10th Smögen Summer Symposium on Virology

August 21-23, 2014

Venue: Smögens Havsbad

Arranged by the Swedish Society for Virology

http://www.swedishvirology.se

Photo: J. Westin

Welcome to the 10th Smögen Symposium on Virology

It is a pleasure for the Organizing Committee to announce that the 10th symposium will be held at Smögens Havsbad, Smögen, Thursday August 21 to Saturday August 23, 2014. This is the same venue as last year.

The scientific as well as the social agenda will be very much alike previous Smögen meetings. One aim is to bring together Scandinavian virologists, representing both clinical and preclinical (or Natural Science) Virology. Smögens Havsbad or "Havsbadet" is a famous spa establishment with traditions from 1903, and with a splendid view of the beautiful archipelago north-west of Smögen. Havsbadet is a fully modern hotel with a high-class cuisine, and an excellent conference facility, including an indoor swimming pool. As the number of expected delegates exceeds the number of beds at Havsbadet we have reserved one excellent alternative: "Makrillviken", a high standard youth hostel, situated less than 200 m from Havsbadet. As an extra fallback we have reserved rooms at "Pensionat Bryggan", situated in the heart of the Smögen Sailing Harbour, should Makrillviken be filled.

We wish you all very welcome in Smögen!

Sigvard Olofsson, Tomas Bergström, Kristina Eriksson, Lennart Svensson

The Venue: Smögens Havsbad

Foto: SVEN LINDWALL

A fully modern hotel with a capacity of more than 100 guests in comfortable single and double rooms. Indoor swimming-pool and exclusive spa services available. The conference facilities include a fully equipped lecture hall taking 100-200 people, and a lot of smaller meeting rooms. The cuisine is excellent, the same overwhelming view of the Skagerrak archipelago as experienced by the first summer guests of Smögens Havsbad in 1903 is still available from the dining room and the western terrace. The hotel is situated close to the heart of Smögen offering excellent sea baths and easy access to the classical wooden quay of the Smögen Sailing Harbour.

Additional Lodgings: Makrillviken & Pensionat Bryggan

These are the ideal accommodation for the young virologists accepting shared rooms, some of which with corridor shower and toilet. Modern standard. Full board, incl. bed cloths and bathroom towels also for the delegates staying at Makrillviken and Bryggan (fallback). All meals and service at Havsbadet. Delegates registering for “Bed in shared” will be checked in at Makrillviken in the first place.

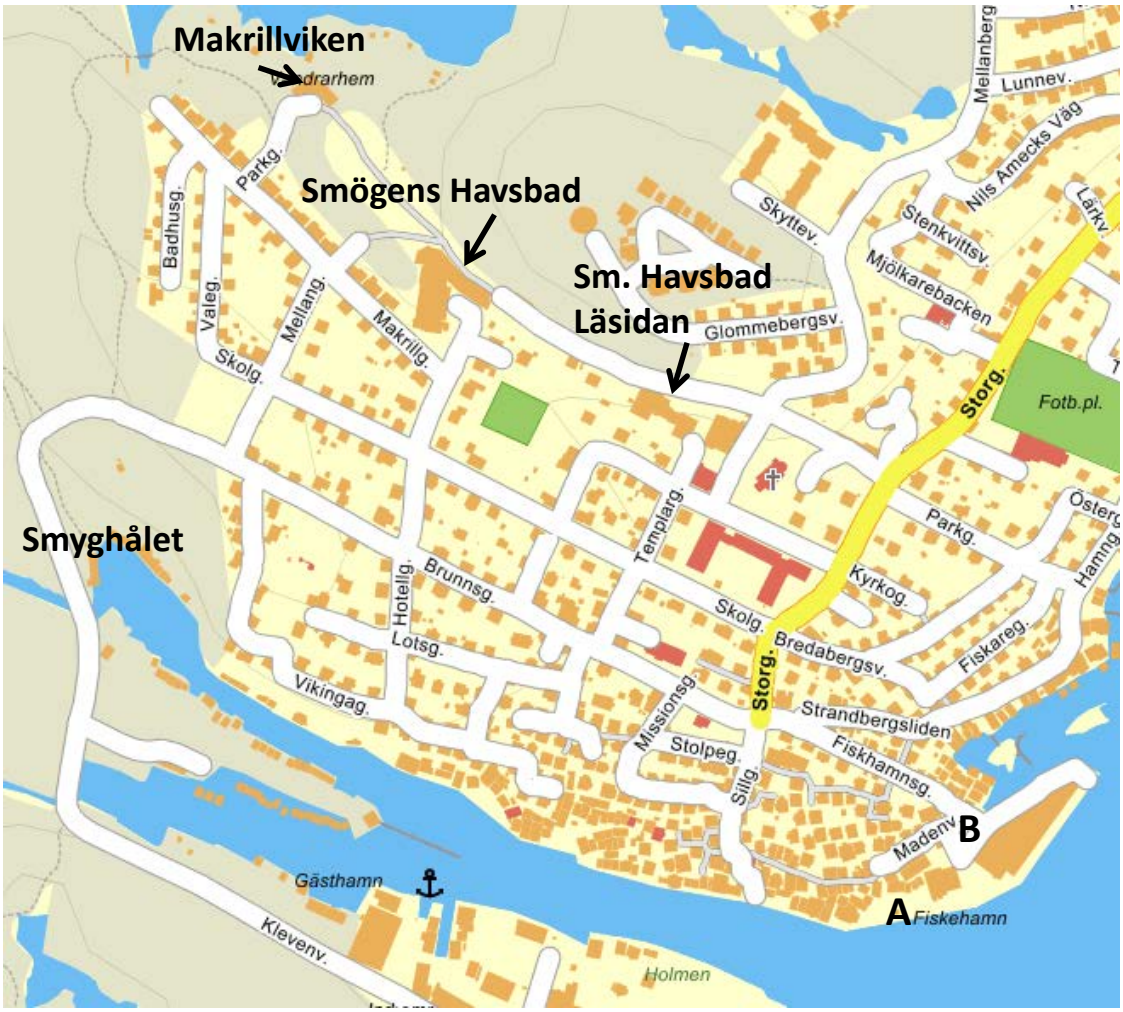
Seafood Buffet on board of M/S Byfjorden during an evening cruise in the Smögen Archipelago

Welcome onboard the "Byfjorden" to a traditional Smögen Seafood Buffet including shrimps, crayfish and a lot of other seafood specialities. "Byfjorden" was built in 1968 at Skaalurens Skibsbyggeri, Rosendal, Norway. The ship was lengthened in 1989 by 5 meters and she is now 31 m long with a cruising speed of 11 knots. She recently became thoroughly restored and modernized and takes up to 120 dining guests. Her large deck offers great possibilities for wonderful veiws of the scenery.

Alternative Friday Night Activity: Gourmet Three Course Dinner

The Gourmet Dinner will take place at Smögens Havsbad as an alternative for delegates not interested in Archipelago Cruises and Seafood mainly based on shellfish. Owing to requests from the 2013 Gourmet Dinner guests the time intervals between the different dishes will be reduced.

Map of Important Places



The positions of the two hotels are indicated. Läsidan is a room annex for Havsbadet

A: Where boarding for the Seafood Cruise on Friday takes place.

B: Place for Welcome Reception Thursday

Program

Thursdag, August 21

All events at Smögens Havsbad unless otherwise stated.

Please note that all information provided during the sessions is considered as privileged. You are therefore not allowed to take any photographs of the slides.

12.00	Registration + Lunch
13.30	Introduction
13.45-14.30	**Keynote 1: Eckard Wimmer** Stony Brook, New York
	Synthetic poliovirus and other designer viruses: what have we learned from them? Moderator: Mikael Lindberg
14.40-15.00	**Special Theme Address: Heléne Norder** Göteborg
	Is it possible to eradicate polio using OPV?

15.00-15.30	**Session 1: Clinical Virology: Diagnostics & Chemotherapy** Chairwoman: Lena Grillner	
Johan Lennerstrand	Uppsala	Analysis by population- and ultra-deep-sequencing of polymorphisms with excessive resistance to NS5A inhibitors in treatment-naive subjects with HCV genotype 1a and 3a
Simon Larsson	Gothenburg	Reduced levels of hepatitis B virus DNA and surface antigen by suppression of episomal DNA and pregenomic RNA but not of S RNA
Lena Serrander	Linköping	The risk of HCV RNA contamination in serology screening instruments with a fixed needle for sample transfer

15.30-16.00 Coffee Break

16.00-16.50	**Session 2: Epidemiology & Evolution** Chairwoman: Lena Serrander	
Győzö Kajan (Victor)	Umeå	Multigene typing of human adenovirus strains in Sweden
Johan Nordgren	Linköping	Predominance of norovirus and sapovirus in Nicaragua after implementation of universal rotavirus vaccination
Magnus Simonsson	Uppsala	Meta-analysis of literature data on symptom frequencies and duration: Development of criteria to determine the causing microorganism of gastrointestinal illness in outbreaks and epidemiological studies
Anders Widell	Malmö/Lund	An HCV Strain probably representing a new major Genotype (Genotype 8), distantly related to Genotype 6.
Michelle Wille	Kalmar	Temporal dynamics, diversity, and interplay in three components of the viriodiversity of a Mallard population: Influenza A virus, avian paramyxovirus and avian coronavirus

16.50-17.20	**Session 3: Tumor Viruses** Chairman: Anders Widell	
Ka-Wei Tang	Gothenburg	The viral landscape in human cancer
Stefan Schwartz	Lund	Regulation of HPV16 late gene expression
Torbjörn Ramqvist	Stockholm	Correlation of LMP10 expression and clinical outcome in human papillomavirus (HPV) positive and negative tonsillar and base of tongue cancer

17.20-18.00	Årsmöte Svenska sällskapet för virologi
18.30	Welcome Reception, *Smögen Fishing Harbour*
20.00	Dinner

Friday, August 22

07.30	Breakfast

08.45-09.30	**Keynote 2: Marco Vignuzzi** Institut Pasteur, Paris, France
	RNA virus population diversity: implications for inter-species transmission Moderator: Tomas Bergström

09.45-10.35	**Session 4: Early Virus-Cell Interactions** Chairwoman: Anna Överby	
Marta Bally	Gothenburg/ Institut Curie, Paris	Artificial cell-membrane mimics to study the role of the influenza virus matrix protein in virus budding and release
Noomi Altgärde	Gothenburg	Binding of glycoprotein C from Herpes Simplex Virus-1 to surface-immobilized sulfated glycosaminoglycans
Waqas Nasir	Gothenburg	Interactions of GII.4 norovirus like particles with membrane bound fucosylated histo-blood group antigens
Gustaf Rydell	Gothenburg	Parvovirus B19 recognizes membrane associated glycosphingolipids
Andres Merits	Tartu, Estonia	Expected and unexpected cellular factors involved in alphavirus replication and recognition

