angio gene, and the antigen T (which includes an event of alternative splicing, yielding a short and a large antigen T transcript). Additionally, it contains the non-coding control region (NCCR), known to be highly repetitive and to vary in number, length, and location of the repeats. Subtyping of BKV has been mainly studied in VP1 and the NCCR. Subtyping and subgrouping of BKV is conducted routinely in diagnostic assays and in epidemiological studies. Recently, Morel et al. published (*Journal of Clinical Microbiology* 2017, 55, 4) a strategy to subtype BKV through 100 bp VP1 amplicon. NCCR diversity is more complex than VP1, as it is configured by 5 repeat blocks (O, P, Q, R, and S). NCCR blocks can vary in number and length, resulting in a gradient of infectivity and replication. Rearranged NCCR have been linked to diverse patient etiologies, although any specific arrangement has failed to correlate with disease outcome or to have any predictive value. Due to the high abundance of BKV individuals and clinical implications for human health that may represent BKV typing, a reliable, automatic, and free typing tool would be of great interest. Here, BKTyper is presented, a whole genome genotyper for polyoma BKV, based on a VP1 typing by Morel's algorithm and NCCR block identification. BKTyper can accept both whole BKV genome or regions of interest in fasta format to generate the typing profile.



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Abstract

38 Cell Surface-Expressed GPI-Anchored Peptides from the CHR Domain of Gp41 Are Potent Inhibitors of HIV-1 Fusion

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Current antiretroviral therapy efficiently suppresses viral replication but cannot eliminate latent HIV reservoirs. Moreover, the associated high costs, side effects, and drug resistance have stimulated a need for the development of alternative methods of HIV-1/AIDS treatment, such as peptide inhibitors or gene editing.

Recently, we have developed SORTS (Surface Oligopeptide knock-in for Rapid Target Selection), a method for the rapid selection of CRISPR/Cas9 gene-edited cells via knock-in of the Flag and HA epitope tags embedded into the shortest GPI-protein, CD52. By targeting the capsid region of the HIV-1 genome, we demonstrate that SORTS can be applied in provirus eradication. However, the cells with inactivated provirus will be susceptible for HIV re-infection. We hypothesized that knocking in one of the peptides from the CHR-domain of *gp41*, which are known potent inhibitors of HIV-1 fusion, instead of the epitope tag, will provide “post-curable” HIV-1 resistance. While these peptides were extensively studied as soluble substances, their inhibitory effects on HIV after expression on cell surfaces via GPI-anchor are largely unknown. In this study, we established HEK293T/CD4/R5 and Raji/CD4/R5 HIV-1 permissive cell lines that stably expressed one of the Gp41 peptides C34, MT-C34, MT-C34-R, MT34-15D or alfa-helix mimetics HP23L, p52, MT-WQ-IDL. For cell surface delivery, the indicated peptides were embedded into CD52 molecule, and upstream GFP was used to select transformed cells. Using a single-cycle replication assay with inLuc reporter vector and different Envs, we demonstrated that C34-based GPI-anchored peptides inhibited both cell-free and cell-to-cell HIV-1 infection by at least two order of magnitude. With the exception of HP23L, the alfa-helix mimetics were less potent inhibitors. Thus, peptides from Gp41 associated with lipid rafts and exerted a strong inhibitory activity which can far exceed that determined for soluble peptides, but this should be tested further.

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39 Characterising Interactions between Influenza A Virus and Respiratory Syncytial Virus During In Vitro Coinfection

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Influenza A virus (IAV) and respiratory syncytial virus (RSV) are important respiratory pathogens that share common epidemiological features and cellular tropism within the respiratory tract. This gives rise to the potential for biological interactions between IAV and RSV during coinfection of hosts. Virus–virus interactions are increasingly recognised for their contribution to viral dynamics during infection, however, the molecular processes underpinning these interactions are unknown.

Here, we developed an in vitro coinfection system to characterise the infection dynamics of IAV (A/Puerto Rico/8/34, H1N1) and RSV (strain A2) in single virus infection or coinfection in lung epithelial cells, with the aim to identify biological processes that drive virus–virus interactions during coinfection. We compared viral replication kinetics at different multiplicities of infection and observed that RSV replication was inhibited during coinfection with IAV, whilst IAV replication was facilitated by coinfection. To further characterise IAV/RSV interactions, we determined the relative proportions of single virus infected or coinfecting cells during early and late timepoints post-infection and observed differences in expression of viral proteins between single virus infection and coinfection. To characterise differences in viral-induced cytopathic effect, we measured cell viability after IAV/RSV infection or coinfection. Compared to RSV infection, cell death is induced at earlier timepoints post IAV infection and coinfection, indicating that different cellular processes are initiated in response to infection.

These studies highlight that both competitive and facilitative ecological interactions occur between IAV and RSV during coinfection and shed light on sources of potential interactions at the cellular and molecular level.



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Abstract

40 Characteristic of a Distant Relative of *Teseptimavirus* Genus Phages that Acquired the Ability to Lysogenize Its Host

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Pseudomonas syringae is a plant pathogen, which groups over 40 pathovars. Climate change and international trade facilitate the worldwide spread of pathogenic *P. syringae* strains. In recent decades, infections with *P. syringae* have been causing large losses in vegetable growing and horticulture. With the aim to look for biocontrol agents that could minimize these losses, we isolated bacteriophages infective for certain *P. syringae* strains. One of these phages, designated by us as vB_PsyP_3MF5 (3MF5), appeared to have atypical properties. It formed clear plaques on the layers of sensitive cells at elevated temperatures but was unable to form plaques at room temperature. It quickly adsorbed to its host cells and had a short latent period and a large burst size at permissive temperature. However, several survivors of phage infection could be isolated in a standard killing assay. They appeared to form a lysis zone when placed on a layer of cells that were not treated with this phage, indicating that they are 3MF5 lysogens. In support of that, their DNA could serve as a template for PCR amplification with 3MF5 specific primer pairs. The analysis of 3MF5 genomic sequence revealed features typical of *Teseptimavirus* genus phages which are obligatorily lytic and are unable to lysogenize cells. Additionally, comparative analysis of predicted 3MF5 proteins excluded the presence of any obvious homolog of a typical phage repressor that inhibits transcription of early phage genes in lysogens. Conceivably, the repression is achieved either by the interaction of a temperature-sensitive host/phage protein with a region controlling the expression of phage early genes or by temperature-induced structural changes in phage RNA, which could act by the occlusion of ribosomal binding sites of early phage genes. Surprisingly, the results of our preliminary studies indicate that despite its conditionally temperate nature, 3MF5 exhibits biocontrol properties.



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Abstract

41 Characterization of Dengue Virus Nonstructural Protein 4A for Its Role in Viral Replication Organelle Biogenesis

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Dengue virus (DENV) constitutes one of the most important arboviral pathogens affecting humans, and causes severe diseases such as dengue fever, dengue hemorrhagic fever, and dengue shock syndrome. The high prevalence of DENV infections, which cause more than twenty thousand deaths annually, and the lack of effective vaccines or direct antiviral drugs make it a global health concern. DENV genome replication occurs in close association with the host endomembrane system, which is remodeled to form the viral replication organelle that originates from ER-derived membranes. Despite recent progress in our understanding of the DENV replication cycle, the viral and cellular determinants responsible for the biogenesis of DENV replication organelles are still poorly defined. The viral nonstructural protein (NS) 4A can remodel membranes and has been recently shown to associate with numerous host factors in DENV replicating cells. In the present study, we used reverse and forward genetic screens and identified sites within NS4A required for DENV replication. In addition, we mapped the determinants in NS4A required for interactions with both viral and cellular factors. Moreover, taking advantage of our newly developed polyprotein expression system, we evaluated the role of NS4A in the formation of DENV replication organelles. Together, our results highlight NS4A as a central hub in facilitating associations between viral and host factors required for the biogenesis of the viral replication organelles.



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42 Characterization of JC Polyomavirus Entry by Serotonin Receptors

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JC polyomavirus (JCPyV) causes a lifelong persistent infection in the kidney in the majority of the population. In severely immunocompromised individuals, JCPyV can become reactivated, spread in the central nervous system, and infect glial cells, astrocytes and oligodendrocytes, which are necessary for myelin production. Viral infection and cytolytic destruction of glial cells leads to development of the fatal demyelinating disease progressive multifocal leukoencephalopathy (PML), for which there are no approved treatment options. In order to develop effective antiviral therapies it is essential to define virus-host cell interactions that drive infection, and virus-receptor interactions are major regulators of tissue tropism and viral disease outcomes. Following attachment to a sialic acid receptors, JCPyV requires the serotonin 5-hydroxytryptamine (5-HT₂) receptors to mediate internalization. However, the mechanism by which JCPyV utilizes 5-HT₂ receptors to invade host cells is poorly understood. Using super-resolution Fluorescence Photoactivation Localization Microscopy (FPALM), we have determined that JCPyV localizes with 5-HT₂ receptors at times consistent with viral entry. Further, we have defined that 5-HT₂ receptor-associated scaffolding proteins beta-arrestin, adaptor protein complex 2 (AP2), and dynamin are required for viral internalization through a clathrin-mediated endocytosis pathway. Additionally, we have identified a beta-arrestin-binding motif in the intracellular loop of the 5-HT_{2A} receptor that is critical for JCPyV entry and infection. These findings highlight the importance of viral receptors in regulating viral infection and illuminate potential targets for antiviral treatments.



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Abstract

43 Characterization of the Neurovirulence of the FNV Vaccine Strain of Yellow Fever Using the BBB-Minibrain Model

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An extremely effective live attenuated vaccine against yellow fever virus (YFV-17D) has been intensively used worldwide since 1936. In very rare cases, side effects of encephalitis (YFV-AND) occur after vaccination, in particular, after vaccination with the YFV-FNV strain, which was withdrawn in the 1980s. YFV-AND may result from the appearance of viral variants that have acquired the ability to invade the nervous system (neuroinvasion), to replicate in neurons (neurotropism), and to alter homeostasis (neurovirulence). To validate this hypothesis, we made an attempt to isolate and characterize such variants using a culture model mimicking the human blood–brain interface, the BBB-Minibrain.

After inoculation of YFV-17D or YFV-FNV in the luminal part of the device, we observed that the YFV-FNV strain crossed the blood–brain barrier (BBB) more efficiently than YFV-17D. We have shown that the viral particles of YFV-FNV that cross the BBB do so by transcytosis. Sixteen neuroinvasive variants were isolated by plaque assay. Their average sequences as established by NGS differed from the average sequence of the parental YFV-FNV strain. This analysis indicates that the YFV-FNV strain contains viral variants that have acquired the ability to cross the BBB. Two variants were selected and amplified to study their neurotropism and their neurovirulence.

Our work will provide a better understanding of the neurovirulence of YFV vaccines.



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Abstract

44 Characterization of the Respiratory Syncytial Virus N⁰-P Complex Paves the Way for Studying the Mechanism of Genome Encapsidation

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Like its counterparts belonging to the *Mononegavirales* order, the RNA genome of respiratory syncytial virus is encapsidated by the viral nucleoprotein N at all steps. This RNA-N complex is the template for the viral RNA-dependent RNA polymerase. Polymerization of N along the genomic and anti-genomic RNAs during replication requires the supply of neosynthesized N that is maintained monomeric and RNA-free, thanks to the interaction with the viral phosphoprotein P, to form a soluble N⁰-P complex. Characterization of this complex is challenging since N has a strong tendency to concomitantly oligomerize and interact with RNA. Using mutated and truncated N recombinant proteins, we managed to isolate different RSV N⁰-P-like complexes, allowing us to gain the main structural information for this complex. We first identified the minimal domain of P (residues 1-30) required to maintain N in monomeric form. By combining biophysical approaches (SAXS, analytical ultracentrifugation) with biochemical and functional analysis of N mutants, we then showed a strong homology between the N⁰-P complexes of RSV and of *Human metapneumovirus* (hMPV). More specifically, our data revealed that (i) the C-terminal acidic and hydrophobic residues of N play a critical role in the folding of the C-arm close to the RNA groove required to impair RNA binding, and (ii) the binding surface of the P peptide on RSV N is similar to the one observed in the crystal structure of the hMPV N⁰-P complex. Based on these data, we recently generated a chimeric construct composed of the full-length N and the N-terminal region of P and purified a complex competent for encapsidation. This complex represents a new tool to study the mechanisms involved in the control and the RNA specificity of encapsidation.



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Abstract

45 Chikungunya Virus Binds Specific Glycans Using Recently Defined Residues in the E2 Glycoprotein

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Chikungunya virus (CHIKV) is an arthritogenic alphavirus that causes a debilitating musculoskeletal disease. Currently, there are no vaccines or antiviral agents licensed to treat CHIKV disease. Studying the host requirements for CHIKV infection, such as cell attachment factors, may aid in the development of therapeutics. Previous work has shown that two CHIKV strains depend on cell surface glycosaminoglycans (GAGs) for efficient infection. However, the specific types of GAGs and other glycans to which CHIKV binds are not fully characterized. To identify the types of glycans CHIKV binds, we conducted multiple glycan microarray analyses using virus-like particles and determined that CHIKV preferentially binds GAGs as compared to nine other glycan groups. The array results also indicate that sulfate groups on GAGs are essential for CHIKV binding and CHIKV binds more strongly to GAGs with longer chains. To determine the requirement of GAGs for efficient cell binding, we assessed GAG use by strains of all three CHIKV clades. We found that all strains displayed dependence on GAGs for efficient cell binding, which varied slightly by strain. Enzymatic cleavage of cell surface GAGs and genetic alterations that diminish GAG expression both result in diminished binding and infectivity. Additionally, we have begun to define the GAG-binding region on CHIKV using alanine mutagenesis of the viral attachment protein. The alanine mutant viruses have been tested for structural conformation, the capacity to directly bind GAGs, and the capacity to efficiently bind cells. Overall, we have identified six E2 residues required for GAG binding. Future work will use these low GAG-binding mutant viruses to test the importance of GAG binding in a mouse model of CHIKV infection. Collectively, these studies provide evidence for a critical function of GAGs in CHIKV infection, begin to define the GAG-binding region on the virus, and contribute new knowledge regarding the engagement of host cells by CHIKV.



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46 Circulation of Toscana Virus in a Sample Population of Corsica, France

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Sandfly-borne phleboviruses pathogenic to humans, such as Toscana virus (TOSV) and sandfly fever Sicilian (SFSV) viruses, are endemic in the Mediterranean region. In France, several autochthonous cases of TOSV infection have been described which cause either meningitis or encephalitis. The aim of the present study was to estimate the seroprevalence of TOSV and SFSV antibodies in a healthy population from Corsica.

In this cross-sectional study, participants were enrolled from the medical staff at University of Corsica Pasquale Paoli (UCPP) and from general practitioners of the Corsican Sentinelles Network. The seroprevalence study was based on virus microneutralization (MN).

A total of 240 patients were tested for TOSV and SFSV. Altogether, 54 serum samples were confirmed for TOSV infection (seroprevalence = 22.5%). None of the samples were positive for SFSV (0/240). The main place of residence was significantly associated with TOSV seropositivity (p -value = 0.005).

The overall rate of TOSV antibody seroprevalence observed in our study suggests a more intense circulation of TOSV in Corsica, with a rate significantly higher than the 8.7% reported in Corsica in 2007 from blood donors. The absence of seropositivity to SFSV seems to confirm the low circulation of this virus in Corsica and in continental France.

The increasing circulation of TOSV reported here should encourage the implementation of surveillance systems to control phlebovirus infection.



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47 Common Occurrence of Belerina Virus, a Novel Paramyxovirus Found in Belgian Hedgehogs

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European hedgehogs (*Erinaceus europaeus*), also known as common hedgehogs, can be found all throughout Western Europe, including the Iberian Peninsula and Italy on the south side and Scandinavia in the north. Hedgehogs are known to carry a variety of bacterial and fungal pathogens, as well as viruses, the pathological and zoonotic potential of which is not always fully elucidated yet. Here, we report the discovery of a novel paramyxovirus, named Belerina virus, in Belgian hedgehogs. Based on its divergence from other known paramyxovirus species, Belerina virus is thought to represent a new species in the family *Paramyxoviridae*. Phylogenetic analysis groups Belerina virus together with members of the genus *Jeilongvirus*, although its genome organization is most similar to that of several yet unclassified bat viruses. Because of several dissimilarities with other Jeilongviruses, these bat viruses have been proposed to represent a new genus, tentatively called ‘Shaanvirus’. Out of 147 animals screened in this study, 57 tested positive for Belerina virus (39%), indicating a wide spread of this virus throughout the Belgian hedgehog population, although the virus’ pathogenic and zoonotic potential remains to be elucidated. In summary, we present here the complete genome sequence of Belerina virus, a putative new paramyxovirus species commonly found in Belgian hedgehogs.



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48 Complete Virus Inactivation Using a Combined Heat and Chemical Treatment

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Validated inactivation protocols are required for the safe handling and disposal of virus samples. This is particularly important for ACDP3 agents and above. Various methods are employed for virus inactivation, some of which include the use of heat or chemical chaotropic agents such as guanidine hydrochloride. It is generally accepted that these processes are sufficient to denature all viruses. While inactivation of certain viruses with such methods have been reported, validation of their activity against a wide range of viruses is required. Here, we examined the inactivation of a panel of ACDP2 viruses (*Rhabdoviridae*, *Togaviridae*, *Peribunyaviridae*, and *Flaviviridae*) using a combination of heat, guanidine hydrochloride-containing buffer, and ethanol.

Viruses were treated with proteinase K and guanidine hydrochloride-containing buffer, and heated at 56 °C for 30 min. This was followed by additional treatment with absolute ethanol. Resulting virus–buffer–ethanol mixtures were column-purified to remove residual ethanol and other toxic reagents, before being introduced to cells. Column purification was confirmed to be insufficient for virus removal. Cultures were incubated at 37 °C for 1 h, after which media supplemented with 2% foetal bovine serum was added. Cultures were then observed daily for cytopathic effects. Samples that showed no evidence of cytopathic effects were passaged thrice to confirm absence of cytopathic effects and complete inactivation of viruses.

Cultures infected with control viruses that had not been treated with buffer, heat, and ethanol but column-purified developed cytopathic effects, while cultures infected with treated viruses showed no cytopathic effects even after three passages, thus confirming complete virus inactivation.

Results from this study provide evidence of the use of a combination of heat, guanidine hydrochloride-containing buffer, and ethanol for the complete inactivation of all members of the four families investigated.



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49 Composition of Herpesvirus Ribonucleoprotein Complexes

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Herpesvirus genomes are decoded by host RNA polymerase enzymes, generating messenger ribonucleotides (mRNA) that are post-transcriptionally modified and exported to the cytoplasm through the combined work of host and viral factors. These viral mRNA bear 5'-m⁷GTP caps and poly(A) tails that should permit assembly of canonical host eIF4F cap-binding complexes to initiate protein synthesis. However, the precise mechanisms of translation initiation remain to be investigated for Kaposi's Sarcoma-associated herpesvirus (KSHV) and other herpesviruses. During KSHV lytic replication in lymphoid cells, activation of caspases leads to cleavage of eIF4G and depletion of eIF4F. Translating mRNPs depleted of eIF4F retain viral mRNA, suggesting that non-eIF4F translation initiation is sufficient to support viral protein synthesis. To identify proteins required to support viral protein synthesis, we isolated and characterized actively-translating messenger ribonucleoprotein (mRNP) complexes by ultracentrifugation and sucrose-gradient fractionation followed by quantitative mass spectrometry. The abundance of host translation initiation factors available to initiate viral protein synthesis were comparable between cells undergoing KSHV lytic or latent replication. Translation initiation factors eIF4E2, NCBP1, eIF4G2, and eIF3d were detected in association with actively-translating mRNP complexes during KSHV lytic replication, but their depletion by RNA silencing did not affect virion production. By contrast, the N6-methyladenosine methyltransferase METTL3 was required for optimal late gene expression and virion production, but dispensable for genome replication. Furthermore, we detected several KSHV proteins in actively-translating mRNP complexes that had not previously been shown to play roles in viral protein synthesis. We conclude that KSHV usurps distinct host translation initiation systems during latent and lytic phases of infection.



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50 Conservation of Genetically-Embedded Virus Assembly Instructions: A Novel Route to Antiviral Therapy

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Many single-stranded RNA viruses, including major viral pathogens, present RNA-encoded virus assembly instructions (VAIs) within their genetic message that can be isolated from the genetic code and repurposed for the design of virus-like particles. These VAIs rely on multiple dispersed RNA secondary structure elements with a consensus recognition motif for the capsid (core) protein, called packaging signals (PSs), which collectively promote capsid assembly. In this talk, I will provide evidence for the evolutionary conservation of the PS-encoded assembly instructions among different viruses in a viral family and discuss the implications of this discovery for viral evolution. I will then demonstrate how the VAIs can be exploited for therapy. In particular, defective interfering particles occur spontaneously in viral evolution as mutant strains lacking essential parts of the viral genome. Their ability to replicate in the presence of wild-type virus at the expense of virally produced resources can be mimicked by therapeutic interfering particles (TIPs). I will introduce a novel approach to the design of such TIPs based on synthetic nucleic acid sequences containing the VAIs but otherwise lacking genetic information. Using multiscale models of a viral infection, I will demonstrate the potential of these particles in both prophylaxis and treatment of RNA viral infections.



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51 CRISPR/Cas9 Editing of Viral Receptors and Biotechnological Approach to Host Resistance

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Avian sarcoma and leukosis virus (ASLV), diversified into seven phylogenetically relative subgroups (A, B, C, D, E, J, and K), present as either exogenous or endogenous viruses in domestic chicken. These subgroups are unequivocally classified by subgroup-specific receptor usage. The ALV-J subgroup enters the cell through a receptor identified as the chicken Na⁺/H⁺ exchanger type 1 (chNHE1) with twelve predicted transmembrane segments and the prominent extracellular loop 1. In multiple galliform species, the single W38 deletion or substitution makes the NHE1 receptor molecule resistant to virus entry. On the other hand, resistant alleles for NHE1 have not been found in chicken.

We recently established a new technique for transgenesis in chicken, which improves the efficiency of gene modification (including gene introduction, CRISPR/Cas9-mediated knockouts and knockins) and skips the chimeric G₀ stage. This technique has been employed to introduce the W38 deletion into the chNHE1 and biotechnologically prepare ALV-J-resistant chicken line. We present the CRISPR/Cas9-mediated deletion of W38 in chicken primordial germ cells and the successful production of the gene-edited birds. The resistance to ALV-J was examined both *in vitro* and *in vivo* and the chickens homozygous in W38 deletion was found to be ALV-J-resistant, in contrast to DW38 heterozygotes and wild-type birds, which were ALV-J-susceptible. Deletion of W38 did not manifest any visible side effects. Our data clearly demonstrate the antiviral resistance conferred by precise CRISPR/Cas-9 gene editing in the chicken. Furthermore, our highly efficient CRISPR/Cas-9 gene editing in primordial germ cells represents a substantial addition to the gene technology available for chicken, an important food source and research model.



