5th Swiss Virology Meeting

September 9-10, 2014
Thun

Organizing and Scientific Committee (in alphabetical order)

Angela Ciuffi (University Hospital Center and University of Lausanne)
Monika Engels (University of Zurich)
Cornel Fraefel (University of Zurich)
Urs Greber (University of Zurich)
Stefan Kunz (University Hospital Center and University of Lausanne)
Darius Moradpour (University Hospital Center and University of Lausanne)
Matthias Schweizer (University of Bern)
Roberto Speck (University Hospital and University of Zurich)
Caroline Tapparel Vu (University Hospitals Geneva and University of Geneva)
Amalio Telenti (University Hospital Center and University of Lausanne)
Alexandra Trkola (University of Zurich)

www.swissvirology.ch
Many thanks to our generous Sponsors:
Tuesday, September 9, 2014

10:00 – 10:30      Arrival and Welcome Coffee

10:30 – 10:35      Welcome Address  Matthias Schweizer & Angela Ciuffi

10:35 – 11:20      PLENARY TALK SWISS VIROLOGIST ABROAD (CUSO)
                   Introduction: Cornel Fraefel
                   Michael Linden - King’s College London, UK

11:20 – 12:30      SESSION I: VIRUSES AND GENE THERAPY (CUSO)
                   Chairs: Cornel Fraefel and Ph.D. students

11:20 – 11:45      Birgit Dreier - University of Zurich
                   Retargeting adenovirus using a modular DARPin-based adapter strategy

11:45 – 12:00      Sandra Ivic - University Hospital Zurich
                   A novel in vitro/vivo selection strategy for lentiviral vectors (abstract 16)

12:00 – 12:15      Bruna Pereira - University of Zurich
                   Lentivirus-mediated targeting of myelin-antigen to dendritic cells induces tolerance of auto-reactive effector CD4+ T cells (abstract 28)

12:15 – 12:30      Vhibu Prasad - University of Zurich
                   Targeting the Secretory Pathway Enhances Human Adenovirus infection and Cancer Cell Killing (abstract 29)

12:30 – 14:00      Sandwich Lunch

12:30 – 14:00      Round table discussion of Ph.D. students with Michael Linden (CUSO)

14:00 – 16:00      SESSION II: VIRUS-CELL INTERACTION AND REPLICATION
                   Chairs: Urs Greber & Philipp Plattet

14:00 – 14:30      Stefan Kunz - Lausanne University Hospital and University of Lausanne
                   Targeting host cell factors to combat pathogenic arenaviruses

14:30 – 15:00      Jovan Pavlovic - University of Zurich
                   New insights into the antiviral function of Mx proteins

15:00 – 15:15      Stefania Luisoni - University of Zurich
                   Lysosomal secretion controls key lipids for adenovirus endocytosis and infection (abstract 20)

15:15 – 15:30      Yohei Yamauchi - ETH Zurich
                   Mechanism of influenza virus uncoating (abstract 42)

15:30 – 15:45      Mirco Schmolke - University of Geneva
                   The nucleoprotein of newly emerged H7N9 influenza A virus harbors a unique motif conferring resistance to human Mx protein (abstract 36)

15:45 – 16:00      Selene Gluck - University of Zurich
                   Is it host cell cycle arrest a pre-set condition for Rotavirus replication? (abstract 15)

16:00 – 18:30      Coffee Break and POSTER SESSION

19:00 – 21:00      Dinner
Wednesday, September 10, 2014

7:30 - 8:45  Breakfast

9:00 – 11:00  SESSION III:  IMMUNE RESPONSE, ANTIVIRAL THERAPY AND VACCINATION
Chairs: Huldrych Günthard & Stefan Kunz

9:00 – 9:30  Matthias Schweizer - University of Bern
Bovine viral diarrhoea: A playground for virologists and a target for eradication

9:30 – 10:00  Christian Münz - University of Zurich
Human tumor virus infection and immune control in vivo

10:00 – 10:15  Stéphanie Anchisi - University of Geneva
RIG-I ATPase activity and the discrimination of self/non-self RNA (abstract 1)

10:15 -10:30  Michel Crameri - University of Zurich
Interference of influenza A virus with type I interferon-mediated signaling (abstract 8)

10:30 – 10:45  Christoph Zürcher - University of Bern
Truncation of the C-terminus of pestivirus Erns strongly reduces its intracellular Activity (abstract 44)

10:45 – 11:00  Lucia Reh - University of Zurich
Degree of efficacy loss of broadly neutralizing antibodies during HIV-1 cell-to-cell transmission is strain and epitope dependent (abstract 31)

11:00 – 12:30  Coffee Break and POSTER SESSION

12:30 – 14:00  Lunch

14:00 – 16:00  SESSION IV:  MEDICAL AND ENVIRONMENTAL VIROLOGY, VIRUS EVOLUTION
Chairs: Laurent Kaiser & Angela Ciuffi

14:00 – 14:30  Nicolas Fasel - University of Lausanne
Metastatic leishmaniasis: the viral beast within!

14:30 – 15:00  Tamar Kohn - EPFL
Solar disinfection of waterborne viruses

15:00 – 15:15  Mojtaba Khosravi - University of Bern
Molecular Basis of Canine Distemper Virus-Mediated Membrane Fusion through SLAM Receptors (abstract 17)

15:15 – 15:30  Viet Loan Dao Thi - Lausanne University Hospital
Investigation of the Polyprotein Encoded by Hepatitis E Virus Open Reading Frame 1 (abstract 9)

15:30 – 15:45  Manuel Schibler - University of Geneva
Experimental Rhinovirus Recombination in the Polyprotein Coding Region (abstract 34)

15:45 – 16:00  Corinna Oberle - University Hospital Zurich
Characterization of transmitted/founder viruses and their sources in HIV-1 transmitter/recipient pairs (abstract 26)

16:00 – 16:15  Best poster awards

16:15 – 16:30  Farewell Address
ABSTRACTS

(by alphabetical order of the presenting author)
**RIG-I ATPase activity and the discrimination of self/non-self RNA**

Stéphanie Anchisi, Jessica Guerra, Dominique Garcin.

*Department of Microbiology and Molecular Medicine, Faculty of Medicine, University of Geneva, Geneva, Switzerland.*

Many RNA viruses are detected by RIG-I, a cytoplasmic sensor that triggers an antiviral response upon binding non-self RNA that contains a stretch of dsRNA bearing a base-paired 5’ppp nucleotide. To gain insight into how RIG-I discriminates between self and non-self RNA, we used duplexes whose 3’ strand contains both ribo- and deoxynucleotides and examined their binding to RIG-I, their relative ability to stimulate ATPase activity, to induce RIG-I dimerization on the duplex, and to induce IFNβ. Although necessary, the precise role of RIG-I ATPase activity remains unknown. We show that the chemical nature of the 3’ strand is not critical for RIG-I binding. However, two key ribonucleotides, at positions 2 and 5 on the 3’ strand, are minimally required for RIG-I ATPase activity that is necessary but not sufficient for IFNβ stimulation. We find that model self RNAs with shorter stretches of dsRNA bind to RIG-I less stably, but nevertheless have an enhanced ability to stimulate the ATPase. Moreover, ATPase activity promotes RIG-I recycling on RIG-I/dsRNA complexes. As pseudo-self-RNAs bind to RIG-I less stably, they are preferentially recycled by ATP hydrolysis that weakens helicase domain binding of dsRNA. Our results suggest that one function of the ATPase is to restrict RIG-I signaling to its interaction with non-self RNA.
Full length quasispecies analysis of bovine viral diarrhoea virus

Claudia Bachofen¹,², Kim Willoughby¹, Ruth Zadoks¹, George Russell¹.

¹ Virology Department, Moredun Research Institute, Edinburgh, United Kingdom.
² Current address: Institute of Virology, Vetsuisse faculty, University of Zurich, Switzerland.

Bovine viral diarrhea virus (BVDV) a pestivirus of the flaviviridae family is one of the most important cattle pathogen in first world countries. The amazingly broad host range of the virus may lead to formation of virus reservoirs in other ruminant species and could thus represent a pitfall for the long-term success of the BVDV eradications that are ongoing in several European countries. Assumingly, the quasispecies nature of this RNA virus facilitates interspecies transmission by providing genetic plasticity. Therefore, analysis of the spectrum of virus variants present in BVDV persistently infected animals may reveal important information on the quasispecies dynamics involved in interspecies transmissions. In order to analyse quasispecies diversity in an unbiased way, a method enabling generation of viral cDNA for next generation sequencing without using specific primers or amplification had to be developed. After testing several methods, the combination of polyadenylation of the viral 3’end and using oligo-dT primers for reverse transcription proved most successful. In a preliminary experiment the quasispecies diversity over the entire length of the BVDV genome (12.5kb) was successfully determined. First analyses using laboratory and field strains revealed distribution and frequency of single nucleotide polymorphisms as well as providing evidence for the surprisingly rapid loss of diversity after only three passages in cell culture. In addition to being an essential pre-requisite for studies on interspecies transmission of BVDV, the method may be applicable to other RNA viruses and thus be helpful to increase our understanding of the molecular basis of interspecies transmissions e.g. of zoonotic viruses.
**HIV-1 adapted to replication on target cells with low CD4 levels shows enhanced neutralization sensitivity, CCR5 usage and pro-inflammatory macrophage infectivity**

David Beauparlant*1, Peter Rusert*1, Corinna Oberle2, Carsten Magnus1, Jacqueline Weber1, Therese Uhr1, Huldrych Günthard2, Alexandra Trkola1.

*Coauthors

1 Institute of Medical Virology, University of Zurich, Zurich, Switzerland.
2 Division of infectious Diseases and Hospital Epidemiology, University Hospital Zurich, Zurich, Switzerland.

A hallmark of HIV infection is the continuously declining number of the virus’ predominant target cells, the CD4 lymphocytes. Throughout the course of the disease the virus thus needs to adapt to an ever changing target cell environment. Changes in co-receptor usage, reflecting the virus’ demand on a wider host cell range (including cells expressing lower CD4 levels), are well documented at later disease stages. How the virus adapts to the changing target cell repertoire has not been conclusively resolved.

Our current study aimed to dissect the evolution of envelope-CD4 interaction in a controlled setting in vitro. Specifically, we mimicked the decline of CD4\textsuperscript{High} target cells, seen in vivo, to explore the consequences that envelope adaptation to replicate in CD4\textsuperscript{Low} cells has on envelope functional characteristics including the shielding of the trimer and interaction with CD4 and coreceptor.

We show that adaptation of a molecular clone of HIV to growth on target cells with decreasing CD4 levels elevates sensitivity to neutralizing Abs targeting the coreceptor-binding V3 loop and the CD4-binding site. Soluble CD4-IgG\textsubscript{2} also neutralizes the adapted viruses with 1000x higher potency. Most interestingly, the adaptation to low CD4 levels was accompanied by an increased ability to use CCR5, and an enhanced ability to infect M1-like monocyte derived macrophages. These severe adaptations are partially reversed when the virus regains access to target cells with normal CD4 levels, suggesting that maintaining an ‘optimal’ level of CD4 binding efficiency is advantageous for the virus.
**Induction of heterosubtypic antibodies against Influenza A in mice**

Matteo Bianchi, Arek Wyrzucki, Ines Kohler, Michael Maurer, Lars Hangartner.

Institute of Medical Virology, University of Zurich, Zurich, Switzerland.

Influenza A viruses can rapidly mutate their antigenic structures as a strategy to escape pre-existing humoral immunity. Vaccines to influenza therefore only protect against the immunizing or closely related strains, and have to be annually reformulated. A universal vaccine that induces broad immunity against influenza A viruses, including newly emerging strains, is a long-sought goal in medical research.

We have designed two version of an experimental antigen based on HA proteins from different influenza subtypes. These antigens have occluded the strain-specific epitopes while prominently exposing the conserved epitopes of the stem. In phage display, one version of the antigen only isolated heterosubtypic antibodies with restricted breadth, while the second version of the antigen, referred to as Ag2, isolated antibodies of much wider breadth. It was therefore chosen for immunization of mice.

In mice, Ag2 could elicit a broad immune response including antibodies recognizing HA from all group 1 and group 2 subtypes tested. When neutralizing titers were assessed, only antiviral activity against the homologous or closely related subtypes was detected, indicating that the majority of the heterosubtypic antibodies induced were not broadly neutralizing. Nevertheless, these heterosubtypic antibodies were found to be binding the HA2 subunit of HA, indicating the presence of at least one additional very conserved epitope in the stem of influenza A hemagglutinin. Moreover, even in the absence of neutralizing titers, antibodies induced by Ag2 were found to provide protection of mice against lethal exposure to a heterologous virus.

*Work financed by Swiss National Science Foundation grant PP00P3_123429.*
Exploring the molecular correlates of attenuation using a clone of Small Ruminant Lentiviruses generated from a field isolate

Laure Blatti-Cardinaux\textsuperscript{1,2}, Marie-Luise Zahno\textsuperscript{1}, Ramsés Reina\textsuperscript{3}, Giuseppe Bertoni\textsuperscript{1}.

\textsuperscript{1} Institute of Virology and Immunology, University of Bern, Bern, Switzerland.
\textsuperscript{2} Graduate School for cellular and biomedical sciences, University of Bern, Bern, Switzerland.
\textsuperscript{3} Instituto de Agrobiotecnología, CSIC-Universidad Pública de Navarra, Mutiliva Baja, Navarra, Spain.

