Fifth annual meeting
of the Belgian Society for Virology
Thursday, December 7, 2017
The Royal Academies for Science and the Arts
Hertogsstraat/Rue Ducale 1, Brussels

8h30 Welcome -Prof. Dr. Hans Nauwynck
General assembly of the Belgian Society for Virology
Election of new Belvir Board members

9h00-9h45 Keynote lecture 1 –Prof. Dr. F.J.M. (Frank) van Kuppeveld (Utrecht University): "Picornaviruses hijack intracellular membrane machinery to build replication organelles"

9h45-11h30 Selected presentations (15 min each)
Chairs: Prof. Zeger Debyser and Prof. Peter Delputte

9h45-10h00 Selected talk 1
Stress-induced unfolded protein response contributes to Zika virus-associated microcephaly
Ivan Gladwyn-Ng, Lluis Cordon Barris, Christian Alfano, Catherine Creppe, Thérèse Couderc, Giovanni Morelli, Nicolas Thelen, Michelle America, Bettina Bessières, Férechté Encha-Razavi, Maryse Bonnière, Ikuo K. Suzuki, Marie Flamand, Pierre Vanderhaeghen, Marc Thiry, Marc Lecuit and Laurent Nguyen

10h00-10h15 Selected talk 2
A gammaherpesvirus provides protection against allergic asthma by inducing the replacement of resident alveolar macrophages with regulatory monocytes
Bénédicte Machiels, Mickael Dourcy, Xue Xiao, Justine Javaux, Claire Mesnil, Catherine Sabatel, Daniel Desmecht, François Lallemand, Philippe Martinive, Hamida Hammad, Martin Guilliams, Benjamin Dewals, Alain Vanderplasschen, Bart N Lambrecht, Fabrice Bureau & Laurent Gillet

10h15-10h45 *Coffee break (Marmer Room)*

10h45-11h00 Selected talk 3
Single virus imaging of HIV-1 entry with fluorescently labeled capsid
Irena Zurnic, Lieve Dirix, Veerle Lemmens, Susana Rocha, Johan Hofkens, Frauke Christ, Jelle Hendrix and Zeger Debyser

11h00-11h15 Selected talk 4
Epigenetic regulation of gga-miR-126 during lymphoproliferative disease in chicken
Gennart I, Parissi L, Pejakovic S, Rauw F and Muylkens

11h15-11h30 Selected talk 5

Role of chemokine CCL20 in angiogenesis in liver cancer caused by hepatitis C virus

Mohammed Benkheil, Sam Noppen, Matthias Van Haele, Tania Roskmas, Johan Neyts, Sandra Liekens

11h30-12h30 Selected short presentations (5 minutes each/without discussion)

Chair: Prof. Laurent Gillet

1. Full protection from fatal Zika virus infection by a novel chimeric live-attenuated Zika virus vaccine

Dieudonné Buh Kum, Niraj Mishra, Robbert Boudewijns, Michael A. Schmid, Suzanne Kaptein, Johan Neyts and Kai Dallmeier

2. Proteomic and functional analyses of the virion transmembrane proteome of cyprinid herpesvirus 3

Vancsok C., Peñaranda M.M.D., Raj V.S., Leroy B., Jazowiecka-Rakus J.,Boutier M., Gao Y., Wilkie G.S., Suárez N.M., Wattiez R., Gillet L., Davison A.J., and Vanderplasschen A.F.C.

3. Contribution of lncRNAs in Establishment of HIV Latency in Central Memory CD4 T Cells

Wim Trypsteen, Cory White, Alberto Bosque, Celsa Spina, Steve Lefever, Pieter Mestdagh, Linos Vandekerckhove, Nadia Beliakova-Bethell

4. Towards the development of an effective vaccine against malignant catarrhal fever

Myster F., Javaux J., Van Campe W., Roels S., Mostin L., Kerkhofs P.,Vanderplasschen A., Dewals B.G.

5. Interrogation of the CHIKV nsP-host interactome in human and mosquito cells.

Koen Bartholomeeusen, Chris Hoffmann, Terence Agombin, Lien De Caluwe, Sandra Coppens, Simon Daled, Maarten Dhaenens, Dieter Deforce, Kevin Ariën

6. Zika virus replication in testicles of mice and impact of viral replication inhibitors

Jacobs S., Kaptein S.J.F., Delang L., Neyts J.

7. Nanopore sequencing as a revolutionizing diagnostic tool for viral enteric disease complexes in pig health management

Sebastiaan Theuns, Quinten Bernaert, Bert Vanmechelen, Ward Deboutte, Piet Maes, Jelle Matthijnssens, Hans J. Nauwynck

8. Theiler's murine encephalomyelitis virus (TMEV) induces accumulation of autophagosomes by impairing autophagic flux

Eric C. Freundt, Lars K. Benner, Jaimie L. Miser, Maximillian P. Ganz

9. Viral suppressive capacity: assessing CD8 T cell responses in HIV-infected individuals under antiretroviral therapy

P.Pannus, P. Adams, E. Willems, Iserentant G., L. Heyndrickx, M. Van Frankenhuijsen, E. Florence, M. Kiselinova, S. Rutsaert, K. Vervisch, W. Despiegelaere, C. Devaux, L. Vandekerckhove, G. Vanham

10. The role of macrophages in the enterovirus A71 (EV71) physiopathogenesis in mice
Carmen Mirabelli, Liang Sun, Aloys Tijsma, Hendrik Jan Thibaut, Johan Neyts

11. Beneficial impact of early treatment on restriction factor expression profile
Clarissa Van Hecke, Magdalena Sips, Eva Malatinkova, Ward De Spiegelaere, Karen Vervisch, Chris Verhofstede, Margaret Johnson, Sabine Kinloch-de Loes, Wim Trypsteen, Linos Vandekerckhove

12. Mechanism of Porcine Circovirus Type 2 uptake in porcine peripheral blood monocytic cells
Ruifang Wei, Bo Yang, Wendy Baetens, Abubakar Garba, Jiexiong Xie, Hans J. Nauwynck

12h30-13h30 *Sandwich lunch*

13h30-14h15 Keynote lecture 2 –Prof. Dr. Xavier Saelens (VIB-UGent Center for Medical Biotechnology): "Prevention and treatment options for human Respiratory Syncytial Virus: very small things considered"

14h15-16h00 Selected presentations (15 min each)
Chairs: Prof. Guido Vanham and Prof. Alain Vanderplasschen

14h15-14h30 Selected talk 6
Macavirus latency-associated protein evades immune detection through regulation of protein synthesis *in cis* depending upon its glycin/glutamate-rich domain
Océane Sorel, Ting Chen, Françoise Myster, Justine Javaux, Alain Vanderplasschen, Benjamin G. Dewals

14h30-14h45 Selected talk 7
Access to a main alphaherpesvirus receptor, located basolaterally in the respiratory epithelium, is masked by intercellular junctions and revealed by pollen proteases
Jolien Van Cleemput, Katrien C.K. Poelaert, Kathlyn Laval, Roger Maes, Gisela S. Hussey, Wim Van den Broeck, Hans J. Nauwynck

14h45-15h15 *Coffee break*

15h15-15h30 Selected talk 8
Myxovirus resistance 1 (Mx1) in the myeloid cell compartment contributes to protection against Thogoto virus infection
J. Spitaels, L. Van Hoecke, K. Roose, X. Saelens

15h30-15h45 Selected talk 9
Role of PDGFRβ and associated gangliosides in influenza virus entry

Pieter Vrijens, Els Vanstreels, Sam Noppen, Roberto Ronca, Evelien Vanderlinden, Annelies Stevaert, Manon Laporte, Marco Presta, Sandra Liekens, Lieve Naesens

15h45-16h00 Selected talk 10
Understanding flavivirus pathogenesis: host factor hijacking by non-coding viral RNA
Sander Jansen, Johan Neyts and Kai Dallmeier

16h00-17h00 Selected short presentations (5 minutes each/without discussion)
Chair: Prof. Thomas Michiels

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Annelies Leemans, Marlies Boeren, Winke Van der Gucht, Isabel Pintelon, Kenny Roose, Bert Schepens, Xavier Saelens, Dalan Bailey, Guy Caljon, Louis Maes, Paul Cos, Peter Delputte

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3. Protection by neuraminidase-specific antibodies against influenza A
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4. Conserved fever pathways across vertebrates: a herpesvirus delays fish behavioral fever through expression of a decoy Tnf-alpha receptor
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5. Cyprinid herpesvirus 3 invades the central nervous system of carp using the olfactory system
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6. Helminth-induced IL-4 expands virtual memory $CD8^+$ T cells for early control of gammaherpesvirus infection
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8. Characterization of a murine model of replicative adenovirus based oral vaccination

Emeline Goffin, Justine Javaux, Michel Bisteau, Eric Destexhe, Laurent Gillet

9. A systematic siRNA study of the hemagglutinin cleavage profiles of influenza A and B virus with relevance for the design of airway protease inhibitors
Manon Laporte, Mohammed Benkheil, Lieve Naesens

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11. New strategies for intradermal delivery of the yellow fever vaccine launched from a plasmid
Sapna Sharma, Michael A. Schmid, Niraj Mishra, Johan Neyts, and Kai Dallmeier

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1.

Co-culturing of immortalized mesenchymal cells derived from nasal mucosa, lungs, spleen, lymph nodes and red bone marrow increase the susceptibility of blood monocytes to PRRSV infection

Abubakar Garba[1*], Hans J. Nauwynck[1]

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Mesenchymal cells are multipotent, non-hematopoietic cells found in the stroma of bone marrow and in many other tissues. They are capable of differentiating into many cell types including osteoblasts, chondrocytes and adipocytes. Porcine reproductive and respiratory syndrome virus has a narrow tropism for cells of the monocytic lineage. Siglec-1 (sialoadhesin) is a crucial receptor involved in attachment and internalization of porcine reproductive and respiratory syndrome virus (PRRSV) into macrophages. Previous work in our laboratory showed that co-culturing blood monocytes with immortalized mesenchymal cells derived from nasal mucosa, lungs, spleen, lymph and red bone marrow triggers the expression of siglec-1 in blood monocytes. The present work aimed to examine if the latter mesenchymal cells increase the susceptibility of co-cultured blood monocytes for PRRSV infection. Immortalized mesenchymal cells, inoculated with PRRSV (LV (subtype 1) and Lena (subtype 3)), were found to be resistant to PRRSV. When co-cultures were inoculated with PRRSV (LV (subtype 1) and Lena (subtype 3)) and analyzed by confocal microscopy at 12 hours post-inoculation up to 7±1%, 18±0.9% and 86±1% of LV-inoculated cells and 33±1%, 45±3% and 88±2% of Lena-inoculated cells were found infected at 24 h, 48 h and 72 h of co-cultivation respectively. In contrast, only 0.8±2-2±1.5% LV-inoculated cells and 1±0.5-3±1% Lena-inoculated cells were observed to be infected in pure monocyte cultures (without mesenchymal cell lines) at 24-72 h of co-cultivation and 12 hpi (hours post-inoculation). In conclusion, immortalized mesenchymal cells isolated from nasal mucosa, lungs, spleen, lymph nodes and bone marrow were able to enhance the replication of PRRSV when co-cultured with monocytes isolated from PBMC.

2.

Interrogation of the CHIKV nsP-host interactome in human and mosquito cells.

Koen Bartholomeeusen[1], Chris Hoffmann[1], Terence Agombin[1], Lien De Caluwe[1], Sandra Coppens[1], Simon Daled[2], Maarten Dhaenens[2], Dieter Deforce[2], Kevin Ariën[1]

[1] Institute of Tropical Medicine Antwerp, Virology Unit, Antwerp, Belgium
[2] Progentomics, UGent

Chikungunya virus replicates in cells of both its vertebrate host and insect vector. To identify cellular pathways that the virus engages to allow optimal replication in these evolutionary distinct organisms we performed AP-MS to identify interaction partners of the viral non-structural proteins (nsPs) in both human and mosquito cells. CHIKV nsPs were C- or N-terminally Strep-tagged and transiently expressed in either HEK293T or C6/36 cells. Mass spectrometric analysis of on-bead-digestions of affinity purifications coupled to MiST analysis allowed sensitive and reproducible identification of a significant number of cellular protein interaction partners of nsP1, -3 and -4. The retrieval of well-established nsP3 interactors, G3BP and Bin1, in both human and mosquito cells validated our approach. Separate nsPs were associated with both shared and unique interaction partners, the latter belonging to different cellular pathways. Comparison of high-confidence interactors of nsP3 in human and mosquito cells identified 25 proteins that associate with nsP3 in both organisms. Functional classification of these shared nsP3 interactors using GO annotation showed engagement of cell-cell adhesion-, Hippo signaling-, ribosomal function- and innate immune signaling pathways by nsP3 in both human and mosquito cells. Interaction of members of each functional group with nsP3 were validated in AP-WB experiments. We will present data on the establishment of the virus-host interactome and the identification of a 'core' interactome of CHIKV in human and mosquito cells. We will further present data on the functional relevance of nsP3-host protein interactions in disrupting epithelial integrity, downstream hippo signaling and suppression of innate immune signaling.

