Abstracts

8th Smögen Summer Symposium on Virology

August 23-25, 2012

Venue: Smögens Havsbad

Arranged by: Swedish Society for Virology

Editor: Kristina Nyström

Introductory address

Thursday, August 23

13:35-13:50

Virology and infectious disease

Jens Ole Nielsen

Department of Infectious Diseases, Copenhagen University Hospital, Hvidovre, Copenhagen

Keynote speaker

Thursday, August 23

13:50-14:35

Intestinal microbiota promote enteric virus replication and systemic pathogenesis

Julie Pfeiffer

Department of Microbiology, University of Texas Southwestern Medical Center, Dallas, TX

Thursday, August 23

14:50-15:40

Epidemiology

Local rhinovirus epidemics may be extended and reflect globally circulating types: A four-year molecular epidemiology study

Martina Sansone

Department of Infectious Diseases/Clinical Virology, Sahlgrenska University Hospital, Gothenburg

Human rhinovirus (HRV) is a highly prevalent pathogen and a major cause of acute respiratory tract infection (ARTI). HRV infection is associated with complications such as exacerbation of asthma and chronic obstructive pulmonary disease, pneumonia, sinusitis and otitis media. HRV express less seasonality than other viral ARTIs, which typically appear as epidemics lasting for 1-2 months. In the present study, we compared HRV-types determined by VP4/VP2 sequencing in respiratory samples from 114 patients over a four-year period with published HRV sequences. HRV-A was found in 64, HRV-B in 11, HRV-C in 37 cases. Overall, 33 different HRV-A types, 9 B types, and 21 C types were found. 21 of the subtypes appeared during several seasons with a maximum time-span of 4 years. Many HRV types appeared during a limited time period, indicating seasonality. Other types were identified during successive seasons, and in some cases phylogenetic analysis indicated extended periods of circulation. Most of the strains were closely related to HRV identified in distant locations during the same time period. Our results suggest that HRV infections are highly globalised with many simultaneously ongoing epidemics. The high frequency of HRV infections may thus be explained by a combination of a broad genetic heterogeneity and the ability of each type to remain in the population over extended periods of time.

Detection of norovirus at high quantities in environmental samples from wards with repeated nosocomial outbreaks of gastroenteritis

Nancy Nenonen[1], Charles Hannoun[1], Thomas Andreasson[1], Lennart Svensson[b], Lars-Magnus Andersson[1], Johan Westin[1], Tomas Bergström[1]

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Norovirus is an important cause of nosocomial outbreaks of gastroenteritis (GE), resulting in high morbidity in patients as well as in staff. As part of a prospective study of such outbreaks in Sahlgrenska University hospital, environmental samples were collected from patient rooms in affected wards, as well as from a control ward without spread of GE. We found that nosocomial infections recurred in several wards, suggesting environmental persistence. Norovirus RNA was repeatedly detected from bedside tables, wash basins and air-vents. Further sampling revealed high quantities of NoV in dust (20/20 samples positive) collected from the top of patient cabinets. Norovirus genotype GII strains predominated, and sequencing revealed similarity to patient GII.4 strains. To further investigate the environmental persistence, a ”virus trap” was employed, collecting charged particles from the air of patient rooms over a period of 3 hours. 6/6 samples were GII-positive, suggesting that air-borne spread of this virus may be possible.

Seroepidemiology, comorbidity and riskfactors for Puumala hantavirus in Northern Sweden

Kristina Bergstedt-Oscarsson*, Alette Brorstad*, Maria Baudin*, Ann Lindberg**, Annika Forssén**, Magnus Evander*, Marie Eriksson** and Clas Ahlm*

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A randomly selected, stratified study population of 1,600 subjects was used to evaluate the prevalence, comorbidity and risk factors of PUUV infection in Northern Sweden.

A health test including a questionnaire was performed, and an enzyme-linked immunosorbent assay (ELISA) against the PUUV nucleocapsid protein was used to test presence of Puumala specific IgG antibodies.

The overall seroprevalence for PUUV in the study population was 13.4%, increasing to 22% in 65-75 years old participants. Identified risk factors were smoking, living in rural areas, agricultural work and owning farmland or forests. No association between previous PUUV exposure as measured by presence of specific IgG antibodies and hypertension, diabetes, asthma, chronic obstructive pulmonary disease, stroke or hospitalisation for acute myocardial infarction was found.

In conclusion, this seroprevalence study supports the hypothesis that Puumala hantavirus infection is increasing in Northern Sweden. Moreover, no long term health effects associated to previous PUUV infection could be found in the present material.

Creating a Baseline for Viral Contamination in Raw Water Using Blue Mussels (Mytilus edulis) as Indicators

Anna-Charlotte Schultz *,Mette Myrmel**, AH Sørensen*, Jakob Ottoson***, Jonas Toljander**** , Ronnie Eriksson**** and Magnus Simonsson****

**National Food Institute, Technical University of Denmark, Denmark*
***Norwegian School of Veterinary Science, Norway*
****National Veterinary Institute, Sweden*
*****National Food Agency, Sweden*

Raw water used for production of drinking water may be contaminated with noroviruses and thereby cause outbreaks of gastroenteritis. Bivalve molluscs, like blue mussels, are filter feeders that accumulate viruses from the surrounding water. Raw oysters and insufficiently cooked mussels contaminated with noroviruses have repeatedly been implicated in outbreaks of gastroenteritis. Direct measurements of noroviruses in water tend to be extremely variable and thereby hamper quantitative microbial risk assessment. However, accumulation of noroviruses in blue mussels can be used as an indirect measurement of the average level of norovirus in the water.

This project aims to create a baseline of norovirus and faecal indicator levels in blue mussels grown in selected marine areas located near raw water sources.

Samples of blue mussels are collected each month for two years from two sampling points in Norway, two in Denmark and one in Sweden. Data for the first year of the study is presented.

Along with the baseline study, a one-year study of the incidence of acute gastroenteritis is conducted in the municipal, Ale, which uses surface water connected to the Swedish sampling point. Samples of mussels are enumerated for noroviruses using real-time RT-PCR detection of viral RNA extracted using proteinase K digestion of shellfish digestive tissue.

Seasonal variation of norovirus levels correlated towards temperature will be presented as well as the correlation of the incidence of acute gastroenteritis in the municipal Ale towards norovirus levels in the Swedish mussels.

Tick-borne encephalitis virus (TBEV) in ticks detached from humans in Sweden and Åland 2008-2009

Pontus Lindblom*, Peter Wilhelmsson*, Linda Fryland*, Sten-Anders Carlsson**, Dag Nyman**, Christina Ekerfelt*, Jan Ernerudh*, Pia Forsberg*, Per-Eric Lindgren*

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In Sweden at present, about 200 cases of tick-borne encephalitis (TBE) are registered each year. During the last decades the number of cases shows an increasing trend at the same time as TBE-cases have spread to new areas. We have collected Ticks, blood samples and questionnaires at 27 and 32 participating helthcare centers in Sweden and Åland from tick-bitten individuals in 2008 and 2009 respectively. Blood samples were collected both when the study subject came to deliver the tick and three months later. The ticks were analyzed using real-time PCR to detect and quantify TBEV, and the blood samples from the tick-bitten individuals were screened for TBEV antibodies. The work flow developed is to a large extent automated. In 2008 and 2009 a total number of 1886 persons participated in the study and they handed in 2188 ticks. The prevalence of TBEV-containing ticks was low both in Sweden and Åland, below 0.4 %.

Poster session

Role of Nora virus VP1 protein in Drosophila melanogaster infection

Sajna Anand Sadanandan*, Jens-Ola Ekström*, Joël van Mierlo**, Ronald van Rij** and Dan Hultmark*,***

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***Department of Medical Microbiology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands*
**** Institute of Biomedical Technology, University of Tampere, Finland*

Nora virus is a picorna-like virus causing persistent, non-pathological infection in Drosophila melanogaster. Nora virus genome consists of four open reading frames (ORF), with ORF 2 encoding the replicative cassette and ORF 4 coding for the capsid proteins. The exact function of ORF 1 encoded VP1 protein is under investigation. Predominantly, immune response against viruses in insects is credited to the RNAi pathway, but previous studies have shown that Nora virus titer in RNAi deficient fruit flies remains unaltered. This suggested the production of a potent virus-encoded RNAi inhibitor. In vitro results indicated that the C-terminus of VP1 encoded this RNAi inhibitor. To confirm these results, titers of Nora virus mutants, harboring mutation or deletion in VP1, were analyzed in wild-type flies and RNAi-deficient flies. The deletions in the C-terminus of VP1 were fatal to virus replication as no viral RNA could be detected in either the wild type or the RNAi-deficient flies. Mutating the four leucines, in the leucine-zipper motif of VP1, to glycines, also proved to be lethal to the virus in both fly stocks. These results indicate that besides the inhibition of RNAi, VP1 is very important in the virus replication cycle.

