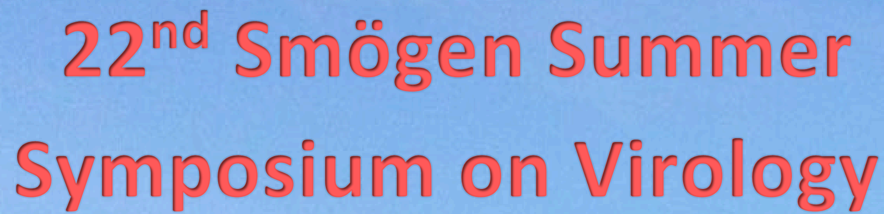

ABSTRACTS

August 21-23, 2025

Arranged by Virus- och pandemifonden –

Swedish Society for Virology

www.swedishvirology.se

Thursday, August 21

15.30-15.50

Epidemiology

Tracing the Genetic Landscape of Puumala Virus in Sweden: Insights from 1990s to Present

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Orthohantaviruses are globally distributed, negative-stranded RNA viruses, primarily harbored by rodents, and pose significant public health risks due to their high morbidity, mortality, airborne transmission, and lack of effective antiviral treatments. In northern Sweden, *Orthohantavirus puumalaense* (PUUV)—the causative agent of Nephropathia epidemica (NE), a mild form of hemorrhagic fever with renal syndrome (HFRS)—is highly endemic, with a case fatality rate of 0.5–6%. NE incidence follows cyclic fluctuations in bank vole populations, with the largest Swedish outbreak recorded in 2006–2007. However, the role of PUUV genetic variation in driving outbreaks remains unclear, partly due to challenges in sequencing wild-type strains from low-titer samples.

Using our custom target-capture sequencing protocol, we recovered PUUV genomes from human and bank vole samples, including isolates dating back to the 1990s. All belonged to the northern Scandinavian lineage and showed strong regional and temporal conservation. Human isolates were phylogenetically interspersed among bank vole sequences, indicating no clade-specific association with human infection. Segment-specific phylogenies revealed incongruent topologies, suggesting historical reassortment. We also observed substantial and uneven homoplasy, potentially indicating recombination. No variant was linked to specific outbreak periods.

These findings suggest that PUUV evolution in Sweden is shaped by long-term geographic stability and segment reassortment, rather than the emergence of new variants during outbreaks.

Distinct Distribution of HEV-3 Subtypes across Humans, Animals, and Environmental Waters in Sweden

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Hepatitis E virus (HEV) remains a public health concern in Europe, yet its transmission is not fully understood. We previously observed a notable discrepancy in the distribution of HEV-3 subtypes between wastewater and clinical samples in Gothenburg. To confirm this observation and comprehensively elucidate HEV-3 circulation patterns across humans, animals, and environmental waters, we analyzed the HEV genetic diversity in archived wastewater samples between late 2016 and early 2018, clinical cases between 2012 and 2024, and all available Swedish sequences from the NCBI Virus database. HEV RNA was detected in all archived wastewater samples, with subtype 3c being the only subtype identified. In typed clinical cases, subtypes 3f (45/126) and 3c (44/126) were nearly equally distributed, though regional dominance varied. When incorporating human sequences from other Swedish studies, subtype 3f became dominant (75/168). Analysis of all available sequences revealed that 3f (113/136) was the dominant subtype in *Sus scrofa* (pigs and wild boars), while 3c (30/33) was dominant in environmental waters. These findings highlight the complex transmission dynamics of HEV-3 in Sweden. The near-absence of 3c in Swedish domestic pigs and wild boars, despite its high proportion in clinical cases, raises the question about the source of human 3c infection. In addition, the near-exclusive detection of 3c in wastewater suggests potential differences in viral shedding, disease severity of HEV-3 subtypes, or alternative host sources. This study emphasizes the importance of integrated One Health surveillance to track HEV circulation across reservoirs.

Thursday, August 21

15.50-16.00

Molecular and Structural Virology

Structural analysis of the herpes simplex virus 1 attachment protein gC: crystallization and prediction by AlphaFold3

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To initiate infection, most, if not all, human herpesviruses binds to sulfated carbohydrates on target cells. Herpes simplex virus 1 (HSV-1) gC is an attachment protein that provides sites for initial virus binding to cell surface heparan sulfate (HS). Such HS-binding viral proteins are often heavily glycosylated, why structural determinations are hampered. Here we present the gC crystal structure (residues 123 to 466, lacking the mucin-like domain and the transmembrane region) at a resolution of 3 Å, with the asymmetric unit comprising a protein monomer. The monomer consists of three distinct domains, each folded into β-sheets stabilized by extensive interstrand hydrogen bonding. These domains are interconnected by loops, some of which have known functional roles. Despite the challenges of crystallizing glycosylated proteins, we successfully resolved the structure, with two N-glycans included.

gC has been previously reported to be present as a oligomer (Kikuchi et al., *Journal of Virology*, 1990). We then generated a dimer using multiple computational tools, including AlphaFold3. The predicted dimer consists of two identical symmetric monomers arranged in a helical conformation. Previous studies from our group have shown that the N-terminal region contains functional residues forming a HS-binding domain (HSBD). We suggest that one cellular HS chain may accommodate to both such HSBDs present on each gC dimer. Furthermore, the epitope of the neutralizing MAb B1C1, an antibody that also inhibits the immuno-evasive property of gC binding to complement factor C3b, was previously found to partly overlap with this HBD. AlphaFold3 predictions show a highly positively charged cleft between the two N-terminal domains that seems to guide the HS binding. Interactions with these residues align with our prior experimental findings.

In conclusion, our results indicate that HSV-1 gC could be a functional dimer, providing HS and C3b binding as well as NT sites in the apical cleft formed by this structure. Furthermore, there was an agreement between the crystal structure and the prediction, which may have bearings to future structural analyses and predictions of HS-binding attachment proteins also from other herpesviruses.

Thursday, August 21

16.30-16.40

Tumor Virology

Deciphering the Evolutionary Enigma of RNA Splicing in HR-HPV Oncogenes E6 and E7

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Background：Human papillomavirus (HPV) comprises over 200 types, with at least 20 high-risk (HR-HPV) types linked to ~4% of global cancers. A defining feature of HR-HPV is the regulation of oncogenes E6/E7 through RNA splicing, a mechanism rarely observed in low-risk (LR-HPV) types. Despite its critical role in carcinogenesis, the cis-elements driving splicing events in HR-HPV remain uncharacterized. Leveraging the close genetic relatedness among HR-HPV, this study aims to decipher the evolutionary origins of E6/E7 splicing and its impact on viral oncogenicity, with implications for antiviral therapy development.

Methods：Full-length E6/E7 sequences from 26 HR-HPV and 5 LR-HPV types were cloned into expression vectors pCL086 and transfected into C33A cells. Splicing events were validated by RT-PCR and Western blotting (WB), while splicing sites (SS) were mapped via Sanger sequencing. Bioinformatics analyses included motif discovery (MEME suite) and phylogenetic tree construction (MEGA) to assess evolutionary relationships.

Findings：

- Splicing Specificity: a) All HR-HPV types produced at least one spliced transcript, whereas no splicing was detected in LR-HPV. b) Group 1 HR-HPV (definitely carcinogenic) exhibited higher splicing efficiency than Group 2 HR-HPV (possibly carcinogenic). c) Most HR-HPV (except HPV59) generated E6*I mRNA, the dominant spliced mRNA-variant for E7 protein expression; HPV59 showed unique splicing patterns and minimal E7 production.
- Splicing Site Conservation: Only 7 types displayed variable SS usage, indicating strong conservation of 5’ and 3’ SS selection across most HR-HPV.
- Evolutionary Correlation: Phylogenetic analysis of E6 sequences revealed clustering of types with identical SS patterns, highlighting a link between splicing specificity and genetic relatedness.
- Genomic Divergence: HR-HPV exhibited distinct genetic architecture compared to LR-HPV, with high intra-group sequence identity among HR-HPV.

Conclusion: HR-HPV uniquely employs E6/E7 pre-mRNA splicing as a conserved mechanism to drive E7 oncoprotein production, potentially contributing to their carcinogenic potential. The correlation between genetic phylogeny and splicing site selection suggests the existence of conserved cis-elements governing this process. We aim to identify these regulatory RNA elements. These findings might provide insights into HR-HPV evolution and oncogenesis, paving the way for targeted therapeutic strategies.

Thursday, August 21

16.40-16.50

Clinical Virology and Diagnostics

Assessing SRLV Load Dynamics and Genetic Diversity in a Swedish Dairy Goat Herd Around Parturition

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Introduction: Small ruminant lentiviruses (SRLVs) are globally distributed retroviruses affecting goats and sheep, often leading to chronic infection, productivity loss, animal suffering and trade restrictions. Accurate detection remains challenging due to the slow replication of the virus and host immune variability, making the combined use of serological and molecular methods essential.

Aims: This study aimed to evaluate changes in SRLV nucleic acid levels across late pregnancy and lactation stages using paired blood and milk samples, and to determine the optimal sampling time for molecular diagnostics. A secondary objective was to characterize the circulating SRLV genotype and explore intra-herd viral diversity.

Methods: Samples were collected from 16 pregnant goats in a seropositive Swedish herd at two time points: around kidding and one month postpartum. SRLV nucleic acid was quantified via nested qPCR. Genetic characterization was performed using partial *pol* gene sequences.

Results: At approximately one month postpartum, 14 of 16 goats exhibited decreased Ct values—indicating elevated proviral loads—and/or converted to qPCR positive status compared to the time around parturition. A statistically significant increase in proviral load was observed between the two sampling points ($p = 0.0042$, two-tailed binomial test). Five goats transitioned from qPCR negative to positive, and among 10 goats that had not yet given birth at the time of the first sampling, nine showed increased proviral load or turned to qPCR positive ($p = 0.0215$) in the second sampling, indicating a post-kidding rise in viral activity. Blood and milk qPCR results showed complete agreement at the second time point (Cohen's $\kappa = 1.00$). Phylogenetic analysis placed both isolates within genotype C. Pairwise comparisons revealed 16% divergence from the reference genotype C strain and 5% intra-herd variability, suggesting potential subtype-level diversity, albeit with low genetic heterogeneity among the goats in the herd.

Conclusion: These findings indicate that proviral load is higher one month after, compared to around parturition, highlighting this period as a more suitable time point for molecular detection. The genetic data point to a relatively homogenous SRLV population, likely derived from a common ancestor, with indications of subtype variation requiring further classification.

Thursday, August 21

16.50-17.10

Antivirals and Chemotherapy

Identification of an MAP4K4 Inhibitor as a basis for development of novel broad-spectrum drugs against human rhino/enteroviruses including poliovirus

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Human rhinoviruses (HRVs) and enteroviruses (HEVs) are dominating as causative agents of viral respiratory tract infections, and are associated with exacerbations of asthma and chronic obstructive pulmonary disease (COPD) (HRV) and with complications from the neuronal system (HEV including poliovirus (PoV)). Despite their significant impact on public health, no approved antiviral drugs specifically target HRV and/or HEV. In this study, we identified an MAP4K4 inhibitor, AZ2953, as a potent antiviral compound against HRV-2 and PoV-1. Screening a kinase inhibitor library in HeLa cells revealed that AZ2953 effectively blocked HRV-2 and PoV-1 infection with an EC_{50} of 0.85 µM. To elucidate the mechanism of action, serial passaging of HRV-2 with the AZ2953 led to a mutant virus with 2-fold increased IC50. Sequencing revealed a mutation in the nonstructural protein 2B (NSP2B), a.a. Ile to Val at position 6 (I6V). Structural modelling predicted that the I6V mutation lied within a hydrophobic N- tail of NSP2B. Western blot analysis revealed that viral protein expression was inhibited in the presence of the AZ2953, further supporting its antiviral mechanism. The time-of-addition experiments demonstrated that AZ2953 remained effective up to 12 hours post-infection, reducing viral titres by 1.5 log10. Interestingly, confocal microscopy showed that HRV-2 infected cells (24 hpi) appeared to exploit MAP4K4 signalling to facilitate cell-to-cell spread. Cells treated with the AZ2953 showed inhibition of MAP4K4 signal and restricted viral infection, with virus limited to isolated cells. These findings suggest that AZ2953 blocks virus-induced MAP4K4 activation and prevents lateral spread to adjacent cells, potentially targeting both viral proteins and host-virus interactions. Importantly, the compound also demonstrated activity against PoV-1, highlighting its potential as a broad-spectrum antiviral also against HEV. Ongoing experiments include generation of escape mutants also for PoV-1. Notably, the compound exhibited potent antiviral activity also in physiologically relevant human airway ALI cultures, reducing viral infection by nearly 2 log10. This study highlights the successful completion of this work with AZ2953 could pave the way for novel treatment strategies against a wide range of enteroviral infections, with significant implications for global public health.

Identification of monocarboxylate transporters as key host factors for adenovirus infections

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Human adenovirus (HAdV) infections are associated with high mortality rates in immunocompromised patients. Today there are no approved nor efficient antiviral drugs available to treat HAdV infections. However, antiviral drugs originally developed against other viral diseases are used for life-threatening HAdV disease but with variable results.