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52 Deciphering the RNA Silencing Suppressor Function in the Potyvirus SPV2

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In most eukaryotes, RNA silencing is a key element in the regulation of gene expression and defense against pathogens. Plants have evolved a defensive barrier against exogenous microorganisms, such as plant-infecting viruses, by specifically targeting and degrading the viral RNAs and thus limiting the negative effects of the diseases caused by them. On the other hand, plant viruses encode for suppressor proteins that repress the host silencing machinery, hence allowing viral replication and infection establishment.

Our current project focuses on the characterization of gene products contributing to the RNA silencing suppressor (RSS) function of *Sweet potato virus 2* (SPV2), genus *Potyvirus*, family *Potyviridae*. SPV2 infects sweet potato (*Ipomoea batatas*, family *Convolvulaceae*), one the most important staple food crops worldwide. Infections by potyvirids result in high yield losses in sweet potato, especially from coinfection with unrelated viruses, and our final goal is to develop efficient control strategies. Our preliminary results analyzing the P1 and HCPro proteases of SPV2, transiently expressed in *N. benthamiana* together with a reporter GFP construct, revealed that HCPro constitutes a strong RSS. This is a novel finding, and we are currently characterizing the functions of other gene products



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Abstract

53 Detection and Quantification of Influenza Virus Defective Viral Genomes from NGS Datasets Obtained after RT or RT-PCR Product Sequencing

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Like most RNA viruses, influenza viruses (IAV) generate defective viral genomes (DVGs) during viral replication. Although there is accumulating evidence of a biological impact of DVGs, the molecular mechanisms leading to their production remain to be unveiled. Various next-generation sequencing (NGS) technologies and detection methods can be used to characterize DVGs. Here, we developed a bioinformatics pipeline called DG-seq to quickly identify and quantify DVGs in influenza viral stocks and compared two processing methods for NGS, with or without PCR amplification.

To evaluate the performance of the DG-seq pipeline, we used either synthetic *in vitro* transcribed DVGs mixed with the full set of synthetic full-length genomic RNAs, or biological RNA samples extracted in duplicate from three IAV stocks: mutant viruses with a R635A or a R638A mutation in the PA subunit of the polymerase that impairs viral transcription, and their wild-type (WT) counterpart. Viral genomic RNAs were reverse-transcribed and either directly subjected to Illumina sequencing (RT-seq) or PCR-amplified prior to sequencing (RT-PCR-seq). Both methods displayed a good reproducibility between batches, with a lower detection rate but a more accurate quantification of DVGs in RT-seq samples.

The PA mutants produced more DVGs than the WT virus, derived mostly from the polymerase gene segments, but also from the NA and HA segments, suggesting that an imbalance between transcription and replication can promote DVG production. Breakpoints occurred near the segment extremities, with no hotspot identified. Interestingly, we observed short direct A/T-rich repeats adjacent to the breakpoint ends at a significantly higher frequency than in the random case.

This work provides the first comparison of DVG detection and quantification from NGS data obtained in the presence or absence of PCR amplification and gives novel insight into the mechanisms of influenza virus DVG production.



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54 Determining the Role of Bacteriophages in the Virulence of *Streptococcus agalactiae*

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Bacteriophages (phage) are viruses that specifically infect bacteria and contribute to their evolution. Nearly all bacterial pathogens contain integrated phage, or prophage, within their genome, and many phage genes are associated with bacterial virulence and/or bacterial fitness. Prophages that are associated with the virulence of human pathogens include those found in *Vibrio cholerae*, *Salmonella* sp., *Staphylococcus aureus*, and *Streptococcus pyogenes*. *Streptococcus agalactiae* (Group B streptococcus or GBS) also contain prophages and, in many cases, multiple prophages in a single strain. GBS is an opportunistic pathogen that is found associated with the mucus membranes of the genitourinary tract in about 25% of the adult population. However, if GBS colonizes an immunocompromised individual (such as a neonate or pregnant mother), it can invade and survive in the bloodstream, resulting in serious morbidity and mortality. Unfortunately, treatment of neonates with antibiotics results in disruption of their normal gut microflora and has major effects on development of their immune system, and there is currently no approved vaccine for GBS. In order to address the threat of GBS to newborns, we need to fully understand the mechanisms by which this pathogen causes disease. We do not yet understand if and how prophages in GBS affect virulence and/or bacterial fitness during an infection. To determine the diversity of prophages found within GBS, we are annotating the sequenced genomes of ~65 clinical isolates of GBS that were isolated from the vaginal tract of women during pregnancy. To determine if prophage genes play a role in bacterial fitness or pathogenicity in GBS, we will cure several known GBS clinical isolates of their resident prophages and compare the cured strains with the wild type strain in a well-established zebrafish infectious disease model. Understanding GBS virulence mechanisms is key to developing novel, effective treatments and vaccines for GBS.



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Abstract

55 Detection of Known and Novel Viral Pathogens in Belgian *Ixodes ricinus* Ticks

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Recent metagenomics studies have revealed several arthropod species to be major reservoirs for RNA viruses. One of these reservoirs is *Ixodes ricinus*, the most prevalent tick species in Europe, which is known to be a vector for many viral and bacterial pathogens. For this study, we decided to investigate the virosphere of Belgian *Ixodes ricinus* ticks. High-throughput sequencing of tick pools collected from six different sampling sites revealed the presence of viruses belonging to many different viral orders and families, including *Mononegavirales*, *Bunyavirales*, *Partitiviridae*, and *Reoviridae*. Of particular interest was the detection of several putative human pathogens, including members of the families *Nairoviridae* and *Phenuiviridae* as well as three new reoviruses, two of which cluster together with members of the genus *Coltivirus*. One of these two viruses represents a new strain of Eyach virus, a known causative agent of tick-borne encephalitis. All genome segments of this new strain are highly similar to those of previously published Eyach virus genomes, except for the fourth segment, encoding VP4, which is markedly more dissimilar, potentially indicating the occurrence of an antigenic shift. Further PCR-based screening of over 200 tick pools for 11 selected viruses showed that most viruses could be found in all six sampling sites, indicating the wide spread of these viruses throughout the Belgian tick population. Taken together, these results illustrate the role of ticks as important virus reservoirs, highlighting the need for adequate tick control measures.



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56 Development of a T7-Independent MARV Minigenome System

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Marburg virus (MARV) is the only known pathogenic filovirus that does not belong to the genus *Ebolavirus*. It causes a severe hemorrhagic fever that is associated with a high mortality rate (>80%). The potential for filoviruses to cause devastating outbreaks, in combination with the lack of licensed therapeutics and vaccines for Marburg virus disease, illustrates the need for more MARV research. However, research involving MARV is hindered by its dependency on access to high-containment laboratories. Virus alternatives such as minigenomes have proven to be a useful tool to study virus replication and transcription at lower biosafety levels and can be used for antiviral compound screening. All currently available MARV minigenomes are dependent on the addition of an ectopic T7 RNA polymerase that can drive minigenome expression. While this allows for high expression levels, ectopic expression of a T7 polymerase is not feasible in all cell types and acts as a confounding factor in compound screening assays. We have developed an alternative MARV minigenome system that is controlled by an RNA polymerase II promoter which is natively expressed in most mammalian cell types. We show here that this novel minigenome can be used in a wide range of cell types and can be easily amended to a 96-well format to be used for high-throughput compound screening, thereby providing a valuable alternative to previously developed MARV minigenomes.



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57 Development of Zika Virus DNA Vaccine Using Envelope Modified Baculoviral Gene Delivery System

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Zika virus (ZIKV) is a mosquito-borne flavivirus, infection of pregnant women can cause a wide range of congenital abnormalities, including microcephaly in the infant. However, there is no vaccine available yet. In this study, we designed to use PrM / E, which is the main target gene of neutralizing antibodies, for the development of DNA vaccine for ZIKV. To enhance the gene delivery, a recombinant baculovirus whose surface was modified to express human endogenous retrovirus (HERV) envelope was constructed. Baculovirus with HERV envelope (AcHERV) showed distinguished higher gene delivery than wild type. Using the AcHERV as a delivery vector, we constructed major antigen (prM-E)-encoding DNA under the CMV promoter, AcHERV-ZIKA. Transducing of prM/E gene in a mammalian cell was confirmed by western blot. Immunization in mice with 10e7 of AcHERV-ZIKA elicited high IgG and neutralizing antibodies. In the challenge test, AcHERV-ZIKA immunized A129 mice showed perfect protection. These results suggest that AcHERV-ZIKA could be a potential vaccine candidate for human application.



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58 Differential Roles of lipin1 and lipin2 in the Hepatitis C Virus Replication Cycle

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Although their origin, nature, and structure are not identical, a common feature of positive-strand RNA viruses is their ability to subvert host lipids and intracellular membranes to generate replication and assembly complexes. Recently, lipin1, a cellular enzyme that converts phosphatidate into diacylglycerol, has been involved in the formation of the membranous web that hosts hepatitis C virus (HCV) replicase. In the liver, lipin1 cooperates with lipin2 to maintain glycerolipid homeostasis. We extended our previous study of the lipin family in HCV infection by determining the impact of the lipin2 silencing on viral replication. In contrast to the specific impact of lipin1 silencing on HCV replication, our data suggest a broader function of lipin2 not only in HCV infection, but also for replication of other RNA viruses. Moreover, lipin2-, but not lipin1-deficient cells display alterations in mitochondrial and Golgi morphology, suggesting that lipin2 contributes to the maintenance of the overall organelle architecture. Coinciding with Golgi fragmentation, our data reveal that lipin2 silencing mainly interferes with HCV virion secretion at late stages of the infection without significantly affecting viral replication or assembly. Overall, this study reveals distinctive functions of lipin1 and lipin2 in cells of hepatic origin, a context in which they are often considered functionally redundant.



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59 Does the Cytoplasmic Tail Matter? Mechanism of Viral Envelope Glycoprotein Targeting by Membrane-associated-RING-CH (MARCH) Proteins

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The MARCH family of RING-finger E3 ubiquitin ligases comprise 11 members that have been reported to play a variety of roles in downregulation of cell-surface proteins involved in adaptive immunity. The RING-CH domain of MARCH proteins is thought to ubiquitinate the cytoplasmic tails (CTs) of target proteins leading to protein degradation through either lysosomal or proteasomal pathways. Three MARCH proteins (MARCH1, 2, and 8) have recently been reported to target the HIV-1 envelope glycoprotein (Env) and vesicular stomatitis virus G glycoprotein (VSV-G), thereby impairing the infectivity of HIV-1 virions bearing HIV-1 Env or VSV-G. However, the mechanism of antiviral activity remains poorly defined. Our data show that MARCH proteins antagonize the full-length forms of HIV-1 Env, VSV-G and Ebola glycoprotein (GP), and impair the infectivity of HIV-1 virions bearing these viral glycoproteins. This Env-targeting activity of the MARCH proteins requires the E3 ubiquitin ligase activity of the RING-CH domain. We observe that MARCH protein targeting of VSV-G is to a large extent CT-dependent. In striking contrast, MARCH-protein targeting of HIV-1 Env and Ebola GP does not require the CT. Confocal microscopy data demonstrate that MARCH proteins are able to trap the viral glycoproteins in an intracellular compartment. We observe that the endogenous expression of *MARCH8* in T-cell lines and PBMCs is inducible by type I interferons (a and b) and is also upregulated by HIV-1 infection. Current studies are aimed at identifying the cellular target for MARCH-mediated ubiquitination in the context of their antiviral activity. These results will clarify the mechanism by which MARCH proteins antagonize viral glycoproteins and provide insights into the antiviral role of cellular inhibitory factors in Env biogenesis, trafficking, and virion incorporation.



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Abstract

60 Drastic Decline of Hepatitis E Virus Detection in Domestic Pigs after the Age of 6 Months, Corsica, France

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Suidae is an important reservoir of hepatitis E virus (HEV) and a source of transmission to humans (direct contact or via consumption of meat products). Our goal was to characterize the epidemiology of HEV infecting domestic pigs in Corsica Island, a French region hyperendemic for HEV. In Corsica, traditional extensive (or semi-extensive) outdoor pig farming systems are common. Sixteen farms were selected according to location and breeding system. Individual pig feces samples were collected, and qRT-PCR for detecting HEV RNA was performed. Nucleic acids from HEV-positive samples were amplified using a specific ORF2 genotyping system. The genotype and subtype of the Corsican HEV sequences were determined by phylogenetic analysis. Among the 919 porcine feces samples tested, 9.2% (n = 85) were positive. The presence of viral RNA was correlated with (i) age (>6 months) adjusted odds ratio (AOR) 0.25 (0.068–0.90) $p = 0.032$; 3–4 months AOR = 4.94 [2.30–10.62] $p = 0.000043$) with the logistic regression model with a random effect at the farm level. Among the 85 positive samples, 83 belonged to genotype 3c and two to genotype 3f. The highest prevalence was observed in the 3–4 months age group, and older age (>6 months) was negatively related to HEV infection, and this suggests that traditional breeding with a late slaughter age may limit the risk of transmission to humans. A kinetics study of pigs from birth to slaughtering would allow ensuring that the type of traditional breeding reported here is very favorable to the absence of the virus in slaughtered pigs and in pork products.



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Abstract

61 Ebola Virus Requires Phosphatidylserine Scrambling Activity for Efficient Budding and Optimal Infectivity

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Ebola virus (EBOV) interacts with cells using multiple categories of cell-surface receptors, including C-type lectins and phosphatidylserine (PS) receptors. PS receptors typically bind to apoptotic cell membrane PS and orchestrate the uptake and clearance of apoptotic bodies. Many viruses coated with PS-containing lipid envelopes, acquired during budding from host cells, can also exploit these receptors for internalization. PS is restricted to the inner leaflet of the plasma membrane in homeostatic cells, an orientation that would be unfavorable for PS receptor-mediated uptake if conserved on the viral envelope. Therefore, it is theorized that viral infection induces host cell PS externalization to the outer leaflet during replication. Cells have several membrane scramblase enzymes that enrich outer leaflet PS when activated. Here, we investigate the role of two scramblases, TMEM16F and XKR8, as possible mediators of cellular and viral envelope surface PS levels during recombinant VSV/EBOV-GP replication and EBOV virus-like particle (VLP) production. We find that rVSV/EBOV-GP and EBOV VLPs produced in *XKR8* knockout cells contain two- to threefold less PS in their outer leaflets. Consequently, rVSV/EBOV-GP produced in delta*XKR8* is 70% less efficient at infecting cells through apoptotic mimicry as compared to the viruses produced by parental cells. In addition, the budding efficiency of both recombinant VSV particles and VLPs was significantly reduced in cells lacking *XKR8*. Our data suggest that virion surface PS acquisition requires *XKR8* activity, whereas the deletion of *TMEM16F* did not affect EBOV-GP-mediated entry of VLP production. Unexpectedly, we observed an additional role of *XKR8* in rVSV/G, rVSV/EBOV-GP, and EBOV VLP budding.



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62 Editing of the Human *TRIM5* Gene Decreases the Permissiveness of Jurkat T Lymphocytic Cells to HIV-1

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TRIM5 α is a cytoplasmic antiviral effector induced by type I interferons (IFN-I) that has the potential to intercept incoming retroviruses by interacting with their capsid core, leading to uncoating induction and partial degradation of core components. Most HIV-1 strains escape restriction by human TRIM5 α due to a lack of interaction between TRIM5 α and its viral molecular target. We previously showed, however, that two point mutations, R332G/R335G, in the capsid-binding region confer human TRIM5 α the capacity to target and strongly restrict HIV-1 upon overexpression of the mutated protein. Here, we explored the possibility to introduce these two mutations in the endogenous human *TRIM5* gene by CRISPR-Cas9-mediated gene editing. For this, we electroporated CRISPR ribonucleoproteins (RNPs) and the donor DNA into Jurkat T lymphocytic cells and isolated clones by limiting dilution. We analyzed 47 clones using specific PCR assays and found that 6 clones (13 %) contained at least one gene-edited allele. One clone (clone 6) had both alleles edited for R332G but only one of the two alleles was edited for R335G. Upon challenge with an HIV-1 vector, clone 6 was significantly less permissive compared to unmodified cells, whereas the cell clones with monoallelic modifications were only slightly less permissive. Following IFN- β treatment, inhibition of HIV-1 infection in clone 6 was significantly enhanced (~50-fold inhibition) whereas IFN- β treatment had no effect on TRIM5 α overexpressed by retroviral transduction. Knockdown experiments confirmed that HIV-1 was inhibited by the edited *TRIM5* gene products, whereas quantification of HIV-1 reverse transcription products confirmed that inhibition occurred through the expected mechanism. In conclusion, we demonstrate the feasibility of potently inhibiting a viral infection through editing of innate effector genes, but our results also emphasize the importance of biallelic modification in order to reach significant levels of inhibition by TRIM5 α .



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63 Elucidating the Role of HIV-2 Viral Protein X

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The human immunodeficiency viruses type 1 and 2 (HIV-1 and HIV-2) are the causative agents of the acquired immunodeficiency syndrome (AIDS). While both viruses share a similar structural and genomic organization, difference in replication dynamics and the clinical course of infection is evident between the two. Patients dually infected were shown to have lower viral loads and generally a slower rate of progression to AIDS than those who are mono-infected. While the roles of the unique accessory proteins have been studied in detail for HIV-1, those of HIV-2, including viral protein X (Vpx), remain largely uncharacterized. In our previous experiments, Vpx of HIV-2 was found to be involved in decreasing infectivity of HIV-1 in dual infection cell culture assays. We set out to elucidate the function of this accessory protein, identifying protein–protein interactions of HIV-2 Vpx with cellular and possibly HIV-1 proteins in dual infection, using *in vitro*, proteomics techniques, and proximity ligation assays. Results showed that wild-type Vpx interacted with many cellular proteins involved in splicing, packaging of pre-mRNA, nuclear export, and translation. Of particular interest was the interaction between HIV-2 Vpx and the pre-mRNA-splicing factor ATP-dependent RNA helicase DHX15; which is required for HIV-1 viral DNA synthesis, and the eukaryotic translation initiation factor 2 subunit 3 (EIF2S3), involved in the early steps of protein synthesis. Additionally, Vpx was also found to interact directly with the cellular transcriptional repressor C-Terminal Binding Protein 2 (CTBP-2). Moreover, Vpx was shown to hinder the function of HIV-1 reverse transcriptase in *in vitro* assays. These findings shed light on the functions of this accessory protein and add to our understanding of the replication dynamics of HIV-2 and its role in dual infection.



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64 Engineering Host-Directed Antibodies against Filoviruses

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Multiple species of filoviruses cause highly lethal hemorrhagic fever in humans for which there are no Food and Drug Administration (FDA)-approved interventions. The increasing incidence of filovirus outbreaks, including the biggest ever 2013–2016 West African epidemic as well as our inability to predict which filovirus will emerge next emphasize the need for broad-spectrum anti-filoviral treatments. Current experimental antibody-based treatments can reverse Ebola virus disease; however, none possess pan-filovirus activity.

Sequence diversity among filovirus glycoproteins (GPs) creates a challenge for the development of broadly protective antibodies. In contrast to most of the GP surface, the interface between viral GP and the endosomal filovirus receptor, Niemann–Pick C1 (NPC1) is highly conserved and provides an attractive antibody target. However, this interface is only exposed in endosomes, a compartment inaccessible to antibodies.

To overcome this limitation, we previously engineered bi-specific antibodies to couple viral GP (mAb-MR72) or NPC1 (mAb-548) specific antibodies with one targeting a GP surface-exposed epitope, thereby using the virion itself for uptake and endosomal delivery into target cells and efficiently block viral entry and infection. Here, we utilized host molecule-mediated intracellular delivery of these antibodies by applying lessons from the lysosomal storage disease field to employ the glycosylation independent lysosomal targeting tag (GILT) as well as Niemann–Pick C2 (NPC2) for delivering enzymes to lysosomes. Using this strategy, we developed a panel of bispecific antibodies that neutralize a highly divergent panel of filoviruses, viz., Ebola, Sudan, and Marburg viruses, with IC₅₀ values in the low nanomolar range. Our results highlight the therapeutic potential of bispecific antibodies to provide pan-filovirus activity.



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65 Enhanced Control of HCMV Infection by Tissue-Resident NK Cells

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Following primary infection, human cytomegalovirus (HCMV) establishes a lifelong infection robustly controlled by the host immune response. Natural killer (NK) cells are an important component of this response evidenced by numerous HCMV-encoded NK immune evasion genes and by individuals with rare NK cell defects who have recurrent herpesvirus infections. Furthermore, HCMV infection has been suggested to drive an expansion of ‘adaptive’ NK cells in peripheral blood characterised by expression of phenotypic markers CD2, CD57 and NKG2C.

To date, the majority of NK cells studies in humans have focused on conventional NK (cNK) that circulate in peripheral blood. However, access to liver perfusates from organ transplant donors provided an opportunity to perform phenotypic and functional studies of tissue-resident NK cells. Using CXCR6 as a marker, we sorted liver-resident NK (LrNK) cells and cNK cells from liver perfusates and used a viral dissemination assay to measure control of HCMV *in vitro*. The data show that NK cells isolated from liver perfusates of seropositive donors were better at controlling HCMV infection *in vitro*. Furthermore, LrNK cells had enhanced capacity to control virus spread compared with cNK cells. Interestingly, the ability of NK cell control from different seropositive donors was variable. Further inspection of the phenotypes of the perfusate NK cells revealed an expansion of NKG2C⁺ NK cells in seropositive donors consistent with published data on circulating NK cells. Interestingly, the functional capacity to control HCMV infection was negatively correlated with the expression of NKG2C but, intriguingly, only in the cNK and not LrNK cell subset.

Thus HCMV infection promotes the accumulation of NKG2C⁺ tissue-resident NK cells in the liver reflecting the circulating NK cell phenotype. Crucially, these data suggest that tissue-resident NK cells are more effective at controlling HCMV replication *in vitro* in a donor dependent manner.