Chikungunya virus replicates in cells of both its vertebrate host and insect vector. To identify cellular pathways that the virus engages to allow optimal replication in these evolutionary distinct organisms we performed AP-MS to identify interaction partners of the viral non-structural proteins (nsPs) in both human and mosquito cells. Comparison of human and mosquito derived interaction partners will allow to define a core cellular machinery that the virus engages in both organisms as well as species-specific interactors.
Separate nsPs were associated with both shared and unique interaction partners. Comparison of high-confidence interactors of nsP3 in human and mosquito cells identified 25 proteins that associate with nsP3 in both organisms. Functional classification of these shared nsP3 interactors using GO annotation showed engagement of cell-cell adhesion-, Hippo signaling-, ribosomal function- and innate immune signaling pathways by nsP3 in both human and mosquito cells.

3.

Role of chemokine CCL20 in angiogenesis in liver cancer caused by hepatitis C virus

Mohammed Benkheil[1], Sam Noppen[1], Matthias Van Haele[2], Tania Roskmas[2], Johan Neyts[1], Sandra Liekens[1].

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Hepatocellular carcinoma (HCC) associated with Hepatitis C Virus (HCV) shows a higher grade of vascularization compared to HCC with other underlying etiologies, suggesting that HCV may play a role in the establishment of a more angiogenic tumor microenvironment. Angiogenesis is a major determinant of tumor malignancy since the newly formed blood vessels allow tumor progression and metastatic outgrowth. Thus, it is important to gain more insight into the potential role of HCV in this process. C-C motif ligand 20 (CCL20), unique ligand for CCR6, is a chemokine originally identified in the liver. By mediating pro-inflammatory processes, CCL20 has been described as being involved in pathological processes (e.g. chronic liver injury and hepatocellular carcinoma) and high serum levels of CCL20 were proposed as specific marker for HCV-elated HCC (HCV-HCC). However, the pro-angiogenic potential of this chemokine is unknown.

We previously showed upregulation of CCL20 in HCV-infected hepatoma Huh7 5.1 cells. Using immunohistochemistry, we showed CCR6 expression on endothelial cells of chronically infected human livers and in HCC-HCC and Alcohol-related HCC. Further, flow cytometry analysis showed CCR6 expression on different types of cultured primary endothelial cells including Human umbilical vein endothelial cells (HUVECs). Moreover, human recombinant CCL20 (hrCCL20) stimulated migration of HUVEC *in vitro*, which was abolished in presence of CCR6 neutralizing antibodies. In addition, CCL20 induced activation of ERK-pathway/signaling in HUVECs. ERK-signaling has been shown to play a crucial role in different steps of angiogenesis, including migration and proliferation. We further assessed the pro-angiogenic potential of CCL20 *in vivo* through matrigel plug formation assay. CCL20 induced blood vessel formation in *NMRI-nu* mice, which was abolished in presence of CCR6 neutralizing antibodies.

Our data show that the pro-inflammatory chemokine CCL20, strongly induced by HCV, has a pro-angiogenic potential. Stimulation of angiogenesis through CCL20 acting on CCR6-positive endothelial residing HCC may be an additional mechanism by which HCV contributes to HCC development and/or malignancy.

4.

Identification of an essential virulence gene of cyprinid herpesvirus 3

Boutier M.[1], Gao Y.[1], Vancsok C.[1], Suarez M. N.[2], Davison A.[2], Vanderplasschen A.[1]

[1.] *Immunology-Vaccinology, FARAH, ULiège, Liège, Belgium*
[2.] *MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom*

The genus *Cyprinivirus* consists of a growing list of phylogenetically related viruses, some of which cause severe economic losses to the aquaculture industry. The archetypal member, cyprinid herpesvirus 3 (CyHV-3) causes mass mortalities worldwide in koi and common carp. A CyHV-3 mutant was described previously that is attenuated *in vivo* by a deletion affecting two genes (ORF56 and ORF57). The relative contributions of ORF56 and ORF57 to the safety and efficacy profile of this vaccine candidate have now been assessed by analysing viruses individually deleted for ORF56 or ORF57. Inoculation of these viruses into carp demonstrated that the absence of ORF56 did not affect virulence, whereas the absence of ORF57 led to an attenuation comparable to, though slightly less than, that of the doubly deleted virus. To demonstrate further the role of ORF57 as a key virulence factor, a mutant retaining the ORF57 region but unable to express the ORF57 protein was produced by inserting multiple in-frame stop codons into the coding region. Analysis of this virus *in vivo* revealed a safety and efficacy profile comparable to that of the doubly deleted virus. These findings show that ORF57 encodes an essential CyHV-3 virulence factor. They also indicate that ORF57 orthologues in other cypriniviruses may offer promising targets for the rational design of attenuated recombinant vaccines.

5

Full protection from fatal Zika virus infection by a novel chimeric live-attenuated Zika virus vaccine

Dieudonné Buh Kum[1#,] Niraj Mishra[1], Robbert Boudewijns[1], Michael A. Schmid[1], Suzanne Kaptein[1], Johan Neyts[1] and Kai Dallmeier[1#]

[1]Laboratory of Virology, Antiviral Drug and Vaccine Development, Rega Institute for Medical Research, KU Leuven – University of Leuven, Belgium

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Introduction: The recent Zika virus (ZIKV) epidemic in the Americas has led to the search of therapeutics and vaccines to curb or prevent the debilitating disease caused by the virus, namely; congenital malformations and abnormalities. A number of replication inhibitors and vaccine candidates have been reported but none has been approved yet. The yellow fever virus (YFV) and ZIKV, together with the dengue, Japaneseencephalitis, West Nile, and tick-borne encephalitis viruses belong to the genus flavivirus.

Aim: Using our recently developed PLLAV (plasmid-launched live-attenuated vaccine) platform and the live-attenuated yellow fever virus (YFV) vaccine strain 17D (YFV-17D) as a vector, we engineered a chimeric vaccine candidate by replacing the antigenic surface glycoproteins of YFV-17D with those of a prototypic Asian lineage ZIKV strain isolated from the Yap Island in 2007.

Methods: The chimeric YF-ZIK virus generated appeared over-attenuated and could not be effectively propagated *in vitro*. In an enforced attempt to propagate the virus in cell culture, we developed a protocol that permitted the replication of the chimera to higher titers for use in experimental animal models and vaccine production.

Results: Our results show that the novel chimeric YF-ZIKprME is highly attenuated *in vitro* and in interferon-deficient AG129 mice, a recently established stringent ZIKV challenge model (Zmurko et al. PLoS NTD 2016). A single-shot of YF-ZIKprME completely protected mice from a lethal challenge with 1x105PFU of a heterologous wild-type ZIKV.

Conclusion: The chimeric YF-ZIKprME vaccine, which resembles the recently licensed chimeric Japanese encephalitis and dengue vaccines Imojev® and Dengvaxia®, respectively, may be developed as a promising ZIKV vaccine.

6

Infection of European eel by Anguillid herpesvirus 1: from basic research to conservation programs

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The European eel (Anguilla anguilla) is a catadromous fish with a complex and fascinating life cycle. Over the last few decades, the number of eels reaching Europe has declined by 99%. The European eel is nowadays classified as a critically endangered species. Among the multiple factors contributing to this decline, viral infection caused by Anguillid herpesvirus 1 (AngHV-1) is thought to play a key role. In addition to its ecological impact, this virus is also causing important economic loses in the eel aquaculture sector. In the present project, we aim to study the infection of the European eel by AngHV-1 as a homologous host-virus model to address fundamental questions related to this member of the *Alloherpesviridae* family. This project is structured around three integrated work packages/objectives. In the first work package, we will use an "In vivo Imaging system" and a recombinant virus expressing firefly luciferase to address various aspects of pathogenesis: (i) the portal of viral entry into the host, (ii) viral spreading within the infected host, (iii) transmission of the virus between subjects and (iv) the effect of water temperature and the developmental stage of the host on the outcome of the infection. The second work package will consist of a multidisciplinary approach aiming to study the effect of the infection on the behavior of the host. Finally, in the third work package, we will investigate the role of ORF35 as a key virulence factor and the potential of ORF35 deleted recombinants as attenuated vaccine candidates compatible with mass vaccination and conservation programs.

A Gammaherpesvirus Infection Protects from Pneumovirus-Induced Immunopathologies

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The hygiene hypothesis postulates that some infections early in life could modulate the later immune responses of their host against heterologous antigens. Gammaherpesviruses (γHV) establish lifelong latency in immune cells and could therefore profoundly imprint the immune system of their host. Pneumovirus infections cause life-threatening pathologies upon primary infections but also after vaccination, explaining the current absence of any licensed RSV vaccine. Studying factors that could influence the development of pneumovirus-mediated immune disorders is therefore highly important. In this study, we showed that a previous γHV infection protects against both pneumovirus vaccine-enhanced disease and heterologous infection. This therefore shed a new light for the understanding of pneumovirus-induced diseases and for the development of new anti-pneumovirus vaccine strategies. Altogether, this study emphasizes the concept of how microbiome can impact immune responses of its host.

8

Cyprinid herpesvirus 3 invades the central nervous system of carp using the olfactory system

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Cyprinid herpesvirus 3 (CyHV-3) causes a lethal disease in common and koi carp causing substantial financial losses to the aquaculture sector. Previous *ex vivo* examination of infected fish had revealed a high level of replication in the olfactory rosettes. In the present project, we investigated using "in vivo bioluminescence imaging" whether CyHV-3 is able to invade the olfactory system of carp. When applied intranasally, a recombinant CyHV-3 expressing luciferase as a reporter gene was able to infect the olfactory neuroepithelium. From this site, the virus then spread via axonal transport through the olfactory bulbs, olfactory nerve tracts to eventually reach the brain. Immunostaining of infected olfactory rosettes suggests that the virus may use both the olfactory neurons and supporting cells as a portal of entry. These findings shade new lights on CyHV-3 pathogenesis.

9

Theiler's murine encephalomyelitis virus (TMEV) induces accumulation of autophagosomes by impairing autophagic flux

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Autophagy is a catabolic pathway that enables the cell to sequester and degrade cytoplasmic contents and also serves as an innate response against intracellular pathogens. Many viruses have evolved to subvert this fundamental pathway for their own replication and spread, including poliovirus, which uses autophagy for non-lytic release. Little is known about whether cardioviruses interact with the autophagic pathway during infection. Here we investigated whether Theiler's murine encephalomyelitis virus (TMEV), a cardiovirus, utilizes autophagy. By immunofluorescence staining and confocal microscopy, we found that the 3D polymerase colocalized with the autophagosomal marker microtubule-associated protein light chain 3 (LC3). Infected cells displayed the presence of numerous autophagosomes as indicated by fluorescent LC3 punctae. The lipidated form of LC3, LC3-II, was also found to increase during the course of infection. Using a tandem fluorescent-tagged LC3 fusion protein that indicates autophagosome maturation, we found that TMEV blocks fusion of autophagosomes with lysosomes. Consistent with inhibition of autophagosome maturation, many GFP-LC3 punctae in infected cells were also found to be Lamp1 positive. Although the viral 3D polymerase was found to localize to autophagosomes, TMEV was found to replicate normally in cells lacking ATG5, a gene essential for canonical autophagy. Thus, inhibition of autophagosome maturation may serve to protect intracellular virus from degradation or may serve to enhance non-lytic release.

10.