10.35	Coffee Break

10.35-11.15	**Poster Presentations during Coffee Break** Chairman: Niklas Arnberg	
Naoko Kajitani	Lund	Expression of Human papillomavirus 16 late genes is affected by Akt/PI3K pathway.
Annasara Lenman	Umeå	Human adenovirus 52 uses sialic acid-containing glycoproteins and the coxsackie and adenovirus receptor for binding to target ce
Kersti Nilsson	Lund	Translation of HPV16 E5 protein
Nadia Peerboom	Gothenburg	Surface-based sensing to probe herpes-glycosaminoglycan interactions with single particle sensitivity
Maria Röhl	Stockholm	Comparable mRNA expression of inflammatory markers in foreskin tissue of HSV-2 seropositive and seronegative asymptomatic Kenyan

11.15-12.15	**Session 5: Pathogenesis** Chairman: Lars Magnus Andersson	
Clas Ahlm	Umeå	Endothelial dysfunction during Puumala virus infection
Charlotta Eriksson	Gothenburg	The anterior commissure is a pathway for contralateral spread of herpes simplex virus type 1 (HSV-1) after olfactory tract infection
Sven Grützmeier	Stockholm	CMV-retinitis in AIDS-patients and its correlation to CMV-encephalitis
Marie Hagbom	Linköping	The vagus nerve and the cholinergic anti-inflammatory pathway attenuates inflammation in rotavirus infection

Jonas Klingström	Stockholm	Consequences of hantavirus-mediated resistance to apoptosis
Anna Gibbs	Stockholm	Localization, phenotype and function of MAIT cells in the female genital mucosa

12.15 Lunch and Free Activities

15.00-15.20	**Special Honorary Address: Erling Norrby**, Stockholm
	Nobel Prizes and the virus concept Introduced by Lennart Svensson

15.20-16.05	**Session 6: Gene Expression and Innate Immunity** Chairwoman: Karin Loré	
Anna Överby	Umeå	Viperin vs. Tick-Borne Encephalitis Virus: Mechanisms of a Potent Antiviral Protein
Johanna Tauriainen	Stockholm	The inhibitory molecule TIGIT is expressed on CD8+ T cells during HIV infection
Ali Mirazimi	Stockholm	Crimean-Congo hemorrhagic fever replication interplays with regulation mechanisms of apoptosis
Anna Lundin	Gothenburg	Potent inhibition of diverse Coronaviruses including MERS by targeting of membrane-bound viral RNA synthesis

16.05-16.25	**Session 7: Emerging Viruses** Chairman: Clas Ahlm	
Magnus Evander	Umeå	Outbreak of Sindbis virus infection, Northern Sweden 2013
Peter Norberg	Gothenburg	Evolution and geographic spread of the tick-borne encephalitis virus

16.25-16.50 Coffee Break

16.50-17.30	**Session 8: Molecular and Structural Virology** Chairwoman: Susann Teneberg	
Lars Magnius	Stockholm	Complete coding regions of the prototypes EV-B93 and EV-C95: Phylogenetic analyses of the P1 and P3 regions of EV-B and EV-C strains.
Ya-Fang Mei	Umeå	Biological properties of novel replication-competent adenovirus 11pGFP vector and strategies of the vector development
Mathilda Sjöberg	Stockholm	Structural changes during maturation and activation of a retroviral spike protein
Anna Sävneby	Kalmar	Efficient replication of recombinant viruses with a coxsackievirus B5 replicative backbone and P1 regions from different enterovirus B types

18.45 Gathering for Archipelago Cruise & Seafood buffet (see map on page 4)

Saturday, August 23

07.30 Breakfast

08.45-10.05	**Plenary Workshop: Trends in Viral Vaccinology** Moderator: Sigvard Olofsson
08.45	Karin Loré, KI, Stockholm Manipulating innate stimulation by distinct adjuvants to improve vaccine responses
09.15	Margaret Liu , KI, Stockholm and San Francisco, CA The Impact of Vector-related Immune Activation on Vaccine Efficacy and Safety
09.45	Anders Fomsgaard, Copenhagen Easy needle-free intradermal delivery and vector optimization of a broad protective polyvalent influenza-A DNA vaccine for pigs
09.55	Matti Sällberg, KI, Stockholm HCV, HBV, and HDV vaccines, which ones do we need?

10-05-10.40 Coffee Break

10.40-11.40	**Session 9: Vaccines & Immunology** Chairwoman: Kristina Broliden	
Britta Wahren	Stockholm	Genetic HIV immunization in children and adults
Marie Borggren	Copenhagen	Rational selection of new immunogens for future HIV-1 DNA vaccine
Robin Löving	Stockholm	Reconstitution of the HIV-1 spike protein into Salipro limited nano-membranes
Karin Granhagen Önnheim	Gothenburg	The challenge dose of herpes simplex virus-2 is critical for protection against neuronal infection in a murine vaccination model

Elisa Crisci	Linköping	Role of complement and specific antibodies during HSV2 infection of immature dendritic cells and epithelial cells
Tina Falkeborn	Linköping	Comparison of the mucosal adjuvant Endocine™ with two well-known adjuvants: cholera toxin and alum

11.40-12.10	**Session 10: Late-Breaking Abstracts** Chairwoman: Britta Wahren	
Ieva Bagdonaité	Copenhagen	A global glycoproteomic analysis of site-specific O-glycosylation in herpes simplex virus virus type 1 glycoproteins
Patrik Medstrand	Lund	Long-term HIV-2 intra-patient evolution in relation to disease progression
Charlotta Polacek	Fredriksberg, DK	Foot-and-mouth disease virus-induced stress granules are disrupted by the viral L-protease

12.10 Lunch

13.00 Bus departure for Göteborg

Abstracts

ANALYSIS BY POPULATION- AND ULTRA-DEEP-SEQUENCING OF POLYMORPHISMS WITH EXCESSIVE RESISTANCE TO NS5A INHIBITORS IN TREATMENT-NAIVE SUBJECTS WITH HCV GENOTYPE 1A AND 3A

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Future interferon-free combo treatments will include HCV NS5A inhibitors for potent pangenotypic effect. Sera from 55 patients with GT1a and 35 patients with GT3a were collected for the NS5A analysis. With the population-sequencing method the NS5A genes were amplified by nested PCR method with degenerated primers to enable a broad genotype analysis (>96 and >97% yield). We chose PCR products from 7 GT1a and 3 GT3a samples, to further study mixes of mutant variants with Pacific Biosciences deep-sequencing; using SAMtools and BCFtools, before being visualized and inspected using the Integrative Genomics Viewer. With the population method (cutoff level of 20%) baseline-resistance variants associated with high resistance were found in 2 (2/55) GT1a samples displaying Q30H or Y93N, and in 1 (1/35) GT3a sample displaying Y93H. These mutations/polymorphisms are correlated with high level of resistance (1500-50000 fold) against NS5A inhibitors daclatasvir and ABT-267. In order to reveal the levels of these resistance variants, deep-sequencing was conducted on the 3 positive samples (GT1a Q30H and Y93N, and GT3a Y93H), and on 5 GT1a and 2 GT3a non-positive samples. The 3 positive samples displayed, respectively, 97.7% (16950/17346) level of Q30H, 99.3% (17444/17560) of Y93N, and 64.2% (12639/19671) of Y93H. Furthermore, the high foldresistant associated variant M28T and Y93C were found in one of the non-positive sample at a level of > 0.3%. Thus, ultra-deep sequencing allows for detection of very low mixes (down to 0,2% in contrast to 20%) of resistant HCV and thus could be used to predict the most cost efficient treatment for an HCV-infected individual before treatment start.

REDUCED LEVELS OF HEPATITIS B VIRUS DNA AND SURFACE ANTIGEN BY SUPPRESSION OF EPISOMAL DNA AND PREGENOMIC RNA BUT NOT OF S RNA

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Hepatitis B virus (HBV) DNA in serum of chronically infected patients declines by 3-4 log10 units at loss of e antigen (HBeAg). The mechanisms behind this decline, and the much smaller decline of surface antigen (HBsAg) levels, are still not well known.

To get a better understanding of this process we analysed levels of HBV DNA and HBsAg in serum, and covalently closed circular DNA (cccDNA), pregenomic RNA (pgRNA) and S-RNA and total intrahepatic HBV DNA in liver biopsies from 84 chronically infected patients (16 positive and 68 negative for HBeAg).

In HBeAg positive patients, reduced HBV DNA levels reflected decreased cccDNA and pgRNA. Further reduction of HBV DNA after loss of HBeAg was due to effects downstream reverse transcription. A reduced HBV DNA/HBsAg ratio corresponded to lower pgRNA/cccDNA ($p=0.02$) and higher S-RNA/cccDNA ($p<0.001$) ratios, indicating that in HBeAg-negative stage transcription of pgRNA but not S-RNA becomes suppressed. The correlation between the viral markers was stronger in HBeAg-positive patients, suggesting a greater influence of other factors in the HBeAg-negative phase of disease.

Conclusion: The much lower HBV DNA levels in serum after loss of HBeAg is due to combined reduction of cccDNA, pgRNA and yet unidentified effects (possibly shorter virion half-life). Increased levels of S-RNA partly explain why HBsAg remain high in the HBeAg-negative phase. The correlation between intrahepatic viral load and serum HBV DNA was poor in HBeAg-negative patients.

THE RISK OF HCV RNA CONTAMINATION IN SEROLOGY SCREENING INSTRUMENTS WITH A FIXED NEEDLE FOR SAMPLE TRANSFER

LENA SERRANDER

Background: Hepatitis C diagnostics involve antibody screening and confirmation of current infection by detection of HCV RNA positivity. In screening instruments with fixed pipetting needle, there is a risk of sample carry-over contamination.

Objectives: The aim of this study was to evaluate the risk of such contamination in a proposed clinical setting.

Study design: In the present study, known HCV RNA positive (n=149) and negative (n=149) samples were analysed by anti-HCV Abbott in an Architect instrument in an alternating fashion in order to test for contamination.

Results: In subsequent retesting of the previously HCV RNA-negative samples, six samples (4%) were positive by the Cobas Taqman assay with a maximum level of 33 IU/mL. The results show that there is a risk for transfer of HCV in the Architect instrument but they also show that the levels of HCV RNA observed are low.