The small ruminant lentiviruses (SRLV) are retroviruses infecting sheep and goats. They are divided into five groups A-E, and further subdivided into subtypes. In spite of a successful eradication campaign that completely eliminated SRLV induced arthritis in Swiss goats, SRLV serological escape mutants are still circulating in Switzerland. Their hallmark is a notably reduced virulence \textit{in vivo}, associated with a very efficient transmission between animals, potentially related to their pronounced tropism for the mammary gland, where SRLV induced lesions were detected.

The aim of this study is to characterize at the molecular level these peculiar attenuated SRLV strains. For this purpose, an infectious molecular clone of a Swiss field isolate was constructed. Since we hypothesized that specific mutations in the long terminal repeats (LTR) were responsible for the reduced virulence, we first used a reporter gene assay to select mutations having an impact on the LTR promoter activity. In a next step, the promising mutations were introduced in the molecular clone. All these mutants were found to be infectious, and we will present and discuss results obtained by infecting different primary cells and cell lines with these viruses.

Additionally, the envelope and/or the LTR of a highly pathogenic SRLV were substituted in the molecular clone, as both env and LTR play an important role in the context of virulence and cell tropism. These mutants were infectious and a series of experiments in different cells are in progress.
Conformational protection of antibody epitopes on the native HIV-1 envelope glycoprotein trimers

Ivan Branislav 1, Oliver F. Brandenberg1, Carsten Magnus1, Roland Regoes2, Peter Rusert1, Alexandra Trkola1.

1 Institute of Medical Virology, University of Zurich, Zurich, Switzerland.
2 Institute of Integrative Biology, ETH Zurich, Zurich, Switzerland.

Antibodies binding non-native forms of HIV-1 envelope glycoprotein (Env) appear very early in HIV-1 infection and are also routinely generated in animal and human vaccination trials. However, such antibodies fail to bind the functional envelope trimer and to neutralize an overwhelming majority of clinical HIV-1 isolates. Understanding the shielding of antibody epitopes on the native, trimeric HIV-1 Env is therefore of consequence for the design of future vaccination strategies. A major driver of resistance to neutralization is the V1V2 domain of gp120 which shields neutralization sensitive epitopes, in particular the V3 loop of gp120 with high efficiency. Two modes of V3 loop shielding by the V1V2 loop have been proposed: I) self-protection where both V3 and V1V2 are located on the same protomer within the Env trimer and II) neighboring-protection where the V1V2 loop from one protomer shields the V3 loop on the neighboring protomer. To date, structural and functional studies of the HIV-1 envelope yielded inconclusive or even seemingly contradicting results concerning the mode of V3 protection by the V1V2 loop. This raises the intriguing possibility that in fact both protection modes may occur and that a given V1V2 may either switch between the two protection modes or alternatively, may use one protection mode preferentially depending on the envelope studied. Here we present a detailed analysis of assay formats and parameters used to determine the shielding mode of V1V2 to derive a strategy that allows best the definition of alternate shielding modes.
Investigating of the Canine Distemper Virus (CDV) Matrix Protein

Fanny Bringolf¹, Nadine Ebert¹, Lisa Alves², Mojtaba Kohsravi¹, Andreas Zurbriggen¹, Philippe Plattet¹.

¹ Division of Experimental Clinical Research, Neurology Unit, DCR-VPH, Vetsuisse faculty, University of Bern, Bern, Switzerland.
² Division of Neurology, Vetsuisse faculty, University of Bern, Bern, Switzerland.

Canine distemper virus (CDV) belongs to the genus Morbillivirus in the family Paramyxoviridae, which also includes the human pathogen Measles virus (MeV) and the newly “eradicated” Rinderpest virus. As all morbilliviruses, CDV is a highly infectious pathogen, which infects lymphatic cells, epithelial tissue of many organs and, eventually, cells of the central nervous system. Cell-to-cell transmission as well as production of viral particle differs highly depending on the viral strain and the type of infected tissues. Interestingly, the matrix protein (M) is proposed to act as a central determinant in regulating these two critical mechanisms (budding and transmission). Although it is well known that M can associate with the plasma membrane and interact with different viral and cellular proteins, the precise molecular mechanisms underlying these key functions remain to be elucidated. Recent high and low resolution structural data suggested that the paramyxovirus M protein also assembles into homo-oligomers that may eventually form supra-molecular structures. The ability of M to homo-oligomerize was subsequently proposed to contribute to the induction of the necessary membrane curvature for the virus to bud. Here, we provide biochemical, cellular and functional evidences that, indeed, the CDV matrix protein readily oligomerizes. Furthermore, using a library of 58 alanine mutants (that span the entire M protein) we identified essential microdomains that controlled functional M-M binding, although not necessarily mapping at the putative dimeric interface. Overall, our preliminary findings suggest that proper M-M assembly is required for an appropriate localization and, potentially, for viral budding and transmission.
Reconstruction of HIV-1 Full-length Reference Genomes from Short Sequence Reads

Nottania K. Campbell1,2*, Corinna S. Oberle1,2*, Francesca Di Giallonardo1,2, Christine Leemann1, Stefan Schmutz1, Yannick Duport1, Huldrych F. Günthard1,2*, and Karin J. Metzner1,2*.

1 Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, Zurich, Switzerland.
2 Life Science Zurich Graduate School, University of Zurich, Zurich, Switzerland.

Current methods in the evolutionary analysis of viral next-generation sequencing (NGS) data employ aligning sequence reads to a reference genome. Although practical this convention results in alignment errors due to the genetic distance between these sequences and the reference, subsequently leading to an under-/overestimation of diversity. De novo assembly as an alternative approach is especially problematic when investigating the diversity of heterogeneous viruses such as the Human Immunodeficiency Virus type 1 (HIV-1). Current assembly algorithms fail to resolve this, consequently a sophisticated workflow is needed for minimizing pseudo-diversity, so as to obtain an accurate overview of the variants present in viral populations.

For this HIV-1 RNA was isolated from patients’ samples as described by F. Di Giallonardo et al. (Nucleic Acids Research. 2014). We analyzed 8 HIV-1 acutely (low diversity) and 8 HIV-1 chronically (high diversity) infected patients via Illumina-MiSeq, performed quality trimming and filtered for reads >100 bp with an average Phred score >30. These were then analyzed with three alignments methods: sequential alignment refinement to HIV-1_HXB2, de novo assembly with a HIV-1_HXB2 scaffold, and de novo assembly.

Sequential alignment entropy ~3.3% acute, and ~4.6% chronic; de novo with HIV-1_HXB2 scaffold alignment entropy: ~2.4% acute, and ~3.7% chronic. De novo assembly alone was not feasible, however, using HIV-1_HXB2 genome as a scaffold for contigs ±1 round of refinement proved to be the best method for deriving accurate reference assemblies.

Using our sophisticated alignment workflows we’ve generated full-length reference genomes, an essential starting point for future evolutionary analyses.
Interference of influenza A virus with type I interferon-mediated signaling

Michel Crameri1, Thomas Ludersdorfer2, Jovan Pavlovic1.

1 Institute of Medical Virology, University of Zurich, Zurich, Switzerland.
2 Institute of Virology and Immunology (IVI), Mittelhäusern, Switzerland.

Type I interferons (IFNs) act as the first line of defense against viral infections. Induced by pattern recognition receptors in infected cells, they are secreted and bind to their cognate receptor in an autocrine or paracrine fashion. Thereby, they initiate the IFN-mediated signaling cascade that eventually leads to the establishment of an antiviral state owing to the production of IFN-induced effector proteins. Type I IFN-mediated signaling involves phosphorylation of signal transducer and activator of transcription 1 (STAT1) and STAT2. Phosphorylated STATs heterodimerize and translocate to the nucleus, where they activate transcription of IFN-responsive genes.

Previous studies have shown that influenza A virus (IAV) disrupts type I IFN-mediated signaling. However, the mechanisms involved are currently only marginally characterized and remain a matter of dispute. In this study, we first investigated which viral factors are responsible for blocking the signaling response to IFN. Using a luciferase-based reporter system, we found that forced expression of the non-structural protein 1 (NS1) derived from an avian IAV strain showed a dramatic negative impact on type I IFN-induced gene expression. However, STAT1 phosphorylation did not appear to be affected in cells overexpressing NS1. At the same time, an avian IAV strain deficient for NS1 was still able to block IFN-induced gene expression. Here, we found that inhibition of the type I IFN-mediated signaling cascade occurred at a step before STAT1 phosphorylation.

Taken together, we suggest that influenza A viruses have evolved multiple strategies to inhibit type I IFN-mediated signaling, relying on both NS1-dependent and NS1-independent functions.
Investigation of the Polyprotein Encoded by Hepatitis E Virus Open Reading Frame 1

Viet Loan Dao Thi, Darius Moradpour, Jérôme Gouttenoire.

Division of Gastroenterology and Hepatology, Lausanne University Hospital (CHUV) and University of Lausanne, Lausanne, Switzerland.

Hepatitis E virus (HEV) is believed to be the most common cause of acute hepatitis and jaundice in the world. However, current knowledge of the molecular virology of hepatitis E is scarce. The HEV positive-strand RNA genome harbours 3 open reading frames (ORFs). Whereas ORF2 and 3 encode the viral structural proteins, ORF1 encodes the functional domains required for viral RNA replication. It is unclear whether these are processed into distinct proteins or act as a large protein with multiple functions.

Using specific antibodies directed against functional ORF1 protein domains, we investigated the putative polyprotein processing i) in a wheat germ-based cell-free expression system, ii) in newly established human cell lines inducibly expressing HEV ORF1, and iii) in selectable subgenomic HEV replicons derived from the HEV genotype 3 Kernow-C1 strain. The latter system is currently also being exploited to evaluate novel antiviral approaches against hepatitis E. The product of HEV ORF1 was detected only as a full-length protein in the three experimental systems investigated, including in a context of genuine viral RNA replication. These results indicate that no or only very inefficient processing of the protein encoded by ORF1 occurs during viral replication.

Our results indicate that HEV may be peculiar among positive-strand RNA viruses in expressing a large protein comprising all necessary functions to exert RNA replication. Efforts are ongoing to validate these findings in a fully infectious system. If confirmed, our findings yield a number of intriguing questions regarding the functional organization, structure and cell biology of the protein encoded by HEV ORF1.
Generic adenovirus delivery system using DARPin-based adapters

Birgit Dreier¹, Annemarie Honegger¹, Christian Hess¹, Gabriela Nagy-Davidescu¹, Peer R. E. Mittl¹, Markus Grütter¹, Markus Schmid¹, Natalya Belousova², Galina Mikheeva², Victor Krasnykh² and Andreas Plückthun¹.

¹ Department of Biochemistry, University of Zurich, Zurich, Switzerland.
² Department of Experimental Diagnostic Imaging, M.D. Anderson Cancer Center, University of Texas, Houston, TX, USA.

Adenoviruses (Ad) have shown promise as vectors for in vivo gene delivery in clinical trials. Efficient viral targeting to a tissue of choice requires both the ablation of the virus’ original tropism and the engineering of an efficient receptor-mediated uptake by a specific cell population. To this end, we have developed a series of bispecific adapters by fusing two modules, both consisting of Designed Ankyrin Repeat Proteins (DARPins): one binding with unprecedented functional affinity to the fiber knob of Adenovirus serotype 5 (Ad5), the other module binding to one of several tumor markers tested (Dreier et al., (2013) PNAS 110:E869-877). By solving the crystal structure of the complex of the trimeric knob with three bound DARPins at 1.95 Å resolution, we could use computer modeling to design a link to a trimeric protein of extraordinary kinetic stability, the capsid protein SHP from the lambdoid phage 21. We arrived at a module which binds the knob like a trimeric clamp and which remains stably bound to the virus’ knob for at least 10 days. When this ‘quasi-covalent’ module was fused to DARPins of varying specificities, it enabled efficient Ad5-mediated delivery of a transgene in a HER2-, EGFR- or EpCAM-dependent manner with transduction efficiencies comparable or even exceeding those of Ad5 itself. By using these adapters, which can be efficiently produced in E. coli, Ad5 can be rapidly converted to new receptor specificities using any ligand as receptor binding moiety. In this way pre-fabricated Ad5 viruses with different payloads can readily be charged and retargeted to many cell types and tissues of choice. We are presently working on an expansion to additional cell specificities for retargeting of Ad5 and detargeting of Ad5 from off-target cell tropism.
**Human Cathepsin W is a host factor required for escape of influenza A virus from late endosomes**

Thomas O. Edinger, Marie-Theres O. Pohl, Silke Stertz.

*Institute of Medical Virology, University of Zurich, Zurich, Switzerland.*

Human Cathepsin W (CtsW) is a lysosomal peptidase, which was identified in a genome wide siRNA screen to be required for influenza A virus (IAV) entry\(^1\). Using a set of different siRNAs directed against human cathepsin W mRNA, we confirm that reducing protein levels results in impaired IAV entry into lung epithelial cells (A549). We observed significant reduction in viral titers after siCtsW treatment but no impact on cell viability or induction of ISGs (interferon stimulated genes). This reduction was stable over time and viral replication was not restored to control levels after more than two days infection. By dissecting the entry process of IAV into different steps, we identified the exact target step where CtsW is playing its crucial role. Using different labeling and imaging techniques, we could eliminate attachment, internalization and early endosomal trafficking as entry steps that require CtsW. In contrast, we found that late endosomal trafficking of IAV is inhibited in siCtsW treated A549 cells. Confocal imaging revealed viral particles trapped in LBPA\(^-\)-(lysobisphosphatidic acid)-compartments representing late endosomes in siCtsW treated cells. To confirm this effect, we performed a fusion analysis with a dual color labeled virus to detect fusion of viral membranes with endosomal membranes. Cathepsin W knockdown cells showed a significant reduction in the number and size of fusion sites compared to control cells. Finally, we established a mutant cell line which expresses an enzymatically inactive form of CtsW. Infection experiments with these cells indicate the necessity of the catalytic triade of CtsW for the entry process of IAV.