Full-length genomic and biologic comparison of Cyprinid herpesvirus 3 strains

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Cyprinid herpesvirus 3 (CyHV-3) is the archetype of fish alloherpesviruses and the etiological agent of a lethal disease in common and koi carp. Since its emergence in the late 1990s, CyHV-3 has spread across the word. To date, the genomes of only four CyHV-3 strains are available in the Genbank. No comparison of the biological properties of these strains have been reported. Here, we sequenced 7 strains of CyHV-3 of different geographical origins and compared their growth *in vitro* as well as their virulence *in vivo*. The major findings were: (i) we confirm the existence of the two genetic lineages previously reported as "European and Asian" but reveal inconsistencies between the geographical origin and the genotype of some strains; (ii) inter-strain recombinations were detected in only one strain (GZ11); (iii) an inverse correlation was observed between viral growth *in vitro* and virulence *in vivo*; (iv) analyses of CyHV-3 disrupted genes suggested that ORF27 is an essential virulent factor; ORF12,16,30,55,64,105, 108 and 128 are non-essential virulent factors; ORF52 and 153 are putative virulent factors.The present study illustrates the interest of coupled genomic and biologic comparison of viral strains to study viral evolution and pathogenesis.

11.

Epigenetic regulation of gga-miR-126 during lymphoproliferative disease in chicken

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The Gallid herpesvirus-2 (GaHV-2) naturally infects chicken and is responsible of Marek's disease (MD). MD is causing important economic losses for the poultry industry and is a robust model for studying virus-induced tumor formation. During the infection, GaHV-2 modulates viral and cellular gene expression and triggers transformation of latently infected cells. The importance of viral and cellular microRNAs (miR) in host-pathogen interactions has been identified recently and additional research is needed to better understand their roles. This study focuses on an intragenic host miR, gga-miR-126, mediating proper angiogenesis. This miR has been reported to impair cancer progression through signaling pathways that control cell proliferation and survival. It was shown to be repressed during the viral infection. The first aim of this study was to confirm the downregulation of this cellular miR during the viral infection. In order to respond to this objective, gga-miR-126 was quantified by quantitative RT-PCR, in several cell types representing the different viral phases: (i) infected chicken embryonated fibroblast (CEF) were used for the replicative infection, (ii) latently infected cell line (MSB-1) were either treated or not with a DNA methyltransferase inhibitor (5-azacytidine (5aza)) or with a Histone deacetylase inhibitor (Sodium butyrate (Nabu)) and they represented the reactivation phase, (iii) finally, *in vivo* samples were used for the tumorigenesis phase (peripheral blood leucocytes (PBL) and T $CD4^+$ lymphocytes). No change of gga-miR-126 expression was observed during the replicative infection. After treatment with the 5aza, a high increase (4 fold) of gga-miR-126 was observed while no modification (1,1 fold) of its expression was shown after nabu treatment. Finally a repression (6, 7 fold) was observed in tumoral T $CD4^+$ lymphocytes compare to uninfected T $CD4^+$ lymphocytes. These results show an impact of DNA methylation on gga-miR-126 expression regulation and a repression during the tumorigenesis phase of GaHV-2 life cycle. To characterize this low expression level, DNA methylation pattern was assessed in MSB-1 cell line and in PBL and T $CD4^+$ cells through Bisulfite Genomic Sequencing Assay in two CpG islands. In MSB-1 cell line, the level of DNA methylation was very high in the two CpG islands (CpG1, 85 % and CpG2, 95 %). *In vivo*, DNA methylation was at a lower level in both CpG islands. Interestingly, a higher percentage of methylation is observed, for PBL and T CD4+ cells, in infected group compare to uninfected group. The repression of gga-miR-126 in infected T CD4+ lymphocytes might be explained by the higher percentage of methylation observed at the two CpG islands for the infected groups.

The third aim of this work is to understand the functionality of gga-miR-126 during tumorigenesis. In order to respond to this objective three main strategies was put in place. The first strategy is to validate some targets of gga-miR-126 by luciferase assay. The second strategy is to assess cell phenotype (growth, viability, apoptosis) after overexpression of gga-miR126 with the use of the tetON system *in vitro*. A stable cell line containing this system was created and the addition of tetracycline allows the overexpression of gga-miR126. The third strategy was the creation of a recombinant virus containing an expression cassette allowing the overexpression of gga-miR-126 *in vivo*. All these experiments are in progress.

12.

Characterization of a murine model of replicative adenovirus based oral vaccination

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Oral vaccination offers many immunological and practical advantages. Nevertheless, oral immunization may be hampered by oral tolerance mechanisms. A solution to this problem lies in the use of viral vectors. Since the 1970s, alive, orally administered adenovirus serotype 4 and 7 vaccines are effectively used to protect United States military personnel from severe respiratory diseases caused by these viruses. Replication-competent adenoviruses appear therefore as promising vectors for the development of oral vaccines. Until now, as human adenoviruses replicate efficiently only in a highly restricted host range, researches on this topic have suffered from the lack of reliable animal models. In this study, we used mouse adenovirus type 1 (MAV-1) to develop a small animal model for oral replicative adenovirus vaccines. We firstly characterized adenovirus oral infection in mice. No clinical signs were observed following MAV-1 oral administration. Nevertheless, viral DNA was detected by qPCR in various organs, showing that the virus efficiently infects by this route. Furthermore, this infection generated a specific and neutralizing humoral response. We then evaluated the protection induced by MAV-1 oral infection against a respiratory homologous challenge. Our observations showed that oral immunization prevent the weight loss due to an intranasal infection. Moreover, histological and qPCR analysis showed a protection against lung inflammation and viral replication. Altogether, these results show that MAV-1 offers a reliable model for oral vaccination based on replicative adenoviruses. This model provides a valuable tool to assess the potential of adenoviruses as oral vaccine platforms.

13.

A favipiravir-resistant chikungunya virus variant showed reduced fitness *in vivo*

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Favipiravir (T-705) is a broad-spectrum antiviral, which has been approved in Japan for the treatment of severe influenza virus infections. T-705 is metabolized intracellularly to its ribofuranosyl 5'-triphosphate form, which was shown to competitively inhibit the incorporation of ATP and GTP by the viral RNA-dependent RNA polymerase (RdRp). We reported earlier that favipiravir inhibits the *in vitro* replication of chikungunya virus (CHIKV) and protects against disease progression in CHIKV-infected immunodeficient mice (Delang et al., J Antimicrob Chemother 2014) and in the acute stage of CHIKV infection in immunocompetent mice (Abdelnabi et al., Antiviral Res 2017). Low-level T-705-resistant CHIKV variants were selected in cell culture. A K291R mutation in the F1 motif of the RdRp was linked to phenotypic resistance against T-705.
In the present study, we wanted to explore the *in vivo* replication fitness and pathogenicity of the T-705-resistant CHIKV variant compared to the wild type virus (WT). To this end, C57BL/6J mice were infected with 10^5 PFU of either the T-705-resistant CHIKV variant, a capping inhibitor-resistant CHIKV variant (MADTP-resistant) or WT in the left hind footpad. The infected mice were euthanized on days 0, 3 and 7 post-infection (p.i.) for quantification of viral RNA and infectious virus titer in different tissues. In addition, the swelling of the inoculated foot was measured with a caliper. Interestingly, the T-705-resistant variant had significantly lower viral RNA levels in lymph nodes and other sampled organs on day 3 p.i., and failed to efficiently infect the ankles. Moreover, the mice infected with the T-705-resistant variant had less swelling in the inoculated foot compared to the ones infected with WT. In contrast, the infectivity of the MADTP-resistant variant was comparable to that of WT. Our data thus suggest that if a T-705-resistant variant would emerge, it would not have the fitness to establish a resistant population with a virulence similar to that of WT virus.

14.

Zika virus replication in testicles of mice and impact of viral replication inhibitors

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Zika virus (ZIKV) is an arthropod-borne virus that belongs to the family of *Flaviviridae*. Its main route of transmission is through the bite of a female mosquito, primarily of the *Aedes aegypti* species. In 2008, a US scientist returning from Senegal infected his wife in what is known as the first documented case of sexual transmission of ZIKV. This alternative route for ZIKV transmission has gained much more attention during the latest outbreak in the Americas, with many more reports on sexual transmission[1]. Although not contributing significantly to the transmission of ZIKV in areas where the *Aedes* mosquitos are abundantly present, it may contribute to a large part of locally acquired cases of ZIKV infection in areas where the *Aedes* mosquitos are absent.

We previously established a robust animal model of ZIKV infection in AG129 (IFN-α/β and IFN-γ receptor knock-out) mice[2]. Infection of AG129 mice with ZIKV MR766 (protoype strain, African lineage) resulted in acute neutrophilic encephalitis with viral antigens accumulating in neurons of the brain and spinal cord. Additionally, high levels of viral RNA were detected in the spleen, liver and kidney. Interestingly, high levels of viral RNA ($6.4\log_{10}$) were also detected in the testis of infected male mice. This finding was recently corroborated by others[3,4] that showed that infectious virus was present in the testis and epididymis of infected mice as early as at day 3 post infection (pi) and persisting for as long as 11 weeks pi. We earlier showed that the nucleoside analog 7-deaza-2'-*C*-methyladenosine (7DMA) shows good efficacy against ZIKV infection in mice[1], hence the ZIKV mouse model is very suitable for assessing the *in vivo* efficacy of novel ZIKV inhibitors. In addition, novel inhibitors should preferably also be efficacious in lowering ZIKV titers in testis and seminal fluid, for which our ZIKV mouse model can also be employed. Indeed, we show that the 'tool' compound 7DMA significantly lowers viral RNA and infectious virus titers in the testis and epididymis of mice infected with a ZIKV isolate from Suriname (SL1602), both at day 3 and day 7pi.We thus propose that novel ZIKV inhibitors that are being developed, and to which we actively contribute, should preferably elicit activity in this model.

[1]Hastings AK. and Fikrig E., Yale J Biol Med, 2017
[2]Zmurko J. et al., PLoSNTD, 2016
[3]Govero J. et al., Nature, 2016
[4]Duggal NK. et al., Cell Reports, 2017

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15.

Sensitive non-viral bioassay for quantification of mouse and human type III interferon in biological fluids

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We developed a specific and sensitive luciferase bioassay for IFN type III detection in biological fluids. IFNAR knocked-out/ Mx1-luciferase/ IFNLR1 expressing cells were derived from the LKR10 epithelial cell line, which is naturally responsive to IFN-λ. Using the CRISPR-Cas9 technology, the IFNAR2c subunit of the IFN type I receptor was first knocked-out. IFNAR-KO/LKR10 cells were further transduced with a lentivirus expressing the *Firefly* luciferase under control of the Mx1-promoter. Finally, lentivirus-mediated overexpression of IFNLR1 receptor subunit led to enhanced induction of the reporter gene. Compared to commercial enzyme-linked immunoabsorbent assay system (ELISA), our test is highly sensitive in detecting IFN-λ in the serum of mice previously infected or treated with IFN-λ. Given the non-species specificity of IFN-λ, this assay allows detection of both mouse and human proteins. It can be used for the quantification of IFN-λ2, IFN-λ3 and likely the divergent IFN-λ4. This test is easy to perform, cheaper and more sensitive than commercially available assays.

16.

Understanding flavivirus pathogenesis: host factor hijacking by non-coding viral RNA

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In an effort to unravel why flavivirus pathogens such as the dengue (DENV) and Zika virus (ZIKV) prove to be so harmful, we focus on the key virulence factor 'subgenomic flaviviral RNA' (sfRNA). These non-coding RNAs derived from the 3'UTR are shown to be highly conserved, essential for pathogenesis and a critical determinant of the epidemic potential of flaviviruses. SfRNAs affect the host cell by binding specific RNA-binding proteins, disturbing a range of cellular pathways.
Using a unique yeast three-hybrid (Y3H) method, a human ORFeome library comprising more than 12.000 ORFs was screened for sfRNA-interaction. In total, 73 putative sfRNA-interacting proteins were identified, including previously known host factors of viral replication as well as novel candidate sfRNA binding cellular proteins. Subsequently, an orthogonal yeast screen was performed, testing 25 of our top hits for their interaction with a larger panel of flavivirus sfRNAs (DENV-2, YFV, Modoc, WNV, ZIKV strain MR766 and a Brazilian clinical ZIKV isolate). Three proteins showed interaction with this full panel of flavivirus sfRNAs. A RNA-pulldown (PAR-CLIP) assay using overexpression of the GFP-labelled candiate proteins in DENV-2 replicon cells and crosslinking to sfRNA has been set up to confirm their specific sfRNA-protein interaction in the mammalian system. Preliminary data confirm interaction between sfRNA and DDX6 (a known interactor/positive control) and our first tested protein(s) which has (have) not yet been described as flavivirus host factors. We will show how our thus identified sfRNA-binding cellular proteins may be fit in the flaviviral lifecycle, and how these pathways and interactions usurped by pathogenic flaviviruses may help to understand viral pathogenesis also towards novel means of antiviral intervention.