Conclusions: We conclude that complementary HCV RNA testing on samples identified as anti-HCV positive by screening can be recommended because the complementary results are reliable in the majority of cases when either HCV RNA is negative or HCV RNA is positive with a level >1000 IU/mL. In a minority of cases, with low HCV RNA after anti-HCV antibody screening, cross-contamination should be suspected and a new sample requested for HCV RNA testing. This strategy would reduce the need for obtaining a new sample from the vast majority of patients with a newly discovered HCV antibody positivity.

MULTIGENE TYPING OF HUMAN ADENOVIRUS STRAINS IN SWEDEN

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Adenoviruses can be used in medical applications such as in the treatment of cancer and infectious diseases, or for gene therapy purposes: vectoring is a very active and developing field in virology. To find potential vector candidates, new human adenovirus types, we typed 282 Swedish human adenovirus isolates originating from Malmö and Umeå using molecular methods. Three PCR systems were applied - targeting the genes of the viral DNA polymerase, the penton base and the hexon - to be able to detect recombinant adenoviruses as well; two of these three PCRs were designed by us. The resulting PCR products were sequenced. Introductory results show, that around 80% of the samples are typeable - the strain gave a positive result with all the three PCR systems used. After analysing the partial DNA polymerase gene sequences it was revealed that 63% of the strains cluster to the species Human mastadenovirus C, but almost all other human mastadenovirus species were found as well, with the exception of species G. After the analysis of the partial hexon gene sequences no new human adenovirus types could be described based on distance methods, none of the samples had high enough divergence from established human adenovirus types in that region of the hexon gene which codes the loop 1 of the protein. By comparing the DNA-polymerase and hexon datasets 34 intertypic recombinant strains were found, from which six were not described as a new human adenovirus type yet. The work was financed by the European Union through the Seventh Framework Programme (grant agreement no.: 324325).

PREDOMINANCE OF NOROVIRUS AND SAPOVIRUS IN NICARAGUA AFTER IMPLEMENTATION OF UNIVERSAL ROTAVIRUS VACCINATION

Filemón Bucardo*, Yahoska Reyes*, Lennart Svensson** and JOHAN NORDGREN**

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**Division of Molecular Virology, Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden

Background: Despite significant reduction of pediatric rotavirus (RV) infections following implementation of RV vaccination (RotaTeq) in Nicaragua, a large burden of diarrhea persists.

Methods: We conducted a community- and hospital-based study of the burden of RV, norovirus (NV) and sapovirus (SV) infections as cause of sporadic acute gastroenteritis (GE) among 330 children ≤ 5 years of age between September 2009 and October 2010 in two major cities of Nicaragua with a RotaTeq coverage rate of 95%.

Results: We found that NV, SV and RV infections altogether accounted for 45% of cases of GE. Notably, NV was found in 24% (79/330) of the children, followed by SV (17%, 57/330) and RV (8%, 25/330). The detection rate in the hospital setting was 27%, 15% and 14% for NV, SV and RV respectively, whereas in the community setting the detection rate of RV was < 1%. Among each of the investigated viruses one particular genogroup or genotype was dominant and found in higher proportions in the hospital settings GII.4 (82%) for NV, GI (46%) for SV and G1P[8] (64%) for RV. The RV found in vaccinated children were genetically similar to the vaccine strains; suggesting host factors to account for vaccine failures.

Conclusions: This study shows that NV has become the leading viral cause of pediatric gastroenteritis at hospital and community settings in Nicaragua after implementation of RV vaccination.

META-ANALYSIS OF LITERATURE DATA ON SYMPTOM FREQUENCIES AND DURATION: DEVELOPMENT OF CRITERIA TO DETERMINE THE CAUSING MICROORGANISMS OF GASTROINTESTINAL ILLNESS IN OUTBREAKS AND EPIDEMIOLOGICAL STUDIES

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Gastrointestinal illness (GI) can be caused by a variety of microorganisms. Vast underreporting of GI cases and low proportion of diagnosed cases, for which the causing agent is identified, hampers the possibility to estimate incidence and contribution of various pathogens in outbreaks and epidemiological studies. To improve this possibility we made a literature review on symptom frequencies and duration for the most common GI causing microorganisms. Salmonella, STEC, ETEC, Caliciviruses (norovirus and sapovirus) rotavirus astrovirus, cryptosporidium and giardia were included. Analysis using Nonmetric Multidimensional Scaling, with symptom frequencies from selected published studies as input, showed clustering of the different causing microorganisms. Further analysis showed that some agents as norovirus, sapovirus and giardia could be separated by simple relations between median duration and specific symptom frequencies. We also show that simple criteria on symptom frequencies and median duration could be used to define specific causing microorganism when a single agent could be suspected to cause an outbreak. We will also discuss the possibility to use meta-analysis data from studies on GI symptom frequencies and duration to estimate the contribution of causing agents in prospective cohort studies on GI incidence.

AN HCV STRAIN PROBABLY REPRESENTING A NEW MAJOR GENOTYPE (GENOTYPE 8), DISTANTLY RELATED TO GENOTYPE 6

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Since 1989, the diversity of hepatitis C virus (HCV) has expanded into 7 major genotypes, where all but two (5 and 7) display many subtypes. However, the discovery of new major genotypes has been exceedingly rare for two decades.

Refugees arriving in Sweden are screened for blood borne infections and if found positive, referred to a specialist in infectious diseases. One such case, a refugee from Central Asia, who tested positive for anti-HCV and for HCV RNA, also underwent genotyping by partial sequencing of the NS5B gene (339nct) and the more conserved core (219nct) gene.
Thereby the virus sequence (provisionally named LK) showed maximum nucleotide similarity of 69% (NS5B) and 89% (core) to all known geno- or subtypes. Subsequent cloning of both PCR products indicated no evidence of mixed infection.
To characterize strain LK further, a full length sequencing project was initiated using high fidelity polymerase, and a combination of long PCRs, cloning and primer walking. To date, a continuous sequence of about 8600 bases has been obtained. The LK sequence was aligned to the recently published dataset of 150 full length HCV sequences, representing the most diverse sequences known (Smith et al, Hepatology, 2014). Using Maximum Likelihood methods implemented in Mega 6 and Garli, the isolate LK very clearly branched off close to the root. The closest related sequences were those of genotype 6, however, LK was as close to genotype 6 as genotype 1 was to genotype 4. Analysis for putative recombinant sequences using the pairwise homoplasy index (PHI; implemented in Splitstree v4.10) and RDP, Geneconv, Bootscan, MaxChi, Chimaera, SiScan, 3Seq, LARD and PhylPro (implemented in the RDP package) did not indicate putative recombinant signals between the LK sequence and 150 full length HCV reference sequences.

Our LK strain may thus represent a new major HCV genotype.

TEMPORAL DYNAMICS, DIVERSITY, AND INTERPLAY IN THREE COMPONENTS OF THE VIRIODIVERSITY OF A MALLARD POPULATION: INFLUENZA A VIRUS, AVIAN PARAMYXOVIRUS AND AVIAN CORONAVIRUS

MICHELLE WILLE, Alexis Avril, Conny Tolf, Anna Schager, Sara Larsson, Olivia Borg and Jonas Waldenström

Multiple infections, or simultaneous infection of a host with multiple parasites, are the rule rather than the exception. Interactions between co-occuring pathogens in a population may be mutualistic, competitive or faciliative, however this is as yet poorly incorporated into practical disease ecology. For example, screening of Mallards for influenza A viruses (IAV) have repeatedly revealed high prevalence and large subtype diversity in the temporal region of the Northern Hemisphere. Other studies have identified avian paramyxovirus type 1 (APMV-1) and coronaviruses (CoV) in Mallards, but without making inferences on the larger viral assemblage. In this study we followed 144 wild Mallards across an autumn season in a natural stopover site and constructed infection histories of IAV, APMV-1 and CoV. There was a high prevalence of IAV, comprising of 27 subtype combinations, while APMV-1 had a comparatively low prevalence, with a peak of 2%, and limited strain variation, similar to what have been described earlier from this study site. Avian CoVs were common, with prevalence
up to 10%, and sequence analysis identified a range of genetic lineages. An investigation of the dynamics of co-infections revealed a syngistic effect between CoV and IAV, whereby CoV prevalence was higher given that the birds were co-infected with IAV. There is growing evidence that incorporating more realistic levels of parasite and pathogen diversity is essential for managing infection disease risk.

THE VIRAL LANDSCAPE IN HUMAN CANCER

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Viruses cause 10-15% of all human cancers. Massively parallel sequencing has recently proved effective for uncovering novel viruses and virus□tumour associations, but this approach has not yet been applied to comprehensive patient cohorts. Here we screen a diverse landscape of human cancer, encompassing 4,433 tumours and 19 cancer types, for known and novel expressed viruses based on >700 billion transcriptome sequencing reads from The Cancer Genome Atlas Research Network. The resulting map confirms and extends current knowledge. We observe recurrent fusion events, including human papillomavirus insertions in RAD51B and ERBB2. Patterns of coadaptation between host and viral gene expression give clues to papillomavirus oncogene function. Importantly, our analysis argues strongly against viral aetiology in several cancers where this has frequently been proposed. We provide a virus-tumour map of unprecedented scale that constitutes a reference for future studies of tumour-associated viruses using transcriptome sequencing data.

REGULATION OF HPV16 LATE GENE EXPRESSION

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HPV16 is a sexually transmitted virus that can establish persistent infections in its host. These infections may in rare cases progress to cancer. As progression to cancer takes many years, HPV16 must avoid detection by the immune system. A strictly controlled and restricted expression of the highly immunogenic late viral proteins L1 and L2 probably contributes to the ability of HPV16 to persist. We are therefore interested in the regulation of HPV16 late gene expression. To identify factors that regulate HPV16 late gene expression, for example cellular proteins and signal transduction pathways, epigenetic marks on the HPV genome or small molecues, we have developed a novel cell based HPV16-reporter cell line. It contains an HPV16 genome with a secreted luciferase gene in place of the HPV16 late L1 gene stably integrated in the genome of cervical cancer cells. Induction of HPV16 late gene expression in this cell line, results in production of secreted luciferase in the cell culture medium. We are using this reporter cell line to identify splicing factors and epigenetic marks on the HPV16 genome that regulate HPV16 late gene expression.

