7th Swiss Virology Meeting

ABSTRACT BOOK

January 30 – 31, 2018

Fribourg

www.swissvirology.ch

Organizing and Scientific Committee (in alphabetic order)

Angela Ciuffi (Lausanne University Hospital and University of Lausanne)
Cornel Fraefel (University of Zurich)
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Program 7th Swiss Virology Meeting January 30/31 2018, Fribourg

Tuesday, January 30, 2018

10:00 – 10:25	Arrival and Welcome Coffee
10:25 – 10:30	Welcome Address *Jérôme Gouttenoire & Angela Ciuffi*
10:30 – 12:00	**PLENARY TALK SWISS VIROLOGIST ABROAD (*CUSO*)** *Introduction: Caroline Tapparel*
10:30 –11:15	**Peter Stäheli** – *University of Freiburg, Germany* Interferon-λ and mucosal antiviral immunity
11:15 –12:00	**Pierre Maillard** - *University College of London, United Kingdom* Antiviral RNAi in mammals: a game of hide-and-seek
12:00 – 13:00	Sandwich Lunch Round-table discussion of Ph.D. students & postdocs with Pierre Maillard and Peter Stäheli
13:00 – 14:45	**SESSION I: VIRUS LIFE CYLE (*CUSO*)** *Chairs:* Jérôme Gouttenoire & Cornel Fraefel and Ph.D. students
13:00 –13:30	**Urs Greber** - *University of Zurich* Rewiring cells in virus entry and egress
13:30 – 14:00	**Silke Stertz** – *University of Zurich* IFITM3 incorporation into influenza A virus particles increases sensitivity to antibody-mediated virus neutralization
14:00 – 14:15	**Philip V'Kovski** – *University of Bern* Subcellular molecular environment of coronavirus replicase complexes revealed by proximity labelling
14:15 – 14:30	**Chiara Fedeli** – *University of Lausanne* The role of receptor tyrosine kinases in cell entry of Lassa virus
14:30 – 14:45	**Jérôme Gouttenoire** – *University of Lausanne* Palmitoylation determines the subcellular localization of hepatitis E virus ORF3 protein
14:45 – 15:15	Coffee Break
15:15 – 17:00	**SESSION II: VIRUS EVOLUTION AND NGS ANALYSIS** *Chairs:* Stefan Kunz & Volker Thiel
15:15 – 15:45	**Jacques Fellay** – *EPFL and Lausanne University Hospital* Detecting host pressure on viral sequence through genome-to-genome analysis
15:45 – 16:05	**Claudia Bachofen** - *University of Zurich* Virome analysis: Possibilities and challenges
16:05 – 16:25	**Ronald Dijkman** - *University of Bern* Characterization of virus - host interaction dynamics within the respiratory epithelium
16:25 – 16:40	**Masako Kaufmann** – *University of Zurich* Co-evolution of broadly neutralizing antibodies and HIV-1
16:40 – 16:55	**Sara Cristinelli** – *University of Lausanne* Single-cell RNA-seq reveals transcriptional heterogeneity in latent and reactivated HIV-infected cells
17:00 – 19:00	Coffee Break and **POSTER SESSION**
19:00 – 21:00	Dinner

Wednesday, January 31, 2018

7:30 - 8:45	Breakfast (at hotel)
9:00 – 11:00	**SESSION III: VIRUS AND HOST DEFENSE** *Chairs:* Matthias Schweizer & Caroline Tapparel
9:00 – 9:30	**Michel Strubin** - *University of Geneva* Smc5/6: a new restriction factor that blocks episomal gene transcription
9:30 – 10:00	**Jovan Pavlovic** - *University of Zurich* New viral targets of human Mx proteins
10:00 – 10:15	**Carmela Lussi** - *University of Bern* Site-directed mutagenesis of pestiviral RNase and its effect on innate immune evasion
10:15 -10:30	**Eva Spieler** – *University of Zurich* Identification of novel ISGs involved in the antiviral response against Influenza A virus
10:30 – 10:45	**Michel Crameri** - *University of Zurich* MxB is an interferon-induced restriction factor of human herpesviruses
10:45 – 11:00	**Nora Schmidt** – *University of Zurich* TRIM28 desumoylation during influenza virus infection
11:00 – 12:30	Coffee Break and **POSTER SESSION**
12:30 – 14:00	Lunch at restaurant
14:00 – 16:00	**SESSION IV: MEDICAL AND ENVIRONMENTAL VIROLOGY** *Chairs:* Michael Huber & Angela Ciuffi
14:00 – 14:30	**Karin Metzner** - *University Hospital of Zurich* Factors affecting the dynamics of the HIV-1 reservoir
14:30 – 15:00	**Philippe Plattet** - *University of Bern* Insights into morbillivirus cell entry
15:00 – 15:15	**Verena Kufner** - *University of Zurich* Virus transmission during kidney transplantation assessed by virome analysis of living donor and recipient
15:15 – 15:30	**Caio Foulkes** - *University of Zurich* Identification of broad and potent neutralizing DARPins that target conserved domains of the HIV-1 envelope
15:30 – 15:45	**Eirini Tseligka** - *University of Geneva* Heparan sulfate binding is a critical determinant of enterovirus 71 dissemination and pathogenesis in humans
15:45 – 16:00	**Soner Yildiz** - *University of Geneva* Influenza A virus infection impacts intestinal microbiota dynamics and causes quantitative enteric dysbiosis
16:00 – 16:15	Farewell Address

ABSTRACTS

(by alphabetical order of the presenting author)

HDAC6, A NOVEL HOST TARGET FOR BROAD SPECTRUM ANTIVIRAL THERAPY

Abstract: 160103 P

Stéphanie Anchisi[1], Alina Rudnicka[2], Samuel Cordey[3], Yohei Yamauchi[2,4], Laurent Kaiser[3], Mirco Schmolke[1]

1 University of Geneva, Department of Microbiology and Molecular Medicine, Geneva, Switzerland
2 University of Zurich, Institute of Molecular Life Sciences, Zurich, Switzerland
3 Hôpitaux Universitaires de Genève, Laboratoire de Virologie, Geneva, Switzerland
4 University of Bristol, School of Cellular and Molecular Medicine, Bristol, United Kingdom

Background: Influenza viruses pose a major threat to human and animal health. The WHO estimates up to 5 Mio severe infections, including up to 500'000 fatal infections, occurring annually on a global scale. Influenza viruses have a segmented RNA genome of negative polarity and an error prone RNA dependent RNA polymerase. Therefore, influenza viruses display a high genetic variability, which combined with selective pressure, leads viruses to evolve rapidly to avoid immune recognition. In line with this constant evolution, the production of „universal" vaccine or therapeutics directed against influenza virus antigens is almost impossible.
Aims: In this study, we are proposing host-directed strategies to counteract virus entry, the first step of viral replication cycle. Due to limited genetic coding capacity, influenza viruses heavily depend on the interaction with host proteins at basically every step of its replication cycle to generate progeny viruses. We have previously identified HDAC6, a histone deacetylase family member, as a proviral host factor supporting influenza virus uncoating and release of genetic material into the host cell cytoplasm. Of note, this study was performed using X31, a lab adapted strain of H3N2 influenza virus.
To better understand the relevance of these findings in clinical settings, we have tested the capacity of different clinical isolates of Influenza viruses to infect human lung epithelial cell in which HDAC6 was knocked-out/knocked-in. This approach allowed us to identify among clinical isolates a strain-dependent sensibility to HDAC6. Next, we tried to define the viral determinants of this sensibility to HDAC6 and preliminary data led us to focus on the matrix protein M.

VIROME ANALYSIS - POSSIBILITIES AND CHALLENGES

Abstract: 160109 O

Claudia Bachofen[1], Jakub Kubacki[1], Julia Lechmann[1], Mathias Ackermann[1], Cornel Fraefel[1]

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The analysis of the entirety of viruses present in a sample, the so-called virome, is becoming a valuable tool not only for environmental studies but also in a clinical setting. However, there are still many challenges to master such as the minute proportion of viral nucleic acid compared to host RNA/DNA as well as the diversity of sample material and - in the veterinary field - host species. After a pilot project analysing the virome of water buffaloes in Switzerland, we have therefore worked on the establishment of a next generation sequencing (NGS) protocol tailored to the use in a veterinary diagnostic setting. Based on relative enrichment of virus particles and sequence independent single primer amplification, the protocol has enabled us to successfully detect spiked-in and naturally occurring viruses of a wide range of taxonomic families and from various sample materials such as faeces, blood, tissue, swabs and even pork sausages.
While we aim at offering virome analysis as a diagnostic tool, it is also the basis for several ongoing research projects such as the study of commensal viruses in pigs and the dynamics of the bovine respiratory and enteric microbiome.

MOUSE ADENOVIRUS REPORTER VIRUSES AS TOOLS TO STUDY THEIR BIOLOGY AND IDENTIFY THEIR COGNATE RECEPTORS

Abstract: 160115 P

Manuela Bieri[1], Rodinde Hendrickx[1], Maarit Suomalainen[1], Urs F. Greber[1], Silvio Hemmi[1]

1 Institute of Molecular Life Sciences, University of Zurich, Zurich, Switzerland

Mouse adenoviruses (MAdVs) are icosahedral, non-enveloped, double stranded DNA viruses which belong to the genus Mastadenoviruses and share common characteristics with the extensively studied human adenoviruses. Since their natural host is the mouse, they are interesting tools to study natural virus-host interactions and allow testing of therapeutic adenoviruses in an immune competent animal model. So far, three mouse adenovirus strains, MAdV-1, -2 and -3 have been isolated. Whereas the biology of MAdV-1 has been studied extensively, MAdV-2 and -3 have remained scarcely investigated. Attachment receptors for MAdV-1 were reported to include αv integrins and heparan sulfate proteoglycans. Here we aimed to characterize potential receptor candidates for the attachment of MAdV-2 and -3 to target cells. To score infection, we generated several MAdV GFP, nano luciferase and gaussia luciferase reporter viruses using recombineering techniques, followed by rescue from transfected mouse cells. Competition experiments performed with soluble fiber knob proteins suggested that MAdV-3 uses similar receptors as MAdV-1, but not MAdV-2. Virus overlay protein blot assays and receptor pull-down studies combined with mass spectrometry analyses gave rise to a list of MAdV-2 receptor candidates, which are currently evaluated. In a collaborative effort, a pioneering nano luciferase reporter MAdV-1 virus is prepared to monitor the course of infection in vivo. If successful, this approach will be used for MadV-2 and -3.

H5N1 INFLUENZA A VIRUS PB1-F2 SUPPRESSES PYROPTOTIC CELL DEATH AND SECRETION OF IL-1β

Abstract: 160106 P

Inés Boal-Carvalho[1], Béryl Mazel-Sanchez[1], Mirco Schmolke[1]

1 Department of Microbiology and Molecular Medicine, University of Geneva, Switzerland

Highly pathogenic avian influenza A viruses cause up to 60% mortality, when infecting human patients and are associated with an overwhelming inflammatory host response, a so-called „cytokine storm“. A major source of cytokines in influenza A virus (IAV) infected hosts are inflammatory monocytes, macrophages and dendritic cells. Interestingly, macrophages in the lungs of human patients were shown to be directly infected by H5N1 IAV. Here we are aiming at understanding by which mechanisms IAV controls the production of pro-inflammatory cytokines. One accessory protein of IAV that was implicated in modulating host responses is PB1-F2. This 90-amino acid protein IAV is highly prevalent in avian virus. To investigate the function of PB1-F2 in H5N1 IAV infected macrophages we took advantage of a H5N1 IAV with a targeted depletion of the PB1-F2 ORF. This mutant virus induces overall enhanced cell death in infected macrophages in comparison to a matching WT H5N1 IAV. Additionally, increased release of macromolecular complexes from the cytoplasm of H5N1 IAV lacking PB1-F2 indicated that these macrophages undergo necrotic rather than apoptotic cell death. Next we determined the secretion of the pro-inflammatory cytokine IL-1 β in these conditions. IL-1β production depends on activation of caspase 1 by the host inflammasome and its secretion was recently shown to rely on gasdermin D dependent pore formation in the host cell membrane, in a process termed pyroptosis. We demonstrate that H5N1 IAV PB1-F2 suppresses caspase 1 cleavage and release of gasdermin D in infected macrophages to control pyroptotic cell death and secretion of inflammatory Il-1β.

