CSV2018:
The 2nd Symposium of the Canadian Society for Virology

June 13-15, 2018
Dalhousie University
Sir Charles Tupper Medical Building
5850 College Street
Halifax, Nova Scotia, Canada

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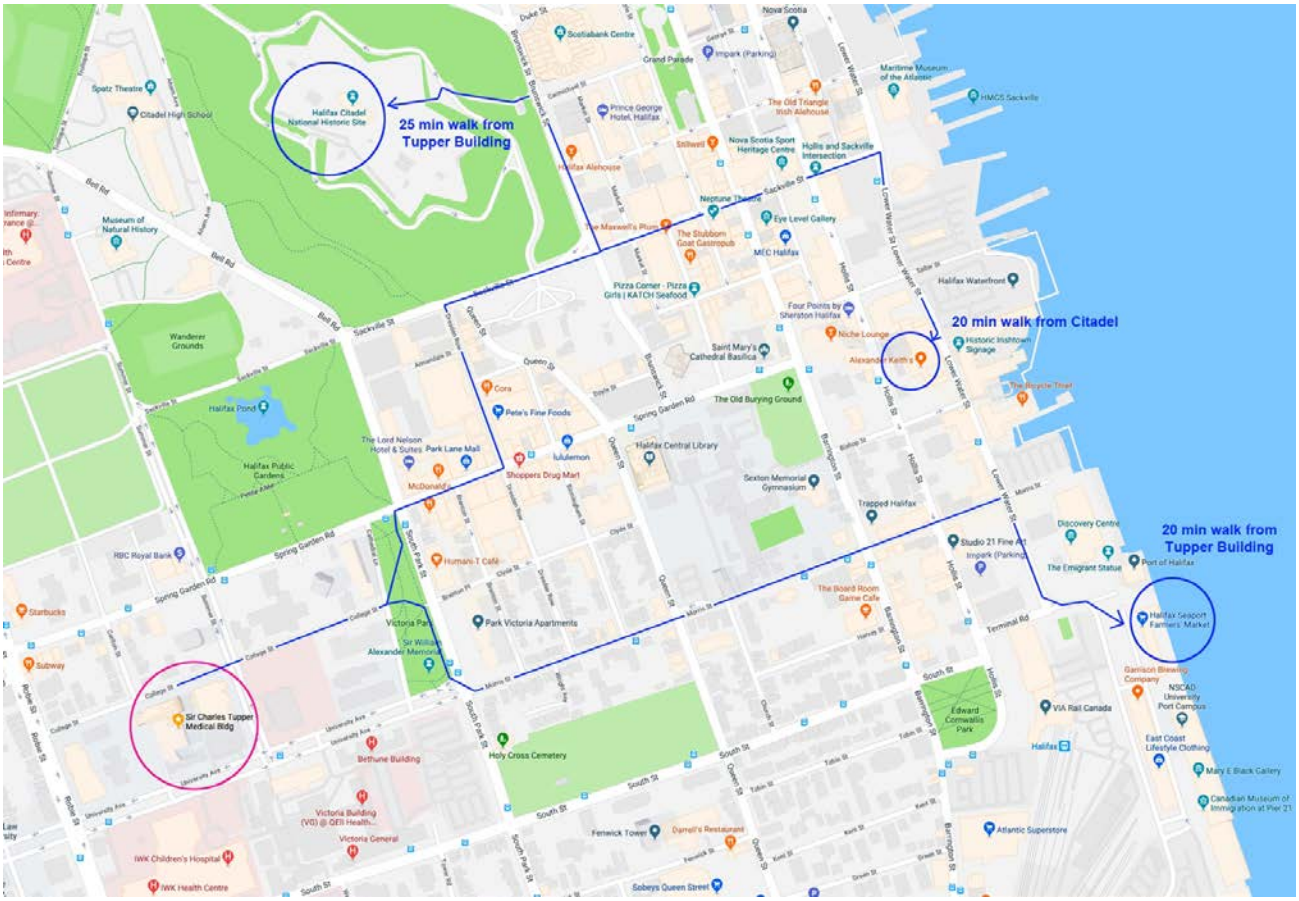