Poster session

Sensitive method for amplification of full-length HIV-1 *env*, applied to construct pseudo-type virus; CRF02_AG/A3 hybrid and CRF02_AG from plasma collected in Guinea-Bissau, West Africa

Sanne Skov Jensen.[1,2], Rosenstierne M. W.[2], Erlandsson L.[2], Da Silva Té D.[3], C. M. Rodrigues[3], Katzenstein T. L.[4], Da Silva Z.[5], Camara C.[5], Aaby P.[6], Leo-Hansen C.[2], Jensen K. J.[2], Gómez Román V. R. [2], Fomsgaard A [2], Vinner L.[2]

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[3]*National HIV Programme, Ministry of Health, Bissau, Guinea-Bissau;*
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[5]*National Public Health Laboratory, Bissau, Guinea-Bissau;*
[6]*Bandim Health Project, Bissau, Guinea-Bissau*

Characterization of the predominant HIV-1 subtypes, sub-subtypes and circulating recombinant forms (CRFs), will enhance our understanding of viral diversity critical to the informed design of vaccines. A panel of pseudo-type viruses based on local strains can give information about neutralization sensitivity of local viral variants. Understanding the neutralization sensitivity of local strains is important for development of a tailor-made antibody inducing vaccine. We have developed a method that facilitates the characterization of full-length *env* sequence-variation from HIV-1 RNA in plasma. This method can be used to develop a panel of *env* subtypes circulating West Africa.

By using a sensitive RT-PCR method, we have successfully amplified full-length *env* (3Kb) sequence from plasma samples, collected from seven Guinea-Bissau individuals with HIV-1 viral loads of ≥5,700 copies per ml. By phylogeny and recombination analysis, we have identified two subtype CRF02_AG *env* sequences and one CRF02_AG/A3 hybrid from three individuals. We have optimized a pseudo-type virus assay and demonstrated functionality of a CRF02_AG and a CRF02_AG/A3 *env* hybrid. These are the first cloned functional *env* sequences available from Guinea-Bissau which can used for HIV-1 neutralization sensistivity assays.

Poster session

Two novel fusion inhibitors of human respiratory syncytial virus

Anna Lundin[1], Tomas Bergstrom [1], Loubna Bendrioua [1], Nina Kann [2], Beata Adamiak [1], Edward Trybala [1]

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To search for novel drugs against human respiratory syncytial virus (RSV), we have screened a diversity collection of 16,671 compounds for anti-RSV activity in cultures of HEp-2 cells. Two of the hit compounds, i.e., the N-(2-hydroxyethyl)-4-methoxy-N-methyl-3-(6-methyl[1,2,4]triazolo[3,4-a]pht halazin-3-yl)benzenesulfonamide (designated as P13) and the 1,4-bis(3-methyl-4-pyridinyl)-1,4-diazepane (designated as C15), reduced the virus infectivity with IC_{50} values of 0.11 and 0.13muM respectively. The concentration of P13 and C15 that reduced the viability of HEp-2 cells by 50% was 310 and 75muM respectively. Both P13 and C15 exhibited no direct virucidal activity or inhibitory effects on the virus attachment to cells. However, to inhibit formation of RSV-induced syncytial plaques P13 and C15 had to be present during the virus entry into the cells and the cell-to-cell transmission of the virus. The RSV multiplication in HEp-2 cells in the presence of P13 or C15 resulted in rapid selection of viral variants that were approximately 1000 times less sensitive to these drugs than original virus. Sequencing of resistant viruses revealed presence of amino acid substitutions in the F protein of RSV, i.e., the D489G for C15-selected, and the T400I and N197T (some clones) for the P13-selected virus variants. In conclusion, we have identified two novel fusion inhibitors of RSV, and the detailed understanding of their mode of antiviral activity including selection for the drug resistant viral variants may help to develop selective and efficient anti-RSV drugs.

Thursday, August 23

16:10-17:10

Clinical Virology and Diagnostics

Real-time PCR detection of human herpesvirus 1-5 in patients lacking clinical signs of a viral CNS infection

Lena Serrander

Klinisk mikrobiologi, Linköping

Background: Infections of the central nervous system (CNS) with herpes- or enterovirus can be self-limiting and benign, but occasionally result in severe and fatal disease. The polymerase chain reaction (PCR) has revolutionized the diagnostics of viral pathogens, and by multiple displacement amplification (MDA) prior to real-time PCR the sensitivity might be further enhanced. The aim of this study was to investigate if herpes- or enterovirus can be detected in cerebrospinal fluid (CSF) from patients without symptoms.

Methods: Cerebrospinal fluid (CSF) samples from 373 patients lacking typical symptoms of viral CNS infection were analysed by real-time PCR targeting herpesviruses or enteroviruses with or without prior MDA.

Results: In total, virus was detected in 17 patients (4%). Epstein-Barr virus (EBV) was most commonly detected, in general from patients with other conditions (e.g. infections, cerebral hemorrhage). MDA satisfactorily amplified viral DNA in the absence of human nucleic acids, but showed poor amplification capacity for viral DNA in CSF samples, and did not increase the sensitivity for herpes virus-detection with our methodology.

Conclusions: Viral pathogens are rarely detected in CSF from patients without signs of CNS infection, supporting the view that real-time PCR is a highly specific method to detect symptomatic CNS-infection caused by these viruses. However, EBV may be subclinically reactivated due to other pathological conditions in the CNS.

Solid Tissue Induced PCR Inhibition and Nucleic Acid Purification in the BioMérieux easyMAG®

Midori Kjellin, Akofa MacKwashie, Bengt Kallin, Anders Bergqvist and Kåre Bondeson

Uppsala University, Dept. Medical Sciences, Virology, Uppsala, Sweden

Objectives: PCR-quantification of viruses from tissue is often associated with poorer analytical performance due to lower yields in purified nucleic acid (NA) and inhibition of RT-polymerase and of the PCR reaction.

A procedure of diluting input amount of material before NA extraction was tested for validity in ten in-house realtime-PCR assays. We have used the NucliSense easyMAG® (bioMérieux) as the automated extraction platform to assess this method.

Methods:All samples were analysed in parallel, with and without addition of a known amount of viral NA (internal quality control), for each of the ten PCR methods. The spiked samples had their NA extracted and analysed both undiluted and diluted 20-fold to evaluate the yield and presence of any inhibitors.

Comparing results from PCR-reactions with high (1:1) and 20-fold (1:20) lower DNA/RNA content generate information on how diluting the samples influences the analytical sensitivity.

Results: *Effect on Ct-delay*: The delay in Ct-response in solid tissue compared to XPNC ranged from 0,3 – 2,1 and 2,6 – 6,7 ct steps for DNA- and RNA-virus PCR assays, respectively.

Analytical sensitivity: In all PCR methods and tissues tested, except in one case, the effect of using the lower amount of tissue decreased the analytical sensitivity.

Uncertainty of measurement: The standard deviation of measurement decreased significantly on analysis of the lower amounts of tissue. The improved uncertainty of measurement was most evident in the RNA assays.

Conclusion: Applying the strategy of dilution before nucleic acid-extraction from solid tissue resulted in reduced rate of inhibition but as a consequence resulted also in lower analytical sensitivity. However, diluting and thus decreasing inhibition improved precision in quantification of viral loads. Moreover, the reproducibility was improved most significantly in the PCR-methods targeting RNA-viruses.

Evaluation of diagnostic criteria for identification of patients with acute viral gastroenteritis in the emergency department

Thomas Andreasson

Department of Clinical Virology, University of Gothenburg

Ionizing air efficiently prevents infection and spread of airborne-transmitted influenza virus

Marie Hagbom[#1], Johan Nordgren[#1], Rolf Nybom[2], Kjell-Olof Hedlund[3], Hans Wigzell[2] and Lennart Svensson[1]*

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#Contributed equally

While many medical important viruses are spread by air, in small droplets through e.g. vomiting, no simple methodology has yet been described to restrict airborne transmitted infections. In this study, we show for the first time that ionizing air can prevent spread and infection of airborne transmitted influenza virus. In an established animal model for aerosol transmission, using guinea pigs and human influenza A virus (strain Pan-99), a novel ionizing device was shown to effectively prevent spread of influenza virus infection between guinea pigs. When using an active ionizer device, none of the 4 uninfected guinea pigs that were exposed to infected animals became infected, whereas 3 of 4 exposed animals became infected when ionizer was inactive. This device, an ionizer that actively collects particles from air by electrostatic attraction can be used to capture and eliminate a wide range of epidemiologically important viruses and other pathogens from air. Airborne transmitted calicivirus, rotavirus and influenza virus were captured with recovery rates between 0.17 to 21% and with 1600-3700 times more virus detected on active- compared to inactive ionizer after 40 to 90 minutes, in a room of 19 m^3 in size. Virus was identified and quantified using real-time PCR and electron microscopy. This device enables unique possibilities to analyze air and prevent the spread of infectious diseases, which provides a wide medical and clinical application.

HBsAg levels as marker for cccDNA and predictor of long-term virological outcome of chronic HBV infection

Simon Larsson, Gunnar Norkrans, Anders Eilard, Magnus Lindh

Department of Infectious Diseases, University of Gothenburg

Background: Quantification of HBsAg has been proposed as a useful diagnostic marker for clinical staging (identification of inactive carrier state) and prognosis of chronic HBV infection.

Aim: The aims of this study were to investigate the correlation between HBsAg levels in serum and i) cccDNA load in liver tissue, ii) liver disease as measured by histological scoring, iii) long-term outcome in terms of HBV DNA and ALT, and iv) loss of HBsAg.