CORRELATION OF LMP10 EXPRESSION AND CLINICAL OUTCOME IN HUMAN PAPILLOMAVIRUS (HPV) POSITIVE AND NEGATIVE TONSILLAR AND BASE OF TONGUE CANCER

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Background: Outcome is better in HPV-positive tonsillar and base of tongue squamous cell carcinoma (TSCC and BOTSCC) compared to matching HPV-negative tumours, with roughly 80% vs. 40% 5-year disease free survival (DFS) with less aggressive treatment than today's chemoradiotherapy. Since current treatment often results in harmful side effects, less intensive therapy, with sustained patient survival would be an attractive alternative. However, before tapering treatment, other markers together with HPV status are necessary to select patients. For this purpose we investigated LMP in relation to tumour HPV status and clinical outcome in TSCC and BOTSCC.

Materials and methods: From 385 patients diagnosed between 2000 and 2007 at the Karolinska University Hospital, 278 formalin fixed paraffin embedded TSCC and BOTSCC biopsies, with known HPV DNA status, were tested for LMP10 nuclear and cytoplasmic expression (fraction of positive cells and staining intensity). The data were correlated to clinical outcome.

Results: An absent/low compared to a moderate/high LMP10 nuclear fraction of positive cells was correlated to a better 3-year DFS in the HPV-positive group of patients (log-rank p=0.005), but not in the HPV-negative group. In the HPV-negative group of patients, in contrast to the HPV-positive group, moderate/high LMP10 cytoplasmic fraction and weak/moderate/high LMP10 cytoplasmic intensity correlated to a better 3-year DFS (p=0.003 and p=0.001) and 3-year overall survival (p=0.001 and 0.009).

ARTIFICIAL CELL-MEMBRANE MIMICS TO STUDY THE ROLE OF THE INFLUENZA VIRUS MATRIX PROTEIN IN VIRUS BUDDING AND RELEASE

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The influenza virus exits from its host by deforming its membrane into a bud before pinching off by membrane fission. The goal of this study is to provide a biophysical understanding of the mechanisms underlying the last steps of the Influenza's replication cycle. In particular, we investigate the contribution of the virus matrix protein M1 to the process.

Our approach relies on the use of artificial lipid assemblies to mimic the physico-chemical properties of the cell membrane. These minimal cell membrane models make it possible to decipher the role of M1 in changing the membrane's organization and morphology. Specifically, sensor-supported lipid bilayers are used, in conjunction with surface-sensitive techniques (surface plasmon resonance and quartz crystal microbalance), to provide insights into the protein's binding affinity and specificity to the membrane as well as into the adlayers architecture and the protein's propensity to self-aggregation when in contact with the lipids. Fluorescence microscopy experiments provide further insights into proteininduced phase separation processes occurring within the membrane. Experiments performed with giant unilamellar vesicles reveal the protein's ability to deform lipid membranes and allow further investigation of membrane abscission processes.

Taken together, our study illustrate the unique potential of cell membrane mimics in providing new fundamental insights into the mechanisms by which a viral protein can deform a membrane and facilitate fission.

BINDING OF GLYCOPROTEIN C FROM HERPES SIMPLEX VIRUS-1 TO SURFACE-IMMOBILIZED SULFATED GLYCOSAMINOGLYCANS

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Introduction: Glycosaminoglycans (GAGs) are long, unbranched polysaccharides present at the cell membrane surface where they, among other functions, serve as receptors and attachment factors for various viruses. For example Herpes Simplex Virus-1 (HSV-1) is known to interact with sulphated GAGs on the cell surface via different glycoproteins in its viral envelope. More specifically, glycoprotein C (gC) is responsible for the initial attachment of the virus to the cell.

Method: The binding of gC proteins from two different HSV-1 strains; KOSc and AC1, was studied in a surface-based assay. The two gCs differ in their molecular structure in that one contains a highly charged mucin-like region (KOSc) and the other does not (AC1). The previously developed surface-based assay platform consists of GAGs end-on immobilized to a SPR Biacore surface via biotin-streptavidin coupling chemistry. Three different GAGs were used: non-sulfated hyaluronic acid (HA), synthetically sulfated hyaluronic acid (sHA), and the naturally sulfated chondroitin sulfate (CS). gCs were added to the GAG platform in the Biacore flow system and the binding was studied.

Results: gC from KOSc and AC1 bound only to sulfated GAGs via the expected binding region, as shown by inhibition experiments using an antibody directed against antigenic site II. The equilibrium binding level on the natural GAG CS was higher for gC from AC1 and no dissociation of the protein was seen. This correlates well with previous cell experiments; viruses with gCs lacking the mucin-like region still binds but does not release and hence impairs the virus to successfully infect the cell. Potentially, the mucin-like region modulates the gC-binding to ensure that the virus can both bind and release.

INTERACTIONS OF GII-4 NOROVIRUS LIKE PARTICLES WITH MEMBRANE BOUND FUCOSYLATED HISTO-BLOOD GROUP ANTIGENS

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GII.4 noroviuses (NVs) are well known viral pathogens causing acute gastroenteritis worldwide. We have used a recently described method based on total internal reflection fluorescent microscopy (TIRFM) to study in detail the binding kinetics of vesicles containing type 1 (H type 1, A type 1, B type 1, Lewis a, Lewis b, A Lewis b) and type 2 chain (Lewis x, Lewis y, A Lewis y) fucosylated HBGAs with Ast6139 GII.4 virus-like particles (VLPs) bound to glycosphingolipids-containing plannar supported lipid bilayers (SLBs).

All type 1 chain HBGAs exhibited binding to the Ast6139 VLPs. For type 2 chain structures, there was no binding observed for Lewis x and A Lewis y in the membrane which could be explained by potential presentation effects of binding epitopes. The results obtained from TIRFM binding experiments were in complete agreement with chromatogram binding assay performed with same HBGAs and VLPs. The detachment kinetics data for each type of GSL-containing vesicles revealed that A Lewis b and Lewis a HBGAs bound to NV like particles with the highest and the lowest detachment activation energies, respectively. For the attachment kinetics, Lewis b HBGA was observed to have the highest rate of attachment. The results presented are of relevance for understanding the NV-HBGA interactions at the membrane surface and are helpful to design novel antiviral therapeutics.

PARVOVIRUS B19 RECOGNIZES MEMBRANE ASSOCIATED GLYCOSPHINGOLIPIDS

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Studies have suggested the glycosphingolipid globoside (Gb4Cer) to be receptor for human parvovirus B19. Virus-like particles have been demonstrated to bind to Gb4Cer on thin-layer chromatograms, but a direct interaction between the virus and lipid membrane-associated Gb4Cer has been debated. Here, we have characterized the binding of parvovirus B19 VP1/VP2 virus-like particles to glycosphingolipids (i) on thin-layer chromatograms (TLCs) and (ii) incorporated into supported lipid bilayers (SLBs) acting as cell-membrane mimics. The binding specificities of parvovirus B19 determined in the two systems were in good agreement: the VLP recognized both Gb4Cer and the Forssman glycosphingolipid on TLCs and in SLBs, compatible with the role of Gb4Cer as a receptor for this virus.

In addition, we are exploring the possibilities to use a total-internal-reflection fluorescence (TIRF) microscopy method to assay the binding of parvovirus B19 from patient serum samples to fluorescent vesicles containing various glycosphingolipids.

EXPECTED AND UNEXPECTED CELLULAR FACTORS INVOLVED IN ALPHAVIRUS REPLICATION AND RECOGNITION

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Alphaviruses are mosquito-transmitted positive-strand RNA viruses. They express one non-structural polyprotein which represents precursor of virus-specific replicase proteins. Viral replication organelles are represented by large cytopathic vacuoles, where replicase complexes bind to membranes of endolysosomal origin. In addition to viral RNAs and proteins, these organelles harbor an unknown number of host proteins.

Semliki Forest virus (SFV)-induced replicase organelles were magnetically isolated. SILAC combined with quantitative proteomics was used to reveal 78 distinct cellular proteins that were at least 2.5-fold more abundant in replicase complex-carrying vesicles than in vesicles obtained from noninfected cells. These host components included the RNA-binding proteins PCBP1, hnRNP M, hnRNP C, and hnRNP K. Silencing of hnRNP M and hnRNP C expression enhanced the replication of three different alphaviruses SFV while PCBP1 silencing decreased SFV-mediated protein synthesis. Notably, the effect of hnRNP K silencing was different in case of different alphaviruses.

The first synthesis carried out by alphavirus replicase is synthesis of negative strand RNA which can be recognized by RIG-I and/or MDA5. However, SFV RNA replicase can also induce IFN-β independently of viral RNA replication as it converts host cell RNA into 5'-ppp dsRNA and induces IFN-β through the RIG-I and MDA-5 pathways. These IFN-inducing modified host cell RNAs are abundantly produced during SFV infection. Similar phenomenon was also observed for polymerase of hepatitis C virus suggesting that host cells can restrict RNA virus replication by detecting the products of unspecific viral replicase RdRp activity.

EXPRESSION OF HUMAN PAPILLOMAVIRUS 16 LATE GENES IS AFFECTED BY AKT/PI3K PATHWAY

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HPV16 is known as the causative agent of cervical cancer. The HPV lifecycle progresses depending on the differentiation of a host epithelial cell. In rare cases, the HPV16 infected cells fail to differentiate and are stuck in the early stage, a situation that may give rise to high-grade cervical lesions that can progress to cervical cancer. In addition to a chronic HPV16 infection, genetic alterations in the cellular genome contribute to carcinogenesis. In cervical cancer, the gain of chromosome 3q consistently results in the increased copy number of PI3KCA, a positive regulator of PI3-kinase and a putative oncogene. Here we have investigated if inhibition of the PI3K pathway can activate HPV16 late gene expression.

An in house HPV16 reporter cell line for HPV16 late gene expression named C33A2 was used for our analysis. Activation of HPV16 late gene expression in the reporter cell results in production of sLuc in the cell culture medium. With the inhibitors of various kinases in the PI3K pathway, most notably PI3K, Akt and mTOR, the inhibition of these, in particular Akt, showed a high induction of HPV16 late gene expression in cervical cancer cells.

These results strongly suggest that signal transduction pathways that promote cell proliferation and carcinogenesis, also actively suppress HPV16 late gene expression. Lack of expression of the highly immunogenic viral late structural proteins may be necessary for establishment of chronicity and progression to cancer. We are currently investigating the mechanism of suppression of HPV16 late gene expression by the PI3K pathway.

