Fourth annual meeting
of the Belgian Society for Virology
Thursday, December 8, 2016
The Royal Academies for Science and the Arts
Hertogsstraat/Rue Ducale 1, Brussels

Theme: 'The battle between viruses and the innate immunity'

8h45: Welcome: Prof. Hans Nauwynck
Belgian Society for Virology - General Assembly

9h00-9h45: Keynote lecture 1 – Prof. Dr. Peter Staeheli (University Medical Center Freiburg): 'The battle between viruses and the innate immunity'

9h45-12h30 Selected presentations (15 min each)
Chairs: Prof. Zeger Debyser and Dr. Benjamin Dewals

9h45-10h00: selected talk 1
Convergent mechanisms used by Cardioviruses, KSHV and Yersinia to activate RSK kinases
Michael Peeters, Frédéric Sorgeloos, Didier Vertommen, Felix Müller-Planitz and Thomas Michiels

10h00-10h15: selected talk 2
Can antiviral drug-resistant chikungunya viruses be transmitted by mosquitoes?
Leen Delang, Pei-Shi Yen, Marie Vazeille, Anna-Bella Failloux

10h15-10h45: *Coffee break (Atrium)*

10h45-11h00: selected talk 3
A7 gene expression is essential for *alcelaphine herpesvirus 1*-driven $CD8^+$ T cell expansion and typical malignant catarrhal fever.
Myster F., Davison A.J., Vanderplasschen A., Dewals B.G.

11h00-11h15: selected talk 4
A respiratory gammaherpesvirus infection protects against allergic asthma through alveolar recruitment of regulatory monocytes.
B. Machiels, M. Dourcy, X. Xiao, C. Mesnil, C. Sabatel, D. Desmecht, H. Hammad, M. Guilliams, A. Vanderplasschen, B. Dewals, B. Lambrecht, F. Bureau, L. Gillet

11h15-11h30: selected talk 5

Characterization of new RNA polymerase III and RNA polymerase II transcriptional promoters in the Bovine Leukemia Virus genome

Anthony Rodari, Benoît Van Driessche, Nadège Delacourt, Sylvain Fauquenoy, Caroline Vanhulle, Anna Kula, Karima Merakchi, Arsène Burny, Olivier Rohr and Carine Van Lint

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

Chair: Prof. Thomas Michiels

1. The Cyprinid herpesvirus 3 – carp model: a unique model to test the roles in anti-viral innate immunity of Zalpha domain proteins detecting unusual nucleic acid conformations

Morvan Léa, Po-Tsang Lee, Rakus Krzysztof, Van Snick Jacques, Athanasiadis Alekos, Michiels Thomas, Vanderplasschen Alain

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8. MALT1, a potential therapeutic target, contributes to the pathogenicity of the virulent rabies virus CVS-11 in mice.

Kip E., Suin V., Staal J. , Kalai M., Beyaert R. and Van Gucht S.

9. First record of Lyssavirus infection in a bat in Belgium
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10. Lactate dehydrogenase-elevating virus alleviates mouse parent to F1 GVHD without preventing donor cell implantation but inhibiting IFNγ and IL-27 production, antigen presentation by dendritic cells and T cell allo-responsiveness in a Type I IFN-dependent process
Mélanie Gaignage, Reece G. Marillier, Catherine Uyttenhove, Nicolas Dauguet, Anubha Saxena, Bernard Ryffel, Jacques Van Snick and Jean-Paul Coutelier

11. Role of Varicella Zoster virus ORF9p in the secondary egress : importance of its interaction with the cellular Adaptin Protein-1.
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12. Pseudorabies virus glycoproteins gE/gI suppress type I interferon production by plasmacytoid dendritic cells.
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12h30-13h30: *Sandwich lunch (Atrium)*

13h30-14h15: Keynote lecture 2 – Prof. Dr. Herman Favoreel (University of Ghent): "Tipping the balance: how alphaherpesviruses deceive Natural Killer (NK) cells"

14h15-17h00 Selected presentations (15 min each)
Chairs: Prof. Johan Neyts and Prof. Alain Vanderplasschen

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Bert Schepens, Liesbeth Vande Ginste, Joanne Langley, Lisa Macdonald, Genevieve Weir, Annasaheb Kolpe, Eef Parthoens, Walter Fiers, Marianne Stanford and Xavier Saelens

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Van der Gucht W., Leemans A., De Schryver M., Heykers A., Cos P., Delputte P.

5. A single nucleoside inhibitor against both norovirus and rotavirus replication.

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6. Induced pluripotent stem cell-derived neuronal cultures as a model to study Zika virus infections and antivirals

Kristina Lanko, Suzanne Kaptein, Kristel Eggermont , Abdulsamie Patel, Christel Claes, Wenting Guo, Philip Van Damme, Ludo Van Den Bosch, Catherine Verfaillie, Johan Neyts, Leen Delang

7. Virulence acquisition in Infectious Salmon Anaemia Virus: the role of mutations in the hemagglutinin-esterase and fusion protein.

Fourrier M., Lester K., Mcbeath A. and Collet B.

8. Type I interferon, crucial in host defence against EHV-1?

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1.

The tale of how an *in silico* designed Coxsackievirus B3 protease inhibitor turned out to be a capsid binder with a novel mechanism instead

Rana Abdelnabi[1], Ajay Kumar Timiri[2], Leen Delang[1], Venkatesan Jayaprakash[2], Pieter Leyssen[1], Johan Neyts[1]

1. KU Leuven – University of Leuven, Department of Microbiology and Immunology, Rega Institute for Medical Research, Laboratory of Virology and Chemotherapy, B-3000 Leuven, Belgium.
2. Department of Pharmaceutical Sciences and Technology, Birla Institute of Technology, Mesra, Ranchi-835215, Jharkhand, India.

An *in silico* molecular docking study on the Coxsackievirus B3 (CVB3) 3C-protease guided the synthesis of a novel benzene sulfonamide derivative (i.e. compound 17) that was shown to inhibit the *in vitro* replication of CVB3. Our aim was to use virus-cell-based assays to confirm the 3C-protease as a target for the compound and to study the particular characteristics of its antiviral activity. Compound 17 proved to inhibit the *in vitro* replication of CVB3 (strain Nancy) as well as of CVB1, CVB4, CVB5 and CVB6 with EC_{50} values ranging between (0.7-37) µM. Surprisingly, the compound did not show any antiviral activity against CVB2 and other viruses from different enteroviruses groups. In contrast to what is expected for a protease inhibitor, a time-of-drug-addition study pointed out that compound 17 interfered with an early step in the CVB3 replication cycle. A thermo-stability assay provided an additional indication of an interaction between compound 17 and the CVB3 virus particle. This latter mechanism of action was confirmed by the genotyping of independently selected compound 17-resistant CVB3 variants, which all carried mutations in the VP1 gene (F76C, E78G, A98V and D133G). Compared to the wild-type (WT), the reverse-engineered VP1 F76C, E78G, A98V and D133G mutants proved to be 18, 21, 3 and 38-fold less sensitive to the antiviral effect of compound 17, respectively. Interestingly, the mutated VP1 residues are located outside the common drug-binding pocket for capsid binders such as pleconaril. Moreover, the D133 residues of all five VP1 units are arranged in the form of ion channel at the 5-fold axis. Further experiments are ongoing to explore the precise mechanism by which compound 17 interacts with CVB3 capsid and to develop more potent and broader-spectrum analogs.

2.

Conservation in HIV-1 Vpr encoding region determines tertiary genomic RNA folding and alternative splicing.

Ann Baeyens1, Evelien Naessens1, Anouk Van Nuffel1, Karin E Weening1, Anne-Marie Reilly1, Eva Claeys1, Wim Trypsteen2, Linos Vandekerckhove2, Sven Eyckerman3, Kris Gevaert3, Bruno Verhasselt1*

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Vpr is a pleiotropic accessory protein, dispensable for HIV-1 propagation in T cell lines, but important to establish infection of resting cells, like macrophages. Despite this apparent redundancy, Vpr is highly conserved among different isolates. To study Vpr-host protein interactions in a fully replicating virus, we constructed an NL4-3 HA/FLAG-Vpr virus. Surprisingly, viral production and replication were defective, due to aberrant splicing of genomic RNA. This defect was not protein- but RNA-based and sequence dependent, suggesting that not only protein, but also RNA sequence conservation is imposed on the Vpr encoding region of HIV. Simulation of genomic RNA folding suggests that introduction of the tag-sequence induced an alternative folding structure in a region enriched in splice sites and splicing regulatory sequences. To test this, alternative tagging strategies were evaluated *in silico* and NL4-3 HA/His6-Vpr was selected as a valid alternative. Indeed, *in vitro* infectivity and mRNA splice pattern improved although did not return to wild-type values. This implies that sequence-specific modifications may interfere with tertiary mRNA folding to skew the alternative splicing balance. To test if tertiary mRNA folding is conserved in the RNA sequence, we studied NL4-3 Vpr U213C, a silent mutation in all three reading frames. The U213 site is 99% conserved and its mutation affected mRNA folding, mRNA splicing balance and infectivity. In long-term culture, this mutation reversed, which restored infectivity. Before reversion, we also observed another secondary mutation Vpr G53A. This mutation could ameliorate the predicted folding structure of HIV NL4-3 Vpr U213C mRNA and was therefore cloned and tested *in vitro*. We found that the G53A mutation alone affected viral infectivity and splicing, but that in combination with Vpr U213C it could partially compensate the defective phenotype of HIV NL4-3 Vpr U213C. As both G53 and U213 are highly conserved in Vpr, we conclude from these results that sequence conservation in Vpr preserves tertiary mRNA folding, important to balance viral splicing and replication.

3.

In search of alphaviral receptors in human and mosquito cells

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Chikungunya Virus is an arthropod borne virus (arbovirus) from the genus alphavirus. Alphaviruses are single positive stranded RNA viruses that produce enveloped virions. Like Dengue, Yellow Fever and Zika virus, Chikungunya virus is transmitted to humans by infected mosquitos during blood meals. Chikungunya virus infection causes acute high fever, rash and joint pains that evolve into chronic arthritic pains in up to 30% of patients. A successful vector-host-vector transmission cycle for arboviruses requires viral replication in both the arthropod and vertebrate host cells. Chikungunya virus enters the cell through receptor mediated endocytosis after engagement of an unknown cellular receptor by the envelope protein. To date, several candidate receptors have been suggested using virus-overlay- and yeast-two-hybrid techniques. However, there is limited overlap between these studies and validation of functionality and direct interactions between envelope and candidate receptor molecules is lacking. To identify cellular receptors and attachment molecules in an unbiased manner in both human and mosquito cells we optimized the expression, processing, functionality and purification of a native envelope protein carrying an internal Strep-tag. Affinity purification of the envelope protein coupled to mass spectrometric detection allows the identification of interacting proteins in both human and mosquito cells. Potential receptor molecules have been identified and are currently being validated structurally and functionally. Unbiased identification of cellular receptors in these evolutionary very distinct organisms will further our understanding of the mechanisms arboviruses employ to allow replication in both mosquito and humans. We will present an update on this ongoing work.

4.

Usutu virus in Belgium, 2016

Benzarti Emna, Garigliany Mutien, Linden Annick, Gilliaux Gautier, Lévy Etienne, Sarlet Michaël, Franssen Mathieu, Derouaux Antoine, Francis Frédéric, Desmecht Daniel

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Abstract
Usutu virus (USUV) is a single-stranded RNA flavivirus and is a member of the Japanese encephalitis virus serocomplex. Its life cycle involves mosquitoes as vectors and birds as amplifying hosts. The USUV was discovered in South Africa in 1959. In Europe, the first demonstration of the virus circulation was reported in Austria in 2001. In subsequent years, USUV was found in several other European countries, including Hungary (2003–2006), Switzerland (2006), Spain (2006–2009), Italy (2009), Germany (2013), and Belgium (2014).
In August-September 2016, an outbreak of wild bird deaths occurred in the northwest European region extending over Belgium, the Netherlands and the eastern border of the German state of North Rhine Westphalia.
Birds found dead in some of the affected Belgian provinces were necropsied. RT-qPCR and immunohistochemical assays investigations demonstrated an infection by USUV. The virus was isolated from a blackbird and its complete genome was sequenced by next-generation sequencing. Phylogenetic analyses revealed a close relationship of the Usutu-LIEGE strain with those from Germany, which form a distinct group within the USUV phylogeny, the so-called *Europe 3* lineage.

5.

Protein-chromatin interactions of LEDGF/p75 as a novel drug target

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LEDGF/p75, a transcriptional co-activator, plays an important role in tethering protein complexes to the chromatin. Through this tethering function LEDGF/p75 is implicated in a diverge set of human diseases including HIV infection and mixed lineage leukemia (MLL). In HIV, LEDGF/p75 is a cofactor for the viral enzyme integrase and tethers the pre-integration complex to actively transcribed genes. After integration, the viral DNA becomes part of the human genome and viral replication is ensured. Even under successful treatment with combinatorial anti-retroviral therapy (cART) and despite decades of research, still no cure for infection with this pandemic virus is available.

The two most important domains in LEDGF/p75 are the integrase binding domain (IBD) and the Pro-Trp-Trp-Pro (PWWP) domain. Others parts of LEDGF/p75 are largely unstructured. The PWWP domain consists of a conserved Pro-Trp-Trp-Pro sequence and can interact with methylated lysine 36 in histone H3 (H3K36me3), a marker of actively transcribed genes. The IBD interacts with a conserved sequence, the integrase binding motif (IBM), available in all known cellular binding partners of LEDGF/p75.

Since LEDGF/p75 plays an essential role in HIV-1 integration, small-molecule inhibitors of LEDGF/p75 can inhibit HIV replication. Up till now, only the IBD has been targeted. This led to the discovery of LEDGINs, molecules binding to the LEDGF/p75 binding site on the CCD of HIV-1 integrase and inhibiting the interaction with LEDGF/p75. Different pharmaceutical companies working on HIV/AIDS have been exploring this novel class of inhibitors. Unfortunately, not much is reported on small-molecule inhibitors of trimethyl-lysine binders but one possible strategy could be to target the hydrophobic pocket in the PWWP domain by mimicking the trimethyl-lysine side chain of histone 3.