In collaboration with Chemical Biology Consortium Sweden, we have screened an AstraZeneca library consisting of around 13,000 target-annotated compounds that target 1,763 known human proteins with the goal to identify host-directed anti-adenoviral compounds. Human erythroleukemia K562 cells were infected with a replication-competent red fluorescent protein (RFP)-expressing vector based on HAdV type 11 and the inhibitory effect of each compound was assessed at three different concentrations, 0,1, 1 and 10 µM. The primary screening yielded a 5 % hit rate and the hits were subjected to extended dose-response analysis and in parallel the host cell toxicity was assessed. Target-enrichment analysis identified a monocarboxylate transporter (MCT), in the SLC16 solute carrier family, as a promising host cell target. The MCT inhibitors had EC_{50} values in the low nanomolar range and low cell toxicity.

MCT´s are plasma membrane transporters of mainly lactate, pyruvate and ketone bodies across membranes and are ubiquitously expressed in human tissues. MCT´s are overexpressed in many cancers, due to the Warburg effect that is commonly observed in cancer cells. Interestingly, the metabolism of many virus-infected cells resembles the Warburg metabolism observed in cancer cells, with increased glucose uptake and glycolysis followed by excessive lactate production and export.

We further evaluated the antiviral activity of MCT inhibitors on against several wild-type HAdVs in several different cell types, including primary human cells, and report a general and potent antiviral activity that is not due to general host cell toxicity. Furthermore, we analyzed the metabolomic profile of infected and non-infected cells treated with MCT inhibitors and observed an expected decrease in lactate export and intracellular pH in treated cells.

To summarize, whole-cell screening of 13,000 target-annotated compounds identified MCT´s as anti-HAdV targets. Available MCT inhibitors gave potent anti-HAdV activity against wild-type HAdV types and displayed low host cell toxicity in several cell types. We will assess the antiviral activity of MCT inhibitors against additional types of viruses and test MCT inhibitors in *in vivo* models to further evaluate MCT´s as antiviral targets.

Friday, August 22

09.45-10.35

Pathogenesis

Innovative Multidisciplinary Strategies to Combat Severe Dengue and Other Mosquito-borne Arboviruses

Ujjwal Neogi[1] on behalf of COMBAT Consortium[2]

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In recent decades, the re-emergence and geographic expansion of arboviral outbreaks have become a significant global health concern. Nearly 4 billion people across approximately 130 countries are currently at risk for arboviral infections, with the public health threat from these diseases continuously escalating. The global burden of arboviruses is substantial, causing up to 700,000 deaths annually. DENV alone accounts for over 390 million cases each year, presents a formidable global public health challenge, with an estimated 100 million symptomatic infections annually. The COMBAT initiative, a pioneering European Research Constellation, addresses this crisis by leveraging advanced technologies and expertise from endemic regions to develop innovative, scalable solutions for primarily *Orthoflavivirus denguei* (Dengue Viruses-DENV), and further expanding to other arboviruses (funding applied). COMBAT advances super-resolution microscopy to visualize rare host-virus interactions at the nanoscale and introduces brain-on-chip technology for modeling neurotropic flavivirus pathogenesis. The project develops novel antiviral strategies to inhibit DENV entry and regulate cytokine storms through host-directed therapies in dengue infection, while applying similar frameworks to other arboviruses to understand and mitigate its neuroinflammatory effects. By leveraging multi-omics approaches, COMBAT aims to identify early predictive biomarkers of severe dengue-related complications. It integrates artificial intelligence and machine learning to model virus-host interactions and predict disease progression, contributing to EU pandemic preparedness frameworks. By using the similar approaches, we have identified Glutamate oxaloacetate transaminase 2 (GOT2) as a novel host factor involved in Japanese encephalitis virus (JEV) pathogenesis, linking viral replication with metabolic and inflammatory pathways. Silencing GOT2 significantly suppressed viral replication, positioning it as a promising therapeutic target. Through its interdisciplinary and translational approach, COMBAT not only enhances our molecular understanding of DENV and other arboviruses but also seeks to deliver affordable, deployable solutions for diagnostics, treatment, and policy. It sets a new benchmark in pandemic preparedness by uniting cutting-edge science with real-world application in at-risk regions and contributing to EU pandemic preparedness frameworks.

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In collaboration with European Virus Archive – Marseille (EVAM)

Whole brain 3D optical imaging of viral infection and Galectin-3 visualizes massive macrophage infiltration in fatal flaviviral encephalitis.

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Orthoflaviviruses consists of several neuroinvasive viruses. Viral brain infections cause severe neurological symptoms that may lead to long-term neurological complications lasting months to years after viral clearance. We have previously showed that intracranial (ic) infection with Langat virus (LGTV), a biosafety level 2 tick-borne orthoflavivirus, causes neuroinflammation and recruitment of macrophages to the brain in wild type mice. Inflammation and recruitment of immune cells is crucial for viral clearance, but excessive immune activation can also be a disease escalating factor in viral encephalitis by promoting extensive neuronal loss. Spatio-temporal visualization and characterization of cells mediating neuroinflammatory responses could therefore significantly improve our understanding of antiviral responses in the brain.

To identify novel neuroinflammation markers, compatible with 3D optical imaging, we relied on our previously published single-nuclei sequencing dataset. Various inflammatory targets were selected among the genes upregulated in the macrophage population and their enhanced expression during infection was confirmed using immunofluorescence microscopy. To study the effects of neuroinflammation in a lethal infection model, wild type and $IPS1^{-/-}$ mice were infected i.c. with LGTV and brains were harvested for downstream analysis.

Galectin 3 (Gal3) was the most promising target as it had high expression in infected brains while being nearly absent in uninfected samples. Gal3 upregulation in the brain is specific to infection as no or low expression is detected by western blot and qPCR in mice receiving i.c. PBS injection. Further characterization of Gal3 expressing cells revealed co-expression of Iba1 and CD45, but not TMEM119, indicating that these are infiltrating macrophages. Light-sheet fluorescent microscopy (LSFM) of brains, dual-labeled for Gal3 and the viral protein NS5, demonstrated high Gal3 expression in infected regions, suggestion macrophage-recruitment to the site of virus replication. Interestingly, increased Gal3 expression was not only found in infected regions but appeared more widespread. This may reflect that inflammation is lingering in regions where the virus has already been cleared, potentially promoting extensive tissue damage.

Uncovering a role for hyaluronan in Puumala virus-induced pulmonary disease

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Hantaviruses are globally distributed zoonotic viruses that cause severe human disease with high case-fatality rates. In Europe, Puumala orthohantavirus (PUUV) is the most prevalent hantavirus with over 2,000 reported cases annually. PUUV infections cause a mild form of haemorrhagic fever with renal syndrome, characterized by increased vascular permeability, acute kidney injury and thrombocytopenia. While the haemorrhagic and renal manifestations are well characterized, several studies highlight pulmonary symptoms in PUUV patients, revealing a critical gap in our understanding of the disease. A potential contributor to these symptoms is hyaluronan, a highly hydrophilic glycosaminoglycan implicated as a driving factor of the pulmonary manifestation in other respiratory viral infections. Here, we investigate the role of hyaluronan in PUUV pathogenesis, by examining its accumulation in the lungs of deceased patients and analysing changes in hyaluronan concentration and fragmentation in bronchoalveolar lavage fluid from infected patients. Additionally, we characterize hyaluronan production and regulation responses of lung fibroblast and epithelial cells following PUUV infection.

As an initial observation, immunohistochemical staining of lung tissue from deceased PUUV patients revealed extensive accumulation of hyaluronan in the alveolar space, with some regions showing complete loss of alveolar morphology. Supporting this, patients infected with PUUV exhibited significantly elevated levels of hyaluronan in bronchoalveolar lavage fluid compared to healthy controls, with hyaluronan levels positively correlating with disease severity. This increase was primarily driven by the accumulation of high-molecular weight hyaluronan, with no detectable change in low-molecular weight hyaluronan. *In vitro*, lung fibroblasts and epithelial cells accumulated hyaluronan following PUUV infection, with peak levels observed at 72- and 96-hours post-infection, respectively. This accumulation was a result of the increased expression of hyaluronan synthase 2 and 3, with fibroblasts and epithelial cells displaying different expression levels of hyaluronan synthases and hyaluronidases.

In summary, we demonstrate extensive accumulation of hyaluronan in the lungs of deceased PUUV patients and identify a correlation between hyaluronan and disease severity. Furthermore, we show that lung fibroblasts and epithelial cells accumulate hyaluronan following PUUV infection as a result of increased expression of hyaluronan synthase. Together, these results provide the first insight into the role of hyaluronan as a potential driver of PUUV lung pathogenesis, highlighting an understudied aspect and possible novel target for symptomatic treatment.

Dysregulated innate epithelial response to norovirus infection in intestinal organoids from patients with common variable immunodeficiency

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Common variable immunodeficiency (CVID) is a primary immune deficiency characterized by impaired B-cell function and reduced immunoglobulin levels, leading to recurrent and long-lasting infections. Norovirus can cause chronic infections in CVID patients, potentially leading to malabsorption and increased mortality. While impaired adaptive immune responses are well-documented in CVID, the role of innate response in gastrointestinal infections is less explored.
To investigate the innate epithelial response, we infected secretor positive human intestinal enteroids from CVID patients (n=4) and immunocompetent individuals (n=2) with norovirus GII.3, GII.4, or stimulated with poly(I:C). Replication was analyzed by RTqPCR and transcriptional responses by whole genome RNA sequencing (RNA-Seq). No significant difference was seen in norovirus replication between CVID and immunocompetent enteroids, or in epithelial response observed after poly (I:C) stimulation. However, the RNA-Seq revealed that immunocompetent enteroids infected with GII.4 upregulated antiviral pathways while CVID enteroids exhibited responses mainly related to metabolic pathways. Several genes important for innate immune response, such as IFITM1, ZBP1, IFIT1 and HERC5, were significantly upregulated in immunocompetent but not in CVID enteroids. In contrast, GII.3 infection upregulated immune-specific genes and pathways in both CVID and immunocompetent enteroids, but the response was substantially lower in CVID.

Altogether, our study identifies several key differences in the epithelial response to norovirus infection, suggesting that an impaired innate immune response may contribute to more severe and prolonged norovirus infection in CVID patients.

Spatiotemporal penetration of zoonotic influenza A viruses through respiratory mucus gel networks

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The ongoing widespread of highly pathogenic avian influenza virus (HPAIV) H5N1 (clade2.3.4.4b) around the globe and the infection of novel hosts like dairy cattle and low frequency spillover into humans on the American continent is concerning. Furthermore, cattle may have potential as a mixing vessel for future influenza A viruses (IAV) and the current route of transmission is yet to be completely understood. For most viral infection routes, mucus is the first barrier against attachment or entry before virus-cell interaction occurs. In the airways, respiratory mucus forms a viscous network that mechanically traps inhaled particles, such as viruses, which are then transported outward from the lungs, airways or nasal cavity through mucociliary clearance. In addition to the mechanical barrier function, mucins possess a high glycan density, able to bind viral lectins. Hence, host and tissue-specific mucus may affect virus-host interaction by influencing virus attachment and antiviral defense. We investigate the interplay between different IAV strains and mucus collected from various species to understand its relevance for viral tropism and host adaptation. We started collecting primary cells and native mucus samples from organs of relevant species (e.g., cattle, swine, birds, humans). Further, we developed standardized biophysical and biochemical techniques to quantify the spatiotemporal virus penetration through obtained species-specific mucus samples. Using these methods, we analyze the penetration properties of IAV particles and their subsequent infection potential. Preliminary results suggest that origin-specific mucus influences viral penetration and infection efficiency of H5N1 and H1N1 influenza strains. Our aim is to investigate the interaction between viral attachment proteins, mucin glycans and the physical properties of mucus that may shape viral penetration efficiency and infectivity. Understanding these mechanisms provides novel insights into the cross-species transmission potential of zoonotic and potentially pandemic IAV strains.

Friday, August 22

11.05-11.15

Emerging viruses

Establishment of PhIP-Seq for Detection of Emerging Pathogens and Characterisation of the Human Virome in Denmark

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Phage immunoprecipitation sequencing (PhIP-Seq) is a novel and modern immunoprofiling assay that offers a multiplex, high-throughput approach to antibody profiling against diverse pathogens across large cohorts. It combines the use of phage display libraries and next-generation sequencing to detect antibody reactivity to >100,000 peptides, simultaneously. One area where this technology can be applied is to characterise better a population's immunity and exposure to both endemic and emerging viruses.

There are currently more than 200 known virus species capable of infecting humans, and with increasing risks of zoonotic spillover events, this number is expected to rise. Additionally, due to climate change and increased global travel, the introduction of exotic or emerging virus infections is being observed more frequently. As such, accurate population-wide surveillance of both endemic and non-endemic viruses will become increasingly important in the future to better inform public health strategies.