ASSESSING THE IMPORTANCE OF INFLUENZA NEURAMINIDASE IN VIRAL REPLICATION

Abstract: 160083 P

Loïc Borcard[1], Samira Locher[1], Gert Zimmer[1]

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The envelope of influenza A viruses contains two major integral membrane glycoproteins. Hemagglutinin (HA), the most abundant envelope glycoprotein, is responsible for virus entry by mediating virus attachment to cellular sialoglycoconjugates and by inducing fusion between the viral and endosomal membrane. Neuraminidase (NA), the second most abundant envelope protein, plays a crucial role in virus release by cleaving sialic acid residues from cellular and viral glycoproteins. The current dogma says that the receptor-binding activity of HA and the receptor-destroying activity of NA needs to be balanced. In the present study, this dogma was revisited by employing recombinant chimeric vesicular stomatitis virus (VSV) expressing the HA of the highly pathogenic avian influenza virus A/chicken/Yamaguchi/7/2004 (H5N1) and NA from different human and avian influenza viruses (HxN1). The VSV vector also encodes for either GFP or secreted Nano luciferase in order to ease virus titration and detection of infected cells. First results suggest that not all NAs do support replication of chimeric VSV equally well. A detailed analysis will show whether differences in enzyme activity or specificity are responsible for this HA/NA imbalance. Future work will also assess the importance of deletions in the NA stalk domain, glycosylation sites, the second sialic acid binding site and other mutations that may affect NA function. In addition, chimeric viruses will be used to see whether HA (H5) could be combined with different NA subtypes. Results obtained with chimeric VSV will be reassessed with recombinant influenza virus reassortants in appropriate animal models.

EVOLUTION OT THE HIV-1 ENVELOPE PROTEIN DURING THE DEVELOPMENT OF A MPER DIRECTED BROADLY NEUTRALIZING ANTIBODY RESPONSE

Abstract: 160045 P

Chris Brunner[1], Nikolas Friedrich[1], Jacqueline Weber[1], Therese Uhr[1], Myène Morin[2], John Robinson[2], Peter Rusert[1], Huldrych Günthard[1,3], Alexandra Trkola[1]* and the Swiss HIV Cohort Study

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3 Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, Zurich, Switzerland
*Corresponding Author

Detailed characterization of the rare patients who develop a broadly neutralizing antibody (bnAb) response against HIV-1 is considered crucial to inform vaccine design. We recently identified in the Swiss 4.5K Screen (Rusert et al. 2016, Nat Med) 239 bnAb inducing individuals. Here we described the detailed evaluation of the longitudinal plasma antibody response and viral escape in a donor who was predicted to have a Membrane Proximal External Region (MPER) bnAb response. The MPER is part of the envelope glycoprotein 41 located close to the lipid bilayer of the virion and is the target of multiple bnAbs. The viral envelopes that give rise to a broadly neutralizing immune response to the MPER domain are of great interest for immunogen design, as well as the investigation of immune-evasion mechanisms of the virus in this patient. Here we present a detailed investigation of the autologous virus Envelope (Env) sensitivity to autologous plasma neutralization and known MPER bnAbs. The plasma neutralization profile showed a prototypic pattern, with plasma neutralizing the autologous viruses from earlier, but not from contemporaneous or later time points. Interestingly, MPER directed bnAbs showed in line with a MPER-escape a distinct increase in IC50s during the time course of about one log. The patient plasma developed neutralization breath from approximately two years after infection onwards, reaching up to 83% of heterologous virus strains. Plasma potency against relatively neutralization resistant Tier-2 HIV-1 strains increased about tenfold up to two years post-infection and then remained constant until the patient started ART five years after infection. We further conducted binding profiling studies using MPER-mimetics, gp41 constructs and soluble env trimers to investigate epitope specificities of the plasma Ab response. Based on this, binding targets will be chosen as baits for memory B cell selection that express MPER-directed bNAbs for subsequent mAb cloning.

A MOLECULAR EPIDEMIOLOGY APPROACH TO IDENTIFY HIV-1 SUPERINFECTION IN THE SWISS HIV COHORT STUDY

Abstract: 160065 P

Sandra E. Chaudron[1,2,3], Karin J. Metzner[1,2], Alex Marzel[1,2], Jürg Böni[2], Sabine Yerly[4], Thomas Klimkait[5], Matthieu Perreau[6], Roger D. Kouyos[1,2], Huldrych F. Günthard[1,2], for the Swiss HIV Cohort Study

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HIV-1 superinfection (SI) is the infection of an HIV-1 seropositive individual with another viral strain. SI has been studied and associated with immune escape, viral recombination and disease progression. However, SI identification remains challenging: 1. SI strain may outcompete or be outcompeted by the first strain. 2. SI is difficult to discern from co-infection. 3. Intra-subtypes SI is difficult to prove, especially if caused by viruses from similar transmission clusters. 4. Finally, sampling frequencies are too low and systematic screenings of large populations to date are missing due to lack of needed longitudinal samples in untreated patients. Here we benefit from historic samples from >19,000 patients in the well characterized longitudinal Swiss HIV Cohort Study (SHCS). We thus aim to perform a large scale screen of the SHCS using longitudinal samples from patients and a molecular epidemiology approach to identify superinfection candidates.

To do so, sequences of the HIV-1 pol gene from 11,738 patients in the SHCS drug resistance database were used for phylogenetic reconstruction. 4558 patients had ≥2 longitudinal sequences available with 931 patients belonging to non-monophyletic clusters that were kept in the subsequent analysis. Then based on our dataset distribution 2 criteria emerged to select HIV-1 superinfected patients: 1. ≥20 patients must be included in the smallest cluster that includes all of the focal patient's sequences and 2. a genetic distance of ≥5% between the focal patient's sequences was also applied. With these criteria, 330 candidates for HIV-1 superinfection were identified and will be further confirmed and studied. Based on these results; our molecular epidemiology approach is so far the largest screen to identify HIV-1 superinfection using longitudinal samples.

BROAD SPECTRUM INVESTIGATIONAL AGENT GS-5734 FOR THE TREATMENT OF EBOLA, MERS CORONAVIRUS AND OTHER PATHOGENIC VIRAL INFECTIONS WITH HIGH OUTBREAK POTENTIAL

Abstract: 160093 P

Tomas Cihlar[1], Alison Hogg[1], Travis Warren[2], Emmie de Wit[3], Timothy Sheahan[4], Michael Lo[5], Veronica Soloveva[2], Jessica Weidner[2], Laura Gomba[2], Friederike Feldmann[3], Jacqueline Cronin[3], Amy Sims[4], Adam Cockrell[4], Joy Feng[1], Iva Trantcheva[1], Darius Babusis[1], Danielle Porter-Poulin[1], Roy Bannister[1], Richard Mackman[1], Dustin Siegel[1], Adrian Ray[1], Robert Jordan[1], Mark Denison[6], Christina Spiropoulou[5], Stuart Nichol[5], Ralph Baric[4], Heinrich Feldmann[3], Sina Bavari[2]

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Recent viral outbreaks with significant mortality such as Ebola virus (EBOV), SARS-coronavirus (CoV) and MERS-CoV reinforced the need for effective antiviral therapeutics to control potential global epidemics. GS-5734 is a novel adenine nucleotide prodrug in development for treatment of EBOV. In vitro antiviral activity of GS-5734 has been established against multiple pathogenic RNA viruses including filoviruses, coronaviruses and paramyxoviruses with EC50 of 40 to 200 nM. In vivo, therapeutic efficacy leading to 80-100% survival was observed in rhesus monkeys infected with lethal doses of EBOV or Marburg virus and treated with once daily intravenous (IV) administration of 5 to 10 mg/kg GS-5734 beginning 3 to 5 days post-infection (p.i.). In all non-human primate filovirus infection models, GS-5734 significantly reduced systemic viremia and ameliorated severe clinical disease signs and anatomic pathology. In mice infected with MERS- or SARS-CoV, twice daily subcutaneous administration of 25 mg/kg GS-5734 beginning 1 day p.i. significantly reduced lung viral load and improved respiratory function. In rhesus monkeys, once-daily IV administration of 5 mg/kg GS-5734 initiated 1 day prior to MERS-CoV infection reduced lung viral load, improved clinical disease signs, and ameliorated lung pathology. Finally, in African green monkeys infected with a lethal dose of Nipah virus, once-daily IV administration of 10 mg/kg GS-5734, starting 1 day p.i. resulted in 100% survival to day 90 without any major respiratory or CNS symptoms. GS-5734 is currently being tested in a phase 2 study in male Ebola survivors with persistent viral RNA in semen. Lyophilized drug formulation has been developed that can be administered to humans via a 30-min IV infusion and does not require cold chain storage. Together, these results support further development of GS-5734 as a broad spectrum antiviral to treat viral infections with high mortality and significant outbreak potential.

MXB IS AN INTERFERON-INDUCED RESTRICTION FACTOR OF HUMAN HERPESVIRUSES

Abstract: 160039 O

Michel Crameri[1], Michael Bauer[2], Nicole Caduff[3], Raphael Walker[1], Fiona Steiner[1], Francesca D. Franzoso[4,5], Cornelia Gujer[3], Karin Boucke[2], Talissa Kucera[1], Andrea Zbinden[1], Christian Muenz[3], Cornel Fraefel[4], Urs F. Greber[2], Jovan Pavlovic[1]

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The human myxovirus resistance proteins MxA and MxB are expressed upon stimulation with type I and type III interferons (IFNs) in response to viral infection. While MxA is well-known to elicit potent antiviral activity against many viruses, MxB has long been unrecognized to serve any antiviral function. Recently, a potent antiviral effect of MxB against human immunodeficiency virus type 1 (HIV-1) and other primate lentiviruses has been discovered. Evolutionary analyses then indicated that MxB is likely to exert inhibitory activities against additional viruses.

To test this hypothesis, we selected herpes simplex virus type 1 (HSV-1) as a representative of nuclear-replicating large DNA viruses. We first utilized T98G cells as a model due to their high endogenous MxB expression. Then, we tested MxB in absence of other IFN-induced genes by generating A549 cells stably overexpressing MxB (A549-MxB).

We observed that silencing of MxB expression in IFN-treated T98G cells partially rescued HSV-1 replication. Moreover, HSV-1 replication was strongly inhibited in A549-MxB cells as compared to control cells. In line with this, HSV-1 replication in A549-MxB cells recovered to a large extent after siRNA-mediated knock-down of MxB. Similar results were obtained using HSV-2 and Kaposi's sarcoma-associated herpesvirus (KSHV). Further experiments revealed that MxB acts early upon infection, i.e. prior to viral immediate-early gene expression. Accordingly, transmission electron microscopy of incoming HSV-1 capsids revealed an impairment of HSV-1 uncoating in cells expressing MxB. Finally, we employed click chemistry to show that MxB inhibits the delivery of HSV-1 genomic DNA to the host cell nucleus.

In conclusion, out data indicate that MxB interferes with herpesvirus infection at the stage of entry, suggesting a gatekeeper function of MxB at the nuclear envelope.

SINGLE-CELL RNA-SEQ REVEALS TRANSCRIPTIONAL HETEROGENEITY IN LATENT AND REACTIVATED HIV-INFECTED CELLS

Abstract: 160059 O

Monica Golumbeanu[1,2], Sara Cristinelli[3], Sylvie Rato[3], Miguel Munoz[3], Matthias Cavassini[4], Niko Beerenwinkel [1,2*], Angela Ciuffi [3*]

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Background: Despite effective treatment, HIV can persist in part in latent reservoirs, which represent a major obstacle towards HIV eradication. Targeting and reactivating latent cells is challenging due to the heterogeneous nature of HIV infected cells. We used a primary model of HIV latency and single-cell RNA sequencing to characterize transcriptional heterogeneity during HIV latency and reactivation, and to understand transcriptional programs leading to successful reactivation of HIV expression.
Methods: Primary human CD4+ T cells were infected with an HIV-based vector encoding the green fluorescent grotein (*gfp*) reporter gene. Successfully infected (GFP+) cells were sorted by FACS, expanded in culture and allowed to return to a resting, latent state. Latently infected cells were either left untreated (control), or exposed to the histone deacetylase inhibitor SAHA, or to T-cell receptor (TCR)-mediated stimulation. Cells were separated using a fluidic technology and subject to single-cell RNA sequencing (scRNA-Seq). TCR-stimulated cells were also monitored for GFP expression upon reactivation.
Results: Principal component analysis of 224 single cell gene expression profiles identified two distinct clusters of cells, for each condition (untreated, SAHA, TCR). Quantitative analysis of GFP expression of TCR-activated cells further discriminated the two cell subsets, with one cell subpopulation being more susceptible to HIV expression reactivation than the other cell subpopulation. A common set of 134 genes was differentially expressed between these two cell clusters, and was enriched for viral processes, translation regulation, and RNA and protein metabolism. This specific 134-gene signature was also recapitulated in primary CD4+ T cells isolated from HIV+ individuals, revealing a similar transcriptional heterogeneity.
Conclusion: Transcriptional analysis of single cells identified two CD4+ T cell populations that might reflect two distinct resting cellular states, and displaying a different potential for cell stimulation and HIV reactivation. Our results identified for the first time a specific cellular signature, associated with success of HIV reactivation. These data should provide a valuable tool to facilitate the identification of successful latency reversing agents and help designing targeted strategies for purging the HIV latency reservoirs.