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66 ER-Shaping Atlastin Proteins Act as Central Hubs to Promote Flavivirus Replication and Virion Assembly

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Members of the *Flavivirus* genus rely extensively on the host cell endomembrane network to generate complex membranous replication organelles (ROs) that facilitate viral genome replication and production of virus particles. For DENV and ZIKV, these ROs included vesicles which are formed through membrane invagination into the ER lumen, termed invaginated vesicles or vesicle packets (VPs), as well as large areas of bundled smooth ER, termed convoluted membranes. Though the morphology of these virus-induced membrane structures has been well characterized, the viral and host constituents that make up flaviviral ROs are still poorly understood. Here, we identified a subset of ER resident proteins (atlastins), normally required for maintaining ER tubule networks, as critical host factors for flavivirus infection. Specific changes in atlastin levels had dichotomous effects on flaviviruses with ATL2 depletion leading to replication organelle defects and ATL3 depletion to changes in viral assembly/release pathways. These different depletion phenotypes allowed us to exploit virus infection to characterize non-conserved functional domains between the three atlastin paralogues. Additionally, we established the ATL interactome and show how it is reprogrammed upon viral infection. Screening of specific ATL interactors confirmed non-redundant ATL functions and identified a role for ATL3 in vesicle trafficking. Our data demonstrate that ATLs are central host factors that coordinate the ER network and shape the ER during flavivirus infection.



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67 Expression Profiles of NOD Type Receptors in Salmonid Cells after Infection with Infectious Pancreatic Necrosis Virus (IPNV)

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Infectious pancreatic necrosis virus (IPNV) is a worldwide etiologic agent of one disease that causes severe economic losses in several species of fish, mainly in young salmonids. Its genome consists of two linear double stranded RNA segments that encode five viral proteins. Teleost fish respond to infectious agents, mainly through the components of innate immunity. This response to viral infections is initiated, conducted, and coordinated by pathogen recognition receptors (PRRs), which can detect the presence of microorganisms through the identification of molecular patterns associated with pathogens (PAMPs). Heterologous PRR molecules have been found in salmonids, even in teleost fish. NOD type receptors (NLR) are a multigenic family of cytoplasmic molecules involved in immunity and apoptosis; these receptors have been little studied in fish. However, they have recently been linked to antiviral defense. There is no information that relates the expression of NOD type receptors with IPNV infection. Thus, the objective of this study was to analyze the gene expression of several members of subfamily A of the NLR (NOD1, NOD2, NLR-C3, NLR-C5, and NLR-X1) in response to IPNV infection by real-time quantitative PCR (RT-qPCR) and cellular models used *in vitro* and *ex vivo*. The expression analysis revealed that CHSE-214 cells, infected with IPNV, show a positive regulation of the NLRs, with the NLRX1 gene being the one with the highest expression. A similar result was obtained when primary cultures of head kidney of rainbow trout were infected with IPNV, but in this case, the most stimulated receptor was found to be NLR-C5. Overall, the results suggest that NLRs could play a key role in the regulation of defense mechanisms of salmonids against viral pathogens and justify the exploration of the precise molecular mechanism related to the immune system of NLRs in these fish.



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Abstract

68 FDA-Approved Drugs Efavirenz, Tipranavir, and Dasabuvir Inhibit Replication of Multiple Flaviviruses In Vitro

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Arthropod-borne flaviviruses like tick-borne encephalitis virus (TBEV), West Nile virus (WNV), Zika virus (ZIKV), Dengue virus (DENV), and yellow fever virus (YFV) cause several serious life-threatening syndromes (encephalitis, miscarriages, paralysis, etc.). No effective antiviral therapy against these viruses has been approved yet. We selected, via in silico modeling, 12 FDA-approved antiviral drugs (paritaprevir, dolutegravir, raltegravir, efavirenz, elvitegravir, tipranavir, saquinavir, dasabuvir, delavirdine, maraviroc, trifluridine, tauroursodeoxycholic acid) for their interaction with ZIKV proteins (NS3 helicase and protease, NS5 RNA-dependent RNA polymerase, and methyltransferase). Only three of them were active against ZIKV, namely, dasabuvir (ABT-333), efavirenz, and tipranavir. These compounds inhibit virus replication of ZIKV (MR-766 and Paraiba_01) in Vero cells; therefore, we tested these compounds against other medically important flaviviruses WNV (13-104 and Eg101) and TBEV (Hypr). Dasabuvir was originally developed as an antiviral drug against hepatitis C virus (HCV); tipranavir and efavirenz are used for treating human immunodeficiency virus (HIV) infection. The antiviral effects of efavirenz, tipranavir, and dasabuvir were tested for ZIKV in HUH-7, astrocytes (HBCA) and UKF-NB-4 cells, where we also identified a significant inhibition effect of these compounds. For Vero cells, efavirenz inhibited all investigated viruses with EC₅₀ ranging from 9.70 to 29.26 μM; the tipranavir inhibition effect was from 16.19 (WNV 13-104) to 27.47 μM (TBEV), while the strongest and most robust antiviral effect was demonstrated in the case of dasabuvir (EC₅₀ values ranging from 9.09 (TBEV) to 10.85 μM (WNV 13-104)). These results warrant further research of these drugs, either individually or in combination, as possible pan-flavivirus inhibitors.



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69 First Report on Detection and Genetic Diversity of Picobirnaviruses in the Small Indian Mongoose (*Herpestes auropunctatus*)

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Picobirnaviruses (PBVs), family *Picobirnaviridae*, are bi-segmented, double-stranded RNA viruses. PBVs are considered as opportunistic enteric pathogens. The gene segment-1 of PBV encodes the capsid protein, whilst gene segment-2 codes for the RNA-dependent RNA polymerase (RdRp). Based on differences in gene segment-2, PBVs are classified into genogroup-I (GI) and GII. Although PBVs have been detected in a wide variety of host species, there are no reports on PBVs from mongoose so far. We report here high rates of detection (35.36%, 29/82) of GI PBVs in fecal samples from the small Indian mongoose (*Herpestes auropunctatus*) on the Caribbean island of St. Kitts. Applying a combination of a non-specific primer-based amplification method and conventional RT-PCR using a newly designed primer targeting the 3'-untranslated region (UTR), we could amplify and sequence the complete/nearly complete gene segment-2 of eight mongoose PBV strains. Except for a single strain, the gene segment-2 of the remaining mongoose PBV strains contained the putative open reading frame encoding the RdRp. The gene segment-2/putative RdRps of the mongoose PBV strains retained the various features that are conserved in other PBVs (5'- and 3'-terminal nucleotide sequences, bacterial ribosomal binding site sequence in 5'-UTR, and the 3 domains in putative RdRps). On the other hand, phylogenetic analysis and sequence identities of the putative RdRps revealed high genetic diversity among the mongoose PBV strains and with those of PBVs from other host species. To our knowledge, this is the first report on detection and genetic diversity of PBVs from the mongoose, expanding the host range of PBVs and providing vital insights into the various features and evolution of putative RdRps of PBVs in a new host species.



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Abstract

70 GB Virus C E2 Inhibits PD-1-Mediated T Cell Signaling Dysfunction during Chronic Viral Infection

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Background: Program death receptor 1 (PD-1) is a co-inhibitory receptor that is upregulated and contributes to T cell dysfunction (exhaustion) during chronic viral infections, including HIV and HCV. GB virus C (GBV-C) is a persistent human virus, and co-infection is associated with reduced immune activation and improved clinical outcomes in HIV- and Ebola-infected individuals.

Methods: PD-1 levels were measured by flow cytometry on CD38⁺ T cells from 45 HIV-infected individuals, 20 of whom were co-infected with GBV-C. Jurkat cell lines that stably express GBV-C E2 protein and vector control were used to purify total cellular RNA before and 24 h following activation by anti-CD3/CD28 treatment. Gene expression was analyzed by RNA-seq and qRT-PCR.

Results: HIV-infected individuals with GBV-C viremia had reduced PD-1 expression on activated CD4⁺ and CD8⁺ T cells compared to HIV-infected GBV-C negative individuals. GBV-C particles and GBV-C E2 protein each inhibited PD-1 expression on T cells in vitro. Consistent with this, GBV-C E2 reduced gene expression of *PD-1* and its ligand, *PD-L1* in both resting and activated T cells. GBV-C E2 regulated transcription of the PD-1 signaling pathway and T cell activation associated genes without downregulation of interferon stimulated and innate immunity related genes needed to resolve viral infections.

Conclusions: Current understanding of chronic RNA virus infections is that upregulation of PD-1 with T cell exhaustion is critical for viral persistence. However, these data demonstrate that GBV-C infection reduced PD-1 expression on activated T cells during HIV infection, and that GBV-C E2 protein inhibits PD-1 signaling in T cells. This may preserve T cell function and contribute to the lack of immune deficiency in people with chronic GBV-C infection. Understanding mechanisms by which GBV-C E2 alters PD-1 signaling may aid in the development of novel immunomodulatory therapeutics to prevent T cell dysfunction (exhaustion) during chronic viral infections.



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71 Generation of Immunogenicity- and Protection-Efficacy-Improved Recombinant Clade 2.3.4.4c H5N6 Vaccine Strains

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Highly pathogenic (HP) clade 2.3.4.4 H5Nx viruses have spread worldwide and can be divided into subclades a to d. In Korea, clade 2.3.4.4a and b H5N8 and clade 2.3.4.4c H5N6 viruses have caused huge economic losses in the poultry industry. In this study, we synthesized consensus hemagglutinin (HA) and neuraminidase (NA) genes of Korean clade 2.3.4.4c H5N6 viruses and generated a PR8-derived H5N6 recombinant virus. The recombinant H5N6 virus showed high virus titer in ECEs, but its immunogenicity was not high in chickens. We introduced a H103Y mutation into the HA gene to increase the antigen yield in ECEs, and an avian PB2 gene to remove mammalian pathogenicity while conserving antigen yield. The oil emulsion vaccine, composed of H103Y-bearing recombinant H5N6 virus, induced higher antibody titer in chickens and ducks. In addition, we tested the effect of internal proteins (NP, M, NS1, and NEP) on protective efficacy of inactivated oil emulsion vaccines in chickens. We compared protective efficacy of two recombinant H5N6 vaccines with PR8 and avian NP, M, and NS genes against a HP H5N6 virus. All the vaccines completely protected chickens from mortality. Although the rates of virus shedding were insignificantly different, the period of virus shedding differed between vaccines. Thus, we could successfully improve immunogenicity and protection efficacy of clade 2.3.4.4c H5N6 vaccines by introducing a H103Y mutation and matching internal genes to field viruses.



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72 Generation of Monoclonal Antibodies against Variable Epitopes of the M Protein of Rabies Virus

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Rabies virus (RABV), the causative agent of rabies, is highly neurovirulent for warm-blooded animals, with a mortality rate of up to 100%. The RABV matrix protein (M) is required for virus particle assembly and budding. However, little is known about antigenic differences in the M protein. In this study, five monoclonal antibodies (mAbs) against the RABV M protein were generated using a recombinant M protein, designated as 3B9, 4A1, 2B11, 2C1, and 4B11. All five mAbs reacted with the CVS-11 strain but showed no reactivity against the HEP-Flury strain in indirect immunofluorescence and Western blotting. The epitope targeted by these mAbs was further identified by peptide scanning using GST-fused peptides. The ²⁵PPYDDD³⁰ peptide was defined as the minimal linear epitope. Alignment of amino acid sequences and phylogenetic analysis of different RABV strains indicated that the variable epitope ²⁵PPDGDD³⁰ is only present in the HEP-Flury and variant Flury strains of clade III, while the other strains resembling ERA and SRVA9 within the clade had another variable epitope, ²⁵PLDDDD³⁰. A Y27D mutation within the epitope was found among the remaining RABV strains distributed in different clades. However, a single D28G mutation eliminated the reactivity of these five mAbs. In addition, the mAbs were able to recognize wild type RABV strain in indirect immunofluorescence and Western blotting and detect RABV-infected brain tissue using immunohistochemistry. The newly established mAbs and identified epitope may facilitate future investigations into the structure and function of the M protein as well as the development of diagnostic methods for the detection of different RABV strains worldwide. Most importantly, the epitope recognized by the mAbs against M protein might serve as a novel target for the development of a vaccine targeting RABV virulent strains.



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73 HCV Infection of Adult Hepatocytes Induces a Temporally Structured Transcriptional Program That Overlaps with the *IRF1* Regulon

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Transcriptional profiling provides snapshots of virus-induced cellular changes, which can differ between cell types and exhibit temporal structuring. Previously, transcriptional responses to hepatitis C virus (HCV) infection of cancer cell lines, fetal hepatocytes, and chronically infected liver biopsies were described. Here, we quantify initial cellular responses to ex vivo HCV infection of authentic target cells: adult primary human hepatocytes (PHHs). PHHs were isolated from livers of donors undergoing partial hepatectomy. Plated PHHs were infected with HCV prior to RNA extraction and RNA-seq. Sampling was coordinated to capture global transcriptional changes associated with two distinct stages of early infection. Identification of differentially expressed genes, gene ontology, and canonical pathway analyses were subsequently performed. Distinct gene programs were dysregulated by HCV at 6 and 72 hours post-infection, which exhibited minimal overlap. Cell metabolism and ribosome biogenesis genes were dysregulated at 6 hours, in addition to early induction of the transcription factor *IRF1* which has the potential to orchestrate a subsequent antiviral program. Contrastingly, HCV-induced gene signatures at 72 hours comprised a program of antiviral effectors, noncoding RNAs, antiviral transcription factors, and chemokines. This program exhibited partial overlap with the *IRF1* regulon, as defined by *IRF1* ectopic expression in Huh-7.5 cells and quantification of dysregulated genes. Furthermore, a subset of *IRF1*-regulated genes had high basal expression in PHHs, but were not further induced upon HCV infection. In conclusion, we describe the adult hepatocyte response to initial HCV infection and detect temporally distinct perturbations in the transcriptional landscape. We also describe an expanded *IRF1* regulon and quantify the intrinsic and inducible component in PHHs.



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74 Heterologous Immunity and Hepatitis C Virus: Impact on Natural Infection, Pathogenesis and Vaccine Design

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Chronic infection with the hepatitis C virus (HCV) afflicts 1%–3% of the world's population and can lead to serious and late-stage liver diseases. Developing a vaccine for HCV is challenging because the correlates of protection are uncertain. Host immune responses play an important role in the outcome of infection with HCV. They can lead to viral clearance and a positive outcome, or progression and severity of the chronic disease. Studies of natural immunity to HCV in humans have resulted in many enigmas. Extensive research in the past >25 years into understanding the immune responses against HCV have still resulted in many unanswered questions, implicating the role of unknown factors and events. Human beings are not immunologically naïve because they are continually exposed to various environmental microbes and antigens, creating large populations of memory T and B cells. This pool of memory T and B cells can cross-react against a new pathogen in an individual and thereby influence the outcome of the new infection.

In our recent studies, we made the surprising discovery that peptides derived from structural and non-structural proteins of HCV have substantial amino acid sequence homologies with various proteins of adenoviruses, and that immunizing mice with a non-replicating, non-recombinant adenovirus (Ad) vector leads to induction of a robust cross-reactive cellular and humoral response against various HCV antigens. We also extended this observation to show that recombinant adenoviruses containing antigens from unrelated pathogens also possess the ability to induce cross-reactive immune responses against HCV antigens along with the induction of transgene antigen-specific immunity. This cross-reactive/heterologous immunity can (a) accommodate the development of dual-pathogen vaccines, (b) play an important role in the natural course of HCV infection, and (c) provide a plausible answer to many unexplained questions regarding immunity to HCV.



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75 Highly Abundant Extracellular Vesicles in Semen Inhibit Zika Virus Infection

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The Zika virus (ZIKV) causes severe birth defects and can be transmitted via sexual intercourse. Semen from ZIKV-infected individuals contains high viral loads and may therefore serve as an important vector for virus transmission. We analyze the effect of semen on ZIKV infection of cells and tissues derived from the anogenital region. We found that ZIKV replicated in all analyzed cell lines, primary cells, and endometrial or vaginal tissues. However, in the presence of semen, infection by ZIKV and other flaviviruses was potently inhibited. We show that semen prevents ZIKV attachment to target cells and that an extracellular vesicle preparation from semen is responsible for this anti-ZIKV activity. These particles are highly abundant in semen with concentrations of $>10^{13}$ per ml. The ZIKV-inhibitory activity was conserved in between semen samples derived from various donors, with a mean IC_{50} of pooled semen against different ZIKV isolates of only ~1%. Our findings suggest that ZIKV transmission is limited by semen. As such, semen appears to serve as a protector against sexual ZIKV transmission, despite the availability of highly susceptible cells in the anogenital tract and high viral loads in this bodily fluid.



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Abstract

76 HIV-1 Envelope Glycoprotein Trafficking and Viral Transmission

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HIV-1 encodes an envelope glycoprotein complex (Env) containing a long cytoplasmic tail (CT) harboring trafficking motifs implicated in Env incorporation into virions. Although the requirement for the Env CT in viral transmission is known, the precise mechanism by which Env is incorporated into nascent virions and localizes to the virological synapse remains poorly defined. To further elucidate the mechanism of Env trafficking we examined three HIV-1 strains: the lab-adapted clade B strain, NL4-3, and a transmitted/founder (T/F) clade C virus, K3016, and a T/F clade B virus, CH077. The HIV-1 Env CT contains two invariant trafficking motifs: tyrosine endocytosis motif, Y⁷¹²SPL, and C-terminal dileucine motif, LL⁸⁵⁵. Virion Env incorporation analysis revealed that Y⁷¹²SPL is necessary for efficient Env incorporation while LL⁸⁵⁵ is dispensable. Spreading infection kinetics were analyzed in various T-cell lines and primary human PBMCs; the results indicated that both endocytic motifs contribute to efficient viral spread in culture. Analysis of Env localization to the T-cell uropod, the portion of the plasma membrane that forms a virological synapse with uninfected cells, was found to be dependent on the Env CT and the Y⁷¹²SPL motif. Cell-to-cell and cell-free transmission assays using T cells infected with HIV-1 bearing Y⁷¹²A or LL⁸⁵⁵AA Env CT mutations are ongoing to establish a role for these motifs in both modes of viral transmission. These studies will significantly enhance our understanding of Env trafficking and viral transmission, providing insights into viral Env-host interactions in physiologically relevant cells.



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77 Identification of a Novel Yellow Fever Virus Cellular Restriction Factor

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Background: We previously identified a yellow fever virus (YFV) genome-derived, short noncoding RNA (vsRNA) generated during infection that targets the 3'UTR of a Src-kinase regulatory phosphatase (PTPRE) reducing PTPRE translation. By contrast, a related flavivirus (Zika) did not regulate PTPRE. We assessed the role of PTPRE in YFV and Zika replication.

Methods: Human PTPRE with a β -Globulin 3'UTR to remove the 3'UTR sequence targeted by the YFV vsRNA was stably expressed in Jurkat and Huh7D cells using a tetracycline-regulated promoter. YFV (17D strain) and Zika (PR strain) were used to infect the different cell lines, and viral replication was measured by viral RNA and infectivity. Attempts to knock out PTPRE by CRISPR were unsuccessful, suggesting PTPRE may be required for cell survival.

Results: Expression of PTPRE with nontargeted 3'UTR led to a 100-fold reduction in infectious virus released from Jurkat and Huh7D cells by infectivity and a 3–10-fold reduction in viral RNA levels. Infectivity was restored in cells grown in doxycycline. By contrast, nontargeted PTPRE expression had no effect on release of infectious Zika virus. PTPRE expression did not alter YFV attachment or entry. Despite reduced infectivity of supernatant virus produced by PTPRE-nontargeted cells, YFV envelope content in culture supernatants was similar to that released by cells not expressing nontargeted PTPRE. Electron microscopy demonstrated large, empty-appearing viral particles in YFV-infected cells expressing nontargeted PTPRE that were not present in cells not expressing nontargeted PTPRE.

Conclusion: PTPRE is a novel YFV restriction factor that appears to increase the proportion of defective YFV particles released into cell culture media, potentially by preventing YFV RNA encapsidation. PTPRE effects on viral replication are not conserved among flaviviruses, as Zika replication was not altered by PTPRE overexpression. Understanding how PTPRE inhibits YFV may provide insight into YFV assembly and release and potentially help to identify novel drug targets.



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78 Identification of Aquarius and Senataxin as Restriction Host Factors for Hepatitis B Virus Infection

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Hepatitis B virus (HBV) represents an important human pathogen causing acute and chronic hepatitis. Over 240 million people are chronically infected, many of whom will die due to complications such as liver cirrhosis and hepatocellular carcinoma. Currently approved therapies are very effective in suppressing virus replication and viremia, but they are not curative because they do not completely eliminate the nuclear episomal DNA form of HBV (cccDNA) that re-establishes infection upon interruption of therapy. Despite our understanding of many aspects of the HBV lifecycle, details of the HBV cccDNA biology remain poorly understood. Our group is pursuing a loss-of-function genetic screening approach to identify cellular factors regulating HBV infection. A lentivirus-delivered shRNA library, composed of 384 shRNAs, was used to interrogate the function of 80 DNA damage repair pathway proteins in the establishment of HBV infection. The primary screening identified 10 cellular factors that regulate the HBV infection both positively or negatively. Two of those proteins, Aquarius (AQR) and senataxin (SETX), were subsequently validated as factors restricting the HBV infection in independent experiments. Silencing of AQR and SETX led to an increased infection efficiency that was characterized by higher intracellular levels of HBV cccDNA, HBV mRNA, and core protein, and increased HBeAg accumulation in the supernatants of infected cells. The expression level, glycosylation pattern, and localization of the HBV receptor, NTCP, in AQR- and SETX-downregulated cells was equivalent to that of control cells. Collectively, our results are compatible with AQR and SETX restricting early steps in the HBV lifecycle and downstream HBV entry that affect the establishment of the HBV cccDNA pool. Experiments to unravel the function of these proteins in the context of HBV infection are currently underway.



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Abstract

79 Identification of Novel Determinants of Neutralization Epitope Shielding for Hepatitis C Virus In Vitro

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Epitope shielding is suggested as an important mechanism mediating escape of hepatitis C virus (HCV) from host neutralizing antibodies (ntAb). Knowledge on determinants of epitope shielding facilitates rational HCV vaccine design.

To identify determinants of epitope shielding, which might have a negative effect on viral fitness, we used an evolutionary approach passing HCV in cell culture in the absence of ntAb.

Culture infectious HCV recombinants with genotype 1-6 Core-NS2 were subjected to 18–51 passages in Huh7.5 cells until peak infectivity titers of ~6 log₁₀ focus forming units/ml were achieved, signifying a 0.5–3 log₁₀ titer increase compared to the original viruses. Per virus, next-generation sequencing revealed 6–23 dominant open reading frame (ORF) substitutions, including 2–4 substitutions in the envelope proteins. Polyclonal passaged viruses and recombinants engineered with the identified substitutions showed increased viral fitness and up to 4-orders-of-magnitude increased sensitivity to human monoclonal antibodies (AR3A, AR4A) and polyclonal antibodies (C211) compared to the original viruses. For genotype 1a, this effect was mediated by envelope substitutions at positions 361, 417, and 532; for 2a, by a position-532-substitution supported by substitutions at positions 410 and 546; and for 3a, by a position-418-substitution. While position 418 and 532 map to glycosylation motifs, the identified position-418-substitution is not expected to affect glycosylation, and different changes at position 532, all expected to disturb glycosylation, showed largely different effects on neutralization sensitivity, suggesting a mechanism not directly involving changes in glycan occupancy.