Conventional techniques used for assessing serological immunity have limitations when characterising emerging pathogens across a community. These methods have limited multiplexing capabilities, low throughput, and a dependency on protein expression and purification. PhIP-Seq can also provide a 'serological time capsule' of not only current but past infections, at an epitope-level resolution, which can better inform diagnostics, vaccine design and preparedness.

At present, there is no widely accessible unbiased meta-serologic assay available in Denmark or in most other European countries. To this end, we will develop and implement a screening platform using PhIP-Seq to define the Danish population's immunity across the entire human virome and emerging viruses within viral families with pandemic potential. This will allow us to better understand the true prevalence and disease burden of viral infections within the population.

Friday, August 22

11.15-11.35

SARS-Coronavirus 2

Identification and Functional Validation of an Essential Host Protein in SARS-CoV-2 Replication

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SARS-CoV-2, the virus responsible for COVID-19, continues to evolve with the emergence of new variants. Between January and May 2025, several shifts in SARS-CoV-2 variant dynamics were observed worldwide. As most mutations occur in the spike protein, comprehensive understanding of the viral life cycle and the identification of essential host factors remain critical for advancing therapeutic strategies. Despite the implication of numerous host proteins in SARS-CoV-2 infection, the key host proteins and their precise functional roles are still not fully elucidated.

In this study, we used a unique haploid cell screening platform to identify an essential host protein involved in SARS-CoV-2 infection. Functional validation showed a substantial reduction in SARS-CoV-2 infectivity, with an average decrease of 50% and 80.5% upon knockdown and knockout of the protein using multiple 2D cell culture models. These findings indicate that this host protein plays a critical role in supporting viral replication, highlighting its potential as a target for therapeutic intervention.

Adaptation of the WHO COVID-19 Clinical Progression Scale for Registry-Based Data: A Whole-Population Study in Sweden

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Purpose
COVID-19 has been extensively researched, however the lack of standardized COVID-19 severity categorization in register-based research complicates comparison of studies. The WHO COVID-19 Clinical Progression Scale is a standardized disease severity tool for clinical data, though not adapted to data available in health registries. We aimed to develop and validate such a novel categorization with international applicability.

Methods
The WHO Clinical Progression Scale was translated to a severity index utilizing ICD- and procedure-codes from outpatient, inpatient, intensive care, and mortality registries using the adult Swedish population and SARS-CoV-2 positive-test data (January 2020 - July 2022). Cox proportional hazards was applied to determine whether increasing severity correlates with mortality in COVID-19 patients compared to the population.

Results
The WHO-Scale was translated to ten categories reflecting increasing need for advanced care, encompassing 8,245,474 individuals including 1,981,946 SARS-CoV-2 infections. Fatal COVID-19 cases were older with more comorbidities. Those receiving mechanical ventilation and ECMO were younger with fewer comorbidities. Among survivors beyond 30 days, 90-day all-cause mortality increased with severity using category zero (no laboratory-verified SARS-CoV-2) as reference. Mortality was lowest for patients without healthcare, adjusted for age, sex, comorbidities and socio-economic variables (adjusted hazard ratio (aHR) 1.18, 95% confidence interval (CI) 1.13–1.22). Those hospitalized >5 days had higher mortality (aHR 5.83, 5.5–6.17). Those requiring ECMO/ECLS had the highest mortality (aHR 593.54, 317.77–1108.65).

Conclusions
The novel COVID-19 severity index associated with all-cause 90-day mortality and aligned with previous literature. This index will enable comparative studies of COVID-19, which is important for public health policies and development of clinical guidelines. This is an innovative epidemiologic tool with potential applicability in all countries with centralized health registers. The index also has potential to be validated and used for other infectious diseases and in real-time data for modelling predictions.

Teamwork makes the dream work - systemic and mucosal immunity in shaping protection against SARS-CoV-2

PhD-thesis by Ulrika Marking, defended at Karolinska Institutet 16 May 2025.
Main supervisor: Charlotte Thålin.

Understanding the mechanisms of protection against SARS-CoV-2 infection, not just severe disease, is essential for comprehending the transition from uncontrolled viral transmission to a low-grade, endemic circulation of SARS-CoV-2, as well as for understanding future emerging viruses.

Through regular screening studies of vaccinated healthcare workers, we demonstrated a strong association between nasal anti-ancestral spike IgA responses and protection against SARS-CoV-2. We identified a temporal relationship between rising nasal spike IgA levels and declining viral load during infection, as well as a trend toward more rapid nasal spike IgA responses in reinfected individuals.

We also found that high levels of vaccine-induced anti-ancestral spike serum IgG were independently associated with both protection against infection and lower viral load in those who became infected. However, this effect diminished as variants with stronger immune evasiveness emerged. The dimeric nasal spike IgA was shown to have a stronger ability to bind viral variants compared to serum spike IgG, and we argue that, with the evolution of immune-evasive strains and the concurrent development of mucosal defences, mucosal spike IgA replaces serum spike IgG as the most relevant correlate of protection against infection. Additionally, through statistical modelling, we found that the increased protection against infection observed in individuals with hybrid immunity is largely mediated by mucosal spike IgA

When investigating factors associated with strong nasal spike IgA responses, we found that prior infection was paramount, and that repeated infections strengthened these responses in a boosting manner. We observed a previously unreported durability of mucosal spike IgA, with an increased likelihood of nasal spike IgA detection for more than 22 months following the most recent infection. We also found that parenteral vaccination was associated with lower nasal spike IgA levels, with an adjusted OR for detectable nasal spike IgA of 0.24 [95% CI 0.08-0.68] among individuals with 1-3 vaccine doses compared to those who were unvaccinated. Additionally, the temporal infection-vaccination sequence influenced nasal spike IgA levels, with higher nasal spike IgA levels in participants infected prior to vaccination compared to those with breakthrough infections as their first viral encounter. This finding is important for both vaccine policy and to mucosal vaccine development, particularly for replicating vaccine platforms where systemic immunity may reduce viral replication and the resulting inflammation that shapes the subsequent mucosal immune response.

The observations in this thesis illustrate the transition from early pandemic control—where serum-derived protection from vaccination was paramount—to the current phase of endemic transmission, where mucosal immune responses play a central role in population immunity. Although parenteral vaccination and systemic responses are crucial for protection against severe COVID-19, a vaccine platform that evokes a robust mucosal response may be more potent in inducing population immunity, with a reduction in viral transmission and variant evolution.

Friday, August 22

11.35-12.05

14.00-14.50

Virus-Cell Interactions I and II

Hazara virus adaptation to Hyalomma tick cells is associated with mutations into the polymerase and glycoprotein precursor encoding genes

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Introduction: Crimean-Congo Hemorrhagic Fever Virus (CCHFV) is a tick-borne pathogen that presents a serious threat to human health, causing a severe hemorrhagic fever with high mortality rate. Ticks, notably those of the genus *Hyalomma*, serve as the principal vector for CCHFV, harboring the virus persistently and acting as its natural reservoir. However, due to biosafety concerns, limited knowledge is available regarding the virus-vector interaction. Host cycling likely contributes to the virus's evolutionary flexibility, however cross-species transmission imposes selective constraints, influencing viral adaptation. This study explores the evolutionary progression of Hazara virus (HAZV), utilized as a model for CCHFV, across different host cell lines, aiming to elucidate how pressure within host environment drives viral adaptation and impacts infectivity.

Materials and Methods: HAZV was grown on human SW13 cells and used to infect *Hyalomma*-derived tick cells (HAE/CTVM8) for 30, 60 days. This first passage was used to perform four additional passages on fresh HAE/CTVM8 cells, each lasting 30 days. We evaluated both phenotypic and genotypic changes by infecting new HAE/CTVM8 cells for 5 days and SW13 for 3 days. Phenotypic changes were assessed by evaluating viral infection in ticks and SW13 cells while genotypic changes by NGS sequencing.

Results: Through analysis of HAZV propagation in HAE/CTVM8 cell line, we determined the emergence of mutations within all three viral genome segments. Notably, the passage of HAZV within this cell line appears to lead to greater stabilization of few mutations, two within the gene encoding the RdRp (Q490Q, G2112R) and four in the glycoprotein precursor (GPC) (A381T, A802T, E849K, K1415K). HAZV-adapted to tick cells demonstrated a host-dependent effect on viral infection efficiency, with higher infectivity in HAE/CTVM8 compared to mammalian cells. Conversely HAZV from SW13, employed as a control, exhibited the opposite trend.

Discussion and Conclusions: Our results showed that mutations appear during the first 30 days and increase slowly until 60 days up to 30-40%. With the following passages in tick cells, mutations on the RdRp increased from 15-30% to 80-95% while on the GPC from 10-20 to 50-75% suggesting a host drive adaptation. Viral replication kinetics indicate that tick-adapted HAZV replicate better in tick cells, suggesting a potential role of the selected mutations in viral adaptation to the invertebrate host. Although genetic changes in passaged HAZV were minimal they seem to lead an increased relative fitness and replicative ability of the virus in the homologous HAE/CTVM8 cell line. Further experiment will be performed after isolation of mutated viruses.

"This research was supported by EU funding within the MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT)"

Identification of TRIM21 and TRIM14 as Antiviral Factors Against Langat and Zika Viruses

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Flaviviruses are usually transmitted to humans via mosquito or tick bites, whose infections may lead to severe diseases and fatality. During intracellular infection, they remodel the endoplasmic reticulum (ER) membrane to generate compartments scaffolding the replication complex (RC) where replication of the viral genome takes place. In this study, we purified the ER membrane fraction of virus infected cells to identify the proteins that were enriched during flavivirus infection. We found that tripartite motif-containing proteins (TRIMs) including TRIM38, TRIM21, and TRIM14 were significantly enriched during infection with mosquitoborne
(West Nile virus strain Kunjin and Zika virus (ZIKV)) and tick-borne (Langat virus (LGTV)) flaviviruses. Further characterizations showed that TRIM21 and TRIM14 act as restriction factors against ZIKV and LGTV, while TRIM38 hinders ZIKV infection. These TRIMs worked as interferon-stimulated genes to mediate IFN-I response against LGTV and ZIKV infections. Restriction of ZIKV by TRIM14 and TRIM38 coincides with their colocalization with ZIKV NS3. TRIM14-mediated LGTV restriction coincides with its colocalization with LGTV NS3 and NS5 proteins. However, TRIM21 did not colocalize with ZIKV and LGTV NS3 or NS5 protein suggesting its antiviral activity is not dependent on direct targeting the viral enzyme. Finally, we demonstrated that overexpression of TRIM21 and TRIM14 restricted LGTV replication.

Metabolic intervention as an antiviral strategy against respiratory Viruses

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Respiratory viruses, such as influenza, are among the most common causative agents of human infectious disease. Influenza A virus (IAV) actively induces certain cellular functions and depends on the host cell metabolism of the infected cell. It has long been known that suppressing glycolysis can restrict virus replication. Moreover, IAV infections alter metabolic pathways like glycolysis, fatty acid synthesis (FAS), the tricarboxylic acid (TCA) cycle, glutaminolysis or oxidative phosphorylation (OXPHOS). We demonstrated that the inhibition of these metabolic pathways led to a significant reduction of viral titers. In the presence of inhibitors targeting glutaminolysis, FAS, and OXPHOS, the cellular glycolysis and respiration network was unbalanced resulting in a prolonged phase of viral messenger RNA transcription while virus replication for production of genome equivalents was reduced. To better understand how metabolic fueling intermediates affect the IAV life cycle, we investigated the effects of oxaloacetate (OAA), a key enzyme of the TCA cycle, on viral growth during glycolysis inhibition. The TCA plays a central role in cellular metabolism and is already known to support IAV infection and replication. Our results proved the virus restricting potential of the glycolysis inhibitor 2-deoxy-d-glucose. Importantly, supplementation of OAA almost complete reserved the inhibitory effect of 2-DG on viral growth. Taken together, the results provided a better understanding of metabolic virus-host interactions and highlight the potential of metabolic interference as an effective antiviral approach.

Transcriptomic Profiling of Host Innate Immune Responses to Batai Virus Infection Using Nanopore Sequencing

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Bataivirus (BATV), a member of the *Peribunyaviridae* family, is a globally distributed mosquito-borne virus infecting e.g., humans, livestock and birds. Serological surveillance in Europe has shown a BATV seroprevalence of up to 46% in ruminants. Although infections in ruminants and humans are typically mild or asymptomatic, neurological disorders have been reported in infected seals. However, the molecular mechanisms underlying host-virus interactions in ruminants remain largely unexplored. Therefore, in this study, Madin-Darby bovine kidney (MDBK) cells were infected with BATV (MOI = 1), and RNA was extracted from both infected and mock-infected cells at 24h, 48h, and 72 hours post-infection. Nanopore cDNA sequencing coupled with bioinformatics analysis was used to characterize transcriptomic changes.