\(^1\)Human host factors required for influenza virus replication. König, R., S. Stertz et al., *Nature* **463**:813-7
**The dynamics of filamentous and globular Mammalian Orthoreovirus factories relies on the microtubule network**

Catherine Eichwald¹,², Max L. Nibert² and Mathias Ackermann¹.

¹ Institute of Virology, University of Zurich, Zurich, Switzerland.
² Department of Microbiology and Immunobiology, Harvard Medical School, Boston, MA, USA.

Mammalian orthoreovirus replicates in cytosolic inclusions called viral factories which, depending on the viral strain, can adopt filamentous or globular morphology. Here we compared the dynamics of formation of filamentous and globular viral factories, directly quantifying the size, number, localization and morphology of viral factories at different times post-infection. Our results show that both types of viral factories are highly dynamic, ultimately condensing at the perinuclear region. We present clear evidence that globular viral factories rely on both the MT-network and the molecular motor dynein for the assembly and maintenance of their structure and also for the perinuclear region condensation. Additionally, we studied filamentous factory-like structures (FLS) formation by co-expressing μ2 and μNS at different ratios and show that μ2-MT association, perinuclear condensation and MT-stabilization are all dependent on μNS expression levels. Additionally, we provide solid evidence showing that both filamentous viral factories and FLS are able to recruit components from the host centrosome such as γ-tubulin and centrin by establishing MTOCs (microtubule organizing centers) inside the viral factories. Our findings provide important insights in the dynamics of formation of both filamentous and globular viral factories, describing a new function for the multipurpose viral protein μ2 as a non-centrosomal attachment factor.
RNA interference screen reveals cellular factors involved in HSV-1-supported AAV2 replication

Francesca D. Franzoso¹, Artur Yakimovich ², Rebecca Vogel¹, Urs Greber², Bernd Vogt¹, Mathias Ackermann¹, Cornel Fraefel¹.

¹ Institute of Virology, University of Zurich, Zurich, Switzerland.
² Institute of Molecular Life Sciences, University of Zurich, Zurich, Switzerland.

Adeno-associated virus type 2 (AAV2) is a non-pathogenic, helpervirus-dependent parovirus that replicates and assembles in nuclear replication compartments (RCs) which recruit viral and host cell proteins. To identify those cellular factors that positively or negatively affect AAV2 replication in cells co-infected with the helper virus HSV-1, we conducted a functional small interfering RNA (siRNA)-based screen in HeLa cells. We used a short library of three independent siRNAs per target gene. The 63 target genes were previously identified as host cell proteins associated with AAV2 RCs in presence of either HSV-1 or adenovirus as the helper virus. Different combinations of viruses were used in the following experimental conditions: i) to define those cellular proteins that have a role in AAV2 replication, the cells were co-infected with recombinant AAV2 encoding EGFP fused with the AAV2 Rep protein and wild-type (wt) HSV-1; (ii) to study early steps of the AAV2 life cell cycle prior to DNA replication, we infected the cells with a self-complementary (sc) AAV vector encoding EGFP in presence or absence of wt HSV-1: (iii) to assess potential roles of these proteins in early steps of HSV-1 infection, we infected the cells with a recombinant HSV-1 encoding EGFP. The results of these screens will be presented and discussed in the context of AAV2 and helpervirus biology.
High tolerance to sequence variation allows precise and rapid definition of ELITE HIV neutralizing antibodies

Nikolas Friedrich¹, Emanuel Stiegeler¹, Carsten Magnus¹, Umut Karakus¹, Jaqueline Weber¹, Therese Uhr¹, Huldrych Günthard², Peter Rusert¹, Alexandra Trkola³.

¹ Institute of Medical Virology, University of Zurich, Zurich, Switzerland.
² Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, University of Zurich, Zurich, Switzerland.

To date ELITE HIV-1 neutralizing antibodies (ENAbs), defined by superior inhibitory breadth and potency, are identified via large panels of HIV-1 isolates comprising 100-200 clones of different subtypes. We propose here an alternative approach to assess the quality of neutralizing Abs and inhibitors taking into account the structural plasticity of the virus envelope spike related to its extraordinary capability to constantly acquire mutations during infection and to evade the humoral immune response while maintaining functionality. Using a gp120 alanine mutant pseudovirus panel of the clade B envelope JR-CSF we have systematically analyzed envelope conformational plasticity and its consequences for the neutralizing activity of antibodies and inhibitors targeting various epitopes on gp120 and gp41. ENAbs including VRC01, PGT121, and PGT128 proved to be largely resistant to conformational changes induced by mutations in gp120. In contrast, mAbs with more limited neutralization breadth, including broadly neutralizing Abs directed against the CD4-binding site (b12), gp120-V3 (447-52D) and gp41-MPER (4E10), showed variable potency across the virus mutant panel and allowed the identification of mutants with generally enhanced neutralization sensitivity. Neutralization data with HIV-1 patient plasma demonstrates that these mutants are sensitive to antibodies commonly developed during infection in humans. In agreement, according to sequence data from genetically diverse clones, variability is restricted in these positions. In most cases general sensitivity can be attributed to compromised epitope shielding by the gp120 subdomain V1V2. Moreover, monitoring sensitivity against the gp41-MPER directed mAbs allowed a systematic analysis of the cross-talk between the two envelope subunits gp120 and gp41.
Is it host cell cycle arrest a pre-set condition for Rotavirus replication?

Selene Glück, Mathias Ackermann, Catherine Eichwald.

Institute of Virology, University of Zurich, Zurich, Switzerland.

The Rotavirus (RV) replication machinery requires a stabilized cytosolic microtubule (MT)-network and also the activity of kinesin Eg5. At the onset of mitosis and interphase the MT-network gets depolymerized, allowing the nucleation of short microtubules at the centrosomes, following the spindle assembly. Here, we hypothesize that rotavirus interferes with the MT-breakdown by arresting the cells before mitosis. To test this hypothesis, we used synchronized MA104 cells, a RV permissive cell line, at the onset of S-phase and monitored the cell cycle progression after infection with RV by staining with propidium iodide, followed by flow cytometry. Our results show that different RV strains, like the simians SA11 and RRV as well as the porcine OSU, arrest cells in S/G2 phase when compared with the non-infected condition. In addition, we found that the cells lines CV-1, Caco-2 and MDCK were also arrested in S/G2 phase upon infection with RV. Our results highly suggest that RV uses a common pathway for the arrest of the host cell cycle that is RV-strain and cell-line independent. Additionally, we found that the molecular motor Eg5, which normally localizes near centrosomes, is disperse in the cytosol surrounding the RV-viroplasms in infected cells. Finally, we show a series of experiments aimed at elucidating the responsible viral component involved in the cell cycle arrest and in the re-distribution of the molecular motor surrounding the viroplasms.
A novel in vitro/vivo selection strategy for lentiviral vectors

Sandra Ivic\(^1\), Renier Myburgh\(^1\), Karl-Heinz Krause\(^2\), Roberto F. Speck\(^1\).

\(^1\) Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, University of Zurich, Zurich, Switzerland.
\(^2\) Department of Pathology and Immunology, Geneva Medical Faculty and Department of Genetic and Laboratory Medicine, Geneva University Hospitals, Centre Médical Universitaire, Geneva, Switzerland.

Genetic engineering of hematopoietic progenitor cells (HPC) by lentiviral-based vectors suffers from their low transduction rate with ~20% when aiming a single integration event. This amount of transgene expression may not be sufficient for the correction of a number of diseases. We propose a novel in vitro/in vivo selection/suicide system based on the expression of the human reduced folate carrier 1 (hRFC1) and the human ribonucleoside-diphosphate reductase subunit M2 (RRM2). hRFC1 is the main transport protein for the uptake of folates which are indispensable for purine synthesis and therefore for DNA synthesis. A greater expression level of hRFC1 should render cell more sensitive to killing by methotrexate (suicide) but resistant against trimetrexate (selection) due to their pharmacological properties - untransduced cells are expected to show the opposite effects with higher resistance to methotrexate but lower threshold killing to trimetrexate. RRM2 catalyzes the reduction of ribonucleotides into deoxyribonucleotides and its overexpression should lead to resistance to its inhibitor hydroxyurea (selection of transduced cells). We expect an additive or even synergistic effect when trimetrexate and hydroxyurea are given together on selection/killing of transduced vs. untransduced cells. We are currently examining the concept proposed in vitro using various cell lines. Provided promising results, we then move forward to gene engineering HPC and their transplantation into humanized mice. Transduced cells would be monitored via GFP in vivo for their proliferation or disappearance depending upon the compounds administered.
Molecular Basis of Canine Distemper Virus-Mediated Membrane Fusion through SLAM Receptors

Mojtaba Khosravi\textsuperscript{1,2}, Fanny Bringolf\textsuperscript{1,2}, Lisa Alves \textsuperscript{2,3}, Maria Bieringer\textsuperscript{4}, Jürgen Schneider-Schaulies\textsuperscript{4}, Andreas Zurbriggen\textsuperscript{5} and Philippe Plattet\textsuperscript{1}.

\textsuperscript{1} Division of Experimental Clinical Research, DCR-VPH, Vetsuisse faculty, University of Bern, Bern, Switzerland.
\textsuperscript{2} Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland.
\textsuperscript{3} Department of Neurology, Vetsuisse faculty, University of Bern, Bern, Switzerland.
\textsuperscript{4} Institute for Virology and Immunobiology, University of Würzburg, Würzburg, Germany.

Despite availability of safe and effective vaccines, both measles virus (MeV) and canine distemper virus (CDV) continue to cause infections with high mortality and morbidity in humans and animals. Both viruses rely on two envelope glycoproteins, the attachment (H) and fusion (F) proteins, and a host cell surface receptor (SLAM or Nectin-4) to execute membrane fusion for cell entry. Although it was recently reported that CDV hardly replicates in human SLAM (hSLAM)-expressing Vero cells, single amino acid substitution in the H-protein (D540G or P541S) was sufficient to trigger productive infectivity. Using transient expression systems, we refined the molecular basis of CDV-H adaptation to hSLAM. Our findings revealed that only tiny amino acid substitutions are tolerated at positions H-540 and H-541 to productively interact with hSLAM. Furthermore, we identified three residues in hSLAM locating at the putative binding interface which, when mutated into the canine SLAM (cSLAM) corresponding amino acid (E71G, E75K and R130H) enabled membrane fusion triggering. Remarkably, covalent H-SLAM molecules were efficiently engineered by substituting residues H-P541 and SLAM-E/G71 into cysteines, thereby confirming their close spatial proximity. Because residue 71 of SLAM locates in a loop region that is predicted to dock slightly differently on CDV-H compared to MeV-H, our findings suggest that the side-chain volume of residue SLAM-71 combined with structural flexibility of the loop microdomain are two essential contributing factors leading to productive interaction with CDV-H. Overall, our data reveal the ease of CDV-adaptation to the human SLAM receptor; a characteristic that spotlights its threatening zoonotic potential.
Characterization of HIV-1 Integration Sites in Macrophages Infected with Autologous Virus

Yik Lim Kok\textsuperscript{1,2}, Valentina Vongrad\textsuperscript{2}, Huldrych F. Günthard\textsuperscript{2}, Karin J. Metzner\textsuperscript{2}.

\textsuperscript{1} Life Science Zurich Graduate School, University of Zurich, Zurich, Switzerland.
\textsuperscript{2} Department of Medicine, Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, Zurich, Switzerland.

Integration site analysis of HIV-1 in macrophages, a relatively under-examined cellular target of HIV-1, has been particularly challenging owing to the fact that it is too invasive to obtain tissue macrophages from HIV-1+ patients directly. To mimic an \textit{in vivo} infection, we have examined HIV-1 integration sites in primary monocyte-derived macrophages (MDMs) extracted from seven HIV-1 infected patients, which were subsequently infected \textit{ex vivo} with autologous primary HIV-1 isolates. Patients’ CD4 T cells and the clonal HIV-1 strain JR-FL were also included for comparison. All patients were on antiretroviral therapy for at least two years to minimize the amount of cells already harbouring HIV-1 proviruses. HIV-1 integration sites were amplified with nrLAM-PCR, and sequenced on the Illumina MiSeq platform. Mapping of the sequencing reads and classification of the integration sites were done using the online bioinformatic tools HISAP and DAVID. In both MDMs and CD4 T cells, a total of 1,692 integration sites (MDM Primary Isolate: 705; MDM JR-FL: 315; CD4 T Cell Primary Isolate: 568 & CD4 T Cell JR-FL: 104) analysed showed distributions in the human genome and associations with physical genomic features that are consistent with previous reports. In MDMs, a consistent enrichment of integration sites in genes with certain gene annotations has been observed with both autologous primary isolates and JR-FL, \textit{e.g.} genes involved in catabolic processes. Such consistency was not observed in CD4 T cells, suggesting that the repertoire of accessible sites in the genome of MDMs could be more restricted for HIV-1 integration.
Diagnosis of an Enterovirus C104 Strain in a Lung Transplant Recipient by High-Throughput Sequencing

Dagmara Lewandowska1*, Michael Huber1*, M. Schuurmans2#, Osvaldo Zagordi1#, Andrea Zbinden1#, Fabienne Geissberger1, Jon Huder1, Peter Schreiber1, Jürg Böni1, Christian Benden2, Nicholas Müller3$, Alexandra Trkola1$.