Therefore, we performed a fragment-based drug discovery screen to develop inhibitors of the LEDGF/p75-chromatin interaction. First, we proved the LEDGF/p75-H3K36me3 interaction in an Amplified Luminescent Proximity Homogeneous assay (Alphascreen). This assay is highly sensitive and was validated to show outcompetition. We also developed two independent assays to screen for binding of small molecules to the PWWP domain of LEDGF/p75. These assays are based on melting temperature and intrinsic tryptophan fluorescence of the PWWP domain. Second, we selected 58 small fragments, all derivatives of N-methylpyrrolidin, and screened these for binding to the hydrophobic pocket. This information together with available NMR-data on the structure of LEDGF/p75 was used to select possible inhibitors. Afterwards, 4 million compounds were virtually screened and 525 compounds were selected to test in our Alphascreen assay. After screening we eliminated false-positives and aspecific protein-chromatin inhibitors by the implementation of a counterscreen. All 32 hits were further tested and analogues for the top 5 hits have recently been made. These hits will be, by the aid of modelers and chemists from the Centre for Drug Design and Discovery (CD3), further developed into lead structures for a novel class of HIV inhibitors.

6.

Harnessing the power of the yellow fever vaccine virus for the development of a therapeutic hepatitis B vaccine

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HBV is the causative agent of hepatitis B, a liver disease that can evolve to chronicity. An estimated 240 million people, mainly in Asia and Africa, are chronically infected with HBV, with more than 686.000 people dying every year due to complications related to chronic HBV infection such as liver cirrhosis and hepatocellular carcinoma. Current treatments based on nucleos(t)ide analogs control viral replication in most patients, but do not act upon the covalently closed circular DNA (cccDNA) that acts as a reservoir, thus making life-long therapy necessary. Most patients control an acute infection efficiently without the appearance of any evident clinical symptoms. However, 5 – 10 % of infected adults (and >90% of infected neonates) are unable to clear the virus and develop chronic hepatitis B. In those who clear the virus, vigorous and multispecific CD4 and CD8 T-cell responses of the Th1 profile (production of IFN-γ) are detected. The CD4 T-cell response that is specific for the HBV nucleocapsid protein [also called HBV core antigen (HBcAg or HBc)] is important in HBV control since this stimulates the activation of CD8 T-cells. In fact, CD8 T-cells, (or CTLs) are the main cellular subset responsible for resolution of the infection as they clear HBV-infected hepatocytes through cytolytic and non-cytolytic mechanisms. In those who do not resolve the infection and develop chronic hepatitis B, the responses are weaker. Indeed, it has been demonstrated that the level of HBV-specific CTLs is correlated to HBV control. The restoration of a strong CTL response is the main goal of a therapeutic HBV vaccine. Such vaccine is urgently awaited so as to be able to cure millions of people with chronic hepatitis B. To this end, we cloned the sequence of HBc into the capsid gene of the yellow fever vaccine virus YFV17D. During maturation, this HBc is cleaved off from the YFV17D polyprotein and released into the cytoplasm. We have been able to show that this virus efficiently expresses HBc in addition to the YFV17D upon infection of BHK cells. After administration of this recombinant virus to IFN type I and II receptor knock-out mice (AG129), these mice produced HBc-specific antibodies as shown by immunofluorescence. Moreover, we have been able to show that the splenocytes of AG129 mice that have been vaccinated with our HBc-expressing YFV17D produce IFN-γ upon stimulation with immunodominant HBc-derived peptides in an enzyme-linked immunospot assay (ELISpot).

7.

A protein conserved in cypriniviruses is a major virulence factor of Cyprinid herpesvirus 3

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Cyprinid herpesvirus 3 (CyHV-3) is the causative agent of a lethal disease in common and koi carp. Since its emergence, CyHV‑3 has caused severe economic losses worldwide creating a need for a safe and efficacious vaccine. In a previous study, we showed that a recombinant strain deleted for ORF56 and ORF57 exhibited a safety/efficacy profile compatible with its use as an attenuated recombinant vaccine.

In the present study, we investigated the relative contribution of the two genes to the attenuated phenotype observed. To reach this goal, a series of recombinants deleted either for ORF56 or ORF57 were produced. These recombinants were characterized *in vitro* for their correct molecular structure. In addition, immunofluorescence staining showed that the deletion of ORF56 did not abrogate the expression of ORF57, and *vice versa*. *In vivo* experiments demonstrated that the deletion of ORF57 explains most of the attenuation observed for the ORF56-57 deletion. Furthermore, we observed that ORF57 deletion induced *in vitro* a growth defect (reduction of both the production of infectious particles and the size of viral plaque). Orthologue of CyHV-3 ORF57 in Anguillid herpesvirus 1 (AngHV-1) has been shown to be a tegumental protein. Interestingly, using both qPCR and western blot based approaches, we demonstrated that the particles produced by the ORF57 deleted mutant are less infectious than those of the wild type virus.

In conclusion, this study demonstrates that ORF57 is a major virulence factor of CyHV-3. Importantly, as ORF57 is conserved in cypriniviruses, its orthologues could therefore represent a target for production of attenuated vaccine against several other major fish pathogens such as AngHV-1 and Cyprinid herpesvirus 2 (CyHV-2).

8.

Can antiviral drug-resistant chikungunya viruses be transmitted by mosquitoes?

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Because of its global (re-)emergence and the high morbidity associated with infection, the chikungunya virus (CHIKV) has become a substantial public health problem. However, no antivirals or vaccines are currently available to treat or prevent CHIKV infections. Chikungunya virus is transmitted by female mosquitoes of the *Aedes aegypti* and *Aedes albopictus* species, mostly present in tropical and subtropical regions. It is expected that in the coming years, CHIKV can induce autochthonous cases in Europe when the mosquitoes transmitting this virus will install a stable population in regions favorable to transmission. Local CHIKV cases reported in Italy (2007) and in France (2010, 2014) stress the potential to establish transmission cycles using the European *Aedes albopictus* populations.
Several molecules were recently discovered that have antiviral activity against CHIKV. For some of these, CHIKV variants could be selected in cell culture that are resistant to the antiviral effect of these molecules. However, no information is available yet about the replication and transmission abilities of these antiviral resistant viruses in mosquitoes.
To study the transmission of antiviral resistant CHIKV by mosquitoes, two different resistant variants were selected: (i) MADTP^{res} CHIKV, containing a mutation in nsP1, and (ii) favipiravir^{res} CHIKV with a mutation in the viral polymerase. *Aedes aegypti* Paea mosquitoes were orally infected with an artificial blood meal containing either wild-type or resistant CHIKV variants. At day 3, 7 and 20 post-infection (pi), CHIKV viral loads were quantified in bodies (infection), heads (dissemination) and saliva (transmission) of individual mosquitoes by a foci-forming assay. The infection rate of the two resistant viruses was mostly similar to that of the WT virus. In contrast, the dissemination rate of the favipiravir^{res} CHIKV was markedly decreased as compared to WT and MADTP^{res} CHIKV. Furthermore, the favipiravir^{res} CHIKV was not transmitted in the saliva at d3 and d7 pi, in contrast to WT. Similar results were obtained in field-collected species (*Ae. aegypti* Pazar F3 and *Ae. albopictus* Nice F10). Quantification of viral titers in mosquito body and head samples showed that the viral titers of the favipiravir^{res} CHIKV were $3\log_{10}$ lower than the titers of WT and MADTP^{res} CHIKV. Deep sequencing of saliva samples is ongoing to determine the presence of resistance mutations.
In conclusion, the replication of the favipiravir^{res} CHIKV variant is severely attenuated in mosquitoes in comparison to that of WT and MADTP^{res} CHIKV and no transmission of this variant could be detected. In contrast, the MADTP^{res} CHIKV could be transmitted, albeit with lower efficiency than WT. It needs to be determined whether the transmitted virus is still resistant against the antiviral drug. Our results thus indicate that different antiviral resistant arboviruses can have a very different behavior in mosquitoes.

9.

The EEL4EVER project: a multidisciplinary initiative aiming to develop an attenuated vaccine against Anguillid herpesvirus 1 compatible with reintroduction program of glass eels

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The stock of European eel (*Anguilla anguilla*) is severely depleted reaching less than 1% of what it was in the 1980s. In 2007, the European commission initiated "the eel management plan" aiming to rebuilt the stock of this diadromous fish species. This plan relies on three main axes: (i) limiting fisheries; (ii) facilitate fish migration through the rivers and (iii) restocking suitable inland waters with young eel (glass eel) captured during their migration from see water to fresh water. Unfortunately, this plan totally neglected the impact of infectious diseases. Anguillid herpesvirus 1 (AngHV-1), a member of the *Cyprinivirus genus* has been suggested as one of the main infectious causes of eel decline. AngHV-1 is phylogenetically related to Cyprinid herpesvirus 3 (CyHV-3 also called Koi herpesvirus (KHV)). Recently, we developed a safe and efficacious attenuated vaccine against CyHV-3. This vaccine is compatible with mass vaccination of young carp. The goal of the EEL4EVER project is to develop a similar vaccine for eel against AngHV-1. This vaccine should be compatible with the reintroduction program of glass eel. Here, we describe the main lines of this ambitious and multidisciplinary project which has been selected recently by the "Fonds européen pour les affaires maritimes et la pêche (FEAMP).

KEYWORDS: Vaccination of wildlife, European eel, Anguillid Herpesvirus-1

10.

A Gammaherpesvirus Infection Protects the Host from Pneumovirus-Induced Immunopathologies

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Gammaherpesviruses are highly prevalent pathogens that establish lifelong latency. However, little is known about how these viruses imprint the immune system of their host. Here we used murid herpesvirus 4 (MuHV-4) to investigate the impact of gammaherpesvirus infections on the development of pneumovirus-induced immunopathologies. Firstly, a respiratory Th2 vaccine-enhanced disease was induced in mice by subcutaneous vaccinations with formalin-inactivated antigens of pneumonia virus of mice (FI-PVM) followed by an intranasal challenge with wild-type PVM. This homologous host-pathogen model was used to mimic the historical human respiratory syncitial virus vaccine-enhanced disease that had occurred in children during past vaccinal trials. We observed that MuHV-4 infection, either before or after the FI-PVM vaccination, prevents the development of the PVM-induced Th2 immunopathology while the vaccinal protection against PVM infection was unaffected. Notably, reduced levels of total leukocytes, eosinophils, and Th2 cytokines were observed in MuHV-4 imprinted mice. This protective impact against the vaccine-enhanced disease was maintained over time and required pulmonary MuHV-4 replication. Furthermore, in non-vaccinated mice, we also observed that MuHV-4 confers striking heterologous clinical protection against the lethal wild-type PVM infection. This protection was associated with a highly improved PVM-specific cytotoxic CD8 T cell response that was observed in lungs of MuHV-4 imprinted mice. Altogether, these results open new perspectives for prevention of pneumovirus associated diseases and highlight that some so-called pathogens could be revealed in the end as beneficial for their host.

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11.

A novel innate immune response induced by murine Norovirus infection

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Human norovirus infection is the main cause of non-bacterial gastroenteritis worldwide. The symptoms include vomiting and diarrhea that typically last for 24 - 48 hours. Although this acute illness suggests a critical role for innate immunity in controlling the infection, little is known about the mechanisms by which Norovirus triggers innate immunity. We use murine norovirus 1 (MNV1) for studying the regulation of innate immunity in response to norovirus infection.

We used STAT1$^{-/-}$ mice, which due to impaired interferon responses display systemic spread of MNV causing lethality,(*1*) to study the viral spread and associated cytokine responses after gastrointestinal MNV1 infection. In doing so, we discovered a novel cytokine cluster induced by MNV1 in several sites of viral replication. In bone-marrow derived macrophages (BMDMs), we could elucidate part of the signaling pathway responsible for this MNV1-induced response. Importantly, MNV1 induced this novel innate immune response independently of currently known MNV-initiated signaling pathways. Indeed, the only receptor known thus far to detect MNV is MDA-5,(*2*) a cytosolic dsRNA sensor that signals through the adaptor protein Mavs. However, the cytokine cluster we identified is induced by MNV1 also in Mavs$^{-/-}$ BMDMs, suggesting that this MNV1-induced response is not triggered by viral dsRNA.

In conclusion, we identified a novel innate immunity response upon MNV infection that is induced by a signaling pathway distinct from the currently known MNV-triggered innate immunity pathways. The detailed results will be presented on the annual BELVIR meeting.

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12.

Structural and functional impact of the H5 haemagglutinin insertion on the recombinant vaccine rNDV-H5

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Currently available Avian Influenza (AI) vaccines demonstrate several shortcomings, prompting the need for the development of alternative, safer and more effective vaccinal approaches. Recombinant Newcastle disease virus vaccines expressing the immunogenic H5 hemagglutinin of AI (rNDV-H5) are one of "the new generation" vaccines addressing some of the classical AI vaccine's drawbacks. One of these rNDV-H5 vaccines under development was investigated more into detail in our laboratory, both structurally and functionally.
Structural analysis by immunogold staining confirmed the expression of the H5-insert on the rNDV-H5 surface (Lardinois et al., 2016). In addition, the H5 insertion was demonstrated to modify the distribution of NDV fusion (F) and hemagglutinin-neuraminidase (HN) glycoproteins on the rNDV-H5 surface. Compared to the parental NDV LaSota, a decrease of the number of F and increase of the number of HN glycoproteins was demonstrated by immunogold staining and neutralisation assay. The new combined representation of H5 and NDV glycoproteins, F and HN, on the rNDV-H5 surface results in increased both the hemagglutinating and neuraminidase activities compared to the parental NDV LaSota.
The functional viral entry pathways of AI and NDV differ, NDV infects the targeted cells by a direct fusion with the plasma membrane while AI virus enters via an endosomal entry pathway. Therefore, the potential functional implications of the H5 insertion at the rNDV-H5 surface on the entry process was investigated *in vitro* by infection of chicken embryo fibroblast (CEF) cells with the rNDV-H5 vaccine. The blocking of endosomal or direct fusion entry pathways of the rNDV-H5 was performed using H5 or F specific neutralising monoclonal antibodies (mAbs), respectively, and analysed by flow cytometry. First, the functionality of the H5 entry pathway was confirmed by a reduced infectivity for rNDV-H5-infected CEFs, while it remained unchanged for the parental NDV LaSota-infected CEFs after H5 mAb neutralisation. Second, the functionality of the NDV fusion entry was confirmed by specific F neutralisation resulting in a strongly reduced infectivity of CEFs for both rNDV-H5 and parental NDV LaSota. The F neutralisation impact was significantly lower on rNDV-H5 infectivity when compared with the parental NDV LaSota confirming the functionality of the H5-endosomal entry pathway.
In conclusion, our results demonstrated the impact of the H5-insert on the structural and functional characteristics of this recombinant NDV vaccine strain that could influence its potential vaccinal efficacy.