Preliminary results revealed a strong innate immune response as early as 24 hours post-infection, with 960 genes differentially expressed (DE) (FDR ≤ 0.05; fold change ≥ 2). Up- and down-regulated genes were evenly distributed; however, functional analysis revealed marked differences in biological processes. Up-regulated genes were predominantly associated with antiviral responses, particularly the type I interferon pathway, and included Interferon Induced Protein 44 (IFI44), Interferon Stimulated Gene 15 (ISG15), Interferon-Induced Protein with Tetratricopeptide Repeats 1 (IFIT1), and 2'-5'-Oligoadenylate Synthase 1Z (OAS1Z), all of which showed high fold changes. In contrast, down-regulated genes were mostly involved in metabolic pathways. However, the most downregulated gene was YPEL3 (FC = 19), a p53-regulated gene known to induce cellular senescence, and its downregulation can lead to reduced apoptosis. Several additional genes associated with apoptosis was also identified among the downregulated genes including BCL2 Interacting Protein 3 (BNIPL3), TNFRSF1A Associated Via Death Domain (TRADD) and PRA1 family protein 2 (PRAF2) suggesting a potential viral strategy to dampen host apoptotic responses. Together these findings provide new insights into the viral-host dynamics of BATV infection in its main host (*Bos taurus*). Understanding the functions of the DE genes can provide insights into BATV pathogenesis and host defense mechanisms. Further functional studies are needed to elucidate their specific roles during infection.

HIV-1 derived replication intermediates/oligonucleotides induce a type I IFN-dependent immune suppression via STING activation that can be restored by targeting IFNARI

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The hallmark of HIV-1 infection is the progressive development of multicellular and systemic immune dysfunction, leading to immune exhaustion, destruction of lymphoid tissues and culminating in AIDS. Dendritic cells (DCs), even though not to any high degree productively infected with the virus, play a pivotal role in HIV dissemination to CD4+ T cells. CD4+ T cells are the main target for HIV infection and are subsequently depleted by the virus leading to disease progression. Type I interferons (IFNs) are critical for host defense during acute infection but contribute to chronic immune activation during later stages of HIV disease. This persistent activation leads to immune cell dysfunction and exhaustion. During the HIV life cycle there will be a production of viral derived oligonucleotides that will end up in the cytosol. These oligonucleotides are detected in DCs by intracellular sensors such as cGAS, RIG-1 and IFI16 leading to an activation of the STING signalling pathway and subsequent type I IFN production. Here, we investigated the underlying mechanisms creating HIV-1-mediated immune dysfunction scrutinizing the role of type I IFNs using an allogenic DC and T cell co-culture model. This model induces a mixed lymphocyte reaction resulting in expansion of antigen specific T cells. This setup is used to mirror in vitro priming of naïve T cells occurring in lymphoid tissues. In this and in previous studies we show that HIV-1 exposure in the DC-T cell co-culture promotes the expansion of suppressive T cells and influences DCs to adopt a more suppressive phenotype resulting in diminished effector functions. The induction of a suppressive phenotype was also seen after treating DCs with ssDNA derived from the HIV-1 genome, an oligonucleotide known to activate STING signalling. These results suggest that the impairment was due to production of type I IFNs and subsequent IFN-α/β receptor signaling which was initiated by the HIV-derived ssDNA activation of IFI16/cGAS followed by STING signaling in the DCs. Targeting IFNAR1 with the specific antibody Anifrolumab restored the immune functions of both DCs and T cells, as well as T cell proliferation and T cell effector function. This finding was also supported by a restoration of the IL-2, IFN-γ, and granzyme B secretion after treatment with Anifrolumab.

Our findings indicate that the immune impairments existing in untreated or antiretroviral therapy (ART) treated HIV-infected individuals are mediated, if not fully, at least in part by the type I IFN's negative effect on DC and T cells. Therapeutics targeting IFN-α/β receptors, such as Anifrolumab, hold potential as combination treatment alongside ART, to achieve a more complete immune restoration and contribute to improved quality of life among people living with HIV.

ISG15 in Viral Immunometabolism: Mechanisms, Models, and Therapeutic Opportunities

Soham Gupta, Team and Collaborators

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Interferon-stimulated gene 15 (ISG15) is a ubiquitin-like protein with emerging importance beyond classical antiviral roles. Originally characterized as an effector of type I interferon (IFN) responses through ISGylation, ISG15 is now recognized as a central immune-metabolic checkpoint, coordinating antiviral signaling with cellular metabolic adaptation. Our work defines ISG15 as a multifunctional regulator of host defense, energy homeostasis, and viral pathogenesis, with functions particularly prominent in human cells.

A key focus of our research is the ISG15-RSAD2 (viperin) axis, which we identify as a critical node in metabolic antiviral immunity. We demonstrate that ISG15 suppresses RSAD2 transcriptional induction during type I IFN stimulation and viral infection. In ISG15 knockout (KO) cells, viperin is markedly upregulated, resulting in increased production of the antiviral nucleotide analog ddhCTP and depletion of the cellular CTP pool. This shift strongly correlates with reduced intracellular SARS-CoV-2 and CCHFV viral gene expression, likely due to ddhCTP-mediated inhibition of viral RNA-dependent RNA polymerases. Importantly, RSAD2 knockdown reverses this phenotype, confirming a functional ISG15-RSAD2 axis that restricts viruses through metabolic reprogramming.

Beyond viperin regulation, ISG15 deficiency disrupts broader metabolic networks. KO cells display altered NAD+/NADH ratios, increased aerobic glycolysis, and elevated lactate levels. Proteomic analyses further identify ISG15 as a regulator of DERA, an enzyme linked to nucleotide salvage and glycolytic flux. Under immune stress, DERA is destabilized in ISG15 KO cells, suggesting that ISG15 safeguards redox and energy balance during infection.

Viruses have evolved mechanisms to counteract ISG15 activity. We are investigating two viral deISGylases: SARS-CoV-2 PLpro and CCHFV-L1 (OTU domain) both of which target ubiquitin- and ISG15-mediated antiviral pathways. Using affinity purification-mass spectrometry (AP-MS/MS), we mapped host interactors of PLpro and L1, identifying pathways linked to immune signaling and metabolism, including the RIG-I-MAVS-IRF3 axis targeted by SARS-CoV-2 PLpro. To further characterize these viral strategies, we are applying di-glycine remnant proteomics to define host ISGylation and ubiquitination substrates affected by PLpro and L1. In parallel, we employ a replicating CCHFV model with mutated ISG15- and ubiquitin-binding domains in L1 (in collaboration with Eric Bergeron, CDC, Atlanta), enabling precise dissection of viral immune evasion mechanisms.

Together, this work establishes ISG15 as a central regulator of viral immunometabolism, uncovering its role in shaping ISG expression, antiviral metabolism, and host–pathogen interactions. Our findings lay the foundation for future therapeutic strategies targeting ISG15-regulated pathways in infection, inflammation, and immune dysregulation.

Enhancement of Respiratory Syncytial Virus Infectivity by a G Protein Derived Peptide: Implications for G-F Interaction and F Activation

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Human Respiratory Syncytial Virus (RSV) is the leading cause of acute lower respiratory tract infections in children and the elderly worldwide. The RSV virion possesses two major surface glycoproteins: the attachment G protein and the fusion F protein. While the F protein is a prime target for both vaccine and therapeutic development. The F protein is synthesized as a precursor with a metastable pre-fusion conformation, requiring furin cleavage and subsequent structural changes to become fusogenic. A significant gap remain in the understanding of RSV entry mechanisms, including the precise molecular events that initiate F activation.

To address this gap, we utilized an RSV variant lacking the G protein, generated by 10 consecutive passages of RSV in the presence of *Erythrina abyssinica* extracts. Our preliminary data demonstrate that, the G-deficient RSV variant exhibited a 300 to 800% increase in infectivity in both HEp-2 and human bronchial airway epithelium (ALI) cells upon supplementation with a 15-mer synthetic peptide derived from the RSV G protein. Further assays showed that this peptide bound irreversibly to viral particles (both G-deficient and G-competent) and promoted both attachment and penetration. The binding of the peptide to the G-deficient variant suggests a G-F interaction, as F is the only major surface glycoprotein present on this variant. We are currently conducting further assays to demonstrate the interaction between G and F, and to identify the domains of F that interact with G. Understanding of this interaction is crucial for elucidating how the G protein triggers F for fusion.

Aedes albopictus produce virus-derived DNA upon Batai virus infection

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Background and objectives: Batai virus (BATV) is a tri-segmented negative-sense single-stranded vector-borne RNA virus belonging to the *Orthobunyavirus* genus. BATV is transmitted by a wide range of mosquito species and has been shown to infect humans and multiple animal species in Africa, Asia, Australia and Europe. In Europe, the livestock seroprevalence (cattle, sheep and goat) has in certain studies reached up to 46%. Novel strategies to control mosquito-borne viruses are needed and one way could be by breaking the immune balance between the mosquitoes and viruses. Viral-derived DNA (vDNA) has been shown to play an essential role in vector survival and virus tolerance and recent studies have shown that mosquitoes that are unable to produce vDNA have died after virus infection. However, there is still a gap in knowledge regarding vDNA molecules in control strategies. This study aims to improve the knowledge about mosquito immune response to orthobunyaviruses.

Material and Methods: For the *in vitro* experiment, mosquito cells (U4.4 *Aedes albopictus* cells) were infected with BATV (MOI 0.1). At 24h, 48h, 72h and 96h after infection, the vDNA production was assessed using PCR. Primers targeting regions across all three viral genome segments (S, M and L). The amplicons were sequenced and the correct amplification was confirmed using BLASTn. The supernatant was tested for virus by a real-time PCR SYBR Green based assay, targeting the S segment.
For the *in vivo* pilot experiment, adult *Aedes albopictus* mosquitoes (n=7) were microinjected with BATV (144.9 PFU/uL). vDNA production and viral replication/dissemination was assessed 7 days and 14 days post infection. The analyses were performed as described for the *in vitro* experiment.

Results: The BATV infected U4.4 cells produced, at all three time points, vDNA from the S, M and L segments, respectively. For each of the segments, vDNA from different regions of the respective segment were produced. The real-rime PCR confirmed viral replication. The mock-infected cells did not produce vDNA and no virus was detected. Our preliminary *in vivo* data show that mosquitoes also produced vDNA upon BATV infection. 7 days after infection, vDNA from 3 regions of the S segment and from 3 regions of the M segment were observed. Also, at 14 days after infection, vDNA from the S segment (1 region) and the M segment (2 regions) were detected. No vDNA produced from the L segment was observed at any of the time point.

Conclusion: Through this study we show that the mosquito *Aedes albopictus* produce vDNA as a response to viral infection, both *in vitro* and *in vivo*. Although preliminary, the *in vivo* results indicate that the vDNA produced is not equally distributed across each individual segment and possibly some of the segments are more targeted than others. The findings provide knowledge about vDNA dynamics and can improve our understanding of immune balance between viruses and mosquitoes.

Friday, August 22

14.50-15.20

Viral Immunology I

HSV-2 Glycoprotein G facilitates neuroinvasion after genital infection and yields protective immunity when administered as a glycosylated vaccine antigen

Ebba Könighofer [1,2], Carolina Gustafsson [2], Lindvi Gudmundsdotter [3], Ekaterina Mirgorodskaya [4], Jonas Nilsson [4], Maria Ekblad [2], Beata Adamiak [5], Eva Jennische [1,2], Stefan Lange[1,2], Per Elias [6], Edward Trybala[1,2], Staffan Görander [1], Tomas Bergström[1,2], Jan-Åke Liljeqvist [1,2], Rickard Nordén [1,2]

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Post translational modification of glycoprotein G of herpes simplex virus type 2 occurs in the secretory pathway of the infected cell. The protein is cleaved into a secreted protein (sgG-2) and a membrane associated protein (mgG-2) which is further modified by the addition of glycan structures. Here, we report a detailed characterization of the glycan profile of mgG-2 and show that the protein is strongly immunogenic, eliciting a glycan dependent humoral immune response in a mouse model, including Th1 phenotypic CD4+ T cell activation. Full protective effect appears to be dependent on intact glycosylation of mgG-2 which is reflected in a poor CD4+ T cell activation after immunization with the de-glycosylated mgG-2. Immunization with the glycosylated mgG-2 protect mice against lethal challenge with HSV-2 and prevents neuronal spread of the virus. Also, we show that a mgG-2 negative HSV-2 mutant virus fail to spread to dorsal root ganglia and the central nervous system of genitally infected mice, despite local viral replication at the primary site of infection. Altogether, our data show that mgG-2 is essential for HSV-2 spread in vivo and that the adaptive immune response directed to the glycosylated protein can inactivate the viral infection. This highlights mgG-2 as a promising vaccine candidate and offers a foundation for future development of glycan dependent vaccine candidates.