Methods: In the first part of the study, HBsAg levels in serum (by Abbott Architect) were related to cccDNA in liver tissue measured by real-time PCR (n=84) and to histological score (n=160); in the second part HBsAg levels at baseline were related to HBV DNA, ALT and HBsAg levels at follow-up after 9.1 years (n=124).

Results: Overall, there was a significant correlation between cccDNA and HBsAg level in serum (r^2=0.42, p<0.001), but this was not observed within HBeAg negative patients. At baseline, HBsAg levels correlated significantly with HBV DNA, ALT, histological inflammation score (r^2=0.47, p<0.001, r^2=0.065, p=0.001 and r^2=0.13, p<0.001 respectively) but not with fibrosis (p=0.15). Baseline levels of HBsAg correlated significantly with follow-up levels of HBsAg, HBV DNA and ALT (r^2=0.18, p<0.001, r^2=0.12, p=0.001 and r^2=.054 p=0.01 respectively), but not with loss of HBsAg. An HBsAg level below 3.5 log IU/mL identified minimal liver damage (normal ALT and inflammation score below 6 in cross-sectional analysis) with a predictive value of 77%, and inactive carrier state (HBV DNA< 4 log IU/mL and normal ALT at follow-up) with a predictive value of 27%.

Conclusion: HBsAg levels are associated with cccDNA load, but not within HBeAg-negative stage, and reflect clinical stage and liver disease, but the clinical utility is uncertain, because HBsAg measurements did not add much to HBV DNA quantifications alone.

Multiplex detection of antibodies to many viruses and bacteria. New possibilities for research and diagnosis.

Jonas Blomberg

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Currently, serology is largely run as immunoassays one antigen at a time. The Luminex system now makes it possible to run many immunoassays at the same time, at a relativley low cost. It offers new opportunities to cover large antigen sets in one test, like the human herpesviruses, picornaviruses, parvoviruses, polyomaviruses, retroviruses, tick-borne microbes and mycobacteria. A judicous combination of whole microbe lysates, recombinant proteins and synthetic peptides, plus a multivariate analysis, allows tailor making sensitivy and specificity to suit specific research and diagnostic purpuses. We have explored the possibilites offered by this versatile system.

Thursday, August 23

17:10-17:30

Molecular Pathogenesis

Hantavirus-infection confers resistance to cytotoxic lymphocyte-mediated apoptosis

Shawon Gupta*, Monika Braun**, Nicole D. Tischler***, Malin Stoltz*, Karin B. Sundström*, Niklas K. Björkström****, Hans-Gustaf Ljunggren**, and Jonas Klingström*

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***** Liver Immunology Laboratory, Division of Gastroenterology and Hepatology, Department of Medicine, Karolinska Institutet, Karolinska University Hospital Huddinge, Stockholm, Sweden*

Hantaviruses cause hemorrhagic fever with renal syndrome (HFRS) and hantavirus cardio-pulmonary syndrome (HCPS). Endothelial cells are the main targets for hantaviruses, and increased vascular permeability is a hallmark of HFRS and HCPS. An intriguing observation in patients with HFRS and HCPS is that the virus-infection leads to strong activation of CD8 T cells and NK cells but no obvious endothelial cell destruction. Here, we provide an explanation for this dichotomy by showing that hantavirus-infected endothelial cells are protected from cytotoxic lymphocyte-mediated induction of apoptosis. When dissecting potential mechanisms behind this phenomenon, we discovered that the hantavirus nucleocapsid protein inhibits the enzymatic activity of both granzyme B and caspase 3. Thus, hantaviruses block cytotoxic granule-mediated apoptosis thereby protecting infected cells from cytotoxic lymphocytes. This finding suggests that cytotoxic cell-killing of infected cells are not responsive for HFRS/HCPS-pathogenesis. In a follow up of this finding we have observed previously unknown mechanisms for pathogenesis, involving hantavirus-mediated deregulation of immune cell functions.

Coxsackievirus A24 variant uses O-linked glycoconjugates with sialic acid as cellular receptors on human corneal cells

Nitesh Mistry*, Hirotoshi Inoue*, Fariba Jamshidi*, Rickard Storm*, Yorihiro Nishimura**, Hiroyuki Shimizu*** and Niklas Arnberg*

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***National Institute of infectious Diseases, Musashimurayama, Tokyo, Japan*
****Tokyo Metropolitan Institute of Medicine Science, Tokyo, Japan*

Coxsackievirus A24 variant (CVA24v) is a causative agent of acute hemorrhagic conjunctivitis (AHC), which is a severe and highly contagious eye infection. AHC occurs as a painful conjunctivitis, but keratitis and upper respiratory symptoms have been observed. Since the first AHC outbreak in 1969, it has caused three pandemics and no antiviral drugs are available to treat this infection. CVA24v has been show to replicate in both conjunctival and corneal cells in vivo, but the early events of this virus' life cycle are still unclear. Previously it has been reported that CVA24v use sialic acid as an attachment receptor on corneal cells and that this sugar residue is most likely linked to a protein on the cell surface. To further characterize the CVA24v receptor, we first treated corneal cells with an inhibitor of ganglioside synthase and metabolic inhibitors of O-linked and N-linked glycosylation membrane glycoproteins. Inhibiting N-linked glycans had no or little inhibitory effect of CVA 24v binding. However, a strong inhibition of binding and infection was observed when inhibiting O-glycosylation on corneal cells. These results suggest that the CVA24v receptor on corneal cells is a sialylated, O-linked membrane glycoprotein.

P-selectin glycoprotein ligand-1 (PSGL-1) was recently shown to function as a receptor for enterovirus 71. To test if PSGL-1 also served as a receptor for CVA24v, we quantified virion binding to native L929 cells and to L929 cells that over-express PSGL-1. CVA24v bound significantly better to PSGL-1 positive cells and the binding to both cell lines was completely abolished upon neuraminidase treatment. These results suggest that CVA24v use O-linked sialic acid-containing glycans on PSGL-1 as cellular receptors.

Keynote speaker

Friday, August 24

09:00-9:45

Human retroviruses: Some lessons from the past and current prospects for their control

Robert Gallo

Institute of Human Virology, University of Maryland School of Medicine, Baltimore, MD

Keynote speaker

Friday, August 24

10:00-10:45

HIV envelope immunogen modifications: Rational for vaccine development

Richard Wyatt

IAVI Neutralizing Antibody Center at The Scripps Research Institute, La Jolla

Friday, August 24

11:25-12:35

HIV: Virology, Vaccine and Treatment

Focus on novel quaternary neutralizing HIV-1 epitopes in Env-based immunogen structures

Holland R. Cheng

Molecular and Cellular Biology, College of Biological Sciences, UC Davis

A time-continuous IgG-model infers HIV-1-incidence: infection does not occur at the midpoint between negative and positive tests in cross-sectional data

Jan Albert

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Knowledge about HIV-1 incidence is vital for informed public health decisions, but is difficult to obtain because most patients have an established infection of unknown duration at diagnosis. Previous studies have used HIV-1-specific immunoglobulin G (IgG) levels to indicate if a patient was infected recently, but a time-continuous model has not been available to translate IgG levels to time since infection. Here we present a logistic model of IgG production in an infected patient. We used previously published metadata from 792 patients in 16 cohorts for whom the HIV-1-specific IgG levels had been longitudinally measured using the IgG capture BED enzyme immunoassay. To account for patient variability, we used mixed effects modeling to estimate general population parameters. The typical patient IgG production rate was estimated at r=6.72[6.17,7.33]×10-3 OD-n units day-1, and the carrying capacity at K=1.84[1.75,1.95] OD-n units, predicting how recently patients were infected in the interval ^t=(31,711) days. Using these parameter values, we validated our model on new BED data from a cross-sectional population of 819 Swedish HIV-1 patients diagnosed in 2002-2010. Overall, our trained model had an accuracy of 94% finding a realistic recency period of the patients in the validation data. We found that while time-of-infection in cohort data on average is the midpoint between last negative and first positive HIV-1 test, time-of-infection is strongly skewed towards the first positive sample in cross-sectional data. Using our IgG model we could infer the "true" incidence of HIV-1 in Sweden. In this study we have developed a time-continuous model to estimate the date of infection for patients infected within the past 711 days. This model opens the door to more accurately reconstructing HIV-1 epidemics, e.g., by differentiating between a real outbreak and simply better uptake of previously unknown HIV-1 cases.

HIV-1 recombinant forms (CRF02_AG and A3/AG) are associated with a faster progression to AIDS-related death than non-recombinant sub-subtype A3

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***REGA Institute, Katholieke Universiteit, Leuven, Belgium*
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*****Department of Laboratory Medicine, Lund University, Malmö, Sweden*

HIV-1 group M includes nine subtypes and numerous circulating recombinant forms (CRFs). Different subtypes may differ in biological properties but it is unclear if these differences affect disease progression. We have sequenced the C2-V3 region of 152 HIV-1 infected individuals with estimated dates of infection from Guinea-Bissau, West Africa. Subtype could be determined for 148 of the 152 sequences. Of the 148 individuals, 78 (53%) were infected with CRF02_AG, 42 (28%) with sub-subtype A3 and 23 (16%) with a recombinant form of A3 and CRF02_AG (A3/AG). Five patients (3%) were infected with non-A subtypes and excluded from further analyses. Mean duration of follow-up (± SD) was 6.0 ± 3.2 years. Median time to AIDS onset was 6.8 years for CRF02_AG, 7.3 years for A3, 7.8 years for A3/AG and 6.8 years for the combined recombinant group of CRF02_AG and A3/AG. The median time to AIDS-related death was 11.3 years for A3, 9.0 years for CRF02_AG, 8.2 years for A3/AG and 8.7 years for CRF02_AG and A3/AG combined. Compared to A3, the adjusted HRs for time to death were 2.34 (95% CI, 1.03-5.30, p=0.042) for CRF02_AG, 3.08 (95% CI, 1.09-8.70, p=0.034) for A3/AG and 2.30 (95% CI, 1.08-4.89, p=0.031) for CRF02_AG and A3/AG combined, when adjusting for age and sex in a Cox proportional hazard model. Our results suggest that there are differences in disease progression between different HIV-1 variants, with recombinant forms displaying a faster progression to AIDS-related death.