1*, # and $ contributed equally

1 Institute of Medical Virology, University of Zurich, Zurich, Switzerland.
2 Division of Pulmonary Medicine, University Hospital Zurich, Zurich, Switzerland.
3 Division of Infectious Diseases, University Hospital Zurich, Zurich, Switzerland.

Enterovirus (EV) and Rhinovirus (RV) species cause a wide spectrum of diseases. Precise discrimination would be of advantage for disease monitoring in immunocompromised individuals; however, accurate differentiation of EV and RV can be problematic because of high homology between the viruses.

We report on a 51-year old individual with a protracted respiratory tract infection 4 months after lung transplantation. Subsequent respiratory samples were analyzed by a commercial, multiplex real-time PCR, which detects 18 respiratory viruses. Due to cross-reactivity issues of this assay, if both EV and RV are positive, samples are recorded EV positive. Following an initial RV only positivity, series of samples proved highly positive for RV but only borderline for EV, suggesting an EV infection that is not properly detected by the kit with RV cross-reactivity or a possible co-infection with both viruses.

To confirm or rule out co-infection, we performed whole nucleic acid high-throughput sequencing of diverse clinical specimens from several time points. De novo assembly revealed infection with a Human Enterovirus C104 (EV-104) closely related to the recently identified AK11 strain. Using reference-based alignment we recovered full-length genomes. The metagenomic approach clearly ruled out RV as source of the highly positive PCR signal, as no RV sequences were detected. EV reactivity in the commercial kit could be partially restored by supplementing with in-house primers and probe.

In summary, the metagenomic approach proved to be successful in supporting the EV diagnosis and clarifying potential viral co-infections, highlighting the potential of the technology in complex diagnostic situations.
Early events in virus infections critically depend on host membrane dynamics, yet how lipids tune the viral life cycle remains poorly understood. Here, we use adenovirus as a model to address lipid biology of viral invasion. High-resolution lipid profiling of infected cells revealed rapid and selective ceramide enrichment during adenovirus entry. Ceramide is composed of sphingosine and a fatty acid, and is a component of sphingomyelin, a major lipid in the plasma membrane. Ceramide is involved in membrane microdomain function, such as formation of vesicles, cell signalling, or cell death. Unlike with wild type HAdV-C2/5, we observed no ceramide increase with the HAdV-C2 mutant TS1, which is impaired at signaling and escape from endosomes, implicating that ceramide increase is linked to infectious events during entry. Chemical perturbation of the ceramide generating lysosomal enzyme acid sphingomyelinase (ASMase) interfered with virus endocytosis, uncoating and membrane penetration. These key events were regulated by a pool of ASMase released extracellularly within the first minutes of infection. ASMase re-localisation correlated with rapid, Ca\(^{2+}\)-regulated lysosomal exocytosis. Spatio-temporal dynamics of Ca\(^{2+}\) fluxes were observed by live cell imaging and revealed that wild type but not TS1 adenovirus triggers the influx of extracellular Ca\(^{2+}\) into the cytosol upstream of lysosomal exocytosis. Together, these data establish a model for how an incoming non-enveloped virus harnesses calcium signaling to hijack host lipid metabolism, produce specific lipid signatures and effectively overcome the host cell membrane.
Systems biology-based investigation of the humoral immune response against various influenza A subtypes

Michael A. Maurer, Matteo Bianchi, Michael Huber, Merle Schanz, Ines Kohler, Lars Hangartner.

Institute of Medical Virology, University of Zurich, Zurich, Switzerland.

In this study we want to gain a deeper insight into the heterosubtypic, i.e. the broadly neutralizing, humoral immune response to influenza A viruses using a dual systems biology approach. One side investigates the B cell receptor repertoire by next generation sequencing while the other side analyzes the heterosubtypic serum antibody repertoire by mass spectrometry. Combining the data from both approaches, the heterosubtypic antibody response to influenza viruses can comprehensively be analyzed in individuals. Based on our large screening study analyzing the heterosubtypic antibody response in healthy donors, we will choose individuals with poor, average and good heterosubtypic activity, and determine their heterosubtypic B cell repertoire with this dual systems biology approach. By comparing the different datasets, we hope to determine patterns that promote heterosubtypic antibody response, and that could be deployed for the development of a pan-influenza vaccine.
**Enterovirus 71 intra-host adaptation and acquisition of neurotropism determinants**

Simon Meister¹, Komla Sobo¹, Caroline Tapparel¹².

¹ Microbiology and Molecular Medicine, University of Geneva, Geneva, Switzerland.
² Division of Infectious Diseases and Division of Laboratory Medicine, University Hospitals of Geneva, Geneva, Switzerland.

Enterovirus 71 (EV71) is a non-enveloped positive stranded RNA virus belonging to the *Picornaviridae* family. This virus is the main cause of hand-foot-and-mouth disease (HFMD) outbreaks in Asia-Pacific countries, and can be associated with severe neurological complication in young children. The mechanism by which the virus reaches the central nervous system as well as its neurotropic determinants remain ill-defined.

We have identified a mutation (VP1L97R) within the VP1 capsid protein of this virus, acquired in the course of a disseminated EV-71 infection in an immunocompromised host, which confers the virus a binding advantage in neural cells. Our goal is to find out whether this mutation alters receptor or co-receptor usage. Two different membrane proteins, scavenger receptor class B, member 2 (SCARB2) and P-selectin glycoprotein ligand-1 (PSGL-1) have been reported as cellular receptors for EV-71. Other molecules such as annexin II, surface heparan sulfate glycosaminoglycan and sialic acid may also play a role in viral attachment.

To evaluate the impact of the VP1L97R mutations on EV-71-receptor or co-receptor interactions and to investigate potential receptor switch that may result in neural cell tropism, we will compare the binding of viral clones bearing or not the L97R substitution, and successively analyze the effects of silencing or digestion of these receptors or co-receptors on viral attachment in different cell types.

Altogether, these experiments will determine whether the VP1 change provides a higher affinity to a given receptor and/or attachment molecule, resulting in a different cell and thus tissue tropism. Knowledge about EV71 tissue tropism determinants will help to define targets for antivirals or vaccine development.
Immune stimulants may reinforce latent EBV

Vanessa Mordasini, Anna Elsener, Michele Bernasconi, David Nadal.

Experimental Infectious Diseases and Cancer Research, Division of Infectious Diseases and Hospital Epidemiology, Children’s Research Center, University Children’s Hospital of Zurich, University of Zurich, Zurich, Switzerland.

Epstein-Barr virus (EBV) infects the host via the oropharynx, and lymphoid tissues of the Waldeyer’s ring including tonsils act as reservoir. The mechanisms governing the balance between the default latent infection and lytic reactivation in B cells are not completely understood. Nevertheless, triggering of innate immune Toll-like receptors (TLRs) expressed by B cells seems to contribute. We assessed the effects of a commercially available immune stimulant (OM85) that contains heat-inactivated bacteria that colonize or infect the upper airways on the balance latent/lytic infection on Akata Burkitt’s lymphoma cells, an established model to study EBV reactivation. Treatment of Akata cells prior to induction of lytic EBV reactivation by cross-linking of the B-cell receptor (BCR) significantly suppressed lytic EBV reactivation similarly to the suppression exhibited by triggering TLR9. Nevertheless, interleukin (IL) 10 as surrogate marker for TLR9 triggering in Akata cells was not induced by OM85 treatment. Furthermore, OM85 was shown not to contain bacterial DNA that is a ligand for TLR9. Interrupting TLR9 signaling using Akata cells with a dominant negative MyD88, the adaptor protein for TLR9, however, did not abolish the suppressive effect of OM85 treatment for lytic EBV. Immune stimulants like OM85 although not containing bacterial DNA may unexpectedly impact on the mode of infection of EBV in a similar way as infecting bacteria releasing DNA via TLR9 triggering. Thus, immune stimulants may lead to reinforcement of the default latent EBV and potentially promote its oncogenic potential. This needs to be considered, especially in the immune compromised host. The signaling pathway is under investigation.
Identification of the Semliki Forest Virus Protein(s) Targeted by the Antiviral Effector Protein MxA

Dominik Müller1,2, Patricia Nigg1,2, Michel Crameri1,2, Fiona Steiner1,2, Jovan Pavlovic1.

1 Institute of Medical Virology, University of Zurich, Zurich, Switzerland.
2 Ph.D. Program in Microbiology and Immunology, University of Zurich, Zurich, Switzerland.

MxA is a key player in the interferon mediated antiviral activity against many RNA viruses including influenza A virus (IAV) and Semliki Forest virus (SFV). For SFV, a positive stranded RNA virus of the togaviridae family, it was shown that MxA restricts viral replication at the level of transcription and inhibits the amplification of a SFV-based replicon system in the absence of all structural viral proteins. The exact molecular mechanism is still unclear. Recently, however, we could show that MxA interacts with two cellular RNA helicases named UAP56 and URH49. UAP56 plays a pivotal role for IAV replication. This helicase is recruited by the IAV polymerase complex and is required for nuclear export of nascent viral RNAs. Moreover, down regulation of UAP56 leads to accumulation of double stranded RNA in the perinuclear region. One possible explanation for the antiviral activity of MxA is thus interference with the activity of RNA helicases essential for viral replication. In contrast to IAV, SFV codes for its own RNA helicase termed nsp2. The SFV helicase is expressed as part of a polyprotein (nsP1-4). It codes for the replication complex and is processed in a well-defined order during replication. Aim of this project is to assess the interaction of MxA with the four non-structural SFV proteins as single proteins and as polyproteins including the naturally occurring polyprotein intermediates. This interplay is studied with help of IAV and SFV mini replicon assays and with co-immunoprecipitation in cell culture as well as in vitro with recombinant proteins in AlphaScreen assays.
**Tetrameric and dimeric MxA proteins recognise influenza A virus nucleoprotein that is not incorporated in vRNPs after 1° transcription**

Patricia E. Nigg¹, Dominik Mueller¹, Linda Brunotte², Dominik Dornfeld², Michel Crameri¹, Martin Schwemmle² and Jovan Pavlovic¹.

¹ Institute of Medical Virology, University of Zurich, Zurich, Switzerland.
² Institute for Virology, University Medical Centre of Freiburg, Freiburg, Germany.

The interferon-induced human MxA protein exerts a broad antiviral activity against negative stranded RNA viruses including influenza A (IAV). The recent atomic resolution of the MxA structure revealed that it can form stable tetramers and oligomers. However, whether these tetramers of MxA or higher ordered oligomeric structures represent the antiviral entity and to bind to components of the viral ribonucleoprotein (vRNP) remains to be shown. Biochemically characterised MxA mutants with a defined oligomerisation state revealed that the tetrameric, dimeric and the monomeric form of MxA exhibited antiviral activity. Since previous studies have shown that the nucleoprotein (NP) of IAV plays an important role in determining sensitivity or resistance to MxA, we also tested whether these MxA mutants interact with NP by transient transfection and co-immunoprecipitation. Intriguingly, only the tetrameric and dimeric forms of MxA were able to bind NP efficiently. To determine whether MxA associates with vRNPs, MxA expressing cells were infected with a recombinant virus expressing a strep-tagged PB2, allowing the purification of vRNPs. Enrichment of vRNPs failed to co-purify detectable amounts of MxA. These results suggest that the antiviral active tetrameric and dimeric form of MxA preferentially recognise free NP. According to these results we suggested a model where MxA captures free NP which consequently leads to the inhibition of IAV replication. To confirm this theory we infected cells pre-treated with cycloheximide. RT qPCR clearly shows that MxA inhibits IAV replication after 1° transcription, supporting our model of MxA preventing IAV replication by capturing newly synthesised NP.
Characterization of transmitted/founder viruses and their sources in HIV-1 transmitter/recipient pairs

Corinna S. Oberle¹, Beda Joos¹, Nottania K. Campbell¹,³, David Beauparlant²,³, Herbert Kuster¹, Corinne Schenkel¹, Peter Rusert², Alexandra Trkola³, Karin J. Metzner³, Huldrych F. Günthard¹.

¹ Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, University of Zurich, Zurich, Switzerland.
² Institute of Medical Virology, University of Zurich, Zurich, Switzerland.
³ Life Science Zurich Graduate School, University of Zurich, Zurich, Switzerland.

In most HIV-1 transmission events only one (or a few) transmitted/founder virus from the genetically diverse virus population of the transmitter is infecting the recipient. Whether HIV-1 transmission is a stochastic event or the transmitted/founder viruses have beneficial properties facilitating transmission and infection is controversially discussed.

To investigate the phenotypes of transmitted/founder viruses and the virus population of the transmitter, we examined virus isolates obtained from the nearest time point of transmission of 9 potential transmitter/recipient pairs. Recipients were enrolled in the Zurich Primary HIV Infection Study and potential transmitters were identified by performing phylogenetic analysis of HIV-1 pol sequences of the Swiss HIV Cohort Study Drug Resistance Database (>19,000 genotypes, distance <1.5% between transmitter and recipient). We characterized these virus isolates in respect to replication capacity in peripheral blood mononuclear cells (PBMCs) and monocyte-derived macrophages (MDM), entry kinetics, sensitivity to entry inhibitors, and replication capacity in the presence of IFN-α.