Defining B cell epitopes of Influenza A Hemagglutinin in DNA Vaccinated Pigs and Ferrets

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Hemagglutinin (HA) is the most abundant surface glycoprotein on the influenza A virion, and it enables virus entry by binding sialic acids on the host cell surface. HA remains the primary target for current licensed vaccines and next-generation influenza vaccines, as HA-directed antibodies may prevent viral infection. However, antibodies targeting the major antigenic sites on HA show species-specific differences, which is particularly relevant for the pre-clinical evaluation of vaccines and the comparability of data between species, including humans. To better understand the antibody recognition of B cell epitopes, including and beyond major antigenic sites, we defined antibody reactivity to epitopes spanning the entire HA protein in a challenge model of pigs and ferrets vaccinated with a DNA vaccine encoding the HA protein of A/California/04/2009, among other antigens. Epitope recognition was assessed post-vaccination and after challenge with influenza A A/California/7/09, and compared to antibody functionality, including virus neutralization and hemagglutination inhibition. Despite receiving the same vaccine, pigs and ferrets displayed distinct B cell epitope pattern recognition, targeting different HA domains and antigenic sites. The pigs exhibited a broader antibody recognition with a preference for the stalk domain, and showed a strong recognition of conserved regions within the receptor-binding site, which may contribute to the observed cross-neutralization of heterologous swine influenza strains in some pigs. In contrast, ferret serum antibodies mainly targeted the globular head domain with fewer epitopes recognized overall, and limited overlap with conserved functional sites. The limited recognition of conserved epitopes did not consistently align with the observed neutralizing activity in ferrets. These results highlight the complexity of vaccine-induced antibody reactivity and associated functionality in various model animals, cautioning against the extrapolation of findings across different host species.

Ancestral HA/NA-Based DNA Vaccine Suppresses Airway Inflammation and Viral Shedding in Pigs

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Influenza A virus (IAV) remains a persistent global health threat due to its ability to rapidly mutate and reassort. Pigs, the key intermediaries in IAV evolution, can host and mix human, avian, and swine strains, as seen in the 2009 H1N1 pandemic. Denmark, producing over 28 million pigs annually, reports more than half its herds positive for swine IAV (swIAV). Novel reassortant strains in Danish swine and recent human infections with swine-origin IAV in Denmark raise concerns about potential zoonotic spillover and highlight the urgent need for effective vaccine development targeting swIAV.

To address this, we developed a novel DNA vaccine (HA/NAanc) based on inferred ancestral hemagglutinin (HA) and neuraminidase (NA) sequences of IAV. Piglets were vaccinated using needle-free delivery with either HA/NAanc or a construct encoding HA and NA of H1N1pdm09 (HA/NApdm09). Control groups received DNA encoding internal IAV proteins, a non-IAV glycoprotein, or PBS. Upon challenge with a zoonotic Danish swine-origin H1N1 strain, both vaccines induced strong, cross-reactive humoral and T-cell responses. HA/NAanc matched HA/NApdm09 in recall and neutralizing antibody responses and showed improved viral clearance from lower lungs and shortened duration of nasal viral shedding compared to the PBS group, indicating better mucosal immunity. Lung gene expression in control animals revealed elevated cytokine signaling, suggesting increased airway inflammation in the absence of effective mucosal protection. These results support the potential of ancestral-sequence DNA vaccine to enhance immune responses and reduce transmission of heterologous IAV in pigs.

Friday, August 22

15.20

Poster Session

New insights into the function of the proline-rich region in the hypervariable domain of CHIKV nsP3

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The chikungunya virus (CHIKV) positive-sense RNA genome encodes the nonstructural proteins nsP1–nsP4 that form the viral replicase complex. Amongst the nsPs, nsP3 has been studied intensely over the last few years and has revealed various functions. In particular, the C-terminal hypervariable domain (HVD) of nsP3 was identified as the major hub for host cell interactions. Within this disordered region, a proline-rich sequence motif (P1-region) is well conserved amongst multiple alphaviruses and is the binding site for Src-homology 3 (SH3) domains of amphiphysin-1 and -2 (BIN1). To further understand the distinct functions and possible virus-host interplay of this highly conserved proline-rich region, we generated a viral ΔP mutant (CHIKV-ΔP) for infection studies. Viral growth assays showed that titers are decreased in infected human osteosarcoma cells (HOS) but are unaffected in BHK-21 cells compared to CHIKV-WT. Moreover, viral protein expression was altered in the CHIKV-ΔP mutant compared to CHIKV-WT. Hypothesizing that this phenotype is linked to the innate immune response, we studied the interferon (IFN) and cytokine response in CHIKV-ΔP and CHIKV-WT-infected HOS cells. We observed that the response is increased in CHIKV-ΔP-infected cells compared to CHIKV-WT. Our data expands the understanding of the CHIKV nsP3-HVD that is likely interfering with the human innate immune response.

NGS library-mining approach to discover nanobodies targeting the CHIKV spike protein.

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Chikungunya virus (CHIKV) is a re-emerged mosquito-borne alphavirus causing acute and chronic disease in humans. Its spread into new regions, driven by its mosquito vector and adaptation to different mosquito species, poses a substantial global public health threat. Nanobodies, single-domain antibody fragments, show promise as diagnostic and therapeutic agents against viral infections. We generated a nanobody library by immunizing a camelid using a DNA-launched replicon prime and recombinant CHIKV spike boost. A bacteriophage display library was screened using a bivariate enrichment approach to identify candidates targeting specific proteins of the CHIKV spike complex. After one round of bio-panning with E1 or CHIKV spike recombinant proteins, the basal and enriched nanobody libraries were analysed by next-generation sequencing, and the enrichment for each nanobody was calculated. We confirmed the binding of 12 nanobodies to the CHIKV spike by flow cytometry analysis of cells infected with the three CHIKV lineages (ECSA, WA, and Asian). The neutralization ability of the nanobodies against all three lineages was analysed by PRNT. Nanobodies showing the highest rate of neutralization were selected to generate Fc-constructs expressed in mammalian cells. Additionally, neutralizing nanobodies with distinct binding sites were functionalized and fused by SPAAC reaction to form nanobody dimers, both as homo- and heterodimers. The resulting functionalized nanobodies were analysed by PRNT. The increase in size and avidity conferred increased neutralization potency for all tested functionalized nanobodies. Dyl010-Fc, Dyl059-Fc, Dyl084-Fc, and Dy201-Fc exhibited the lowest $PRNT_{50}$ values against the WA lineage with values of 4.3 nM, 11.5 nM, 2.13 nM, and 14.3 nM, respectively. Functionalizing Dyl084 as homo- or heterodimers increased its neutralization potency, underscoring its potential as a therapeutic tool. These nanobodies enhance the research toolkit for CHIKV and could aid in developing therapeutic or diagnostic tools.

Dynamics of SARS-CoV-2 Variants and Mutations in Central Sweden Between 2023 and 2024 and their Potential Implications on Monoclonal Antibodies Pemivibart and Sipavibart as PrEP in the Region

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Background: Monoclonal antibodies (mAbs) are an important option against SARS-CoV-2, especially as pre-exposure prophylaxis (PrEP) for patients with immune system impairment. PrEP mAbs like sipavibart and pemivibart have been approved for limited use in several countries. Certain SARS-CoV-2 variants carry mutations in the spike (S) protein, conferring resistance to these mAbs.

Objectives: We aimed to examine the relative abundance of different circulating SARS-CoV-2 variants/mutations in central Sweden between 2023 and 2024, and to predict the effectiveness of sipavibart and pemivibart in the region as a PrEP for COVID-19.

Methods: An amplicon-based Nanopore sequencing method was used for sequencing SARS-CoV-2 samples. Coronapp was used to identify mutations in these sequences. Using the published in vitro resistance data for sipavibart and pemivibart, the effectiveness of these mAbs in the region was inferred.

Results: We have observed that the relative abundance of the KP.3.1.1 variant and the Q493E mutation started to increase in the later part of 2024 in the region. Also, since April 2024, the relative abundance of the F456L mutation reached 100% during many weeks until the end of the study period. The KP.3.1.1 variant is significantly resistant to pemivibart. Further, the presence of the F456L mutation in the Omicron subvariants confers high fold resistance towards sipavibart.

Conclusion: The use of sipavibart or pemivibart as PrEP for COVID-19 in the region may currently not be effective. However, new mAbs under development as PrEP for COVID-19 can be effectively used by routinely sequencing SARS-CoV-2 in patients to identify variants and resistance mutations.

Viral Vector Delivery of a Therapeutic Protein for Tendon Healing following Injury: Immunologic and Therapeutic Outcomes

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Tendon and ligament injuries can cause both temporary and permanent disabilities. These injuries occur during daily activities ranging from walking to cooking, to weekend athletes, all the way to professional athletes, whether human or equine. Flexor tendon lacerations (FTLs) significantly disable >100,000 people each year in the US including for example, "guacamole hand" with ruptured hand tendons when the knife slips while cutting avocados. Over 33 million tendon injuries occur in the US each year, with >1 million tendon tears; 400,000 Anterior Cruciate Ligament (ACL) tears alone occur, not including other ligaments.

Surgical repair, needed for complete transections of tendons can result in poor healing due to the poor blood supply, low growth factor activity, and sparse cellularity of tendons. Rupture of the repair, i.e., *re-rupture* occurs in 20-30% for tendon repairs, requiring repeat surgery or with potential complete loss of function. Repair of the ACL, as an example of ligament repair, is also suboptimal as it, uses cadaver ligaments or a ligament taken from another location.

We have developed a unique treatment for ruptured/torn/cut tendons and ligaments using a safe adeno-associated virus type 2 (AAV2) vector that delivers the gene for a Growth Factor, Vascular Endothelial Growth Factor (VEGF) directly into the tendon (or ligament) during surgical repair allowing it to produce the VEGF hyper-locally where it is needed. The VEGF is produced by tendon cells for a few weeks optimizing the healing, specifically by causing the production of Type 1 collagen rather than the Type 3 (scar) collagen that would otherwise be made. In the best animal model of flexor tendon healing, administration of the AAV2-VEGF improves the healing of flexor tendon repairs by 210% at 4 weeks and with less scar (which should result in greater flexibility and less pain).

We recently completed a double-blinded GLP (Good Laboraboty Practices) IND-enabling study demonstrating the lack of toxicity of the vector, including evaluating immune responses directed against the vector and the encoded protein. We additionally performed a dose-range finding study to determine the efficacy of a range of viral vector doses. Data to be presented demonstrating proof-of-concept include the strength of the tendon post-op, with and without the AAV2-VEGF injections, frequency of tendon rupture post-treatment, duration of transgene production, and relevant safety data and dose-range finding data to determine the optimal dose for treatment. The immunological and transgene aspects of using a modified virus to deliver a therapeutic gene to a discrete location provide a novel perspective for virologists who otherwise focus on the disease-related aspects of the virus itself.

Structural Investigation of CVB3 Non-structural Protein 2C

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Coxsackievirus B3 (CVB3) is a positive-sense RNA virus of the enterovirus genus, family *Picornaviridae*. Positive-strand RNA virus replication organelles (ROs) act as platforms for viral RNA replication. These organelles are derived from host cytoplasmic membranes, with their structure and origin varying between virus types. For enteroviruses, ROs are formed from the endoplasmic reticulum (ER), Golgi, or autophagosomes and evolve throughout infection. RNA replication occurs on the outer surface of ROs, The process begins with the synthesis of a negative-sense RNA template, which is then used to generate more positive-sense RNA. Capsid assembly and RNA loading also occur on ROs. In previous research by our group [1], a protein complex called “tether” is observed to localize the capsid on the ROs’ surface by tethering capsid assembly intermediates to the RO membrane. Considering the membrane binding activity of 2C and its interaction with capsid protein VP3, this tether is suggested to be a 2C hexamer[1-3]. This project aims to characterize the structure of the tethered complex on membranes through in vitro reconstruction of purified protein on liposomes and cryo-electron tomography (cryo-ET). We have successfully purified WT 2C and a mutant containing charged residues at the N-terminus to reduce protein aggregation. Negative-stain EM and mass photometry confirm reduced aggregation in the mutant. Flotation assays suggest minimal changes in membrane binding, consistent with previous observations from N-terminal truncation of poliovirus 2C[3]. 2C-decorated liposomes have been reconstructed, and the sample is currently being optimized for cryo-ET.

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Non-canonical roles of autophagy in enterovirus infection

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Enteroviruses are a diverse group of RNA viruses with over 100 human-infecting members, causing diseases ranging from myocarditis and pancreatitis (e.g., Coxsackievirus B3, CVB3) to poliomyelitis (poliovirus) and the common cold (rhinoviruses). These viruses extensively remodel intracellular membranes to form replication organelles that support viral genome replication and assembly. Intriguingly, these membranes are decorated with the autophagy protein LC3.

Autophagy, or "self-eating," is a degradative cellular process that maintains homeostasis by recycling cellular components. A key step in autophagy is LC3 lipidation—the covalent attachment of LC3 to cellular membranes. While LC3 lipidation is critical for canonical autophagy, it also functions in damage-responsive membrane remodeling, termed as non-canonical autophagy (NCA). This damage responsive NCA is mediated by two distinct LC3 lipidation complexes. However, it remains unknown whether and how enteroviruses exploit NCA to support their replication.

In this study, we employed a combination of advanced cell biology and molecular virology techniques to elucidate the role of NCA in enterovirus replication. We first assessed viral infection across a panel of NCA-related knockout cell lines to determine the contribution of specific NCA factors. To dissect the specific role of these proteins in replication independent of viral entry, we employed a CVB3-GFP replicon system. Furthermore, cryo-electron tomography will be used to visualize how NCA factors contribute to cytoplasmic remodeling during infection. This work aims to uncover detailed mechanistic insights into how enteroviruses hijack specific NCA functions, possibly unveiling new targets for antiviral treatments.