1a, 2a, and 3a viruses with the identified envelope substitutions showed decreased dependency on HCV co-receptor SR-BI, decreased temperature stability, and decreased viral breathing compared to the original viruses, suggesting “open” envelope states with increased epitope accessibility.

Thus, we identified novel determinants of epitope shielding mediating “closed” envelope states resulting in a dramatic decrease in ntAb sensitivity with implications for HCV vaccine design.



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80 In Situ Capture RT-qPCR Method for Detection of Human Norovirus in Food and Environmental Samples

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Human noroviruses (HuNoVs) are the major cause of non-bacterial acute gastroenteritis worldwide. RT-qPCR is a widely used method to detect HuNoVs. However, the method is unable to enrich virus from environmental samples and to discriminate between infectious and non-infectious viruses. In this study, we explored a new in situ capture RT-qPCR (ISC-RT-qPCR) methodology to estimate the infectivity of HuNoV in environmental and food samples. This assay was based on capturing encapsidated HuNoV by viral receptors, followed by in situ amplification of the captured viral genomes by RT-qPCR. We demonstrated that ISC-RT-qPCR did not capture and enable signal amplification of heat-denatured Tulane virus (TV) and HuNoVs. Therefore, ISC-RT-qPCR provides better estimates for infectivity of HuNoV than RT-qPCR. We then utilized the ISC-RT-qPCR to detect HuNoV in environmental water samples and food samples as compared to a conventional RT-qPCR procedure. The presence of HuNoV was examined in 36 oyster samples from retail markets using by both assays for detection. The detection rates of GI HuNoV in gill, digestive glands, and other tissues were 33.3%, 25%, and 19.4% by ISC-RT-qPCR; and were 5.6%, 11.1%, and 11.1% by RT-qPCR. ISC-RT-qPCR is more sensitive than RT-qPCR for the detection of HuNoV in oyster. By contrast, the HuNoV detection rate by ISC-RT-qPCR is lower for environmental samples. Of the 72 water samples that tested positive for HuNoV by RT-qPCR, only 20 (27.8%) of these tested positive by ISC-RT-qPCR, suggesting that 72.2% of RT-qPCR-positive samples were unlikely to be infectious. A better detection rate by ISC-RT-qPCR in oyster samples indicates the likelihood of infectious HuNoV that accumulated in oyster and a lower detection rate of HuNoV in environmental water by ISC-RT-qPCR, indicating that the majority of RT-qPCR-positive samples were from non-infectious viral RNA.



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81 PSGL-1 Restricts HIV-1 Infectivity by Blocking Virus Particle Attachment to Target Cells

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P-selectin glycoprotein ligand-1 (PSGL-1) is a dimeric, mucin-like, 120-kDa glycoprotein that binds to P-, E-, and L-selectins. PSGL-1 is primarily expressed on the surface of lymphoid and myeloid cells and is up-regulated during inflammation to mediate leukocyte tethering and rolling on the surface of endothelium for migration into inflamed tissues. Although it has been reported that PSGL-1 expression inhibits HIV-1 replication, the mechanism of PSGL-1-mediated anti-HIV activity remains to be elucidated. Here, we report that PSGL-1 in virions blocks the infectivity of HIV-1 particles by preventing the binding of particles to target cells. This inhibitory activity is independent of the viral glycoprotein present on the virus particle; binding of particles bearing the HIV-1 envelope glycoprotein, vesicular stomatitis virus G glycoprotein, or lacking a viral glycoprotein, is impaired by PSGL-1. Mapping studies show that the extracellular, N-terminal domain of PSGL-1 is necessary for its anti-HIV-1 activity, and the PSGL-1 cytoplasmic tail contributes to inhibition. In addition, we demonstrate that the PSGL-1 related monomeric E-selectin binding glycoprotein CD43 also effectively blocks HIV-1 infectivity. HIV-1 infection, or expression of either Vpu or Nef, downregulates PSGL-1 from the cell surface; expression of Vpu appears to be primarily responsible for enabling the virus to partially escape PSGL-1-mediated restriction. Finally, we show that PSGL-1 inhibits the infectivity of other viruses such as murine leukemia virus and influenza A virus. These findings demonstrate that PSGL-1 is a broad-spectrum antiviral host factor with a novel mechanism of action.



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Abstract

82 Independent Inhibition of the Polymerase and Deubiquitinase activities of the Crimean–Congo Hemorrhagic Fever Virus Full-Length L-Protein

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The Crimean–Congo hemorrhagic fever virus (CCHFV) is a segmented negative-sense RNA virus that can cause severe human disease. The World Health Organization (WHO) has listed CCHFV as a priority pathogen with an urgent need for enhanced research activities to develop effective countermeasures. We report on the expression, characterization, and inhibition of the CCHFV full-length L-protein that provides an important tool in this regard. The requirements for high biosafety measures hamper drug discovery and development efforts with infectious CCHFV. Hence, we decided to adopt a biochemical approach that targets the viral RNA-dependent RNA polymerase (RdRp). The CCHFV RdRp activity is part of a multifunctional L protein that is unusually large, with a molecular weight of ~450 kDa. The CCHFV L-protein also contains an ovarian tumor (OTU) domain that exhibits deubiquitinating (DUB) activity. Previous studies have shown that DUB activity interferes with innate immune responses and viral replication. Here, we utilized the baculovirus expression system and generated a full-length CCHFV L protein. RdRp activity was seen in the presence of divalent metal ions, and inhibition of RNA synthesis was demonstrated with nucleotide analogues. The ubiquitin analogue CC.4 inhibits the CCHFV-associated DUB activity of the full-length L protein and the isolated DUB domain to a similar extent. We have finally shown that RdRp and DUB activities are functionally independent. The full-length CCHFV L-protein provides an important tool for the discovery of antiviral agents. High-throughput screening (HTS) campaigns are now feasible. The same enzyme preparations can be utilized to identify polymerase and DUB inhibitors.



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83 Influenza A Virus Disturbs the Host Cell Protein Homeostasis by Inducing the Accumulation of Insoluble Proteins

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Influenza A virus (IAV) is the causative agent for most of the annual respiratory epidemics in humans and the major influenza pandemics in the last century and is associated with high morbidity and mortality, especially in the elderly. In order to efficiently replicate, this virus hijacks the host cellular machinery and requires precise interactions with host components. However, cells have evolved specific defense mechanisms to counteract the effects induced by the virus. In fact, upon IAV infection, several processes within the cytosol and the endoplasmic reticulum related to protein synthesis and processing have shown to contribute either as part of an effective replication cycle or as part of an effective cellular antiviral response.

Recent reports show contradictory findings regarding the control of the cellular proteostasis mechanisms by both the virus and the host cell. With this study, we aim to further unravel the interplay between IAV and the host cell proteostasis-related mechanisms at early time points post-infection. Our results suggest that the virus disturbs host cell protein homeostasis by inducing the accumulation of insoluble proteins in a process that seems to be related to viral RNA processing. We have further analyzed the interplay between IAV infection and the endoplasmic reticulum unfolded protein response. Our results may lead to a better understanding of the interplay between IAV and the host cell and, furthermore, contribute to the development of novel antiviral strategies.



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84 Influenza D Virus: A Potential Threat for Humans?

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Influenza D virus (IDV) is a novel influenza virus first isolated from swine in 2011 in Oklahoma. Several studies have isolated IDV in cattle from multiple geographic areas, suggesting cattle as a possible primary natural reservoir for the virus. To date, few studies have been performed on human samples and there is no conclusive evidence that IDV has the ability to infect humans.

This serological study aimed to assess the prevalence of antibodies against IDV in the human population.

The IDV used in the serological analysis was influenza D/bovine/Oklahoma/660/2013.

The human serum samples, collected in Italy between 2005 and 2017, were randomly selected from the laboratory internal serum bank and tested by hemagglutination inhibition assay (HI). HI positivity has been confirmed using a virus neutralization assay (VN).

Based on HI positivity (HI titers ≥ 10), a low prevalence (5%–10%) was observed between 2005 and 2007. There was a sharp increase since 2008 resulting in two main peaks in 2009–2010 and 2013–2014, a finding confirmed by the statistical trend analysis.

The same pattern and trends can be seen with higher HI titers of >20 and ≥ 40 .

The prevalence of antibodies against IDV has increased in the human population in Italy from 2005 to 2017. Low prevalence values between 2005 and 2007 suggest that IDV most probably circulated before its detection in 2011, and maybe even before 2005.

In Italy, IDV has been shown to circulate among swine and bovine herds. It is therefore possible that prevalence peaks in humans follow the infection epidemics in animals and do not to persist in the population, resembling a spillover event from the animal reservoir and showing that the virus may not circulate consistently in the human population. However, IDV showed the ability to elicit an immune response in humans.



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Abstract

85 Influenza Virus Usurps an Interferon-Induced Translational Program to Promote Viral Replication

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Hosts mount prudently tuned responses to viral infection in an attempt to block nearly every step of the replication cycle. Viruses must adapt to replicate in this hostile antiviral cellular state. Interferon stimulation or pathogen challenge robustly induces expression of IFIT (interferon-induced proteins with tetratricopeptide repeats) proteins. IFITs are a family of proteins that bind RNA and play antiviral roles during infection. Thus, we were surprised to identify the IFIT family as top candidate proviral host factors for influenza A virus (IAV) in a genome-wide CRISPR–Cas9 knockout screen. We validated the proviral activity of IFIT2 by showing that IFIT2-deficient cells support lower levels of IAV replication and exhibit defects in viral gene expression. The molecular functions of IFIT2, let alone how they are used by influenza virus, are unknown. Using CLIP-seq, we showed that IFIT2 binds directly to viral and cellular mRNAs in AU-rich regions largely in the 3'UTR, with a preference for a subset of interferon-stimulated mRNAs. IFIT2 also associates with actively translating ribosomes in infected cells to facilitate the translation of viral messages. IFIT2-responsive elements from an IAV mRNA were sufficient to confer translational enhancement to exogenous transcripts in *cis*. Conversely, mutation of these elements or the use of an IFIT2 RNA-binding mutant ablated stimulation of viral gene expression. Together, these data link the RNA-binding capability of IFIT2 to changes in translational efficiency of target viral mRNAs and the stimulation of viral replication. They establish a model for the normal function of IFIT2 as an antiviral protein affecting the post-transcriptional fate of cellular mRNAs and explain how influenza virus repurposes IFIT2 to support viral replication. Our work highlights a new node for the regulation of translation during interferon responses and highlights how canonical antiviral responses may be repurposed to support viral replication.



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Abstract

86 Inhibition of HCMV Replication by Small Molecules Interfering with the Dimerization of DNA Polymerase Processivity Factor UL44

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Human cytomegalovirus (HCMV) is a leading cause of severe disease in immunocompromised individuals and in congenitally infected newborns. Despite the availability of several drugs, pharmacological treatment is associated with toxicity and the emergence of resistant strains. Therefore, it is essential to identify new potential targets of therapeutic intervention. One of those is the dimerization of HCMV DNA polymerase processivity factor UL44. Indeed, UL44 plays an essential role in viral replication by tethering the DNA polymerase holoenzyme to the DNA and its dimerization is absolutely required for DNA binding and OriLyt-dependent DNA replication, since point mutations prevent DNA binding and abolish viral replication. Therefore, the aim of this study is to identify small molecules (SMs) that hinder viral replication by interfering with UL44 homodimerization. To this end, we first validated the UL44 crystal structure *in vitro* by GST-pulldown and thermal shift, and in cells using fluorescence and bioluminescence resonance energy transfer assays, which were used to perform a virtual screening to identify SMs potentially interfering with UL44 homodimerization. A total of 18 out of the 140 identified SMs were tested for their ability to impair replication of the TB4-UL83-EYFP recombinant HCMV. Four SMs reproducibly inhibited viral replication in the absence of evident cytotoxicity. The 3 compounds with the highest selectivity index (ranging from ~5 to ~20), as assessed by MTT and FRA assays, were subsequently further tested for their ability to inhibit the replication of an AD169-GFP recombinant virus and a GCV-resistant derivative, resulting in similar ED50. The most active compound inhibited AD169 replication as assessed by plaque reduction assays, with an ED50 of ~15 μ M, and specifically impaired the expression of late genes as assessed by Western blotting assays. Overall, our data suggest that SM-mediated impairment of UL44 dimerization and viral replication could be employed as a valuable therapeutic approach for HCMV infection and treatment of HCMV-resistant strains.



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87 Inhibition of Influenza A Virus Infection by Toosendanin from *Melia Fructus*

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Toosendanin (TSN) is a major bioactive component of *Melia Fructus* (MF) with anti-inflammatory, anti-botulinum, anti-microbial, and analgesic efficacy. Our previous study demonstrated that MF has anti-influenza A virus activity; however, the contribution of TSN is still unclear. In this study, we found that TSN suppressed influenza A virus infection when administered before or concurrently with the virus, but not after infection. TSN pretreatment inhibited viral hemagglutinin (HA), nucleoprotein (NP), polymerase acidic (PA) protein, and matrix protein 2 (M2) mRNA synthesis as well as NP, PA, M2, and nonstructural protein 1 (NS1) expression, but had no effect on HA or neuraminidase (NA) activity. In addition, TSN induced cytoplasmic localization of PA protein, disrupting nuclear translocation. Pretreatment with TSN also suppressed infection-induced phospho-AKT expression but not the host immune response. Thus, TSN may be a promising candidate for anti-influenza agent targeting the PA protein of the influenza A virus RNA polymerase complex.



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88 Interferon-Stimulated SAMHD1 Restricts Hepatitis C Virus Replication

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Human SAMHD1 is an IFN-induced dNTP triphosphatase that is able to restrict HIV-1 replication, whereas its role in innate immunity against virus infection remains largely unexplored. In this work, we provided evidence that SAMHD1 functions as an anti-HCV host factor. We found that overexpression of *SAMHD1* resulted in significant inhibition on the replication of HCV, but not other RNA viruses including influenza A virus and EV71. SAMHD1 knockdown partially relieved the inhibitory effect of IFN on HCV, suggesting its important role in the innate immune response against HCV. Mechanistic studies revealed that SAMHD1 targets viral RNA replication without impact on both protein translation and virus entry. Transcriptome analysis showed a broad inhibitory effect of SAMHD1 on host genes involved in cholesterol and fatty acid biosynthesis. In particular, SAMHD1 was shown to downregulate the mRNA abundance of *SREBP1*, a master transcriptional regulator of de novo lipid biosynthesis, impairing the formation of lipid droplets. Restoring intracellular lipid levels by either exogenous lipid addition or *SREBP1* overexpression counteracted the restriction of HCV by SAMHD1, providing evidence that SAMHD1 inhibits the replication of HCV by suppressing host cholesterol and fatty acid biosynthesis. Together, these data unveil, for the first time, a novel antiviral mechanism of SAMHD1 and open new avenues for the development of novel anti-HCV therapeutics.



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89 Investigating the Interaction of HMGB1 Protein with the Dengue Virus Genome and Its Role in Dengue Virus Replication

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Dengue virus, the most prevalent arthropod-borne human pathogen belonging to the *Flaviviridae* family, causes millions of infections annually. Approximately half of the world's population is living in a dengue-endemic area. The genome of dengue virus is positive-sense, single-stranded RNA, which is flanked by untranslated regions known as 5' UTR (~100 nucleotides) and 3' UTR (~500 nucleotides). The secondary structures of UTRs are crucial for determining infectivity, translation, viral replication, and immune modulation. Of note, dengue virus infection induces the secretion of various pro-inflammatory cytokines, including HMGB1 protein. The HMGB1 (high-mobility group box 1) protein is a highly conserved, ubiquitous nuclear protein which performs various functions based on its cellular localization. The cytoplasmic translocation and secretion of HMGB1 protein are implicated in various chronic and inflammatory diseases, metabolic disorders, cancer, and viral infections, including by flaviviruses. In the present work, we focused on the participation of HMGB1 in dengue virus pathogenesis. By using two independent methodologies, we have demonstrated the critical interaction of HMGB1 protein with dengue UTRs. RNA electrophoretic mobility shift assay and tryptophan fluorescence assay indicated that the HMGB1 protein binds to dengue UTRs. Furthermore, Western blot and confocal immunofluorescence analysis showed the correlation between the HMGB1 protein and viral RNA in the dengue-infected A549 cells. Our findings suggest that HMGB1 protein translocates to the cytoplasm and interacts with viral UTRs, modulating dengue virus replication in A549 cells. HMGB1 appears to be an important host factor which plays a crucial role in the severity of dengue disease, though further investigations are needed to elucidate the specific action. The complicated pathogenesis of severe dengue disease can be better understood by resolving the complex and intricate relationship between dengue virus and its host cell.



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90 Investigating the Role of Signal Transducer and Activator of Transcription 3 (STAT3) in Dengue Virus Propagation

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Dengue fever is one of the most significant arthropod-borne viral diseases affecting at least 50 million people per year in tropical countries. Like other viruses, dengue virus (DV) has co-existed and evolved along with its host to antagonize host cell biological processes for its own propagation, while evading alleged elimination by the immune system. IFN-activated STAT1, STAT2, and STAT3 are the key mediators of host innate antiviral response in the case of DENV infection. It is reported that DENV modulates the interferon response either by inhibiting the phosphorylation of STAT1 or by STAT2 degradation via its ubiquitination. However, so far, the strategies employed by DENV to modulate the STAT3 signaling cascade remain unknown. It is possible that DV may strategically tweak STAT3 signaling to enhance its replication and survival within the host. In this study, we attempt to understand how DENV circumvents STAT3 signaling or modulates STAT3-mediated interferon signaling to its own advantage. We have employed various techniques like confocal microscopy, Western blot, RT-PCR, and luciferase assays to determine the expression level of STAT3 in DENV-infected cells and the effect of STAT3 inhibition on viral replication. Our results show that there is an overall increase in STAT3 expression as well as its tyrosine phosphorylation status in DV-infected cells, while its inhibition by chemical inhibitors leads to downregulation of viral proteins post-infection. However, the mechanism and implication of STAT3 modulation need to be deciphered for a more profound and comprehensive understanding. We propose to further investigate the role of STAT3 modulation in DENV replication by silencing STAT3 to establish a formidable co-relation between the two. There is a need to determine the effect of activation or inhibition of STAT3 on DV replication to have a better understanding of the underlying mechanisms employed by DV in modulating the STAT3 pathway.



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91 Isolation and Elimination of Latent and Productive Herpes Simplex Virus from the Sacral and Trigeminal Ganglions

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There is an immediate need for alternative anti-herpetic treatment options effective for both primary infections and reoccurring reactivations of herpes simplex virus types 1 (HSV-1) and 2 (HSV-2). Existing options include antivirals that have been approved for clinical administration and a limited number of nucleoside analogues. The present article tests a new treatment based on a systemic understanding of how the herpes virus affects cell inhibition and breakdown, and targets different phases of the viral cycle, including the entry stage, reproductive cross mutation, and cell-to-cell infection. The treatment consisted of five immunotherapeutic core compounds (5CC), which were hypothesized to be capable of neutralizing human monoclonal antibodies. These 5CC are effective inhibitors of herpes viral DNA synthesis and interferon (IFN)-induced cellular antiviral response, and they were here found to neutralize antiviral reproduction by blocking cell-to-cell infection. Antiviral activity of the 5CC against HSV-1 and HSV-2 was tested on RC-37 cells in vitro using a plaque reduction assay. The 50% inhibitory concentration (IC₅₀) of 5CC was 0.0009% for HSV-1 plaque formation and 0.0008% for HSV-2 plaque formation. Further tests comprising of a phenotypic assay, PEA, were performed to evaluate the susceptibility of HSV-1 and HSV-2 to antiherpetic drugs in Vero cells after virus entry. Indicators of the 5CC found that the combination exhibited high levels of virucidal activity against HSV-1 and HSV-2 in viral suspension. These concentrations of the 5CC are nontoxic and reduced plaque formation by 98.2% for HSV-1 and 93.0% for HSV-2. Virus HSV-1 and HSV-2 titers were reduced significantly by 5CC to the point of being negative, ranging 0.01–0.09 in 72%. These results suggest that the 5CC are strong alternative candidates for treating herpes simplex.



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92 Laninamivir-Resistant Substitutions in N3 to N9 Avian Influenza Neuraminidase Subtypes and Correlation between In Vitro and In Vivo Antiviral Efficacy

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Laninamivir (LAN), a neuraminidase inhibitor (NAI) that has been recently approved in some countries, has fundamentally similar binding moieties with the other NAIs including oseltamivir (OS), zanamivir (ZA), and peramivir (PER) and possibly shares some common resistance markers. Therefore, we further screened the substitutions in N3 to N9 NA subtypes that conferred resistance against OS, ZA and PER in our previous study and profiled the substitutions that are resistant to LAN. In addition, protective efficacy in mouse model was performed using two multi-NAI resistant viruses to determine the correlation of antiviral efficacy between in vitro and in vivo model. Among the 73 substitutions previously found during OS and ZA selection, we discovered 20 substitutions conferring resistance to LAN which ranged from 12.0 to 550.5 in fold-change of IC_{50} against LAN compared to their parental virus. V116D substitution (N8) conferred resistance only to LAN and 9 substitutions conferred multidrug resistance to the four available NAIs. Because of the similarities in the structures of LAN and ZA, LAN also shares most of the resistance substitutions with ZA (14 of 20 substitutions). Interestingly, N3 variant possessing R292K substitution exhibited susceptibility to LAN alone while N8 variant possessing Q136K substitution showed susceptibility only to OS. These multi-NAI resistant NA genes, R292K in N3 and Q136K in N8 were introduced into the mouse-adapted avian influenza H5N2 genetic backbone and showed the similar susceptibility to the NAIs as observed. The H5N3-R292K and H5N8-Q136K showed the increased survival rate and decreased lung viral titer in the infected mice when only treated with LAN or OS, respectively. This addition in the NAIs-resistant profiling of NA substitutions not only provides a comprehensive understanding of NAI-resistance mechanism but also contributes to the drug prescription management for NAI-resistant influenza viruses that may emerge in humans.