Lardinois, A., et al. *Avian Diseases*, *60*(1s), 191–201.

13.

Phylogenetic analysis of rabies virus isolated from herbivores in Minas Gerais and São Paulo border (2000-2009), Brazil

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Rabies transmitted by the hematophagous bat *Desmodus rotundus* represents a public health concern and a burden for the Brazilian livestock industry. Current evidence suggests that rabies occurrence is related to landscape characteristics, topography, hydrography, animal production systems and land use. However, a few studies have analyzed the possible connections among geographic factors and the molecular diversity of the rabies virus, furthering the understanding of the spatial and temporal dynamics of outbreaks. A study reported that the latest rabies epizootics in herbivores reported in the eastern region of São Paulo (close to the Minas Gerais border) occurred in two epidemic waves; the first was before 1998, and the other occurred after 1999. Using this evidence, the aim of the present study was to analyze cases of rabies in herbivores in the southern region of Minas Gerais (2000-2009) and their possible relationship with the aforementioned epidemics, considering the geographic characteristics of the region. Partial sequences of glycoprotein (539 nt) and nucleoprotein genes (414 nt) were obtained from 31 rabies virus isolates from herbivores. A phylogenetic tree was proposed for each genomic region using the Neighbor joining method, fixing the Kimura 2-parameter evolution model with a bootstrap level of 1,000 replications. Genetic sublineages were plotted on maps, considering rabies risk areas for herbivores in São Paulo, as well as topographic characteristics and hydrographic basins, to visualize any apparent distribution pattern influenced by those features. The phylogenetic trees had concordant topologies, suggesting a possible common origin for rabies outbreaks in herbivores along the SP/MG border, surrounding the less elevated portions of the Serra da Mantiqueira and along the hydrographic basins of Piracicaba/Jaguarí, Paranaíba do Sul, Grande, Pardo and Mogi-Guaçu rivers.The co-circulation of several viral lineages was observed in some municipalities, possibly due to an overlapping of rabies outbreaks. Inferred protein sequences of both genes showed synonymous mutations, except among residues 20 to 200, corresponding to the external domain of the glycoprotein. This information prompted cooperation among the animal health services of both states to reinforce rabies control in the border area.

14.

Virulence acquisition in Infectious Salmon Anaemia Virus: the role of mutations in the hemagglutinin-esterase and fusion protein.

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Infectious Salmon Anaemia (ISA) is a viral disease which has resulted in substantial economic losses to the salmon industry worldwide. It is caused by an orthomyxovirus with a genome consisting of eight RNA segments. Segments 6 and 5 encode the two major surface glycoproteins, the Haemagglutinin-esterase (HE) and Fusion (F) protein, respectively. The HE is the receptor binding protein while the F protein orchestrates viral membrane fusion.

Since the discovery of an avirulent HPR0 variant and the fact that deletions in the segment 6 Highly Polymorphic Region (HPR) are systematically associated with pathogenic strains, there has been a strong suspicion that ISAV surface glycoproteins play an important role in determining virulence. However, functional analyses of these surface proteins are lacking which has hindered our ability to understand the mechanisms leading to virulence acquisition.

We recently performed two extensive studies using point mutations on both the HE and F proteins from wild types ISA strains and fusion assays based on ghost erythrocyte techniques. Results indicated that both segment 6 HPR deletions and segment 5 mutations work in conjunction to promote viral fusion. More accurately, these mutations influence specific stages of this viral process, such as the activation of the F protein by the HE or proteolytic cleavage.

These findings provide the first indication that avirulent HPR0 ISAV constitute a reservoir from which pathogenic strains arise in farmed Atlantic salmon. This work also sheds some light on how the combined effect of HE and F protein mutations may determine ISAV virulence.

15.

Lactate dehydrogenase-elevating virus alleviates mouse parent to F1 GVHD without preventing donor cell implantation but inhibiting IFNγ and IL-27 production, antigen presentation by dendritic cells and T cell allo-responsiveness in a Type I IFN-dependent process

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Lactate dehydrogenase-elevating virus (LDV), a mouse nidovirus, was reported years ago to attenuate spleen destruction during GVHD. As neither the consequences on survival nor the underlying mechanisms were studied in this early report, we re-examined the effect of LDV on the acute GVHD induced by transfer of B6 spleen cells into B6D2F1 recipients. LDV infection resulted in a 3-fold increase in survival rate with reduced weight loss and liver inflammation but with the establishment of permanent chimerism that correlated with decreased IL-27 and IFNγ plasma levels. Infected mice showed a transient elimination of splenic CD11b+ and CD8α+ conventional dendritic cells (cDC) required for allogeneic CD4 and CD8 T cell responses *in vitro*. This impaired Ag stimulation was not observed in TLR7-deficient APC. A second effect of the virus was a decreased T cell proliferation and IFNγ production during MLC without detectable changes in $Foxp3^+$ Treg cell numbers. Both cDC and responder T cell inhibition were Type I IFN-dependent. These suppressive effects were very transient but had long-lasting effects in GVHD inhibition, suggesting that timely alterations of immune responses could have permanent therapeutic consequences for bone marrow transplantation.

16.

Computer-assisted study of animal behavior: a powerful technique for the development of an attenuated vaccine against Testudinid Herpesvirus 3 causing a lethal disease in several endangered tortoise species

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Testudinid herpesvirus 3 (TeHV-3) is the causative agent of a lethal disease affecting several endangered species of tortoises. The threat that this virus poses to endangered animals is focusing effort on the development of vaccines. As various inactivated vaccines were proved to be ineffective, we aimed to develop a live attenuated vaccine. With that goal in mind, the genome of different strains and subclones of TeHV-3 was sequenced recently (Gandar *et al.*, 2015, *Journal of Virology*, 89:11438-11456). This approach revealed the existence of mutants exhibiting deletions ranging from 12.5 to 22.4 kb. Importantly, inoculation of Hermann's tortoises suggested the potential of some of these mutants as attenuated vaccine candidates. Attenuated vaccines must be safe and efficacious. Here, we developed a platform to investigate the safety of vaccine candidates using computer-assisted study of animal behavior. In this system, tortoises are housed individually in longitudinal arena with a floor encompassing a temperature gradient of 24°C to 32°C. The behavior of the animals is monitored constantly using adapted automatized video-tracking software. This system allows to measure and to analyze statistically various behavioral parameters for each subject according to time. Examples of these parameters are the thermal preferendum of the animals and their mobility (distance covered, mean speed, top speed, …). In the near future, this powerful multi-translation approach will be used in addition to clinical examinations to test the safety of different attenuated vaccine candidates.

17.

Genome sequencing of Cyprinid herpesvirus 3: a basis for the study of its evolution and of the pathogenesis

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Since its emergence in the late 1990s, CyHV-3 has had great ecological impact and induced severe economic losses in the common and koi carp industries. Until now, the genomes of 4 different strains of CyHV-3 have been available in the Genbank. Even if these strains represent the two existing lineages (Asian and European) described for CyHV-3, genome sequencing of additional strains is required to estimate the genetic diversity within this viral species. Here, we sequenced the complete genome of 7 additional strains. The 11 genome sequences were then used to investigate the phylogeny of the strains and the genetic diversity of different genes studied in our laboratory (16 genes encoding envelope transmembrane proteins, ORF112 encoding a Zalpha domain protein, ORF56 and ORF57 loci which are deleted in the attenuated recombinant vaccine developed by our laboratory). For most of these genes, the conservation of the expression of a protein product was investigated in cells infected by 6 representative strains. The data obtained confirmed the non-essentiality of some ORFs and different levels of conservation for the others. Finally, full length genome analyses revealed interesting features on the evolution of CyHV-3.

Keywords: Cyprinid herpesvirus 3, genome sequencing, viral evolution

18.

The HBZ RNA targets the Polycomb repressive complex and affects the HOTAIR lncRNA network

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In contrast to 5'-LTR promoted sense transcripts, the HBZ gene is consistently highly expressed in ATL cells. The HBZ protein exerts a series of important functions such as inhibition of Tax-directed transcriptional activation. Furthermore, the cytotoxic response specific to HBZ is the most effective in controlling the proviral loads. On the other hand, the HBZ protein is poorly immunogenic and is hardly detectable in fresh ATL cells. In this context and considering that the HBZ RNA, in the absence of HBZ protein, promotes proliferation of human lymphocytes, we were interested in the functions specifically associated with the HBZ ribonucleic acid.

On the basis of an in silico prediction model, we identified the transcriptional repressor Ezh2 as a potential interactor of HBZ mRNA. Ezh2 is member of the Polycomb-group (PcG) family that acts as a gene silencer by histone modification leading to chromatin condensation. Further RNA immunoprecipitation experiments highlighted the specific interaction of the HBZ mRNA with the Ezh2 protein. We next showed that the HBZ RNA unable to express the protein interferes with the Tax-mediated transactivation of viral gene transcription directed by the 5‘LTR. Finally, we demonstrate that the HBZ ribonucleic acid affects interaction of Ezh2 with the HOTAIR lncRNA.

In summary, our data indicate that the HBZ ribonucleic acid, independently of the HBZ protein, acts at the interface of the chromatin-modifying machinery to regulate epigenetic-dependent mechanisms.

19.

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.

20.

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.

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21.

RSK may control stress granules assembly through PKR inhibition

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Objectives

Theiler's Murine encephalomyelitis virus (TMEV) belongs to the *Theilovirus* species within the *Cardiovirus* genus. The leader (L) protein encoded at the N terminus of viral polyprotein is multifunctional and was shown to antagonize the innate immune response. We reported that wild type but not mutant L protein inhibits stress granules (SG) assembly in infected cells. Recent results suggested that this inhibition was consequent to L-mediated inhibition of the double-stranded RNA (dsRNA)-activated protein kinase R (PKR). However, we found no evidence for interaction between L protein and PKR or viral dsRNA suggesting that PKR inhibition by L might be indirect. Recently, we identified cellular kinases of the RSK family as binding partners of the L protein. In this study, we evaluated the influence of RSK in inhibition of PKR activation and SG assembly by TMEV L protein.

Results

L-Mutant (LZn) but not wild type TMEV induces SG formation in HeLa cells from 8 hours post-infection.

PKR-KO HeLa M cells were generated using the CRISPR/Cas9 technology. Unlike wild type HeLa M, these cells failed to produce SG after infection with LZn TMEV although they readily formed SG after sodium arsenite treatment.

To analyze the involvement of RSK kinases on SG assembly, eIF3 (SG marker) immunolabeling was performed on cells treated with the RSK inhibitor: BID1870. BI-D1870 treatment, however, influenced neither SG assembly nor TMEV infection. In contrast, knocking down RSK1 and/or RSK2 using shRNAs led to the induction of small SG-like eIF3 dots in the cytoplasm, even after infection with the wild type virus. Western blot analysis revealed that PKR was activated by Lwt TMEV infection in the RSK knocked down cells.

Conclusions

In this study, we confirmed that PKR has an important role in SG assembly after TMEV infection. Interestingly, knock down of RSK kinases (that are activated by L) led to the emergence of SG in cells infected with the wild type virus. Thus TMEV may utilize RSK kinases to inhibit SG assembly through PKR inhibition.

22.

Evaluation of cellular immune responses induced by vectored recombinant HVT vaccines using Gumboro disease as a model

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Recombinant Herpesvirus of Turkey (rHVT)-IBD vaccines are successfully used to induce an immune protective response against Infectious Bursal Disease (IBD). Low titers of rHVT-IBD-induced antibodies are detected in vaccinated chickens before 14 days post-vaccination (dpv) (Gelb et al. 2016). In addition, IFN-γ production by splenocytes can be measured after specific stimulation at 3 weeks after rHVT-IBD vaccination (Ingrao et al. 2016, submitted), suggesting that protection may also partially be attributed to cell-mediated immunity. In the present study, by a phenotypic analysis of the peripheral blood and splenic T cells subsets of rHVT-IBD-vaccinated chickens, we observed an increase of CD4-CD8+ and CD4+CD8+ cells populations at 7 dpv. In a second experiment, a boost with a live attenuated IBDV was done to specifically restimulate *in vivo* the rHVT-IBD vaccinated chickens one week after the first immunization. The induction of immune responses was characterized by analyzing the transcriptional levels of several immune-related genes in spleen, bursa and lung at 2-5-7 days post-boost (dpb), and was compared to three control groups of chickens, namely rHVT-IBD alone, live attenuated IBDV and unvaccinated. The expression level of IFN-γ in rHVT-IBD/IBD chickens was significantly higher at 7 dpb in bursa and lung when compared with live attenuated IBD group. In lung, CD8 expression was upregulated in rHVT-IBD/IBD group at all time points tested, suggesting a reactivation of CD8+ T cells in response to live IBDV. An increased expression of Granzymes A and K was also observed in lung at 7 dpb for this rHVT-IBD/IBD group, implying a specific activation of cytotoxic response to rHVT-IBD vaccines. In spleen, an upregulated CD8 expression was only observed at 2 dpb in rHVT-IBD/IBD group and was not measurable at any other time points, suggesting a homing of cytotoxic T cells out of this lymphoid organ. As observed in lung, an upregulated CD8 expression is detected in bursa of rHVT-IBD-vaccinated birds.
These data strongly support the involvement of cell-mediated response components in triggering effective rHVT-IBD-induced immunity to IBDV.