A UNIQUE N332 SUPERSITE DIRECTED HIV-1 BROADLY NEUTRALIZING ANTIBODY FROM A SLOW PROGRESSOR SHARES CHARACTERISTICS WITH BOTH CD4BS AND V3-GLYCAN bnAbs

Abstract: 160051 P

Hanna Ebner[1], Merle Schanz[1], Peter Rusert[1], Michael Huber[1], Claus Kadelka[1], Thomas Liechti[1#], Branislav Ivan[1], Herbert Kuster[2], Jacqueline Weber[1], Therese Uhr[1], Katherine Doores[3], Osvaldo Zagordi[1], Dominique Braun[2], Huldrych F. Guenthard[1,2], Alexandra Trkola[1]

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A comprehensive understanding of broadly neutralizing antibody (bnAb) development is critical to inform HIV-1 vaccine design. We previously identified in the frame of the Swiss 4.5K Screen a cohort of 239 bnAb inducers that provided information on crucial determinants of bnAb evolution. Here we report on the bnAb response of Swiss 4.5K participant Z91 an individual with slow progressing, HIV-1 subtype B infection that was contained at modest viral loads for 9 years. A V3 glycan specific bnAb plasma activity was detectable relatively early in infection (13% breadth by week 46 post infection) and steadily increased until 7 years post infection (78% breadth). A monoclonal antibody, ZPHI-12, isolated at week 258 (58% breadth) showed unique binding and neutralizing features that resemble both V3 glycan/ 324GDIR327 and CD4bs bnAbs. Early viral escape from ZPHI-12 involved loss of N332 but was dominated later by rare mutations in the 324GDIR327 motif that allowed restoration of N332. Altogether, our findings unravel a novel solution for bnAbs in recognizing the N332 supersite and exemplify unique HIV-1 escape strategies that allow perseverance of N332 aiding our understanding of how these antibodies evolve and may be elicited by vaccination.

THE DYNAMICS OF BOTH FILAMENTOUS AND GLOBULAR MAMMALIAN REOVIRUS VIRAL FACTORIES RELY ON THE MICROTUBULE NETWORK

Abstract: 160102 P

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Mammalian reovirus viral factories (VFs) form filamentous or globular structures depending on the viral strains. Here, we demonstrate that both kinds of VFs condense into a perinuclear position. However, filamentous VFs reach this position faster than globular ones. These dynamic differences correlated with the capacity of a filamentous virus strain to transit its VFs from globular to filamentous morphologies. Interestingly, we show that globular VFs coalesce with each other, thereby gaining in size and decreasing in numbers. Moreover, globular VFs rely on intact microtubule network and dynein for their structural assembly, maintenance and perinuclear condensation. By using different transfection ratios of *μ*NS and a filamentous version of *μ*2, to mimic filamentous VFs at various infection stages, we determined the dependency on MT network for the perinuclear condensation of VF-like structures (VFLSs). Also, increased amounts of *μ*NS promote the perinuclear positioning of VFLSs, which directly correlates with an increase in acetylated tubulin levels.

FUNCTIONAL CHARACTERIZATION OF THE ROLE OF NUCLEOPORINS IN IAV INFECTION

Abstract: 160041 P

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In contrast to most other RNA viruses, influenza viruses replicate their genome in the host cell nucleus. Upon endosomal fusion and uncoating, the viral ribonucleoproteins (vRNPs) are imported into the nucleus through the nuclear pore complex (NPC), yet the exact mechanism remains to be elucidated. The NPC is a large macromolecular complex consisting of multiple copies of around 30 different nucleoporins (NUPs), which controls the bi-directional nucleocytoplasmic trafficking of RNA and proteins. Post-translational modifications of NUPs, such as phosphorylation, regulate the assembly of the NPC and the efficiency of nucleocytoplasmic transport. In a genome-wide phosphoproteomic screening previously conducted in our lab, several nucleoporins were found to be dephosphorylated upon minutes of infection. The phosphorylation of Nup96, which is co-transcribed with Nup98 resulting in one mRNA encoding both proteins, was shown to be strongly reduced upon viral infection. In an attempt to characterize the role of these proteins in IAV replication, we observed that silencing of both Nup96 and Nup98 dramatically reduced viral replication and, more precisely, the nuclear import of parental vRNPs. Overexpression of siRNA-resistant Nup98 or Nup96 was performed in combination with silencing of the endogenous proteins as an approach to study their individual role in virus replication. However, it became apparent that removal of one of these structural components of the NPC, leads to mislocalization of one or more other nucleoporins. Interestingly, overexpression of Nup98 significantly increased the replication of a Renilla-encoding reporter IAV, indicating its pro-viral activity early in infection. Although the infection-induced change in phosphorylation of the NUPs remains to be confirmed and its contribution to virus replication elucidated, our results suggest that both Nup96 and Nup98 play an important role in IAV replication.

THE ROLE OF RECEPTOR TYROSINE KINASES IN CELL ENTRY OF LASSA VIRUS

Abstract: 160066 O

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The Old World arenavirus Lassa (LASV) is the causative agent of a severe viral hemorrhagic fever with high mortality in human and is currently considered as one of the most important emerging pathogens by WHO. LASV is endemic in Western Africa, and human infection is caused mainly by zoonotic transmission from infected rodent host Mastomys natalensis via aerosols of contaminated excreta. Due to the lack of a licensed vaccine and limited treatment options the molecular mechanisms underlying LASV infection have been widely investigated in recent years. The principal LASV receptor is dystroglycan (DG), an ubiquitously expressed extracellular matrix receptor. However, virus binding depends on DG's tissue-specific post-translational modification with the O-linked sugar polymer matriglycan, suggesting the usage of alternative receptors in tissues lacking glycosyilated DG. The broadly expressed phosphatidylserine receptor Axl has been recently identified as an alternative LASV receptor candidate, through an entry mechanism known as apoptotic mimicry. I investigated Axl-dependent LASV entry in cells lacking functional DG using recombinant lymphocytic choriomeningitis virus expressing LASV glycoprotein (rLCMV-LASVGP) as a BSL2 model, and found that virus infection was strongly reduced by Axl knock-down and antibody blocking. Moreover, I show that rLCMV-LASVGP induces Axl phosphorylation, and that the highly specific Axl tyrosine kinase inhibitor R428 can abolish rLCMV-LASV infection (IC50 < 1 μM). Axl-mediated LASV entry is facilitated by heparan sulfate, involves a pathway resembling macropinocytosis, and depends on the late endosomal protein LAMP-1 as an intracellular receptor. In addition to Axl, inhibitors and blocking antibodies against hepatocyte growth factor receptor (HGFR) and epithelial growth factor receptor (EGFR) likewise perturbed LASV cell entry. Together, my studies implicate several receptor tyrosine kinases that contribute to LASV entry in a non-redundant manner, making them promising targets in LASV antiviral therapy.

SUBGENOMIC RNA IS A DETERMINANT OF FLAVIVIRUSES HOST ADAPTATION, FITNESS AND EPIDEMIC POTENTIAL

Abstract: 160112 P

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Specialized RNA structures in the 3‘ untranslated region (UTR) of flaviviruses impede the degradation of non-coding subgenomic flaviviral RNA (sfRNA) resulting in their accumulation in host cells, suppressing antiviral mechanisms and increasing viral fitness and cytopathogenicity. While our understanding of this mechanism arises from particular cases of Flaviviruses, such as Dengue and West Nile, the diversity of 3‘UTR, the presence of RNA structures and function across flaviviridae is not known, including in devastating viruses such as Yellow Fever, Japanese Encephalitis and Zika. Through a comprehensive analysis of > 6000 3‘UTR sequences representing all known viruses in Flaviviridae and implementation of state-of-the-art non coding RNA bioinformatics, we reveal the presence of homologous RNA structures in all flaviviruses, which share essential secondary and tertiary interactions for sfRNA production. In several viruses the RNA structures have duplicated, with differential selection pressures between the structures. The later deletion of one duplicated RNA structure seems to have contributed to speciation in some of these viruses, including widely spread pathogenic flaviviruses. These structures are also known for their ability to bind host proteins and increase replicative fitness, highlighting their central role in host adaptation and fitness. Based on these results and currently available experimental data we postulate that sfRNA are highly evolving viral weapon and serve as „protein sponge“ that exert their function through sfRNA-host protein interactions as a long-standing strategy in flaviviruses to facilitate host adaptation and increase viral fitness. We urge the investigation of their role in vaccine development as well as in epidemiological and clinical outcome.

IDENTIFICATION OF BROAD AND POTENT NEUTRALIZING DARPINS THAT TARGET CONSERVED DOMAINS OF THE HIV-1 ENVELOPE

Abstact: 160047 O

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Broadly neutralizing antibodies (bNAbs) targeting the HIV-1 envelope protein are a major focus of vaccine development, treatment and prevention. There is a concentrated search for immunogens that elicit bnAbs and strategies for application of passively transferred bnAbs in prevention and in cure strategies are intensively investigated. In the crossroad of these approaches, we utilize the Designed Ankyrin Repeat Protein (DARPin) technology to define DARPins that bind to the HIV-1 envelope proteins and neutralize genetically diverse HIV-1 strains as potently as bnAbs. DARPins are like antibodies proteins that can be developed for high binding affinities but differ from antibodies in their high physical stability, small size and often conformation specific binding mode. We previously identified broadly neutralizing DARPins targeting the V3 loop in gp120 and the membrane proximal external region (MPER) we thus focused here specifically on the characterization of DARPins that bind to alternative regions on Env.
Here we utilized DARPin libraries to select HIV-1 envelope (Env) specific DARPins by high throughput ribosome display. Functional HIV binding and neutralizing DARPins were selected, determining binding to trimeric, pre-fusion conformation Env constructs in enzyme-linked immunosorbent assay (ELISAs) and by performing HIV-1 pseudovirus neutralization assays.
By this, we successfully selected 14 novel DARPins with interesting profiles of three different categories. The first group binds variable loops 1 and 2 (V1V2) in a closed conformation of Env trimer, the second category are DARPins that bind to the envelope protein independently of its open or closed plasticity and the third category represents neutralizing DARPins that recognized domains on open conformations of Env that do not involve the V3 loop. Epitope characteristics and neutralization capacity of these novel inhibitors of HIV-1 will be presented.

PALMITOYLATION DETERMINES THE SUBCELLULAR LOCALIZATION OF HEPATITIS E VIRUS ORF3 PROTEIN

Abstract: 160095 O

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Background and aim: Hepatitis E virus (HEV) is a positive-strand RNA virus encoding 3 open reading frames (ORF), namely ORF1, ORF2 and ORF3. HEV ORF3 protein is a small, hitherto poorly characterized protein involved in viral particle secretion and possibly other functions. Here, we investigate the structure and function of this essential viral protein.
Methods: A panel of ORF3 constructs and the full-length protein expressed by infectious HEV were investigated by confocal laser scanning microscopy and immunoblot using GFP fusion proteins and newly established recombinant antibodies. Oligomerization was studied by coimmunoprecipitation and fluorescence resonance energy transfer. A wheat germ-based system was used for the cell-free expression of ORF3 protein. Posttranslational modifications were probed by site-directed mutagenesis and different biochemical assays.
Results: HEV ORF3 protein forms membrane-associated oligomers. HEV ORF3 proteins produced in cell-free and mammalian cell expression systems displayed different apparent molecular weight. Sequence analyses revealed the presence of 8 conserved cysteine residues within the first 21 amino acids which were found to be palmitoylated, as corroborated by 3H-palmitate labeling, the investigation of cysteine-to-alanine substitution mutants and treatment with the palmitoylation inhibitor 2-bromopalmitate (2-BP). Abrogation of palmitoylation by site-directed mutagenesis or 2-BP treatment relocalized ORF3 protein from the plasma membrane to the cytoplasm and decreased stability of the protein. Moreover, we found, using selective permeabilization conditions coupled to immunofluorescence, that HEV ORF3 protein is entirely exposed to the cytosolic side of the membrane. The functional consequences of palmitoylation are currently being investigated using cell-culture derived infectious HEV.
Conclusions: HEV ORF3 protein forms membrane-associated oligomers and is palmitoylated at conserved N-terminal cysteine residues. This posttranslational modification determines the subcellular localization, stability and likely also the function of HEV ORF3 protein. These findings provide new insights into the life cycle of HEV and may yield new angles for therapeutic intervention.