In situ analysis of CD8+ T cells and NK cells in ectocervical tissue from HIV-1 infected Kenyan individuals

Anna Petrova*, Taha Hirbord*, Joshua Kimani**, Terry B. Ball***,**** , Kristina Broliden*, Annelie Tjernlund*

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***Medical Microbiology, University of Nairobi, Kenya*
****Medical Microbiology, University of Manitoba, Winnipeg, MB, Canada*
*****National Microbiology Laboratory, Public Health Agency of Canada, Winnpeg, MB, Canada*

Background: The majority of all HIV transmissions, world-wide, occur through heterosexual transmission, through mucosal surface of the genital tract. The genital mucosa is considered to be the portal of entry for HIV since and hence it is importance to understand the immunological milieu of the genital tract.

Aim: To define the number, localization and function, ex vivo, of CD8+ T cells and NK cells in genital tissue samples from HIV-1 infected Kenyan individuals in relation to HIV uninfected individuals.

Study cohort and Material: Ectocervical biopsies were collected from HIV seropositive female sex workers and HIV seronegative lower risk women (LR) from Nairoby, Kenya.

Results: Preliminary qPCR data show that HIV-infected women have a significant higher mRNA expression of CD3, CD8 and HLA-DR as compared to the LR group (p=0.03, p=0.0001 and p=0.04 respectively). Further qPCR experiments will be performed to detect IFN-, IL-10, IL-17, IL-22 and NK cells (NKp46). Immune histochemistry will be performed to detect and characterize the CD8+ T cells and NK cells at the protein level and to visualize their spatial location

Conclusion: The level of immune activation at genital mucosa of HIV-infected women is higher as compared to the LR-group. All individual data within the HIV positive group will be correlated to individual viral load.

Low immune activation state in the ectocervical mucosa of HIV exposed seronegative commercial sex workers

Annelie Tjernlund*, Karin Bohman* Joshua Kimani**, Terry B Ball***, ****, Taha Hirbod*, Kristina Broliden*

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Heterogeneity in susceptibility to HIV infection has been demonstrated in several risk groups of HIV-exposed seronegative (HESN) individuals. We hypothesized that a low immune activation state of the cervical mucosa contributes to relative host resistance to HIV infection. Cervical biopsies were collected from HESN commercial sex workers (n=17) and low risk (LR) control women (n=22). Immunohistochemistry analysis showed that the HESN group had significantly lower expression of CD4 cells in the submucosal compartment. At the epithelial level the HESN group had significantly more CD8 cells and FOXP3 cells whereas the expression of CD3, CD45RO and HLA-DR were similar between the groups. The mRNA measurements were concordant with the immunohistochemical staining, except for a higher level of CD25 cells in the HESN group as compared with the LR group. The level of immune activation was further evaluated by mRNA expression of selected pro- and anti-inflammatory immune molecules and cytokines but no significant differences were found between the study groups.

Despite active commercial sex work the HESN women did not have an elevated immune activation state of the cervical mucosa as assessed by selected immune markers and compared with a LR control group. Lower CD4 cell numbers and higher expression of CD25 and FOXP3 in the genital mucosa of these HESN women may contribute to host resistance against HIV infection.

Nanovaccine HIV, preclinical and clinical

Britta Wahren

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HIV plasmid nanovaccines are under clinical development for prophylaxis and treatment of HIV/AIDS. They contain plasmid DNA (pDNA) consisting of only CpG motifs asdjuvant and are developed to be delivered by electroporation to skin cells. To develop a stable, commercializable nanovaccine that maintains the structure and physico-chemical properties of the desired HIV plasmid DNAs, we have developed several plasmid mixtures, encoding either the env or the gag specificities of HIV-1, following a finding of immunocompetence with a full mix of plasmids. However, delivery of these nanoplasmids has permitted overriding both dose limitation as well as immunodominance limitation by delivery of the plasmids by both a needlefree device, Bioject and/or an electroporation device, Dermavax. It is generally accepted that the efficiency of plasmid DNA-based drugs is related to a higher level of structural forms of pDNA. The stability of the plasmid DNA and the extent of its biological activity is the amount of supercoil forms compared to the open circular forms, which are generated due to a chemical or physical degradation force. We therefore investigated the effect of the chemical environment and shearing forces on the stability of biologically active superhelical forms of pDNA. In addition we have been able to study in vivo continuous expression of immune nanoplasmid DNA by IVIS, and the resulting immune reactivity by the plasmid-expressed genes. These concerns resulted in an optimal delivery schedule for gene immunization, in this case anti-HIV immune responses.

Friday, August 24

12:35-12:55

Tumor virology

HPV16 E2 induces late gene expression from HPV16 episomes in primary keratinocytes

Anki Mossberg

Lunds Universitet, Inst Lab med avd med mikrobiologi, BMC B13, Lund

During a persistant human papillomavirus infection, there is continued expression of the early regulatory viral genes, which drives progression to cancer. At the same time, the late structural and highly immunogenic genes are suppressed allowing the infected cell to hide from the immune system of the host. We speculate that inhibition of HPV16 late gene expression is a requirement for establishment of persistence and development of cancer. Viral gene expression is regulated at the level of RNA processing; i.e. splicing, polyadenylation and RNA stability and we have previously identified several cellular proteins involved in this regulation.

We have recently shown that HPV16 E2 induces late gene expression by inhibiting early polyadenylation resulting in a readthrough into the late region of the HPV16 genome. This is a conserved property of E2 of both mucosal and cutaneous HPV types.

E2 inhibits polyadenylation in vitro by preventing the assembly of polyadenylation factors at the polyA signal. E2 binds to members of the cleavage and polyadenylation specificity factor (CPSF) complex and overexpression of the CPSF30 subunit counteracts the effect of E2 in transfected cells. The effect required the N-terminal transactivation domain and hinge domain of E2. Finally, overexpression of HPV16 E2 induced late gene expression from a full-length genomic clone of HPV16 as well as from episomal copies of the genome in primary cells.

Together, these data indicate that E2 induces late gene expression through inhibition of early polyadenylation by targeting the CPSF complex. As overexpression of E1 counteracts the effect of E2, we speculate that accumulation of E2 in the upper layers of an HPV16 infected epithelium during the viral life cycle, not only turns off the expression of the pro-mitotic viral E6 and E7 genes, but also initiates the switch from early to late genes expression once a critical level of E2 is reached.

Splicing inhibitory elements that regulate HPV-16 late 5 -splice site SD3632

Li Xiaose

Lunds Universitet, Inst Lab med avd med mikrobiologi, BMC B13, Lund

Suppression of HPV-16 late gene expression may allow infected cells to escape the immune system. Identification of inhibitory factors of HPV-16 late gene expression may be important to understand the progression of HPV-16 infection to cervical cancer. SD3632 and SA5639 are two splice sites on the HPV-16 genome used exclusively by the late L1 mRNA. We have identified a 238 nucleotide cis-acting RNA element located immediately upstream of SD3632. We have constructed subgenomic HPV-16 plasmids that are driven by a CMV promoter and contain a CAT report gene as surrogate marker for L1. The plasmids contain 238 nucleotides inhibitory element upstream of SD3632, while others contain a variety of mutant sequences. We transfected these plasmids into HeLa cells and measured CAT levels by CAT ELISA. Our results showed that deletion of the 238 nucleotides upstream of SD3632 resulted in high CAT production compared to the plasmids containing this sequence, A 32-nucleotides sequence immediately upstream of SD3632 was the smallest inhibitory sequence. It contained two copies of the AUAGUA. A number of nucleotide substitutions in these motifs alleviated inhibition of SD3632. Introduction of two nucleotide substitutions in each AUAGUA motif in a full length HPV-16 genome resulted in induction of late gene expression from HPV-16 episomes in primary human keratinocytes. We speculate that the AUAGUA motifs are binding sites for cellular factors that regulate HPV-16 L1 expression during the viral life cycle.