23.

MALT1, a potential therapeutic target, contributes to the pathogenicity of the virulent rabies virus CVS-11 in mice.

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Rabies virus is a highly neurovirulent RNA virus, which causes about 59000 deaths in humans each year. The paracaspase MALT1 is crucial for immune and inflammatory cell activation by different receptors, including antigen receptors, lectin receptors, and some GPCRs. MALT1 acts both as a scaffold signalling protein and a cysteine protease that cleaves several substrates, promoting NF-κB signalling and specific mRNA stabilization. As a result, MALT1 activation drives the expression of multiple immunoregulatory genes. Mepazine, an inhibitor of MALT1 proteolytic activity, proved recently to have therapeutic effects in B lymphoma and experimental autoimmune encephalomyelitis in mice.

Here, we examined the role of MALT1 in the development of rabies disease using MALT1 knock-out (KO) mice or mepazine treatment after infection with the virulent rabies strain CVS-11.

The development of rabies disease was significantly slowed down in MALT1 KO mice compared to wild-type (WT) mice (mortality at 12 days instead of 8 days post virus inoculation). Moreover, MALT1 KO mice exhibited reduced expression of IL-1β, IFN-γ, TNF-α and iNOS compared to WT mice which might explain the delay in the appearance of clinical signs. Viral RNA loads were also decreased, suggesting that inhibition of MALT1 somehow reduces virus replication and/or spread of the virus in the brain. Stronger T lymphocyte infiltration, macrophage infiltration and microglia activation were observed in WT mice compared to KO mice, which suggests that these cells contribute to pathogenicity rather than protection of the host. Daily mepazine treatment also delayed the onset of disease, showing an involvement of the MALT1 proteolytic activity. We are currently further examining the impact of MALT1 deficiency in mice with conditional KO in specific cell types to better understand the role of MALT1 in rabies pathogenesis.

Our study shows that MALT1 and mepazine have a significant impact on the progression of rabies virus infection and disease, which emphasizes the importance of inflammatory/immunological mechanisms on rabies disease development.

24.

Pseudorabies virus glycoproteins gE/gI suppress type I interferon production by plasmacytoid dendritic cells

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Plasmacytoid dendritic cells (pDC) play a central role in the antiviral immune response, both in the innate response and in shaping the adaptive response, mainly because of their ability to produce massive amounts of type I interferon (TI-IFN). Here, we report that cells infected with the live attenuated Bartha vaccine strain of the porcine alphaherpesvirus pseudorabies virus trigger a dramatically increased TI- IFN response by porcine primary pDC compared to cells infected with wild type PRV strains (Becker and Kaplan). Since Bartha is one of relatively few examples of a highly successful alphaherpesvirus vaccine, identifying factors that may contribute to its efficacy may provide insights for the rational design of other alphaherpesvirus vaccines. The Bartha vaccine genome displays several mutations compared to the genome of wild type PRV strains, including a large deletion in the US (unique short) region, encompassing the glycoprotein E (gE), gI, US9 and US2 genes. Using recombinant PRV Becker strains harboring the entire Bartha US deletion or single mutations in the four affected US genes, we demonstrated that the absence of the viral gE/gI complex contributes to the observed increased IFN response. Furthermore, we showed that the absence of gE leads to an enhanced ERK1/2 phosphorylation in pDC, which correlates with a higher TI-IFN production by pDC. In conclusion, the PRV Bartha vaccine strain triggers strongly increased TI-IFN production by porcine pDC, which possibly may contribute to the successful antiviral immune response generated by this vaccine. Our data further indicate that the gE/gI glycoprotein complex suppresses TI-IFN production by pDC, which represents the first pDC evasion mechanism described for alphaherpesviruses.

25.

Induced pluripotent stem cell-derived neuronal cultures as a model to study Zika virus infections and antivirals

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Background:
Since the Zika virus (ZIKV) has been associated with foetal abnormalities and neurological complications, relevant models are urgently needed to study the particular characteristics of the infection in neuronal cells and the inhibition thereof by antiviral molecules.

Methods:
Wild-type human induced pluripotent stem cells (IPSC) were differentiated into respectively cortical neurons, motor neurons and astrocytes. Cells were infected with ZIKV strain MR766 or with PRVABC59 from the African and Asian lineage respectively and monitored for the development of a cytopathogenic effect (CPE). The activity of three antiviral molecules, i.e. T-705 (favipiravir), 7-deaza-2'-*C*-methyladenosine (7-DMA) and ribavirin, was assessed in these cultures. The viral load in culture supernatants was quantified by qRT-PCR and end-point titrations.

Results:
All three cell types proved susceptible to ZIKV infection, with full CPE observed as of day 6 post infection in cortical neurons and astrocytes and day 8 p.i. in motor neurons. All cell types produced high titers of infectious virus (up to 10^7 $TCID_{50}$/ml). Cortical and motor neurons could be infected with ZIKV at a MOI as low as 10^{-5}; for astrocytes this was MOI 10^{-4}. The kinetics of infection in motor neurons was slower than in the other cell types with the first signs of CPE occurring at day 6 post infection, whereas for cortical neurons and astrocytes full CPE is observed by that time. By the end of experiment the PRVABC59 strain, which is an isolate from the current ZIKV outbreak in Latin America, resulted in higher viral loads ($1Log_{10}$ higher), as compared to MR766 strain. The nucleoside analogue 7-DMA (at 10 µg/ml) reduced viral loads and delayed the virus-induced CPE in all three cell types: in astrocytes the viral load was reduced to undetectable levels for both strains; in cortical and motor neurons the reduction in viral titers of 2-3Log was observed. Despite the fact that both T-705 and ribavirin inhibited ZIKV replication in Vero E6 cells, these compounds proved inactive in the stem cell-derived neuronal cultures.

Conclusion:
We demonstrate that ZIKV productively infects stem cell-derived cortical and motor neurons as well as astrocytes. Interestingly, whereas all three studied antivirals block ZIKV replication in Vero E6 cells, only 7-DMA does so in the neuronal cultures employed here. Together our results demonstrate that human IPSC-derived neuronal cells represent a relevant *in vitro* model to study ZIKV neurotropism and to assess the potential efficacy of inhibitors of viral replication.

26.

Role of Varicella Zoster virus ORF9p in the secondary egress : importance of its interaction with the cellular Adaptin Protein-1.

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ORF9p (HSV-1 VP22 homolog) is an essential VZV tegument protein.
We have shown that ORF9p interacts with the clathrin adaptor Protein-1 (AP-1) complex and have identified the ORF9p motif important for this interaction. The mutation of this motif strongly impairs VZV secondary envelopment. We have demonstrated that some viral glycoproteins also co-immunoprecipitate with AP-1. We are testing the hypothesis that ORF9p might bridge this interaction. Moreover, we have shown that the deletion of the amino-terminal region of OR9p also strongly impacts the secondary envelopment. This region contains a cysteine predicted to be palmitoylated. So far, all cargo known to interact with AP-1 are membrane proteins. We are investigating the possibility that ORF9p is indeed palmitoylated. The impact of this potential palmitoylation on ORF9p interaction with AP-1 and with glycoproteins will be evaluated.
We believe that it would be interesting to test the infectivity of our ORF9p-mutant VZV strains in dendritic or T cells as well as their capacity to enter neurons, establish latency and reactivate. Finally, it would be relevant to test our mutants in the post herpetic neuralgia (PHN) model developed by. P. Kinchington in which some ORF9p mutants have been shown to display a less severe phenotype.

27.

Modulation of virally-induced immunopathology by type I and II interferons.

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Viruses may modulate the immune microenvironment of their host, with consequences on the course of concomitant diseases with an immune component. Using lactate dehydrogenase-elevating virus (LDV) as a mouse experimental model, we observed severe exacerbation of endotoxinic shock and of autoantibody-mediated autoimmune diseases such as hemolytic anemia and thrombocytopenia. LDV induced a strong macrophage activation, which resulted in enhanced cytokine production after lipopolysaccharide exposure, and in increased phagocytosis of opsonized target cells. Macrophage activation after LDV infection was induced by gamma-interferon production by natural killer cells. In contrast, type I interferons inhibited gamma-interferon production, macrophage activation and the resulting immunopathology. Thus LDV infection provides a useful model to analyse the mechanisms by which viruses may modulate macrophage-mediated diseases through a balance in the production of type I and II interferons.

28.

Effects of murid herpesvirus 4 infection on the mouse B-cell repertoire

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To confer protection, the adaptive immune system produces a highly diversified repertoire of antibodies that are selected and expanded in response to specific antigens. While pathogens can affect the antibody repertoire of responding B cells, their effect on the whole repertoire is mostly unknown. This is especially true for persistent viruses such as Gammaherpesviruses (γHVs) which are ubiquitous in human and animal populations. Indeed, although γHVs induce a polyclonal B cell activation as a normal part of their life cycle, the consequences of these infections on the host's antibody repertoire are still largely unknown. Recent advances in high-throughput DNA sequencing technologies enabled characterization of the antibody repertoire. In this study, we used murid herpesvirus 4 (MuHV-4), a γHV infecting laboratory mice, to study the imprinting of a γHV infection on the antibody diversity of its host. Briefly, we developed in mice a consensus sequencing approach that incorporates unique barcode labels on each starting RNA molecules and therefore allows us to reduce rate of sequencing error and to quantify transcripts. Based on this technique, the subsequent bioinformatics analysis of antibody heavy chain sequences allowed us to compare the diversity, the isotype frequency, the level of somatic hypermutation and the lineage structure of the antibody repertoire in MuHV-4-infected and uninfected mice. Using a reporter virus, we also investigated the antibody repertoire of MuHV-4-infected and uninfected cells. Altogether, this study highlights that deep sequencing of immunoglobulin transcripts provides direct insight into the imprinting of γHVs infection on the immune system of their hosts.

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29.

In vivo infectivity and *in vitro* replicative fitness regain of a recombinant murine norovirus

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Recombination can create considerable changes in viruses, allowing for antigenic shifts, host jumps and pathogenesis and fitness modifications. Mathijs et al. recently isolated a viable recombinant murine norovirus (RecMNV) of reduced replicative fitness *in vitro* after coinfection of murine norovirus strains MNV1-CW1 and -WU20 in RAW 264.7 cells. We (1) evaluate the infectivity of RecMNV *in vivo* and (2) follow its replicative and genetic adaptations over serial *in vitro* passages.

(1) *In vivo* infectivity of RecMNV was compared to its parental strains in Balb/cByJ mice via weight loss measurement and viral load estimation in faeces, tissues and organs 48 and 72 hours post-infection using plaque assay and RT-qPCR. Average body weights of RecMNV-infected mice were generally higher than those of MNV-1- or WU20-infected mice. Viral loads were detected in all examined organs, suggesting that, like its parental strains, RecMNV can disseminate beyond the digestive tract to produce a systemic infection.

(2) RecMNV progenies resulting from the first (RecE) and tenth (RecL) *in vitro* passages were compared and their sequences determined. RecL showed a significant increase of lysis plaque diameters and faster replication kinetics than RecE. Molecular analysis of RecE and both parental strains showed seven nucleotide changes in the RecE genome, comprising two non-silent mutations. In ORF3, a mutation at position 7245 introduced a stop codon, resulting in a 20 amino-acid shorter VP2 for both RecE and RecL. Comparison of RecE and RecL revealed twelve nucleotide changes, comprising five non-silent mutations.

Our data suggest that recombination occurring *in vitro* between two homologous murine norovirus strains can give rise to a chimeric strain, which shows similar biological properties to its parental strains and is capable of productive *in vivo* infection. In addition, we provide evidence of viral adaptation and *in vitro* replicative fitness regain after a recombination event.

30.

A respiratory gammaherpesvirus infection protects against allergic asthma through alveolar recruitment of regulatory monocytes.

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The hygiene hypothesis postulates that augmentation of allergic diseases in developed countries could be linked to reduced exposure to infections during childhood. Surprisingly, the potential protective role of herpesvirus infections against allergy development has never been addressed in details. Here, we investigated how a gammaherpesvirus infection affects the subsequent development of allergic asthma. Our results demonstrate that respiratory infection by Murid herpesvirus 4 (MuHV-4) inhibits the development of House Dust Mites (HDM)-induced asthma by modulating functions of lung innate immune cells. Specifically, MuHV-4 infection induced the replacement of resident alveolar macrophages (AMs) by monocytes educated to display markers associated with regulatory functions. These monocyte-derived AMs blocked the ability of dendritic cells to trigger a Th2 response against HDM. Collectively, our results reveal that replacement of embryonic AMs by regulatory monocytes is a major mechanism underlying the long-term training of the lung immunity after infections.

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31.

Evaluation of a human *in vitro* bronchial epithelium model for the study of respiratory syncytial virus polymerase inhibitors.

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Respiratory syncytial virus (RSV) is a major cause of upper and lower respiratory tract infections and the most common viral infection detected in hospitalized infants under the age of six months. Currently, a series of compounds targeting viral polymerase are in pre-clinical or clinical trial: the nucleoside inhibitors ALS-8112, ALS-8176 and the non-nucleoside inhibitors AZ-27, PC-786. We aimed here to study RSV infection in the physiologically relevant model of a reconstituted 3D-human airway epithelium (HuAEC) and to assess the activity of the RSV-polymerase inhibitors.
HuAEC of bronchial origin derived from a single healthy donor were retrieved from Epithelix (Switzerland). RSV-A Long-strain was used to infect the apical surface of HuAEC inserts in duplicate. Three days after infection, ALS-8112, ALS-8176, AZ-27 and PC-786 were added at a concentration of $100xEC_{50}$ for 5 days by daily refreshing the basal medium of HuAEC inserts. Apical samples were collected every day (D1-D7) and starting from D10, every 5 days to monitor the viral genome copy number shed from the apical surface. Tissue integrity was measured at D10 and D15 by quantifying lactate dehydrogenase (LDH) and by measuring the trans-epithelium electric resistance (TEER).
HuAEC showed a high and reproducible rate of RSV-A replication. Overall, the nucleoside inhibitors showed a more than 2 Log_{10} (>99%) inhibition of RSV-A infection and the replication rate was still controlled at D10 and D15 (3 and 8 days after stop of treatment). RSV-infected inserts treated with AZ-27 showed a virus rebound at D10 and D15. We are currently studying the genetic sequence of RSV at D10 and D15 to detect the eventual arise of resistance mutations due to this sub-optimal treatment. PC-786 was tested at a concentration of 0.1 mg/ml and at 0.1 µg/ml and at both concentrations, the antiviral activity was comparable. LDH and TEER values collected at D10 were in line with the observed inhibition of RVS-A replication.
Overall, our first results demonstrated that the HuAEC is a robust and reliable model to study RSV infection. Interestingly, the treatment with some specific antiviral compounds could cure HuAEC inserts whereas with other polymerase inhibitors, a viral rebound was observed. Taken together, these first evidences suggest that the 3D-human airway epithelium model could constitute a valuable tool to study antiviral development.