HUMAN ADENOVIRUS 52 USES SIALIC ACID-CONTAINING GLYCOPROTEINS AND THE COXSACKIE AND ADENOVIRUS RECEPTOR FOR BINDING TO TARGET CELLS

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Most adenoviruses attach to host cells by means of the protruding fiber protein that binds to host cells via the coxsackie and adenovirus receptor (CAR). Human adenovirus type 52 (HAdV-52) is one of only three gastroenteritis-causing HAdVs that are equipped with two different fiber proteins, one long and one short. Here we show by means of virion-cell binding experiments and infection experiments that HAdV-52 can also attach to host cells via CAR, but most of the binding depends on sialic acid-containing glycoproteins. Flow cytometry, surface plasmon resonance and ELISA analyses revealed that the terminal knob domain of the long fiber (52LFK) binds to CAR, and the knob domain of the short fiber (52SFK) binds to sialic acid-containing glycoproteins. X-Ray crystallography analysis of 52SKF in complex with sialylated glycans combined with functional studies of knob mutants revealed a unique interaction as compared to other, known adenovirus:glycan interactions. We believe that these findings may shed light on adenovirus biology and may improve targeting of adenovirus based vectors for gene therapy.

IDENTIFICATION OF HPV16 E5 MRNA

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Cervical cancer is the second most common malignancy in women worldwide and, more than 99% of the tumors contain human papillomavirus (HPV). While the vast majority of all HPV infections are cleared by the immune system of the host, chronicity may be established. Such infection may progress to cervical cancer. Chronic HPV infections continues to express the early HPV genes, in particular, E6 and E7 that drive cell proliferation and prevents apoptosis. The HPV E6 and E7 have been termed viral oncogenes. The HPV E5 protein cause tumors in mice and enhance E6- and E7-driven immortalization and proliferation of human cells in vitro. Thus, E5 may contribute to carcinogenesis but its role in that process remains elusive. As a matter of fact, it is not known when E5 is expressed during the viral infections since the protein has been difficult to monitor. In addition, the E5 gene is not the first open reading frame (ORF) on any of the alternatively spliced mRNAs produced by HPV, raising some doubt about expression during an HPV infection.

The sequence of HPV16 E5 protein is present on many alternatively spliced mRNAs, but is always preceded by other ORFs. To investigate how the HPV16 E5 protein is expressed, we created HPV cDNA plasmids representing all HPV mRNAs encoding E5. These plasmids were transfected into cervical cancer cells and their ability to express E5 was determined. Since an immunological tool that detects E5 is unavailable, we replaced the E5 ORF with the secreted luciferase reporter gene.

By transfecting these cDNAs that reflects the population of alternatively spliced mRNAs, we show that E5 is only produced in significant amounts from one early mRNA on which no larger ORFs are present upstream of E5. This mRNA is produced from the early promoter p97 and is singly spliced between HPV16 SD226 and SA3358. It contains the upstream E6 AUG, but lacks that upstream E1 and E7 AUGs. Knock-out of both E1 and E7 start codons on HPV16 mRNAs that failed to produce E5 resulted in a large increase in translation of E5, demonstrating that these upstream ORFs suppress expression of E5 on the majority of the HPV16 mRNAs. These results were confirmed in the context of the full HPV16 genome, which produced increased levels of E5 when both E1 and E7 AUGs had been inactivated. To lend further support to the idea that the early HPV16 mRNA that is spliced between SD226 and SA3358 is the best producer of E5, we combined optimized splice signals sites in such a way, that production of this mRNA was enhanced in full HPV16 genome. This was done by combining enhancement of the SD226 with a knock-out of SA409, increasing the frequency of splicing from SD226 to SA3358, causing an even stronger increase in E5 translation as this splicing event excludes larger, upstream ORFs. These results identify the early mRNA produced from the p97 promoter and spliced between SD226 and SA3358 as the main HPV16 E5 producing mRNA.

SURFACE-BASED SENSING TO PROBE HERPES-GLYCOSAMINOGLYCAN INTERACTIONS WITH SINGLE PARTICLE SENSITIVITY

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A number of viruses have learned to exploit glycoconjugates present at the cell surface for the initial step of cell infection. One example is the Herpes Simplex Virus (HSV), an enveloped DNA virus, which is recruited at the host surface through interactions between its glycoproteins and cell-surface sulfated polysaccharides, called glycosaminoglycans (GAGs).

In our work we develop surface-based platforms mimicking the cell surface, to study herpes - glycosaminoglycan interactions. A first approach is based on the creation of an artificial cell coat by immobilizing GAGs on a sensor surface [1]. Here, we focus on studying the binding of the HSV-1 virus particles to two different types of GAGs: synthetically sulfated hyaluronic acid and chondroitin sulfate. The aim is to gain insights on binding affinity and specificity to these adlayers. In addition to this, we are developing a second, more complex, sensor platform based on the surface-immobilization of native membranes extracted from cells. This assay will allow the characterization of the initial attachment of the herpes simplex virus to cell membranes in a more native-like environment.

To investigate the interaction between live viruses and cell-surface mimics we use quartz crystal microbalance with dissipation monitoring and total internal reflection fluorescence microscopy. The former technique is used for the characterization of the cell-surface mimic properties, while the latter can probe binding of live viruses with single particle sensitivity, providing detailed information on interaction kinetics.

Taken together, these platforms will provide fundamental understanding on the mechanisms by which the virus recognizes and specifically binds to the cell membrane, giving important insights for the development of anti-viral drugs and vaccines.

[1] Altgärde et al. Acta Biomaterialia, 9(9), 8158–8166.

COMPARABLE MRNA EXPRESSION OF INFLAMMATORY MARKERS IN FORESKIN TISSUE OF HSV-2 SEROPOSITIVE AND SERONEGATIVE ASYMPTOMATIC KENYAN MEN

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Objectives: Skin biopsies from local sites of HSV-2-induced ulcers can show infiltrates of inflammatory cells several months after macroscopic healing. We here hypothesized that foreskin tissue samples of asymptomatic HSV-2 seropositive men had remaining signs of inflammation at the molecular level. Even in the absence of clinical lesions, genital inflammation may contribute to increased HIV susceptibility upon sexual exposure to the virus.

Methods: Tissue samples (n=86) collected from men undergoing circumcision were stratified into study groups based on HSV-2 serology and assessed for mRNA expression of inflammatory markers. Markers of interest were further assessed by immunohistochemical staining within the tissue samples.

Results: The two study groups had comparable levels of all molecular markers (E-cadherin, ZO-1, occludin, CD3, CD4, CD8, CD69, CCR5, HLA-DR, Langerin, DC-SIGN, Mannose Receptor 1, IL-1, IL-6, TNF-α, β7, IgA, IFN-α and CCL5), except for lower mRNA levels of the epithelial junction protein claudin-1 in the HSV-2 seropositive group (p=0.008). Although mRNA levels of claudin-1 were lower in HSV-2 seropositive individuals, the corresponding protein could be visualized in the foreskin epithelium of all samples tested.

Conclusion: Whereas no general inflammation was demonstrated in the foreskin of asymptomatic HSV-2 seropositive individuals, a decreased expression of claudin-1 indicates a less robust genital epithelial barrier. An intact epithelial barrier is essential for blocking mucosal entry of genital infections including HIV.

ENDOTHELIAL DYSFUNCTION DURING PUUMALA VIRUS INFECTION

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Ischemic cardiovascular diseases such as acute myocardial infarction (AMI) and stroke are significant causes of morbidity and death globally. In a recent study (Connolly-Andersen et al. 2014, Circulation) we identified hemorrhagic fever with renal syndrome (HFRS) caused by Puumala virus, as a risk factor for AMI and stroke supporting previous findings that infectious diseases, e.g. influenza, trigger acute cardiovascular events. However, the underlying mechanisms have yet to be clarified.

We hypothesize that viral infections may induce endothelial dysfunction and excessive endothelial surface layer (ESL) degradation leading to coagulopathy and platelet adhesion that increases the likelihood of infarctions in the heart and brain.

In the present study, we have analysed markers for endothelial dysfunction and vascular repair in HFRS patients during the course of infection.

Most of the studied markers were elevated during the acute phase of the infection and associated with disease outcome. Interestingly, syndecan-1, the marker for ESL degradation, was significantly associated with thrombocytopenia, hypoxia, decreased blood pressure and disease severity.

In conclusion, Puumala virus infection causes endothelial dysfunction which is one of the pathogenic mechanisms of HFRS. Furthermore, the degradation of ESL may cause thrombogenic environment facilitating cardiovascular events.

THE ANTERIOR COMMISSURE IS A PATHWAY FOR CONTRALATERAL SPREAD OF HERPES SIMPLEX VIRUS TYPE 1 (HSV-1) AFTER OLFACTORY TRACT INFECTION

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Herpes simplex encephalitis (HSE) is the most common cause of sporadic viral encephalitis in the Western world. Two pathways for viral entry to the central nervous system (CNS) in HSE have been suggested, either via the trigeminal nerve or via the olfactory tract, but this question remains unsettled. Studies of the viral spread between the two brain hemispheres are scarce also in animal systems, but might be of relevance to the location and development of clinical manifestations of HSE.

We have developed a rodent model in which we have investigated the viral spread of HSV-1 in the CNS of rats after intranasal instillation of virus in the right nostril. Rats were sacrificed after 1-6 days post-infection and tissues were analyzed by immunohistochemistry, and viral DNA load was quantified by qPCR.

In this model HSV-1 penetrated lamina cribrosa to infect the mitral cells of the olfactory bulb (OB) on the right side only. Interestingly we found that the anterior commissure, a bundle of nerve fibers extending between the two brain hemispheres, contained clusters of HSV-1 positive cells in oligodendrocytes. The anterior commissure appeared to convey a rapid transmission of virus between the right and the left OB. This made us suggest that the anterior commissure appears to act as contralateral shortcut for transmission of HSV-1 between the OBs and probably also between the olfactory cortices. Earlier studies have indicated an affinity of HSV-1 for the limbic system of the brain, based on MR findings and clinical manifestations in HSE, and our results indicate that the anterior commissure may assist in viral spread to these locations.

CMV-RETINITIS IN AIDS PATIENTS BEFORE COMBINATION ANTIRETROVIRAL THERAPY (C-ART): TREATMENT, COMPLICATIONS AND RELATION TO CMV-ENCEPHALITIS

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Background: Cytomegalovirus (CMV)-infection is an important opportunistic infection in patients with HIV-infection. It is reactivated in these patients when the CD4 count is < 100 x 106/mL. Before the introduction of combination antiretroviral therapy (c-ART) 1996 CMV-retinitis (CMVR) was considered the most common manifestation of CMV-reactivation in these patients. Today CMVR is still the most common cause of blindness in developing countries. However there are no cohort studies correlating it to other CMV-manifestations in patients with HIV.