Role of NS protein in the formation of the Replication Complex in Langat Virus

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Flaviviruses are positive-sense RNA viruses whose intracellular replication is marked by endoplasmic reticulum (ER) membrane remodelling. The genome replication step is one of the least understood processes in the viral life cycle. Upon flavivirus infection, the viral genome is translated as a single polyprotein comprising both capsid and non-capsid ("non-structural", NS) proteins. The polyprotein, upon protease cleavage, forms inverted buds on the ER, called replication organelles (ROs). ROs are the site of viral genome replication. Inside the membrane bud, the NS proteins co-localise with the viral genomic RNA and bring about its replication. But, how the non-enzymatic NS proteins (NS2A, NS2B, NS4A, NS4B) initiate the formation of ROs or partake in genome replication is yet to be deciphered. Using cryo-ET, we have recently studied the RCs *in situ* in Langat virus, a BSL2 model organism for TBEV. The tomograms highlighted the occurrence of macromolecular densities at the membrane neck of ROs, which on segmentation yielded a molecular mass of approximately 500 kDa. In this study, we investigate the probable role of NS4B in the formation of this 'neck'. NS4B, has been successfully expressed in mammalian and insect expression systems and affinity purified using Flag-tag in the presence of detergent. Also, we have used Fluorescence Size Exclusion Chromatography (FSEC) with a GFP tag to assess the protein's oligomeric state, which aligns well with our AlphaFold multimer model. In the future, we aim to investigate the molecular details of NS4B using cryo-EM and establish its role in the formation of ROs.

Optimizing SFV Replication Organelle Purification for Cryo-ET of the Neck Complex

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Alphaviruses are mosquito-borne, enveloped viruses that are small and spherical, with a positive-sense single-stranded RNA genome. *Semliki Forest Virus* (SFV), a close relative of the re-emerged *Chikungunya virus*, is one of the most extensively studied model alphaviruses. SFV can infect humans, causing fever, severe headaches, and muscle and joint pain. Following entry into host cells, alphaviruses dramatically remodel intracellular membranes to form specialized structures known as replication organelles (ROs), or "spherules", which provide a protected microenvironment for viral RNA replication. The megadalton-sized neck complex of these ROs is assembled from viral non-structural proteins (nsP1–4) and currently unidentified host factors. Our aim is to purify active SFV spherules from infected mammalian cells using native cellular fractionation methods and elucidate the architecture of their “neck complex” using cryo-electron tomography (cryo-ET). In parallel, we will perform proteomic profiling of membrane fractions to identify host proteins that interact with viral replication machinery, providing mechanistic insights into host–virus interplay. High resolution structural insight into the spherule neck complex will uncover molecular mechanisms of alphavirus genome replication and facilitate the development of targeted antiviral strategies against emerging alphaviruses.

Surveillance of enteroviruses and parechovirus in wastewater

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Wastewater surveillance has recently emerged as an effective tool for monitoring the circulation of enteric viruses at the population level, offering early warning signals for potential outbreaks. Enteroviruses (EVs) and parechoviruses (PeVs), both members of the *Picornaviridae* family, are of particular concern. EVs are excreted in feces and nasopharyngeal secretions, and in severe cases, such as meningitis, encephalitis, paralysis, and sepsis can also be detected in cerebrospinal fluid (CSF) and blood. Both EV and PeV infections can cause sepsis and meningoencephalitis in infants. While *PeV-1* and *PeV-6* infections are typically mild, *PeV-3* can lead to severe neurological illness in infants. In climates with distinct seasons, EV and PeV infections usually peak in late summer and early autumn, particularly among children and young adults. This study aimed to identify and compare circulating EV and PeV types in wastewater and clinical samples to assess their application on outbreak prediction. Clinical data were collected from feces, CSF, nasopharyngeal secretions, and blood samples that tested positive for EV or PeV between 2016 and 2017. Additionally, 39 biweekly wastewater samples from Gothenburg, collected between week 40 of 2016 and week 2 of 2018, were analyzed using both Sanger and next-generation sequencing (NGS). Most EV and PeV infections were reported during summer and fall, with *PeV-3* being the most frequently detected type. EVs patients were not yet typed. Preliminary wastewater surveillance revealed EV peaks during weeks 6 and 18–30, while PeVs showed relatively high levels during weeks 4, 26, 44, and 48. Further sequencing identified the predominant circulating EV types were *CV-B4, CV-B5, CV-A16, CV-A1,* and *EV-A76, which accounted* (n = 8, 20%). In addition, the analysis revealed a previously underestimated presence of PeVs (n = 30, 77%). *PeV-1* was present throughout the year, while *PeV-3* and *PeV-6* were more commonly observed in spring and winter. Overall, EV-B and EV-C types predominated in spring and summer, with EV-B types more frequent in the fall in wastewater. All detected types have the potential to cause widespread outbreaks. Monitoring EVs and PeV in wastewater is related to findings in patient samples and can help fill knowledge gaps regarding the dynamics and seasonality of these viruses supporting efforts to predict and prevent future outbreaks.

The Influence of Outer Membrane Vesicles of *Vibrio cholerae* and *Escherichia coli* on Human Enteric Adenovirus Infection

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Human adenovirus types 40 and 41 (HAdV-40/41) are leading causes of acute gastroenteritis in children, yet no targeted antivirals or vaccines are currently available. Recent research has highlighted bacterial membrane vesicles (BMVs) – nano-sized particles secreted by both commensal and pathogenic gut bacteria – as potential modulators of viral infections. Depending on their bacterial origin, these vesicles can alter host immune responses and suppress or promote viral infectivity. However, their specific influence on HAdV-40/41 remains poorly understood.

This study investigated how BMVs derived from commensal *Escherichia coli* and pathogenic *Vibrio cholerae* affect HAdV-40/41 infection in human intestinal epithelial cells. BMVs were isolated and characterised using biochemical and functional assays. Their impact on viral infectivity was assessed in the human large intestinal cell lines Caco-2 and HCT-8 across three experimental conditions: pre-incubation (to evaluate immune priming effects), co-incubation (to test direct interactions with the virus), and post-infection (to examine late-stage modulation). Infection levels were quantified using immunofluorescence.

The results suggest a modulatory role for BMVs in enteric viral infections. Notably, *V. cholerae* BMVs enhanced HAdV-40 infectivity when pre-incubated with the virus, indicating a potential priming effect that may facilitate viral entry or replication. These findings open new avenues for exploring how bacterial components influence viral pathogenesis in the gut and highlight the potential of targeting BMVs in developing novel strategies against adenoviral gastroenteritis.

Kan screeningen för livmoderhalscancer förbättras genom testning för metylering av *FAM19A4/miR124-2?*

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HPV-test har högre känslighet än cytologi för att upptäcka livmoderhalscancer och dess förstadier (1). Men specificiteten är låg, då de flesta HPV-positiva kvinnor inte kommer att utveckla cancer. Primär HPV-testning har ökat andelen som behöver följas upp med kolposkopi, med risk för ökade väntetider och försenade diagnoser (2,3). Cytologi, som hittills har använts som ett tilläggstest för att avgöra vem som behöver uppföljning, har begränsad känslighet och låg specificitet (4). Testning för DNA-metylering kan vara en metod att öka specificiteten i screeningen (5-7). Vi har utvärderat metylering av de mänskliga generna *FAM19A4/miR124* (QIAsure™ Methylation Test) för triagering av HPV mRNA-positiva kvinnor 30 år och äldre, som deltog i screeningen i Skåne under 2017. Totalt 4 227 kvinnor (6,8%) var positiva för högrisk HPV mRNA med metoden Aptima™ (Hologic). Av dessa fick 680 (16%) diagnosen höggradig intraepitelial skivepitellesion (HSIL), adenocarcinoma in situ (AIS), adenocarcinom (ADC) eller skivepitelcancer (SCC) inom tre år (histologisk diagnos), varav 88% (601) hade avvikande cytologi (ASCUS+, atypical squamous cells of undetermined significance or worse) i screeningprovet. Avvikande cytologi sågs hos totalt 46% av alla HPV-positiva i screeningen, vilket innebar uppföljning av nästan 2000 kvinnor. Ca 38% av kvinnor utan histologiskt bekräftad höggradig dysplasi eller cancer hade avvikande cytologi. Metyleringstestet var positivt hos ca 12% (6/50, 95% KI: 4,5-24%) med normal cytologi och ca 27% (15/56, 95% KI: 16-40%) med lätt skivepitelatypi (ASCUS) som läkt ut HPV-infektionen senast 2020, vilket talar för att färre kvinnor skulle behöva uppföljning om metylering skulle ersätta cytologi som triagetest. Cellprov från kvinnor som inom tre år utvecklade SCC, ADC, AIS och HSIL var positiva i metyleringstestet i 88% (7/8), 83% (5/6), 68% (19/28) respektive 55% (44/80) av fallen och visade avvikande cytologi i 89% (8/9), 63% (5/8), 83% (24/29) respektive 74% (59/80) av fallen. Metyleringstestet förefaller vara lika känsligt som cytologi för att förutsäga cancer, men mindre känsligt än cytologi för detektion av HSIL (p=0,014 Fischers exakta test). Eftersom rekommendationen är att HPV-positiva kvinnor med HSIL ska behandlas, är det i nuläget inte rimligt att byta triagemetod från cytologi till detta metyleringstest.

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Lassa virus glycoprotein GP-based virus-like particles as a potential vaccine candidate.

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Lassa mammarenavirus (LASV) is a highly pathogenic virus, which is endemic to West African countries and the causative agent of Lassa fever (LF), a severe hemorrhagic fever in humans. LF disease is associated with high morbidity and mortality rates, and until now no approved vaccines or specific antiviral therapies are available, highlighting the urgent need for the development of effective therapeutic and preventive strategies. This project aims to evaluate a novel LASV vaccine candidate based on virus-like particles (VLPs) that display structurally authentic trimeric glycoprotein spikes (GP) of the prototypic LASV strain Josiah. These LASV GP VLPs, produced in stably transfected mammalian cells, are non-infectious and highly immunogenic. Previous studies have shown that polyclonal antibodies induced by LASV GP VLPs possess broadly cross-reactive neutralizing activity and confer protective efficacy in a lethal LF mouse model, laying the foundation for evaluating GP VLPs as a potential LASV vaccine candidate.

Preliminary data suggest that LASV GP VLPs can activate various T cell subsets, particularly those exhibiting an effector phenotype. Moreover, immunization with GP VLPs in mice elicited GP-specific IgG antibody responses. Challenge studies in a lethal LASV mouse model represent a critical next step in evaluating the protective capacity of our VLP-based immunization strategy and will significantly contribute to advancing this vaccine candidate toward future clinical application.

ISG15 modulates viperin expression and metabolic adaptation during immune responses

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Interferon-stimulated gene 15 encodes a ubiquitin-like protein (ISG15) that plays diverse roles in antiviral immunity, functioning both as a covalent modifier (ISGylation) and in its free intracellular or secreted form. Although ISG15 is typically associated with antiviral defense, paradoxically, ISG15-deficient human cells display enhanced resistance to several viruses, suggesting a more complex and potentially pro-viral function.

To gain an understanding of ISG15's regulatory effects, we performed TMT-labeled quantitative proteomics from Huh7 wild-type and ISG15 knockout cells. This analysis revealed a broad upregulation of interferon-stimulated genes (ISGs) upon interferon beta treatment in the absence of ISG15. These findings led us to hypothesize that ISG15 modulates antiviral responses by actively restraining ISG induction.

We focused on the ISG viperin (RSAD2), an enzyme that converts CTP into the chain-terminating antiviral nucleotide analog ddhCTP. We show that ISG15 suppresses the transcriptional induction of viperin in an ISGylation-dependent manner. Loss of ISG15 led to an enhanced viperin induction following exogenous interferon beta treatment or SARS-CoV-2 infection, correlating with reduced viral gene expression. This phenotype was reversed by viperin knockdown. Mechanistically, elevated viperin activity resulted in depletion of the cellular CTP pool and accumulation of ddhCTP, an antiviral metabolite known to inhibit several viral RNA polymerases. Consistent with this, metabolite extracts from interferon-treated ISG15 knockout cells inhibited T7 RNA polymerase activity in vitro.

Our results position ISG15 as a critical regulator of nucleotide metabolism during infection, acting not only through direct ISGylation but also by tuning the expression of antiviral ISGs. Altogether, we uncovered a previously underappreciated immunomodulatory role for ISG15, whereby it fine-tunes immune activation to preserve metabolic homeostasis. This regulatory mechanism may represent a broader principle by which ISG15 shapes host-virus interactions, underscoring a potential therapeutic target.