The replication capacity in PBMCs revealed similar growth curves for all transmitters’ and recipients’ virus isolates. Transmitters’ and recipients’ virus isolates replicated to variable degrees in MDMs. Differences in entry kinetics were observed between different transmitter/recipient pairs but not within the pairs. The sensitivity to entry inhibitors was similar between transmitters and recipients. Moreover, resistance to IFNα was variable within and between pairs.

The phenotypic characteristics of transmitted/founder viruses and the virus population of the transmitter are very similar in our 9 transmitter/recipient pairs. No distinct phenotypic pattern could be attributed to transmitted/founder viruses by the assays used.
**Characterization of arenavirus envelope glycoprotein cleavage by subtilisin kexin isozyme 1 (SKI-1)/site 1 protease (S1P) using as molecular sensor**

Joel Oppliger¹, Joel Ramos da Palma¹, Dominique J. Burri¹, Nabil G. Seidah², Antonella Pasquato¹, and Stefan Kunz¹.

¹ Institute of Microbiology, University Hospital Center and University of Lausanne, Lausanne, Switzerland.
² Laboratory of Biochemical Neuroendocrinology, Clinical Research Institute of Montreal, University of Montreal, Montreal, Canada.

Several arenaviruses are the causative agent of severe hemorrhagic fevers characterized by high morbidity and mortality. No vaccine or drugs are available to treat or prevent arenaviral infections with the exception of the nucleoside analog Ribavirin, which has limited efficacy. During the life cycle of arenaviruses in the host cell, the membrane viral glycoprotein precursor GPC must be cleaved by the cellular protease the Subtilisin Kexin Isozyme-1 (SKI-1) / Site-1 Protease (S1P) to be incorporated into the viral particle and allow fusion with the host membrane. The ability of newly emerging arenaviruses to hijack human SKI-1/S1P appears therefore as a crucial determinant for their zoonotic potential. The advent of next-generation sequencing has greatly accelerated the discovery of novel arenaviruses frequently without isolation of infectious virus and complete GPC sequences. Here, we implement a newly developed molecular sensor to characterize the processing of known and putative arenavirus GPC-derived sequences by human SKI-1/S1P. Our sensor accurately recapitulates the efficiency and subcellular location of GPC processing of the Old World arenaviruses lymphocytic choriomeningitis virus (LCMV) and Lassa virus (LASV). The sensor further allowed to correctly predict the efficient processing of the GPC of the newly emerged pathogenic Lujo virus by human SKI-1/S1P and to define the cleavage site. The human disease potential of NW arenaviruses critically depends on their ability to use human transferrin receptor 1 (hTfR1) for cell entry. Using our sensor, we found efficient processing of NW arenavirus GPC sequences by human SKI-1/S1P, independent of their ability to use TfR1 for entry. These data indicate a significant zoonotic potential of currently non-pathogenic viruses, once they have overcome the barrier at the level of the cellular receptor.
Lentivirus-mediated targeting of myelin-antigen to dendritic cells induces tolerance of auto-reactive effector CD4+ T cells

Bruna de Andrade Pereira¹, Mathias Ackermann¹, Shahid Chaudhary², Rebecca Vogel¹, Bernd Vogt¹, Christiane Dresch³, Cornel Fraefel¹.

¹ Institute of Virology, University of Zurich, Zurich, Switzerland.
² Institute of Immunology, University Children’s Hospital, Zürich, Switzerland.
³ Department of Immunology, University of Washington, Seattle, WA, USA.

Lentiviral vectors are efficient gene transfer vehicles, and are used for both research and gene therapy applications. In this project we used the self-inactivating (SIN) lentivirus to transduce hematopoietic stem cells due to the ability of lentiviral vectors to stable integrate into the genome of the transduced cells resulting in long-term transgene expression. The SIN-lentivirus vectors express the myelin oligodendrocyte glycoprotein (MOG), an antigen involved in experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis. The MOG-transgene is under the control of a dendritic cell (DC) specific promoter to transcriptionally target antigen expression to DCs. After re-infusion, the vector-transduced HSC will give rise to MOG-expressing DCs that, in a non-inflammatory condition, are expected to tolerize self-reactive T cells. Here, we demonstrate that all mice that had received HSC transduced with MOG-lentivirus vector (tolerized group) were protected after adoptive transfer of MOG-specific activated CD4+ T cells, while all mice that received HSC transduce with a control lentivirus vector (non-tolerized group) developed the disease. In contrast to non-tolerized group, the MOG-specific activated CD4+ T cells adoptive transferred into tolerized mice became unresponsive after isolation and in vitro re-stimulation and up-regulated regulatory molecules associated with anergy and regulatory T cells. In addition, the in vivo depletion of regulatory T cells resulted in disease susceptibility of the tolerized animals, suggesting that these cells play indeed a role in tolerance induction/maintenence. The ability to revert the pathogenic MOG-specific CD4+ T cells highlights the potential of this strategist for a therapeutic approach in an established autoimmune disease.
Targeting the Secretory Pathway Enhances Human Adenovirus Infection and Cancer Cell Killing

Vibhu Prasad\(^1\,^2\), Maarit Suomalainen\(^1\), Mirjam Pennauer\(^3\), Artur Yakimovich\(^1\), Vardan Andriasyan\(^1\,^2\), Silvio Hemmi\(^1\), Urs Greber\(^1\).

\(^1\) Institute of Molecular Life Sciences, University of Zurich, Zurich, Switzerland.
\(^2\) Molecular Life Sciences Graduate School, ETH and University of Zurich, Zurich, Switzerland.
\(^3\) Institute of Molecular Biosciences, University of Graz, Graz, Austria.

Oncolytic viruses infect and kill cancer cells but not normal cells. Human adenoviruses (HAdVs) are widely used oncolytic agents, engineered to produce progeny within the tumor, and thereby elicit bystander effects. To search for host factors enhancing bystander effects, we conducted a targeted RNA-interference screen against guanine-nucleotide exchange factors (GEFs) of small GTPases. The knock-down of Golgi Brefeldin-A Resistant-Guanine-Nucleotide-Exchange Factor 1 (GBF1) stimulated HAdV infection. GBF1 is a GEF for ADP-ribosylation factors (Arfs) regulating ER to Golgi and intra-Golgi transport. Cells treated with the GBF1 inhibitor Golgicide A (GCA) enhanced HAdV-induced cytopathic effects in epithelial and melanoma cancer but not normal cells, if applied several hours prior to HAdV inoculation. GCA-treated cells contained fewer incoming HAdV than control cells, but enhanced viral gene expressions, or green fluorescent protein under cytomegalovirus promoter from transgenic B- or C-species HAdVs. GCA boosted HAdV titers and spreading in cancer cells, but did not enhance viral E1A expression in non-infected cell lines, or cells transfected with plasmid reporter DNA, indicating that GCA induced a metabolic state in infected cells conducive for enhanced cell killing. The results show that targeted interference with host factors leads to virus-enhanced cytotoxicity in cancer cells.
Characterization of the activity of an innate immunity protein, the Apolipoprotein L6 (APOL6)

Nitisha Pyndiah, Angela Ciuffi, Amalio Telenti.

Institute of Microbiology, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland.

Host restriction factors are proteins hampering virus replication, and share common features including positive selection, viral counteraction, interferon-inducible expression and differential expression among HIV+ patients. APOL6 was identified in a screen aiming at identifying novel HIV-1 candidate host restriction factors. APOL6-mediated restriction was validated in a co-transfection assay with an HIV-1 LTR-EF1-GFP, showing up to 10-fold reduced GFP expression in APOL6-expressing cells compared to control cells. Species-specific restriction of APOL6 primate orthologs co-transfected with HIV-1 LTR-EF1-GFP revealed a higher GFP inhibition mediated by hominids and new world monkey APOL6 compared to old world monkeys APOL6. A similar APOL6-mediated restriction was observed when APOL6 was co-transfected with alternate GFP expression vectors (SIV LTR-GFP, SIV LTR-CMV-GFP, EIAV LTR-CMV-GFP, FIV LTR-CMV-GFP and MLV LTR-CMV-GFP). In contrast, APOL6 was not able to inhibit HIV-based vector transduction nor adenovirus or LCMV. All together, these data suggest that APOL6-mediated restriction is not virus-specific, but rather displayed a broad action against various promoter constructs. This points out to a specific APOL6 mechanism, potentially acting at the level of nucleic acid (DNA or RNA) sensing and/or degradation. Through co-transfection analyses using human and rhesus APOL6, respectively displaying high and low restriction ability, we identified a specific APOL6 domain and residue mediating APOL6 effects. We are currently identifying APOL6 cellular interactants by Mass spectrometry to elucidate the mechanism of APOL6-mediated restriction.
Degree of efficacy loss of broadly neutralizing antibodies during HIV-1 cell-to-cell transmission is strain and epitope dependent

Lucia Reh, Carsten Magnus, Merle Schanz, Jaqueline Weber, Therese Uhr, Peter Rusert, Alexandra Trkola.

Institute of Medical Virology, University of Zurich, Zurich, Switzerland.

In contrast to free-virus entry, HIV-1 cell-to-cell transmission allows for a high efficacy of virus infection to which neutralizing antibodies (nAbs) and even highly potent broadly neutralizing antibodies (bnAbs) have been shown to dramatically lose neutralization potency. Since the latter are considered as main leads for vaccine design and as therapeutics for passive immunization, a precise definition of their inhibitory potential during cell-to-cell transmission is needed. To this end, we tested a range of bnAbs for their inhibitory capacity during cell-to-cell transmission of HIV-1 strains from subtypes A, B and C. Overall, the potency to inhibit HIV-1 cell-to-cell transmission was strongly decreased compared to free-virus transmission across all bnAbs tested. However, activity loss varied considerably between virus strains and high potency against free-virus did not ensure lower loss in activity during cell-to-cell transmission. Intriguingly, inhibition capacities of certain bnAb-virus combinations proved comparable for both transmission modes. Yet, this preserved activity was highly strain dependent and no bnAb that potently blocked cell-to-cell transmission over a range of HIV-1 strains could be identified.

To estimate the consequences of activity loss during cell-to-cell transmission, we conducted detailed mathematical analyses that showed an increased probability of viral resistance mutations to bnAbs to arise through cell-to-cell transmission rather than free-virus spread.

In sum, our data suggest that efficacy of bnAbs during cell-to-cell transmission cannot be predicted by their free-virus activity. Potent inhibition of both transmission routes will only be possible through a combination of bnAbs, either by multi-component vaccines or antibody cocktails in passive immunization.
Newly emerging pathogenic viruses represent a serious challenge for Global Health. Hantaviruses are an important group of viruses that can cause serious diseases in humans and poses an increasing threat. Human pathogenic hantaviruses are associated with two severe diseases: Hemorrhagic Fever with Renal Syndrome (HFRS) in Asia and Europe and Hantavirus Cardiopulmonary Syndrome (HCPS) in the Americas, with case mortality rates up to of 15% and 40%, respectively. The geographical distribution of hantaviruses is determined by their natural primary host, rodents, shrews, moles and bats, that carry the virus as asymptomatic persistent infection. Human infection occurs accidentally, mainly by inhalation of aerosolized rodent excreta. Currently no effective preventive vaccine, immunotherapeutics, or antiviral drugs are licensed for specific treatment of hantavirus diseases.

Replication and transcription of the viral RNA genome represent crucial steps of viral multiplication, which are highly conserved among hantaviruses and therefore represent promising drug targets for the development of effective broad-spectrum antivirals. Over the past decade, minireplicon systems have emerged as powerful experimental surrogates to study replication and transcription of many highly pathogenic RNA viruses. The major goal of our study is to develop a Nanoluciferase reporter-based minireplicon system for the prototypic Hantaan virus that can be used in high-throughput screening (HTS) of synthetic small molecules libraries. After their initial discovery, novel candidate anti-viral drugs will be validated with live pathogenic hantaviruses in the new high containment facilities at Spiez Laboratory.
The oxysterol-binding protein mediates cholesterol recruitment via phosphatidylinositol 4-kinase III beta to enhance human rhinovirus replication

Pascal S. Roulin1,2, Mark Lötzerich1, Federico T. Torta3, Lukas B. Tanner3, Frank J.M. van Kuppeveld4, Markus R. Wenk3, Urs F. Greber1.

1 Institute of Molecular Life Sciences, University of Zurich, Zurich, Switzerland.
2 Life Science Zurich Graduate School, Molecular Life Sciences Program, Zurich, Switzerland.
3 Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore.
4 Department of Infectious Diseases and Immunology, University of Utrecht, Utrecht, Netherlands.