Methods: At Venhälsan, we followed all patients with HIV-infection from diagnosis till death 1989-1996. When they had CD4 < 100 x 106/mL and got fever and/or other symtoms an investigation for reactivated CMV-infection by CMV-PCR in blood, CMV organ disease and other opportunistic infections (OI) was performed. An ophthalmologic examination was done when they got disturbances of vision. After diagnosis of CMVR they had continuous treatment with foscarnet, gancyclovir or both till they died.

Results: Of the 221 patients that died with CD4 < 100 x 106/mL 84 (38%) got CMVR , 60 unilateral and 24 bilateral. CD4 count at diagnosis was 17. There were no other OI in the eyes besides one patient with toxoplasmosis. CMV-PCR in blood was positive in 94%. After two weeks of treatment 56% improved, 27% had progression, 10% were unchanged and 6% had died. Eventually all progressed. Retinal amotio was seen in 17 (20%) and blindness in 21 (25%). Mean survival time was six months with Foscarnet or Gancyclovir as single drug, but eight months with combination therapy. CMV-encephalitis (CMVE) developed in 46% of the patients. There was no statistical difference between unilateral, bilateral, central or peripheral CMVR and development of CMVE. Other OI of the CNS was lymphoma (10%), toxoplasmosis (5%) and PML (4%).

Conclusion: CMVR was before c-ART a very severe manifestation of CMV infection. Half of the patients also had CMVE. After initial regress on treatment it slowly progressed. CMVE should be suspected and further investigated in all patients with CMVR.

THE VAGUS NERVE AND THE CHOLINERGIC ANTI-INFLAMMATORY PATHWAY ATTENUATES INFLAMMATION IN ROTAVIRUS INFECTION

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While rotavirus (RV) is the major cause of acute gastroenteritis in young children, with pronounced diarrhea, vomiting and lesions of the small intestine, the inflammatory response is most limited. Tracey and coworkers (Science, 2002) reported a paradigm in immunology, that CNS interacts and attenuates the innate inflammatory response through the cholinergic antiinflammatory pathway, a circuit consisting of the vagus nerve, acetylcholine and the α7-nicotinic acetylcholine receptor (α7-nAchR). As RV stimulates the enteric nervous system, enterochromaffin cells, vagal afferents and the vomiting centra in CNS, we raised a novel hypothesis that limited inflammatory response is partly due to stimulation of this pathway.

To assess the role of vagus and α7-nAChRs in the inflammatory response to RV infection three different animal models were orally infected with murine RV; α7-nAChR knockout (KO) mice, vagotomized mice and wild-type (wt) mice treated with a α7- nAChR antagonist. In vitro, mouse intraperitoneally macrophages and human blood macrophages, were stimulated with the RV toxin NSP4 to investigate if the α7-nAChR agonist nicotine can block pro-inflammatory cytokine release from macrophages.

Our results shows that RV-infected vagotomized, α7-nAChR antagonist treated and α7-nAChR KO mice have increased levels of pro-inflammatory cytokines. Vagotomized infected adult mice, had at 48 hours post infection elevated levels of IL-1, TNF-α and IL-6 in serum, and both vagotomized and α7-nAChR KO mice showed elevated levels of IL-6 in all segments (duodenum, jejenum and ileum) of the small intestine duodenum/jejenum, $p \leq 0.05$). Moreover, macrophages, treated with nicotine could attenuate RV toxin stimulated release of TNF-α and IL-6 ($p \leq 0.05$).

These results shows that the cholinergic anti-inflammatory pathway contributes to attenuate inflammation following RV infection, an observation that further supports the gut-brain communication in RV disease.

POSSIBLE CONSEQUENCES OF HANTAVIRUS-MEDIATED RESISTANCE TO APOPTOSIS

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Hantaviruses cause hemorrhagic fever with renal syndrome (HFRS) and hantavirus cardiopulmonary syndrome (HCPS), two human diseases with high case fatality rates. Endothelial cells are the main targets for hantaviruses and vascular permeability is a hallmark of HFRS/HCPS. An intriguing observation in patients with HFRS/HCPS is that the virus infection leads to strong activation of cytotoxic lymphocytes, CD8 T cells and Natural Killer cells, but no obvious destruction of infected endothelial cells. We recently provided a possible explanation for this dichotomy by showing that hantavirus-infected endothelial cells are protected from cytotoxic lymphocyte-mediated killing [Gupta et al., PLoS Pathogens 2013]. Hantaviruses were also able to inhibit chemically induced apoptosis in both endothelial and epithelial cells. When dissecting potential mechanisms behind this phenomenon, we discovered that the hantavirus nucleocapsid (N) protein contains multiple granzyme B sites and at least one caspase 3 site and that the N-protein inhibits the enzymatic activity of both granzyme B and caspase 3. These findings provide a tentative explanation for the hantavirus-mediated block of cytotoxic granule-mediated killing, and hence the protection of infected cells from cytotoxic lymphocytes. These findings may explain why infected endothelial cells in hantavirus-infected patients are not destroyed by the strong cytotoxic lymphocyte response.

Here, novel data regarding possible consequences of the observed hantavirus-mediated inhibition of apoptosis will be presented.

LOCALIZATION, PHENOTYOPE AND FUNCTION OF MAIT CELLS IN THE FEMALE GENITAL MUCOSA

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Background: Mucosa-associated invariant T (MAIT) cells are a recently described innate-like T cell population, which recognize bacterial products and display antimicrobial activity. MAIT cells have been shown to be abundant in blood and in the gut associated mucosa. However, their presence in the female genital tract is unknown. The aim of this study is thus to define number, localization and functional phenotypes, ex vivo, of MAIT cell in the female genital mucosal (FGM) tissue samples.

Methods: Cervical and endometrial tissue samples were collected from women undergoing hysterectomy (n=15). Flow cytometry was used to characterize and enumerate MAIT cells as well as to determine their functional profile. In situ staining was performed to define the distribution of the MAIT cells in the tissue samples.

Results: The majority of the MAIT (Va7.2+, CD161+) cells were CD8+Tcells and they represented about 2% out of the total amount of mucosal T cells, both in the cervical and in the endometrial compartment. MAIT cells furthermore produced high levels of proinflammatory cytokines after E. coli stimulation. Individual MAIT cells were mainly located close to the cervical basal membrane and in the submucosal compartment of the endometrium.

Conclusions: MAIT cells were present in the FGM and they have the capacity to produce cytokines known to be involved in both antimicrobial activity as well as in regulating homeostasis and integrity of the mucosal barrier. Thus, MAIT cells may play a role in defending the barrier integrity and preventing dissemination of local infections in the FGM.

VIPERIN VS. TICK-BORNE ENCEPHALITIS VIRUS: MECHANISMS OF A POTENT ANTIVIRAL PROTEIN

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Tick-borne encephalitis virus (TBEV; family Flaviviridae), is the medical most important arbovirus in Europe and Russia. TBEV has a significant impact on public health as it causes ~10,000 annual cases of encephalitis and meningitis, and no antiviral treatment is available. We are investigating TBEVs interactions with the interferon (IFN) system. Therefore, TBEV was tested for its sensitivity to IFNa pre-treatment, measuring virus replication in cells and virus load in the supernatant. TBEV turned out to be very sensitive to IFN, as viral RNA and titres were reduced by up to three logs after treatment with 100 units IFN alpha. To identify the cellular protein mediating the anti TBEV effect of IFN alpha, a screen of interferon inducible genes was performed. This screen identified viperin as a very strong inhibitor of virus replication.

Viperin is an interferon-induced protein with a broad antiviral activity. However, the mechanism of action is not clear. Since TBEV is extremely viperin sensitive it is a perfect model virus for investigating the antiviral mechanism of viperin. This protein contains a radical S-adenosyl-L-methionine (SAM) domain with a [4Fe-4S] cluster. We have shown that wt viperin requires ER localization for full antiviral activity, interacts with the cytosolic Fe/S protein assembly factor CIAO1, and incorporates 55Fe. We have also set up a number of assays to determine which step of the virus lifecycle that viperin is targeting and screened a set of mutants to determine which domains are responsible for the antiviral activity of viperin.

THE INHIBITORY MOLECULE TIGIT IS EXPRESSED ON CD8+ T CELLS DURING HIV INFECTION

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During chronic viral infections, such as HIV-1, CD8+ effector T cells display a loss of function and up-regulate inhibitory molecules, e.g. PD-1, CD160 and 2B4, a process referred to as T cell exhaustion. Recently a new inhibitory molecule, T cell immunoglobulin and ITIM domain (TIGIT), expressed on T and NK cells was shown to inhibit the function of these cells. We set out to investigate the expression of TIGIT on CD8+ T cells during HIV infection as well as its correlation to markers of T cell activation and exhaustion and the transcription factors T-bet and Eomes.

Samples were gathered from the HIV clinics in Stockholm and advanced multi-color flow cytometry was used to investigate the expression of TIGIT on CD8+ T cells in untreated HIV + individuals (n=39), HIV+ individuals on long-term therapy (n=12) and healthy controls (n=20). Expression of TIGIT was significantly increased on bulk and HIV-specific CD8+ T cells during chronic HIV infection, and correlated with a higher level of T cell activation (CD38, HLA-DR), a specific transcriptional phenotype (T-betdimEomeshi) and markers of T cell exhaustion (PD-1, CD160 and 2B4). Furthermore, TIGIT expression was decreased after long-term therapy, however it did not reach the levels seen in healthy controls.

Our preliminary data shows that the inhibitory molecule TIGIT is up-regulated on bulk and HIV-specific CD8+ T cells during chronic HIV infection, possibly contributing to T cell dysfunction. Investigating the role of this inhibitory molecule is of importance to understand more about the molecular mechanisms concerning T cell exhaustion.

CRIMEAN-CONGO HEMORRHAGIC FEVER REPLICATION INTERPLAYS WITH REGULATION MECHANISMS OF APOPTOSIS

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Pathogenesis of viral hemorrhagic fevers (VHF) is associated with alteration of vascular barrier function and hemorrhage. To date, the specific mechanism behind this is unknown. Programmed cell death and regulation of apoptosis in response to viral infection is an important factor for host or virus survival but this has not been well-studied in the case of Crimean-Congo hemorrhagic fever virus (CCHFV).