32.

Individual monitoring of salmonid immune responses during immunisation and infection

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Aquaculture currently provides nearly half of all fish consumed globally and is the fastest growing sector of food and animal production. Infectious diseases are the most significant threat to the future expansion of this industry. Vaccination is widely considered the best prevention strategy and much effort is focussed upon the development of new and more efficacious fish vaccines. Most research groups use a vaccination-challenge strategy to evaluate immune protection in terminally-acquired tissue samples. However this approach requires large numbers of animals to obtain sufficient statistical power providing limited information on the kinetics of the protective response. To address this, we established a non-lethal sampling method (by withdrawing small amounts of blood repeatedly from the same individual) to monitor salmonid immunity during viral infection or immunisation. This enabled us to monitor (by real-time PCR and ELISA) key immune markers from a small number of animals during pathogen challenge. Furthermore, due to the limitations of mRNA-level validation, we are currently developing a high-throughput proteomics platform to allow the rapid and accurate quantification of immune-responsive proteins in plasma samples during immunisation. Thus, we are optimising targeted and shotgun mass spectrometry approaches, performed on a Q Exactive hybrid quadrupole-Orbitrap, using rainbow trout (*Oncorhynchus mykiss*) as our study model. By applying a non-lethal sampling protocol we were able to individually monitor changes in immune markers during the course of an immune response. Such information will allow a better understanding of fish immunity and might be applied in the future to reduce the number of animals required in vaccine development.

33.

The Cyprinid herpesvirus 3 – carp model: a unique model to test the roles in anti-viral innate immunity of Zalpha domain proteins detecting unusual nucleic acid conformations

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The innate immune system relies on numerous molecules that act as sensors of nucleic acids. Nucleic acids are detected based on their structure, subcellular localization or sequence. Zalpha domains are 66 aa long domains which bind to left-handed dsDNA or dsRNA (Z-DNA/Z-RNA). The description of Zalpha domains in proteins belonging to the host innate immune system but also in viral proteins suggests that even the conformation of the nucleic acid could be exploited by the innate immune system as a PAMP or DAMP.

The present project aims to test this interesting and original hypothesis both in vitro and in vivo taking advantage of the Cyprinid herpesvirus 3 (CyHV-3)/ Carp model. Carp, like all cypriniform fish, encodes PKZ, a paralogue of the dsRNA-dependent protein kinase (PKR) expressed by all vertebrates. While PKR possesses dsRNA binding domains, PKZ has Z-DNA/Z-RNA binding domains. Both proteins, once activated by binding to the appropriate nucleic acids, phosphorylate eIF-2α thereby blocking protein synthesis. This leads to accumulation of preinitiation complexes and later, to the organization of these into stress granules.

Recently, it was demonstrated that ORF112 of CyHV-3 encodes a Zalpha domain protein (pORF112) over-competing the binding of PKZ to Z-DNA. Alignment of the ORF112 sequence demonstrated remarkable conservation throughout various CyHV-3 strains, hinting at an important and conserved function of the protein. This protein is abundantly expressed in all 7 tested strains. In addition, it has been shown that only the full-length ORF112 protein is detected in infected cells. The ORF112 gene is essential for replication of the virus in cell culture: it is the Zalpha domain, and not the rest of the ORF112 protein (of unknown nature), that is responsible for this observation. Furthermore, the Zalpha domain of ORF112 can be replaced by Zalpha domains of various origins (viral, eukaryotic) and in varying numbers (one or two). The distribution of pORF112 has previously been shown to be cytoplasmic and nuclear, with perinuclear granules that become more diffuse over the course of infection. In this context, pORF112 has been shown to co-localize with Z-DNA (in the nucleus) on one hand and with dsRNA (in the cytoplasm) on the other.

34.

A7 gene expression is essential for *alcelaphine herpesvirus 1*-driven CD8$^+$ T cell expansion and typical 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. Our recent data have demonstrated that AlHV-1 latently infects CD8$^+$ T-cells resulting in their proliferation and MCF lesions. How AlHV-1 infects CD8$^+$ T-cells and induce their proliferation remain however unknown. To better understand MCF pathogenesis, we first used Illumina high-throughput sequencing to obtain the full genomic sequence of the highly passaged attenuated strain WC11. Sequencing data revealed a genomic sequence very similar to the pathogenic strain C500 with only four ORFs that were not conserved in WC11, 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. Likewise, A7 has been predicted to encode a glycoprotein containing a C-type lectin-like domain. The absence of A7 in the high passaged attenuated strain could participate in the attenuation of WC11 through a defect of virus entry into target 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*. Next, we infected rabbits intranasally with the A7^{STOP} virus. While the A7^{STOP} virus induced hyperthermia with a significant delay compared to the WT virus, neither enlargement of lymphoid organs nor typical expansion of CD8$^+$ T cells could be detected in the absence of A7 expression. Interestingly, viral DNA loads and antibody responses were significantly reduced in lymphoid tissues of A7^{STOP} infected animals. Finally, the infiltrations of lymphoid cells typically observed in WT-infected animals were severely reduced in absence of A7. In conclusion, these findings demonstrated that the lack of A7 significantly alters the development of MCF, likely through deviating CD8$^+$ T cell tropism.

35.

Quantifying and characterizing the HIV reservoir in virally suppressed patients

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Background

Secondary control of HIV infection likely occurs when a patient's specific immune responses are sufficiently strong and the latent viral reservoir has become sufficiently small after a certain period of antiretroviral treatment. In preparation of a treatment interruption study, we are fine-tuning our assays to measure these immune responses as well as the viral reservoir and investigate any correlations that might exist with clinical parameters.

Subjects and Methods

Fifty-three patients on cART were recruited, 83% were male with median: age 45.7 years, time on cART 70 months [IQR: 41 – 130], nadir CD4 count 283 cells/µL [IQR: 191 – 390]. All patients had suppressed viremia.

Total HIV DNA and unspliced mRNA were quantified in PBMCs using digital droplet PCR. The novel 'tat/rev induced limiting dilution assay' (TILDA) was used to quantify viral transcriptional activity, in a sub-group of 21 patients infected with HIV subtype B. Assays to measure immune responses will be performed in the coming months. Linear regression and student t-test were used to study correlations.

Results

Total HIV DNA was detected in 50 out of 53 patients, ranging from 0 to 866 copies per million PBMCs (median: 270). Unspliced HIV RNA was detected in 35 out of 53 patients (quantitative data not normalized yet). TILDA results ranged from 0 to 313 cells [IQR: 1.4 – 55.8] with detectable HIV RNA transcripts per million CD4 T cells after stimulation.

Total HIV DNA correlated directly with usRNA levels and both inversely correlated with nadir CD4 ($p < 0.05$). Correlations with actual CD4, zenith VL and time on treatment were not significant. TILDA results did not significantly correlate with any other parameters but appeared higher for patients with a detectable versus an undetectable plasma viral load ($p = 0.071$).

Conclusion

In this group of virally suppressed patients, heterogeneity in terms of reservoir size was observed. Total DNA and usRNA correlated with each other and with pretreatment nadir CD4 but not significantly with actual CD4 or zenith viral load. Studies on in depth phenotypical and functional immune parameters are ongoing.

36.

Convergent mechanisms used by *Cardioviruses*, KSHV and Yersinia to activate RSK kinases

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BACKGROUND & AIM: Theiler's virus (TMEV) belongs to the *Cardiovirus* genus. As other *Cardioviruses*, it expresses a Leader (L) protein, which is implicated in the escape of the host immune response. Amongst its functions, L protein interacts with and maintains RSK kinases activated by inhibiting their dephosphorylation by phosphatases. Strikingly, two other very different pathogens (Kaposi's Sarcoma-associated Herpes Virus [KSHV] and bacteria *Yersinia*) express proteins (respectively ORF45 and YopM) that interact with and activate RSK exactly as the L protein of TMEV does (1,2). This work aims at defining the interface between L and RSK in order to understand the basis of RSK activation by pathogens.

RESULTS: By mutagenesis and co-immunoprecipitation, we identified a DDVF motif of the L protein implicated in the interaction with RSK2, where the Phenylalanine is critical for the interaction. Very interestingly, ORF45 and YopM possess the same DDVF motif. In the case of KSHV ORF45, the Phenylalanine residue of this motif was also shown to contribute to RSK binding (3). We showed that the YopM Phenylalanine residue was also involved.

By competition experiments, we showed that L and YopM interact with the same region of RSK2. Thus, the RSK activation mechanism used by the different pathogens is likely identical.

Cross-linking experiments identified a putative binding site of pathogen's protein on RSK2. This suggests a model for activation of the kinase. The DDVF motif could tilt an alpha helix, which is close to the activation loop of RSK2 and thereby maintain the kinase in an active conformation.

CONCLUSION: To conclude, we highlighted an evolutionary convergence of three very different pathogens (RNA virus, DNA virus and bacteria). They activate RSK kinases via a common DDVF motif, probably via the same mechanism. Current efforts are now exerted to confirm the RSK binding site, the RSK activation mechanisms, and RSK targets that are involved in host-pathogen interaction.

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37.

Type I interferon, crucial in host defence against EHV-1?

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Equine herpesvirus-1 (EHV-1) is one of the main causes of respiratory disease, late-term abortion and neurological disorders in horses worldwide. EHV-1 replicates in the epithelial cells of the upper respiratory tract (URT), and reaches its target organs, the pregnant uterus and central nervous system, via a cell-associated viremia in monocytic cells. Different EHV-1 strains circulating in the field have been characterized as neurovirulent and non-neurovirulent phenotypes. It was demonstrated that a single nucleotide polymorphism in the catalytic subunit of the viral DNA polymerase is associated with the neurovirulence of EHV-1 strains. Previous studies showed that both phenotypes replicate in a plaque-wise manner in the epithelium of the URT. Non-neurovirulent strains induce a higher amount of larger viral plaques than neurovirulent strains. Further, neurovirulent strains cross the basal membrane (BM) much earlier (24 hpi) in infection compared to non-neurovirulent strains (36 hpi). Based on these results, we hypothesize that differences in sensitivity for the innate immunity could partly explain the differences in viral replication in the URT. Interferon (IFN) type I is an important cytokine that acts in the first line of defence against viral infections. It is shown that herpesviruses have evolved mechanisms to deal with the host defense strategies. Here, we investigated if IFN type I is involved in the different behaviour of both EHV-1 phenotypes in the URT. First, we measured the IFN levels in equine nasal and tracheal mucosa *ex vivo* explants inoculated with either the non-neurovirulent 97P70 or neurovirulent 03P37 EHV-1 strains. We found that similar levels of IFN (51,7 ± 62,2 U/ml versus 32,9 ±21,7 U/ml) were detected in *ex vivo* explants inoculated with both EHV-1 strains starting from 48hpi. Second, we tested if pre-treatment of mucosa explants with increased concentrations of recombinant equine IFN affected EHV-1 replication and growth. We showed that EHV-1 replication of both strains was decreased in an IFN concentration dependent manner. However, we observed that the non-neurovirulent EHV-1 97P70 strain induced higher virus titers (4,9 ± 2,0 $\log_{10}$ $TCID_{50}$/ml) and plaque sizes (155 ± 32,5 μm) compared to the neurovirulent EHV-1 03P37 strain (3,7 ± 1,9 $\log_{10}$ $TCID_{50}$/ml and 106,5 ± 36,1 μm) after IFN pre-treatment at a physiological concentration of 100 U/ml. In conclusion, both EHV-1 strains induce the production of IFNα in respiratory mucosae at the same level but the non-neurovirulent strain replicated more easily in the epithelial cells of the URT in the presence of IFN. Based on the results, we hypothesize that the non-neurological strain has evolved a strategy to replicate in the URT in the presence of IFN, in contrast to the neurological strain, indicating that the neurological strain only recently appeared in the horse population.

38.

First results in the use of bovine ear notch tag for bovine viral diarrhoea virus detection and genetic analysis

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Background: Infection due to bovine viral diarrhoea virus (BVDV) is endemic in most cattle-producing countries throughout the world. The key elements of a BVDV control programme are biosecurity, elimination of persistently infected animals and surveillance. Bovine viral diarrhoea (BVD) is a notifiable disease in Belgium and an official eradication programme started from January 2015, based on testing ear notches sampled during the official identification and registration of calves at birth. An antigen-capture ELISA test based on the detection of BVDV Erns protein is used. Ear notch sample may also be used to characterize the genotype of the calf when appropriate elution/dilution buffer is added. Both BVDV antigen-ELISA analysis and animal traceability could be performed.

Methodology: With regards to the reference protocol used in the preparation of ear notch samples, alternative procedures were tested in terms of BVDV analytic sensitivity, diagnostic sensitivity and specificity, as well as quality and purity of animal DNA.

Principal Findings/Significance: The Allflex DNA Buffer D showed promising results in BVDV diagnosis and genome analyses, opening new perspectives for the livestock industry by the exploitation of the animal genome. Due to the high number of cattle involved in the Belgian official BVDV eradication programme based on ear notch tags sample, a large database on both BVDV status of newborn calves and cattle genome could be created for subsequent different uses (e.g. traceability, determination of parentage, genetic signatures throughout the genome associated with particular traits) evolving through a more integrated animal health.