Positive-strand RNA viruses form cytosolic membrane-associated replication complexes composed of host and viral proteins, viral RNA and cellular membranes of various origins. Like other enteroviruses, human rhinoviruses (HRVs) rearrange Golgi membranes including the ER-Golgi-intermediate compartment and trans-Golgi network. The enteroviral 2B, 2C and 3A proteins have been implicated in reorganizing intracellular membranes. They are, however, not sufficient to give rise to the endomembrane network observed during enterovirus replication, implying that additional host factors are involved. Here, we show by chemical inhibitors and RNAi-mediated knock-down approaches that phosphatidylinositol 4-kinase III beta (PI4K3β) and oxysterol-binding protein (OSBP) are required for rhinovirus replication in cultured and primary human airway cells. PI4K3β catalyze the production of phosphatidylinositol 4-phosphate (PI(4)P), which controls vesicular traffic and membrane biogenesis on Golgi membranes. PI4K3β is recruited at the sites of rhinovirus replication leading to a local enrichment of PI(4)P lipids. Binding PI(4)P via its PH domain, OSBP shuttles cholesterol present on ER membranes to virus-derived membranes. Indeed, cholesterol level increased during late rhinovirus replication correlating with a decreased level of cholesteryl-esters. Collectively, our data suggest a model where a balanced composition of PI(4)P and cholesterol on Golgi-derived membranes support the establishment and maintenance of rhinovirus replication domains.
Experimental Rhinovirus Recombination in the Polyprotein Coding Region

Manuel Schibler¹, Isabelle Piuz¹, Caroline Tapparel².³.

¹ Laboratory of Virology, Division of Infectious Diseases and Division of Laboratory Medicine, University Hospitals of Geneva, Geneva, Switzerland.
² Microbiology and Molecular Medicine, University of Geneva, Geneva, Switzerland.
³ Division of Infectious Diseases and Division of Laboratory Medicine, University Hospitals of Geneva, Geneva, Switzerland.

Recombination is a widespread phenomenon ensuring both the stability and the variation of RNA viruses genomes. Intraspecies recombination is frequently described among enteroviruses but appears to be sporadic in rhinoviruses, while both virus groups belong to the same Enterovirus genus. Interspecies recombination is even rarer for rhinoviruses and seems to be related to ancient events, which contributed to the speciation of these viruses. We recently reported that artificially engineered 5'UTR interspecies rhinovirus/rhinovirus or rhinovirus/enterovirus recombinants are fully viable.

Using a similar approach, we demonstrate here that exchanges in the polyprotein region between members of the same rhinovirus species, but not between members of different species, can give rise to fully competent chimeric viruses.

To further study rhinovirus recombination experimentally we used non-replicative recombination, induced by co-transfection of 5’ end-deleted and 3’-end deleted and replication-deficient rhinovirus genomes. In this system, intraspecies recombination also resulted in viable viruses, and recombination sites could be mapped, whereas no interspecies rhinovirus recombinant could be recovered.

Our results suggest that only intraspecies recombination gives rise to viable rhinovirus chimeras in the polyprotein coding region.
**Generic DARPin-mediated adenovirus delivery system approaching systemic applications**

Markus Schmid, Birgit Dreier, Andreas Plückthun.

*Department of Biochemistry, University of Zurich, Zurich, Switzerland.*

Efficient *in vivo* gene delivery using adenoviruses to a tissue of choice requires both the engineering of an efficient receptor-mediated uptake by a specific cell population and the ablation of the virus’ original tropism. For the retargeting, we have developed a series of bispecific adapters by fusing three modules: one trimerizing subunit of a phage protein of extraordinary kinetic stability and two modules both consisting of *Designed Ankyrin Repeat Proteins* (DARPins). One DARPin is binding with unprecedented functional affinity to the fiber knob of Adenovirus serotype 5 (Ad5), the other DARPin binding to one of several tumor markers tested (Dreier et al., 2013 *PNAS* 110:E869-877). When this ‘quasi-covalent’ module was fused to DARPins of varying specificities, it enabled efficient Ad5-mediated delivery of a transgene in a HER2-, EGFR- or EpCAM-dependent manner with transduction efficiencies comparable or even exceeding those of Ad5 itself. Motifs in the viral capsid mediating the native viral tropism and interactions with host factors where removed using viral genome engineering. To block further epitopes of *e.g.* neutralizing antibodies by a stealth coat, capsid-binding DARPins have been selected. These capsid binders will be further engineered and extended to generate an efficient cover of the viral capsid protecting from factors responsible for the clearance of wt Ad5 in an *in vivo* environment. In this way pre-fabricated genetically modified Ad5 viruses with different payloads can readily be charged and retargeted to many cell types and tissues of choice.
The nucleoprotein of newly emerged H7N9 influenza A virus harbors a unique motif conferring resistance to human Mx protein

David Riegger¹*, Rong Hai²,³*, Dominik Dornfeld¹, Benjamin Mänz¹, Victor Leyva-Grado⁷, Maria T Sánchez-Aparicio², Peter Palese²,³, Otto Haller¹, Martin Schwemmle¹, Adolfo García-Sastre²,³,⁴, Georg Kochs¹,⁶*, Mirco Schmolke²,³,⁵,⁶*.

*These authors contributed equally to the work.
¹ Institute for Virology, University Medical Center, Freiburg, Germany.
² Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, USA.
³ Global Health and Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai, New York, USA.
⁴ Division of Infectious Diseases, Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, USA.
⁵ Current address: Department of Microbiology and Molecular Medicine, University of Geneva, Geneva, Switzerland.

Interferon-induced Mx proteins show strong antiviral activity against influenza A viruses. We recently demonstrated that the viral nucleoprotein (NP) determines resistance of seasonal and pandemic human influenza viruses to Mx, while avian isolates retain Mx sensitivity. We identified a surface exposed cluster of amino acids in NP of pandemic A/BM/1/1918 (H1N1) comprising isoleucine-100, proline-283 and tyrosine-313 that is essential for reduced Mx sensitivity in cell culture and in vivo. Accordingly, two substitutions in NP of PR/8(mut) to the avian Mx-sensitive amino acids (P283L and Y313F) led to attenuation in Mx1-positive mice. Serial lung passages of PR/8(mut) in Mx1 mice resulted in a single exchange of tyrosine to asparagine at position 52 in NP. This residue is in close proximity to the 100, 283 and 313 cluster and partially compensates the loss of Mx resistance in PR/8(mut). Intriguingly, NP of the newly emerged avian-origin H7N9 virus also contains an asparagine at position 52 and shows reduced Mx sensitivity. N52Y substitution in NP results in increased sensitivity of the H7N9 virus to human Mx, indicating that this residue is a critical determinant to upgrade Mx resistance in mammals. Our data strengthen the hypothesis that the human Mx protein represents a potent barrier against zoonotic transmission of avian influenza viruses. However, the newly emerged H7N9 viruses overcome this restriction by harboring a NP that is less sensitive to the Mx-mediated host defence. This might contribute to zoonotic transmission of H7N9 and to the severe to fatal outcome of H7N9 infections in humans.
AAV2 Rep68 can bind to consensus Rep-binding sites on the HSV-1 genome

Michael Seyffert, Daniel L. Glauser, Kurt Tobler, Oleg Georgiev, Rebecca Vogel, Bernd Vogt, Leticia Agúndez, Michael Linden, Hildegard Büning, Mathias Ackermann, and Cornel Fraefel.

1 Institute of Virology, University of Zurich, Zurich, Switzerland.
2 Suisselab AG, Zollikofen, Switzerland.
3 Institute of Molecular Life Sciences, University of Zurich, Zurich, Switzerland.
4 Department of Infectious Diseases, King’s College London School of Medicine at Guy’s, King’s and St. Thomas Hospital, London, United Kingdom.
5 Center for Molecular Medicine Cologne, University of Cologne, Cologne, Germany.

Adeno-associated virus type 2 (AAV2) has previously been shown to inhibit the replication of its helper virus herpes simplex virus type 1 (HSV-1). The inhibitory activity has been linked to the expression of the AAV2 Rep proteins, more precisely, to the combined helicase and DNA binding activity of Rep68 and Rep78. The exact mechanism of inhibition however is not known. Here, we show that the helicase activity of Rep52, a Rep protein without the DNA-binding domain, is sufficient to prevent the replication of a DNA template if binding is facilitated. Moreover, we found that the AAV2 Rep68 protein can bind to consensus Rep-binding sites (RBS) on the HSV-1 genome in silico, in vitro and in cell culture. The functionality of the HSV-1 consensus RBSs was tested and verified with a Rep68/78-specific replication assay. These results corroborate our hypothesis that the mechanism of Rep68/78 mediated inhibition of HSV-1 DNA replication may involve direct binding of Rep proteins to the HSV-1 genome and modification of the bound DNA via the helicase activity.
Neutralization of Diverse HIV Strains by V3 Specific DARPinS with Different Structural Preferences in Epitope Recognition

Nikolas Friedrich$^{1a}$, Emanuel Stiegeler$^{1a}$, Axel Mann$^{1a}$, Yufan Wu$^{2}$, Alexander Batyuk$^{2}$, Peter Rusert$^{1}$, Jacqueline Weber$^{1}$, Birgit Dreier$^{3}$, Melissa Robbiani$^{3}$, John Robinson$^{4}$, Andreas Plückthun$^{2}$, Alexandra Trkola$^{1}$.

$^{a}$ equal contribution

$^{1}$ Institute of Medical Virology, University of Zurich, Zurich, Switzerland.
$^{2}$ Institute of Biochemistry, University of Zurich, Zurich, Switzerland.
$^{3}$ Center for Biomedical Research, Population Council, New York, NY, USA
$^{4}$ Department of Chemistry, University of Zurich, Zurich, Switzerland.

The HIV-1 envelope gp120 subunit variable loop 3 (V3) is essential for viral entry by interacting with the co-receptor. While V3 specific neutralizing antibodies (nAbs) have highlighted the potential of V3 directed entry inhibitors for long, the recent discovery of V3 specific highly potent, broadly neutralizing Abs (bNAbs) funneled a renewed interest in designing V3 specific entry inhibitors. Here we derived V3 directed Designed Ankyrin Repeat proteins (DARPinS) from molecular libraries by ribosome display. Selections were focused on V3 utilizing a structurally arrested V3 loop mimetic peptide as panning target. Six DARPinS differing in V3 structural requirements for binding were obtained and probed for inhibitory activity against genetically diverse envelope pseudotyped viruses on TZM-bl cells. Neutralization breadth varies among the V3 DARPinS reaching up to 75% (12 out of 16) for clone 63_B7 against modestly neutralization resistant (Tier 2) subtype B envelopes. The V3 DARPinS are affected to different degree by the V1V2 envelope subdomain protecting V3. Of particular interest, access of 63_B7 is less restricted by V1V2 compared to classical V3 antibodies commonly elicited during infection in humans. Using gp120 alanine mutant pseudoviruses we identified amino acid positions within and outside of V3 contributing to DARPin resistance for clones 63_B7, 5m3_D12 and SE4. The interaction of 63_B7 and 5m3_D12 with V3 loop mimetics was resolved at the atomic level by x-ray crystallography and lead to the delineation of a neutralization hot spot on V3. Together the DARPinS provide valuable information towards the further design of V3 reactive inhibitors.
Identification of OCIAD1 as Cellular Substrate of the Hepatitis C Virus NS3-4A Protease

Huong T.L. Tran, Kenichi Morikawa, Rose Zibi, Viet Loan Dao Thi, François Penin, Markus H. Heim, Manfredo Quadroni, Jérôme Gouttenoire, Darius Moradpour.

1 Division of Gastroenterology and Hepatology, Lausanne University Hospital (CHUV) and University of Lausanne, Lausanne, Switzerland.
2 Protein Analysis Facility, University of Lausanne, Lausanne, Switzerland.
3 Institut de Biologie et Chimie des Protéines, University of Lyon, Lyon, France.
4 Division of Gastroenterology and Hepatology, University Hospital Basel, Basel, Switzerland.

The NS3-4A protease plays a central role not only in the viral life cycle but also in the persistence and pathogenesis of hepatitis C virus (HCV). It has been reported to cleave several host factors and to thereby modulate cellular signaling pathways. Here, we describe OCIAD1 (ovarian cancer immunoreactive antigen domain containing 1) as novel cellular substrate of the HCV NS3-4A protease. OCIAD1 was identified as substrate of the NS3-4A protease by a quantitative proteomics approach involving SILAC coupled with mass spectrometry. OCIAD1 is a poorly characterized membrane protein potentially involved in cancer development. It is cleaved by NS3-4A at Cys 38, close to a predicted transmembrane segment. Cleavage was observed in heterologous overexpression systems, the replicon and HCVcc systems, as well as in liver biopsies from patients with chronic hepatitis C. In addition, NS3-4A-mediated cleavage of OCIAD1 was found in a wheat germ-based cell-free expression system. The subcellular localization of OCIAD1 on mitochondria was not altered by NS3-4A-mediated cleavage. Interestingly, OCIAD2, a homolog of OCIAD1 with a Cys residue in a similar position and identical subcellular localization, was not cleaved by NS3-4A. Domain swapping experiments revealed that the sequence surrounding the potential cleavage sites as well as the predicted transmembrane segment contribute to substrate selectivity. Overexpression as well as knock down and rescue experiments with siRNA-resistant versions of OCIAD1 did not reveal any effect on the viral life cycle in the HCVcc system in vitro, raising the possibility that OCIAD1 may be involved in the pathogenesis of hepatitis C.

In conclusion, OCIAD1 represents a novel cellular substrate of the HCV NS3-4A protease. It does not appear to be involved in the viral life cycle but may play a role in the pathogenesis of hepatitis C, which shall be explored in further studies.
The Role of RNA Interference in HIV-1 Infected Primary Human Monocyte-Derived Macrophages


* Contributed equally to this work
1 Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, University of Zurich, Zurich, Switzerland.
2 Institute of Pharmaceutical Sciences, ETH Zurich, Zurich, Switzerland.
3 Department of Biosystems Science and Engineering, ETH Zurich, Basel, Switzerland.
4 Computational and Systems Biology, University of Basel, Basel, Switzerland.