Viruses and the Ubiquitin System: Mapping Host-Virus Interactions via High-Throughput Proteomics and Molecular Approaches

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The ubiquitin system is a widely implemented post-translational modification (PTM) that remains crucial in the innate immune defenses against pathogens. Ubiquitination and ISGylation, the covalent conjugation of ubiquitin (Ub) and interferon-stimulated gene 15 (ISG15), play key roles in recognizing viral products and regulating critical antiviral signaling pathways. Viruses have evolved sophisticated mechanisms to disrupt host antiviral defenses to facilitate infection and persistence. Virus-encoded deubiquitinases (DUBs) and deISGylases (DISGs) reverse Ub and ISG15 modifications, dysregulating cellular antiviral responses and altering cellular metabolism.

This study focuses on viral DUBs/DISGs, PLpro and L1, encoded by RNA viruses SARS-CoV-2 and CCHFV, and DUBs encoded by DNA viruses EBV, HCMV, KSH, and HSV1. We have implemented high-throughput analysis such as affinity purification-mass spectrometry (AP-MS/MS) to identify key cellular targets of these viral proteases at steady-state and during active infection for select viruses, which has providing insight into conserved and virus-specific strategies of immune evasion. In this data, we identified substrates of PLpro such as MAVS in the RIG-I-like receptor signaling pathway. We validated some of the data using overexpression of tagged-PLpro catalytic domain. Here, we show that PLpro targets the activation of RIG-I and disrupts the integrity of the RIG-I signalosome. PLpro readily interfered with the ubiquitination of the RIG-I 2CARDs, which was not observed by the PLpro catalytic mutant. Immunoprecipitation of PLpro showed interactions with the E3-ligase TRIM25 and adaptor molecule 14-3-3, and using NEM/Iodoacetamide we concluded that these interactions were Ub-mediated. PLpro also reduced the ubiquitination state of MAVS, which may inhibit aggregate formation thus disrupting MAVS-dependent signal transduction. Downstream, we observed that PLpro dampened IFN response using a luciferase-based reporter assay.

Using diglycine-based AP-MS/MS, we further investigate the distinct role of the DUB vs DISG activities of PLpro and L1. We have established ISG15-knockout cell lines that we will use to distinguish between ubiquitinated and ISGylated substrates targeted by PLpro and L1. Additionally, we have established plasmids encoding for catalytic mutants and Ub/ISG15-binding mutants of PLpro and L1 which we overexpress in different cell lines to further investigate functional capacities of the viral proteases. Our goal with this research is to gain a deeper mechanistic insight into these viral deubiquitinases to lay a better foundation in designing broad-spectrum antivirals against both emerging and re-emerging viral pathogens.

Development of Langat virus infectious clones as a platform for live-attenuated tickborne encephalitis vaccine

Naveed Asghar[1], Rita Jaafar[1], Anna Valko[1], Olivia Merinder[2], Karl Ljungberg[3], Mårten Lindqvist[4] and Magnus Johansson[1]

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Tick-borne encephalitis (TBE) is one of the most important tick-transmitted diseases in Europe and Asia. TBE virus (TBEV) infections lead to a diversity of disease outcomes ranging from mild flu-like symptoms to severe neurological disorders. With no specific antiviral treatment available, vaccination remains the most effective protective strategy for TBE. Currently, only inactivated TBE vaccines are available on the market, which require repeated booster doses to sustain immunity. In addition, vaccine breakthroughs are reported in some patients, especially in the elderly. In contrary to inactivated vaccines, live-attenuated viral vaccines could provide long-term, or even lifelong immunity after a single dose. Langat virus (LGTV) is a naturally attenuated strain of TBEV, which makes it a potential candidate for a live-attenuated TBE vaccine. In this study, we engineered and rescued four infectious clones (ICs) of LGTV using RNA- and DNA-based reverse genetics methods. Next generation sequencing of the rescued ICs showed that the viruses rescued by DNA-based methods were more similar to the parental LGTV sequence and showed higher genetic stability after passaging in cell culture. One of the DNA-launched LGTV IC was further evaluated *in vitro* and *in vivo* which exhibited growth kinetics and immune profile comparable to the LGTV strain in our laboratory. This reverse genetics platform will be utilized to introduce targeted mutations within the LGTV genome to develop a live-attenuated TBE vaccine.

Distinct antiviral T cell immunity patterns against common viruses and its correlation with SARS-CoV-2 seroconversion

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The importance of the cell-mediated immunity for protection against severe disease was emphasised during the COVID-19 pandemic caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). In addition to their longevity and antiviral functions, T cells promote immunoglobulin (Ig) G seroconversion, whose link to infection clearance is well known. Yet, the interplay between SARS-CoV-2-specific T cells and seroconversion is not well established. Thus, the aim of this study is to characterise antigen-specific memory (m) $CD4^+$ T cells against SARS-CoV-2 and other common viruses in healthy young adults (n=80; 24-27 years old) in relation to SARS-CoV-2 seroconversion status.

Using a high-parameter spectral flow cytometry panel, we characterised antigen-specific $mCD4^+$ T cells against five recirculating respiratory viruses, including human coronavirus (HCoV)-OC43 and SARS-CoV-2, and two chronic viruses. Our results show that robust antigen-specific T cells against common respiratory viruses and herpesviruses are present in over 90% and 75%, respectively, of healthy young adults. Distinct phenotypical and functional qualities were associated with each of the antigen-specific T cell populations. When investigating the role of SARS-CoV-2 seroconversion, the SARS-CoV-2-specific $mCD4^+$ T cells expanded and matured alongside IgG seroconversion by demonstrating greater effector functions. Notably, the frequency of $mCD4^+$ T cells against the closely related HCoV-OC43 was unaffected by serostatus. However, the frequency of IFN-γ^+IL-$2^+$$TNF^+$ HCoV-OC43-specific $mCD4^+$ is significantly lower, and conversely IFN-$\gamma^+$$TNF^+$ HCoV-OC43-specific $mCD4^+$ T cells trends to be higher, in SARS-CoV-2-seropositive donors compared to SARS-CoV-2-seronegative donors.

These findings highlight the heterogeneity within the $mCD4^+$ T cell compartment, while simultaneously showing the interaction between SARS-CoV-2 immune responses and HCoV-OC43-specific $mCD4^+$ T cell immunity. Despite their prevalence and socioeconomic burden, T cell immunity against common respiratory viruses is rarely studied, making our study one of the few to describe these immune responses in detail.

Deciphering the TRIM5α-induced innate immune response blocking LINE-1 retrotransposons

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Retrotransposition events of endogenous LINE-1 elements are associated with impaired genome stability and genetic disorders. To keep retrotransposition at bay, numerous countermeasures have emerged within hosts. As such, the human restriction factor TRIM5α has been identified as cytosolic sensor and potent inhibitor of LINE-1 elements, thereby protecting the human genome from harmful retrotransposition events. We previously demonstrated that the restriction of LINE-1 elements through TRIM5α occurs at the promotor level through initial sensing of cytosolic LINE-1 complexes and subsequent induction of NF-κB and AP-1 innate immune signaling. To identify and characterize novel factors that contribute to the TRIM5α-mediated activation of AP-1 and NF-κB we utilized RNA sequencing. 293T shTRIM5α cells were transfected with expression plasmids encoding TRIM5α and LINE-1 or an empty vector control followed by RNA extraction, library preparation, and NGS sequencing. Of multiple up-regulated genes, we chose four candidates for follow-up investigations. We show that the expression of these four hits is indeed elevated upon TRIM5α and LINE-1 expression as assessed by Western blotting and quantitative PCR, thus, validating our RNA-seq data. Using our established systems for analyzing LINE-1 gene expression and the frequency of retrotransposition *in vitro* we demonstrate that two of the candidates potently impair LINE-1 promotor activity and diminish the retrotransposition rate from moderate to strong extent upon exogenous overexpression. Performing such assays in cells lacking one or multiple of the candidate genes in combination with mutagenesis and interactome techniques will help to pinpoint key regulators that govern the TRIM5α-induced AP-1 and NF-κB innate immune signaling. In the future, it will also be intriguing to investigate whether these factors might be dysregulated in disease settings such as auto-inflammation and cancer, which are disorders that frequently correlate with elevated LINE-1 transcript or protein abundance.

Immune Profiling of Mammarenavirus Infection in Human and Reservoir-Derived Dendritic Cells and Macrophages

Nicolas Corrales, Angelika Lander, Katharina Hansen-Kant, Joseph Prescott

Introduction: *Mammarenaviridae* is a genus of ambisense RNA-viruses, with members that cause viral hemorrhagic fever, namely Lassa virus (LASV) and Lujo virus (LUJV) and members that have not been associated with disease, such as Mopeia virus (MOPV). LASV infection results in 5000 to 10,000 deaths annually in sub-Saharan Africa, and LUJV has a case-fatality rate of up to 80%. The Natal multimammate mouse (*Mastomys natalensis*, NMM) has been identified as a natural reservoir of LASV and MOPV. However, these animals do not show clinical signs upon infection, suggesting coadaptation between the viruses and the natural reservoir´s immune system. In humans, LASV targets dendritic cells (DCs) and macrophages (MΦs), among other cells, and induces activation and maturation of DCs, but downregulates interferon (IFN) response and thus fails to induce robust T-cell responses in co-culture experiments.

Objectives: Given the lack of pathology of LASV infection in MM, and the pivotal role of DCs and MΦs in the disease development in humans and non-human primates, the purpose of our study is to compare the immune profile of human and NMM-derived DCs and MΦs responses to pathogenic (LASV & LUJV) and non-pathogenic (MOPV) *Mammarenavirus* infection.

Materials & Methods: We differentiated DCs and MΦs from bone-marrow cells isolated from adult NMM or human monocytes and infected them with LASV, LUJV or MOPV at a MOI of 2. We sampled cells and supernatants at 2 days post-infection. As controls for the activation of the innate immune response in these cells, we stimulated the cells Sendai virus (SeV). We performed a characterization of immune profile of the cells the cells, measured the transcript levels of immune-related genes and quantified viral loads.

Results: Here we show that the cells are permissive to the *Mammarenavirus* infection, and we describe the comparative immune-profile resulting of the infection with pathogenic vs. non-pathogenic *Mammarenaviruses*, in human and in a natural reservoir species. This gives us insights on the mechanisms of pathogenesis in humans and protection for the natural reservoir species.

Conclusion: The co-evolution of virus-host pairs plays a key role in defining the pathogenicity of a viral infection, and by further characterization of natural reservoir's responses to highly pathogenic viruses, we can gain insights on the immune mechanism that lead to a non-pathogenic infectious phenotype.

HCV evades innate immune-mediated translational shutdown through RNA non-canonical capping

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Objective: We previously identified that HCV 5' caps its RNA with the cellular metabolite flavin adenine dinucleotide (FAD), protecting it from RIG-I-mediated innate immune sensing. Here, we investigate the role of 5'FAD and innate immune factors in regulating viral translation.

Methods: Translation efficiency was measured using HCV reporters and replicons in cells and cell-free systems. The ratios of actively translated vs. total HCV RNA was determined using polysome fractionation. RIG-I, IFIT1/2/3/5, and PKR knock-out (KO) cell lines were used to determine the role of these factors in translational repression. Proteome-wide affinity purification and mass spectrometry (MS) identified proteins with 5'-specific RNA-binding.

Results: HCV IRES driven translation was significantly higher for 5'FAD-capped compared to 5' triphosphorylated (5'ppp) RNA. This was not due to enrichment of 5'FAD compared to 5'ppp HCV RNA among actively translated RNA present in polysome fractions, and both exhibited similar translation efficiency in a cell-free system. Co-transfection of 5'FAD or canonical m7G-capped HCV RNA with 5'ppp non-translating IRES mutant RNA resulted in a trans-acting suppression of translation. A number of proteins specifically binding 5'ppp RNA potentially mediating this effect, including IFITs and RIG-I, were identified by RNA 5'end specific affinity purification. Translational repression was independent of the 5'ppp RNA sensor RIG-I, and the KO of PKR and IFIT1/2/3/5 had only minor impacts on HCV RNA translation.

Conclusions: Our results suggest that the 5'FAD cap, in addition to evasion of RIG-I sensing, enhances viral RNA translation by shielding the HCV RNA from yet unidentified sensors, thereby preventing global cellular translational repression. The identification of the responsible host factor(s) using proteome-wide affinity purification and MS will be critical to elucidate this mechanism. These findings extend the role of 5'FAD capping in innate immune evasion, highlighting its potential as a tool for RNA-based therapeutics and viral immune evasion studies.

Saturday, August 23

09.45-11.00

Viral Immunology II and III

Evaluating the long-term efficacy and immunogenicity of COVID-19 vaccines

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The CoVacc clinical trial was established to investigate how different COVID-19 vaccination regimens shape long-term immune responses. In Sweden, the initial two-dose program included the adenoviral vector-based Vaxzevria (AstraZeneca) and the mRNA-based Comirnaty (Pfizer–BioNTech) or Spikevax (Moderna), followed by mRNA-only boosters.

We conducted a longitudinal cohort study within CoVacc, including 578 individuals who received varying vaccine regimens. SARS-CoV-2-specific IgG responses against spike (S), nucleocapsid (N), and receptor-binding domain (RBD) were measured after the first, second and third vaccine doses.