Keywords: Bovine; Ear notch tag; Bovine; bovine viral diarrhoea virus; detection; genetic analysis.

39.

TRIM24 suppresses infection by Murid Herpesvirus 4 infection

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Gammaherpesviruses (γHV) are archetypes of persistent viruses that have been identified in a range of animals from mice to man. They are host-range specific and establish lifelong latency of immunocompetent hosts. Most of these viruses are associated with neoplastic diseases, where the best studied γHV are human herpesvirus 4 and 8 that are respectively associated with Burkitt's lymphoma and Kaposi's sarcoma. By opposition to its human counterparts, murid herpesvirus-4 (MuHV-4) is able to replicate to high titres in cultured cells and is therefore an excellent candidate for studying the cycle of these viruses. Identification of host factors involved in a viral infection will provide a better understanding of host-virus interactions including host genes subverted by the virus for its replication as well as genes activated for host defense and protection. Here, we applied a genome wide RNAi screen to identify key host factors involved in MuHV4 infection. We identified 65 genes that appeared to be necessary for the completion of the MuHV-4 cycle and likely the initial steps of infection. In parallel, silencing of 110 genes increased MuHV-4 infection suggesting a possible antiviral function. Among these, TRIM24, a member of the RI NG-type E3 ligase family, was shown to have antiviral activity against MuHV-4. This was then substantiated in a separate experiment and interestingly, silencing of TRIM24 expression appeared to significantly increase MuHV-4 binding to the host cell. In the future, we want to investigate how TRIM24 exerts its antiviral effect and if it also blocks infection by other viruses.

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40.

Characterization of new RNA polymerase III and RNA polymerase II transcriptional promoters in the Bovine Leukemia Virus genome

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Bovine leukemia virus (BLV), the etiologic agent of enzootic bovine leucosis, is a B-lymphotropic oncogenic retrovirus closely related to the human T-cell leukemia virus I and II (HTLV-I and II). It is widely accepted that BLV latency, due to the RNA polymerase II (RNAPII) 5'LTR-driven transcriptional and epigenetic repression, is a viral strategy used to escape from the host immune system and contribute to tumor development. However, by deep sequencing and bioinformatics analysis, a highly expressed BLV micro-RNA (miRNA) cluster has been recently reported, suggesting that the silencing dogma in BLV transcriptional regulation is only partially correct. In addition, these viral miRNAs are produced through a non-canonical process, involving RNA polymerase III (RNAPIII).

In this report, we used chromatin immunoprecipitation assays to demonstrate the *in vivo* recruitment of a *bona fide* RNAPIII complex to the BLV miRNA cluster both in BLV-latently infected cell lines and in ovine BLV-infected primary cells, through a canonical type 2 RNAPIII promoter. In addition, by specific knockdown of the RPC6 RNAPIII subunit, we showed a direct functional link between RNAPIII transcription and BLV miRNAs expression. Furthermore, in BLV-latently infected cell lines and in ovine BLV-infected primary cells, we showed that both the tumor- and the quiescent-related isoforms of RPC7 RNAPIII subunits were recruited to the miRNA cluster, consistent with previous studies showing that the viral miRNAs are transcribed at all stages of BLV disease. Epigenetically, we demonstrated that the BLV miRNA cluster was enriched in positive epigenetic marks in agreement with the high expression level of the viral miRNAs previously reported.

Interestingly, we also demonstrated the *in vivo* recruitment of RNAPII at the 3'LTR/host genomic junction, associated with positive epigenetic marks. Functionally, we showed that the BLV LTR exhibited a strong antisense promoter activity and identified *cis*-acting elements of an RNAPII-dependent promoter. Finally, we provided evidence for an *in vivo* collision between RNAPIII and RNAPII convergent transcriptions.

Taken together, our results provide new insights into alternative ways used by BLV to counteract silencing of the viral 5'LTR promoter.

41.

Helminth-induced inflammation enhances control of γ-herpesvirus acutre infection

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In developing countries, helminth infections are highly prevalent. This is also the case for other infectious diseases such as malaria, tuberculosis or AIDS. Coinfections are therefore frequent and a better understanding of how our immune system faces multiple aggressions is essential. Studies showed a negative impact of helminth infections on vaccination or on the immune response against numerous microbial pathogens. A recent study has highlighted that exposure to helminths induced the reactivation of latent murid herpesvirus 4 (MuHV-4) infection. However, it is not known how pre-exposure to helminth could affect MuHV-4 host colonization. In this study, we have investigated how helminth induced Th2-type inflammation influence the control of a γ-herpesvirus acute infection. We have monitored viral replication by *in vivo* imaging and observed that pre-exposure to *Schistosoma mansoni* (Sm) eggs enhanced the control of acute pulmonary infection and weight loss induced by MuHV-4. Interestingly, the enhanced protection was associated with a higher $CD8^+$ T cell lung response, including higher number of cells and higher effector capacities. The improvement of $CD8^+$ T cell response was dependent on Th2-type inflammation, as signalling through interleukin-4 receptor α chain was essential to boost this response. CD8+ T cells genes expression profil will be investigated in order to understand how Th-2 type inflammation influence CD8+ T cells activation. Despite this enhanced protection against MuHV-4 infection, Sm eggs exposure doesn't influence MuHV-4 latency establishment. Therefor, future experiments will aimed at studying the impact of Sm eggs exposure on memory formation, genital reactivation of latent MuHV-4 and transmission.

42.

Effect of hepatitis E virus infection on the human hepatic innate immune response in human liver chimeric mice

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Introduction: The hepatitis E virus (HEV) is an emerging pathogen in developed countries. The study of HEV infection and pathogenesis is hampered due to the lack of robust cell culture systems and practical, ethical and financial limitations associated with the use of non-human primates and pigs. Recently, we succeeded to infect human liver chimeric uPA/SCID mice with different HEV preparations, mainly of genotypes 1 and 3. Since an adaptive immune response is lacking in our model, it is a unique tool to study the direct influence of HEV infection on the transcriptome of the infected human hepatocytes without the influence of a cellular immune response.

Materials and Methods: Liver fragments were collected from non-infected and HEV infected mice 4 and 9 weeks after inoculation of the genotype 1 strain Sar-55. Total DNA and RNA were purified and the expression profile of 48 innate immune response-associated genes was analyzed using human-specific RT-qPCR.

Results: Many interferon-stimulated genes were strongly up-regulated in the liver of infected mice, such as IFI27, IFI44L and RSAD2 (fold change up to 14,09 compared to uninfected control). RSAD2, aka Viperin plays an important role in the cell antiviral state induced by type I and II interferons and has activity against many RNA and DNA viruses. We detected an up to 6,31-fold increase in expression of IFIT protein encoding transcripts (IFIT1, IFIT2, IFIT3), while a 2 to 3,39-fold increase of HLA-A, HLA-B, HLA-F, HLA-J, TRIM-22 and ISG20 was observed after 9 weeks of infections**.**
In addition, HEV infection strongly induced chemokine expression: the expression of CXCL9 and CXCL10 was increased 24.3-fold and 8.7-fold respectively after 9 weeks of infection, while there was only a 5.52 and 3.08-fold increase after 4 weeks, indicating a more pronounced effect at a later stage of infection. The latter two chemokines are mainly induced by interferon gamma and are involved in leucocyte stimulation, trafficking, and cell adhesion molecule expression. Numerous genes involved in the innate immune response were not significantly affected by the infection (<twofold change). These include USP18, TLR3,

ISG15, STAT1, STAT2, OAS1, IRAK1, MX1, MAVS, IRF1, IRF3, IRF7, IFI6, IFI44 and DDX60.

Conclusion: HEV infection directly triggers the expression of interferon-stimulated genes in the infected human hepatocyte. Our humanized mouse model can be used to study virus-host interactions and HEV pathogenesis.

43.

Preclinical and Clinical development of a new HRSV vaccine based on the Small Hydrophobic protein that instructs macrophages to clear infected cells

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Human Respiratory Syncytial Virus (RSV) is the major cause of lower respiratory tract infections in infants and pediatric hospitalization worldwide. HRSV infections do not evoke long-living protective immunity, so reinfections occur lifelong. HRSV can also cause severe disease in elderly with a burden that is only slightly below that of epidemic influenza in this age group. HRSV bronchiolitis likely results from massive infection of the bronchiolar epithelium and subsequent sloughing of infected cells that ultimately clump and obstruct the very narrow airways of young infants. Despite decades of vaccine research that has been focusing mainly on the RSV fusion protein, the key target for neutralizing antibodies, there is currently no vaccine for RSV. A major obstacle in the development of an effective RSV vaccine is the ability of this virus to infect humans in the presence of circulating neutralizing antibodies. In addition it is not clear whether such antibodies can also promote clearance of infected cells.

We have developed an alternative vaccination strategy based on the ectodomain of the small hydrophobic protein of HRSV (SHe). We demonstrated that SHe-specific antibodies can protect mice and cotton rats against HRSV infection. SHe-specific antibodies, which do not neutralize the virus, strongly depend on Fcγ Receptors and alveolar macrophages to protect mice against infection. So most likely, SHe-specific antibodies control HRSV by engaging alveolar macrophages to eliminate virus infected cells. In line with this, we could demonstrate that SHe-specific antibodies can indeed engage macrophages to phagocyte HRSV-infected cells and take up viral antigen form these cells in vitro.

We recently have performed a randomized, observer-blind, controlled, dose escalation phase I trial to evaluate the safety and immunogenicity of the SHe antigen formulated in DepoVax^{TM} (DPX-RSV) in healthy older adult volunteers. Interim analysis demonstrate that the DPX-RSV vaccine is well tolerated and induces SHe antigen specific responses in 75 percent of subjects vaccinated with the lower dose, and 100 percent of those vaccinated with the higher dose. Although the vast majority of the volunteers had high levels of neutralizing antibodies, very few subjects had detectable antibodies directed against SHe before DPX-RSV vaccination. Functional analysis revealed that DPX-RSV-induced SHe-specific serum

antibodies were able to engage macrophages to phagocytose SH-expressing cells. Remarkably, despite the high levels of pre-existing HRSV-specific antibodies sera of healthy adults are poor in inducing phagocytosis of HRSV infected cells. However vaccination with DPX-RSV significantly boosted phagocytosis of HRSV infected cells. Since a failure to clear HRSV infected cells is a hallmark for HRSV-associated lung pathology, our findings suggest that the ectodomain of the Small Hydrophobic protein is a highly relevant HRSV vaccine antigen.

44.

Dendritic cells and stromal cells are the initial targets for replication of the live-attenuated yellow fever vaccine in the dermis

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Yellow fever virus (YFV), which is a member of the *Flaviviridae* family, is transmitted via *Aedes aegypti* mosquitoes in tropical regions of Africa and South America and can cause high fever, hemorrhage and life-threatening liver disease, with a case fatality of 20-60%. Despite successful use of the live-attenuated YFV vaccine (YFV-17D) for ~80 years, the mechanism underlying long-lasting protective immunity induced by this vaccine remains unknown. Unraveling the cellular tropism of the YFV-17D vaccine is an important step towards understanding which cells produce viral antigen for the subsequent induction of protective virus-specific memory T and B cell responses. Previous studies showed that YFV-17D infects *in vitro* human fibroblasts as well as monocyte-derived dendritic cells (DCs) that can restimulate vaccine-specific $CD8^+$ memory T cells. However, even though various subsets of DCs exist that carry specialized functions in stimulating T cells populations, the targets for YFV-17D replication *in vivo* have not been identified. We set out to determine these initial targets for YFV-17D infection in the skin following intradermal inoculation, as a relevant route for vaccination. We therefore constructed novel YFV-17D reporter viruses to track cellular tropism and dissemination in (IFN-receptor deficient) AG129 mice that are highly susceptible to YFV-17D infection. Intradermal inoculation of AG129 mice with a mCherry-expressing YFV-17D reporter virus and analysis via 12-color flow cytometry revealed that non-hematopoietic stromal cells (likely fibroblasts) as well as skin-resident classical DCs and macrophages are the initial targets of YFV-17D replication. In addition, YFV-17D replication led to substantial recruitment of inflammatory monocytes to the dermis one day after inoculation and differentiation into monocyte-derived DCs after 3-5 days. Further, we constructed a luciferase-expressing (nLuc) YFV-17D reporter to determine virus replication and dissemination via *in vivo* luminescence imaging (IVIS Spectrum). Intradermal inoculation of AG129 mice led to localized replication of nLuc-YFV-17D in the inoculated ear skin and draining lymph nodes after 5 days. Using these thus established tools, we are currently dissecting the dynamics of infection, virus dissemination, and targets for replication of YFV-17D within various tissues, depending on the route of vaccination. These data can provide crucial information for strategies that target specific cell types and tissues for optimized vaccination. Overall, we expect that a deeper insight into YFV-17D tropism will allow to optimize delivery of an entirely novel YFV-17D-derived vaccine platform that we are currently developing and that allows, among others, to store and transport the vaccine without a cold-chain.

45.

A RNAi screen of host kinases at the host-Respiratory syncytial virus interphase reveals pitfalls of the RNAi technology

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Human respiratory syncytial virus (HRSV) is an enveloped, negative-stranded RNA virus belonging to the *Paramyxoviridae* family and a common human respiratory pathogen. Although an HRSV 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 HRSV at least once, requiring hospitalization in 1-2% of the cases. At present, very little is known about the cellular factors that counteract or are needed for HRSV infections. Therefore, we focused on host kinase-controlled signaling pathways that either enhance or counteract HRSV infections. Elucidating these pathways will very likely help to understand the HRSV infection process in more detail and serve as a guide to discover novel therapeutic targets.