Friday, August 24

15:00-15:50

Pathogenesis

Novel reporter for in vivo detection of virus-infected cells

Jens-Ola Ekström*, Emma C. Nilsson**, Sajna Anand Sadanandan* and Dan Hultmark*, ***

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**** Institute of Biomedical Technology, University of Tampere, Finland*

The fruit fly, Drosophila melanogaster, is an important model for innate immunity, but surprisingly few virus studies have been performed in this experimental system. Underdeveloped virological techniques may be one of the reasons. We addressed the fundamental issue of a precise and convenient detection of virus-infected cells inside live fruit flies. For this we constitutively expressed a target to the viral protease in transgenic flies. The target protein is composed of the yeast transcription factor Gal4 fused to a transmembrane domain via a linker with a cleavage site for the viral protease. The transmembrane domain anchors Gal4 in the cytoplasmic space, thus preventing it from entering the nucleus. Upon infection, the linker is cleaved by the viral protease, allowing Gal4 to enter the nucleus to drive the expression of a red fluorescent protein. Using this reporter design we detected infection of two different picorna-like viruses, Nora virus and Drosophila C virus, in live fruit flies. Our reporter opens the possibilities for detailed study of molecular interactions between viruses and the fruit fly, using the wealth of genetic tools available for the Drosophila model. Potentially, it could also be adapted for other model systems, including vertebrates and cell cultures.

Semliki Forest virus non-structural protein 3 sequesters G3BP into viral replication complexes to inhibit stress granule assembly

Marc D. Panas, Gunilla Karlsson Hedestam and Gerald McInerney

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Dynamic, mRNA-containing stress granules (SGs) form in the cytoplasm of cells under environmental stresses including viral infection. Many viruses appear to employ mechanisms to disrupt the formation of SGs on their mRNAs, suggesting that they represent a cellular defence against infection. Here we report that in Semliki Forest virus (SFV) infected cells, the C-terminal domain of the viral non-structural protein 3 (nsP3) binds to Ras-GAP SH3-domain binding protein (G3BP), a cellular protein necessary for SG formation. This binding is mediated by a domain of previously unknown function in the C-terminus of nsP3 which contains two 7 amino acid repeat motifs, L/ITFGDFD and is conserved in the Old World alphaviruses. Early in SFV infection, G3BP is sequestered by nsP3 into viral RNA replication complexes in a manner that inhibits the formation of SGs on viral mRNAs. A viral mutant carrying a C-terminal truncation of nsP3 induces more persistent SGs and is attenuated for propagation in cell culture. We also show that the efficient translation of viral mRNAs containing a translation enhancer sequence also contributes to the disassembly of SGs in infected cells, even in the absence of the nsP3/G3BP interaction. Further, we show that the nsP3/G3BP interaction alone also blocks SGs induced by other stresses than virus infection. This is one of few described viral mechanisms for SG disruption and underlines the role of SGs in anti-viral defence.

TLR3 expression and susceptibility to HSV-2

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Lack of TLR3 functional activity predisposes children to HSV-1 encephalitis and mice to HSV-2 meningoencephalitis. In this study, we evaluated if there is any link between TLR3 expression patterns and the susceptibility to HSV-2 infection in humans. Firstly, we studied genetic variations in 239 patients with genital HSV-2 infection and 162 uninfected controls. Two SNPs in the *TLR3* gene (rs13126816 and rs3775291) were associated with an enhanced expression of TLR3 mRNA and a reduced incidence of HSV-2 infection, which suggests that polymorphisms in TLR3 confer natural resistance to HSV-2 in humans. Secondly, since human newborns are highly susceptible to HSV-2 infection, we investigated if they have an impaired TLR3 expression profile. Blood mononuclear cells from adults expressed high levels of TLR3 mRNA, particularly in NK cells. Cord NK cells on the other hand had low to undetectable TLR3 mRNA levels and no detectable TLR3 protein expression. The cytotoxic function of cord blood NK cells was impaired and cord NK cells (as opposed to adult NK cells) did not respond to the TLR3 ligand poly(I:C), showing that the immune system of newborns does not react normally to dsRNA. There were no differences in the TLR3 mRNA levels between men and women, including pregnant women, implying that TLR3 is not under sex hormonal control. However, decidual NK cells were also devoid of TLR3 mRNA, suggesting that TLR3 expression might be modulated locally in the pregnant uterus. Taken together, these data suggest that individuals with low TLR3 levels are more susceptible to HSV-2, and that newborns are particularly poor at expressing TLR3.

Hantavirus RNA and specific antibodies in patients with hemorrhagic fever with renal syndrome

Lisa Pettersson*, Therese Lindgren**, Joacim Rocklöv***, Johan Hansson**, Magnus Evander*, Clas Ahlm**

**Department of Clinical Microbiology/Division of Clinical Virology*
***Division of Infectious Diseases, Norrlands University Hospital and Umeå University*
****Department of Public Health and Clinical Medicine; Umeå University*

Objectives: Members of the genus Hantavirus (family Bunyaviridae) are rodent-borne pathogens, and virus is transmitted to humans by inhalation of infected rodent excreta. In parts of Europe, Puumala virus (PUUV) is endemic in bank voles (Myodes glareolus). PUUV infection in humans cause nephropathia epidemica (NE), a relatively mild form of hemorrhagic fever with renal syndrome (HFRS). The pathogenesis of HFRS, e.g. the role of viral load and immune response, is still not completely understood. To determine whether levels of antibodies and RNA is a valuable clinical tool to predict disease outcome we wanted to investigate the duration and level of viremia and antibody response correlate to clinical picture, and disease severity in HFRS patients.

Methods: In this prospective study 105 verified HFRS patients were followed in regards to symptoms, clinical outcome, and different laboratory parameters. PUUV RNA level in plasma was measured with real-time RT-PCR, PUUV specific primers and probe from the S-segment was used. The antibody response was measured using immunofluorescence test (IF) for PUUV specific IgM and IgG.

Results: All patients´ first serum samples were analyzed with IF; 93% were IgM positive, 86% were IgA positive and 80% were IgG positive.

90% of the samples collected at day 0-3 after onset of disease were PUUV RNA positive (mean 1.2 x 105 PUUV RNA copies/ml). 81% of the patients were RNA positive day 4-7. . In some patients the viremia lasted for more than 2 weeks, up to 20 days after disease onset (even after day 20 a couple of samples was low positive).

A low platelet count (below 50 x 10¬9/L) was associated with a significantly lower IgG titer. This significance remained when adjusted for age and sex.

More data on antibody and viral RNA kinetics in associations to clinical symptoms will be presented.

Conclusions: A vast majority of patients have detectable RNA in their plasma samples, it implies that an ongoing virus infection and replication as well as the immune response

can be of importance for the pathogenesis of NE. The level and duration of viral load implicate that effective antiviral treatment might be beneficial in PUUV infection. Both serology and PUUV PCR are valuable diagnostic tools to confirm HFRS.

CMV-associated adrenalitis and ACTH-stimulation test in AIDS patients

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****MTC, KI, Stockholm*

Background: Cytomegalovirus (CMV)-infection is an important opportunistic infection in patients with immunodeficiency. In HIV-infected patients CMV-infection is often diagnosed when the CD4-count is < 100 x 106/L. CMV-adrenalitis is a common CMV-manifestation but seldom diagnosed before death.

Patients and Methods: We have followed all patients with HIV-infection from diagnosis till death from 1989-1996 at our clinic. All patients with CD4-counts below 100 x 106/L were investigated for CMV-adrenalitis, as soon as they had fever, fatigue, and if they had other manifestations of CMV-disease. CMV was diagnosed by PCR-technique, pp65-antigen detection, and/or virus isolation in peripheral blood. Adrenalitis was diagnosed by ACTH-stimulation test (Synactentest), and other diseases according to symptoms. At autopsy the adrenals and other organs were investigated by hematoxylin and eosin staining and immunohistology. The features of CMV-infection in the cortex and the medulla of the adrenals was evaluated by a standardized protocol. All patients were treated with cortisone after pathological Synactentest and later received treatment with Foscarnet or Ganciclovir.

Results: In 217 of 221 (98%) of the patient , who were CMV-seropositive, 180 (83%) had detectable CMV polymorphonuclear cells. Synactentests were performed in 142 patients and of these 82 (58%) were pathological. Adrenals were analysed postmortem in 46(22%). Of these 46 adrenals 32 (67%) showed inflammation, typical CMV-infected cells and atrophy of both marrow and cortex. They were also positive by immunohistochemistry of the adrenals. 29 with atrophy also had a pathological Synactentest. Three patients with presence of only inflammation and CMV-infected cells had normal or borderline Synactentests.

Conclusion: Synactentest is a useful tool in diagnosing CNV adrenalitis. The normal Synactentest in patients with severe inflammation suggests a clinical course of the CMV-infection with inflammation, destruction and then atrophy of the adrenal glands.

The findings were typical although all had antiviral therapy against the CMV-infection before death.

Friday, August 24

15:50-17:20

Viral Immunology

Galectins, glycoprotein cross-linkers and guides of intracellular trafficking: what could they do in virus infection?

Hakon Leffler*, Enas Sheik Kahlil**, Tavan Fattah*, Michael Carlsson*, and Marianne Jansson**

**Section Microbiology, Immunology, Glycobiology (MIG), and ** Section Medical Microbiology, Department of Laboratory Medicine, Lund University*

Galectins are small soluble proteins with affinity for β-galactoside-containing glycoconjugates. They are synthesized and reside in the in the cytosol, separated from their potential ligands by the plasma membrane or vesicle membranes. Upon an unknown signal they can cross either or both of these membranes by a non-classical secretory mechanism. By this they gain access to glycoproteins, which they may cross-link, with various functional results. One relevant for virus infections is to enhance interaction between a particle (virus) coming from outside binding to the cell surface, as shown for galectin-1 and HIV. Another is to direct intracellular trafficking of glycoproteins (as shown in many studies) or the virus particle after uptake into the cell. A third is reaction between cytosolic galectins and luminal glycoproteins after lysis of endocytic vesicles (as shown for bacteria, but maybe also by virus). Other effects include induction of signaling and of apoptosis by cross-linking cell surface receptors, which may contribute to bystander effects and to immune regulation (desired or aberrant) during virus infection.