BACKGROUND
Micro RNAs (miRNAs) and other small noncoding RNAs (sncRNAs) are key players in post-transcriptional gene regulation. We and other groups have described the presence of HIV-1 derived sncRNAs in different experimental setups; however, so far their biological function remained to a large extent unknown. Here we used a global, comprehensive approach to investigate whether viral small RNAs may play a role in the RNA interference (RNAi) pathway in primary human monocyte-derived macrophages (MDMs). Specifically, we aimed to characterize the profiles of host and HIV-1 derived small RNAs and the possible impact of these RNAs on the viral life cycle.

METHODOLOGY
We applied Argonaute 2 (Ago2) photoactivatable ribonucleoside-enhanced crosslinking and immunoprecipitation (PAR-CLIP) to MDMs infected with HIV-1JRFL for 14 days from two different donors. It has been previously demonstrated that this approach enables identification of Ago2-bound miRNAs as well as of the miRNA-targeted mRNAs. Illumina sequencing was performed on PAR-CLIP and small RNA (18 to 30 nt) samples from the same donors, and small RNA sequencing was performed on HIV-1 infected MDMs from two additional donors.

RESULTS
The analysis of PAR-CLIP data demonstrated the absence of viral RNAs in Ago2-RISC, suggesting that viral sncRNAs do not enter the canonical RNAi pathway in MDMs. However, small RNA sequencing on samples from the same MDM cultures confirmed the presence of HIV-1 sncRNAs, although expressed at low levels (< 0.5 % of total small RNA fraction). Most host miRNAs revealed no significant change in expression levels between infected and non-infected MDMs.

CONCLUSIONS
Our data indicate that it is unlikely that viral sncRNAs are incorporated as functional miRNAs or resemble targets for host miRNAs in Ago2-RISC. The presence of HIV-1 sncRNA as detected by small RNA sequencing implies alternative functional roles or biogenesis pathways. Future efforts are needed to uncover potential functions of HIV-1 derived sncRNAs in MDMs or other HIV-1 host cell types.
Plaque2.0: Image-based High-Throughput Virus Replication Quantification Framework


* equal contribution
Institute of Molecular Life Sciences, University of Zurich, Zurich, Switzerland.

Developed in 1950s, plaque assay started the era of the quantitative virology, allowing precise tittering and subsequent purification of infectious units from inoculae of various etiologies. However, more than a means to titer viruses, plaque assay delivers phenotypes potentially bearing yet unexplored information about the life cycle and spreading mechanism of viruses. Recent advance in automated high-throughput imaging and sCMOS camera technologies together with state-of-the-art image analysis algorithms can help harnessing this information. Here we present Plaque2.0 – an assay framework bridging classical assay, high-throughput approach and high resolution midrange magnification imaging. Plaque2.0 is accompanied by image analysis software aimed at enabling researchers to maximize information they obtain from their plaque assays, while making the analysis robust and fully automated. We show that Plaque2.0 is a replication assay suitable for species ranging from large DNA to small RNA viruses.
Mechanism of Influenza Virus Uncoating

Yohei Yamauchi1, Indranil Banerjee1,2, Yasuyuki Miyake3, Laure Decamps16, Hung Ho Xuan17, Peter Horvath4, Amalio Telenti5, Manfred Kopf6, Patrick Matthias3, Ulrike Kutay1, Ari Helenius1.

1 Institute of Biochemistry, ETH Zurich, Zurich, Switzerland.
2 Neurobiology, Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland.
3 Epigenetics, Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland.
4 Synthetic and Systems Biology Unit, Biological Research Center, Szeged, Hungary.
5 Institute of Microbiology, University Hospital Center and University of Lausanne, Lausanne, Switzerland.
6 Institute for Molecular Health Sciences, ETH Zurich, Zurich, Switzerland.
§ Present address, Freiburg University, Germany.
† Present address, University of Regensburg, Germany.

Influenza A virus (IAV) is a serious human pathogen with great medical, social, and economic impact. The capsids of incoming IAVs undergo uncoating after fusion at late endosomes (LEs), followed by import of viral ribonucleoproteins (vRNPs) into the nucleus. During endosomal transit the M2 channel primes the viral core for uncoating. Here we show that to initiate the disassembly of its stable capsid shell in the crowded environment of the cytosol, IAV takes advantage of cytosolic histone deacetylase 6 (HDAC6) and the host cell’s aggresome formation and disassembly machinery.

The IAV core mimics a misfolded protein aggregate by carrying unanchored ubiquitin chains that serve as key signaling molecules to activate a HDAC6-dependent aggresome processing pathway. We observed that the free ubiquitins in the virus core interact with the ubiquitin-binding ZnF-UBP domain of HDAC6 and this is essential for uncoating and infection. A further requirement for the other components of the aggresome processing machinery such as dynein, dynactin, myosin II, microtubules and actin, indicated that physical force generated by these motors is crucial for IAV uncoating at LEs and successful host cell entry. (Banerjee et al.)

Our current working model is that IAV uses mechanical forces provided by cytoskeletal motors (and nucleocytoplasmic transport factors) in order to uncoat its capsid. The clarification of such cell-assisted virus uncoating mechanisms can provide insights into new anti-viral strategies.
Phosphorylation events in early steps of influenza A virus infection

Emilio Yáñez, Ralph Nüssli, Dario Andenmatten, Silke Stertz.

Institute of Medical Virology. University of Zurich, Zurich, Switzerland.

Influenza A virus (IAV) entry activates signalling pathways that reprogram the cell and have been shown to be crucial for the outcome of viral infection. We find that EGFR and MAPK1/8 are activated upon IAV infection and positively influence viral replication. However, the complexity of these signalling cascades, tightly connected with each other, require a broader analysis to identify the main routes and the key mediators involved in these processes. First, we have performed an ELISA-based multiplex analysis for the identification of Receptor Tyrosine Kinases (RTKs) that are activated upon IAV entry. We have observed that the ErbB family, a group of RTKs closely related with EGFR, is consistently activated in response to the virus infection. Currently, we are trying to characterize the role of these proteins in the early steps of the viral infection. Moreover, and in order to obtain a more comprehensive view of the signalling events induced during IAV entry, we are conducting a quantitative phosphoproteomics screen of A549 cells after short times post-infection with IAV. The aim of this analysis is the identification of signalling networks that could play a crucial role in the early steps of the viral infection. The key proteins of these cascades, for which specific inhibitors are already available, could be used as clinical targets for future antiviral interventions.
Truncation of the C-terminus of pestivirus E<sup>ns</sup> strongly reduces its intracellular activity

Christoph Zürcher<sup>1,2</sup>, Kay-Sara Sauter<sup>3</sup>, Matthias Schweizer<sup>3</sup>.

<sup>1</sup>Institute of Virology and Immunology, University of Bern, Bern, Switzerland.
<sup>2</sup>Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland.
<sup>3</sup>Institute of Virology and Immunology, Vetsuisse Faculty, University of Bern, Bern, Switzerland.

E<sup>ns</sup> is a glycoprotein encoded exclusively by pestiviruses. Whereas it is a structural protein, a significant portion of E<sup>ns</sup> is secreted. The main function of the protein has been attributed to its ribonuclease activity, which can block interferon (IFN) induction by extracellularly added viral RNA. However, the exact function and site of action are still under debate.

Soluble E<sup>ns</sup> binds to glycosaminoglycans on cell membranes via a binding site in the C-terminus. We have shown that cell-bound, but yet un-internalized extracellular E<sup>ns</sup> is easily detached when treated with the soluble glycosaminoglycan heparin. The effect is lost within one hour at 37°C, but not at 4°C, which indicates that E<sup>ns</sup> is taken up by an energy-dependent pathway.

Here we show that a mutant E<sup>ns</sup> protein lacking the C-terminal part (∆C-term) has a one order of magnitude less efficient intracellular activity against ss- and dsRNA-induced IFN synthesis without loss of ribonuclease activity. Binding by the mutant RNase to the cell membrane is weak and cell-bound ∆C-term E<sup>ns</sup> is easily removed by washes with medium at 4°C. Different to the wild-type form, the intracellular activity of the ∆C-term mutant is not affected by heparin pre-treatment or washes at 37°C, suggesting an alternative route of entry into the cells.

Thus, we propose that E<sup>ns</sup> acts as an enzymatically active decoy receptor that degrades extracellularly added viral RNA mainly in endolysosomal compartments. This efficiently prevents the activation of intracellular pattern recognition receptors (PRRs) and maintains a state of innate immunotolerance in persistently infected animals.
LIST OF PARTICIPANTS

(by alphabetical order)
ANCHISI Stéphanie 1*
Microbiology and Molecular Medicine
University of Geneva
Michel-Servet 1
Geneva
Switzerland
stephanie.anchisi@unige.ch

AVILA SANCHEZ Mislav
Institute of Virology
University of Zurich
Winterthurerstrasse 266a
Zurich
Switzerland
mislav81@yahoo.com

BACHOFEN Claudia 2*
Institute of Virology, Vetsuisse Faculty
University of Zurich
Winterthurerstrasse 266a
Zurich
Switzerland
claudia.bachofen@uzh.ch

BEAUPARLAND David 3*, 27
Institute of Medical Virology
University of Zurich
Winterthurerstrasse 190
Zurich
Switzerland
beauparlant.david@virology.uzh.ch

BIANCHI Matteo 4*, 22
Institute of Medical Virology
University of Zurich
Winterthurerstrasse 190
Zurich
Switzerland
bianchi.matteo@virology.uzh.ch

BLATTI-CARDINAUX Laure 5*
Institute of Virology and Immunology
University of Bern
Länggassstrasse 122
Bern
Switzerland
laure.cardinaux@vetsuisse.unibe.ch

BRANISLAV Ivan 6*
Institute of Medical Virology
University of Zurich
Winterthurerstrasse 190
Zurich
Switzerland
ivan.branislav@virology.uzh.ch
BRINGOLF Fanny
Department of Clinical Research and Veterinary Public Health
University of Bern
Bremgartenstrasse 109A
Bern
Switzerland
fanny.bringolf@vetsuisse.unibe.ch

BÜCHI Werner
ViiV Healthcare GmbH
Talstrasse 3-5
Münchenbuchsee
Switzerland
werner.u.buechi@viivhealthcare.com

CAMPBELL Nottania Kay
Division of Infection Diseases and Hospital Epidemiology
University Hospital of Zurich
Rämistrasse 100
Zurich
Switzerland
nottaniakay.campbell@usz.ch

CIUFFI Angela
Institute of Microbiology
Lausanne University Hospital (CHUV) and University of Lausanne
Bugnon 48
Lausanne
Switzerland
angela.ciuffi@chuv.ch

CRAMERI Michel
Institute of Medical Virology
University of Zurich
Winterthurerstrasse 190
Zurich
Switzerland
crameri.michel@virology.uzh.ch

DA PALMA Joel
Institute of Microbiology
Lausanne University Hospital (CHUV) and University of Lausanne
Bugnon 48
Lausanne
Switzerland
joel.palma@chuv.ch

DAO THI Viet Loan
Division of Gastroenterology and Hepatology / Institute of Microbiology
Lausanne University Hospital (CHUV) and University of Lausanne
Bugnon 48
Lausanne
Switzerland
viet-loan.dao-thi@chuv.ch
DREIER Birgit
Department of Biochemistry
University of Zurich
Winterthurerstrasse 190
Zurich
Switzerland
bdreier@bioc.uzh.ch

EDINGER Thomas
Institute of Medical Virology
University of Zurich
Winterthurerstrasse 190
Zurich
Switzerland
edinger.thomas@virology.uzh.ch

EICHWALD Catherine
Institute of Virology
University of Zurich
Wintherturerstrasse 266a
Zurich
Switzerland
ceichwald@vetvir.uzh.ch

EROGLU Mustafa
Institute of Medical Virology
University of Zurich
Winterthurerstrasse 190
Zurich
Switzerland
mustafacan.ero glu@uzh.ch

FAHRNY Audrey
Division of Infection Diseases and Hospital Epidemiology
University Hospital of Zurich
Rämistrasse 100
Zurich
Switzerland
audrey.fahrny@usz.ch

FASEL Nicolas
Department of Biochemistry
University of Lausanne
Boveresses 155
Epalinges
Switzerland
nicolas.fasel@unil.ch

FRAEFEL Cornel
Institute of Virology
University of Zurich
Winterthurerstrasse 266a
Zurich
Switzerland
cornel.fraefel@access.uzh.ch
FRANZOSO Francesca
Institute of Virology
University of Zurich
Winterthurerstrasse 266a
Zurich
Switzerland
francesca.franzoso@vetvir.uzh.ch

FRIEDRICH Nikolas
Institute of Medical Virology
University of Zurich
Winterthurerstrasse 190
Zurich
Switzerland
friedrich.nikolas@virology.uzh.ch

GALAN NAVARRO Clara
Institute of Microbiology
Lausanne University Hospital (CHUV) and University of Lausanne
Bugnon 48
Lausanne
Switzerland
clara.galan@chuv.ch

GEISSBERGER Fabienne-Désirée
Institute of Medical Virology
University of Zurich
Winterthurerstrasse 190
Zurich
Switzerland
geissberger.fabienne@virology.uzh.ch

GERS-HUBER Gustavo
Product Development
Hutman Diagnostics AG
Mattenstrasse 22
Basel
Switzerland
g.gers-huber@hutmandiagnostics.com