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Abstract

93 Lyophilized Matrix Containing Ready-to-Use Primers and Probe Solution for Standardization of Real-Time PCR and RT-qPCR Diagnostics

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Real-time molecular techniques have become the reference methods for direct diagnosis of pathogens. The reduction of steps is a key factor in order to decrease the risk of human errors resulting in invalid series and delayed results. We describe here a process involving preparation of oligonucleotide primers and hydrolysis probe in a single tube at predefined optimized concentrations that are stabilized via lyophilization (Lyoph-P&P). Lyoph-P&P was compared to the classic protocol using extemporaneously prepared liquid reagents assaying (i) sensitivity, (ii) long-term stability at 4 °C, and (iii) long-term stability at 37 °C, mimicking transportation without cold chain. Two previously published molecular assays were selected for this study. They target two emerging viruses that are listed on the blueprint of the WHO to be considered for preparedness and response actions: *chikungunya virus* (CHIKV) and *Rift Valley fever phlebovirus* (RVFV). The results of our study demonstrate that (i) Lyoph-P&P is stable for at least 4 days at 37 °C, supporting shipping without the need of cold chain, (ii) Lyoph-P&P rehydrated solution is stable at +4 °C for at least two weeks, (iii) the sensitivity observed with Lyoph-P&P is at least equal to, often better than, that observed with liquid formulation, (iv) the validation of results observed with low-copy specimens is rendered easier by higher fluorescence levels. In conclusion, Lyoph-P&P holds several advantages over extemporaneously prepared liquid formulations and merits consideration as a novel real-time molecular assay for implementation into a laboratory with routine diagnostic activity.



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94 Taking Advantage of Nature's Benefits: Soluble and Stable Antigen Straight Out of the Pathogen

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Integral membrane proteins (MP) exhibit specific tridimensional conformation and topology that define their various functions. Pathogen surface antigen, encompassing many MP, are the forefront of the viral strategy which is broadly targeted by the host immune response. These antigens are present in equilibrium under different oligomeric forms, with distinctive epitopes, and to obtain them under soluble form and/or stable constitutes a real stake.

Solubilization of a full-length MP directly from a pathogen to rapidly obtain a native antigen mimicking the original conformation of MP at the pathogen surface is the process development reported in this work. Rabies virus (RABV) was used as a model for this demonstration and its fulllength glycoprotein G was stabilized in amphiphatic polymers (A8-35 amphipols). The stability of the soluble RABV-G was evaluated under various stress conditions (temperatures, agitation and light exposures) and a long-term stable RABV-G formulation, suitable for freeze-drying process, was defined using a design of experiment approach.

RABV-G/A8-35 in liquid form was shown to be antigenically stable at 5°C and 25°C for one month, and a dedicated kinetic modelling predicted its stability up to 1 year at 5°C. To mitigate RABV-G/A8-35 sensitivity to mechanical stress, a solid form of RABV-G/A8-35 and a freeze-drying process were considered resulting in a 2-year thermally stable product at 5°C, 25°C and 37°C.

To the best of our knowledge, this is the first time that a natural full-length MP extracted from a virus, trapped in amphipols was kept antigenically stable in the long term, in a defined freeze-dried form out of any refrigerated storage conditions. These results described an easy process to obtain a pure, well conformed native-like antigen of interest, from a circulating pathogen which is of concern for diagnostic (quantification/characterizations assays), therapeutic and vaccine strategies. After protein physical characterization, identification of RABV G/A8-35 neutralizing epitopes has been underway before *in vivo* testing.



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95 Mapping the Interface between New World Hantaviruses and Their Receptor, PCDH1

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Hantaviruses are found throughout the world and can cause deadly diseases in humans, specifically hantavirus cardiopulmonary syndrome (HCPS) in the New World, and hemorrhagic fever with renal syndrome (HFRS) in the Old World. Currently, no FDA-approved, specific antiviral drugs or vaccines are available. Recently, we showed that New World hantaviruses utilize protocadherin-1 (PCDH1) for endothelial cell entry and infection by directly engaging its first extracellular cadherin repeat (EC1) domain. Knockout of *PCDH1* also greatly reduced pulmonary infection and was highly protective in a Syrian hamster model of lethal challenge with Andes virus (ANDV). To further understand PCDH1's role in hantavirus entry, we sought to map the binding interface between hantavirus Gn/Gc and PCDH1-EC1. Accordingly, we screened a panel of EC1 proteins, bearing point mutations in solvent-exposed residues, for their capacity to recognize Gn/Gc and block viral entry. EC1 mutations defective at Gn/Gc binding were engineered singly, and in combinations, into full-length PCDH1, expressed in PCDH1-knockout cells, and evaluated for their capacity to complement viral infection. We identified a surface in the PCDH1-EC1 domain, comprising contiguous residues, which was required for virus-PCDH1 recognition and PCDH1-dependent viral entry. However, this region does not overlap with the EC1–EC4 heterodimer interface recently described by Modak and Sotomayor. In addition, through the use of recombinant vesicular stomatitis viruses bearing chimeric hantavirus Gn/Gc glycoproteins, we were able to pinpoint the importance of N-terminal domain of the Gn subunit for PCDH1-mediated entry. With these taken together, identifying the location of the interface could provide a direction for the development of host-directed antiviral drugs, which do not interfere with PCDH1's endogenous function, as well as help to map an antigen target on Gn/Gc for antiviral antibodies.



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Abstract

96 MARCH8 Restricts Ebola Virus Replication by Blocking the Viral Glycoprotein Processing and Glycosylation

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Ebola virus (EBOV) glycoprotein (GP) is a class I fusion protein whose maturation is dependent on furin-mediated processing. EBOV-GP is heavily glycosylated, with glycans constituting ~50% of its molecular mass. Compared to 15 *N*-linked glycosylation sites, EBOV-GP₁ has ~80 potential *O*-linked glycosylation sites in the mucin-like domain (MLD), suggesting that *O*-linked glycans are dominated. The membrane-associated RING-CH (MARCH) family consists of 11 members that are RING-finger ubiquitin E3 ligase. Recently, human MARCH1, MARCH2, and MARCH8 were reported to inhibit HIV-1 replication by targeting its Env. Here, we show that human MARCH8 also inhibits EBOV replication by blocking GP incorporation into virions via downregulating its cell surface expression. To understand how the downregulation occurs, we investigated EBOV-GP subcellular localization, processing, glycosylation, and intracellular trafficking in the presence of human MARCH8. We find that MARCH8 interacts with GP and retains GP in the Golgi. MARCH8 also interacts with the homob domain of furin that blocks its convertase activity. In consequence, MARCH8 blocks GP processing in an MLD-independent manner. Consistently, MARCH8 also blocks the *O*-linked, but not the *N*-linked glycosylation of GP. Importantly, in the presence of MARCH8, the shedding of GP₁ but not the secreted GP (sGP) is blocked, suggesting that MARCH8 targets the GP₁ C-terminal region. The MARCH8 activity is extended to its orthologs from *Bos taurus* and mice, and its paralogs MARCH1 and MARCH2. In addition, MARCH8 inhibits the processing of another two class I fusion proteins, including HIV-1 Env and IAV HA, and it triggers the degradation of a class III fusion protein VSV-G. We conclude that MARCH8 exerts a very broad and conserved antiviral activity by inhibiting the maturation of class I fusion proteins, which blocks their secretion to cell surface and incorporation into virions. It should also target class III fusion proteins by triggering their degradation.



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97 Metabarcoding-Like Approach for High Throughput Detection and Identification of Viral Nucleic Acids

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Next generation sequencing (NGS) technologies have greatly enhanced our ability to identify new viral pathogens in various types of biological samples. This approach has led to the discovery of new viruses and has revealed hidden associations of viromes with many diseases. However, unlike the 16S rRNA that allows for bacterial detection by metabarcoding, the diversity and variability of viral genome render the creation of universal oligonucleotides for targeting all known and novel viruses impossible. Whereas whole- genome sequencing solves this problem, its efficiency is inadequate due to the high cost per sample and relatively low sensitivity. Furthermore, the existing approaches to designing oligonucleotides for targeted PCR enrichment are usually incomprehensive, being oriented at detecting a particular viral species or a genus based on the presumption of its presence in the sample. In this study, we developed a computational pipeline for designing genus-specific oligonucleotides that would simultaneously cover a multitude of known viruses from different taxonomic groups. This new tool was used to design an oligonucleotide panel for targeted enrichment of viral nucleic acids in different types of samples and demonstrated its applicability for detection of multiple viral genera at once. Next, we created a custom protocol for NGS library preparation adapted to the new primer panel, which have been tested together on a number of samples and proved highly efficient in pathogen detection and identification. Since a reliable algorithm for bioinformatic analysis is crucial for rapid classification of the sequences, in this work, we developed an NGS-based data analysis module and demonstrated its functionality both for detecting novel viruses and analyzing the virome diversity. This work was supported by an RSF grant (№17--74--20096).



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98 MicroRNAs Are Predicted to Control the Ubiquitin/Proteasome system in *Carica papaya* Plants Infected by the Papaya Meleira Virus Complex

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Papaya sticky disease (PSD) is a severe disease that can destroy papaya trees. PSD is associated with a complex formed between a toti-like virus, papaya meleira virus (PMeV), and an umbra-like virus, papaya meleira virus 2 (PMeV2). PSD symptoms only appear after flowering, indicating that at the pre-flowering stage, there is a host stress response associated with tolerance to sticky disease symptoms. Transcriptomic and proteomic analysis of symptomatic plants revealed the modulation of protein turnover, suggesting the involvement of the ubiquitin/proteasome system (UPS) in this pathosystem. In parallel, the analysis of microRNAs modulated during the infection showed that microRNAs predicted to target UPS genes were specially altered. This study aimed to evaluate the importance of UPS for *C. papaya*-PMeV complex interaction by revisiting transcriptomic and proteomic datasets obtained from infected plants at different developmental phases. In the referred datasets, 1074 transcripts and 80 proteins were related to the UPS pathway. Among the 42 UPS-related genes responsive to PSD, 22 were detected at transcript and 21 at protein level. Also, the microRNAs predicted to target UPS-related genes were identified, especially those altered during papaya infection by PMeV complex. A total of 106 miRNAs assigned to 33 miRNA families and targeting 146 gene transcripts were found. Among them, 22 miRNAs were predicted to target 4 genes (ETS_124.37, ETS_223.21, ETS_56.53, and ETS_66.17) that were observed to be modulated at transcript level at pre-flowering stage and one gene (ETS_33.158) modulated at protein level at post-flowering stage. Experimental evidence support the idea that miR837 and miR844 were especially relevant in controlling UPS during *C. papaya* response to PMeV complex. The miRNA expression and the consequent reduction of transcripts levels could result in increased PMeV complex tolerance in *C. papaya*. The results presented here add up to the knowledge of UPS involvement during virus infection in plants.



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99 Mixed Infections of Plant Viruses in Crops: Solo vs. Group Game

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Plant diseases are responsible for considerable economic losses in agriculture worldwide. Recent surveys and metagenomics approaches reveal a higher than expected incidence of complex diseases, like those caused by mixed viral infections. Particularly, frequent cases of mixed infections are co-infections or superinfections of plant viruses belonging to different genera in the families *Potyviridae* (*Ipomovirus* or *Potyvirus*) and *Closteroviridae* (*Crinivirus*). The outcome of such multiple infections could modify viral traits such as host range, titer, tissue and cell tropisms, and even vector preference and transmission rates. Therefore, we believe that understanding the virus–virus, virus–host, and virus–vector interactions would be crucial for developing effective control measures.

Since there is still limited knowledge about the molecular mechanisms underlying the different interactions, and how they might contribute to specific diseases in mixed infection, we are analyzing ipomovirus–crinivirus and potyvirus–crinivirus pathosystems to better understand single and mixed infections in selected susceptible hosts (Cucurbitaceae and Convolvulaceae plants), also incorporating in the study the interactions with insect vectors (whiteflies and aphids). Among other strategies, we are engineering new biotechnological tools to explore the molecular biology and transmission mechanisms of several viruses implicated in complex diseases, and we are also addressing the possibility to produce virus-like particles (VLPs) through transient expression of the CP of different viruses in *Nicotiana benthamiana* plants, with the aim to study requirements for virion formation and determinants of transmission.

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100 Morin Hydrate Has Antiviral Effects on Influenza Virus Infection In Vitro and In Vivo

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Influenza A virus, which is the major cause of seasonal or pandemic flu, is increasing in prevalence worldwide. Currently, oseltamivir, an anti-influenza drug which is potent and selective inhibitor of neuraminidase protein found in influenza A and B viruses, has been widely used in treatment of infected patients. However, the efficacy of oseltamivir is limited by the recent emergence of oseltamivir-resistant influenza viruses.

Morin hydrate (3, 5, 7, 2', 4'-pentahydroxyflavone) is a flavonoid originally isolated from the branches of *Morus alba* L., and is a constituent of many herbs, fruits, and red wine. It shows antioxidant, anti-inflammatory, neuroprotective, and anticancer activity through inhibition of the NF- κ B signaling pathway. However, the antiviral activity of morin hydrate toward influenza A/PR/8 viruses has not been studied.

In the present study, morin hydrate showed significant antiviral activity against influenza A/PR/8 (PR8) and inhibited hemagglutination of PR8 in vitro. In addition, the disease symptom of PR8-infected mice was significantly alleviated by oral treatment with morin hydrate, which reduced systemic production of inflammatory cytokines and chemokines such as TNF- α and CCL2. Furthermore, coadministration of morin hydrate with oseltamivir improved protection of PR8-infected mice and reduced the inflamed area in lung tissues. Coadministration of oseltamivir with morin hydrate also significantly decreased the levels of CCL2, TNF- α , and IL-1 β .

Our results suggest that the increased protection against PR8 infection from treatment with morin hydrate could be explained by its antiviral and anti-inflammatory activities, and also confirm that the antiviral effect was enhanced in combined administration with oseltamivir.



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101 Nationwide Screening for Important Bee Viruses in Belgian Honey Bees

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The ecological and economic importance of bees for pollination and biodiversity is well established. The health of bees is, however, threatened by a multitude of factors, including viruses, bacteria, parasites, and pesticides. In this study, we screened 562 colonies from 193 beekeepers distributed all over Belgium to monitor the prevalence and distribution of seven important viruses in Belgian honey bees (*Apis mellifera*). Several of these viruses have been linked with an increased risk for colony loss. Although these viruses can severely impact honey bees and can even cause the death of larvae or adults, colonies with a low virus titer usually appear asymptomatic (covert infection). The presence of viruses was determined by real-time RT-PCR and sequencing was performed as a confirmatory test. The three most prevalent viruses in Belgian honey bees are *Deformed wing virus B* (DWV-B or VDV-1), *Black queen cell virus* (BQCV), and *Sacbrood virus* (SBV). These viruses were found in more than 90% of the honey bee colonies, but often with a high Ct value, which indicates that they are present at low virus titers. In certain colonies, however, DWV-B, BQCV, or SBV was detected with a low Ct value, representing a high virus titer (in some cases, more than 7 log₁₀ genome copies per bee) and an increased likelihood of development of clinical symptoms. *Deformed wing virus A* (DWV-A), *Acute bee paralysis virus* (ABPV), and *Chronic bee paralysis virus* (CBPV) were found in less than 40% of the colonies. *Kashmir bee virus* (KBV) was not found in any of the analyzed Belgian honey bees. Most of the honey bee colonies are infected with more than one virus, albeit with low virus titers. The impact of viruses can however become critical in the presence of other detrimental factors such as parasites (*Nosema* sp., *Varroa* sp.) and pesticides.



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Abstract

102 Neutralizing Antibodies against Crimean–Congo Hemorrhagic Fever Virus Derived from a Human Survivor

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Crimean–Congo hemorrhagic fever virus (CCHFV) is an arbovirus belonging to the *Nairoviridae* family. The virus as well as ticks of the *Hyalomma* genus, which serve as its reservoir host, are found in parts of Africa, western Asia, and southern Europe. Following sporadic zoonotic or human-to-human transmission, infection is characterized by fever, fatigue, vomiting, diarrhea, and in fatal cases, often also hemorrhagic symptoms. There are currently no vaccines or targeted treatments available against CCHFV, leading the WHO to declare it a Blueprint priority pathogen in 2017.

Here, we report the isolation and characterization of a panel of human monoclonal antibodies (mAbs) against CCHFV. Using a novel soluble Gn/Gc sorting antigen, we were able to isolate memory B cells specific for CCHFV from four convalescent donors. From each patient sample, we were able to derive several potentially neutralizing antibodies with IC₅₀ in the nanomolar range as determined by neutralization of CCHFV virus-like particles. Neutralization by candidate hits was also confirmed using authentic CCHFV. We further show that several of the most potentially neutralizing mAbs possess a breadth of neutralization spanning 3 clades of CCHFV strains. These broadly neutralizing mAbs are currently being tested in a mouse model of CCHFV infection, with preliminary results indicating that they have protective potential.



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103 New Insights into Influenza A Virus Nonstructural Protein 1-Mediated Immune Evasion

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Influenza A virus (IAV) is a major respiratory pathogen causing epidemics every year or pandemics sporadically. The mammalian innate immune system employs several RNA sensors, including cytosolic RIG-I, endosomal TLR3, and TLR7, to detect IAV infection to trigger type I interferon (IFN)-mediated antiviral immunity. Meantime, IAV has evolved diverse strategies to subvert type I IFN-mediated antiviral defenses. Of note, the IAV nonstructural protein 1 (NS1) plays a crucial role in counteracting the RIG-I pathway in an RNA binding-dependent and -independent manner. It is of our interest to further explore if IAV NS1 may employ other mechanisms in impairing innate immune responses to facilitate IAV propagation in the host. Our recent work reveals a novel mechanism by which IAV NS1 not only counteracts RIG-I signaling but also other RNA sensing pathways through targeting TRAF3, a common regulator linking several DNA and RNA sensors to type I IFN production. An IAV NS1 mutant virus, which harbors an NS1 mutant unable to bind TRAF3, was generated for ex vivo and in vivo studies. Our ex vivo studies indicated that IAV NS1 mutant virus, compared to IAV WT virus, induced higher IFN- β production from mouse conventional dendritic cells (cDCs) and plasmacytoid DCs. Together, our data suggest that IAV NS1 mediates immune evasion through subverting multiple RNA sensing pathways to promote viral infection. Work is ongoing to further assess the in vivo role of this IAV NS1 mutant virus in mice.



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104 Novel Insights for Biosurveillance of Bat-Borne Viruses

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Bats are rich reservoirs of viruses, including several high-consequence zoonoses. In this study, high throughput sequencing is used to characterize the virome through a longitudinal study of a captive colony of fruit nectar bats, species *Eonycteris spelaea*, in Singapore. This study utilized viral RNA extracted from swabs of four body sites per bat per timepoint. Swabs of the exterior of the bat (head and body) were used to evaluate virus populations and demonstrate utility as a sample site for future surveillance to extrapolate population-level infection. Through unbiased shotgun and target-enrichment sequencing, we identified both known and previously unknown viruses of zoonotic relevance and defined the population persistence and temporal patterns of viruses from families that have the capacity to jump the species barrier. We observed population persistence of three zoonotic-related viral families that are known to be associated with spillover from bats to humans: *Paramyxoviridae*, *Reoviridae*, and *Coronaviridae*. To our knowledge, this is the first study that combines probe-based viral enrichment with high-throughput sequencing or to create a viral profile from multiple swab sites on individual bats and their cohort. This work demonstrates temporal patterns of the *E. spelaea* virome, including several novel viruses. Noninvasive surveillance methods that target the body of bats not only detect viruses shed within the colony but can also represent viral populations dispersed throughout the entire colony. New knowledge of persistent viral families should inform future directions for biosurveillance of viruses that have the potential to cross the species barrier from bats to humans or other amplifying hosts.



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105 Novel Methodology for the Detection of Enveloped Viruses

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Viral infections in humans cause a huge burden in worldwide healthcare that has increased due to the emergence of new pathogenic viruses, such as in the recent EBOV outbreaks. Viral particles in body fluids are often at very low levels, making diagnosis difficult. In order to address this problem, we have developed a new detection platform to isolate and detect different enveloped viruses.

We have recently identified that sialic acid-binding Ig-like lectin 1 (Siglec-1/CD169) is one cellular receptor used by EBOV and HIV-1 to enter myeloid cells, key target cells for infection and pathogenesis. For viral uptake, the V-set domain of this myeloid cell receptor recognizes the gangliosides of viral membranes that were dragged during viral budding from the plasma membrane of infected cells.

We took advantage of this specific interaction between Siglec-1 and viral gangliosides to develop a new detection methodology. We have generated a recombinant protein that contains the V-set domain of Siglec-1 fused to the human IgG Fc domain for anchoring in latex beads. These coated beads allow the isolation of viral particles and their measurement by flow cytometry. We have tested its efficacy to detect HIV-1 and EBOV and its specificity by using anti-Siglec-1 antibodies that prevent the interaction and serve as a negative control. To test the capacity of our method, we used synthetic liposomes to assess the effect of ganglioside concentration in membranes as well as the size of viral particles.

This methodology would facilitate the diagnostic of infections by concentrating viral particles in a fast and direct method. At a time when global human mobility facilitates dissemination of infectious agents, our approach represents a rapid and effective method to maximize the identification of both known and emerging enveloped viruses as part of public health viral surveillance strategies.



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Abstract

106 Pathobiological and Genomic Characterization of a Cold-Adapted Infectious Bronchitis Virus (BP-caKII)

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We established a cold-adapted infectious bronchitis virus (BP-caKII) by passaging a field virus through specific pathogen-free embryonated eggs 20 times at 32 °C. We characterized its growth kinetics and pathogenicity in embryonated eggs, and its tropism and persistence in different tissues from chickens; then, we evaluated pathogenicity by using a new premature reproductive tract pathogenicity model. Furthermore, we determined the complete genomic sequence of BP-caKII to understand the genetic changes related to cold adaptation. According to our results, BP-caKII clustered with the KII genotype viruses K2 and KM91 and showed less pathogenicity than K2, a live attenuated vaccine strain. BP-caKII showed delayed viremia, resulting in its delayed dissemination to the kidneys and cecal tonsils compared to K2 and KM91, the latter of which is a pathogenic field strain. A comparative genomics study revealed similar nucleotide sequences among BP-caKII, K2, and KM91 but clearly showed different mutations among them. BP-caKII shared several mutations with K2 (nsp13, 14, 15, and 16) following embryo adaptation but acquired multiple additional mutations in nonstructural proteins (nsp3, 4 and 12), spike proteins, and nucleocapsid proteins following cold adaptation. Thus, the establishment of BP-caKII and the identified mutations in this study may provide insight into the genetic background of embryo and cold adaptations, and the attenuation of coronaviruses.