REWIRING CELL FUNCTIONS IN VIRUS ENTRY AND EGRESS

Abstract: 160018 O

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Virus infections are unpredictable and threaten humans and livestock. Acute infections occur on a regular basis. They cause morbidity and mortality, or may be cleared and remain undetected for years. For example, species C adenoviruses cause acute respiratory disease, are suppressed by host immunity and lead to viral persistence in infected individuals for years without striking pathology. How viruses break out of persistence and cause disease is important for treating viral disease and gene therapy, yet is poorly understood. A virus particle carries a genome, and contains information in its sugar, lipid or protein moieties, which enables it to enter a host cell or assemble new virions in the infected cell. The particles receive chemical, enzymatic and mechanical cues from the host, which triggers a stepwise uncoating process culminating with the dissociation of the viral genome from the particle, for example at the cell nucleus. If virion uncoating occurs prematurely, e.g. by catastrophic uncoating in the cytoplasm, or viral genomes are misdelivered, interferon and inflammation responses ensue and preclude infection. Immediate early viral gene products critically overcome innate immunity. For example, the short-lived adenovirus E1A proteins drive the cell cycle into S-phase, and control the transcription of all viral promoters, thereby acting as the pacemaker for infection. E3 proteins protect the infected cell against immune clearance, for example by down-regulating receptors from the cell surface. Remarkably, the E3 promoter of species C adenoviruses responds to the activation of T cells in absence of E1A expression, activates anti-apoptotic gene expression, and promotes the survival of persistently infected T lymphocytes. Here we discuss latest insights into how viral activation of the stress sensor kinase inositol-requiring enzyme 1 (Ire1) in the endoplasmic reticulum boosts E1A transcription, and drives the cell into lytic replication.

INITIAL VIRAL LOAD INFLUENCES INNATE IMMUNE RESPONSE AGAINST NEW WORLD ARENAVIRUS

Abstract: 160086 P

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Arenaviruses are a large family of emerging negative single stranded RNA viruses, whose RNA-dependent RNA polymerase of arenaviruses lacks proofreading activity, resulting in a high mutation rate. Arenavirus populations present therefore as complex quasispecies, which are subject to selection and adaptation. The prototypic Old World arenavirus lymphocytic choriomengitis virus (LCMV) displays auto-interference activity and super-infection exclusion, which influence population dynamics. These phenomena may involve generation of defective interfering (DI) particles, which inhibit the viral life cycle and/or accumulation of the viral matrix protein Z, which inhibits viral replication. In contrast to LCMV, auto-interference activity and super-infection exclusion remain controversial for New World arenaviruses such as the highly pathogenic Junin (JUNV) and the non-pathogenic Tacaribe (TCRV) virus. In this study we found that both JUNV and TCRV exhibit auto-interference with inhibition of viral progeny production during infections performed at high multiplicities of infection (MOI >1 PFU/cell). The phenomenon was more pronounced for JUNV compared to TCRV and was not cell-type restricted, suggesting an intrinsic viral property. Infections at high MOI lowered the amount of viral genomes per infected cell, which correlated with reduced interferon (IFN)-I production. Viral propagation was likewise affected, evidenced by the absence of acutely infected cells with high viral load that are readily observed at low MOI. Our data indicate that the viral dose markedly affects and influences overall virus production and hence the properties of the viral progeny. In vivo, this may limit viral propagation within an infected host and minimize the innate immune response against the viral infection.

A COMPARATIVE STUDY ON THE BIOLOGY AND ANTI-HIV EFFECT OF IFN-α SUBTYPE, IFN-α14, IFN-α MUTANT, YNS, WITH ELEVATED BINDING AFFINITY TO IFNAR AND PROTOTYPE IFN-α SUBTYPE, IFN-α2, IN VIVO IN HUMANIZED MICE

Abstract: 160069 P

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The type I Interferons (IFN-α family) are the most potent anti-viral cytokines. They are released subsequent to encounter with pathogens and orchestrate the immune response to fight the invaders. IFN-α comprises of 13 subtypes which all bind to the same interferon-α/β receptor (IFNAR) consisting of two chains, IFNAR1 and IFNAR2, but with different affinity. Currently, a controversy exists whether the difference in biological activity of IFN-α subtypes is quantitative or qualitative, *i.e.,* is it only a function of dose and binding affinity or activation of different signaling cascades all together? Data we have generated *in vitro* show that IFN-α subtypes with high affinity to IFNAR display significant anti-HIV effects already at low doses while those with low affinity need higher doses (manuscript in preparation). The transcriptomics of tissue specimens treated with IFN-α14 and -α2 were rather identical, which is consistent with identical biological properties. In the latter work, we also found that the IFN-α mutant, YNS, with extremely high affinity to the IFNAR displayed potent anti-HIV activities.
Our data about the biological properties of IFN-α subtypes have been generated using primary human cells treated *ex vivo*. However, to take into account the complex activities of IFNs on the variety of cells of the lympho-reticular system, and in turn the interplay of the various cells with each other, we need a thorough investigation of IFN-α subtypes' biological properties *in vivo* using humanized mice. As IFNs have a very short half-life (2-8h), we have obtained PASylated subtypes of interest with 10-times longer half-lives *in vivo* compared to the wild-type ones.

NON-CONSERVATIVE MUTATION IN THE CANINE DISTEMPER VIRUS ATTACHMENT PROTEIN DISRUPTS CELL INVASION BY INCREASING THE INTERMOLECULAR INTERACTION

Abstract: 160082 P

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Host cell entry by morbilliviruses (*e.g.* measles virus (MeV) or canine distemper virus (CDV)) is coordinated by two interacting envelope glycoproteins; a tetrameric attachment (H) protein and a trimeric fusion (F) protein. Upon receptor engagement, it is assumed that H- and F-proteins undergo series of conformational changes ultimately leading to membrane merging and fusion pore formation. More specifically, the ectodomain of H-tetramers consists of stalk, connector and head domains that adopt „F-triggering-permissive“ native structures. While the central region of the stalk is proposed to interact and activate F, the heads carry the receptor binding activity. In contrast, the precise functional role of the C-terminal module of the stalk (termed „linker“) and the following connector domain, although hypothesized to assume flexible structures to support putative receptor-induced head-stalk structural rearrangements, remains largely unexplored. In this study, to gain mechanistic insights, we conducted a thorough „non-conservative“mutagenesis-scan analysis of the MeV and CDV H-linker/connector domains. Our data provided evidence that substituting the hydrophobic isoleucine residue encompassed within the linker module (H-I146) into any amino acids with charged chemical properties translated into the assembly of over-stabilized H-tetramers, which correlated with fusion promotion-deficiency. Since H-I146 mutants remained entirely competent in intracellular trafficking, F-interaction, receptor binding activity and activating a highly destabilized F-mutant, our findings suggest that the morbillivirus H-stalk C-terminal linker module requires some structural freedom to enable the generation of fully bioactive H-tetramers. Altogether, our data demonstrate that the H-stalk linker module regulates the folding of loosely-assembling functional H-tetramers. Noteworthy, stabilized H-tetramers may offer useful probes for structural determination and ensuing antiviral drug design.

MONITORING THE INNATE IMMUNE RESPONSE USING TRANSGENIC HUMAN AIRWAY EPITHELIAL CELL CULTURES

Abstract: 160014 P

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The respiratory epithelium is the main entry port of respiratory viruses in humans and is an important barrier to infection. Herein, the innate immunity is the first line of defence against intruding pathogens and plays a crucial role in the clinical outcome of respiratory infections. However, the dynamics of the interferon response towards respiratory pathogens at the main entry port is not well understood. To characterize these complex dynamics, we established transgenic primary human Airway Epithelial Cell (hAEC) cultures harboring a reporter cassette with an enzymatic reporter protein under the control of a promoter element known to be induced during late (e.g. Mx1) stage of the innate immune response. For functional characterization, we stimulated the Mx1 reporter hAECs with different TLR 3,7/8 or 9 agonists as a proxy for viral infection. Basolateral medium was collected every 3 hours for 24 hours. In addition, we compared the transcriptional response with that of naive hAEC 24 hours post stimulation.
Both the luciferase activity and transcriptional response results revealed that only TLR3 induces an innate immune response in both naive and transgenic AECs, which is in accordance to the described literature. More important, the luciferase activity results demonstrate that we can actively monitor the innate immune response in our reporter hAECs, both over time and in amplitude. Thereby providing a robust method to gain detailed knowledge on virus-host interaction dynamics within the respiratory epithelium.

SEQUENTIAL RECEPTOR-INDUCED CONFORMATIONAL STATES OF NATIVE MEMBRANE-EMBEDDED HIV-1 ENV

Abstract: 160062 P

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During the entry process, the HIV-1 envelope glycoprotein (Env) trimer undergoes a sequence of conformational changes triggered by both primary receptor CD4 and coreceptor engagement. Resolution of the conformation of these transient entry intermediates have proven challenging leaving certain aspects of the entry process unresolved, including stoichiometry of Env-receptor interactions. Employing antibody epitope mapping we show that increasing CD4 engagement alone leads to the sequential adoption of different conformational states of the Env trimer. Maximal accessibility of the coreceptor-binding site was detected below Env saturation by sCD4 whereas the exposure of the fusion peptide and heptad repeat 1 required higher sCD4 occupancy. Of particular note, several broadly neutralizing antibodies (bnAbs) displayed preference for binding to CD4 triggered Env indicating that they may neutralize also CD4 bound virions.

THE ROLE OF AUTOPHAGY INDUCTION AND ACIDIC VESICLE ACCUMULATION IN ADENO-ASSOCIATED VIRUS 2 (AAV2) INFECTION

Abstract: 160108 P

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AAV2 is a small, non-pathogenic, helper virus-dependent parvovirus with a biphasic life cycle. In absence of a helper virus, the AAV2 genome can integrate at a preferred site on human chromosome 19 or persist as episome in the host cell nucleus. In this study, we addressed the questions whether (i) autophagy can enhance AAV2 transduction efficiency in normal human fibroblasts (NHFs) and hepatocytes (HepG2s) and (ii) AAV2 can enhance autophagy. To address the first question, cells were treated with an activator (rapamycin) or inhibitors (chloroquine and 3-methyladenine) of the autophagy pathway. In addition, we treated cells with sucrose, which previously has been shown to enhance AAV2 transduction (Sutter et al., unpublished data) and to induce acidic vesicle formation (Higuchi et al., 2015). Cells treated with activators or sucrose showed a significantly higher AAV2 transduction rate compared to untreated cells, whereas a significant decrease in transduction rate was observed when inhibitors were used. We also show that the autophagosomal marker LC3-II accumulated to high levels in AAV2-infected cells as well as in sucrose treated cells, indicating that both AAV2 and sucrose can induce autophagy. We also performed immunofluorescence and fluorescence in situ hybridization assays to monitor AAV2 capsids, AAV2 genomes, and acidic vesicles to investigate whether AAV2 particles accumulate in vesicles of the autophagy pathway.
Overall, the results indicate that AAV2 can induce autophagy and that the transduction rate of AAV2 is significantly higher when the autophagy pathway is activated.