We performed a siRNA-based knockdown screen of the complete human kinome (719 genes) in A549 cells that were subsequently infected with HRSV. Viral replication was quantified by determining plaque numbers and size. Transfection of siRNAs directed against one particular kinase, *i.e.* MYLK4, consistently resulted in reduced HRSV replication and this reduction was evident after knockdown with two different siRNAs. This suggested that MYLK4 plays an important proviral role during a HRSV infection. However, when MYLK4 expression was rescued using a overexpression vector containing multiple wobble base mutations in the siRNA target regions, HRSV replication was still reduced by the MYLK4 siRNAs. Therefore, the reduced HRSV replication was likely mediated by off-target silencing by the two MYLK4 siRNAs. In an attempt to identify the gene(s) that are responsible for reduced HRSV replication after knockdown, we performed mRNA Seq (a deep sequencing method to study the transcriptome) on cells that had been transfected with either of the two MYLK4 siRNAs, scrambled siRNA or an HRSV Nucleoprotein-specific siRNA. Remarkably, hundreds of genes were deregulated by each MYLK4 siRNA, and 35 of these were common. Currently we are selecting deregulated genes by function for further validation. Our results show that siRNA-based knock down can elicit an antiviral effect that is not in line with the intended target.

46.

Synthetic virus-like particle – endocytosis and processing by dendritic cells for vaccine delivery

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Dendritic cells (DCs) employ several endocytic routes for processing antigens, driving forward adaptive immunity. Different nano/micro scale particulate vaccine carriers have been proven to be promising vaccine or drug delivery vehicles. However, information on the process of particle interaction with DCs and the endocytic routes utilised for their trafficking and processing within DCs is limited. In order to characterize the interaction of such particles with DCs we employed synthetic virus like particles (SVLPs). These self-assembling SVLPs efficiently induce adaptive immunity without requirement for an adjuvant, raising the question of how DCs interact with them. DCs rapidly bind SVLPs within 1 minute. Confocal microscopy demonstrated an association of internalized SVLPs with ovalbumin, high molecular weight dextran, and cholera toxin B, implying a clathrin independent mode of endocytosis. While SVLPs were readily internalised by immature DCs, with maturing DCs the uptake was reduced but not abolished. These results demonstrated that SVLPs are internalised via different endocytic routes, of which caveolin-independent, lipid raft-mediated macropinocytosis is identifiable as primary. We further evaluated if SVLPs can be structurally modified in order to achieve both targeting and activation of DCs. DCs rapidly and efficiently bound and internalised all types of SVLPs, implying that structural manipulations did not impact interaction of SVLPs with DCs. SVLPs carrying P3Cys or P2Cys moieties were capable of DC activation, measured by up-regulation of CD86. The present characterization allows for a definition of how DC handle virus-like particles showing efficacious immunogenicity, elements valuable for novel vaccine design in the future.

Key words
Dendritic cells
Endocytosis
Synthetic virus like particles

47.

Outbreak investigations and molecular characterization of foot-and-mouth disease viruses circulating in southwestern Niger

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Abstract: In Niger, the epidemiological situation regarding foot-and-mouth disease (FMD) is unclear and most outbreaks are not reported. This study aimed i) to identify current FMDV strains circulating in cattle herds, ii) to identify exposure risk factors associated with FMD seropositive animals in clinical outbreaks and iii) to test the relationship between one FMDV isolate from southwestern Niger and reference vaccine strains. Epithelial tissues (n=25) and sera (n=227) were collected from cattle in eight districts of the western part of Niger. 158 (70%) out of the 227 tested sera were positive for the presence of antibodies against FMDV, using the non-structural protein (NSP) test. Using the Liquid Phase Blocking ELISA (LPBE) test, 136 (86%) of 158 sera tested for antibodies to structural proteins of four FMDV serotypes (A, O, SAT 1 and SAT 2) were positive for one or more serotypes. The multivariate logistic regression analysis with NSP positives revealed that only the herd composition (presence of both cattle and small ruminants) was significantly associated with FMDV seropositivity (P-value = 0.006). Antigen-ELISA and VP1 coding sequence analysis revealed the presence of FMDV serotype O that was characterised within the O/WEST AFRICA topotype. The antigenic relationship between one of the FMDV isolates from Niger (O/NGR/4/2015) and 3 reference vaccine strains was determined by the two-dimensional virus neutralization test (2dmVNT), revealing a close antigenic match between the field isolate from Niger and three FMDV serotype O vaccine strains. This study provides an updated knowledge on the epidemiological situation of FMD in the southwestern of Niger and these results highlight the complex nature of the FMD epidemiology in Africa reflected by the transboundary mobility of the virus. Therefore, these findings may help to develop effective control and preventive strategies for the disease in Niger as well as in other countries of West Africa.

Keywords: Foot-and-Mouth Disease Virus; Identification, Molecular Characterization; Serology; Risk factors, southwestern Niger.

48.

First record of lyssavirus infection in a bat in belgium

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Rabies is a fatal encephalitic disease that is caused by a rhabdovirus of the Genus Lyssavirus. Most of the cases are caused by the classical rabies virus, which circulates mainly in dogs and wild carnivores, but other lyssaviruses can also cause similar symptoms and are known to circulate in bats [1]. In Europe, two main lyssaviruses are known to circulate in bats: European Bat Lyssavirus-1 and -2 (EBLV-1 and EBLV-2). Both of these have been shown to circulate in neighbouring countries [2], but so far no cases were detected in Belgium. Here, we present the first case of a local bat infected with EBLV-1. On September28th2016, the Belgian National Reference Laboratory for Rabies (Brussels, Belgium) received a serotine bat found by a hiker in Bertrix. The bat, that was unable to fly, had bitten the hiker when he attempted to move the animal from the main road. The bat was tested by Fluorescent Antibody Test (FAT) and PCR to detect lyssavirus. The brain tissue tested positive in both tests, confirming the diagnosis of rabies in the animal. The patient received a complete post-exposure prophylaxis treatment (administration of rabies immunoglobulins and vaccination). Sequencing of the nucleoprotein and polymerase genes allowed us to determine that the bat was infected with EBLV-1b.
This is the first confirmed case of EBLV in a local bat. Since the virus has been shown to circulate in serotine bats in neighbouring countries, this finding confirms the suspicion that the virus is also endemic in certain bat species (*Eptesicus serotinus*) in Belgium. It should be noted that in contrast to other rabid mammals, infected bats do not tend to approach or actively attack humans. The risk for public health thus remains limited. It is however advisable never to approach (sick) bats. People who do handle bats should be vaccinated against rabies and use thick gloves. Any bite or scratch from a bat should be reported to a medical doctor for advice and follow up.

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49.

Intercellular bridges in the equine respiratory mucosa: a crucial innate barrier against EHV-1 infection

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Equine herpesvirus type 1 (EHV1) is one of the most important virus in horses. Every year, several EHV1 outbreaks lead to the loss of valuable foals because of abortion. In addition, elder horses occasionally need to be euthanized due to EHV1-associated neurological disorders. Because vaccines and antivirals do not sufficiently work, there is a need for better insights in EHV1 pathogenesis to find new targets for the development of therapeutics and to adapt vaccines. Until present, it is still unknown which factors contribute to the development of EHV1 respiratory, neurological and reproductive disorders. EHV1 pathogenesis consists of (1) primary replication in the respiratory mucosa, (2) infection of respiratory mucosa leukocytes, (3) viremia and (4) adhesion of infected leukocytes to endothelial cells in the uterus and the central nervous system with transfer of the virus to surrounding cells. Compared to other alphaherpesviruses, EHV1 replication in the respiratory mucosa epithelium is limited, but the virus can still infect diapeding leukocytes. This implies that the horse's respiratory epithelium consists as a strong barrier against EHV1, but that the early immune system fails to protect leukocytes from infection. Intercellular bridges form a crucial innate immune barrier by preventing virus particles from passing through the epithelium. The objective of this study was to investigate the importance of equine respiratory cell junctions in the protection against EHV1 infection. Polarity of EHV1 infection was examined in primary equine respiratory epithelial cells (ERECs) *in vitro*. ERECs were seeded onto collagen-coated transwells and cell polarity was monitored by measuring the transepithelial cell resistance. EHV1 preferentially infected ERECs at the basal surface (423±122 plaques per 1.5 x 10^5 ERECs), compared to the apical surface (29±13 plaques per 1.5 x 10^5 ERECs). Disruption of the cell junctions by treatment with ethylene glycol tetra-acetic acid (EGTA) partially restored susceptibility to EHV1 at the apical surface (149±34 plaques per 1.5 x 10^5 ERECs), but had no impact on basal infection (360±139 plaques per 1.5 x 10^5 ERECs). Destruction of intercellular bridges by the use of EGTA was also visualized in equine respiratory mucosa *ex vivo* explants. Likewise, EHV-1 infection was enhanced in both nasal and trachea mucosa explants after EGTA treatment (18±2 plaques per 0.8 mm^2 nasal epithelium and 51±31 plaques per 0.8 mm^2 trachea mucosa epithelium), compared to mock treated controls (3±2 plaques per 0.8 mm^2 nasal epithelium and 13±6 plaques per 0.8 mm^2 trachea mucosa epithelium). Overall, our results clearly demonstrate that equine respiratory cell junctions protect ERECs from EHV1 infection. Disruption of these bridges *in vivo* might predispose the horse for a more severe primary EHV1 infection. As a next step, identification of the precise mechanism of this enhanced infection could unravel a big question mark in EHV1 pathogenesis and eventually lead to the development of efficient vaccines and antivirals.

50.

Respiratory syncytial virus (RSV) entry is inhibited by serine protease inhibitor AEBSF when present during early stage infection

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Background: Host proteases have been shown to play important roles in many viral activities such as uncoating, viral protein production and disease induction. Therefore, these host proteins are putative targets for the development of antivirals via inhibition of cellular proteases. For viruses such as Ebola, HCV, HIV and Influenza, host proteases have been described to play essential roles allowing specific protease inhibitors to reduce infection. RSV has also been reported to utilize host proteases in its replication cycle but the knowledge on their exact role and potential as antiviral target is limited. Therefore, we evaluated the effect of protease inhibitors on RSV infection.

Methods: To measure the sensitivity of a RSV infection to protease inhibitors, immortalized cells were infected with RSV and incubated for 16h (one cycle of infection) in the presence or absence of the inhibitors. Cells were fixed, stained and studied using fluorescence microscopy.

Results: With a one infection cycle experiment, inhibitory effects can be easily detected. Several protease inhibitors were tested (AEBSF, Pepstatin A, E-64, TPCK, PMSF and aprotinin) for inhibitory effects, starting 1h prior to inoculation and treatment was continued for 16h during the assay. AEBSF treatment resulted in a significant decrease of RSV infection. Afterwards, HEp-2, A549 and BEAS-2B cells were treated with AEBSF (0.3mM) to determine similar effects in other cell lines. An almost complete block in the number of RSV infected cells after 16h of incubation was demonstrated in all three cell lines and the effect was also clearly dose-dependent. The AEBSF treatment was then applied over the different phases of an infection cycle (pre-, peri- and post-inoculation treatment) to determine the phase in which treatment of AEBSF blocks infection in immortalized cells. AEBSF treatment resulted in a significant decrease in RSV infected cells when present during all phases, during peri- and post-inoculation combined or during only the entry phase of RSV. The inhibitory effect was not only observed using a RSV A2 strain but also with clinical isolates A1998/3-2, A2000/3-4 and B1.

Conclusion: AEBSF is able to inhibit RSV infection and based on our time of addition studies, this block occurs during virus entry. Ongoing experiments will provide more information on the inhibitory mechanism of AEBSF and its potential antiviral activity on the RSV infection in immortalized cells, primary bronchial epithelium and *in vivo* RSV models.

51.

A single nucleoside inhibitor against both norovirus and rotavirus replication.

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Noroviruses and rotaviruses are the two most important viral pathogens responsible for severe childhood diarrhea. Worldwide, diarrhea is the second leading cause of death due to infections among children under the age of five. Of these 10% are caused by noroviruses and 30% by rotavirus (in spite of the availability of two vaccines), the vast majority occur in developing countries. These children often experience multiple episodes of diarrhea per year which can lead to nutritional deficits and long-term consequences, such as growth stunting. Both noro- and rotaviruses can easily spread causing large outbreaks in hospitals, schools, etc. Specific antivirals to treat and prevent noro- and rotavirus infections are of upmost importance. Specifically, a safe and highly efficient single treatment would be desirable to treat patients as quickly as possible upon a noro- or rotavirus infection.
We here demonstrated that a number of nucleoside analogues are able to inhibit the *in vitro* replication of both noro- and rotavirus by targeting the viral polymerase. The most potent nucleoside has an *in vitro* antiviral effect against norovirus (both human and murine) with an EC_{50} of ~7 μM and rotavirus (both human and simian) with an EC_{50} of ~1 μM. The antiviral activity was confirmed by quantification of viral RNA with qRT-PCR. The anti-rotavirus activity was further characterized in immunofluorescence studies with antibody-labeled rotavirus infected MA104 cells by high content imaging. The compound is well tolerated in mice and has excellent oral bioavailability. The *in vivo* efficacy in mouse infection models will soon be studied. Enzymatic studies will confirm the direct inhibition of the noro- and rotavirus polymerases by the 5'-triphosphate metabolites of the nucleoside analogue. Molecular modeling studies may serve as a starting point for the design of novel small molecule inhibitors of these two major pathogens with a safe profile and increased potency.
This is, to the best of our knowledge, the first time that a nucleoside analogue is shown to be active against a highly pathogenic dsRNA virus. Given the dual antiviral activity of the molecule, this study opens the door for the development of a truly broad-spectrum antiviral to treat and prevent viral diarrhea in children.

52.

The ORF27 gene of Cyprinid herpesvirus 3: a case of opposite selective pressures in vitro and in vivo

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Sequencing of CyHV-3 genome revealed the ORF25 family: a family of 6 paralogous sequences encoding type I transmembrane proteins containing an immunoglobuline-like domain. Using mass spectrometry approaches, four ORF25 family members were identified as envelope proteins. ORF27, initially described as a pseudogene, was not detected in the laboratory strains but was subsequently identified as a structural protein in field strains. The focus was made on this **ORF27**, based on the assumption that it **could negatively influence viral growth *in vitro*, and positively influence viral growth and/or transmission *in vivo***.

Sequence alignments performed with nine strains of CyHV-3 revealed that **mutations occurring in ORF27 during *in vitro* culture mainly lead to its non-expression**. This conclusion was confirmed by indirect immunofluorescent staining using polyclonal and monoclonal antibodies raised against pORF27.