17.

Protection by neuraminidase-specific antibodies against influenza A

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There is increasing evidence to suggest that antibodies directed towards influenza A virus (IAV) neuraminidase (NA) are an important correlate of protection against influenza in humans. Moreover, the potential of NA-specific antibodies to provide broader protection than conventional hemagglutinin (HA) antibodies has been recognized. We have isolated two monoclonal antibodies, N1-7D3 and N1-C4, directed towards the N1 NA. N1-7D3 binds to a conserved linear epitope in the membrane distal, carboxy-terminal part of the NA and reacted with the NA of seasonal H1N1 isolates ranging from 1977 till 2007 the 2009 H1N1pdm virus as well as A/Vietnam/1194/04 (H5N1). N1-7D3 lacked NA inhibition (NI) activity and the ability to protect BALB/c mice against a lethal challenge with a range of H1N1 viruses. Conversely, N1-C4 bound to a conformational epitope that is conserved between the 2009 H1N1pdm and H5N1 IAV and displayed potent *in vitro* antiviral activity mediating both NI and plaque size-reduction. Moreover, N1-C4 could provide heterosubtypic protection in BALB/c mice against a lethal challenge with 2009 H1N1pdm or H5N1 virus. Glutamic acid residue 311 in the NA was found to be critical for the NA binding and antiviral activity of monoclonal antibody N1-C4. Next, we examined if the contribution of Fcy receptor mediated mechanisms could contribute to protection by NA-specific IgG antibodies. N1-C4 and polyclonal anti-NA sera, passively transferred to mice, were able to significantly protect against weight loss in mice genetically deficient in (i) the Fc receptor common y-chain (gene: *Fcer1g*) or (ii) FcγRI (gene: *Fcgr1*) and FcγRIII (gene: *Fcgr3*) when challenged with 1 LD50 of A(H1N1)pdm09. Polyclonal anti-NA sera was also able to protect (i) *Fcer1g*$^{-/-}$ mice, (ii) *Fcgr1* and *Fcgr3* double knockout mice. Additionally, treatment of mice with N1-C4 or polyclonal anti-NA sera could reduce viral lung loads in WT mice, however in *Fcer1g*$^{-/-}$ and *Fcgr1* and *Fcgr3* double knockout mice there was a trend for increased lung viral titres in comparison to WT mice. This work indicates that direct NA inhibitory activity plays a dominant role in the control of influenza virus by antibodies directed against NA and provides further evidence on cross-protective epitopes within the N1 subtype and highlight the potential of NA as an important target for vaccine and therapeutic approaches.

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18.

Impact of M2e-specific monoclonal antibodies on influenza A virus spread *in vitro* and in a mouse transmission model

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Influenza is a global threat to public health. Currently available influenza vaccines are effective against strain-matched influenza A and B viruses but do not protect against novel pandemic viruses. Vaccine candidates that target conserved B or T cell epitopes of influenza viruses could circumvent this shortcoming. The conserved extracellular domain of matrix protein 2 (M2e) of influenza A is an example of such a broadly protective vaccine candidate. Protection by M2e-based vaccine candidates largely depends on M2e-specific IgG antibodies. Here we show that the M2e-specific IgG2a monoclonal antibody 65 (mAb 65) can reduce influenza A/Udorn/72 (H3N2) virus replication *in vitro*. This effect was specific to Udorn/H3N2 virus and not seen with WSN/H1N1. We have observed that budding of filamentous influenza A/Udorn virus particles can be readily visualized by immunofluorescence microscopy. Furthermore, using confocal and correlative stochastic optical reconstruction microscopy (STORM), we show that M2e-specific monoclonal antibodies disturb the filament morphology, length, width, and branching pattern that is associated with influenza A/Udorn/301/1972 (H3N2) virus replication. These results suggest that M2e-specific monoclonal antibodies reduce the level of influenza virus particle formation in the case of A/Udorn virus replication. In a mouse model of influenza A virus transmission, we show that passive transfer of mAb 65 of index mice significantly reduces the transmission of Udorn/H3N2 virus to cohoused, unimmunized contact mice. This reduction was evident based on significantly reduced viral loads in snouts and lung tissues of both immunized index and unimmunized contact mice. Passive transfer of mAb 65 reduced Udorn/H3N2 viral titers in lungs of wild type and to a lesser extend in $Fcgr1^{-/-}$ $Fcgr3^{-/-}$ mice, suggesting that *in vivo* protection provided by mAb 65 against Udorn/H3N2 virus is dependent on Fcγ receptor-mediated antibody effector mechanisms. We conclude that M2e-based antibody immune therapy could be used not only to protect the immunized host but also exposed contacts and thus contribute to reduce the spread of a new pandemic virus.

19.

Towards the development of direct-acting antivirals for the treatment of infections with human parechoviruses

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Parechoviruses (HPeV, *Picornaviridae*) are human pathogens causing sepsis-like illness and neurological complications in infants. There are no antivirals available for the treatment of infection with parechoviruses. To address this unmet medical need, we established antiviral assays and validated this for the identification of inhibitors of these viruses.

We first selected a cell line that is well suited for phenotypic antiviral screenings. All cell lines tested i.e. Vero A, SH-SY-5Y, A549 and BGM proved susceptible to infection with HPeV1 and HPeV3. However, cytopathic effect induced by HPeV1 was most pronounced in BGM cells and this cell line was used for subsequent studies. Next the kinetics of replication of HPeV1 and HPev3 were studied in BGM cells: intracellular viral RNA production peaked at 6h post infection for HPeV1 and at 12h p.i. for HPeV3, at 3 days p.i. the extracellular genome copy numbers were comparable for HPeV1 and HPeV3.

We then studied whether a panel of compounds that have earlier been shown to inhibit the replication of picornaviruses are able to inhibit HPeV replication. The broad-spectrum antiviral Favipiravir (T-705), the nucleoside analogue 7-deaza, 2' *C* methyladenosine (7DMA)and ribavirin were found to inhibit both HPeV1 and 3 replication with 7DMA being most effective (EC_{50} 11,5 μM). Enterovirus-specific inhibitors, i.e. the capsid binders, 2C and 3C inhibitors were devoid of activity against HPeV.

We next performed a screen in BGM cells against HPeV1 infection of a library of ~2000 compounds, obtained from the national cancer institute (NCI, USA). A total of 43 hits were selected by microscopic evaluation, confirmation of activity (>1 log10 inhibition) was done by means of qRT-PCR, and the activity of 10 compounds was confirmed (three of which are approved anticancer drugs, two target mTOR and 5 compounds belong to the chemically diversity library). The particular characteristics and mechanism of antiviral action of these compounds is now being studied against HPeV3.

In conclusion, we established an antiviral assay platform for medium-throughput screening for HPeV inhibitors; identified reference inhibitors and identified 10 inhibitors of in vitro HPeV1 replication.

20.

A systematic siRNA study of the hemagglutinin cleavage profiles of influenza A and B virus with relevance for the design of airway protease inhibitors

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Replication of influenza virus in human airways requires cleavage of the hemagglutinin precursor HA0 by host cellular proteases. This cleavage is indispensable for viral infectivity as it primes the hemagglutinin for fusion during virus entry. Diverse trypsin-like proteases (TLPs) have been implicated, in particular Transmembrane Serine Proteases (TMPRSSs) which can activate certain HA subtypes in cell lines that overexpress the respective proteases (Böttcher et al, J Virol 2006, 80: 9896-98). For transmembrane protease serine 2 (TMPRSS2), an association with the replication of influenza A/H1N1 and A/H7N9 has been clearly established. TMPRSS2- knock-out mice survive an A/H1N1 or A/H7N9 infection that is lethal in wild-type mice (Tarnow et al, J Virol, 88: 4744-51), and humans showing higher TMPRSS2 expression are more susceptible to these two influenza subtypes (Cheng et al, J Inf Dis, 212: 1214-21). For A/H3N2 and influenza B, the protease dependency is unresolved and more research is needed to precisely define their protease cleavage profiles (Laporte and Naesens, Curr. Op. Virol. 2017, 24:16–24).

We performed a systematic siRNA-based analysis in the human airway epithelial Calu-3 cell line, to establish the role of 40 different TLPs in influenza virus replication.

For A/H1N1 and A/H3N2, TMPRSS2 knockdown led to a marked (>10-fold) reduction in virus titers and cytopathic effect (CPE). For influenza B, only a marginal effect was seen after TMPRSS2 knockdown, consistent with the report that TMPRSS2-knockout mice are not protected against influenza B infection (Sakai et al, Sci Rep 2016, 6: 29430). Several siRNAs yielded partial inhibition of influenza B CPE, however none of the tested siRNAs achieved an effect that was as strong as that seen for TMPRSS2 siRNA versus influenza A. Immunostaining for viral nucleoprotein confirmed the complete suppression of influenza A replication after TMPRSS2 knockdown. For influenza B, immunostaining revealed that, upon treatment with siRNA for kallikrein 13 (KLK13), the infection rate was two-fold reduced when compared to the non-targeting siRNA control. The observation that several siRNAs produced some, but in all cases, incomplete inhibition of influenza B virus, indicates that either the most relevant protease(s) were not addressed by our siRNA panel, and/or redundancy exists among several TLPs.

To design successful protease inhibitors, we first need a clear insight into the protease dependency of different human influenza (sub)types. While TMPRSS2 is clearly linked with A/H1N1 and A/H3N2, a more complex picture emerges for influenza B.

21.

Removal of the N-glycosylation sequon at position N116 located at p27 of the respiratory syncytial virus fusion protein elicits enhanced antibody responses after DNA immunization

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Prevention of severe lower respiratory tract infections in infants caused by the human respiratory syncytial virus (hRSV) remains a major public health priority. The development of a safe and effective prophylactic vaccine is very important in the control of RSV disease burden. Currently, the major focus of vaccine development relies on the hRSV fusion (F) protein since it is the main target protein for neutralizing antibodies induced by natural infection. Furthermore, hRSV F is highly conserved among RSV strains. Additionally, the protein conserves 5 N-glycosylation sites, two of which are located in the F2 subunit (N27 and N70), one in the F1 subunit (N500) and two in the p27 peptide (N116 and N126). To our knowledge, improvements can be made to the understanding of the contribution of N-glycosylation in the immunogenicity of the RSV F protein. Here, we developed expression vectors encoding RSV F glycosylation mutants. *In vitro* characterization of the expressed RSV F glycomutants was performed after transfection of these vectors in BSR T7/5 cells and revealed that all mutants are transported to the cell surface, though reductions in surface expression were observed for F N70Q, F N500Q, F N27-70-500Q and F N27-70-116-126-500Q. Furthermore, it was shown that *in vitro* RSV F fusion activity is determined by N-glycan at position N500. To study the influence of the loss of one or more N-glycosylation sites in RSV F on immunogenicity, BALB/c mice were immunized with the different plasmids encoding the RSV F glycomutants. In comparison with F WT DNA immunized mice, higher neutralizing titers were observed following immunization with F N116Q. Moreover, RSV A2 L19F challenge of mice that had been immunized with mutant F N116Q DNA was associated with lower RSV RNA levels compared with those in challenged WT F DNA immunized animals. Since p27 is assumed to be post-translationally released from the mature F trimer after cleavage and thus not present on the mature RSV F protein, it remains to be elucidated how deletion of this glycan can contribute to enhanced antibody responses and protection upon challenge. These findings provide new insights to improve the immunogenicity of RSV F in potential vaccine candidates.

22.

Study of the interplay between a gammaherpesvirus infection and innate lymphoid cells in the context of type 2 immunity.