In this study, we demonstrated that CCHFV infection suppresses cleavage of poly (ADP-ribose) polymerase (PARP), triggered by staurosporine at early post infection. We also demonstrated that CCHFV infection suppresses activation of caspase-3 and caspase-9. Most interestingly, we found that CCHFV N can suppress induction of apoptosis by Bax and inhibit the release of cytochrome-c from the inner membrane of mitochondria to cytosol. However, CCHFV infection induces activation of Bid at late post infection, suggesting the activation of extrinsic apoptotic signaling. Consistently, supernatant from late post-infected cells stimulated was found to induce PARP cleavage, most probably through the TNF-α dead receptor pathway. In summary, we found that CCHFV has strategies to interplay with apoptosis pathways and thereby regulate caspase cascade. We suggest that CCHFV suppresses caspase activation at early stages of the CCHFV replication cycle, which perhaps benefits the establishment of infection. Furthermore, we suggest that the host cellular response at late post infection induces host cellular pro-apoptotic molecules through the death receptor pathway. External host-derived stimuli most probably initiate the apoptotic process, and the route continues either by crosstalk between the death receptor and mitochondria routes, or separately.

POTENT INHIBITION OF DIVERSE CORONAVIRUSES INCLUDING MERS BY TARGETING OF MEMBRANE-BOUND VIRAL RNA SYNTHESIS

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We screened a collection of 16671 diverse compounds for anti-human coronavirus 229E activity and identified an inhibitor, designated K22, that specifically targets membrane-bound coronaviral RNA synthesis. K22 exerts most potent antiviral activity after virus entry during an early step of the viral life cycle. Formation of double membrane vesicles (DMVs), a hallmark of coronavirus replication, was greatly impaired upon K22 treatment accompanied by near-complete inhibition of viral RNA synthesis. K22-resistant viruses contained substitutions in non-structural protein 6 (nsp6), a membrane-spanning integral component of the viral replication complex implicated in DMV formation, corroborating that K22 targets membrane bound viral RNA synthesis. K22 inhibits a broad range of coronaviruses, including Middle East respiratory syndrome coronavirus (MERS-CoV), and efficient inhibition was achieved in primary human epithelia cultures representing the entry port of human coronavirus infection.

OUTBREAK OF SINDBIS VIRUS INFECTION, NORTHERN SWEDEN 2013

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The mosquito-borne Sindbis virus (SINV) has birds as amplifying hosts. The infection, named Ockelbo disease in Sweden, is characterized by fever, exanthema and arthritis-like symptoms that may persist for years. At the end of August 2013, an unexpected outbreak of SINV disease appeared in Sweden, north of the endemic region.

SINV IgM and IgG were analyzed by immunofluorescence, viral RNA by SINV specific qRTPCR. Patients answered a web-based questionnaire. Mosquitoes were captured from a hotspot region and identification of SINV positive mosquitoes was done by SNP-analysis followed by sequencing the barcoding region of the cytochrome oxidase I (COI) gene.

63 SINV cases were confirmed with an additional 30 probable cases (IgM only). Most cases appeared during 3 weeks (Aug-Sept). 63% were women, 37% men, median age 53 (7-85). Disease symptoms were rash (81%), arthritis (93%), fever (67%). Three months after acute symptoms 29 % had severe ongoing arthralgic symptoms and 21 % reported milder symptoms. During the outbreak 1,600 mosquitoes were captured. We detected SINV RNA in a Culiseta morsitans mosquito and isolated and sequenced a new strain, SINV Lövånger. According to phylogenetic analyses, SINV Lövånger displayed a high degree of similarity to Finnish SINV isolates.

This is the first time a large outbreak of SINV disease has been documented in northern Sweden. The outbreak raises questions regarding the origin of the virus and future surveillance strategies. Furthermore, the weather was unusually warm just before and during the outbreak, favoring conditions for a prolonged mosquito season and stay of migrating birds. Although not a life-threating disease, the outbreak resulted in human suffering, sick leave, concern in the society and, in several cases, a prolonged arthritis.

BIOLOGICAL PROPERTIES OF NOVEL REPLICATION-COMPETENT ADENOVIRUS 11PGFP VECTOR AND STRATEGIES OF THE VECTOR DEVELOPMENT

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In this study, we reported a strategy to introduce a GFP cassette into the E1 region of the full adenovirus 11p genome (RCAd11pe1GFP), and a counterpart vector in which the same insertion was constructed to the E3 region. Kinetic studies showed that RCAd11pe1 initially expressed GFP in A549 cells at 2 hrs p.i., whereas RCAd11pe3 started to express GFP at 4-5 hrs p.i. Expression peaked after 36 hrs p.i. and caused CPE as the Ad11pwt. Dog (MDCK), hamster (CHO) and mouse cell lines (McCoy, C127) manifested different susceptibility to infection by these replicating vectors. The repression of viral DNA synthesis illustrated by a low GFP-expression by RCAd11pe3GFP was attributed to the species-specific properties of mouse or hamster cells. To study the biological properties, GFP expression mediated by RCAd11pe1 and RCAd11pe3 vectors was monitored and implied viral transcription in monkey and human cells. Monkey CV-1 and MA104 cells efficiently expressed higher lever of GFP as like as human A498 cells, in contrast, slightly reduced expressing GFP in Vero and J82 cells were also observed. Vero cells were shown to have greater capacity for adenovirus production than CV-1 and MA104 cells. Furthermore, a ratio of infectious particles to physical particles (IP/PP) produced from Vero cells was comparable to the ratio of IP/PP isolated from A498 cells. In contrast, CV-1 and MA104 cells released ten folds less infectious particles relative to Vero cells. Consequently, all the AMGK cell lines studied can be applied for evaluation of RCAd11p mediated gene delivery and Vero cells have additional preference to be used as a safety cell line to produce RCAd11pGFP virion, in order to avoid a potential oncogen contamination from using carcinoma cells for virion propagation.

STRUCTURAL CHANGES DURING MATURATION AND ACTIVATION OF A RETROVIRAL SPIKE PROTEIN

MATHILDA SJÖBERG

A retroviral infection starts with a membrane fusion event directed by the viral spike protein, Env. In Moloney murine leukemia virus Env is a trimeric complex of a two disulfide linked subunits; the receptor binding surface subunit (SU) and the fusion active transmembrane subunit (TM). The newly synthesized Env matures by two proteolytic cleavages. First, furin cleaves the Env precursor into the SU and TM subunits in the Golgi region of the infected cell and then the viral protease cleaves the R-peptide from TM in newly formed virus. Upon receptor binding a free thiol-group of a CXXC-motif in the SU subunit is activated to attack and break the disulfide bond linking SU to TM. This releases SU and allows the metastable TM to continue folding, resulting in fusion peptide insertion in the cell membrane, and a jackknife like back folding of TM forcing the viral and the cell membrane together and finally complete fusion of the two membranes. We have isolated several intermediate forms of Env, the furin precursor, the R-precursor, the mature and an isomerization arrested state of Env and studied the structures by cryo-electron microscopy and image reconstruction. Together this provides a view of the domain movements during Env maturation and activation.

EFFICIENT REPLICATION OF RECOMBINANT VIRUSES WITH A COXSACKIEVIRUS B5 REPLICATIVE BACKBONE AND P1 REGIONS FROM DIFFERENT ENTEROVIRUS B TYPES

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Phylogenetic studies of enteroviruses have revealed that the capsid genomic region (P1) is type-specific, while the parts of the genome coding for the non-structural proteins (P2-P3) have been shown to be species-specific. The genome may be regarded as two modules that evolve independently. In this study we investigated whether the non-structural coding part of the genome in one type could support replication of a virus with a P1 region from another type of the same species. We used a cassette vector (pCas) contaning a full-length cDNA copy of coxsackievirus B5 (CVB5) as a replicative backbone. The P1 region of pCas was replaced with the corresponding part from other enterovirus B types. The replication efficiency after transfection with clone derived in vitro transcribed RNA was studied and compared to that of pCas. With the exception of echovirus 30, all viruses showed comparable replication capacities to pCas directly after transfection of viral genomes. The echovirus 30 chimera needed adaptation prior efficient replication using the CVB5 replicating backbone. We conclude that the replicative backbone of the CVB5 cassette vector supports replication of interspecies constructs with P1 regions derived from other members of the enterovirus B species.

THE IMPACT OF VECTOR-RELATED IMMUNE ACTIVATION ON VACCINE EFFICACY AND SAFETY

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Vectors composed of, or derived from, bacterial plasmids, RNA, viruses, and even bacteria have been developed as delivery systems for prophylactic vaccines and immunotherapy. The emphasis has been upon utilizing the vector as a means to deliver the gene encoding an antigen or multiple antigens in order to elicit the desired immune response against key parts of the pathogen without having the potential infectivity that could occur if an attenuated version of the pathogen were utilized instead (viz., HIV). While much of the rationale for the selection of different vectors was based upon issues such as insert capacity or ease of manufacture, it has become increasingly clear that the immunogenicity of the vectors themselves and differences in the immunity against the encoded antigens affects not only the efficacy of vectored-vaccine, but possibly even the safety. This presentation will compare the types of immune activation of certain vectors to highlight the issues that should be considered for gene-based vectored vaccines and immunotherapies.

EASY NEEDLE-FREE INTRADERMAL DELIVERY AND VECTOR OPTIMIZATION OF A BROAD PROTECTIVE POLYVALENT INFLUENZA-A DNA VACCINE FOR PIGS

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Influenza vaccines inducing a broad cross-reactive immune response would be of great advantage for improved protection against both seasonal and emerging influenza viruses in humans and pigs. We have developed an alternative influenza vaccine based on DNA expressing 6 selected influenza proteins of pandemic origin (H1,N1,H3,N2,NP,M). Intradermal immunisation with electroporation induced HI antibodies >40 HAI/ml between 7 -10 days after second vaccination in pigs and induced protection against challenge with virus homologous and heterologous to the HA/NA DNA vaccine. Subsequently, we aimed to optimize and ease delivery suitable for pig herds using needle-free delivery to the skin. We successfully adapted our DNA formulation to the IDAL (Intra Dermal Application of Liquids) device (MSD) designed for pigs and obtained antibody responses comparable with those obtained by i.d. electroporation when tested in the rabbit model. We further aimed to enhance the DNA vaccine performance, production yield, and safety by changing our 1st generation DNA vaccine vector backbones (pSSI and wrg7079) to the antibiotic-free 3nd and 4rd generation vectors NTC8385 and NTC9385 (Nature Technologies), respectively. The improvements encourage for clinical trials.

HCV, HBV, AND HDV VACCINES, WHICH ONES DO WE NEED?