OPTIMIZING HIV-1 VIRUS TEST PANELS FOR THE EFFECTIVE DETECTION OF NEUTRALIZATION BREADTH

Abstract: 160068

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Potent broadly neutralizing antibodies (bnAbs) are a key focus of HIV-1 vaccine and therapy development but are only elicited at low frequency in natural infection. The identification of bnAb responses requires virus-screening panels that effectively capture neutralization breadth. Varying procedures and thresholds to define breadth are applied in the literature calling for standardization efforts to enable unbiased assessment of vaccine responses. The Swiss 4.5K Screen identified 239 bnAb inducers (Rusert, Kouyos Nat Med 2016), which provided an ideal set of patients to investigate the composition of optimized virus panels for the detection of HIV-1 neutralization breadth.
The Swiss 4.5K Screen was based on a multi-clade panel of 8 HIV-1 strains. 729 plasma samples, which showed > 80% inhibition of at least one of these viruses, were screened against 15 additional viruses thus yielding neutralization information for 23 viruses. For 162 plasma samples with the highest predicted bnAb activity (based on the 23-virus panel), we obtained ID50 values against a multi-clade 40-virus panel. The median ID50 served as a proxy for true neutralization breadth.
A larger screening panel generally led to more accurate neutralization breadth predictions. However, the gain in predictive strength decreased with the number of included viruses. A comparison of various transformation functions for the % inhibition values revealed max(value-10%,0) as best, yielding significantly better predictions than the frequently used 20-50-80 rule. As expected, virus panels of diverse subtypes performed better than single-clade panels. Intriguingly, inclusion of certain viruses like TRO_clone11 led to consistently better average predictions than others, independent of panel size. General neutralization sensitivity of the 23 viruses had no impact on our findings.
Collectively, our systematic survey of virus screening panels provides a basis for standardized HIV-1 neutralization breadth assessment and prediction methods, which will be particularly important in the evaluation of forthcoming vaccine efficacy trials.

CHARACTERIZATION OF BAT INFLUENZA VIRUS ENTRY

Abstract: 160029 P

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Two novel influenza A-like virus sequences have been isolated from two South American bat species in recent years. Initial studies revealed that these bat influenza viruses, classified as H17N10 and H18N11, are highly divergent from known influenza A virus (IAV) strains. In line with their inability to bind the canonical IAV receptor sialic acid, bat influenza viruses show a restricted cell type-specific tropism. Little is known about target cells and the entry characteristics of the bat influenza viruses. We have developed different reporter assays to investigate entry of virus-like particles (VLPs) pseudotyped with the bat influenza virus glycoproteins H17 or H18 on different cell lines. Aiming at the identification of the cellular receptor of H18N11, we have performed a comparative transcriptome analysis of three pairs of cell lines differing in H18N11 susceptibility. Thus, we can test potential receptor candidates by silencing or ectopic expression experiments. Altogether, we want to study bat influenza virus entry and uncover their cellular receptor to get a deeper insight into the biology of the newly discovered IAV subtypes.

CO-EVOLUTION OF BROADLY NEUTRALIZING ANTIBODIES AND HIV-1

Abstract: 160052 O

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A clear understanding of viral and antibody coevolution that leads to the induction of HIV-1 broadly neutralizing antibodies (bnAbs) is considered a prerequisite for developing bnAb based vaccines. A recent systematic survey of the neutralizing antibody activity in 4'484 HIV-1 infected individuals of the Swiss HIV Cohort Study (Swiss 4.5K Screen) conducted by our group identified 181 broad and 58 elite neutralizers (Rusert, Kouyos Nat Med 2016).
Here we present an analysis of an elite neutralizer (patient SHCS-Pat-50206) with subtype D infection with a predicted variable loop 1 and 2 (V1V2) bnAb activity. We followed the autologous virus evolution and plasma neutralization activity in patient SHCS-Pat-50206 over a period of 12.5 years during which the patient was partially on antiretroviral treatment (ART). In total, 42 bulk amplified envelope genes were isolated from twelve off-ART time points. Plasma neutralization in the patient developed breadth after 1.5 years of infection (30%) and reached maximum breadth (81%) by 12.5 years.
We successfully cloned three antibody variants of a bnAb lineage, termed SHCS-13 from cultured patient B cells. Clones SHCS-13.1, SHCS-13.2 and SHCS-13.3 showed highly related CDRH3 sequences. Two of the antibodies, SHCS-13.1 and SHCS-13.2, displayed high neutralization capacity and breadth and showed a remarkably high degree of correlation (r=0.77 and r=0.52) with the patient plasma activity. Interestingly, neutralization activity of the bnAb against the autologous viruses was weak despite a steady increase in breath. This indicates that the bnAb must have maintained a sufficient capacity to bind the autologous virus that allowed maturation to thrive. Assessment of SHCS-13 Env binding capacity are currently underway.
Overall, our data highlight that bnAb maturation does not require a potent neutralization capacity against the autologous strain opening new avenues for defining immunogens that induce bnAbs similar to SHCS-13.

VIRUS TRANSMISSION DURING KIDNEY TRANSPLANTATION ASSESSED BY VIROME ANALYSIS OF LIVING DONOR AND RECIPIENT

Abstract: 160028 O

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Background: Before kidney transplantation, donors and recipients are routinely screened for a number of active or latent viral infections using specific tests. Little is known about other untested, apathogenic viruses a donor might carry and likely will transmit. Here, we aimed to characterize the viral metagenome of both the donor and the recipient using metagenomic sequencing at time of transplantation as well as up to one year after.
Methods: Recipients of kidney grafts and the corresponding donors were enrolled at the time of transplantation. Follow-up study visits for recipients were scheduled 4-6 weeks and 1 year thereafter. At each visit, plasma, urine and stool samples were collected and patients were evaluated for signs of infection or transplant-related complications.
For metagenomic analysis, blood and urine samples were enriched for viruses, amplified using an anchored random PCR system and sequenced using high-throughput metagenomic sequencing. Viruses detected by sequencing were confirmed using real-time PCR.
Results: We analyzed a total of 30 living kidney donor/recipient pairs with a follow up of at least 1 year. Post transplant routine virus diagnostics mainly detected cytomegalovirus (CMV) and BK polyomavirus (BKPyV) replication in blood and respiratory viruses in throat swabs.
In addition to routine diagnostics, metagenomic sequencing detected JC polyomavirus (JCPyV) in urine of 7 recipients as well as all corresponding donors. Phylogenetic analysis confirmed that donor and recipient were infected with the same strain in 6 cases, suggesting a transmission from transplant donor to recipient. Moreover, Torque teno virus (TTV) was found frequently in time points after transplantation, as expected in patients under immunosuppression.
Discussion: Using metagenomic sequencing, we detected transmission of JCPyV from kidney transplant donors to recipients in several cases. Future studies within larger cohorts are needed to define the relevance of the donor's virome for the recipient, enabling the prediction of transplant outcomes.

SITE-DIRECTED MUTAGENESIS OF PESTIVIRAL RNASE AND ITS EFFECT ON INNATE IMMUNE EVASION

Abstract: 160016 O

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Bovine virus diarrhea virus (BVDV), a pestivirus in the family Flaviviridae, causes persistent infection (PI) in cattle by entering the fetus early in gestation prior to development of adaptive immunity. The inhibition of interferon (IFN) type I synthesis plays an important role in escaping innate immunity and is a prerequisite for the birth of PI calves. Thus, pestiviruses express two IFN antagonists, N^{pro} and E^{rns}. In infected cells, the non-structural protein N^{pro} inhibits IFN induction by inducing the degradation of the transcription factor IRF-3. By contrast, the structural envelope glycoprotein E^{rns} gets also secreted from infected cells in a soluble form and, by virtue of its endoribonuclease activity, it is able to degrade its own viral RNA in order to prevent the activation of an IFN response also in non-infected cells.
Here, we show that the glycosaminoglycan (GAG) binding site located at the C-terminus of E^{rns} is important for its IFN antagonism. We previously showed that E^{rns} is endocytosed via clathrin-mediated endocytosis and that this cellular uptake is required to prevent IFN induction. Substitution of all four positive charged lysine residues in the GAG binding site of E^{rns} to neutral alanines resulted in a complete loss of its activity against IFN induction. In addition, this mutant could also not be detected in cells by immunofluorescence staining. This effect was only moderate when mutating only one or two lysines instead of all four. As all of the mutants were still able to degrade dsRNA in an in vitro RNase assay, we conclude that in addition to its RNase activity, the correct localization of E^{rns} inside the cells is crucial for its IFN antagonistic effect.
With this data, we predict that in a PI animal, E^{rns} is secreted from infected cells followed by endocytosis also into non-infected cells in order to prevent aberrant induction of an innate immune response by N^{pro} and E^{rns} in infected and non-infected cells, respectively.

DARPINS TARGETING THE HIV ENVELOPE PROTEIN AS TOOLS TO DESIGN IMMUNOGENS AND THERAPEUTICS

Abstract: 160044 P

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Highly active antiretroviral therapy (HAART) is successful in treating HIV-1 infection but cannot cure it. Broadly neutralizing antibodies (bnAbs) that potently neutralize genetically diverse HIV-1 strains are considered important components of vaccines, preventive therapies and cure approaches that may fill the gaps in prevention and treatment that currently exist. While bnAb based therapies are moving along in development, HIV-1 vaccine development struggles thus far in designing immunogens that are able to evoke bnAbs. Designed Ankyrin Repeat Proteins (DARPins) are non-immunoglobulin scaffold binders that differ from antibodies in size and structure and allow high affinity and conformation specific binding to epitopes with a well-defined structure. We are developing DARPins targeting the HIV-1 envelope protein as they offer numerous applications ranging from characterization of envelope immunogens to therapeutic applications such as HIV entry inhibition or targeting of drugs to HIV-1 infected cells. Screening for HIV-1 envelope reactive binders from high diversity DARPin libraries by high-throughput ribosome display recently identified DARPins with neutralization breath equaling bnAbs that are specific for the variable loop 3 (V3) and the membrane proximal external region (MPER) of the HIV-1 envelope protein. Intriguingly, we observed that the MPER DARPins target the MPER region differentially from bnAbs. We are currently investigating what role the alpha helical orientation of the MPER domain plays for envelope functionality and neutralization by MPER DARPins and bnAbs. In additional experiments, we seek to expand the HIV-DARPin toolbox to obtain panels of DARPins that enable envelope immunogen characterization and mapping of plasma responses of HIV-infected individuals. Towards this end, we are performing ribosome display selections using probes that allow to specifically target selections towards the helical domains of gp41 and the CD4 binding site (CD4bs) to define DARPins specific for these functionally important and conserved domains.

PHOSPHATIDYLSERINE RECEPTORS TIM-1 AND AXL PROMOTE HANTAVIRUS INFECTION

Abstract: 160067 P

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Hantaviruses are emerging human pathogens responsible for severe human diseases: hemorrhagic fever with renal syndrome in Asia and Europe and Hantavirus cardiopulmonary syndrome in the Americas. Currently, there are no effective antivirals or licensed vaccines against hantaviruses. Viral attachment and entry represent the first steps in virus transmission and are promising targets for anti-viral therapeutic intervention. Here we investigated the largely unknown receptor use of hantaviruses in human respiratory epithelial cells. We focused on Old World Hantaan virus (HTNV) and New World Andes virus (ANDV), the most important pathogens of the major hantavirus Clades. We established and validated a hantavirus pseudotype system based on replication competent and propagation deficient recombinant vesicular stomatitis virus as a suitable BSL2 surrogate model to study hantavirus entry into the host cell. The human alveolar II epithelial cell line A549, a classical immortalised tissue model for human respiratory epithelia, was highly susceptible for infection with both HTNV and ANDV pseudotypes, in line with the known aerosol transmission of the viruses. A range of emerging enveloped viruses can use phosphatidylserine (PS) receptors of the human T cell immunoglobulin and mucin domain containing protein (TIM) and the Tyro3/Axl/Mer (TAM) families to enter human cells via „apoptotic mimicry“. Flow cytometry and Western blot revealed expression of TIM-1 and Axl on A549 cells, consistent with their known expression pattern in human lung epithelia in vivo. Overexpressed TIM-1 and Axl in HEK293 cells that naturally lack both receptors markedly increased entry of HTNV and ANDV. In a complementary approach, we treated A549 cells with blocking antibodies to TIM-1 and Axl and found a major contribution of TIM-1 and to a lesser extend Axl in entry of HTNV and ANDV. With this, we provide first evidence that HTNV and ANDV may hijack the phosphatidylserine receptors TIM-1 and Axl to enter human epithelial cells during airborne transmission.