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Environmental conditions are a key factor in shaping the host immune system. In particular, the early microbiome orientates subsequent immune responses later in life. Thus, we recently observed that infection by Murid herpesvirus 4 (MuHV-4) inhibits the development of House Dust Mites (HDM)-induced allergic asthma in mice through the alveolar recruitment of regulatory monocytes. Group 2 innate lymphoid cells (ILCs) play a major role in the initiation, the maintenance and the memory of type 2 immune responses. Their activation can be triggered by allergens as well as viruses such as influenza, rhinovirus and respiratory syncytial virus. Their plasticity may exacerbate anti-viral immunity, which may have adverse consequences in respiratory diseases. Here, we investigated the different subset of ILCs in mock and MuHV-4 infected mice upon HDM treatment. Interestingly, we observed that MuHV-4 infection affected the number of ILC2 and their capacity to secrete type 2 cytokines. Our results support the hypothesis that modification in numbers, proportions and functions of ILC2 following some viral infections could profoundly impact subsequent development of asthma.

23.

A gammaherpesvirus provides protection against allergic asthma by inducing the replacement of resident alveolar macrophages with regulatory monocytes

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The hygiene hypothesis postulates that the recent increase in allergic diseases such as asthma and hay fever observed in Western countries is linked to reduced exposure to childhood infections. Here we investigated how infection with a gammaherpesvirus affected the subsequent development of allergic asthma. We found that murid herpesvirus 4 (MuHV-4) inhibited the development of house dust mite (HDM)-induced experimental asthma by modulating lung innate immune cells. Specifically, infection with MuHV-4 caused the replacement of resident alveolar macrophages (AMs) by monocytes with regulatory functions. Monocyte derived AMs blocked the ability of dendritic cells to trigger a HDM-specific response by the TH2 subset of helper T cells. Our results indicate that replacement of embryonic AMs by regulatory monocytes is a major mechanism underlying the long-term training of lung immunity after infection.

24.

The role of macrophages in the enterovirus A71 (EV71) physiopathogenesis in mice.

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We previously established that EV71-812 strain replicates in adult immunocompromised (SCID) mice. EV71-812 showed a respiratory tropism (viral RNA in lungs) at early times post infection (pi), before the onset of disease. At later times (10-14 days pi), neuroinvasion was also observed (viral RNA in brain and spinal cord), with associated weight loss and paralysis/death in 80% of the infected-mice. The virus isolated from lungs and brain/spinal cord differed in one amino acid in the "puff loop" of the capsid protein VP2. This mutation conferred this 812-MA strain an exclusive neurotropism, associated with a faster (4 days pi) and higher mortality rates (100%) in infected mice. Since in EV71-812 infected mice, both type II pneumocytes and alveolar macrophages stained positive for EV71 antigens, we here investigated the role of alveolar macrophages in the physiopathogenesis of the EV71-812 and -812MA strain.

SCID mouse bone marrow-derived macrophages were differentiated *in vitro* with M-SCF (7-10 days of treatment), after which these were infected with either the EV71-812 or EV71-812-MA strains. Viral titers were quantified at 2, 4, 8, 24 and 48 h pi by end-point titration. There was no increase in infectious virus titers over-time, which suggested that infection of macrophages was not productive. However, viral antigens were detected by immunofluorescence in infected-macrophages.

Next, SCID mouse macrophages were depleted from the lung compartment by treatment with clodronate liposomes (by intra-nasal inoculation), after which mice were infected with either the EV71-812 or the EV71-812-MA strain. Interestingly, when infection with either strain was performed one day after clodronate-treatment, i.e. when macrophages were already depleted from the lung compartment, 50-75% of the infected-mice died in one day without developing signs of disease. In contrast, when mice were simultaneously treated with clodronate and virus-infected, there was no difference in disease and/or mortality between the EV71-812 and EV71-812-MA infected mice, either in the macrophage-depleted or control groups.

Altogether, these results suggest that macrophages do not fully support viral replication but can internalize the virus. This internalization could prevent an overstimulation of the immune system leading to the septic-like death observed *in vivo* in macrophage-depleted mice. Moreover, macrophages were not the major players in the differential physiopathogenesis observed with EV71-812 and EV71-812MA infection.

25.

Evaluation of the chimeric Japanese encephalitis vaccine (IMOJEV) for protection against yellow fever

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Introduction: Yellow fever virus (YFV) 17D is among the most effective vaccines available today, protecting against YFV-induced devastating liver disease. However, the recent urban YFV outbreaks in Angola and Brazil highlight the severe vaccine shortage in an emergency setting. Further, potential spreading of YFV to Asia, where the *Aedes aegypti* vector mosquitoes are abundant, poses a serious future concern. Japanese encephalitis virus (JEV) is a related mosquito-transmitted Flavivirus that is circulating in South-East Asia and can cause severe neurological symptoms. The chimeric YFV 17D-derived live-attenuated Japanese encephalitis vaccine (IMOJEV), in which the pre-membrane and envelope genes of YFV 17D are replaced with those of JEV, is approved for human use against JEV.
Methods: Other than protecting against infection with JEV, we here evaluated the additional protective effect of IMOJEV against YFV 17D in a stringent lethal challenge model using AG129 mice. These mice are deficient in the interferon-α/β, and -γ receptors and thus are highly susceptible to YFV 17D infection.
Results: Almost all (n=30/31) IMOJEV-vaccinated animals survived the challenge with a 1000-fold lethal dose-50 of YFV 17D and did not show any signs of disease. In contrast, this dose was 100% lethal in non-vaccinated animals (n=31), with mean days to death of 16 ± 3. Already 7- or 14-days post vaccination, IMOJEV provided 60% or 100% protection (n=5) against the YFV 17D challenge, respectively. IMOJEV vaccination of AG129 mice induced neutralizing antibodies only against the JEV but not against YFV 17D. These results are in line with the fact that IMOJEV encodes for YFV 17D prM and E, mediating virus infection.
Conclusion: IMOJEV protects AG129 mice against YFV 17D independently of neutralizing antibodies. This cross-protection may be mediated via immunity to the YFV 17D non-structural proteins that are encoded by IMOJEV. Consequently, our data suggest that, due to its chimeric nature, IMOJEV may serve as efficient dual vaccine against, both, JEV as well as YFV.

26.

Towards the development of an effective vaccine against malignant catarrhal fever

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Alcelaphine herpesvirus 1 (AlHV-1) persists in wildebeest asymptomatically but induces malignant catarrhal fever (MCF), a fatal lymphoproliferative disease upon transmission to several ruminants, including cattle. The significant socio-economic impact of MCF in East-Africa urges for the development of new vaccination strategies. We have previously shown using the experimental rabbit model that the latency-associated gene ORF73 was essential for MCF induction while a ORF73-deficient (ORF73^{-}) strain C500 could induce sterile immunity against a wild-type (WT) challenge. In the present study, we first infected 4-month-old calves with WT or ORF73^{-} virus. Intranasal infection with the WT virus induced typical MCF clinical signs and lesions. However, ORF73^{-} virus did not cause any clinical sign but induced a complete protection against an intranasal challenge with the WT virus. These results were encouraging for future prospects of vaccination against MCF. However, AlHV-1 is highly cell-associated and viral titres remain low, potentially hampering effective vaccine production. Interestingly, attenuated strain WC11 of AlHV-1 displays increased viral growth and cell-free infectious particles. Whole-genome sequencing of strain WC11 revealed few genomic changes including full deletion of the gene A7. A7 is a positional homolog of Epstein-Barr virus (EBV) BZLF2 encoding the C-type lectin-like glycoprotein gp42. Gp42 is expressed in the envelope of EBV virions and mediates entry into B cells. Hence, we used the C500 BAC clone to generate an A7^{STOP} recombinant strain. We observed that a lack of A7 expression resulted in significant increased viral growth in fibroblasts *in vitro*. Also, the plaque size over time and the morphology of the plaques were modified in absence of A7. Finally, infection of rabbits demonstrated that A7 is essential for the development of MCF. In conclusion, the lack of A7 significantly alters the replication of the virus *in vitro* and the development of MCF in rabbits. Thus, joined impairments of A7 and ORF73 could lead to an optimized vaccine.

27.

Stress-induced unfolded protein response contributes to Zika virus-associated microcephaly

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Abstract

Accumulating evidence support a causal link between Zika virus (ZIKV) infection during pregnancy and congenital microcephaly. However, the mechanism of ZIKV-associated microcephaly remains unclear. We combined analyses of ZIKV-infected human fetuses and cultured human neural stem cells with mouse embryos to understand how ZIKV induces microcephaly. After intracerebral and intraplacental inoculation of ZIKV in mouse embryos, we show that it triggers endoplasmic reticulum stress in embryonic brains *in vivo*. This perturbs a physiological unfolded protein response within cortical progenitors that controls neurogenesis. Thus, ZIKV-infected progenitors generate fewer projection neurons that eventually settle in the cerebral cortex whereupon sustained ER stress leads to apoptotosis. Furthermore, we demonstrate that administration of pharmacological inhibitors of UPR counteracts these pathophysiological mechanisms, and prevents microcephaly in ZIKV-infected mouse embryos. Such defects are specific to ZIKV as they were not observed upon intraplacental injection of other related flaviviruses in mice.

28.

Viral suppressive capacity: assessing CD8 T cell responses in HIV-infected individuals under antiretroviral therapy

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Background
Potent HIV-specific immune responses and a small latent viral reservoir are likely required to control viral rebound after treatment interruption. Here we investigate the associations between the viral suppressive capacity of CD8+ T-cells, their immune phenotype and the HIV reservoir in a cohort of antiretroviral therapy-treated (ART) patients.
Methods
Thirty-six patients under ART with suppressed viremia and six healthy donors were recruited. Total HIV-1 DNA and unspliced mRNA (usRNA) levels were measured in PBMCs using digital droplet PCR. In a sub-group of 21 patients infected with HIV subtype B, viral transcriptional activity was quantified with the TILDA assay. Viral suppressive capacity of CD8+ T cells was assessed by p24 ELISA running the viral inhibition assay (VIA): a co-culture system of HIV-superinfected CD4 and autologous CD8 T cells. Immunophenotyping of T cells (cytokine production, cytotoxicity and immune checkpoint markers) was executed before and after peptide stimulation using 18 color flow cytometry. Cytometry data was clustered using viSNE and SPADE in the Cytobank environment. Extracted data was analyzed for subsets correlating with suppressive responses. Linear regression and student t-test were used for statistical analysis.
Results
Total HIV DNA and usRNA levels (median: 187 and 2.2 cps/million PBMCs respectively) correlated well with each other ($p < 0.05$). TILDA values ranged from 0 to 313 cells [IQR: 1.4 – 55.8] with detectable HIV RNA transcripts per million CD4+ T cells after stimulation. However, no correlation with CD8+ T-cell suppressive capacity was observed. CD8+ T-cell suppressive capacity was significantly ($p < 0.01$) increased after HIV peptide stimulation. Viral suppressive capacity correlated with HLA-DR expression in CD8+ T cells during co-culture ($p < 0.005$). At baseline, the suppressive capacity correlated with CD160/PD-1 co-expression in CD8 TEMRA ($p < 0.001$).
Conclusions
Despite heterogeneity in terms of immune responses, phenotype and reservoir size, T-cell exhaustion markers CD160 and PD-1 were predictive for the suppressive capacity of CD8+ T cells. Specific CD8 T cells subsets were identified in responders as compared to non-responders individuals. This opens future prospects for therapeutic interventions restoring immune responses in HIV-1 infected patients.

29.

Epigenetic regulation of the viral telomerase RNA subunit promoter over-expressed during Marek's Disease Virus induced oncogenesis

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Marek's disease virus (MDV) is a chicken oncogenic alphaherpesvirus that induces the rapid onset of a highly malignant T-lymphoma. Disease pathogenesis begins with a semi-productive infection of B-lymphocytes, followed by a switch to latent infection in CD4+ cells and integration in the host telomeres. MDV encodes two copies of a viral telomerase RNA subunit (vTR) that are highly expressed during all stages of the MDV life cycle. This study focuses on the epigenetic regulators involved in the switch between productive and latent phase of MDV life cycle. Objectives were to establish methylation (5mC) and hydroxymethylation (5hmC) patterns, to measure impact of methylation on the telomerase activity and on response elements (E-boxes specific of Myc transcription factor) in the vTR promoter. E-boxes were mutated obtaining E2, E3 and E2E3 mutants. Specific 5mC levels were found *in vitro* during latency, up to 90%. Following induction of the viral reactivation methylation levels dropped to 70%. The results showed that global 5hmC percentage on the vTR promoter was lower compared to 5mC. During latent phase, 2% of 5hmC was observed, what did not change significantly after the reactivation. However, change in 5hmC positions was observed between these two different phases of the viral cycle. For *in vivo* samples, global methylation was significantly lower, from 0.4% up to 2%, depending of the cell type and the day post infection. Measuring telomerase activity showed that reactivation considerably increases its activity. Reporter assays showed significantly lower activity of methylated promoters compared to non-methylated. On non-methylated promoter E2 mutation had no repressive effect, but E3 and E2E3 mutations showed repression of vTR promoter. These results showed that the E3 box is involved in regulating transcription of the vTR. After methylation, mutant forms of the E2/E2 boxes showed higher activity when they were compared to the wild-type promoter. This result indicates that methylation process masked the effect of site-directed mutagenesis of c-myc binding site.