S-specific IgG levels were significantly higher in individuals primed with mRNA vaccines compared to those primed with Vaxzevria, both after the second and third doses. Despite these initial differences, all groups responded robustly to the third mRNA dose, and by day 30 post-dose 3, antibody levels were comparable between homologous mRNA and Vaxzevria-primed groups.

Serological analysis revealed that around half of all samples were positive for N-specific IgG, indicating widespread exposure of SARS-CoV-2. Individuals with evidence of prior infection exhibited markedly higher S-specific IgG responses following the first vaccine dose compared to naïve individuals.

Detailed analyses in a subset of 39 participants showed that mRNA-primed individuals developed higher frequencies of spike-specific memory B cells and greater IgG avidity after two doses, with differences diminishing after the third dose.
Multivariable analysis confirmed that antibody levels increased significantly with each vaccine dose, and prior infection was associated with stronger responses. Age showed a weak negative association with antibody levels, while sex, BMI, and comorbidity burden had no significant impact.

The CoVacc trial confirms the superior immunogenicity of mRNA vaccines and their strong boosting capacity, even after heterologous priming. Age may modestly influence antibody responses, whereas other demographic factors appear to play a limited role in shaping vaccine-induced immunity in a generally healthy population.

Innate and adaptive anti-HIV-1 immune responses during therapeutic immunization

Britta Wahren[1], Ljungberg K[2], Gudmundsdotter L[3], Hinkula J[4], Barnabas S[5], Robb M[6], Rossi P[7], Palma P[7].

Karolinska Institutet, Stockholm with the HIVIS-DNA study group[1]; International Vaccine Institute, Stockholm County[2]; Simplexia AB, Gothenburg[3]; University of Linköping[4], SE; Stellenbosch University, Cape Town[5], SA; Walter Reed Army Institute of Research and Henry M. Jackson Foundation for the Advancement of Military Medicine, MD[6], USA; and H. Bambino Gesù and University of Rome Tor Vergata, Rome, Italy with the HVRRICANE study group[7].

Despite world-wide use of excellent antiviral drugs, over 100.000 prenatally HIV-infected children are born each year, and 1.3 million children are living with HIV. Without continuous antiretroviral treatment the mortality is 100% and eradication is not possible. Cure attempts with various means have thus far failed. Children born by HIV-infected mothers mount weak, if any, immune responses to their virus, generating a state similar to immunological tolerance. Adults with HIV-infection usually have both antibody and cellular immune responses to HIV, although these responses are weak and have developed late in response to the infection. In addition, the genetic variability of HIV-1 is extreme, and the immune system therefore cannot cope with the constant stream of new and variable viruses. To address the high genetic heterogeneity of HIV-1 strains, we developed a multigene, multi-subtype genetic vaccine HIVIS containing gene sequences from subtypes A, B and C. Priming with the HIVIS multigene, multi-subtype DNA vaccine induced broad natural killer and cellular and humoral adaptive immune responses in experimental and prophylactic clinical studies. The pox-virus-based HIV-MVA included sequences of HIV-1 strains A and E and has had remarkable priming and boosting effects.

Therapeutic immunization. A series of clinical studies were performed to provide better immune responses in HIV-infected adults and recently in children. In a Swedish cohort of 12 HIV-infected adults it was found that new HIV-specific immune responses were induced. During therapy interruption (STI), the virus relapsed, but the strains were still susceptible to ART. The first childhood study of 12 children in Italy was safe and elicited augmented NK-and HIV-specific T- and B-cells. However, the therapy did not completely suppress viral replication as a few short-lived viral blips were observed although no STIs were performed in this group. In a second childhood study of 25 adolescents in South Africa, everybody responded with new HIV-specific cellular immunity and monocytes of long-term classical type. There were no viral blips and no safety concern.

Conclusions. We succeeded in giving rise to new multifaceted HIV-specific immune responses in children born with HIV infection who do not generally develop such responses while on antiretroviral treatment. In addition, cells of the innate immune system demonstrated added reactivities during a prolonged time such as NK reactivities and novel non-anticipated monocyte reactivities. We noted no signs of heightened viral load levels and no viral blips of concern, but also no sign of lowering of viral set points. This lends to hope, that a sequential immunization strategy with varied genetic vaccine compositions will sustain and improve innate and adaptive responses to complement antiretroviral therapy and perhaps permit antiviral drug holidays.

Neri A, Olivieri G, et al. Therapeutic HIV vaccine TLR4 adjuvant impact on monocyte subsets in early treated youths with HIV. Abstract 0430, CROI San Fransisco, March 9-12, 2025

Viral Trojan Horses: non-human papillomavirus capsids as mucosal vaccine vector against RSV

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The respiratory syncytial virus (RSV) is a leading cause of severe lower respiratory tract infections such as bronchiolitis, bronchitis, and pneumonia, particularly in infants, the elderly and immunocompromised individuals. Since 2023, the first intramuscularly administered protein-based vaccines against RSV targeting the major immunogenic RSV-F protein have been approved. Nevertheless, mucosal vaccines offer distinct advantages by inducing local immune responses at the primary site of viral entry, which is particularly relevant for respiratory pathogens such as RSV, Influenza, and others.

Previously studies have demonstrated that mucosal vaccination against RSV results in improved protection against RSV compared to conventional intramuscular immunization. Additionally, we demonstrated that a novel vector platform based on non-human papillomaviruses (nhPVs) enables efficient and safe gene delivery. Among the tested nhPV *Macaca fascicularis* papillomavirus-11 (Mf11) and *Puma concolor* papillomavirus-1 (PuC) capsids showed the highest transduction efficacy *in vitro*. Notably, PuC facilitated long-term expression of a luciferase reporter gene highlighting its potential as a vaccine vector.

Building on these findings, we evaluated Mf11 and PuC capsids for the mucosal delivery of a plasmid encoding for RSV-F. In the present study these two nhPV vectors were applied in a heterologous vaccination regimen and compared with the intramuscular application of the formalin inactivated RSV (FI-RSV) in terms of immunogenicity, and protective efficacy after RSV challenge.

All vaccine candidates induced RSV-binding and neutralizing antibodies. However, only mucosally administered nhPV-based vectors elicited robust RSV-specific IgA-antibody responses. While both strategies significantly reduced viral load, pronounced differences were observed in the immunological responses, including cytokine profiles, immune cell composition in bronchoalveolar lavage fluid, and lung tissue damage. Mucosal nhPV vaccination induced a more balanced immune response, whereas FI-RSV immunization was associated with a pronounced Th2-skewed profile and increased pulmonary inflammation.

In conclusion, our preclinical data demonstrate that nhPV-based vectors represent a promising platform technology for mucosal vaccination. These nhPV-vectors support the safe and effective induction of protective immunity against RSV and may be broadly applicable to other respiratory pathogens. Their versatility and suitability for mucosal delivery make them promising candidates for future vaccine development.

Pre-clinical development of a mucosal live-attenuated vaccine for tick-borne encephalitis using the Langat virus platform

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Tick-borne encephalitis (TBE) is a significant disease in Europe and Asia, with a rising incidence due to the spread of the TBE virus (TBEV) and its vectors. Current TBE vaccines provide good protection, but they have a complex immunization schedule and lower efficacy in the elderly, leading to occasional vaccine failures. We aim to develop a novel TBE vaccine to provide better protection with fewer doses through mucosal immunization. The current work covers a pilot study that evaluates live-attenuated TBE vaccine *in vivo*, using Langat virus (LGTV) platform that we developed based on a rescued LGTV infectious clone (LGTV IC).

In the current mouse study, LGTV IC was administered via intranasal and intramuscular routes at two doses (10^3 and 10^5 PFU). The study assessed tolerability, viremia profile, and induced immunogenicity.

As a result, we show that intranasal immunization with LGTV IC induced strong immune responses and revealed a favorable safety profile in a dose-dependent manner. Low-dose intranasal administration was well tolerated, with no clinical signs, weight loss, or viral presence in the central nervous system. It elicited robust anti-TBEV IgG antibodies that successfully neutralized both LGTV and TBEV and induced strong cellular immunity, characterized by TBEV NS3-specific IFNγ and IL-2 secreting cells. Notably, low-dose mucosal immunization outperformed both high-dose intranasal and intramuscular administration in generating a balanced immune response. In contrast, high-dose intranasal immunization caused significant weight loss and minimal viral detection in CSF, indicating potential adverse effects at elevated doses.

These findings support the potential of low-dose mucosal immunization with LGTV IC as a safe and effective TBE vaccination strategy. Further attenuation of LGTV IC is underway to enhance safety for future development.

Sustained Antibody Response to Puumala Hantavirus: A 20-Year Serological Perspective

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Puumala hantavirus (PUUV) is a zoonotic pathogen that causes hemorrhagic fever with renal syndrome (HFRS). Currently, no vaccine or specific antiviral treatment is available, and understanding of protective immunity against re-infection is incomplete. Here, we utilized two distinct cohorts, with aim to investigate the development and maintenance of humoral immunity over decades after a PUUV infection; The Northen Sweden Health and Disease Study (NSHDS), where longitudinal sampling had been performed over more than 20 years of the ”general population” in Västerbotten, and also a cohort of individuals that had clinically verified PUUV-infection and longitudinally sampling for more than a decade. We utilized ELISA and multiplex technology to assess quantity and quality of PUUV-specific IgG, IgA and IgM in these cohorts. We could determine that the overall seroprevalence in the NSHDS cohort was 12%, and that once IgG or IgA to the PUUV GcGc was detected in a sample, respective individual had remained positive over the course of the sampling. In contrast, GnGc- directed IgM was only detected during the acute phase or convalescent phase of the disease (2-8 weeks post infection). The magnitude fo GnGc- or N-specific IgG increased over the first year after infection and then remained at high levels for years or decades. In contrast, GnGc-specific IgA increased over the first 1-2 weeks post infection and then remained at steady-state levels over the study timeframe. Avidity measurements to the GnGc suggested continuous maturation or immunofocusing occurred during the first 6 months after disease onset. By dissecting the GnGc-specific responses into respective subunit (Gn or Gc), we found that the kinetics were relatively similar to both subunity as for the GnGc, but that Gc-directed antibodies reached a higher affinity than Gn-directed antibodies. Similar binding kinetics and avidity increases was found for Nucleocapsid (N)-directed IgG and IgA. Of note, N-directed IgG or IgA had approximately 2-fold higher avidity index than IgG or IgA towards GnGc. As expected, the avidity of GnGc- or N-directed IgM was low at all timepoints. Our initial data suggests that even though PUUV-specific IgG and IgA is maintained over time, the neutralization capacity of isolated IgG is reduced at late timepoints, as compared with those weeks after the infection. Collectively, our data suggests that PUUV-specific humoral immune responses are maintained over decades after an initial infection, either by re-exposure to antigen or by robust memory formation during the first year after infection.

A novel viral vector platform for mucosal immunizations

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Emerging evidence suggests that mucosal immunity mediated by IgA and tissue-resident memory T cells (T_{RM}) plays a pivotal role in the control of respiratory pathogens. Eliciting such responses requires mucosal vaccination, but appropriate vaccine platforms are currently lacking.

In a rational screening approach for novel viral vector platforms with promising characteristics for mucosal delivery, a bat-derived virus with a segmented dsRNA genome emerged as a top candidate. Human infections with this virus have only rarely been recorded and were restricted to certain areas of Southeast Asia where extensive bat populations live in proximity to humans. These infections revealed a respiratory tropism of the virus, among a mild, self-resolving illness, which is not known to have caused any fatality. Genetic manipulation of this virus is possible by an established reverse genetics system.

As a proof of concept, we created packaging capacity by deleting specific regions of the viral attachment protein and inserted GFP as a reporter transgene into the viral genome. We observed GFP expression in viral plaques, but detected deletions of the GFP gene by the virus at early passages. Since the original viral genome shows a remarkable low GC content, we speculated that adapting the GC content of the GFP transgene to that of the viral genome would result in a more stable transgene expression. To investigate this hypothesis, we matched the GFP codon usage to the viral codon usage, which resulted in a lower GC content and a stable reporter virus. Using this reporter virus, we characterized the tropism of the vaccine platform. To this end, several human airway cell lines were successfully infected with the virus, which demonstrated a sustained ability of it to replicate *in vitro* in human airway cells. We then infected murine precision-cut lung slices with the vector as a more physiological model of the lung. We also observed replication in this *ex vivo* system, further validating the potential of the viral vector as mucosal vaccine platform.
To investigate the immunogenicity of the novel a vector platform, a well-established model antigen was inserted and a first proof-of-principle immunization conducted in BALB/c mice. This experiment revealed a robust induction of antigen-specific $CD103^{+}$ $CD69^{+}$ T_{RM} in the lungs. To evaluate the functional profile of the T cells, they were restimulated with vaccine peptides and responding populations identified by intracellular staining for effector cytokines IFNγ, TNF and IL-2. The analyses demonstrated that functional effector populations were induced by the mucosal vaccination in lungs and spleens.

In the future, we want to deliver SARS-CoV-2 antigens by our mucosal vaccine platform and also exploit it as cancer vaccine against lung cancer. This work therefore contributes to address the current lack of mucosal vaccine technologies.