H5N1 INFLUENZA A VIRUS PB1-F2 RELIEVES HAX-1 MEDIATED RESTRICTION OF AVIAN VIRUS POLYMERASE IN HUMAN LUNG EPITHELIAL CELLS

Abstract: 160098 P

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Influenza A viruses (IAV) are highly promiscuous pathogens, frequently crossing species barriers among avian reservoir species or (less frequently) from avian to mammalian hosts. Highly pathogenic IAV from avian hosts were first reported to directly infect humans 20 years ago. However, these are rare events and our understanding of factors promoting or restricting zoonotic transmission is still limited. One accessory protein of IAV, PB1-F2, was frequently associated with pathogenicity of pandemic and zoonotic IAV. This enigmatic, 90-amino-acid-short peptide does not harbor an intrinsic enzymatic function. We thus identified host factors interacting with H5N1 PB1-F2 that could explain its importance for virulence. PB1-F2 binds and co-localizes with HCLS1 associated protein X1 (HAX-1), a recently identified host restriction factor of the IAV polymerase complex. We demonstrate that the polymerase complex of a mammalian adapted H1N1 IAV is resistant to HAX-1 imposed restriction while the polymerase complex of an avian origin H5N1 IAV remains sensitive. Sensitivity to HAX-1 can be alleviated by direct competition through PB1-F2. In summary, our data suggest that PB1-F2 is a critical factor reducing restriction of the viral polymerase complex by HAX-1. We thus propose that PB1-F2 plays a key role in zoonotic transmission of avian IAV into humans.

LOW PREVALENCE OF TRANSMITTED HIV-1 DRUG RESISTANCE DETECTED BY A DRIED BLOOD SPOT BASED NGS METHOD IN NEWLY DIAGNOSED INDIVIDUALS IN CAMEROON IN THE YEARS 2015/16

Abstract: 160034 P

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Background: In resource-limited settings, many individuals receiving first-line drugs develop virological failure and may transmit drug resistant viruses. In order to determine the most recent prevalence, risk factors and transmission patterns of transmitted drug resistance mutations (TDRMs), we monitored HIV-1 drug resistance using a novel next-generation sequencing assay applicable to dried blood spot (DBS) samples.
Methods: DBS samples and questionnaires were collected from 360 newly HIV-1 diagnosed individuals in four hospitals in urban areas in Cameroon in the years 2015-16. We developed an HIV-1 protease and reverse transcriptase drug resistance genotyping assay applicable to DBS and all HIV-1 subtypes of at least group M, called DBS-NGS-GRT. The World Health Organization (WHO) 2009 list of mutations for transmitted drug resistant HIV-1 strains was used to analyze TDRMs.
Results: Applying our DBS-NGS-GRT, baseline HIV-1 drug resistance data were obtained from 298/360 (82.8%) newly diagnosed individuals. Of those, 63% were female and the median CD4 count was 303 cells/µl blood. Samples that failed amplification were significantly lower in cDNA copy number than those which were successfully amplified (p=0.0001). The predominant HIV-1 subtype was CRF02_AG (63.4%). At frequencies above 15%, TDRMs to nucleoside reverse transcriptase inhibitors (NRTI) were observed in 3.0% (9/298), to non-NRTIs in 4.0% (12/298), and to protease inhibitors in 1.3% (3/240). The NNRTI-mutation K103N was the most commonly detected (2.7%). Expanding the analysis to low abundant TDRMs, i.e. 3-15%, 12 additional individuals (4.0%) were found. Having unprotected sex with a known HIV-1 positive person was significantly associated with the transmission of TDRMs (adjusted odds ratio 9.6 (95% C.I. 1.79 - 51.3, p=0.008).
Conclusion: Transmitted HIV-1 drug resistance is currently low in the study sites in Cameroon. Evidence of some risky sexual behaviors depicts a public health problem with possible implications on the prevention of new HIV-1 infections in the country.

AAV2 DNA PULL-DOWN ASSAY: IDENTIFYING THE KEY PLAYERS IN CELL CYCLE DEPENDENT VIRAL GENE EXPRESSION

Abstract: 160100 P

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The adeno-associated virus type 2 (AAV2) is a small, non-enveloped single-stranded DNA virus. Its 4.6 kb genome encodes two gene clusters, rep and cap, which are flanked by self-annealing inverted terminal repeats that form double-hairpin structures. AAV2 belongs to the genus Dependovirus and as the name implies only replicates in presence of a helper virus (e.g. an adenovirus, a herpes virus or a papilloma virus).
AAV2 depends not only on a helper virus for productive replication but also on a specific cell cycle phase. We recently reported that AAV2 DNA replication/gene expression occurs preferentially in the S/G2 phases of the cell cycle. The aim of the present project is to identify the cellular licensing factors that support AAV2 gene expression in the S/G2 phases or the inhibitors that block it in the G1 phase of the cell cycle. Since one of the recombinant AAV2 vectors that showed cell cycle dependent gene expression did not encode any viral genes, the key for the AAV2 cell cycle preference must lie in the structure of the viral genome. To investigate this hypothesis, we developed an assay to directly pull-down AAV2 genomes in order to identify by mass spectrometry the interacting cellular proteins that may play a role in cell-cycle dependent AAV2 DNA replication/gene expression.

VARIABILITY IN DISINFECTION RESISTANCE WITHIN AND BETWEEN CURRENTLY CIRCULATING ENTEROVIRUS B SEROTYPES

Abstract : 160080 P

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Human enteric viruses are a leading cause of waterborne disease. Their environmental persistence and resistance to disinfection influences their dissemination and the risk of transmission. Susceptibility to disinfection is known to vary between viruses and even between related strains, yet the extent of this variation and the underlying reasons remain unknown. Here, we implemented a method to isolate single strain enteroviruses from wastewater. Nine viruses belonging to three serotypes were isolated, namely six strains of coxsackievirus B5, two strains of coxsackievirus B4 and one strain of coxsackievirus B1. Five disinfection treatment were applied to each virus: two inducing genome damage (UV254 and sunlight), two targeting both viral proteins and genomes (free chlorine and chlorine dioxide), and one inducing non-oxidative protein denaturation (heat). Then, the disinfection resistance of the different viruses was compared. Additionally, the resistance of environmental isolates was compared to that of a corresponding lab strain. The relative resistance of the tested viruses differed according to the treatment, with genome-damaging disinfectants inducing the least and oxidative disinfectants inducing the greatest variability in resistance. For most treatments, the extent of resistance was roughly associated with serotype. However, even among strains of a single serotype differences in resistance could be observed, and the response of the lab strain to disinfection frequently did not correspond to that of the environmental isolates. Finally, we partly rationalized the observed patterns by linking resistance to differential viral features, including capsid structure and stability, presence of oxidizable residues in genome and proteins, and replicative fitness. Overall, our results demonstrate that even small differences in the genome and protein composition of a virus can lead to phenotypic features that affect their response to disinfection. This implies that disinfection requirements for any given virus family need to be evaluated based on multiple and currently circulating strains.

CHARACTERIZATION OF THE INNATE IMMUNE RESPONSE AGAINST NEW WORLD ARENAVIRUSES

Abstract: 160050 P

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The New World Arenaviruses (NWA) include several severe human zoonotic pathogens, including Junin (JUNV), Machupo, and Guanarito virus that cause viral hemorrhagic fevers with high mortality. All pathogenic NWA belong to Clade B, where they are phylogenetically interspersed with closely related non-pathogenic viruses. The highly pathogenic JUNV is e.g. genetically closely related to the non-pathogenic Tacaribe Virus (TCRV). The hostâ€™s innate immune response represents a major line of anti-viral defense and is a key determinant for NWA disease potential. Previous studies suggest inherent differences in the capacity of JUNV and TCRV to suppress innate immunity, linked to properties of the viral nucleoprotein (NP) and matrix protein (Z). Here we performed an in-depth comparison of the innate immune response against JUNV and TCRV in human respiratory epithelial cells. Infection of these important target cells resulted in a stronger interferon (IFN)-I response for TCRV when compared to JUNV, which correlated with more efficient viral production and stronger apoptosis triggering. Rather unexpected, quantitative transcription profiling of 80 key innate immunity genes revealed a strikingly similar qualitative pattern for both viruses. The higher amplitude of the IFN-I response in TCRV infected cells correlated with elevated cellular concentrations of viral genomic RNA. Our data indicate that JUNV and TCRV activate similar innate signaling pathways and argue against differential inhibition of innate signaling by JUNV and TCRV NP and Z. The different amplitude but strikingly similar pattern of innate immune gene expression in cells infected with JUNV and TCRV rather suggest that the absolute amount of viral RNA synthesized is a major determinant of the innate immune response.

IN VIVO INVESTIGATION OF BROADLY NEUTRALIZING ANTIBODIES IN HUMANIZED MICE

Abstract: 160035 P

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Combined anti-retroviral therapy (cART) is the cornerstone of HIV treatment resulting in rather all cases in undetectable viremia, reduced morbidity and mortality. However, daily drug intake, side effects and the risk of emergence of resistance in patients with poor drug adherence make the development of new therapeutic strategies necessary. Over the last years, broadly neutralizing antibodies (bNAbs) against the HIV-envelope have turned out being promising for new treatment approaches.
We, here, investigate the anti-HIV effects of bNAbs in HIV infected humanized mice. The bNAbs we are exploring are VRC07, PG9 and 10-1074 targeting either the CD4 binding site, V1/V2 region or the glycan V3 region and a novel bNAb LN01, targeting the membrane proximal external region of gp41.
The half-life and serum concentration of the bNAbs ranged from 1.97-9.58d and 7-132 µg/ml. Using a single bNAb resulted in a significant but temporally limited reduction of viremia 7d after starting therapy. We assume that viral rebound was due to the emergence of escape mutants.
Delayed rebound occurred when using two bNAbs, whereas three bNAbs resulted in long-term suppression of viremia within 1-7 weeks and in reduced expression of activation markers on T cells when compared to the untreated control.
Furthermore, bNAbs not only have the ability to neutralize free virus but also are capable of killing already infected cells via Fc gamma receptor mediated mechanisms such as antibody-dependent cell mediated cytotoxicity or phagocytosis. Therefore, we are investigating the efficacy of the tested bNAbs to induce Fc-dependent clearance of infected cells in vitro and the contribution of this mechanism during bNAb therapy in vivo.
We conclude that bNAbs are a very promising alternative to cART, since small molecule drugs lack the ability to induce Fc gamma receptor mediated mechanisms, and since bNAbs may be modified for increasing their half-life permitting an extended dosing schedule.

INVESTIGATING THE ROLE OF TETRASPANINS CD151 AND CD63 IN THE HEPATITIS E VIRUS LIFE CYCLE

Abstract: 160081 P

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Background: Hepatitis E virus (HEV) is a positive-strand RNA virus encoding 3 open reading frames (ORF). ORF1 encodes the viral replicase, ORF2 the viral capsid and ORF3 a small protein involved in virion secretion. Recent data indicates that virus egress involves the exosomal pathway. A published yeast two-hybrid screen identified tetraspanins CD151 and CD63, known exosomal factors, as interactors of ORF3 protein (Geng Y et al. PLoS One 2013;8:e56320). Our study aims at validating CD63 and CD151 as interacting partners of ORF3 protein and at defining their roles in the HEV life cycle.
Methods: The subcellular localisation of CD151 and CD63 as well as viral proteins was investigated by confocal laser scanning microscopy. Replicon and full-length constructs were used to investigate HEV entry, RNA replication and virus production following siRNA- or CRISPR/Cas9-mediated gene silencing of CD151 and CD63.
Results: Immunofluorescence analyses revealed that CD151 co-localised with HEV ORF3 and CD63 partially co-localised with HEV ORF2 protein in cells producing infectious virus. Furthermore, CD151 is localised to bile canaliculi-like structures together with ORF3 protein. In addition, functional analyses revealed the involvement of CD151 in HEV entry. However, neither CD151 nor CD63 were found to be involved in HEV RNA replication. Efforts to delineate a potential role of CD151 and/or CD63 in virus production are ongoing.
Conclusions: Our results demonstrate that CD151 co-localises with HEV ORF3 in cells producing infectious virus at bile canaliculi-like structures, suggesting a role in virus production. Ongoing functional studies shall further characterize the role of CD151, as well as CD63, in the HEV life cycle. Altogether, this work should yield new insights into the function of viral proteins and virus-host interactions required for productive HEV infection.

THE SMALL COMPOUND INHIBITOR K22 DISPLAYS BROAD ANTIVIRAL ACTIVITY AGAINST DIFFERENT MEMBERS OF THE FLAVIVIRIDAE FAMILY AND OFFERS POTENTIAL AS PAN-VIRAL INHIBITOR

Abstract: 160015 P

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The family of Flaviviridae encompasses several viruses, which cause widespread morbidity and mortality throughout the world. We have previously described the identification of a small compound inhibitor, K22, which exerts a potent antiviral activity against a broad range of coronaviruses upon targeting of the membrane-bound RNA replication step. To analyze the antiviral spectrum of this inhibitor we tested several members of the Flaviviridae family, which encode positive-strand RNA genomes and are assumed to replicate in tight association with reorganized host cell membranes. Virus-induced host cell membrane reorganization is believed to be an evolutionary conserved strategy which facilitates highly efficient viral genome replication and might at the same time contribute to evasion from host cell defense mechanisms. We could show that K22 is able to inhibit replication of several members of the Flaviviridae family, including emerging zika virus and yellow fever virus. It is tempting to speculate that K22 exerts its broad antiviral activity against several positive-strand RNA viruses via the same mechanism thereby presenting an attractive target for the development of a pan-viral inhibitor. Future studies analyzing the exact mode-of-action might present the first step towards generation of a new antiviral drug, which could be used for broad therapeutic use in animals and humans.