30.

Equine herpes virus 1 infection of CD4$^+$ T-lymphocytes

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Equine herpes virus 1 (EHV-1) causes respiratory disease, late-term abortion and neurological disorders in horses worldwide. Abortigenic and neurovirulent phenotypes are circulating in the field. Both EHV-1 phenotypes replicate in the epithelial cells of the upper respiratory tract (URT), and reach its target organs, the pregnant uterus, and central nervous system, via a cell-associated viremia in leukocytes. Laval *et al*., (2015) demonstrated that cells of the myeloid lineage (CD172a$^+$) play an important role in the pathogenesis of both EHV-1 phenotypes. Furthermore, Vandekerckhove *et al*., (2010) showed in the nasal explant model that not only monocytic cells but also T-lymphocytes have an unexplained role in the pathogenesis of EHV-1. The aim of this study was to investigate the interplay of EHV-1 with T-lymphocytes. First, blood-derived T-lymphocytes were cultured in lymphocyte medium containing 4 U/ml IL-2 and inoculated with either abortigenic 97P70 or neurovirulent 03P37 EHV-1 strains. The expression of immediate early proteins (IEP) and the late gB proteins in T-lymphocytes were determined at 1, 3, 6, 9, 12, 24 and 48 hpi. IEP positive cells were detectable starting from 1 hpi for the abortigenic strain and 3 hpi for the neurovirulent strain. From 1 hpi to 3 hpi, the percentage of IEP-cells increased from 1.5% to 3.0% in 97P70-inoculated cells and 1.0% to 1.4% in 03P37-inoculated cells. A maximum of 8.7% and 3% IEP-positive cells was reached at 6 hpi. The late gB proteins were initially detected at 3 hpi, in 0.4% and 0.1% of EHV-1 97P70 and 03P37-inoculated T-lymphocytes. The expression of gB increased in time, while the IEP decreased starting from 12 hpi. EHV-1-inoculated T-lymphocytes showed no significant decrease in cell viability during cultivation, compared to mock-inoculated cells. Moreover, extracellular virus titers did not increase in time, which might indicate an immune-evasion strategy of EHV-1 to stay longer in the host. However, co-cultivation of T-lymphocytes with rabbit kidney cells, a cell line permissive for EHV-1, could induce a transfer of infectious virus.
Overall, we can conclude that abortigenic and neurovirulent EHV-1 strains can replicate in blood-derived T-lymphocytes. This leads to the hypothesis that not only monocytic cells but also T-lymphocytes have an important role in the viral dissemination in the horse. EHV-1 is able to infect and hijack T-lymphocytes early in infection. In this way, the virus can be transferred to monocytic cells and the endothelial cells of the pregnant uterus and central nervous system.

31.

Development of a propidium-monoazide-based method to correlate positive RT-qPCR signals to infectivity of murine norovirus particles

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Human noroviruses, genus *Norovirus*, family *Caliciviridae*, are a major viral cause for gastroenteritis outbreaks. Molluscs, which filter contaminated water and accumulate noroviruses in their hepatopancreas, are a typical vector for human infection. The *in vitro* culture of human noroviruses is not viable for routine analysis. Thus, the murine norovirus is used as surrogate. RT-qPCR, the established molecular method for detection of human noroviruses in food, does not allow the distinction of infectious and non-infectious viruses. Our study addresses this issue by combining the use of an intercalating agent, propidium monoazide (PMA)-pretreatment and RT-qPCR on murine noroviruses. PMA has been shown to enter only viruses with compromised capsids, subsequently binding to nucleic acids, and inhibiting PCR amplification. Viruses with intact capsids nevertheless remain impermeable and detectable via RT-qPCR. A protocol for the inactivation of norovirus samples via different methods and the subsequent set-up of a PMA-qPCR has been achieved. Initial PMA-RT-qPCR results obtained for the murine norovirus model in a simple matrix (PBS), will be further adapted for detection in a complex matrix (mussel hepatopancreas), from which intact viruses must be extracted before analysis. The current ISO-norm for extraction incorporates a capsid-damaging treatment with proteinase K and impairs the recovery of infectious viruses. An optimisation of a newer approach implementing glycine-elution and PEG-concentration is currently ongoing for better infectious virus recovery. The final objective is to detect and quantify only infectious norovirus in naturally contaminated mussels by PMA-RT-qPCR.

32.

Helminth-induced IL-4 expands virtual memory CD8$^+$ T cells for early control of gammaherpesvirus infection

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Chronic infections with helminths can imprint the immune system, Notably, helminths can affect bystander CD8$^+$ T cell responses. Virtual memory CD8$^+$ T cells (T_{VM}) are a population of antigen-inexperienced T cells displaying memory properties and that can be expanded through responsiveness to IL-4 in the thymus but also in secondary lymphoid organs. However, it is not clear how helminth-induced inflammation that is governed by IL-4 actually affects the T_{VM} compartment. In this study, we showed that helminths can induce CD44^{high}CD62L^{high}CXCR3^{high}CD49d^{low} T_{VM} expansion in the lymphoid organs depending on direct IL-4 signaling on CD8$^+$ T cells that expressed high levels of the T-box transcription factor Eomesodermin. Importantly, helminth-induced T_{VM} expansion was associated with early control of acute respiratory infection with the gammaherpesvirus murid herpesvirus 4 (MuHV-4). Such early control of MuHV-4 infection could further be explained by increased antigen-specific CD8$^+$ T cell effector responses in the lung and was directly dependent on IL-4 signaling. These results suggest that parasitic helminths can condition CD8$^+$ T cells through innate mechanisms to acquire virtual memory properties and subsequently respond more effectively to antigen-specific activation, resulting in enhanced control of viral infection.

33.

An Interactome analysis of the RSV non-structural proteins 1 and 2

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Human respiratory syncytial virus (RSV) is an enveloped, negative-stranded RNA virus belonging to the *Pneumoviridae* family and a common human respiratory pathogen. Although an RSV infection is commonly associated with only mild respiratory symptoms, very young children and the elderly are at risk for developing (severe) bronchiolitis or pneumonia. By the age of 2 years, almost all children have been infected with RSV at least once, requiring hospitalization in 1-2% of the cases. A remarkable feature of RSV infections is the near absence of type I interferons. Two unique non-structural proteins encoded by RSV, NS1 and NS2, have been shown to independently and cooperatively suppress both the induction and signaling of type I and III interferons. Recently, evidence is arising that antiviral functions of interferon-stimulated genes (ISGs) may also be suppressed by NS1 and/or NS2. In order to suppress the interferon response, NS1 and/or NS2 interfere with several key signaling molecules, *i.e.* RIG-I, MAVS, TRAF3, IKKε, TBK1, IRF3/7, STAT1 and STAT2. The expression of some of these proteins is reduced by NS1 and/or NS2, suggesting that NS1 and/or NS2 can selectively degrade proteins. In line with this, it has been proposed that NS1 and NS2 assemble into a so called "NS degradasome" complex of 300-750 kDa, which may possess proteasome-like activity. To better understand the complex interplay between the host and NS1/NS2, we investigated the interactomes of NS1 and NS2. Therefore, we used three different protein-protein interaction (PPI) mapping techniques, *i.e.* proximity-based protein biotinylation (BioID), Virotrap and antibody-mediated affinity purification. BioID and Virotrap allow the identification of PPIs in their natural cellular environment before cell lysis. As such, we could identify 796 and 322 proteins in the NS1 and NS2 proxeomes, respectively, whereas 40 and 54 proteins were identified in the NS1 and NS2 interactomes identified by Virotrap, respectively. Further analysis of these candidate NS1 and/or NS2 interactors revealed that several subunits of the proteasome complex were identified with both BioID and Virotrap, but not with affinity purification. Our results suggest that NS1 and NS2 interact with (subunits of) the proteasome. Our findings may help to characterize the RSV "NS degradasome" complex. Follow-up experiments with simultaneous overexpression of NS1 and NS2 and with endogenously expressed NS1 and NS2 during a RSV infection are planned to confirm the interaction of NS1 and/or NS2 with the proteasome.

34.

New strategies for intradermal delivery of the yellow fever vaccine launched from a plasmid

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Background: The live-attenuated yellow fever virus vaccine (YFV 17D) has been introduced more than 80 years ago and is still used efficiently, but an old fashion production protocol (using embryonated chicken eggs) and the need of a cold chain for transport and storage results in vaccine shortage and ~300,000 cases and ~80,000 fatalities every year worldwide. To overcome this problem, DNA-launched delivery of live-attenuated vaccines is an ideal platform to develop and manufacture cost-effective vaccines, even allowing that individuals from low- and middle-income countries, who are at the greatest risk of infection, can receive life-saving immunizations. However, a significant issue with the use of canonical DNA vaccines is that the cellular uptake of naked DNA is very inefficient in vivo and the bulk of the injected DNA remains extracellular. The route of plasmid DNA delivery can significantly influence its efficacy and immunogenicity.
Aim: The objective of our present study is to optimize the route and device for delivery of a novel DNA-launched YFV 17D vaccine using time resolved live virus imaging in mice.
Approach: We engineered a stable YFV 17D cDNA that launches replication from our plasmid-launched live-attenuated vaccine (PLLAV) platform. Knowledge about the site and dynamics of YFV 17D replication is a prerequisite for optimizing vaccination to prime adaptive immune responses. Using our PLLAV technology for ready cloning, we generated a YFV 17D reporter virus that drives expression of nanoLuciferase in live animals for *in-vivo* imaging. Different devices and different routes were compared for efficient delivery of the DNA-launched vaccine. Following intravenous inoculation of the specific substrate, luminescence emission from anesthetized mice was recorded using the IVIS Spectrum imaging system. Inoculation of YFV 17D nanoLuciferase reporter virus served as positive control compared to PLLAV DNA delivery.
Results: Subcutaneous injection of AG129 mice with nanoLuciferase YFV 17D virus or PLLAV induced luminescence signals *in vivo*, indicating active replication of YFV 17D in the skin. PLLAV efficiently launches virus replication, with a peak of luminescence for PLLAV on day 7 and for virus on day 5 following inoculation. Also, transdermal needle-free jet injection was found more efficient than subcutaneous inoculation but only in the presence of transfection reagent. In contrast, intradermal laserporation followed by topical application successfully delivered vaccine virus but failed to initiate YFV 17D replication from PLLAV. In its current formulation, launching of PLLAV replication is successful in 67% and 73% of sites following subcutaneous or jet inoculation, respectively.
Conclusion: Subcutaneous needle delivery or transdermal needle-free jet injection are efficient routes of PLLAV delivery, and the addition of transfection reagent boosts jet delivery of PLLAV. This novel plasmid-launched technology opens new avenues to secure the worldwide YFV 17D vaccine stockpile for outbreak scenarios and can serve to clone a broad panel of transgenic vaccines, expressing custom-made antigens via the save and highly efficient YFV 17D platform.

35.