GLUCK Selene
Institute of Virology
University of Zurich
Winterthurerstrasse 266a
Zurich
Switzerland
selene_gluck@access.uzh.ch

GREBER Urs
Institute of Molecular Life Sciences
University of Zurich
Winterthurerstrasse 190
Zurich
Switzerland
urs.greber@imls.uzh.ch
GUERRA Jessica
Microbiology and Molecular Medicine
University of Geneva
Michel-Servet 1
Geneva
Switzerland
jessica.guerra@unige.ch

GÜNTHARD Huldrych
Division of Infection Diseases and Hospital Epidemiology
University Hospital of Zurich
Rämistrasse 100
Zurich
Switzerland
huldrych.guenthard@usz.ch

HERRADOR Antonio
Institute of Microbiology
Lausanne University Hospital (CHUV) and University of Lausanne
Bugnon 48
Lausanne
Switzerland
antonio.herrador-fernandez@chuv.ch

HERRMANN Sybille
Medical Affairs
Gilead Sciences
Turmstrasse 28
Zug
Switzerland
sybille.herrmann@gilead.com

HUBER Michael
Institute of Medical Virology
University of Zurich
Winterthurerstrasse 190
Zurich
Switzerland
huber.michael@virology.uzh.ch

IVIC Sandra
Division of Infection Diseases and Hospital Epidemiology
University Hospital of Zurich
Rämistrasse 100
Zurich
Switzerland
sandraivic@usz.ch

KAISER Laurent
Laboratory of Virology, Division of Infectious Diseases and Division of Laboratory Medicine
University Hospitals of Geneva
Gabrielle-Perret-Gentil 4
Geneva
Switzerland
laurent.kaiser@hcuge.ch
KHOSRAVI Mojtaba
Department of Clinical Research and Veterinary Public Health
University of Bern
Bremgartenstrasse 109A
Bern
Switzerland
mojtaba.khosravi@vetsuisse.unibe.ch

KOHN Tamar
Environmental Chemistry Laboratory
EPFL School of Architecture, Civil and Environmental Engineering
Station 2
Lausanne
Switzerland
tamar.kohn@epfl.ch

Kok Yik Lim
Division of Infection Diseases and Hospital Epidemiology
University Hospital of Zurich
Rämistrasse 100
Zurich
Switzerland
yiklim.kok@usz.ch

KUNZ Stefan
Institute of Microbiology
Lausanne University Hospital (CHUV) and University of Lausanne
Bugnon 48
Lausanne
Switzerland
stefan.kunz@chuv.ch

LEWANDOWSKA Dagmara
Institute of Medical Virology
University of Zurich
Winterthurerstrasse 190
Zurich
Switzerland
lewandowska.dagmara@virology.uzh.ch

LINDEN Michael
Department of Infectious Diseases
King’s College London School of Medicine
Guy’s Hospital, Great Maze Pond
London
United Kingdom
michael.linden@kcl.ac.uk

LUISONI Stefania
Institute of Molecular Life Sciences
University of Zurich
Winterthurerstrasse 190
Zurich
Switzerland
stefania.luisoni@uzh.ch
LUSSI Carmela
Institute of Virology and Immunology
University of Bern
Länggassstrasse 122
Bern
Switzerland
carmela.lussi@vetsuisse.unibe.ch

MAURER Michael
Institute of Medical Virology
University of Zurich
Winterthurerstrasse 190
Zurich
Switzerland
maurer.michael@virology.uzh.ch

MAZEL-SANCHEZ Beryl
Microbiology and Molecular Medicine
University of Geneva
Michel-Servet 1
Geneva
Switzerland
beryl.mazel-sanchez@unige.ch

MEISTER Simon
Microbiology and Molecular Medicine
University of Geneva
Gabrielle-Perret-Gentil 4
Geneva
Switzerland
simon.meister@hcuge.ch

METZNER Karin
Division of Infection Diseases and Hospital Epidemiology
University Hospital of Zurich
Rämistrasse 100
Zurich
Switzerland
karin.metzner@usz.ch

MORDASINI Vanessa
Experimental Infectious Diseases and Cancer Research
University Children’s Hospital of Zurich
August Forel Strasse 1
Zurich
Switzerland
vanessa.mordasini@kispi.uzh.ch

MÜLLER Dominik
Institute of Medical Virology
University of Zurich
Winterthurerstrasse 190
Zurich
Switzerland
mueller.dominik@virology.uzh.ch
MÜNZ Christian
Institute of Experimental Immunology
University of Zurich
Winterthurerstrasse 190
Zurich
Switzerland
christian.muenz@uzh.ch

NIGG Patricia
Institute of Medical Virology
University of Zurich
Winterthurerstrasse 190
Zurich
Switzerland
nigg.patricia@virology.uzh.ch

OBERLE Corinna
Division of Infection Diseases and Hospital Epidemiology
University Hospital of Zurich
Rämistrasse 100
Zurich
Switzerland
corinna.oberle@usz.ch

OPIPLGER Joël
Institute of Microbiology
Lausanne University Hospital (CHUV) and University of Lausanne
Bugnon 48
Lausanne
Switzerland
joel.oppliger@chuv.ch

PASQUATO Antonella
Institute of Microbiology
Lausanne University Hospital (CHUV) and University of Lausanne
Bugnon 48
Lausanne
Switzerland
antonella.pasquato@chuv.ch

PAVLOVIC Jovan
Institute of Medical Virology
University of Zurich
Winterthurerstrasse 190
Zurich
Switzerland
pavlovic.jovan@virology.uzh.ch

PEREIRA Bruna
Institute of Virology
University of Zurich
Winterthurerstrasse 266a
Zurich
Switzerland
brunavrb@gmail.com
PERREAUD Jérémie
Institute of Microbiology
Lausanne University Hospital (CHUV) and University of Lausanne
Bugnon 48
Lausanne
Switzerland
jeremie.perreaud@unil.ch

PLATET Philippe
Department of Clinical Research and Veterinary Public Health
University of Bern
Bremgartenstrasse 109A
Bern
Switzerland
philippe.platet@vetsuisse.unibe.ch

PRASAD Vibhu
Institute of Molecular Life Sciences
University of Zurich
Winterthurerstrasse 190
Zurich
Switzerland
vibhu.prasad@uzh.ch

PYNDIAH Nitisha
Institute of Microbiology
Lausanne University Hospital (CHUV) and University of Lausanne
Bugnon 48
Lausanne
Switzerland
nitisha.pyndiah@chuv.ch

QUENNEVILLE Simon
Institute of Microbiology
Lausanne University Hospital (CHUV) and University of Lausanne
Bugnon 48
Lausanne
Switzerland
simon.quenneville@chuv.ch

RATO Sylvie
Institute of Microbiology
Lausanne University Hospital (CHUV) and University of Lausanne
Bugnon 48
Lausanne
Switzerland
sylvie.ferreira-rato@chuv.ch

Reh Lucia
Institute of Medical Virology
University of Zurich
Winterthurerstrasse 190
Zurich
Switzerland
reh.lucia@virology.uzh.ch
ROTHENBERGER Sylvia
Institute of Microbiology
Lausanne University Hospital (CHUV) and University of Lausanne
Bugnon 48
Lausanne
Switzerland
Sylvia.Rothenberger-Aubert@chuv.ch

ROULIN Pascal
Institute of Molecular Life Sciences
University of Zurich
Winterthurerstrasse 190
Zurich
Switzerland
pascal.roulin@imls.uzh.ch

SCHENKEL Corinne
Division of Infection Diseases and Hospital Epidemiology
University Hospital of Zurich
Rämistrasse 100
Zurich
Switzerland
corinne.schenkel@usz.ch

SCHIBLER Manuel
Laboratory of Virology, Division of Infectious Diseases and Division of Laboratory Medicine
University Hospitals of Geneva
Gabrielle-Perret-Gentil 4
Geneva
Switzerland
simon.meister@hcuge.ch

SCHMID Markus
Department of Biochemistry
University of Zurich
Winterthurerstrasse 190
Zurich
Switzerland
m.schmid@bioc.uzh.ch

SCHMOLKE Mirco
Microbiology and Molecular Medicine
University of Geneva
Michel-Servet 1
Geneva
Switzerland
mirco.schmolke@unige.ch

SCHMUTZ Stefan
Division of Infection Diseases and Hospital Epidemiology
University Hospital of Zurich
Rämistrasse 100
Zurich
Switzerland
stefan.schmutz@usz.ch
SCHWEIZER Matthias
Federal Food Safety and Veterinary Office
University of Bern
Länggassstrasse 122
Bern
Switzerland
matthias.schweizer@vetsuisse.unibe.ch

SEYFFERT Michael
Institute of Virology
University of Zurich
Winterthurerstrasse 266a
Zurich
Switzerland
mseyffert@vetvir.uzh.ch

SIEBEN Christian
Institute of the Physics of Biological Systems, Experimental Biophysics Laboratory
EPFL School of Basic Sciences
Sorge
Lausanne
Switzerland
christian.sieben@epfl.ch

STALDER Hanspeter
Institute of Virology and Immunology
University of Bern
Länggassstrasse 122
Bern
Switzerland
hanspeter.stalder@vetsuisse.unibe.ch

STEINER Fiona
Institute of Medical Virology
University of Zurich
Winterthurerstrasse 190
Zurich
Switzerland
steiner.fiona@virology.uzh.ch

STERTZ Silke
Institute of Medical Virology
University of Zurich
Winterthurerstrasse 190
Zurich
Switzerland
stertz.silke@virology.uzh.ch

STIEGELER Emanuel
Institute of Medical Virology
University of Zurich
Winterthurerstrasse 190
Zurich
Switzerland
stiegeler.emanuel@virology.uzh.ch
STROUVELLE Victoria  
Division of Infection Diseases and Hospital Epidemiology  
University Hospital of Zurich  
Rämistrasse 100  
Zurich  
Switzerland  
victoria.strouvelle@usz.ch

SUTTER Sereina  
Institute of Virology  
ETH Zurich  
Winterthurerstrasse 266a  
Zurich  
Switzerland  
sutterse@student.ethz.ch

TAPPAREL Vu Caroline  
23, 35  
Microbiology and Molecular Medicine, University of Geneva  
Division of Infectious Diseases and Division of Laboratory Medicine, University Hospitals of Geneva  
Gabrielle-Perret-Gentil 4  
Geneva  
Switzerland  
caroline.tapparel@hcuge.ch

THOMPSON Danielle  
Institute of Microbiology  
Lausanne University Hospital (CHUV) and University of Lausanne  
Bugnon 48  
Lausanne  
Switzerland  
danielle.thompson@unil.ch

THORBALL Christian  
Institute of Microbiology  
Lausanne University Hospital (CHUV) and University of Lausanne  
Bugnon 48  
Lausanne  
Switzerland  
christian.thorball@chuv.ch

TORRIANI Giulia  
33  
Institute of Microbiology  
Lausanne University Hospital (CHUV) and University of Lausanne  
Bugnon 48  
Lausanne  
Switzerland  
giulia.torriani@unil.ch

TRAN Huong T.L.  
40*  
Division of Gastroenterology and Hepatology / Institute of Microbiology  
Lausanne University Hospital (CHUV) and University of Lausanne  
Bugnon 48  
Lausanne  
Switzerland  
thi-lan-huong.tran@chuv.ch
TRIPA Ion
Institute of Medical Virology
University of Zurich
Winterthurerstrasse 190
Zurich
Switzerland
tripa.ion@virology.uzh.ch

UHR Therese
Institute of Medical Virology
University of Zurich
Winterthurerstrasse 190
Zurich
Switzerland
uhr.therese@virology.uzh.ch

URSPRUNG Stephan
Institute of Medical Virology
University of Zurich
Winterthurerstrasse 190
Zurich
Switzerland
stephan.ursprung@uzh.ch

VETTER Beatrice
Institute of Medical Virology
University of Zurich
Winterthurerstrasse 190
Zurich
Switzerland
vetter.beatrice@virology.uzh.ch

VKOVSKI Philip
Institute of Virology and Immunology
University of Bern
Länggassstrasse 122
Bern
Switzerland
philip.vkovski@vetsuisse.unibe.ch

VONGRAD Valentina
Division of Infection Diseases and Hospital Epidemiology
University Hospital of Zurich
Rämistrasse 100
Zurich
Switzerland
valentina.vongrad@usz.ch

WEBER Jacqueline
Institute of Medical Virology
University of Zurich
Winterthurerstrasse 190
Zurich
Switzerland
weber.jacqueline@virology.uzh.ch
YAKIMOVICH Artur
Institute of Molecular Life Sciences
University of Zurich
Winterthurerstrasse 190
Zurich
Switzerland
artur.yakimovich@imls.uzh.ch

YAMAUCHI Yohei
Department of Biology
ETH Zurich
Otto-Stern-Weg 3
Zurich
Switzerland
yohei.yamauchi@bc.biol.ethz.ch

YÀNGÜEZ Emilio
Institute of Medical Virology
University of Zurich
Winterthurerstrasse 190
Zurich
Switzerland
yanguez.emilio@virology.uzh.ch

ZAGORDI Osvaldo
Institute of Medical Virology
University of Zurich
Winterthurerstrasse 190
Zurich
Switzerland
zagordi.osvaldo@virology.uzh.ch

ZÜRCHER Christoph
Institute of Virology and Immunology
University of Bern
Länggassstrasse 122
Bern
Switzerland
christoph.zuercher@vetsuisse.unibe.ch