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107 Persistent Transcriptional Alterations after Hepatitis C Virus Elimination in Cell Culture

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Chronic hepatitis C virus (HCV) infection causes liver inflammation and fibrosis, which can lead to development of cirrhosis and hepatocellular carcinoma (HCC). Recent approval of direct-acting antiviral (DAA) drug combinations has revolutionized antiviral therapy against HCV. These drugs enable virus eradication in virtually all treated patients regardless of the genotype and liver disease status. Based on clinical parameters, it has been proposed that elimination of infected cells by reactivated immune responses may be dispensable for virus eradication in contrast to previously used interferon-based therapies. It is thus formally possible that the patients that are declared cured do indeed carry formerly infected cells that display irreversible alterations due to prolonged chronic HCV infection.

Although transcriptional profiles of biopsies from cured patients have been previously studied, it is difficult to determine the precise mechanisms by which permanent alterations are established in the context of a heterogeneous tissue often in patients with an underlying liver disease. Thus, we used cell culture models of persistent HCV infection to determine if HCV infection causes permanent transcriptional alterations in host cells after virus eradication. In these models, HCV infection causes profound alterations of host cell transcriptome that aim at regaining cellular homeostasis in the context of intracellular membrane rearrangements, interference with homeostatic processes, and persistent stress conditions, and permit cell survival even though the virus has colonized the host cell. In this context, we asked the question of whether the original homeostasis and original transcriptomic profile are regained in formerly infected cells after DAA treatment-mediated virus eradication. Our results indicate that a minor subset of transcriptional alterations persists even after virus eradication, suggesting that DAA-mediated eradication does not ensure normalization of formerly infected cell homeostasis. Combined analysis of the transcriptional profiles in proliferating and growth-arrested cells suggest that several mechanisms underlie the establishment of permanent alterations.



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Abstract

108 Pioneering siRNA-Mediated Protection of Mammalian Cells against Zika Virus (MR-766) Infection

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Here, we present empirical data documenting the siRNA-mediated protection of cells after Zika virus (ZIKV) infection. siRNAs were designed to target well-conserved sequences across the ZIKV genome. Several delivery technologies were utilised. After the electroporation of 100 nM siRNA into human hepatocyte-derived carcinoma (Huh7) cells, the Feron Zv-2 sequence (specific to the ZIKV NS3 gene) yielded a cell viability of $150.3\% \pm 7.4\%$ (SEM: n = 4) ($p = 0.0004$) relative to cells treated only with virus ($33.9\% \pm 12\%$, SEM: n = 4). Further, 100 nM siRNA Feron Zv-4 (specific to ZIKV 3'UTR) resulted in $119.1\% \pm 11.2\%$ cell viability (SEM: n = 4) relative to the control cells treated with ZIKV ($p = 0.0021$). Cells were electroporated with siRNA prior to ZIKV infection and viability was monitored 4 days after this. Additionally, two novel siRNA delivery systems were tested. The first utilized recombinant *Bacillus anthracis* PA83 (octamer-forming mutants) co-incubated with the N-terminal 255 amino acids of *B. anthracis* lethal factor (LFn) fused in-frame with the RNA binding domain for human protein kinase R (LFn-PKR) at a concentration of 50 $\mu\text{g}/\text{mL}$ (each). Here, baby hamster kidney (BHK) cells treated with 100 nM siRNA Feron Zv-1 yielded $79.0\% \pm 4.0\%$ viability relative to the control ($50.2\% \pm 1.7\%$, SEM: n = 3) 3 days after exposure to ZIKV ($p = 0.0096$). Finally, HeLa exosomes loaded with siRNA Feron-Zv2 were incubated with Huh7 cells prior to ZIKV infection. For the siRNA-exosome treated cells, a viability of $123\% \pm 46\%$ (SEM: n = 18) relative to $8\% \pm 16\%$ (SEM: n = 18) for the same concentration of control HeLa exosomes was recorded ($p = 0.0416$). In each instance, 0.3 mol was used and cell viability monitored using the Pierce™ Firefly Luciferase Glow Assay Kit by Thermo Scientific™. Here, we show for the first time that siRNA can significantly reduce ZIKV-induced cell killing. Future work will require quantitating ZIKV mRNA in relation to siRNA treatment as well as testing the siRNAs and delivery systems within more complex models.



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Abstract

109 Porcine Respiratory Cell and Tissue Co-Infections and Superinfections with Porcine Reproductive and Respiratory Syndrome and Swine Influenza Viruses

Georges saade ¹, Caroline HERVET ¹, Déborah Ménard ¹, Patricia Renson ², Céline Deblanc ², Juliette Bougon ², Gaëlle Simon ², Olivier Bourry ², Nicolas Bertho ¹ and François Meurens ¹

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Porcine reproductive and respiratory syndrome virus (PRRSV) and swine influenza type A virus (swIAV) are major contributors to the porcine respiratory disease complex that is still threatening porcine farming around the world. Understanding the interactions between these two viruses and the effect of their co-infection on the porcine immune response will lead to better preventive and therapeutic measures. In vivo studies of these co-infections showed contradictory results while in vitro studies showing slight interferences between the viruses were limited due to the fact that the two viruses do not share the same host cells. SwIAV infects mainly epithelial cells, while PRRSV infects only cells expressing CD163, such as alveolar macrophages. In an attempt to evaluate the effect of PRRSV infection on the replication of swIAV, the possible induction of trained immunity, and the host antiviral response, we carried out several co-infections and superinfections using local viral strains. Our infection protocols were performed using a tracheal epithelial cell line and precision cut lung slices (PCLS) combining different cell types mimicking infections in real conditions. The expression of viral and cellular transcripts involved in the recognition of the virus and in the antiviral response was assessed by quantitative PCR. Immunostaining was also carried out to monitor the virus distribution in the pulmonary tissue. Interestingly, without infecting epithelial cells, PRRSV was able to interfere with swIAV infection and inhibit the antiviral response of the hosting cell. The mechanisms of this interference are still unclear and need to be investigated to ultimately apply improve preventive and therapeutic approaches.



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110 Prophage BPs Alters Mycobacterial Gene Expression and Antibiotic Resistance

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Diseases caused by mycobacteria, such as *Mycobacterium tuberculosis*, are the leading cause of death worldwide. With the emergence of strains that are resistant to first-line anti-tuberculosis drugs and naturally drug-resistant pathogens such as *M. abscessus*, there is a need to increase our understanding of mycobacterial fitness and virulence and identify new targets for drugs. The majority of pathogenic species of the bacterial genus *Mycobacterium*, including *M. tuberculosis*, carry integrated viral genomes (prophage) that are hypothesized to contribute to virulence. Though we know many of the ways in which phage genes directly contribute to pathogenesis, e.g., the CTX phage encodes the toxin in *Vibrio cholera*, we know little about the impact of phage that encode no obvious toxin or virulence gene. Using an RNAseq approach, our lab recently showed for the first time that the presence of a prophage alters expression of 7.4% of genes in the pathogenic mycobacterial species, *M. chelonae*. The presence of prophage BPs increased expression of genes in the *whiB7* regulon, including *whiB7*, *eis2*, and *tap*, and decreased expression of a *padR*-family transcription factor. BP lysogens were more resistant to aminoglycosides (kanamycin and amikacin) and tetracycline than wild-type strains of *M. chelonae*. To determine how the BP prophage drives changes in bacterial gene expression and phenotype, we are testing the effect of individual BP genes expressed during lysogeny, such as the immunity repressor, on bacterial gene expression and antibiotic resistance phenotypes.



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111 Protection of Chickens with Maternal Avian Influenza Virus (AIV) Immunity after Vaccination with a Recombinant AIV-Newcastle Disease Vector

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Highly pathogenic avian influenza virus (HPAIV) belongs to the family *Orthomyxoviridae* and causes a systemic and highly lethal disease in poultry.

Vaccination with recombinant Newcastle disease vector viruses (NDV) expressing the hemagglutinin (HA) of HPAIV H5N1 induces high antibody titers in chickens free of specific pathogens, conveying protection against a lethal infection with HPAIV H5N1. Protection of chickens possessing maternally derived NDV immunity was achieved after the replacement of the surface proteins of NDV, the fusion (F), and the hemagglutinin-neuraminidase protein (HN) against those of avian paramyxovirus serotype 8. However, maternal AIV antibodies (α AIV-MDA+) still interfere with vaccine virus replication, resulting in inefficient protection.

For our study, recombinant rNDVsolH5_H5 was generated. Insertion of a transgene encoding a truncated soluble HA between the NDV phosphoprotein and matrix protein genes, in addition to the gene encoding a membrane-bound HA inserted between NDV F and HN of lentogenic NDV Clone 30, was expected to increase the total amount of HA expressed by the recombinant virus. Western blot and mass spectrometry analyses confirmed the increase in HA expression compared to the parental rNDVH5 expressing only the full-length HA.

The protective efficacy of the newly generated recombinant NDV was tested in an animal experiment. α AIV-MDA+ chickens were vaccinated on either day 7, 14, or 21 after hatching. A homologous challenge infection was carried out three weeks later. Although the youngest chickens showed the highest titer of α AIV-MDA, there were no AIV antibodies detectable 21 days after vaccination. However, 40% of vaccinated chickens were protected, while 85% and 100% protection was observed in the middle-aged and oldest chickens, which had low and no detectable levels of α AIV-MDA, and moderate and high AIV antibody levels after vaccination. Challenge infection of non-vaccinated chickens resulted in high mortality.



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112 In Vivo Editing of *Rotavirus* Double-Stranded RNA Genome Mediated by the CRISPR-Csy4 Endoribonuclease

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CRISPR nucleases have produced several important scientific discoveries due to their wide applications in editing cellular and viral genomes, including DNA and single-stranded RNA viruses. However, nuclease-mediated genome editing of double-stranded RNA (dsRNA) viruses has not been described so far.

Here, we achieved the first in vivo CRISPR nuclease-mediated editing of defined *Rotavirus* genome segments. Using the *Pseudomonas aeruginosa* CRISPR-Csy4 enzyme fused to the rotavirus nonstructural proteins NSP5 or NSP2, we localised the Csy4 endoribonuclease to cytoplasmic viral factories (viroplasm) fostering site-specific cleavage of genome replication intermediates. Csy4 nuclease-mediated cleavage generated defined deletions of the targeted genome segment in a single replication cycle, paving the way for extensive use of CRISPR nucleases for genome editing of dsRNA viruses. Taking advantage of the defined editing events, we visualised, for the first time, the product of secondary transcription by the newly assembled viral particles. We used engineered recombinant RVs which, only upon deletion events on the genome of the progeny virus, either express a new tag on a viral protein or light up EGFP expression, thus permitting monitoring and quantification of secondary transcripts and their translation products in living cells during the replication cycle. We demonstrate that this step significantly contributes to the overall production of viral proteins in rotavirus-infected cells. Our approach, allowing the in vivo generation of a new progeny of recombinant fluorescent rotaviruses, paves the way for harnessing of this tool for the screening of drugs targeting pathways directly associated to secondary transcription and/or the assembly of newly made particles. We anticipate that the nuclease-mediated cleavage of dsRNA virus genomes will promote a new level of understanding of dsRNA viral replication mechanisms and host–pathogen interactions.



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113 Recombinant Rotaviruses Rescued by Reverse Genetics Reveal the Essential Role of NSP5 Hyper-Phosphorylation in the Assembly of Viroplasms

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Rotavirus (RV) is a non-enveloped virus with a segmented dsRNA genome that replicates in round-shaped cytoplasmic viral factories termed viroplasms. Formation of these viral factories requires co-expression of the two non-structural viral proteins, NSP5 and NSP2. During RV infection, NSP5 undergoes a complex hyper-phosphorylation, which is primed by the phosphorylation of a single serine residue (Ser67). Despite extensive studies on NSP5 phosphorylation, the role of this post-translational modification in the formation of viroplasms and its impact on the virus replication remains obscure. Taking advantage of a modified fully tractable reverse genetics system, we shed light on the role of NSP5 during RV infection. An NSP5 trans-complementing cell-line was used to generate and characterize several recombinant rotaviruses (rRVs) with mutations in NSP5. We demonstrate that a rRV lacking NSP5 (NSP5-KO) was completely unable to assemble viroplasms and to replicate, confirming its pivotal role in rotavirus replication. A number of mutants with impaired NSP5 phosphorylation were generated to further interrogate the function of this post-translational modification in the assembly of replication-competent viroplasms. We show that the rRV mutant strains, such as one carrying the S67A mutation, exhibit impaired viral replication and the ability to assemble round-shaped viroplasms in MA104 cells. Furthermore, we investigated the mechanism of NSP5 hyper-phosphorylation using NSP5 phosphorylation-negative rRV strains, as well as MA104-derived stable transfectant cell lines expressing selected NSP5 deletion mutants. Our results indicate that NSP5 hyper-phosphorylation is a crucial step for the assembly of functional round-shaped viroplasms and highlight a key role of the C-terminal tail of NSP5 in the formation of replication-competent viral factories. Such a complex NSP5 hyper-phosphorylation cascade may serve as a paradigm for the assembly of functional viral factories in other RNA viruses.



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Abstract

114 Risk Factors Associated with Positive IgG Anti-HEV Detection among Different Adult Subpopulations in Corsica, France, 2019

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Hepatitis E virus (HEV) is a major cause of acute hepatitis worldwide. In France, hyperendemic areas, including Corsica, have IgG anti-HEV seroprevalence higher than 60%. The aim of this study was to determine the seroprevalence of IgG anti-HEV in three different adult populations in Corsica and to assess activity and alimentary risk factors of infection. A total of 930 individuals, including 467 blood donors, 393 student or staff of university, and 70 patients from general practice, were tested between 2017 and 2019. All obtained sera were tested for the presence of IgG anti-HEV antibodies using an ELISA Wantai assay, and each serology was associated with a specific questionnaire. The association between seropositivity and potential risk factors was tested with univariate and multivariate analysis. Out of the 930 samples, 52.3% (486/930) were seropositive, corresponding to 54.4% (254/467) among blood donors, 47.6% (187/393) among university students or staff, and 64.3% (45/70) among patients of general practice. Two main risk factors were determined: depiecing and evisceration activity (adjusted odds ratio = 2.76 95%CI [1.51–5.37]; *p*-value = 0.00077) as well as consumption of a local raw pork sausage (Fittonu) (AOR = 1.95 95%CI [1.45–2.64]; *p*-value = 0.00001). Increasing age was also associated with higher seroprevalence (*p*-value = 0.00272). The analysis of exposure between the different populations seemed to be homogeneous after age stratification. This study provides relevant information for control and preventive strategies as well as concrete advice to risk groups in Corsica.



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Abstract

115 Role of Cytoplasmic N6-Methyladenosine (m6A) Readers on HIV-2 Protein Synthesis

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Human immunodeficiency virus type-2 (HIV-2) establishes reservoirs at levels comparable to HIV-1 but its infection is associated to very low rates of progression to AIDS. A hallmark of HIV-2 patients is a very low, usually undetectable viral load, which has been explained by a strong immune activity against HIV-2 but also by a post-transcriptional repression exerted at the levels of protein synthesis. Since the presence of the N6-methyladenosine (m6A) in the HIV-1 full-length RNA was shown to promote Gag synthesis and hence viral replication, we investigated whether HIV-2 Gag expression is regulated by the m6A RNA modification. Our results indicate that, in contrast to what was reported for HIV-1, knock down and overexpression of the m6A readers YTHDF1, 2 and 3 exerts different effects on HIV-2 Gag synthesis. As such, while YTHDF3 negatively regulates Gag expression, YTHDF1 and YTHDF 2 have the opposite effect. We also observed that YTHDF proteins relocalize together with the HIV-2 gRNA in stress granules-like structures, sites where the viral genome is stored in the absence of active translation. Interestingly, YTHDF3 knock down results in increased Gag synthesis and a decrease in the viral accumulation in stress granules-like structures.

Together, these data suggest that HIV-2 protein synthesis is negatively regulated by the m6A reader YTHDF3 and that the association between YTHDF3 and the HIV-2 gRNA is responsible, at least in part of the accumulation of the viral RNA in stress granules-like structures during viral replication.



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116 SAMHD1 Enhances Chikungunya and Zika Virus Replication in Human Skin Fibroblasts

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Chikungunya virus (CHIKV) and Zika virus (ZIKV) are emerging arboviruses that pose a worldwide threat to human health. Currently, neither vaccine nor antiviral treatment to control their infections is available. As the skin is a major viral entry site for arboviruses in the human host, we determined the global proteomic profile of CHIKV and ZIKV infections in human skin fibroblasts using stable isotope labelling by amino acids in cell culture (SILAC)-based mass spectrometry analysis. We show that the expression of the interferon-stimulated proteins MX1, IFIT1, IFIT3 and ISG15, as well as expression of defense response proteins DDX58, STAT1, OAS3, EIF2AK2, and SAMHD1 was significantly upregulated in these cells upon infection with either virus. Exogenous expression of IFITs proteins markedly inhibited CHIKV and ZIKV replication which, accordingly, was restored following the abrogation of IFIT1 or IFIT3. Overexpression of SAMHD1 in cutaneous cells or pretreatment of cells with the virus-like particles containing SAMHD1 restriction factor Vpx resulted in a strong increase or inhibition, respectively, of both CHIKV and ZIKV replication. Moreover, silencing of SAMHD1 by specific SAMHD1-siRNA resulted in a marked decrease of viral RNA levels. Together, these results suggest that IFITs are involved in the restriction of replication of CHIKV and ZIKV and provide, as yet unreported, evidence for a proviral role of SAMHD1 in arbovirus infection of human skin cells.



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117 Sequential Emergence and Wide Spread of Neutralization Escape MERS-CoV Mutants during the 2015 Korean Outbreak

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An unexpectedly large outbreak of MERS swept South Korea in 2015. This outbreak was initiated by an infected traveler and amplified by several superspreading events. Previously, we reported the emergence and spread of mutant MERS-CoV bearing spike mutations (I529T or D510G) with reduced affinity to human receptor CD26 during the Korean outbreak. To assess the potential association of spike mutations with superspreading events, we collected viral genetic information reported during the Korean outbreak and systemically analyzed the relationship of spike sequences and epidemiology. We found sequential emergence of the spike mutations in two superspreaders. In vivo virulence of the mutant viruses seems to decline in human patients, as assessed by fever duration in affected individuals. In addition, neutralizing activity against these two mutant viruses in sera from mice immunized with wild-type spike antigen were gradually reduced, suggesting an emergence and wide spread of neutralization escapers during the Korean outbreak.



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118 Serologic Analysis of Hepatitis E Virus Infection in Patients with Kidney-Related Illnesses

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Hepatitis E is a globally distributed human disease caused by hepatitis E virus (HEV). HEV is a positive-sense, single-stranded RNA virus that belongs to the family *Hepeviridae*. Within the genus *Orthohepevirus*, seven HEV genotypes infect various mammalian hosts. HEV genotypes HEV-1 to HEV-4 and HEV-7 can infect humans. HEV-3 is zoonotic with domestic pig, wild boar, deer, and other mammal species as reservoirs. HEV-3 is an underestimated emerging threat which is spread in Europe. It is transmitted through undercooked pork meat or other products and with blood components through transfusions. HEV-3 infection in immunocompetent patients is self-limiting and clinically asymptomatic. However, immunocompromised individuals are at a high risk of developing chronic hepatitis E. Chronic infection may lead to life-threatening liver cirrhosis. Patients with kidney transplants or kidney-related illnesses are in this risk group. In this study, a serologic analysis of blood samples obtained from kidney transplant recipients, patients with chronic kidney disease, patients under dialysis, and healthy controls was performed. A prevalence of anti-HEV antibodies was assessed by commercial and in-house ELISAs.



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119 Similarities in Antiviral Humoral Immune Response to Nucleocapsid Proteins of Hazara and Crimean–Congo Hemorrhagic Fever Virus

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Hazara virus (HAZV), a tick-borne agent of the nairoviruses, is closely related to Crimean–Congo hemorrhagic fever virus (CCHFV). Hazara virus has not been reported as a pathogen for humans and can be studied under BSL-2 conditions, whereas CCHFV causes severe hemorrhagic diseases, with up to 30% mortality rate in humans, and requires BSL-4 facilities to be handled. Serologic and phylogenetic similarities between two viruses would therefore be an interesting area of research. In this study, we evaluated the immunological similarities between these two viruses using nucleocapsid protein as a model. Here, we evaluated cross-reactivity between CCHFV and HAZV rNP, which forms virus-like particles when expressed in *Pichia pastoris*. In Western blot assays using CCHFV-infected human and immunized mice and rabbit sera, cross-reactions were detected between the nucleoproteins of both viruses. Virus-like particles were visualized by transmission electron microscopy (TEM) and monitored by dynamic light scattering (DLS). These results suggest that nucleocapsid proteins of HAZV and CCHFV share similarities regarding the antiviral humoral response in both species.



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Abstract

120 Single-Particle Tracking Porcine Epidemic Diarrhea Virus Moving along Microtubules in Living Cells

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Porcine epidemic diarrhea virus (PEDV), a member of the genus *Alphacoronavirus*, has caused severe damage to the swine industry. Although viruses are believed to hijack the microtubule-based transport system, the exact manner of PEDV moving along microtubules has not been fully characterized. In this study, PEDV was labeled with quantum dots, which have great brightness and photostability. By using quantum dot-labeled PEDV and single-particle tracking, we were able to systematically dissect the dynamic behaviors of PEDV moving along the microtubules in living cells. We found that PEDVs maintained a restricted motion mode with a relatively stable speed in the cell membrane region while displaying a slow–fast–slow velocity pattern with different motion modes in the cell cytoplasm region and near the microtubule-organizing center. The return movements of small amount of PEDVs were also observed in living cells. Collectively, our work is crucial for understanding the movement of PEDV in living cells, and the proposed work also provides important references for further analysis and studies of the infection mechanism of PEDV.