MODIFICATION OF THE SPIKE GENE ATTENUATES HIGHLY VIRULENT PORCINE EPIDEMIC DIARRHEA VIRUS IN PIGLETS

Abstract: 160032 P

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Porcine epidemic diarrhea (PED) represents an important disease of swine worldwide. It is caused by porcine epidemic diarrhea virus (PEDV), member of the family Coronaviridae, genus Alphacoronavirus. While lowly virulent viruses usually lead to harmless infections, the highly virulent strains induce severe disease in newborn piglets with high mortality rates, causing huge economic losses to the swine industry. After severe outbreaks in Europe during the 1980s and 1990s, PEDV became endemic in Asia. Lately, besides lowly virulent strains, highly virulent PEDVs were detected for the first time in the USA and in some European countries. To minimize the economic losses caused by highly virulent variants, enhanced focus on PEDV research is required.

Our aim is to identify viral genes contributing to the development of the severe form of the disease using well-defined recombinant viruses generated by reverse genetics. Accordingly, we established a vaccinia virus-based reverse genetic system for the PEDV field strain Minnesota (MN).
In order to assess the involvement of the S gene in PED pathogenesis, 7-days-old piglets were inoculated with the recombinant PEDV strain MN. Serious clinical signs as vomitus and diarrhea were observed already twelve hours post infection. In contrast, piglets infected with the same recombinant virus but containing the S gene of the cell culture-adapted strain CV777 did not show any clinical signs. These clinical findings are supported by RNA titer measurements following a newly established real-time RT-PCR protocol as well as by histopathological, immunohistochemical and histomorphometrical analyses.

In summary, our results demonstrate the importance of the S gene on PEDV virulence, but also point to other pathogenicity factors which are likely to be involved.

TRIM28 DESUMOYLATION DURING INFLUENZA VIRUS INFECTION

Abstract: 160058 O

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During influenza virus infections, a massive retargeting of the small ubiquitin-like modifier (SUMO) to a subset of host proteins occurs, which regulates many nuclear functions. In a mass spectrometry approach, TRIM28 was identified as factor that is strongly deSUMOylated during influenza A (IAV) and B virus infections. TRIM28 is a multifunctional protein that is involved in the regulation of different signalling pathways and the DNA damage response, and has transcriptional co-repressor activity, which is dependent on its SUMOylation. The aim of this study was to understand the triggers and consequences of TRIM28 deSUMOylation during infection.

Using transfection-based polymerase reconstitution systems, we show that deSUMOylation of TRIM28 is triggered by nuclear IAV polymerase activity. Co-expression of TRIM28 and a SUMO mutant that cannot be cleaved by SUMO-specific proteases (SENPs) prevents deSUMOylation of TRIM28 in this assay, which hints at a role of SENPs in performing the deSUMOylation of TRIM28. Furthermore, deSUMOylation of TRIM28 during IAV infection does not require proteasome activity, and is not mediated by Ser824 phosphorylation of TRIM28, which is known to be the case during the DNA damage response. Screening a library of SENPs in an overexpression system revealed that several SENPs could potentially act on TRIM28 during infection.

In order to investigate the effect of TRIM28 deSUMOylation on IAV replication, we established a CRISPR-Cas9-edited TRIM28 knockout cell line. In cells lacking TRIM28, viral titres were reduced >10-fold in multicycle growth analyses. Restoring wild-type TRIM28 expression in the knockout cells completely rescued viral titres, while expression of a SUMOylation deficient TRIM28 mutant did not support viral replication. Taken together, our data may suggest that infection triggered deSUMOylation of TRIM28 creates a cellular environment restrictive to efficient IAV replication. Further studies are currently being undertaken to assess whether this represents a novel cellular defence mechanism to limit viral infections.

IDENTIFICATION OF NOVEL ISGS INVOLVED IN THE ANTIVIRAL RESPONSE AGAINST INFLUENZA A VIRUS

Abstract: 160040 O

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Interferons (IFNs) are cytokines that have strong antiviral effects and play a central role in the immune defense of the host cell against virus infections. Interferon stimulated genes (ISGs) are the mediators of IFNâ€™s antiviral activity, and hundreds of different ISGs are known to be upregulated by IFN. However, only a few have been found to be antivirally active against influenza A virus (IAV). Our hypothesis is that the currently known factors alone cannot explain the strong effect of IFN against IAV, and we aim to find novel ISGs which have yet to be fully described. For this purpose, an siRNA library of 102 different human ISGs was created. A robust and efficient assay was established whereby the siRNA library could be screened to identify ISGs that impact the IFN-mediated antiviral response against a synthetic reporter IAV expressing Renilla luciferase. Seven candidates were chosen for further characterization due to their strong and reproducible impact on IAV replication in multiple human cell-lines. qPCR was used to validate the IFN-inducibility of the selected ISGs and the efficiency of siRNA knockdown. Notably, the seven ISGs have diverse functional properties, including RNA-binding, G-protein signaling, metallochaperone activity, or transmemembrane localization, suggesting that each ISG may exhibit distinct antiviral functions against IAV. We are currently establishing IAV replication stage-specific assays to dissect the detailed mechanisms of action of the selected ISGs, and their antiviral breadth. Overall, our work has uncovered several previously unappreciated human host factors that contribute to the antiviral action of IFN against IAV infection.

ASSESSMENT OF THE ANTIVIRAL ACTIVITY OF MxA AGAINST INFLUENZA VIRUS

Abstract: 160042 P

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Human MxA protein belongs to the family of dynamin-like large GTPases and exerts antiviral activity primarily against negative-stranded RNA viruses, including influenza A (IAV). MxA is able to form higher order oligomeric structures. However, the mode of action of MxA remains unknown. There is increasing evidence that MxA targets the IAV nucleoprotein (NP) in conjunction with UAP56, a cellular DEAD-box RNA helicase, which is required for efficient IAV replication due to its chaperone activity for viral NP. In order to better understand the molecular mechanism of action of MxA we analyzed in detail the interplay between MxA, UAP56 and the viral target NP.

By performing co-immunoprecipitation (co-IP) as well as split-GFP experiments we were able to show that NP binds (the dimeric form of) MxA as well as UAP56. In order to narrow down the interaction domains of MxA and UAP56 with NP, we introduced N- and/or C- terminal deletions and point mutations to identify the binding regions of the trimeric complex. Furthermore, we were able to identify the residues in NP important for binding the MxA-UAP56 complex.

From a mechanistic point of view, we are investigating the importance of the oligomeric state of MxA as well as its GTPase function. We have evidence that a functional G-domain is important for proper localization of MxA but is dispensable for its binding to UAP56. The oligomeric state on the other hand is crucial for the binding of MxA to NP since this binding is increased if MxA is rendered dimeric. We are additionally testing whether binding of MxA to UAP-NP dimers leads to the sequestration of UAP56 and hence inhibition of IAV replication.

INVESTIGATING THE ROLE OF STRESS GRANULES AS AN INNATE CELLULAR ANTIVIRAL DEFENCE MECHANISM

Abstract: 160017 P

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Stress granules (SG) are highly dynamic ribonucleoprotein complexes that form in the cytoplasm in the event of cellular stress, such as oxidative stress, amino acid deprivation, heat shock and virus infection. They contain aggregates of stalled 48S translation initiation complexes and translationally silenced mRNAs. SG appear to be sites of mRNA storage under stress conditions and are responsible for the triage of mRNAs to either resume translation if the stress is resolved or direct them to RNA decay pathways if the stress persists. SG are frequently observed in virus infections and many viruses have developed means to modulate stress granule formation. However, it is unclear if SG play an active role in the innate host cell immune response. We aim to analyse to what extent SGs are in integrated into the innate cellular antiviral defence in the well-established models of coronavirus and Semliki Forest virus. In preliminary experiments, we were able to visualize SG during infection with the human coronavirus 229E and with the mouse hepatitis coronavirus (MHV). We will assess the overall composition of SG during a virus infection by using a proximity based APEX2 biotinylation approach coupled to mass spectrometry analysis. The functional impact of these SG components on virus replication will be further analysed by employing a siRNA based screening method. As an additional approach to elucidate if SG are involved in antiviral defence, we will investigate if viral RNA is detectable within SG or if the viral RNA manages to escape accumulation and translational silencing. SG components that display an antiviral effect in our screening methods will be further analysed in primary target cells of virus infection and we will assess if our findings can also be applied to the highly pathogenic severe respiratory syndrome coronavirus (SARS-CoV) and middle east respiratory syndrome coronavirus (MERS-CoV).

48 HOURS IN THE LIFE CYCLE OF PATIENT-DERIVED HIV-1 ISOLATES IN PRIMARY CD4+ T CELLS

Abstract: 160084 P

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The HIV-1 life cycle is very difficult to investigate *in vivo*. Experimental models have been invented to better understand the dynamics and single steps in the HIV-1 life cycle; however, they were carried out using laboratory-adapted HIV-1 strains and/or cell lines.
To further investigate the HIV-1 life cycle in a model closer to the *in vivo* conditions, we conducted a series of 48-hour long time course experiments using patient-derived HIV-1 isolates and primary CD4+ T cells from separate HIV-1 negative donors as inter-donor variability has been previously noted. Two HIV-1 isolates were selected according to their profound difference in viral fitness as measured by p24 ELISA. After infection with HIV-1, cell samples were collected at the time of infection (T0); six hours post infection (T6), and every 2 hours subsequently until 48 hours post infection. HIV-1 p24 antigen was measured to confirm infection, cellular HIV-1 DNA and RNA was isolated, and q(RT-)PCR assays carried out for various HIV-1 DNA and RNA forms associated with different steps of the HIV-1 life cycle.
Total HIV-1 DNA is detectable at hour 6 post infection followed by the appearance of 2-LTR DNA circles at hour 8 post infection. Early gene expression explored by multiple spliced HIV-1 RNA forms is first detected at hour 14 post infection, followed by late gene expression, i.e.,unspliced HIV-1 RNA, starting at hour 24 post infection. Our preliminary results reveal that the HIV-1 life cycle in primary CD4+ T cells infected with patient-derived HIV-1 isolates takes longer than observed in cell lines infected with laboratory-adapted HIV-1 strains. Furthermore, our model might be applicable to study differences in replication competence of distinct HIV-1 isolates in more detail.

DISSECTING VIRAL FEATURES LEADING TO BROADLY NEUTRALIZING ANTIBODY DEVELOPMENT IN AN HIV-1 INFECTED TRANSMISSION PAIR

Abstract: 160048 P

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The elicitation of broadly neutralizing antibodies (bnAbs) targeting the HIV-1 envelope (Env) glycoprotein is the ultimate goal for HIV-1 antibody-based vaccine design due to their ability to inhibit diverse heterologous HIV-1 strains. However, no immunization regimen tested thus far has successfully elicited bnAbs in humans. A comprehensive understanding of the precise determinants of bnAb induction and the pathways to their development is hence vital in order to recapitulate the natural bnAb response by immunization.

We recently identified 239 bnAb inducers in a cohort of 4,484 HIV-1 infected individuals within the framework of the Swiss HIV-1 Cohort Study and the Zurich Primary Infection Study. Among these we identified a linked subtype B female (North African) to male (South Asian) transmission pair where both partners developed a strong bnAb response with similar neutralization profiles as tested against a heterologous 42-virus panel. The high similarity in the bnAb response despite differences in patient demographic characteristics could potentially indicate that the envelope (Env) of the infecting virus harbored a particular capacity to imprint bnAb responses. The aim of the current study is to longitudinally dissect Env features that drove the development of neutralization breadth in this transmission pair.

Neutralization fingerprint analysis of longitudinal plasma from the transmitter and the recipient revealed that both individuals developed a CD4bs bnAb response. The transmitter developed in addition a V3 loop bnAb activity. Both CD4bs and V3 loop bnAb activity was confirmed by mutant virus testing. Autologous Envs from multiple time points have been cloned by single genome amplification and probed for sensitivity to autologous plasma, the partner plasma, heterologous plasma and defined bnAbs to explore the inhibitory resistance. Further genotypic and phenotypic characterization is underway to potentially define Env variants that may have stimulated the bnAb response in these donors.