Methods for characterization of the viral o-glycopeptidome

Ola Blixt

Department of Cellular and Molecular Medicine, Faculty of Health Sciences, University of Copenhagen

Glycans on viral envelope proteins play important roles in attachment and entry to host cells as well as viral protein folding, formation of infectious particles, and shielding from host's immune system. Most functions have been attributed to N-linked glycans of viral glycoproteins whereas the role of O-glycosylation remains poorly explored. By employing the state-of-the-art Zink Finger Nuclease gene targeting technologies (1) and synthetic glycopeptide microarrays (2) we are able to systematically analyze the site-specific O-glycosylation patterns of viral glycoproteins as well as immune responses to these epitopes respectively.

(1). Mining the O-glycoproteome using zinc-finger nuclease-glycoengineered SimpleCell lines. Steentoft C, Vakhrushev SY, Vester-Christensen MB, Schjoldager KT, Kong Y, Bennett EP, Mandel U, Wandall H, Levery SB, Clausen H. Nat Methods. 2011;8(11):977-82.

(2). Characterization of the viral o-glycopeptidome: a novel tool of relevance for vaccine design and serodiagnosis. Cló E, Kracun SK, Nudelman AS, Jensen KJ, Liljeqvist JÅ, Olofsson S, Bergström T, Blixt O. J Virol. 2012; 86(11):6268-78

Soluble CD26 Impacts Treatment Outcome and Functionality of HCV-Specific T Cells in Chronic Hepatitis C Virus Genotype 1 Infection

Jonas Söderholm[1*§], Jesper Waldenström[1*], Massimo Pilli[2], Pierre-Yves Bochud[3], Francesco Negro[4], Galia Askarieh[1], Jean-Michel Pawlotsky[5], Stefan Zeuzem[6], Carlo Ferrari[2], Avidan Neumann[7], Bart L. Haagmans[8], Gabriele Missale[2], Kristoffer Hellstrand[1], and Martin Lagging[1] for the DITTO-HCV Study Group.

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Background & Aims: Combination therapy with interferon and ribavirin for chronic hepatitis C virus infection entails sustained virological response (SVR) rates of 50-80%. Several factors such as non-1 genotype, beneficial *IL28B* single nucleotide polymorphisms (SNPs), low baseline IP-10, and a higher frequency of functional HCV-specific T cells are predictors of SVR. With the recent introduction of Direct-Acting Antiviral (DAA) agents, new biomarkers potentially may be useful for tailoring treatment. Furthermore, membrane-bound CD26 expression previously has been implicated to be a marker of a T helper I–skewed immune response.

Methods: Baseline plasma from 153 genotype 1 patients enrolled in an international multicenter HCV phase III treatment trial (DITTO-HCV) previously analyzed for pretreatment plasma IP-10 concentration and *IL28B* polymorphisms, were evaluated regarding baseline soluble CD26 (sCD26) concentration. Additionally the functionality of HCV-specific $CD8^+$ T cells was evaluated by quantifying interferon-gamma (IFN-γ) positive cells after stimulation with HLA-A2 or HLA-A3 restricted HCV genotype 1a peptides.

Results: Genotype 1 infected patients achieving SVR had lower sCD26 concentration (P=.001) compared with those who did not. Interestingly, an inverse correlation between baseline sCD26 concentrations and pretreatment HCV-specific IFN-γ^+ CD8 T cells was also noted (r_s=-0.65, P=.02).

Conclusions: Lower baseline sCD26 concentration predicts favorable treatment outcome, possibly secondary to being associated with the HCV-specific $CD8^+$ T cell functionality.

TLR7/8 stimulation protects human myeloid dendritic cells but not monocyte-derived dendritic cells from Influenza A virus infection

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Dendritic cells (DCs) play a crucial role in the initiation of immune responses that control infections including Influenza A virus (IAV). IAV is an important human pathogen that primarily targets respiratory epithelial cells. However, also residing immature DCs are potential targets of IAV. During infection, respiratory DCs are exposed to pro-inflammatory cytokines and mature, a process that modifies the phenotype and function of DCs. We wanted to address whether the maturation status after toll-like receptor (TLR) stimulation of different human DC subsets affected their susceptibility to IAV. We showed that immature myeloid DCs (MDCs) are highly susceptible to IAV. Here, we found that immature monocyte-derived DCs (MDDCs) were also readily susceptible to infection. However, upon maturation MDDCs were partially protected against IAV infection after LPS (TLR4) stimulation but not after TLR7/8 ligand (7/8L) stimulation. In contrast, MDCs showed reduced susceptibility to IAV infection after maturation with LPS or 7/8L. LPS stimulation induced expression of the type I interferon susceptible gene MxA in both MDDCs and MDCs, while 7/8L stimulation only upregulated MxA in MDCs. The difference between IAV infections in LPS- vs. 7/8L-matured MDDCs could be that TLR4, unlike TLR7/8, signals through TRIF, a pathway known to induce potent type I interferon response. Our data suggest that MDDCs and MDCs respond differently to TLR stimulation thus showing different susceptibility to viral infection, which may have implications for the design of DC-based immunotherapy.

Mouse models for Hepatitis C with HCV RNA replication

Matti Sällberg

Clinical Microbiology, F68, Karolinska Univ Hospital, Huddinge

Will present new data on mouse models for HCV.

Saturday, August 25

09:00-10:05

Antivirals and Vaccines

Therapeutic vaccination of untreated HIV-1 infected individuals in Denmark and Guinea-Bissau: two phase I/II studies

Anders Fomsgaard [1], Karlsson I [1], Roman-Gómez VR[1], Jensen KJ [1], Jensen SS [1], Leo-Hansen C [1], Brandt L [1], Jespersen S [2], Da Silva Té D [3], Rodrigues CM [3], Janitzek CM [1], Vinner L [1], Katzenstein TL[4], Andersen P [5], Kromann I [6], Andreasen LV [6], Gerstoft J [4], Kronborg G [7]

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[7] *Department of Infectious Diseases, University Hospital Hvidovre, Denmark*

We designed a universal vaccine composed of conserved subdominant CTL epitope peptides restricted to the 5 most common HLA-supertypes. The GMP vaccine produced is based on 18 predicted epitopes (15 CTL epitopes plus three T helper epitopes) mixed with a novel SSI adjuvant CAF01. Two single-blinded phase I/II therapeutic HIV-1 vaccine trials were conducted; one in Denmark (11 patients) and one in Guinea-Bissau (18 patients). Treatment-naïve HIV-1-infected individuals >18 years of age where immunized i.m. (week 0, 2, 4, 8) and followed for 6 months. Placebos received saline. T cell responses were evaluated by flow cytometry (IFNg ;TNFa, IL-2, MIP-1b and CD107a) and/or ELISpot (IFNg).

The vaccine was safe and well tolerated. One non-vaccine-related serious adverse event was reported in the Guinea-Bissau study. No overall or sustained changes in viral-load or CD4+ T cell counts were observed; however, new T cell responses specific for one or more vaccine epitopes were induced. We show that it is possible to generate new T cell responses in treatment-naïve HIV-1-infected individuals using peptides in adjuvant and thereby redirecting immunity to target selected subdominant conserved CTL epitopes. Moreover we demonstrate that it is possible to design a vaccine that is immunogenic in two very different geographic regions, West-Africa and Northern Europe. This broad T cell vaccine is an important proof-of-concept for further development.

2-[4,5-Difluoro-2-(2-fluorobenzoylamino)-benzoylamino]benzoic Acid, an Antiviral Compound with Activity Against Acyclovir-resistant Isolates of HSV-1 and -2

Mårten Strand*, Md. Koushikul Islam*, Karin Edlund*, Christopher T. Öberg**, Annika Allard*, Tomas Bergström***, Ya-Fang Mei*, Mikael Elofsson** and Göran Wadell*

** Department of Virology, Umeå University, Umeå, Sweden*
*** Department of Chemistry, Umeå University, Umeå, Sweden*
**** Department of Virology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden*

Herpes simplex viruses (HSV-1 and HSV-2) are responsible for life-long latent infections in humans, with periods of viral reactivation associated with recurring ulcerations in the orofacial and genital tract. In immunosuppressed patients and neonates, HSV infections are associated with severe morbidity, and in some cases even mortality. Today, acyclovir is the standard therapy for management of HSV infections. However, the need for novel antiviral agents is apparent since HSV isolates resistant to acyclovir therapy are frequently isolated in immunosuppressed patients. In this study, we assessed the anti-HSV activity of the anti-adenoviral compounds 2-[2-(2-benzoylamino)-benzoylamino]benzoic acid, (Benzavir-1) and 2-[4,5-difluoro-2-(2-fluorobenzoylamino)-benzoylamino]benzoic acid, (Benzavir-2) on HSV-1 and HSV-2. Both compounds were active against both viruses. Importantly, Benzavir-2 had similar potency to acyclovir against both HSV types and it was active against clinical acyclovir-resistant HSV isolates.