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There has been significant advance in the development of vaccines against viral hepatitis in the past three decades. The HBV vaccine protects naive individuals against both HBV and HDV. However, for those infected by HBV there is no protection against HDV superinfection, and there is no good therapy for chronic HDV infections. Although HBV therapy is effective in supressing the viral HBV replication (but not HDV replication), the therapy is lifelong. Thus, new vaccines are needed for both HBV and HDV. For HCV new antivirals has completely changed the landscape. Several clinical trials with therapeutic HCV vaccines have suggested that immune activation may have an effect when combined with the previous standard of care, interferon and ribavirin. However, todays and tomorrows antiviral therapies are highly effective against the dominant HCV strains, with cure rates of up to 95%. However, for certain variants, and certain stages of disease, therapeutic vaccines may still have a role. These issues will be discussed in the current presentation.

GENETIC HIV IMMUNIZATION IN CHILDREN AND ADULTS

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A multigene, multisubtype A, B, C HIV-DNA vaccine (HIVIS) has been used in clinical trials in both children and adults with the aim to improve and broaden the infected individuals' immune responses. Safety data showed good tolerance of the vaccine in children and adults. Neither group experienced either virological failure or a decline of CD4+ counts from baseline. In children, increased HIV-specific cellular immune responses were noted transiently to Gag and RT but not to Env. Compared to baseline, the percentage of HIVspecific CD8+ lymphocytes releasing perforin in the vaccinated group was higher after the vaccination schedule had been completed. In the HIV-infected adults, IFN-γ ELISpot showed improved cellular immune CD8+ reactivity, particularly to Gag peptides; primarily CD8+ responses related to MHC class I alleles were noted.

The present study demonstrates the feasibility, safety and moderate immunogenicity of genetic vaccination in vertically HIV-infected children and adults. The immunological profiles indicated a better CD4+ response in children, paving the way for amplified immunotherapeutic approaches.

RATIONAL SELECTION OF NEW IMMUNOGENS FOR FUTURE HIV-1 DNA VACCINE

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Development of therapeutic and prophylactic HIV vaccines for African countries is urgently needed, but what immunogens to use is a question that needs to be solved. One approach is to use envelope immunogens derived from patients with plasma harboring broad anti-viral activity. Such immunogens could potentially be used in a universal vaccine. A different approach is to construct a regionally tailored HIV vaccine, where the idea is that local immunogens potentially induce higher immunological response to a limited pool of local circulating HIV-1 strains. In order to address if there is any basis for a regional vaccine, we have screened two regionally separate cohorts from the Republic of Guinea-Bissau (RGB) and Denmark (DK) for antibody-dependent cell-mediated cytotoxicity (ADCC) and neutralizing activity against local and non-local circulating HIV-1 strains. Neither neutralization nor ADCC activity demonstrated higher potential against local circulating strains, according to clade determination. However, patient plasma from DK demonstrated significantly higher inhibitory activity than patient plasma from RGB, against both local and non-local virus strains. This suggests that there is no basis for a regionally tailored vaccine. Instead, envelope immunogens derived from patient material with broad inhibitory activity in both neutralization and ADCC might be a better option in future HIV vaccines.

RECONSTITUTION OF THE HIV-1 SPIKE PROTEIN INTO SALIPRO LIMITED NANO-MEMBRANES

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Despite extensive efforts, an HIV-1 vaccine is still elusive. Recent advances in monoclonal antibody cloning from patients have lead to the discovery of numerous highly effective, broadly neutralizing Abs (bNAbs) capable of blocking infection by a very broad array of circulating HIV-1 strains. Notable is that all known bNAbs target the virus glycoprotein trimers (spikes) assembled in the viral lipid membrane and these spike proteins are therefore critical for an antibody-based vaccine.

A grand challenge for the field is to engineer soluble HIV-1 spike mimetics that can be used as vaccine antigens for presentation of natively folded spikes to the immune system. To meet this challenge we have developed a novel approach to reconstitute the HIV-1 spike protein into Salipro limited nano-membranes. The Salipro nanoparticle system has been developed by Jens Frauenfeld, working in Professor Pär Nordlunds research group at KI and we are now setting up a the Salipro-HIV-Spike production together. Very encouragingly, our preliminary data show that it is possible to produce, purify and concentrate Salipro-HIVSpike particles. Significantly, purified Salipro-HIV-Spikes were found to be extremely stable at 37°C with preserved function and structure, as judged by epitope mapping before and after CD4 receptor binding. Future applications within structural biology and the potential as immunogen will be discussed.

THE CHALLENGE DOSE OF HERPES SIMPLEX VIRUS-2 IS CRITICAL FOR PROTECTION AGAINST NEURONAL INFECTION IN A MURINE VACCINATION MODEL

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Herpes simplex virus-2 (HSV-2) infects the genital mucosa and establishes latency into the sensory dorsal root ganglia. HSV-2-infections are common and more than 500 million persons are estimated to be infected. The HSV-2 murine genital model has been the first option to study the effect of vaccine candidates. Despite promising data in the preclinical evaluations of HSV-2 proteins gB-2 and/or gD-2 vaccine candidates, protective immunity could not be reproduced in clinical vaccine trials.

We here addressed the difficulties in the translation of results gained from the murine model to humans. In the vaccination model, genital disease progression and survival are usually used as end-points implying that mice are challenged with a lethal dose of HSV-2. We focused on two issues in the vaccination model: i) Is infection in sensory ganglia and spinal cord a more relevant end-point? ii) Is the challenge dose of HSV-2 of importance for protection of neuronal infection? Mice were vaccinated with the gD-2 protein and adjuvant followed by an intravaginal challenge with 25 × LD50 or 0.25 × LD50 of HSV-2. Although the lethal challenge protocol significantly reduced the levels of HSV-2 DNA, the vaccine did not completely protect from neuronal infection. Using the low challenge virus dose all mice were asymptomatic. Surprisingly, the vaccine did not reduce HSV-2 DNA in sensory ganglia and spinal cord, as compared with unvaccinated controls. We conclude that when using neuronal infection as end-point, data from both animal vaccination models and clinical vaccine trials are consistent; the sub-unit vaccine candidates do not protect from neuronal infection. In humans, the viral dose of HSV-2 sufficient for transmission is unknown. If the results from low dose challenge protocol in mice is relevant for humans, new vaccine strategies are warranted.

ROLE OF COMPLEMENT AND SPECIFIC ANTIBODIES DURING HSV2 INFECTION OF IMMATURE DENDRITIC CELLS AND EPITHELIAL CELLS

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Herpes virus type 2 (HSV2) is one of the most common sexually transmitted pathogens and the estimated seroprevalence in Swedish women is around 11-20%. It causes genital ulcer disease and is a co-factor in the transmission and acquisition of HIV-1. Mucosal DCs are one of the first cell types that encounter HSV2 virus since they are localized in and under the mucosa epithelial layer. So far, only few studies have been performed on the interaction between HSV2 and human DCs. Complement factors are detected in vaginal secretions and they are part of the innate defense that recognize infectious agents. HSV2 can be covered with complement and antibodies that can influence the outcome of immune responses. Additionally, some studies suggested the possibility that pre-existing HSV antibodies play some role in the protecting individuals from the acquisition or clinical manifestation of a subsequent HSV infection.

The main aim of our work will be to elucidate HSV2 and different forms of opsonization of HSV2 (complement and/or specific antibodies) affect immature dendritic cell and cervical epithelial cell (ME180) functionality. For these studies we standardized an in vitro system for immature DC infection by HSV2 and assessing the effect of different types of opsonization, using human serum, seronegative or seropositive for HSV antibodies. As complement- HSV2-DC interactions is unexplored, this work will pave the way for new insights concerning our defense against HSV2 and what is required for the immune defense to hinder the infection.

COMPARISON OF THE MUCOSAL ADJUVANT ENDOCINE™ WITH TWO WELL-KNOWN ADJUVANTS: CHOLERA TOXIN AND ALUM

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To enable efficient mucosal vaccination with split or subunit antigens, an adjuvant is often needed. To date, no mucosal adjuvants have been approved for human use, however, there are a variety of mucosal adjuvants in development, including the liposome-based adjuvant Endocine™. The aim of this study was to evaluate split influenza antigen together with Endocine™ as an intranasal vaccine candidate (Immunose™ FLU). In order to assess the potency of Endocine™ as a mucosal adjuvant, we analyzed the induction of humoral immune responses in mice following vaccination with Endocine™, cholera toxin (CT), or aluminum salt (alum) as adjuvants. We show that Endocine™ significantly enhances influenza-specific immune responses in intranasally immunized mice compared to the nonadjuvanted vaccine. Furthermore, vaccines adjuvanted with Endocine™ evoked comparable serum IgG and virus neutralizing (VN) antibody titers as CT. Compared to alum, Endocine™ triggered significantly higher mucosal and serum IgA titers, and similar VN titers. Taken together, these results suggest that intranasal vaccination with Endocine™-adjuvanted influenza vaccine induces mucosal and systemic immune responses comparable to, or higher than, that of the two well-known adjuvants CT and alum.

FOOT-AND-MOUTH DISEASE VIRUS-INDUCED STRESS GRANULES ARE DISRUPTED BY THE VIRAL L-PROTEASE

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The foot-and-mouth disease virus (FMDV) infects cloven-hoofed animals such as cattle and pigs, and is considered one of the most economically important viruses worldwide. It is an 8 kb positive stranded RNA virus that belongs to the Picornavirus family, with members like polio-, rhino- and hepatitis A viruses.

We found that FMDV induces the formation of G3BP1- and TIA-1 containing stress granules (SGs) early during infection in porcine kidney cells, but that these SGs disperse over time. The transient nature of the SGs is due to the cleavage of the G3BP1 by the viral L-protease (Lpro). This was further confirmed using an infectious FMDV mutant lacking Lpro, which did not cleave G3BP1 and led to the accumulation of SGs in the cells. We then analyzed different, naturally occurring variants of Lpro for G3BP1 cleavage efficiency in a coupled cell-free transcription-translation assay. Full length Lpro, in the context of the downstream capsid proteins, showed the most efficient processing of G3BP1 and, as expected, Lpro active site mutants strongly inhibited the cleavage. Finally, the Lpro-directed G3BP cleavage is not dependent on virus replication, as investigated by transfecting FMDV RNAs lacking a functional viral RNA polymerase.

The fact that other picornaviruses, such as poliovirus, also target G3BP1, but via a different protease (White et al, Cell Host Microbe, 2007), suggests a strong pressure on these viruses to develop countermeasures against the host cell SG response.

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