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Abstract

121 Specific Lipid Recruitment by the Retroviral Gag Protein upon HIV-1 Assembly: From Model Membranes to Infected Cells

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The retroviral Gag protein targets the plasma membrane of infected cells for viral particle formation and release. The MA domain of Gag is myristoylated for membrane anchoring but also contains a highly basic region that recognizes acidic phospholipids. Gag targets lipid molecules at the inner leaflet of the plasma membrane including phosphatidylinositol (4,5) bisphosphate (PI(4,5)P2) and cholesterol. Here, we addressed the question whether HIV-1 Gag was able to trap PI(4,5)P2 and/or other lipids during HIV-1 assembly *in silico*, *in vitro* on reconstituted membranes and *in cellulo* at the plasma membrane of the host CD4⁺ T cells. *In silico*, we could observe the first PI(4,5)P2 preferential recruitment by HIV-1 MA or Gag while protein docking on artificial membranes. *In vitro*, using biophysical technics, we observed the specific recruitment of PI(4,5)P2 and, to a less extend cholesterol, and the exclusion of sphingomyelin, using HIV-1 myr(-)Gag on LUVs and SLBs. Finally, in infected living CD4⁺ T cells, we determined lipid dynamics within and away from HIV-1 assembly sites using super-resolution STED microscopy coupled with scanning Fluorescence Correlation Spectroscopy (sSTED-FCS). Analysis of HIV-1 infected CD4⁺ T lymphocytes revealed that, upon virus assembly, HIV-1 is able to specifically trap PI(4,5)P2, and cholesterol, but not phosphatidylethanolamine (PE) or sphingomyelin (SM) at the cellular membrane. Furthermore, analysing CD4⁺ T cells expressing only HIV-1 Gag protein shows that Gag is the main driving force to restrict mobility of PI(4,5)P2 and cholesterol at the cell plasma membrane. Our data provide first direct evidence showing that HIV-1 Gag creates its own specific lipid environment for virus assembly, by selectively recruiting lipids to create a PI(4,5)P2/cholesterol enriched nanodomains favouring virus assembly, and that HIV-1 does not assemble on pre-existing lipid domains.



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122 Structural and Functional Studies of Chikungunya Virus nsP2

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Chikungunya virus (CHIKV) is transmitted to humans through mosquitoes and causes Chikungunya fever. Nonstructural protein 2 (nsP2) contains an N-terminal RNA helicase with both nucleotide triphosphatase and RNA triphosphatase activities, and a C-terminal cysteine protease that is responsible for polyprotein processing. Both N-terminal RNA helicase and C-terminal cysteine protease are connected through a flexible linker. Although the structure of the C-terminal cysteine protease has been solved, the structure and the conformational arrangement of full-length nsP2 remains elusive. Here, we determined the crystal structure of the helicase part of the CHIKV nsP2 (nsP2h) bound to the conserved 3'-end of the genomic RNA and the nucleotide analogue ADP-AIF₄. The structure of this ternary complex revealed the molecular basis for viral RNA recognition and ATP hydrolysis by the nsP2h. Unique hydrophobic protein–RNA interactions play essential roles in viral RNA replication. We also determined the solution structure of full-length nsP2 using small-angle X-ray scattering (SAXS). The solution architecture of the nsP2 was modeled using the available high-resolution structures and program CORAL (complexes with random loops). The CORAL model revealed that nsP2 is partially unfolded and the N-terminal protease domain is arranged near the N-terminal domain of the helicase domain. These findings expand our knowledge of CHIKV and related alphaviruses and might also have broad implications for antiviral and vaccine developments against pathogenic alphaviruses.



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123 Study of Orthonairoviral RNPs Using Clonable Tags in a HAZV Reverse Genetics System

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Crimean-Congo hemorrhagic fever orthonairovirus (CCHFV) is one of the most widespread medically important tick-borne arboviruses, causing outbreaks of human infections with up to 30% mortality rates. Laboratory handling of CCHFV requires high biosafety level containment (BSL-4), but its closely related homologue Hazara virus (HAZV) can be studied using less restrictive BSL-2 protocols. HAZV forms part of the CCHFV serogroup, and they share many structural and biochemical properties. A reverse genetics system for HAZV has been previously described, allowing the engineering of mutant viruses for the study of the orthonairoviral cycle under more amenable BSL-2 conditions.

We used the HAZV reverse genetics system for the incorporation of clonable tags in the nucleoprotein of HAZV, in order to study the morphology and intracellular trafficking of orthonairoviral ribonucleoproteins (RNPs). A c-terminal 6xhis tag was engineered in the nucleoprotein of HAZV for the purification of native viral RNPs from infected cell lysates using Ni²⁺-NTA affinity chromatography. A structural analysis of purified RNPs was done using electron microscopy, revealing the structure of native orthonairoviral RNPs.

We also used the split-green fluorescent protein (split-eGFP) system to tag and visualize the nucleoprotein of HAZV in infected live cells. The 16-c-terminal amino acids of the eGFP were fused to the nucleoprotein of HAZV, and the large trans-complementing eGFP fragment was supplied by transient expression. This system allowed the characterization of the intracellular dynamics of HAZV nucleoprotein by confocal microscopy and the visualization of the intracellular trafficking of orthonairoviral RNPs in live infected cells.

Our study establishes the potential of introducing small clonable tags in the nucleoprotein of HAZV using a reverse genetics system and opens up the possibility of incorporating new clonable tags for further studies of the viral cycle and the virus–host cell interactions of orthonairoviruses under BSL-2 biosafety levels.



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124 Study of the Retrotransposon-Derived Human PEG10 Protease

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Paternally expressed gene 10 (*PEG10*) is a human retrotransposon-derived imprinted gene. Previous works have demonstrated that mutation in the coding sequence of the gene is lethal in embryological age due to defects of placental development. In addition, *PEG10* is implicated in several malignancies, such as pancreatic cancer and hepatocellular carcinoma. The gene of *PEG10* encodes two protein isoforms, which are translated by a typical retroviral frameshift mechanism. The Gag-like protein ($RF1_{PEG10}$) is encoded by reading frame 1, whilst reading frames 1 and 2 account for the Gag-Pol-like polyprotein ($RF1/RF2_{PEG10}$). The protease (PR) domain of $RF2_{PEG10}$ contains an -Asp-Ser-Gly-sequence, which refers to the conservative -Asp-Ser/Thr-Gly- active-site motif of retroviral aspartic proteases. The function of the aspartic protease domain of $RF2_{PEG10}$ remains unclear. In order to further investigate the function of *PEG10* protease (PR_{PEG10}), a frameshift mutant was generated ($fsRF1/RF2_{PEG10}$) for comparison with the $RF1/RF2_{PEG10}$ form. To study the effects of PR_{PEG10} on cellular proliferation and viability, mammalian HEK293T and HaCaT cells were transfected with plasmids encoding for either the frameshift mutant ($fsRF1/RF2_{PEG10}$) or a PR active-site (D370A) mutant $fsRF1/RF2_{PEG10}$. Based on our findings, $fsRF1/RF2_{PEG10}$ overexpression resulted in an increased cellular proliferation, compared to the mutant form. Interestingly, transfection with $fsRF1/RF2_{PEG10}$ had a detrimental effect on cell viability. We hypothesize that PR_{PEG10} may play a cardinal role in the function of this retroviral remnant, possibly implicated in cellular proliferation and inhibition of apoptosis.



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125 Teicoplanin Derivatives Impact on West Nile Virus Pathogenesis

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West Nile virus (WNV) is an emerging arbovirus that causes infections worldwide. Clinical manifestations of the infection vary from asymptomatic to fatal illness when it reaches the central nervous system. To date, vaccine and specific antiviral treatment are not available. Teicoplanin is already used to treat Gram-positive bacterial infections. Furthermore, it has been reported to block the entry of pseudotyped Ebola, Middle East respiratory syndrome coronavirus and severe acute respiratory syndrome coronavirus. Moreover, teicoplanin derivatives showed anti-influenza virus, anti-human immunodeficiency virus, anti-hepatitis C virus, and anti-dengue virus activity. In total, 12 teicoplanin derivatives have been tested against our West Nile virus isolate. Vero E6 cells were simultaneously treated with 50 μ M of teicoplanin derivatives and infected with WNV at the same time. Virus-induced cytopathic effect and cytotoxicity were examined 4 days post-infection. One compound completely blocked virus pathogenesis, while five compounds reduced the viral titer. Further studies will be conducted to unravel the mode of action of promising derivatives.



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126 The AP-1 Transcription Factor is a Key Determinant of Human Cytomegalovirus Latency and Reactivation

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Human cytomegalovirus (HCMV) is a ubiquitous pathogen that latently infects hematopoietic progenitor cells (HPCs). Individuals with a competent immune system are, for the most part, asymptomatic for disease. However when a latently infected individual becomes immunosuppressed, HCMV can reactivate causing severe morbidity and mortality. While much of the viral genome is transcriptionally silenced during latency, some genes are expressed, including the HCMV-encoded G-protein coupled receptor *US28*. We showed *US28* expression is required for latency, as it suppressed the activator protein-1 (AP-1) transcription factor by attenuating the AP-1 subunit, *fos*. In turn, this prevents AP-1 from binding and activating the major immediate early promoter (MIEP), the key promoter regulating the latent-to-lytic transcriptional “switch”. Our new data suggest *US28*-mediated signaling during latency attenuates the Src-MAPK signaling axis to regulate AP-1. We find *US28* expression suppresses Src, MEK, and ERK, as well as *fos* phosphorylation and AP-1 binding to the MIEP. Conversely, pharmacological inhibition of Src, MEK, or ERK in *US28* Δ -latently infected HPCs suppresses infectious virus production, demonstrating the important role for this signaling axis during latency. Our recent data also reveal regulating AP-1 is a key determinant in balancing HCMV latency and reactivation. Infection with a virus in which we disrupted the proximal AP-1 binding site in the MIEP (*AP-1* Δp) leads to reduced AP-1 binding and inefficient viral reactivation compared to wild type. Further, AP-1 is critical for derepression of MIEP-driven transcripts and downstream early and late genes, while other immediate early genes remain unaffected. Collectively, these data suggest AP-1 binding to the MIEP is suppressed during latency, but is required for efficient transactivation of the MIEP during reactivation. We are currently elucidating *US28*'s involvement in recruiting AP-1 to the MIEP during reactivation.



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127 The ATGL Lipase Cooperates with ABHD5 to Mobilize Lipids for HCV Assembly and Lipoprotein Production

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Lipid droplets are essential cellular organelles for storage of fatty acids and triglycerides. The hepatitis C virus (HCV) translocates several of its proteins onto their surface and uses them for production of infectious progeny. We recently reported that the lipid droplet-associated a/b hydrolase domain-containing protein 5 (ABHD5/CGI-58) participates in HCV assembly by mobilizing lipid droplet associated lipids. However, ABHD5 itself has no lipase activity, and it remained unclear how ABHD5 mediates lipolysis critical for HCV assembly. Here, we identify adipose triglyceride lipase (ATGL) as an ABHD5 effector and a new host factor involved in the hepatic lipid droplet degradation as well as in HCV and lipoprotein morphogenesis. Modulation of ATGL protein expression and lipase activity controlled lipid droplet lipolysis and virus production. Mutation of the predicted ABHD5 protein interface to ATGL ablated ABHD5 functions in lipid droplet lipolysis and HCV assembly. Congruently, grafting ABHD5 residues critical for activation of ATGL onto ABHD4, an ABHD5 paralog unable to activate ATGL or support HCV assembly and lipid droplet lipolysis, bestowed these functions onto the engineered protein. Interestingly, minor alleles of ABHD5 and ATGL associated with neutral lipid storage diseases in humans are also impaired in lipid droplet lipolysis and their proviral functions. Collectively, these results show that ABHD5 cooperates with ATGL to mobilize triglycerides for HCV infectious virus production. Moreover, viral manipulation of lipid droplet homeostasis via the ABHD5-ATGL axis, akin to natural genetic variation in these proteins, emerges as a possible mechanism by which chronic HCV infection causes liver steatosis.



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Abstract

128 The Characteristics of New SSB Proteins from Metagenomic Libraries and Their Use in Biotech Applications

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Single-stranded DNA binding proteins (SSBs) bind to single-stranded DNA in a sequence-independent manner to prevent formation of secondary structures and protect DNA from nuclease degradation. These ubiquitous proteins are present in prokaryotes, eukaryotes, and viruses, and play a pivotal role in major cellular processes: replication, recombination, and repair of genetic material. In DNA replication, SSB proteins specifically stimulate DNA polymerase, increase fidelity of DNA synthesis, assist the DNA polymerase to advance forward, and organize and stabilize replication forks.

Here, we present our characterization of four SSB proteins of different origin. One of them was isolated from *Clostridium* sp. phage phiCP130 (SSB C1: 124 aa, Mr = 13,905). Three others (SSB M2: 136 aa, Mr = 15,009; SSB M3: 144 aa, Mr = 16,106; and SSB M5: 138 aa, Mr = 15,851) were isolated from metagenomics libraries. They show high similarity to SSB proteins from *Caldanaerovirga acetigigens*, *Caldanaerobius fijiensis*, and *Fervidobacterium gondwanense*. The recombinant proteins were overproduced in *E. coli* Rosetta (pRARE), except for SSB M5, which was overproduced in *E. coli* BL21. Proteins were purified using a metal-affinity chromatography as His-tagged fusion proteins. Electrophoretic mobility shift assay was used to examine their DNA binding activity with fluorescein-labeled oligonucleotide (dT40) used as a substrate. Thermal stability analysis revealed that they are stable at elevated temperatures with exception of SSB protein C1 which loses its activity above 65 °C. The other proteins are active at high temperatures, SSB M3 up to 85 °C, while SSB M2 and SSB M5 are active at up to 98.7 °C. The subunit structure of proteins was analyzed by gel filtration on Superdex 75 column (AKTA). This allowed us to conclude that in solution, the analyzed proteins exist in oligomeric form, a feature which is characteristic of other SSB proteins. Purified SSB proteins were tested to improve specificity of PCR-based DNA amplification.



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129 The European Virus Archive Goes Global: A Growing Resource for Research

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The European Virus Archive (EVA) was created in 2008 with funding from the FP7-EU Infrastructure Programme, in response to the need for a coordinated and readily accessible collection of viruses that could be made available to academia, public health organisations and industry. Within three years, it developed from a consortium of nine European laboratories to encompass associated partners in Africa, Russia, China, Turkey, Germany and Italy.

In 2014, the H2020 Research and Innovation Framework Programme (INFRAS projects) provided support for the transformation of the EVA from a European to a global organization (EVAg). The EVAg now operates as a non-profit consortium, with 26 partners and 20 associated partners from 21 EU and non-EU countries. In this presentation, we outline the structure and goals of the EVAg, and how EVAg has helped to respond to Viral Public Health Emergencies. Our aim is to bring to the attention of researchers the wealth of products it can provide and to illustrate how they can gain access to these resources, free of charge.



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130 The Intrinsic Link between Metabolic and Antiviral States of the Cell

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Acute treatment of cells with Rapamycin promotes gene delivery by facilitating lentiviral vector entry into cells. We recently showed that mTOR inhibitors lower an intrinsic antiviral barrier mediated by the interferon-induced transmembrane (IFITM) proteins, in particular IFITM3. Rapamycin-mediated degradation of IFITM3 requires endosomal trafficking, ubiquitination, endosomal sorting complex required for transport (ESCRT) machinery, and lysosomal acidification. Lentiviral transduction of diverse human and murine cells, including CD34⁺ hematopoietic stem progenitor cells and fibroblasts, was enhanced following acute Rapamycin treatment and enhancement was diminished upon *IFITM* knock-down or knock-out. In addition to enhancing infection by lentiviral vectors, we found that Rapamycin increased infection by replication-competent Influenza A virus by more than 10-fold. We have subsequently shown that Rapamycin downregulates interferon-induced IFITM3 in the microglial cell line CHME and in monocyte-derived macrophages, which results in increased permissiveness to HIV-1 infection. Here, we describe our efforts to characterize the signaling pathway(s) that result in the negative regulation of IFITM3 during mTOR inhibition. We found that mTOR complex 2 (mTORC2) is the likely complex controlling IFITM3 levels in cells because cell starvation had minimal effect on IFITM3 while it was downregulated following knockdown of Rictor, an mTORC2 component. This result suggests that mTORC2 activity promotes the stability and function of IFITM3, linking a central metabolic regulatory network to the cell-intrinsic antiviral response. Interestingly, we found the knockdown of IFITM3 in HeLa cells resulted in decreased levels of p-Akt Ser473, suggesting that IFITM3 positively regulates Akt activation. This finding suggests a functional interdependence of IFITM3, Akt, mTORC2. Our data may suggest that (1) IFITM3 promotes Akt function by positively regulating mTORC2 signaling, and (2) a negative feedback mechanism exists which results in IFITM3 degradation following mTORC2 inactivation. We are now in the process of identifying other cellular factors that populate these interconnected pathways. Future work will include how IFITM3 influences Akt signaling during oncogenesis, since the upregulation of both are associated with cancers. Lastly, mechanistic studies of rapamycin and other mTOR inhibitors are important because these clinically important drugs, while beneficial in the setting of gene therapy ex vivo, may be deleterious to antiviral immunity in vivo.



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Abstract

131 The Role of DNA Repair Complex DNA-PK in HIV-1 Transcription

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The human DNA-dependent protein kinase (DNA-PK), composed of the heterodimeric protein Ku and catalytic subunit DNA-PKcs, is a sensor of double-strand DNA breaks in the non-homologous end-joining DNA repair pathway. The key role of DNA-PK in the post-integrational repair of HIV-1 has been shown. It has also been suggested that DNA-PK can participate in the regulation of HIV transcription, although the mechanism is unclear. To clarify the impact of each DNA-PK subunit on the transcription of HIV-1, HEK 293T cells in which each of the DNA-PK components was depleted were transfected with reporter vectors containing firefly luciferase under the control of HIV LTR promoter. We detected a positive influence of both Ku subunits, but not DNA-PKcs, on the transcription from the HIV promoter. Ku is known to interact with HIV-1 TAR RNA, playing an essential role in viral transcription; nonetheless, the deletion of the TAR-coding region from LTR did not alter the Ku effect. Human small noncoding 7SK RNA participates in HIV-1 transcription. Direct binding of recombinant Ku and in vitro transcribed 7SK RNA was demonstrated using EMSA. In addition, we identified interactions of endogenous Ku with proteins HEXIM1 and Cdk9 from the 7SK RNP complex. These results suggest Ku exerts its effects on HIV-1 transcription via interaction with the 7SK RNP complex. However, we cannot rule out an indirect effect of Ku on transcription via regulation of the levels of some transcription factors participating in HIV-1 transcription. We performed a transcriptome analysis of wild type HEK 293T cells and those with depleted DNA-PK subunits. The genes regulated by each subunit were defined, and the genes that were mainly dependent on Ku subunits were selected. Among them, we identified transcription factors enhancing HIV-1 transcription, whose levels were downregulated in Ku-depleted cells. The study was supported by RFBR grants №18-34-00-393, №18-04-00542.



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Abstract

132 The Role of Site-Specific Ubiquitination of the Influenza A Virus Polymerase

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Influenza A viruses (IAV) are responsible for periodically occurring epidemics of respiratory disease and can also give rise to devastating pandemics. The IAV genome consists of eight negative-sense single-stranded viral RNA (vRNA) segments. In the viral particle, each segment is associated to a copy of the heterotrimeric viral polymerase, which consists of the subunits PB2, PB1, and PA, as well as many copies of the viral nucleoprotein (NP). In the cell nucleus, the viral polymerase is responsible for the synthesis of viral mRNAs (transcription) and amplification of the viral genome (replication). Both processes underlie complex spatiotemporal and structural regulations and, as recently reported, also imply phases of polymerase dimerization. The activity of the polymerase was shown to be sensitive to post-translational modifications (PTMs) by ubiquitin linkage. However, the sites of ubiquitin-linkage and the direct functional consequences have not been unraveled until today.

To identify ubiquitinated lysines in the viral polymerase during viral infection, we made use of the PTMScan® technology and MS analysis to enrich and identify modified peptides in H1N1 (A/WSN/33) infected cells 5 h p. i. In total, we identified 55 distinct modified lysines in the proteins of the viral polymerase distributed on all three subunits. To study the relevance for viral replication and polymerase function, each lysine was mutated to alanine and/or arginine, and the activity of the viral polymerase was assessed in the polymerase reconstitutions assay.

Our results suggest that ubiquitination at several lysines in the viral polymerase serves important regulatory functions, apart from conventional proteasomal protein degradation, during viral replication. Further studies to unveil the distinct mechanisms of ubiquitination in the viral polymerase are ongoing.



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133 The Structure of Zika Virus NS5 in a Complex with a 5'UTR RNA Promoter

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Zika virus (ZIKV) infection constitutes an important public health concern worldwide. As in other members of the Flaviviridae family, the ~11 kb genome of ZIKV is a positive sense single stranded RNA molecule composed of one open reading frame (ORF) flanked by highly structured 5' and 3' untranslated regions (UTR). The ORF encodes a polyprotein which is processed to generate the mature viral proteins. The non-structural protein 5 (NS5) is responsible for viral genome replication. It comprises an N-terminal methyltransferase (MTase) domain and an RNA-dependent RNA polymerase (RdRP) domain at the C-terminus.

The 5' UTR possesses a crucial structure for NS5 binding and initiation of viral genome replication called stem loop A (SLA). In addition, the conserved structural 3' stem loop (3'SL) located at the terminus of the 3'UTR is required together with 5'UTR to initiate negative-strand RNA synthesis. For this purpose, the viral genome adopt a circularized panhandle-shaped structure formed through long-range interactions between UTRs. This conformation allows positioning both UTRs in close proximity and transferring SLA-bound NS5 from the 5'UTR to the 3'SL to generate the genomic negative strand. The relevance of the interaction between the genomic ends and NS5 make it an attractive target for structural studies and drug design. However, to date, there is no 3D model available for these interactions.

In this work, we present the cryo-EM analysis of the recombinant ZIKV NS5 in a complex with an *in vitro* transcribed SLA RNA. The obtained 3D model was validated by point mutations in NS5, biophysical, and biochemical studies. This ZIKV NS5–SLA complex structure not only constitutes a challenging target overcome by single particle analysis cryo-EM but is also the first structure of a flavivirus–RNA promoter complex shedding some light onto the mechanism of ZIKV genome replication.



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134 Transferrin Receptor 1 Is a Supplementary Receptor That Assists Transmissible Gastroenteritis Virus Entry into Porcine Intestinal Epithelium

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Background: Transmissible gastroenteritis virus (TGEV), the etiologic agent of transmissible gastroenteritis, infects swine of all ages, causing vomiting and diarrhea. In newborn piglets, the mortality rate is near 100%. Intestinal epithelial cells are the primary target cells of TGEV. Transferrin receptor 1 (TfR1), which is highly expressed in piglets with anemia, may play a role in TGEV infection. However, the underlying mechanisms of TGEV invasion remain largely unknown.

Results: Our study investigated the possibility that TfR1 can serve as a receptor for TGEV infection and enable the invasion and replication of TGEV. We observed that TGEV infection promoted TfR1 internalization, clustering, and co-localization with TfR1 early in infection, while TfR1 expression was significantly downregulated as TGEV infection proceeded. TGEV infection and replication were inhibited by occluding TfR1 with antibodies or by decreasing TfR1 expression. TGEV infection increased in TGEV-susceptible ST or IPEC-J2 cell lines and TGEV-resistant Caco-2 cells when porcine TfR1 was overexpressed. Finally, we found that the TGEV S1 protein interacts with the extracellular region of TfR1, and that pre-incubating TGEV with a protein fragment containing the extracellular region of TfR1 blocked viral infection.