IFI16 IS A RESTRICTION FACTOR OF AAV2 INFECTION

Abstract: 160099 P

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Adeno-associated virus (AAV) is a small, non-pathogenic, helper virus-dependent parvovirus with a single-stranded (ss) DNA genome of 4.6 kb. In absence of a helper virus, AAV establishes a latent infection either by integrating its genome site preferentially at the AAV pre-integration site (AAVS1) on human chromosome 19 or by persisting as an episome in the host cell nucleus. In the presence of a helper virus, such as herpes simplex virus type 1 (HSV-1), AAV can enter a lytic replication cycle. We have previously performed a global gene expression analysis (RNAseq) of AAV2 infected normal human fibroblasts (NHFs), resulting in 44`175 annotations. The screening revealed 1`929 genes that were differentially expressed (DE; $p < 0.01$, number of reads ≥ 40) in AAV2-infected and mock-infected cells. To further explore the 1`929 DE genes, a Gene Ontology (GO) term biological process analysis was performed using DAVID and graphically visualized as enrichment map using Cytoscape resulting in eight distinct clusters of biological processes, including DNA replication, DNA damage response (DDR), and cell cycle regulation. Among the top 50 DEs of the DDR cluster was IFI16, a cytosolic and nuclear sensor of ds- and ssDNA. IFI16 has been shown to be a restriction factor of many different viruses through various mechanisms, including interferon response, inhibition of transcription, and epigenetic modification.

In this study we addressed the question whether IFI16 is a restriction factor for AAV2 infection. Indeed, the post-transcriptional knockdown of IFI16 resulted in a significant increase in transduction efficiency of both ss and double-stranded self-complementary (sc) AAV2 vectors. Moreover, by combining multicolor immunofluorescence (IF) with fluorescent in situ hybridization (FISH) in AAV2 infected NHF cells, IFI16 was observed to accumulate in nucleoli together with AAV2 capsids and genomes. Overall, the data indicate an influence of IFI16 on AAV2 infection, which might be linked to its sub-nucleolar localization.

A FUNCTIONAL INSERTION SCREEN FOR HEPATITIS E VIRUS

Abstract: 160094 P

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Background: Hepatitis E virus (HEV) infection is believed to be the most common cause of hepatitis and jaundice in the world. Our current understanding of the molecular virology and pathogenesis of hepatitis E is scarce, especially due to the limited availability of functional tools. Our study aims at developing tagged HEV genomes for live cell imaging and as a tool to investigate the viral life cycle.
Methods: A selectable subgenomic HEV replicon served as a template for random 15-bp sequence insertion using transposon-based technology. Viable insertions mapping along the replicase sequence were selected in a permissive cell line. The identified insertion sites were further investigated and tagged with different fluorescent proteins to monitor HEV replicase expression.
Results: HEV replicons harbouring viable transposon insertions in the Y domain, the hypervariable region (HVR), and the helicase domain of the open reading frame 1 (ORF1) protein have been selected and further characterised. HA and GFP tag insertions allowed for the successful detection of the replicase in a context of genuine HEV RNA replication. Moreover, a full-length HEV genome harbouring an HA tag allowed for the production of infectious particles. These tagged HEV genomes are now being used to further characterise HEV replication sites in cultured cells and to investigate interactions with host proteins and cellular structures.
Conclusion: The development of tagged functional HEV genomes should allow to track viral replication complexes in live cells and should facilitate the identification of host factors, yielding new insights into the HEV life cycle.

IDENTIFICATION OF CELLULAR FACTORS INVOLVED IN HANTAVIRUS ENTRY INTO HUMAN RESPIRATORY EPITHELIAL CELLS AND DEVELOPMENT OF NEW THERAPEUTICS

Abstract: 160053 P

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Hantavirus are emerging rodent-borne viruses of the Bunyaviridae family that can be associated with severe human diseases with high mortality. The current lack of a licensed vaccine and the limited therapeutic options make the development of novel efficacious anti-viral agents to combat hantaviruses an urgent need. The identification of the cellular factor hijacked by hantaviruses in order to enter host cells is essential for the development of novel strategies to combat pathogenic hantaviruses.
Considering the biosafety restrictions linked to work with live pathogenic hantaviruses and that virus cell attachment and entry are mediated exclusively by the viral envelope, we established a pseudotype platform based on recombinant Vesicular stomatitis reporter virus (VSV) bearing the glycoprotein of the Old World prototypic hantavirus Hantaan virus (HTNV) and the South American Andes virus (ANDV).
In a first approach, we treated A549 cells with specific inhibitors against cellular factors implicated in macropinocytosis-related pathways. Cells were then infected and luciferase activity measured to assess productive infection. Our studies reveal significant inhibition of both VSV-HTNVGP and VSV-ANDVGP cell entry in presence of amiloride drugs that target sodium-proton exchanges, which are involved in macropinocytosis. Infection decreased also in presence of actin inhibitors, another essential feature of macropinocytosis. We found that with inhibitors acting on GTPases Cdc42 and Rac1 and their downstream effectors, VSV-ANDVGP infection was significantly inhibited while VSV-HTNVGP was not. Our results provide first evidence for a role of macropinocytosis in hantavirus entry into human respiratory epithelial cells with important virus-specific differences. In a second approach, we employed a semi high-throughput screening format of 96 well-defined kinase inhibitors in order to discover novel anti-viral therapeutic agents against hantaviruses. The screen yielded candidate broad-spectrum and virus-specific antiviral molecules. Interestingly, ANDV specific inhibitors were all associated with macropinocytosis-related cellular factors. Promising candidate anti-viral drugs are currently followed up and will be validated using live pathogenic hantaviruses in the new high containment facilities at Spiez Laboratory, Switzerland.

HEPARAN SULFATE BINDING IS A CRITICAL DETERMINANT OF ENTEROVIRUS 71 DISSEMINATION AND PATHOGENESIS IN HUMANS

Abstract: 160087 O

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Enterovirus 71 (EV71) causes hand, foot and mouth disease, a mild and self-limited illness that is sometimes associated with severe neurological complications. EV71 neurotropic determinants remain ill-defined to date. We previously identified a mutation in the VP1 capsid protein (L97R) that was acquired over the course of a disseminated infection in an immunocompromised host. The mutation was absent in the respiratory tract but was present in the gut (as a mixed population) and in blood and cerebrospinal fluid (as a dominant species). In this study, we demonstrated that this mutation enables EV71 to bind to the ubiquitously expressed heparan sulfate (HS) attachment receptor and modifies viral tropism in cell lines and in respiratory, intestinal and neural tissues. Variants with VP197L or VP197R were able to replicate to high levels in intestinal and neural tissues and, to a lesser extent, in respiratory tissues, but their preferred entry site (from the luminal or basal tissue side) differed in respiratory and intestinal tissues and correlated with HS expression levels. These data account for the viral populations isolated from the patients respiratory and intestinal samples and suggest that improved dissemination, resulting from an acquired ability to bind HS, rather than specific neurotropism determinants, enabled the virus to reach and infect the central nervous system. Finally, we showed that iota carrageenan, a highly sulfated polysaccharide, efficiently blocks the replication of HS dependent variants in cells and 2D neural cultures. Overall, the results of this study emphasize the importance of HS binding in EV71 in vivo pathogenesis and open new avenues for the development of antiviral molecules that may prevent this virus dissemination.

SUBCELLULAR MOLECULAR ENVIRONMENT OF CORONAVIRUS REPLICASE COMPLEXES REVEALED BY PROXIMITY LABELLING

Abstract: 160013 O

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Replication of coronaviruses and other positive-stranded RNA viruses is supported by a network of modified endomembranes. Coronaviruses derive endoplasmic reticulum membranes into double-membrane vesicles and convoluted membranes to which RNA synthesis and replication are closely associated. However, the molecular mechanisms that entail the generation of coronaviral replicative organelles remain elusive.

We employed a sensitive biotin ligase-based proximity labelling approach to identify host factors and molecular pathways involved in the establishment and sustainment of coronavirus replicative structures. By using a recombinant Murine Hepatitis Virus (MHV-BirA*-nsp2) containing a promiscuous biotin ligase embedded in its replicase complex, factors residing in close vicinity of the viral RTC during the whole course of infection become biotinylated and can be efficiently affinity purified for mass spectrometry identification.

Our results demonstrate that viral replicase gene products are significantly enriched over control conditions while structural components are not in proximity to the viral RTC during replication. Furthermore, we provide a catalogue of more than 500 candidates comprising putative proviral factors that assist the establishment of viral replication, and antiviral proteins, such intrinsic restriction factors that target the coronavirus RTC and trigger the first steps of an antiviral innate immune response. In order to clarify the functional involvement of candidate factors during coronavirus replication, we performed a systematic siRNA knockdown of each individual factor significantly enriched at coronavirus replication sites. Altogether, our results shed light on previously uncharacterized molecular events associated with the coronavirus replication and transcription complex and identify novel, critical and conserved virus-host interactions during coronavirus infection.

INFLUENZA A VIRUS INFECTION IMPACTS INTESTINAL MICROBIOTA DYNAMICS AND CAUSES QUANTITATIVE ENTERIC DYSBIOSIS

Abstract: 160105 O

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Microbiota integrity is essential for a growing number of physiological processes. Importantly, commensal microbiota provide a shield against invading bacterial pathogens. However, the impact of viral infections, on host microbiota composition and dynamics is poorly understood. Influenza A viruses (IAV) are common respiratory pathogens causing acute infections. Here, we characterized dynamics of intestinal microbiota following IAV infection using 16S next generation sequencing on a mouse model of IAV infection. Remarkably, we found transient change in composition of microbiota, as well as quantitative depletion of total bacteria in small intestine after IAV infection. We further prove that the number of culturable, viable bacteria residing small intestine decreased significantly, whereas it remained the same in stool samples. In parallel to depletion of bacterial content, we also observed IAV induces disruption of mucus integrity in small intestine, which in turn increased the risk of pathogen invasion in a mouse model of bacterial superinfection with S. typhimurium following IAV infection. We propose that qualitative and quantitative changes in microbiota in the course of an IAV infection could increase the risk of bacterial super-infection, by reducing the threshold this natural shield of commensals poses to invading bacterial pathogens. Mechanisms that lead to this phenomena, and causal links between microbiota and bacterial superinfection need further characterization.

THE CRITICAL ROLE OF HEMAGGLUTININ (HA) IN TRANSMISSION OF LOW-PATHOGENIC AVIAN INFLUENZA VIRUSES IN CHICKENS

Abstract: 160114 P

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Avian influenza viruses (AIV), which primarily replicate in the gastrointestinal tract of wild waterfowl, are shed into the environment at large quantities facilitating transmission to domestic poultry. Continuous circulation of H5 and H7 AIVs in domestic poultry may favour the evolution of highly pathogenic AIVs (HPAIV). To understand the adaptation process of AIV in poultry, we experimentally infected chickens via the intratracheal route with a number of low-pathogenic AIV (LPAIV) H5 and H7 isolates from wild waterfowl. While most H7 viruses were transmitted to contact animals without prior adaptation, none of the H5 LPAIV was passed to the sentinels. In contrast, a HPAIV H5N1 isolate was transmitted to contact birds but failed to do so when the HA proteolytic cleavage site was changed from a polybasic to a monobasic motif. Genetic reassortment between a non-transmittable H5N1 and a transmittable H7N7 revealed that the hemagglutinin (HA) was critical for cloacal shedding and transmission. Interestingly, a 7:1 reassortant harbouring the HA from a low-pathogenic chicken-adapted H5N2 virus was shed from the respiratory tract and transmitted to sentinel chickens. Further analysis using chimeric HA revealed that the globular head domain was sufficient to confer transmission. In contrast, mutations changing the pH threshold of fusion, the proteolytic cleavage site or potential glycosylation sites did not lead to virus transmission. These findings suggest that the HA globular head which contains the receptor-binding domain is a key factor for transmission of LPAIV in chickens. We are currently using defined synthetic oligosaccharides in order to understand the differential receptor-binding activity of duck-origin and chicken-adapted influenza viruses.

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Meeting Desk will be open only January 30th from 9 – 17h with Karin Dietze and Liv Lüscher.
In case of questions or help please call: 079 349 79 86.

On January 31st, the Meeting Desk will be closed.
For help please ask the hotel reception.

Wishing you all a pleasant 7th Swiss Virology Meeting!