Macavirus latency-associated protein evades immune detection through regulation of protein synthesis *in cis* depending upon its glycin/glutamate-rich domain

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Alcelaphine herpesvirus 1 (AlHV-1) is a γ-herpesvirus (γ-HV) belonging to the macavirus genus that persistently infects its natural host, the wildebeest, without inducing any clinical sign. However, cross-transmission to other ruminant species causes a deadly lymphoproliferative disease named malignant catarrhal fever (MCF). AlHV-1 ORF73 encodes the latency-associated nuclear antigen (LANA)-homolog protein (aLANA). Recently, aLANA has been shown to be essential for viral persistence *in vivo* and induction of MCF, suggesting that aLANA shares key properties of other γ-HV genome maintenance proteins. Here we have investigated the evasion of the immune response by aLANA. We found that a glycin/glutamate (GE)-rich repeat domain was sufficient to inhibit *in cis* the presentation of an epitope linked to aLANA. Although antigen presentation in absence of GE was dependent upon proteasomal degradation of aLANA, a lack of GE did not affect protein turnover. However, protein self-synthesis *de novo* was downregulated by aLANA GE, a mechanism directly associated with reduced antigen presentation *in vitro*. Importantly, codon-modification of aLANA GE resulted in increased antigen presentation *in vitro* and enhanced induction of antigen-specific $CD8^+$ T cell responses *in vivo*, indicating that mRNA constraints in GE rather than peptidic sequence are responsible for *cis*-limitation of antigen presentation. Nonetheless, GE-mediated limitation of antigen presentation *in cis* of aLANA was dispensable during MCF as rabbits developed the disease after virus infection irrespective of the expression of full-length or GE-deficient aLANA. Altogether, we provide evidence that inhibition *in cis* of protein synthesis through GE is likely involved in long-term immune evasion of AlHV-1 latent persistence in the wildebeest natural host, but dispensable in MCF pathogenesis.

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36.

Myxovirus resistance 1 (Mx1) in the myeloid cell compartment contributes to protection against Thogoto virus infection

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The antiviral myxovirus resistance protein 1 (Mx1) is an interferon-induced GTPase that plays an important role in the defense of mammalian cells against influenza A, Thogoto and other viruses. Although the induction of the *Mx1* gene is widely used as a readout for the activation of the type I interferon pathway, most inbred laboratory mouse strains have defective *Mx* alleles (due to exon deletions or nonsense mutations). Because of their rapid induction by interferon and their antiviral function, Mx proteins are considered an important part of the innate immune response in stromal cells. If or how Mx proteins in the myeloid cell compartment play a role in the control of Thogoto virus infection has not been investigated in detail. To study this, we performed bone marrow chimera experiments using congenic B6.A2G $Mx1^{-/-}$ and B6.A2G $Mx1^{+/+}$ mice. Bone marrow chimeric mice were challenged with Thogoto virus by intraperitoneal injection of the virus, and the effect of the allogeneic myeloid cell compartment within the $Mx^{+/+}$ or $Mx^{-/-}$ background was assessed using different readouts. Irradiated B6.A2G $Mx1^{+/+}$ recipient mice that had been reconstituted with $Mx1^{+/+}$ bone marrow were highly resistant to Thogoto virus infection, whereas B6.A2G $Mx1^{+/+}$ recipient mice that had received $Mx1^{-/-}$ bone marrow showed significant morbidity. The latter chimeric group also showed a strong interferon response in the liver. However, in both chimeric groups the liver viral loads were below the detection limit. On the other hand, irradiated B6.A2G $Mx1^{-/-}$ recipient mice show high viral loads, and strong interferon responses in the liver irrespective of whether these mice had been reconstituted with myeloid cells with a functional or non-functional Mx1. Nevertheless, B6.A2G $Mx1^{-/-}$ recipient mice reconstituted with $Mx1^{-/-}$ bone marrow showed more morbidity than B6.A2G $Mx1^{-/-}$ recipient mice that had received $Mx1^{+/+}$ bone marrow. Thus, Mx1's role in the stromal cell compartment prevails for the suppression of Thogoto virus replication. However, in the case of Thogoto virus infection, lack of a functional Mx1 in the myeloid cell compartment correlates with increased disease.

37.

Nanopore sequencing as a revolutionizing diagnostic tool for viral enteric disease complexes in pig health management

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Diarrhea is a major cause of health and economic problems in young piglets. Porcine epidemic diarrhea virus (PEDV) and porcine rotavirus A (RVA) are known causes of enteric disease, but several novel viruses have been discovered in the feces of piglets using metagenomics sequencing. Here, diagnostic capacities of the MinION (Oxford Nanopore Technologies) for detection and characterization of pig viral enteric disease complexes were investigated.

First, cell culture grown PEDV and RVA were pooled at loads mimicking shedding quantitities during an acute infection. The suspension was clarified by filtering and nuclease treatment. Viral nucleic acids were purified and cDNA was generated with random primers before sequencing library preparation (1D Ligation Sequencing Kit). Random amplification of viral nucleic acids was not conducted to avoid sequencing bias and to explore the MinION's sensitivity. Sequencing was performed on a MinION R9.4 MIN106 flowcell for 24h. Next, a diarrheic sample (one week old piglet) for which no etiological diagnose was made with traditional diagnostics, was evaluated on the MinION during 3h. Basecalling was done using Albacore and read statistics were generated with Poretools and NanoPlot. Taxonomical classification of viral reads was done using tBLASTx and Krona. Assembly was done de novo (Canu) or by mapping (Samtools).

In the first run, a total of 242,000 reads were generated, of which 6169 and 987 were assigned to PEDV and RVA, respectively. The first read of each viral species was already detected at 7 and 24 seconds after the start of sequencing, respectively. The complete PEDV genome (28kb) was assembled and showed 99% identity to the reference sequence in GenBank. All eleven RVA gene segments were detected with variable coverage rates. The diarrheic sample generated an output of 6287 viral sequences. The majority of reads (n=6250) were classified as bacteriophages within the order *Caudovirales*. Mammalian viral reads of a kobuvirus (n=16), an enterovirus G (n=5) and a porcine astrovirus (n=4) were found at low abundancy, but which could be part of an enteric disease complex.

In conclusion, it has been shown that the MinION is a useful tool for rapid diagnosis and characterization of the porcine viral enteric disease complex and can be directly applied to sequence unamplified viral nucleic acids. Research is needed to obtain higher sequencing yields (number and length of reads). A kobuvirus was discovered for the first time in diarrheic feces of Belgian piglets and is now being investigated further using longitudinal field studies and infection studies to elucidate its role in the enteric disease complex. Given the very high abundance of bacteriophages, their influence should also be questioned and gain more attention by the research community.

38.

Contribution of lncRNAs in Establishment of HIV Latency in Central Memory CD4 T Cells

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HIV cure research has been hampered by the existence of a latent viral reservoir that persists in infected individuals receiving antiretroviral therapy. To date, most of the cure research has focused on protein-coding genes but recently the interest in the study of long non-coding RNA (lncRNA) has risen, as these molecules could provide insight in new therapeutic strategies and further complete insight in the HIV life cycle.

Transcriptome profiling was performed (total RNA-Seq) in two primary HIV latency models of central memory CD4 T cells (T_{CM}) to investigate changes in lncRNA expression. Subsequently, differentially expressed mRNAs and lncRNAs were identified in both models and a guilt-by-association analysis was implemented to infer biological roles for the lncRNAs in HIV latency.

In the primary HIV latency models, we respectively identified 826 & 471 mRNAs (87.8% & 76.2%) and 115 & 147 lncRNAs (12.2% & 23.8%) that were significantly differentially expressed (FDR<0.05) between uninfected and latently infected T_{CM} cells. Between models, 10 lncRNAs were overlapping (oa. NEAT1 and PVT1) and many of these lncRNAs were associated with pathways involved in cell cycle regulation and pathways with a link to HIV latency: IL-7, PTEN, CSK and CCR5. In addition, a cluster of 17 lncRNAs was associated with the p53 pathway and corroborate earlier findings in this T_{CM} model that illustrated p53-dependent latency establishment. One of these upregulated p53-linked lncRNAs, 7SLRNA, has a characterized inhibitory role in the p53 pathway and would suit as a possible new therapeutical target.

Altogether, this study demonstrates that several lncRNAs play a role in HIV latency and can be linked to biological pathways with importance in HIV latency establishment and maintenance. Some of these lncRNAs, i.e. NEAT1, PVT1 or 7SLRNA, represent possible targets for reversing HIV latency and contribute to a HIV cure.

39.

Access to a main alphaherpesvirus receptor, located basolaterally in the respiratory epithelium, is masked by intercellular junctions and revealed by pollen proteases

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The respiratory epithelium of humans and animals is frequently exposed to alphaherpesviruses, originating from either external exposure or reactivation from latency. However, the long-term evolutionary relationship between alphaherpesviruses and their hosts has led to the development of several innate mucosal barriers in the latter. Intercellular junctions (ICJ) form a morphological and functional barrier by preventing virus particles from passing through the epithelium. In addition, they are responsible for maintaining a strict polarity in the epithelium. Virus binding and subsequent entry may occur selectively at either apical or basolateral domains of polarized cells, due to specific sorting of cell surface receptors. In respiratory epithelial cells, the primary target cells of most alphaherpesviruses, polarity of infection and the importance of ICJ has not been studied. Therefore, the objective of this study was to investigate polarity of infection by a well-known member of the alphaherpesvirus family, equine herpesvirus type 1 (EHV1), in the respiratory epithelium and the role of epithelial integrity herein. In horses and wild equids, EHV1 is widespread and economically very important and induces respiratory disorders, abortion and neurological symptoms. Tissue tropism of EHV1 resembles that of the closely related varicella zoster virus and herpes simplex virus 1, since these viruses all replicate well in upper respiratory epithelia.
EHV1 was used to infect equine respiratory mucosal explants and primary equine respiratory epithelial cells (EREC), grown at the air-liquid interface. EHV1 binding to and infection of mucosal explants and EREC was greatly enhanced upon destruction of the respiratory epithelium integrity with EGTA or N-acetylcysteine.
Since disruption of respiratory epithelium integrity leads to enhanced EHV1 binding, we hypothesized that its primary binding/entry receptor is located basolaterally in polarized epithelial cells. EREC were inoculated at either the apical or the inverted basolateral surfaces and EHV1 preferentially bound to and entered EREC at basolateral cell surfaces.
Next, we showed that after enzymatic treatment basolateral, but not apical EHV1 infection of EREC was dependent on cellular N-linked glycans, and not on sialic acids or on heparan sulphate. Finally, the results obtained with these *in vitro* and *ex vivo* models most likely reflect what happens *in vivo* when epithelial integrity is lost. It has been shown that ICJ

integrity is affected by several factors including pollens. Pollens exhibit proteolytic activities for efficient pollination, but when inhaled by humans or animals, these proteases can destruct tight junction proteins. Indeed, by using zymography we found that pollens of Kentucky bluegrass, white
birch and hazelnut exhibit proteolytic activities. Treatment of equine respiratory mucosal explants with these proteases led to the destruction of ICJ and a subsequent increase in EHV1 infection of the epithelium. Overall, our findings demonstrate that integrity of the respiratory epithelium is crucial in the host's innate defence against primary alphaherpesvirus infections. Inhaling pollens into the respiratory tract damages local ICJ and therefore increases the risk of an alphaherpesvirus infection. Finally, by targeting a basolaterally located receptor in the respiratory epithelium, alphaherpesviruses have generated a strategy to efficiently escape from host defence mechanisms during reactivation from latency.

40.

Beneficial impact of early treatment on restriction factor expression profile

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Background:
Host restriction factors become upregulated early on in HIV-1 infection as part of the innate immune response to suppress viral infectivity and activity of some of them, e.g. SLFN11 has been linked to a non-progressive phenotype of HIV-1 infection. Early treated cohorts comprising of patients treated during acute seroconversion are considered a promising group to reach functional cure by acquisition of a non-progressive phenotype. We evaluated HIV-1 host restriction factors and cofactors in early and late treated cohorts and compared their profile with progressive and non-progressive HIV-1 infection to further characterize their role in controlling infection.

Methods:
The expression profile of seven HIV-1 restriction factors and two cofactors (*APOBEC3G, SAMHD1, BST2* (encoding TETHERIN), *TRIM5, MX2, SLFN11, PAF1, PSIP1* (encoding LEDGF/p75) and *NLRX1*) was evaluated by qPCR in 104 HIV-1 infected patients: patients treated during seroconversion (Early treated) or chronic infection (Late treated), long term non-progressors (LTNP), recent ART-naïve seroconverters, ART-naïve chronically infected patients and non-infected controls. Patients were recruited in the Royal Free Hospital London and Ghent University Hospital. Principal Component Analysis (PCA) and Kruskal Wallis (KW) statistical analysis were performed.

Results:
Both, univariate and PCA analysis demonstrated completely distinctive expression pattern of restriction factors in early- and late-treated cohorts. Restriction factor and cofactor levels of early treated HIV-1 patients were significantly upregulated in comparison to late treated patients (APOBEC3G: $p<0.001$; SAMHD1: $p<0.001$; NLRX1: $p<0.05$; SLFN11: $p<0.001$; BST2: $p<0.001$). Interestingly, further analysis demonstrated similarities between early treated patients and LTNP, such as upregulation of *SLFN11* and *BST2*. Furthermore, a negative correlation found in LTNP between *SLFN11* expression and integrated HIV-1 DNA, total HIV DNA and viral load (Spearman r: -0.55; -0.42; -0.7) is indicative of the role of SLFN11 in restricting the HIV-1 reservoir.