For functional studies, four CyHV-3 ORF27 recombinant strains were produced encoding a wild-type (WT) OR truncated (TR) ORF27; with OR without luciferase expression cassette (inserted in a non coding intergenic region) allowing *in vivo* detection of infected cells using *In Vivo* Imaging System. Co-cultures (ORF27 WT – ORF27 TR) performed *in vitro* showed a progressive increase of the proportion of ORF27 TR genotype (from 20% at passage 0 to 98% at passage 4). Multiple growth curve assay and plaque size assay performed *in vitro* led to the observation that **ORF27 WT genotype produces less infectious particles than ORF27 TR but produces larger plaque size**. Finally, comparison of the titres of infectious particles obtained after normal incubation versus spinoculation revealed a significantly higher increase for the ORF27 TR genotype, **suggesting a possible function of pORF27 in viral attachment**.

53.

LEDGINs hamper the establishment of a reactivation competent HIV reservoir in CD4+ T-cells

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Background

Despite the success of combination antiretroviral therapy, the presence of a latent HIV-1 reservoir remains the major impediment towards a cure. Persistence is a consequence of proviral integration. LEDGF/p75 is a cellular co-factor of HIV integrase (IN) that targets integration into active transcription units. LEDGINs, developed as bona fide antivirals, inhibit the interaction between LEDGF/p75 and IN. Residual HIV integration events are retargeted out of transcription units ('early effects'). Moreover, when LEDGINs are added during virus production, progeny viral particles display morphological irregularities and replication defects ('late effects'). Recently we showed that LEDGIN treatment increases the fraction of quiescent integrations in cell lines refractory to reactivation (Vranckx et al., 2016). We now evaluated the potential of LEDGINs to hamper the establishment of a reactivation-competent latent reservoir in primary CD4+ T-cells. We also investigated whether LEDGIN treatment during virus production (late effect) alters the establishment of a latent infection.

Methods

Reactivation of the quiescent pool in primary cells was studied in a chemokine induced model of latency. Resting CD4+ T-cells were stimulated with CCL19, or activated with IL-2/PHA and were infected with NL4.3 in the presence and absence of LEDGIN$_{CX014442}$ or Raltegravir (Ral). Reactivation was induced with PMA and PHA and was determined by p24 ELISA. To evaluate the late effect of LEDGINs on progeny viruses, a double-reporter virus (OGH, Chavez et al., 2015) was produced in the presence of CX014442. SupT1 Cells were infected and reactivated with TNFα and measured by FACS analysis.

Results

Both CX014442 and Ral reduced NL4.3 infection in CCL19 and IL2/PHA treated CD4+ T-cells. The residual latent provirus established under LEDGIN treatment was hampered for reactivation with PMA/PHA in a dose-dependent manner, with hardly no reactivation observed for cells treated with 2x IC_{50} ($IC_{50\ early\ effect}$=3.7μM). The reduction in reactivation competence was corroborated both in resting and activated primary T-cells but not observed with Ral. Infection of SupT1 cells with OGH produced in the presence of LEDGINs resulted in an increase of the quiescent reservoir fraction. Whereas more than 40% of the latent reservoir in the control shifted to a productive infection upon stimulation with TNFα, only 10% produced in presence of 8x IC_{50} of CX014442 ($IC_{50\ late\ effect}$=0.01μM).

Conclusions

LEDGIN-mediated inhibition of the LEDGF/p75-IN interaction blocks replication and reduces the latent reservoir. Our data indicate that LEDGINs result in quiescent proviruses

refractory to reactivation, a phenotype not observed for Ral. Addition of LEDGINs early during acute infection can influence the formation of the latent reservoir. Rendering the majority of residual proviruses defective for reactivation might represent an attractive approach to achieve an HIV remission.

54.

Absence of zoonotic hepatitis E virus infection in Flemish dairy cows

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Introduction Hepatitis E virus (HEV) is a worldwide underdiagnosed virus responsible for at least 20 million infections yearly. It is a positive single-stranded RNA virus and isolates that can infect mammals are classified in the *Orthohepevirus* genus of the *Hepeviridae* family. *The Orthohepevirus A* species is subdivided into seven major genotypes, all belonging to a single serotype. Genotype 1 and 2 are endemic in many developing countries. The zoonotic genotypes 3 and 4 cause sporadic infections in industrialized countries and are mainly associated with food-borne infections due to the consumption of raw or undercooked contaminated meat products. A recent Chinese study reported the detection of HEV in cows. Fecal and milk samples showed high prevalence of active HEV infection. Furthermore infectivity experiments in rhesus macaques showed that the milk contained infectious particles that were resistant to pasteurization. These results indicate that cows might constitute a new zoonotic risk towards humans. Since raw or pasteurized milk is also a consumption product in many European countries, we performed a large-scale screening study in dairy cows reared in Flanders, Belgium.

Material and methods A total of 1,792 milk samples were obtained from different institutions. We collected 65 individual cow milk samples from patients of the Ghent University Faculty of Veterinary Medicine and 56 individual milk samples from the 'Proefhoeve', a research dairy farm of the Ghent University Faculty of Bioscience Engineering. A total of 504 bulk milk samples and 1,105 individual milk samples were provided by the Flanders Milk Control Center (MCC). MCC is a non-profit organization acknowledged by the Belgian government for the investigation of the quality and composition of raw milk produced by Flemish farms with intended use in the dairy industry. The 504 bulk milk samples originated from 460 different farms, randomly selected according to the distribution of farms over the provinces, covering about 10% of the total amount of Flemish farms. The selection comprised 120 mixed farms housing more than 10 pigs. Finally we collected 62 individual cow milk samples and 80 pig fecal samples at a mixed farm in West-Flanders. The milk samples encompass 31 individual samples collected at 2 different time points.

All milk samples were screened for the presence of HEV RNA using a one-step real-time RT-qPCR method. A random selection of 245 bulk milk samples and 31 individual milk samples

from the mixed farm in West-Flanders were tested for the presence of HEV-specific IgG using an in-house adapted version of a commercial ELISA kit (Wantai).
Results HEV RNA could not be detected in any of the milk samples collected during this study. In addition, although we found proof for active HEV infection in the pig herd of the mixed farm, all cow samples tested negative for HEV RNA and HEV-specific IgG, a marker for past exposure.
Conclusion Our results indicate that HEV is not circulating among Flemish dairy cows and that cows are probably not part of the viral reservoir in our region.

55.

Implication of DNA methylation in HIV-1 post-integration latency.

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Background :

The persistence of Human Immunodeficiency Virus type 1 (HIV-1) in a latent state in patients under combined antiretroviral therapy (cART) seriously challenges viral eradication. The transcriptional silencing of the latent HIV-1 provirus is a multifactorial phenomenon including notably a tight epigenetic control[1]. In this regard, the role of DNA methylation in chromatin silencing of HIV-1 promoter, the 5' long terminal repeat (5'LTR), remains controversial. Indeed, two CpG islands flank the HIV-1 transcription start site and have been reported to be hypermethylated *in vitro*, in latently-infected model T-cell lines[2,3]. However, the DNA methylation profile of the HIV-1 promoter is still controversial *in vivo* in patient cells[4–6]. Here, we further investigate the molecular mechanisms underlying DNA methylation status at the HIV-1 promoter in an *in vitro* HIV-1 latently-infected model T cell line.

Material and methods :

Reactivation of HIV-1 expression following treatment with the different demethylating agents was assessed by flow cytometry. Reactivation of HIV-1 production following treatment by these demethylating agents was assessed by p24 ELISA experiments. DNA methylation profiling at the 5'LTR was carried out by bisulfite sequencing. The probability of demethylation at each CpG dinucleotide and associated p-value (Fisher's exact test) was calculated.

Results :

We studied the reactivation potential of several DNA methylation inhibitors, both nucleosidic and non-nucleosidic, in different clones of the HIV-1 latently-infected J-Lat T-cell model line and showed that the treatment by cytidine analogs induced the highest reactivation of HIV-1 expression and production. Off note, the reactivation levels following demethylating treatment differed in the different clones of the J-Lat cells studied, thereby suggesting that the cellular context of HIV-1 integration influences the reactivation potential of the epigenetic drugs. We then characterized the profiles of demethylation following cytidine analogs treatment at the 5'LTR in these different J-Lat cells and showed that the CpG dinucleotides were either uniformly demethylated or presented precise and significant hotspots of demethylation. *In silico* analyses at these hotspots of demethylation showed the presence of binding sites for transcription factors. We are currently investigating the specific molecular mechanisms implicated at these hotspots of demethylation in order to identify new determinants of HIV-1 post-integration latency.

Conclusion :

Our results highlight that the demethylation profile at the HIV-1 promoter and the associated reactivation in HIV-1 gene expression and production vary in different *in vitro* latently-infected model T cell lines. This suggests that different molecular mechanisms could be responsible for the establishment of the methylation pattern at the HIV-1 promoter, thus providing a molecular explanation on the controversy of the implication of DNA methylation in HIV-1 post-integration latency *in vivo*.

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56.

Chemical-controlled activation of Myxovirus Resistance 1 rapidly inhibits influenza A virus RNA polymerase activity

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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 viruses. Mouse MX1 interacts with the influenza ribonucleoprotein complexes (vRNPs) and can prevent the interaction between polymerase basic 2 (PB2) and the nucleoprotein (NP) of influenza viruses. However, it is unclear if mouse MX1 disrupts the PB2-NP interaction in the context of pre-existing vRNPs or prevents the assembly of new vRNP components. Here, we describe a conditionally active mouse MX1 variant that only exerts antiviral activity in the presence of a small molecule drug. Once activated, this MX1 construct phenocopies the antiviral and NP-binding activity of wild type MX1. Influenza A PB2-NP interactions are disrupted within minutes after the addition of the small molecule activator in the presence of the conditional Mx1 variant. These findings support a model in which mouse MX1 interacts with the incoming influenza A vRNPs and inhibits their activity by disrupting the PB2-NP interaction.

57.

Molecular cloning of porcine Siglec-3, Siglec-5 and Siglec-10 and identification of Siglec-10 as an alternative receptor for PRRSV

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Over the past years, several entry mediators have been identified for porcine reproductive and respiratory syndrome virus (PRRSV). Porcine sialoadhesin (pSn, also known as Siglec-1; Sialic acid-binding immunoglobulin-type lectins) and porcine CD163 (pCD163) are identified as two important host entry mediators that fully coordinate PRRSV infection in alveolar macrophages. However, recent isolates exhibited a cellular tropism for not only sialoadhesin positive but also sialoadhesin negative cells, which in part could be explained by the existence of additional receptors that can support PRRSV binding. In a search for new receptors, newly identified Siglecs (Siglec-3, Siglec-5 and Siglec-10), members from the same family as sialoadhesin were cloned and characterized. Only Siglec-10 was able to considerably improve infection and production of PRRSV type 2 strain MN-184 in a non-permissive cell line together with CD163. Siglec-10 functioned as performant as sialoadhesin, whereas, with PRRSV type 1 strain LV, Siglec-10 was clearly inferior to sialoadhesin. Siglec-10 was involved in the endocytosis of PRRSV, confirming the important role of Siglec-10 in the entry of PRRSV. In conclusion, it can be stated that PRRSV may use several Siglecs to enter macrophages, which may explain strain differences in the pathogenesis.

58.

Sexual transmission of Murid Hepresvirus-4 is reduced by the use of the nucleotide analogue cidofovir

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The use of antiviral drugs to limit clinical signs associated with herpesviruses has been extensively documented in the past. In contrast, their capacity to block transmission remains unclear as herpesviruses are mostly shed and transmitted without any symptom and as most herpesvirus animal models do not allow transmission. Recently, we have shown that Murid Herpesvirus-4 (MuHV-4), a model for the highly prevalent human Epstein-Barr virus and Kaposi's Sarcoma associated Herpesvirus, transmits sexually from female to male in laboratory mice. This model is therefore a unique opportunity to evaluate the use of antiviral drugs to limit spread of herpesviruses that transmit sexually. In this study, we tested the capacity of the nucleotide analogue cidofovir to decrease MuHV-4 sexual transmission. On the one hand, we showed that a daily treatment of infected females can reduce genital shedding by 75%. On the other hand, a daily preventive treatment of naive males was sufficient to block viral transmission. Finally, a single post-exposure treatment reduced transmission but failed to block completely infection. Altogether, our results show that cidofovir is a powerful weapon to fight herpesviral infections as it can both reduce clinical signs and limit the transmission capacity of these viruses.

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59.

CCL2 and CCL5 driven attraction of CD172a+ monocytic cells during an equine herpesvirus type 1 (EHV-1) infection in equine nasal mucosa and the impact of two migration inhibitors, rosiglitazone (RSG) and quinacrine (QC)

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Equine herpesvirus type 1 (EHV-1) causes respiratory disease, abortion and neurological disorders in horses. Besides epithelial cells, $CD172a^+$ monocytic cells become infected with EHV-1 in the respiratory mucosa and transport the virus from the apical side of the epithelium to the lamina propria en route to the lymph and blood circulation. Whether $CD172a^+$ monocytic cells are specifically recruited to the infection sites in order to pick up virus is unknown. In our study, equine nasal mucosa explants were inoculated with EHV-1 neurological strains 03P37 and 95P105 or the non-neurological strain 97P70 and the migration of monocytic cells was examined by immunofluorescence. Further, the role of monokines CCL2 and CCL5 was determined and the effect of migration inhibitors rosiglitazone (RSG) or quinacrine (QC) was analyzed. It was shown that with neurological strains but not with the non-neurological strain, $CD172a^+$ cells specifically migrated towards regions of EHV-1 infection and that CCL2 and CCL5 were involved. CCL2 started to be expressed in infected epithelial cells at 24hpi and CCL5 at 48hpi, which corresponded with the $CD172a^+$ migration. RSG treatment of EHV-1-inoculated equine nasal mucosa had no effect on the virus replication in the epithelium, but decreased the migration of $CD172a^+$ cells in the lamina propria. Overall, these findings bring new insights in the early pathogenesis of EHV-1 infections, illustrate differences between neurological and non-neurological strains and show the way for EHV-1 treatment.