Conclusions: Our results support the hypothesis that TfR1 is an additional receptor for TGEV and assists TGEV invasion and replication.



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Abstract

135 Ts2631 Endolysin from the Extremophilic *Thermus scotoductus* Bacteriophage vB_Tsc2631 as an Antimicrobial Agent against Gram-Negative Multidrug-Resistant Bacteria

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Bacteria that thrive in extreme conditions and the bacteriophages that infect them are sources of valuable enzymes resistant to denaturation at high temperatures. Many of these heat-stable proteins are useful for biotechnological applications; nevertheless, none have been utilized as antibacterial agents. Here, we demonstrate the bactericidal potential of Ts2631 endolysin from the extremophilic bacteriophage vB_Tsc2631, which infects *Thermus scotoductus*, against the alarming multidrug-resistant clinical strains of *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and pathogens from the Enterobacteriaceae family. A 2–3.7 log reduction in the bacterial load was observed in antibacterial tests against *A. baumannii* and *P. aeruginosa* after 1.5 h. The Ts2631 activity was further enhanced by ethylenediaminetetraacetic acid (EDTA), a metal ion chelator (4.2 log reduction in carbapenem-resistant *A. baumannii*) and, to a lesser extent, by malic acid and citric acid (2.9 and 3.3 log reductions, respectively). The EDTA/Ts2631 combination reduced all pathogens of the Enterobacteriaceae family, particularly multidrug-resistant *Citrobacter braakii*, to levels below the detection limit (>6 log); these results indicate that Ts2631 endolysin could be useful to combat Gram-negative pathogens. The investigation of *A. baumannii* cells treated with Ts2631 endolysin variants under transmission electron and fluorescence microscopy demonstrates that the intrinsic antibacterial activity of Ts2631 endolysin is dependent on the presence of its N-terminal tail.



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Abstract

136 Ubiquity, Diversity, and Genomic Complexity of Cyanophages in Freshwater Environments

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Cyanophages are viruses that infect cyanobacteria (also known as blue-green algae) and are ubiquitous in marine and freshwater environments. In recent years, freshwater cyanophages have attracted more attention because they can affect global freshwater ecosystems. Spatial distribution and morphological diversity of cyanophage populations were examined in Lake Donghu with three trophic regions: hypertrophic, eutrophic, and mesotrophic regions. The surprisingly high viral abundance (ranging from 10^8 to 10^9 phage mL^{-1}) and morphological diversity were detected. Most of them have tails and belong to the families *Siphoviridae*, *Myoviridae*, and *Podoviridae*. Various morphotypes were observed, such as prolate-headed virus-like particles and lemon-shaped virus-like particles. In addition, some cyanophages have been studied by virological experiments and whole-genome analyses, combined with morphological observation. For example, three cyanophages were isolated and their whole genomes were sequenced. Contractile tail myonophage MaMV-DC infects bloom forming cyanobacterium *Microcystis aeruginosa*. Tailless cyanophage *Planktothrix agardhii* virus isolated from Lake Donghu (PaV-LD) infects filamentous cyanobacterium. Short tail podovirus A-4L can infect the model cyanobacterium *Anabaena sp.* strain PCC 7120.

The MaMV-DC genome contains 169,223 bp encoding 170 putative ORFs. The PaV-LD genome possesses 95,299 bp encoding 142 putative ORFs. The genome of short tail podovirus A-4L has 41,750 bp encoding 38 putative ORFs. There are significant differences in their genomic size and encoded tail proteins, but all three cyanophages contain genes that are not commonly found in phages. By studying the vast biodiversity of viruses in freshwater environments, these novel findings of cyanophages broaden our insights, and allow us to gain more useful knowledge about the global impact of these viruses in freshwater ecosystems.



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137 Ultrastructural Characterization of the Frontal Lobe in the Case of Human Herpes Virus-6 Infection

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Generally, a vast majority of the world's population is exposed to beta-herpesviruses at the time of early childhood. After the primary infection, human herpesvirus-6 (HHV-6) can establish a lifelong persistence. The role of HHV-6 in the development of neurodegenerative disorders is not completely clarified.

Postmortem samples of brain tissue obtained from 24 elderly subjects with unspecified encephalopathy were used in the study. Nested (nPCR) and real-time polymerase chain reaction (RT PCR) were used for the qualitative and quantitative detection of viral genomic sequences in isolated DNA from frontal lobe samples. For ultrastructural examination, transmission electron microscopy (TEM), nPCR, and immunohistochemically confirmed HHV-6-positive tissue samples were used. Immuno-gold (IG) labeling using anti-HHV-6 (20) mouse monoclonal antibody, raised against viral lysate (Santa Cruz Biotechnology, dilution 1:30), was performed.

The HHV-6 DNA was detected in 38% (9/24) of the frontal lobe tissue samples. HHV-6 load in the nPCR-positive samples ranged from 10 to 3878.5 (copies/10⁶ cells). TEM examination of the frontal cortex revealed lipofuscin containing neurons, glial cells, unmyelinated and small myelinated axons, and symmetric synapses. Subcortical brain regions revealed glial cells interspersed by myelinated axons. Expression of viral proteins was found in the nuclei of neurons demonstrating disarranged chromatin. HHV-6-positivity was detected between the adjacent cisternae of the rough endoplasmic reticulum of neurons displaying IG-labeling. Furthermore, products of IG-labeling were found in nuclei and cytoplasm of oligodendrocytes. The cytoplasm of astrocytes was IG-labeled as well.

IG-labeling was used to determine the presence and intracellular localization of HHV-6 proteins in the human brain. HHV-6 possibly contributes to the demyelination process by entry and affection of oligodendrocytes. Finally, neural susceptibility to HHV-6 may be linked to invalid cellular immune response followed by the development of persistent viral infection.



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138 Usutu Virus NS5: Characterization of Polymerase Activity, Protein–Protein Interaction and Cellular Localization %MCEPASTEBIN%

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Usutu virus (USUV) is a mosquito-borne arbovirus that has rapidly propagated in birds across several European countries over the last two decades, leading to substantial avian mortalities. USUV infection in humans has been associated to a growing number of cases of neurological disease in the last years, underlining the need for increased awareness and suitable treatments. Our group is working on the characterization of the NS5 protein of USUV. This protein is responsible for the replication activity of the viral genome and can be a suitable viral target to treat the infection. NS5 contains a RNA-dependent RNA polymerase (RdRpD) and a methyltransferase domains.

Recombinant NS5 and RdRpD proteins expressed in bacteria were purified and biochemically characterized to determine the best conditions for their polymerase activities. Both proteins showed de novo and primer extension activities. Optimal RNA–polymerase reaction conditions included low NaCl (less than 20 mM), 2.5 mM MgCl₂ and 5 mM MnCl₂, 30 °C, and pH 7.25. Polymerase activity was cooperative for the polymerase domain (Hill-coefficient = 5.8) but not for the complete NS5 (Hill-coefficient = 1.2). To study their subcellular location, suitable sequences were cloned into a pcDNA3 vector and expressed in Huh7.5 and HEK293T cells. Both proteins were preferentially located in the cytoplasmic region, although a significant amount was found in the nucleus. Preliminary results showed that the concentration of sofosbuvir (SOFTP) necessary to achieve its incorporation by NS5 in 50% of the nascent RNA is higher than 100 μM, as already observed for DENV.

In this work, we describe the main features of the full-length USUV NS5, including the polymerase activity as well as the effect of protein-protein interactions and subcellular localization. Our results will be very useful for the study of this viral enzyme as a suitable target against the infection and the effect of antiviral drugs.



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Abstract

139 Vaccination with Single Plasmid DNA Encoding IL-12 and Antigens of Severe Fever with Thrombocytopenia Syndrome Virus Elicits Complete Protection in *IFNAR* Knockout Mice

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Severe fever with thrombocytopenia syndrome (SFTS) is an emerging tick-borne disease caused by SFTS virus (SFTSV) infection. Despite the gradual increase in SFTS cases and high mortality in the endemic region, no specific viral therapy nor vaccine is available. Here, we developed a single recombinant plasmid DNA encoding SFTSV genes *Gn* and *Gc* together with NP-NS fusion antigen as a vaccine candidate. The viral antigens were fused with Fms-like tyrosine kinase-3 ligand (Flt3L) and the *IL-12* gene was incorporated into the plasmid to enhance cell-mediated immunity. Vaccination with the DNA can provide complete protection of *IFNAR* KO mice upon lethal SFTSV challenge, whereas immunization with a plasmid lacking *IL-12* gene resulted in partial protection. Since we failed to detect antibodies against the surface glycoproteins Gn and Gc in the immunized mice, antigen-specific cellular immunity, as confirmed by enhanced antigen-specific T cell responses, might play a major role in protection. Finally, we evaluated the degree of protective immunity after protein immunization that was provided by individual glycoproteins Gn or Gc. Although both protein antigens induced significant levels of neutralizing activity against SFTSV, Gn vaccination resulted in relatively higher neutralizing activity and better protection than Gc vaccination. However, both antigens failed to provide complete protection. Given that the DNA vaccines have failed to induce sufficient immunogenicity in human trials when compared to protein vaccines, optimal combinations of DNA and protein vaccine, proper selection of target antigens, and incorporation of efficient adjuvant need to be further investigated for SFTS vaccine development.



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Abstract

140 VANIR—NextFlow Pipeline for Viral Variant Calling and de Novo Assembly of Nanopore and Illumina Reads for High-Quality dsDNA Viral Genomes

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Human cytomegalovirus (HCMV), like other herpes and dsDNA viruses, possesses unique properties derived from their genome architecture. The HCMV genome is composed of two unique domains: long (L) and short (S). Each domain contains a central unique region (U; thus, U_L and U_S, respectively) and two repeated regions (thus, TR_L/IR_L and TR_S/IR_S). Recombination between repetitive regions is possible, yielding four possible genomic isomers, found in equimolar proportion in any viral infective population. Frequent recombination and an altered selective landscape can give rise to the persistence, if not fixation, of diverse variants in culturized HCMV isolates. This phenomenon has already been discovered in AD169 and Towne strains, characterizing a 10 kbp deletion (Δ UL/b') in commonly used viral stocks. Other dsDNA viruses are known for their structural rearrangements and frequent recombination. VANIR (viral variant calling and de novo assembly using nanopore and illumina reads) is a novel analysis pipeline that benefits from both short-read (Illumina) and long-read sequencing technologies (Oxford Nanopore Technologies Ltd., Oxford, UK) to assemble high-quality dsDNA viral genomes and detection of variants. Illumina and nanopore sequencing provide complementary information to the assembly and variant discovery. Assembly contiguity, structural variant, and repeat calling are greatly improved by nanopore read-length and base-calling and base confidence by Illumina reduced error rate and increased yield. This specialized bioinformatic analysis pipeline is encoded in the NextFlow pipeline manager and containerized in a Singularity image. This set-up allows for improved traceability, reproducibility, transportability, and speed. Through VANIR, novel point mutations and structural genome rearrangements are called from sequencing data, benefiting diversity research with attenuated lab-strains and wild-type viruses.



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141 Viruses during Evolution of Life on Early Earth as a Model for Exoplanets?

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The recent discovery of exoplanets with putative habitable zones which may be as frequent as 10^{25} stimulates the interest in the origin of life on the exoplanets but also on the Early Earth (EE). Meteorites and missions to Mars or the Moon teach us about their composition and make us think about the origin of life in general. Prebiotic molecules such as amino acids, nucleosides, and fullerenes exist in space and on EE. Molecules which replicate, mutate, and evolve are signatures of life.

The simplest such biomolecules on Earth may be non-protein-coding (nc)RNA catalytic RNA, ribozymes, and viroids, which fulfill many functions of life, including cleaving, joining, replication, evolution, and are a prerequisite for peptide synthesis and life. Ribozymes/viroids may have evolved to higher complexity, including developing coding RNAs. All of today's RNA viruses are flanked by ncRNA ends around their coding sequences. Giant viruses can be "almost" bacteria with many signatures of life, suggesting a continuous transition from dead to living matter. Even eukaryotic genomes consist of up to about 50% of virus-like sequences. The bacterial/phage world and the virus world show many similarities. Both viruses and phages protect their host by superinfection exclusions—which is equivalent to an antiviral defense of their host, or immunity. Viruses protect against viruses.

During evolution, increase of complexity is considered as a main driving force. However, gene reduction or gene loss also contributes to evolution as a frequently underestimated force. Under laboratory conditions, protein-coding RNA can become non-coding, suggesting "back-evolution" under paradise-like conditions described as "Spiegelman's Monster", while scarcity leads to increase of complexity. Today's viruses may have given up autonomous life as intracellular parasites.

Viroids are discussed here as potentially living structures without a genetic code as models for potentially early forms of life. A model derived from M. Eigen's Hypercycles will be proposed.

Ref: Broecker and Moelling, *Geosciences* (2019), *Annals NY Acad Sci.* (2019), *Frontiers Microbiol* (2019). Moelling K: Viruses more friends than foes, WSP Singapore 2017



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Abstract

142 Visualizing HCV Core Protein via Fluorescent Unnatural Amino Acid Incorporation

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INTRODUCTION: Unnatural amino acids (UAAs) share the same basic structure as proteinogenic amino acids. However, UAAs permit additional functions and applications to proteins due to their different side chains. Recent UAA applications include using fluorescent UAAs to label proteins. The UAA system provides an alternative method to traditional protein labeling mechanisms (antibodies, GFP and tags, such as HA and HIS), which can affect protein functionality and topology. The purpose of this study was to visualize the Hepatitis C Virus (HCV) Core protein using the fluorescent UAA Anap (3-[(6-acetyl-2-naphthalenyl)amino]-L-alanine).

METHODS: Huh-7.5 cells were co-transfected with HCV Core plasmids containing amber stop codons at various positions throughout the coding sequence and a second plasmid encoding the orthogonal tRNA/synthetase pair that facilitates Anap incorporation. Three days post-transfection, cells were stained for Core protein and lipid droplets (LDs) and visualized using immunofluorescence or confocal microscopy.

RESULTS: We have optimized transfection protocols for efficient expression of the tRNA/synthetase pair required for Anap incorporation and are able to visualize our Core mutant proteins containing Anap. We have successfully substituted Anap into 11 different positions within Core, including substitutions for tryptophan, tyrosine, and phenylalanine residues. In addition, we have shown that our core mutants associate with cellular LDs, suggesting that the incorporation of the UAA did not disrupt Core protein expression, stability or cellular localization.

CONCLUSIONS: We have demonstrated the establishment of a UAA incorporation system in an HCV protein without any obvious impact on Core protein function. The ability to label viral proteins using fluorescent UAAs eliminates the requirement of antibodies or tags for protein visualization. In conclusion, the UAA system is a useful method to study HCV proteins and can potentially be used to label viruses for live cell and animal studies.



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Abstract

143 Yellow Fever Virus Vaccine Reduces T Cell Receptor Signaling and the Levels of Phosphatase PTPRE In Vivo

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Background: A Src kinase activating phosphatase (PTPRE) is targeted by a yellow fever virus (YFV) genome-derived, short noncoding RNA (vsRNA) in vitro. The vsRNA reduces PTPRE translation which leads to reduced TCR signaling. vsRNA point mutations restore PTPRE expression and T cell function. We examined TCR signaling and PTPRE levels in individuals before and after YFV vaccination (YFVax).

Methods: Fourteen individuals receiving YFVax ($10^{4.7-5.6}$) IM for travel prophylaxis provided written informed consent for these studies. Blood was obtained before and four times after vaccination (days 3 to 28). Serum and PBMCs were purified and YFV quantified by RNA and infectivity. PBMCs were assessed for activation following anti-CD3 stimulation by measuring phospho-tyrosine-394-Lck and IL-2 release. PBMC PTPRE levels were determined by immunoblot analyses (normalized to actin). YFV neutralizing antibody was determined by PRNT.

Results: YFVax was administered alone (6/14 subjects) or in combination with other vaccines (8/14). All subjects demonstrated reduced resting PBMC PTPRE levels and post-TCR stimulation had reduced IL-2 release between days 4 and 21 compared to pre- and day 28 samples. Phospho-Lck was reduced in all but 2 subjects on the same days, and both of these subjects also received influenza vaccine. Low-level viremia was detected in 10/14 subjects, with infectious titers 100/mL. Viremia was not detected in 4/14 subjects. All recipients developed neutralizing antibodies by day 21.

Conclusion: YFV vaccination regulates PBMC PTPRE levels 4–21 days after infection, despite low to absent infectious YFV detected in serum, suggesting that enough YFV vsRNA is produced and released from cells to have a functional (and measurable) effect on T cell function. Studies are underway to determine if this is mediated by exosomes or defective particles containing the vsRNA that targets PTPRE. Further, the association between PTPRE and TCR signaling confirms a role for PTPRE in TCR function.



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144 Zika Virus Diversity Is Maintained during Transmission from Placenta to Fetal Periphery But Restricted in Fetal Brains

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Since emerging in French Polynesia and Brazil in the 2010s, Zika virus (ZIKV) has been associated with fetal congenital disease. Previous studies have compared ancestral and epidemic ZIKV strains to identify strain differences that may contribute to vertical transmission and fetal disease. However, within-host ZIKV variation during vertical transmission has not been well studied. Here, we used the established anti-interferon treated Rag1^{-/-} mouse model of ZIKV vertical transmission to compare ZIKV populations in matched placentas, fetal bodies, and fetal brains via RNASeq. ZIKV transmission from the placenta to the fetal periphery involved a loose population bottleneck. There was a restriction in the amount of virus entering the fetus from the placenta but not in ZIKV diversity, as the ZIKV population structures were similar in placentas and fetal bodies. In contrast, ZIKV transmission from the fetal periphery to the brain involved a sharp reduction in ZIKV diversity. All fetal brain ZIKV populations were comprised of either one of two variants as largely homogenous populations. In most cases, the predominant variant present in the fetal brain was also the majority variant present in the placenta. However, in two of ten fetal brains the predominant ZIKV variants were undetectable in the matched placental ZIKV population, suggesting possible evidence of selection for certain variants during ZIKV transmission to fetal brains. Thus, certain variants may influence ZIKV's ability to enter the fetal brain and cause disease.



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145 Zika Virus Epidemiology in Selected West African Countries between 2007 and 2012

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Before its recent spread, serological investigations conducted between the 1960s and the 1990s showed the wide presence of Zika virus in Africa. According to the World Health Organization, the entire Africa continent is at risk of Zika outbreak due to the presence of the virus, competent vectors, and the low capacity for surveillance and containment of an epidemic. However, limited data are available on the recent prevalence in the African population.

The aim of this study was to evaluate the immunity against Zika virus in samples of a selected cohort from West Africa, in order to investigate the circulation of the virus in the region during the first years of its emergence in the Pacific.

Human serum samples were collected in 2007 and between 2011 and 2012 from a cohort of subjects from Mali, Senegal, and The Gambia. Samples were tested using an ELISA detection kit and positives were further confirmed by microneutralization test.

Results indicate that Zika virus is present and actively circulating in Senegal and The Gambia, with prevalence values of 13.7% and 6.9% in 2012, respectively. Although no significant differences in prevalence were found for the considered time period, seroconversion of some subjects showed the active circulation of Zika virus in the West African area. Analysis by age showed an increase in immunity in relation with increasing age, demonstrating that the population is consistently exposed to the virus throughout life and with a high possibility to be infected during reproductive age.

In conclusion, the obtained results allow for better knowledge of the circulation of Zika virus within three different ecological and demographic contexts, and represent an update to the limited data currently available.



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Abstract

146 Zika Virus sfRNA Plays an Essential Role in the Infection of Insects and Mammals

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Similar to other flaviviruses, Zika virus (ZIKV) produces abundant noncoding RNA (sfRNA) derived from the 3' untranslated region. The molecular mechanisms that determine the functions of sfRNA are currently not completely understood. Here, we created ZIKV mutants deficient in sfRNA production and employed them to investigate the role of this RNA in virus interactions with mammalian and insect hosts.

We found that in mosquitoes, sfRNA facilitates virus replication and is required for ZIKV dissemination into saliva and virus transmission. Production of sfRNA was found to have no effect on RNAi pathway, but instead downregulated the expression of genes involved in regulation of apoptosis. The TUNEL staining of histological sections from infected mosquitoes confirmed that sfRNA prevents apoptotic death of infected cells thus identifying inhibition of apoptosis as a novel mechanism of sfRNA action in mosquitoes.

We also found that sfRNA facilitates ZIKV replication in mammalian cells, mice, and human brain organoids. Moreover, ZIKV mutants deficient in sfRNA production were unable to form plaques, cause death of human brain organoids, and establish infection in mouse foetal brain. We then found that proviral activity of sfRNA in mammalian cells relies on its ability to suppress type I interferon signalling. We showed that this is achieved via inhibition of phosphorylation and nuclear translocation of STAT1. In addition, we found that production of sfRNA in ZIKV infection of human brain organoids is associated with suppression of multiple genes involved in brain development, indicating that sfRNA can be involved in disruption of brain development associated with ZIKV infection.



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147 Expression of Two Foreign Genes from the Optimal Insertion Sites of Newcastle Disease Virus Vector for Use as a Multivalent Vaccine and Gene Therapy Vector

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Several strains of Newcastle disease virus (NDV) have been developed as vectors to express a foreign gene (FG) for vaccine and gene therapy purposes. A majority of these NDV vectors express only a single FG or two FGs from suboptimal insertion sites in NDV genome. In the present study, we generated NDV LaSota vaccine strain-based recombinant viruses to express two FGs, green fluorescent protein (GFP) and red fluorescent protein (RFP) genes, from the identified optimal insertion sites through a combination of the independent transcription unit (ITU) and the internal ribosomal entry site (IRES) expression approaches. Biological assessments showed that these recombinant viruses maintained the similar growth kinetics and viral yields *in vitro* and *in vivo* with slightly attenuated pathogenicity when compared with their parental viruses. The expression levels of GFP and RFP by the viruses vectoring two FGs were comparable to those by the viruses vectoring only a single FG through the same expression approach. The expression of FGs through the ITU approach was approximately 4-fold more efficient than that through the IRES approach. These results demonstrated that the NDV LaSota vector could efficiently express two FGs from the identified optimal insertion sites through the combination of the ITU and IRES expression methods. The ITU tactic could be used for expression of a higher amount of FG products, whereas the IRES strategy for expression of a lower amount of FG products to meet the requirement for vaccine or gene therapy purposes.



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