Conclusion:
Early treatment potentially prevents depletion of innate antiviral responses in comparison to late treated subjects. Elevated expression of *SLFN11* and *BST2* in LTNP and early treated subjects implies that these restriction factors actively contribute to the non-progressive phenotype in these cohorts.

41.

Proteomic and functional analyses of the virion transmembrane proteome of cyprinid herpesvirus 3

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Virion transmembrane proteins (VTPs) mediate key functions in the herpesvirus infectious cycle. Cyprinid herpesvirus 3 (CyHV-3) is the archetype of fish alloherpesviruses. The present study was devoted to CyHV-3 VTPs. Using mass spectrometry approaches, we identified 16 VTPs of the CyHV-3 FL strain. Mutagenesis experiments demonstrated that eight of these proteins are essential for viral growth *in vitro* (ORF32, ORF59, ORF81, ORF83, ORF99, ORF106, ORF115, and ORF131), and eight are non-essential (ORF25, ORF64, ORF65, ORF108, ORF132, ORF136, ORF148, and ORF149). Among the non-essential proteins, deletion of ORF25, ORF132, ORF136, ORF148, or ORF149 affects viral replication *in vitro*, and deletion of ORF25, ORF64, ORF108, ORF132, or ORF149 impacts plaque size. Lack of ORF148 or ORF25 causes attenuation *in vivo* to a minor or major extent, respectively. The safety and efficacy of a virus lacking ORF25 were compared to those of a previously described vaccine candidate deleted for ORF56 and ORF57 (Δ56-57). Using quantitative PCR, we demonstrated that the ORF25 deleted virus infects fish through skin infection and then spreads to internal organs as reported previously for the wild-type parental virus and the Δ56-57 virus. However, compared to the parental wild-type virus, the replication of the ORF25 deleted virus was reduced in intensity and duration to levels similar to those observed for the Δ56-57 virus. Vaccination of fish with a virus lacking ORF25 was safe but had low efficacy at the doses tested. This characterization of the virion transmembrane proteome of CyHV-3 provides a firm basis for further research on alloherpesvirus VTPs.

42.

Conserved fever pathways across vertebrates: a herpesvirus delays fish behavioral fever through expression of a decoy Tnf-alpha receptor

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When infected by pathogens, endotherms and ectotherms can both increase their body temperature to limit the infection. Ectotherms do so by moving to warmer places, hence the term “behavioral fever”. We studied the expression of behavioral fever by common carp infected by cyprinid herpesvirus 3 (CyHV-3) using multi-chamber tanks encompassing a 24°C-32°C gradient. We showed that carp maintained at 24°C all died from the infection, whereas those housed in multi-chamber tanks all survived as a consequence of their transient migration to the warmest compartment. As the expression of behavioral fever occurred only at an advanced stage of the disease, we hypothesized that the virus might delay this phenomenon in order to promote its replication. This hypothesis was proved correct, and the delay mechanism was found to rely on the expression of a soluble viral decoy receptor for Tnfα encoded by CyHV-3 ORF12. This conclusion relied on three complementary observations: (i) a CyHV-3 ORF12 deleted recombinant induced an early onset of behavioral fever in comparison to wild-type CyHV-3; (ii) ORF12 expression product binds and neutralizes carp Tnfα; and (iii) injection of anti-Tnfα neutralizing antibodies suppressed behavioral fever, and decreased fish survival in response to infection. This study provides a unique example of how viruses have evolved to alter host behavior to increase fitness. It demonstrates that behavioral fever in ectotherms and fever in endotherms are evolutionarily and functionally related through common cytokine mediators that originated more than 400 million years ago. Finally, this study stresses the importance of the environment in the host-pathogen-environment triad.

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43.

Limited cross-neutralization between the mumps vaccine and a recent outbreak strain in sera from different age cohorts of the Belgian population

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Mumps (*parotitis epidemica*) is known as a vaccine-preventable childhood disease, which usually affects children between the ages of 2 to 15 years old. The trivalent measles-mumps-rubella (MMR) vaccine is given at the age of 12 months and 10 to 12 years. Recently, a recurrence of mumps is seen around the world in highly MMR vaccinated young adults. The cause of these new mumps outbreaks in vaccinated communities is still subject to debate. A mismatch between the vaccine and the circulating mumps virus strains, might be a possible explanation. On the other hand, waning immunity (secondary vaccine failure) could also be a cause for the new mumps outbreaks.
A total of 572 serum samples representative for different age groups of the Belgian population were tested for mumps neutralizing antibodies using a Rapid Fluorescent Focus Inhibition Test (RFFIT). We checked whether there was a difference in neutralizing capacity against the vaccine strain (genotype A, Jeryl Lynn) and a recent outbreak strain (genotype G). Additionally, we checked if waning immunity could be detected.
We could find a significant difference between the neutralization titers of the two virus genotypes for nearly all age groups. Antigenic differences between the vaccine strain and the circulating G strain may thus account for some degree of reduced vaccine efficacy. In addition, we also found evidence of waning immunity in highly vaccinated populations.

44.

Role of PDGFRβ and associated gangliosides in influenza virus entry

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Like all viruses, the influenza virus crucially depends on numerous host cell factors for its replication. These cellular factors are potential antiviral targets, since they are less likely to mutate and, consequently, viral resistance is less likely to emerge. In this project, we focused on inhibitors of cellular protein kinases as potential influenza blockers. Through screening of a protein kinase inhibitor library, we identified Ki8751 as a compound with robust and broad activity against influenza A and B viruses and relatively low cytotoxicity. Ki8751 is a known inhibitor of the receptor tyrosine kinases (RTKs) VEGFR2, FGFR2 and PDGFR [Kubo et al. *J Med Chem* 2005, 48, 1359-1366]. First, we established that Ki8751 blocks influenza entry in diverse cell lines, by interfering with post-binding endocytic uptake of the virus. We next aimed at identifying which RTK is involved. Experiments with FGFR4-transfected CHO cells contradicted the proposed role for FGFR4 [König et al. *Nature* 2010, 463, 813-817]. On the other hand, we saw considerably higher virus uptake in CHO-K1 compared to CHO-wt cells. While both cell lines show prominent expression of PDGFRβ, they differ in ganglioside expression, since CHO-K1 are $GM1^-/GM3^+$ whereas CHO-wt are $GM1^+/GM3^-$. The role for GM1/GM3 was confirmed by our observation that virus uptake was reduced when CHO-K1 cells were transfected with β-1,4-N-acetylgalactosaminyltransferase 1 (GalNAcT), an enzyme involved in the conversion of GM3 to GM1. Since gangliosides are known to have diverse regulating effects on RTK signaling [Julien et al. *Cells* 2013, 2, 751-767], we next analyzed the PDGFRβ activation pathway. First, we established that Ki8751 causes complete shut-off of PDGFRβ signaling, confirming that this RTK is the target for the robust anti-influenza activity of Ki8751. Second, while phosphorylation of the PDGFRβ receptor was much more prominent in CHO-wt than CHO-K1 cells, the two downstream effector pathways Raf/MEK/Erk and PI3K/Akt showed an opposite pattern in the two CHO cell lines. Upon influenza virus activation, Erk activity was strongly increased in CHO-K1 but not in CHO-wt cells. This strongly suggest that Erk has a major role in influenza virus endocytosis.
In combination, our findings point to the PDGFRβ receptor as an important cofactor in influenza virus endocytosis, hence PDGFRβ inhibition should be considered in the context of host cell-targeting influenza therapy. This function of PDGFRβ is reduced by GM1 and enhanced by GM3, two gangliosides that are located in different lipid raft compartments. This intriguing finding opens the way towards unraveling the poorly understood role of gangliosides in the influenza virus entry process.

45.

Mechanism of Porcine Circovirus Type 2 uptake in porcine peripheral blood monocytic cells

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Porcine Circovirus Type 2 (PCV2) is associated with post-weaning multisystemic wasting syndrome. Monocytic cells are known to be important target cells in PCV2-infected pigs. They are responsible for taking up and eliminating PCV2 particles. However, the mechanism of PCV2 uptake by these cells is unknown. Therefore, porcine peripheral blood mononuclear cells were isolated by a standardized Ficoll-Paque method. Then, monocytic cells were isolated by plastic adherence. Afterwards, cells were pre-treated with different endocytic inhibitors for 30 min at 37 °C, before they were inoculated with PCV2 in the presence of different inhibitors for 1 h at 37 °C to allow the uptake of the virus particles. Then PCV2 antigens and cell contours were visualized by double immunofluorescence staining, followed by evaluating the level of PCV2 uptake by Image J software. It was found that, inhibiting clathrin-mediated endocytosis with chlorpromazine decreased the uptake of PCV2 to 16%. Besides, inhibiting actin polymerization with cytochalasin D lowered the uptake of PCV2 to 18%. Inhibition of the GTPase activity of dynamin 1 and 2 with dynasore limited the uptake of PCV2 to 50%. In contrast, the disruption of lipid rafts by removing or binding to cholesterol in the plasma membrane with methyl-β-cyclodextrin or filipin did not affect the PCV2 uptake significantly. No significant effect on PCV2 uptake was also obtained by inhibiting macropinocytosis using amiloride. Together, these data indicate that PCV2 virus particles are taken up by monocytic cells predominantly via clathrin-mediated, dynamin-dependent endocytosis. Further research is necessary to know the fate of the virion after uptake: disassembly and transport of the viral genome to the nucleus (start of an infection) or full disintegration.

46.

Single virus imaging of HIV-1 entry with fluorescently labeled capsid

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HIV-1 uncoating is a tightly regulated step in the HIV-1 replication cycle. At present, a consensus uncoating model is lacking, mostly due to conflicting results on intracellular capsid distribution. Resolving the dynamics of capsid uncoating thus necessitates a robust method of imaging functional viruses containing labeled CA. We fluorescently labeled CA within an NL4.3-based molecular clone and evaluated replication of these labeled viruses. Dually labeled VSV-G pseudotyped particles containing C-terminally eGFP-tagged CA (CA-eGFP) and Vpr-transincorporated integrase with a C-terminal mCherry fusion (IN-mCherry) were generated. Since the construct encoding CA-eGFP by itself did not allow viral particle release, we mixed it with a plasmid encoding WT CA at two different ratios (1:2 and 1:10) during virus production. These mixed CA particles were infectious to comparable levels as particles containing only IN-mCherry, used as a reference. Using confocal microscopy, we investigated the cellular distribution and intensity of fluorescent, particle-associated CA and IN in fixed HeLaP4 cells at 6 h post infection. Only 25% to 30% of IN-mCherry containing viruses incorporated between two and four CA-eGFP monomers per particle. At 6 hrs post infection, colocalisation of CA and IN was observed in 20-30% of all cytosolic complexes. CA-eGFP containing complexes accumulated in the perinuclear area, but only 10-15% of these complexes also contained IN-mCherry. Using both eGFP-labeled CA and immunocytochemistry, we confirmed the presence of CA in the nucleus. Labeled capsid was associated with IN in only 1 to 5% of nuclear complexes. Complexes with a high CA-eGFP content were mainly detected in the cytoplasm and to a lower extent in the nucleus (25% and 10%, respectively). Under PF74 treatment, the number of nuclear complexes containing labeled CA decreased 5-fold, suggesting a nuclear import block. Strikingly, the CA-eGFP intensity increased at the nuclear envelope compared to the condition without inhibitor treatment, which is indicative of an uncoating block. Importantly, the intracellular distribution and fluorescence intensity of IN-mCherry complexes was independent of CA-eGFP labeling. PF74 treatment reduced nuclear import of IN-mCherry complexes by 15-fold. The inhibition of CA-eGFP labeled viruses with PF74 suggests that at least some of the dually labeled particles undergo bona fide uncoating.
Directly labeled CA allows single virus imaging of HIV-1 pre-integration steps and provides insights in the cytosolic and nuclear distribution of CA. Therefore, virions carrying labeled IN and CA represent a suitable system to address HIV-1 uncoating following both the viral PIC and the fate of the surrounding capsid.