Anti-HSV-2 activity of PG545 in a mouse model of genital herpes simplex virus type 2 infection

Joanna Said, Edward Trybala, Staffan Görander and Tomas Bergström

Department of Infectious Diseases/Clinical Virology, Sahlgrenska Academy, University of Gothenburg

In humans herpes simplex virus type 2 (HSV-2) infects epithelial cells of the genital mucosa, and then via sensory nerve endings establishes latent infection in dorsal root ganglia. Moreover, genital HSV-2 infection is associated with an increased risk of human immunodeficiency virus type 1 (HIV-1) acquisition. We have previously shown that the lipophile-conjugated sulfated oligosaccharide PG545 effectively inhibited infection of both HSV-2 and HIV-1 in cell culture and that the compound exhibited virucidal properties in vitro. To elucidate the effect of PG545 in vivo, a model using genital HSV-2 infection in C57BL/6 female mouse was selected. Two main experiments were conducted (i) HSV-2 (1×10^5 pfu) was treated with 10 μg (500 μg/mL), 2 μg or 0,4 μg of PG545 15-20 min prior to vaginal administration of the virus/compound mixture, and (ii) PG545 (500 μg/mL) was administered at three different time points (-2 h, 0 h, +2h) relatively to HSV-2 vaginal infection. In (i) 10 and 2 μg completely protected mice (n=7 per group) from HSV-2 infection as revealed by the absence of both vaginal inflammation and CNS infection throughout the 3 weeks observation period, and the lack of infectious virus and viral DNA in vaginal lavages collected 3 days after infection. Mice that recieved HSV-2 pre-treated with 0.4 μg of PG545 developed no symptoms from CNS infection and survived the observation period, however 5 out of 7 exhibited a transient vaginal inflammation. Mice in the control group (no PG545) were euthanized at days 6-9 post-infection. In the time-of-addition experiment (ii) 3 mice out of 8 survived in the group which was infected with HSV-2 just minutes (0 h) before administration of PG545. In the group that recieved PG545 at 2 h after HSV-2 inoculation 1 mouse out of 8 survived the infection. All 8 mice in the group that were treated with PG545 before (-2 h) HSV-2 infection were sacrificed 9-11 days post-infection as opposed to the controls (8 mice) that were euthanized 7-9 days after HSV-2 inoculation. These results suggest that the prior short incubation of HSV-2 with PG545 inactivate viral infectivity and completely protect mice from genital HSV-2 infecion, while treatment of mice with PG545 before and after viral infection delays the developmet of the disease. These data warrants further investigation of PG545 as a microbicide against genital HSV-2 infection.

Evolution of Endocine™, a nasal adjuvant, together with influenza A vaccination - clinical and pre-clinical results

Tina Falkeborn

IKE, Institutionen för klinisk och experimentell medicin, Molekylär Virologi Linköpings Universitet

Production of foot-and-mouth disease virus capsid proteins using the Semliki Forest virus expression system

Maria Gullberg*, Gerald McInerney**, Graham J. Belsham* and Charlotta Polacek*

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During a foot-and-mouth disease virus (FMDV) infection, the virus-encoded 3C protease (3Cpro) cleaves the structural protein precursor P1-2A into the functional capsid proteins VP0, VP1 and VP3 (and 2A). These structural proteins can assemble into empty capsids, which could potentially be used as non-infectious vaccines and diagnostic reagents, circumventing the handling of large amounts of infectious virus. In this study, cDNA expression cassettes were constructed, which encode P1-2A (from FMDV serotypes O and A) and 3Cpro as either P1-2A-3C, or with the 3Cpro expression dependent on the FMDV IRES (P1-2A-IRES-3C). Since the 3Cpro can be toxic for the cells, we used constructs with either a mutant 3Cpro that has reduced protease activity or a defective IRES (the wild-type GNRA tetraloop sequence GCGA converted to GTTA) to obtain an optimal relative expression level. To obtain efficient co-expression of the FMDV proteins, we used the single-round infectious Semliki Forest virus (SFV) expression system. The FMDV cDNA sequences were inserted into a vector encoding the SFV non-structural proteins, which in combination with helper vectors that expresses the structural proteins constitute the SFV expression system. RNA transcripts from these constructs were then co-transfected into baby hamster kidney cells, in which recombinant SFV particles were produced. These virus-like particles were able to perform a single round of infection, during which the FMDV capsid precursor (P1-2A) plus 3Cpro were expressed and correctly processed VP0, VP3 and VP1 were obtained. These results contribute towards the development of improved, safe, FMDV vaccines and facilitate future studies on capsid assembly.

Are host genetic factors the reason for low efficacy of rotavirus vaccines in Africa?

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Rotavirus (RV) is the most common cause of severe acute diarrhea in young children and causes ~230,000 deaths in Africa every year. Due to the high mortality and morbidity of the disease, WHO has recommended the inclusion of two licensed RV vaccines, Rotarix and RotaTeq, in the national immunization schedule of vulnerable countries. However, recent studies have demonstrated low efficacy of these vaccines (~18-60%) in many African countries and the reasons are as of yet unknown.

Through a study investigating viral pathogens in children with diarrhea in Burkina Faso, we have obtained strong indications and raised a hypothesis that human histo-blood group antigens (HBGAs) contribute to susceptibility for common RV strains. As of yet, no host genetic factors have been associated with resistance to human RV infections, nor have *in vivo* receptors for RV been identified. The RV VP4 protein (P-genotype), which is essential for virus attachment and entry, was recently shown to bind to human HBGAs *in vitro* which further strengthen our hypothesis.

In Burkina Faso, we found a high prevalence (34%; 41/122) of the Lewis-negative phenotype (Le^{a-b-}) in children which is rare in Europe and North America (3-5%). Children having the Lewis-negative phenotype were only infected with RV of genotype P[6] which is a regionally common genotype (17/17; $p<0.01$). The globally common RV P-genotype (P[8]) which constitute the basis for the current two RV vaccines, were common in Burkina Faso (58%) but most surprisingly did not infect Lewis-negative children (0/17; $p<0.01$), providing a plausible explanation for the lesser efficacy of the current vaccines in Africa. We are now further investigating this observation with functional *in vitro* studies. Identification of host genetic factors associated with resistance to RV infection and vaccination is most urgent and merit further investigations.

Saturday, August 25

10:05-10:35

Molecular and Structural Biology

How old are the HBV genotypes? Data obtained using the relaxed clock model.

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Background: It may be assumed that the phylogenetic relation between the different hepadnaviruses reflects that of their respective host species, since the host species involved will with time become too divergent to allow cross species transmission. This was used in the present approach. However, this is apparently not valid for the HBV genotypes of the higher primates considering that genotypes F and H represent an earlier branch than the ape hepadna viruses.

Methods: Modeltest was used for identifying general time reversible (GTR) as the suitable method for estimation of genetic distances of complete hepadnaviral genomes the Paup and Puzzle programs were used for determining the GTR distances. The lognormal relaxed clock model in the beast program package was used to trace the time for the major HBV genotype bifurcations. Thereby we made use as reference points the estimated time for the splits between mammals/reptiles-birds, primates/rodents and old/new world monkeys being 310, 65 and 40 Myr respectively.

Results: We confirmed our previous finding of genotypes F/H making up the first split from the human/ape HBV clade and had occurred already 10-14 Myr ago. The next split between genotypes A/B/C on one side and D/E on the other occurred 8-12 Myr ago. Genotype A diverged from B and C 6-11 Myr ago while the two latter diverged 5-8 Myr ago. With this calculation D and E diverged 4-7 Myr ago. In this tree all ape HBV were monophyletic. However, using other parameters gibbon and orangutan HBV split of before homo, pan and gorilla HBV. The former apes are plant feeders why HBV is less likely to cross their species barriers, why these splits are in agreement with current taxonomic classification.

Conclusion: HBV is older than homo sapiens why the genetic diversity of HBV is explained most likely by our species having acquired HBV strains diversity from hominid predecessors.

Maturation cleavage of the retrovirus Mo-MLV spike precursor splays the transmembrane subunits to prime it for receptor triggering

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**equal contribution*

The spike protein of murine leukemia virus, MLV, matures by two cleavage events. First cellular furin separates the receptor binding SU subunit from the fusion active transmembrane subunit, TM and then, in the newly assembled particle, the viral protease removes a 16 residues peptide, the R-peptide, from the endodomain of TM. Both cleavage events are required to prime the spike for receptor triggered activation. Cryo-electron microscopy (cryo-EM) analyses have shown that the mature spike forms an open cage-like structure composed of three SU-TM complexes. In this the SU subunits formed a side lobe with its C-terminal domain and a common cage roof with its N-terminal receptor binding domain (RBD), whereas the TM subunits shaped spike legs, with a peripheral location. Here we have studied the structure of the R-peptide precursor spike by cryo-EM. The TM cleavage in Moloney-MLV was inhibited by amprenavir and the spikes solubilized in Triton X-100 and isolated by sedimentation in a sucrose gradient. We found that the legs of the R-peptide spike were held together by trimeric interactions at the very bottom of the spike. This suggested that the R-peptide ties the TM legs together and that this prevents the activation of TM for fusion by the receptor. The model was supported by further cryo-EM studies using an R-peptide spike mutant, which was fusion competent despite an uncleaved R-peptide. The spike legs of this mutant were found to be separated like in the mature spike. This shows that it is the TM leg separation, normally caused by R-peptide cleavage, which primes the spike for receptor triggering.

The structure of Nucleocapsid protein of Crimean Congo Hemorrhagic fever virus

Ali Mirazimi

Linköpings universitet, SVA och SMI

Crimean-Congo hemorrhagic fever virus (CCHFV) is one of the few deadly pathogens with broad geographical distribution. Due to the requirement of a biosafety level 4 (BSL-4) laboratory, the knowledge behind the virus replication cycle of this virus has been very limited. In this study, we represent a novel hypothesis for replication cycle of CCHFV. In our study, we have determined the structure of CCHFV N in two different forms: a monomeric and a superhelical oligomeric form. Most interestingly, we found that incubating CCHFV N with primer-length ssRNA would convert CCHFV N into its monomeric form and be released from the superhelical structure, which may suggest the first step of replication process. Our study aslo demonstrates that CCHFV N, in its superhelical form, hides the caspase-3 cleavage site at the tip of the stalk region and host cell caspase-3 is unable to cleave CCHFV N. When CCHFV N is released as a monomer, the caspase-3 cleavage site is exposed to cleavage by caspase-3. We believe that exposure of the cleavage site to host cell caspase-3 is crucial in controlling viral replication and transcription by host cells during an infection and represents an important host defence mechanism against CCHFV infection.

Saturday, August 25

11:00-11:50

Virus-cell interactions

Do CCHFV infected human monocyte-derived dendritic cells affect human epithelial cell permeability?

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***Division of Medical Microbiology, Faculty of Health Sciences, Linköping*

Crimean Congo Hemorrhagic Fever virus (CCHFV) is an arthropod-borne pathogen that in humans causes a severe disease, Crimean Congo hemorrhagic Fever (CCHF) characterized by hemorrhage and vascular leakage and with high case fatality rate. The mechanisms determining the pathogenicity of this virus is however largely unknown.

We have previously shown that CCHFV entry and release occur basolaterally and that infection does not affect permeability of polarized MDCK-1 cells. We have also shown that CCHFV can infect PBMCs resulting in increased release of Il-6, Il-10 and TNF-alfa and that infection activates endothelial cells resulting in release of higher levels of IL-6 and IL-8. In this study, we have been interested to set up an in vitro model system to study the effect of proinflammatory factors on the tight and adherens junctions during CCHFV infection. Human epithelial cells, Caco2 cells were grown on 3.0um transwell membranes and resistance measurements were made to ensure confluence before infection. We also isolated human monocyte-derived dendritic cells (DCs) from buffy coats and checked for purity by CD14-positive FACS. Uninfected DCs or supernatant from uninfected DCs were added to the Caco2 model either apically or basolaterally and the resistance was measured.

We have succeeded to set up an in vitro model system for studying the role of proinflammatory factors during CCHFV infection and our preliminary data demonstrate that Caco2 cells can be infected with CCHFV. Furthermore we found that CCHFV infection per se does not have an effect on the permeability of the cell layer. We are currently investigating the effect that infected DCs and their supernatant could have on tight and adherens junctions.

Mechanisms of adenovirus type 37 attachment to and entry into human ocular cells.

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Adenoviruses (Ads) can infect a broad spectrum of human cells in eyes, respiratory tract, gastrointestinal tract and other tissues. Adenovirus type 37 (Ad37) is together with Ad8 and Ad19 the major causative agents of epidemic keratoconjunctivitis (EKC), which is a severe infection of the eyes. Roughly, 20-30 million individuals suffer from EKC every year. We have previously demonstrated that these viruses use sialic acid (SA)-containing glycans as cellular receptors. Glycan array experiments pointed out a glycan that is present in GD1a ganglioside as a receptor candidate. GD1a contains a branched di-sialylated glycan. We found that soluble GD1a glycans inhibited Ad37 binding to and infection of human corneal cells (HCE). The GD1a glycan was shown to bind to a protein through an O-glycosidic linkage. Cocrystallization of the Ad37 fiber knob and the GD1a glycan showed that both sialic acids interacted with two of the three sialic acid-binding sites in the trimeric Ad37 knob. Whereas sialic acid-containing proteins support attachment, we have also data suggesting that Ad37 entry into human ocular cells depend on αV-, α3- and/or α4-integrins. These findings contribute to the understanding of adenovirus tropism and may be useful for development of antiviral drugs.

Selectin ligands in Herpes simplex virus infected T cells

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We have previously shown that herpes viruses induce host genes, involved in the synthesis of distinct carbohydrate structures (e.g. sialyl Lewis x) on the cell surface, upon infection of fibroblasts. As there is evidence that infected T cells play a role during herpes virus dissemination we used HSV1 as a model system to investigate if the virus could exert the same effect in this cell type.

The natural mechanism by which T cells leave the circulation and access adjacent tissue is dependent on an initial interaction between carbohydrate binding selectins and their ligands, e.g. sialyl Lewis x (sLex). Normally the expression of sLex on T cells is strictly regulated and limited by the activity of fucosyltransferases which act as an on-off switch.

We used flow cytometry for the detection of carbohydrates and HSV1 antigens. The gene expression studies were performed by real time PCR.

It was found that HSV1 indeed cause an induction of several genes encoding fucosyltransferases (FUT1, FUT3, FUT5, FUT6 and FUT7) already 3 hours post infection in the H9 T cell linage. This induction was accompanied by expression of the carbohydrate sLex on the surface of the infected cells. In addition, $CD3^+$ T cells isolated from blood donors that were infected with HSV1 exhibited increased activity of FUT gene (FUT1, FUT3, FUT5 and FUT6) transcription and increased expression of sLex.

It is possible that HSV1 and other herpes viruses can circumvent the regulation of FUT genes and thereby actively start sLex synthesis which contributes to its transmission within the host.

A 3D organotypic tissue model for hantavirus infection of the human airway mucosa

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[2]*MTC, Karolinska Institutet, Stockholm, Sweden*
[3]*CIM, Karolinska Institutet, Stockholm, Sweden*

In this study we use an in vitro 3D- model of the human lung to study hantavirus infection in man. So far, most in vitro studies on hantaviruses have been performed with cells in monolayer. Using this 3D-model we are able to study hantavirus infection in a lung microenvironment, including interactions between infected cells and immune cells. This model will render it possible to analyze how an infection influences e.g. the cytokine environment in the lungs, how infected cells affect their surroundings, and the involvement of for example monocytes during hantavirus infection.

Initial experiments shows that this model is highly susceptible for hantavirus-infection, and we will present data regarding specific as well as common responses after infection with HFRS and HCPS-causing hantaviruses.

Hantaviruses cause in two serious and often deadly zoonotic diseases in man: Hemorrhagic fever with renal syndrome (HFRS) and Hantavirus cardiopulmonary syndrome (HCPS). Humans are normally infected via inhalation of virus-contaminated rodent excreta, and the initial site of infection is consequently the lungs. The virus then spread systemically, and HFRS/HCPS is believed to be caused by increased vascular permeability. However, hantaviruses are not cytopathic, and it is currently not known if the virus directly cause the observed increased vascular permeability and/or if it is a consequence of virus-induced immune-pathogenesis. We believe that a better understanding of initial responses at the primary site for hantavirus infection will hopefully increase knowledge regarding mechanisms behind HFRS/HCPS.

Persistent and cytolytic replication of coxackievirus B2 in cultured cells

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The common view of an enterovirus replication cycle is that new virions are released by cytolytic mechanisms. There are however exceptions and whether the replication strategy involves cytolysis or not sometimes differs between cell lines. Group B coxsackievirus (CVB) uses the coxsackie- and adenovirus receptor (CAR) for attachment and entry. Another receptor known to be used by some CVBs is the decay-accelerating factor (DAF/CD55), however it is only used for attachment. In this study green monkey kidney (GMK) cells that express both of these receptors and (RD) cells that do not express detectable levels of CAR, but do express DAF/CD55 was used.

We have previously described a model system where the coxsackievirus B2 prototype, strain Ohio (CVB2O), efficiently replicate in RD cells without cytolysis. A single mutation in VP1, Q164K, changes the replicative strategy resulting in a cytolytic outcome that includes activation of apoptotic mechanisms. Both of these variants cause cytolytic infections in GMK cells.

Three clinical strains of CVB2 have now been included in our studies of replicative strategies of CVB2. The complete P1 region of the clinical strains CVB2-1293, CVB2-1885 and CVB2-2644 were sequenced and all strains had a Q at position 164 in VP1. They were found to cause persistent replication in RD cells and cytolytic replication in GMK cells, thus showing the same phenotype as the prototype strain CVB2O.

The lack of CAR on RD cells indicates that another receptor is being used by CVB2. Preliminary data suggests that viral infection is affected by heparin treatment, which may indicate the use of heparan sulfate as a receptor.


Map of Smögen
Smögenön
Läskärsgatan
Mellanberg
Vallgatan
Kummelgatan
Nils Ahlecks Väg
Skyttevägen
Badhusgatan
Makrillgatan
Glömmebergsvägen
Parkgatan
Storgat
Fotb.pl.
Östergatan
Oskar Fredriksgatan
Tambjörnsgatan
Skolgatan
Bredabergsvägen
Strandbergsliden
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Sjögatan
Madenvägen
Gästhamn
Klevenvägen
Smöghålet
160 m
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