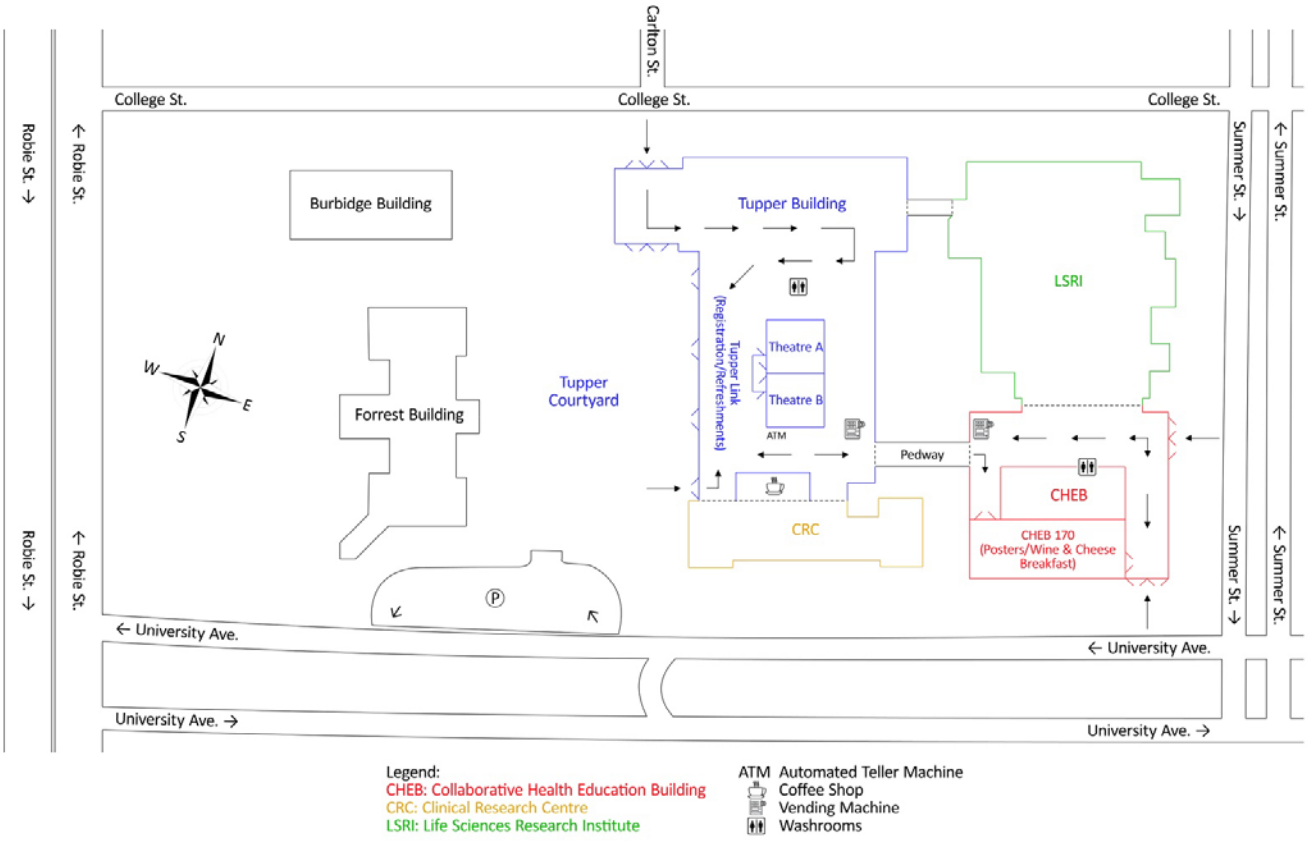

SYMPOSIUM PROGRAM

Wednesday, June 13th

15:00	**Registration and refreshments** (Tupper Link, Ol' School Donut Bus (Tupper Courtyard)) **Poster setup** (CHEB 170)
17:00 - 17:20	**Welcome remarks** (Theatre B) **Craig McCormick** (Dalhousie U) CSV co-founder CSV2018 co-organizer
17:20 - 18:00	**Keynote Lecture sponsored by CIHR** (Theatre B) **David Kelvin** (Dalhousie U.) "1918 to 2018, One Hundred Years on the Pandemic Road"
18:00 - 18:40	**Keynote Lecture sponsored by DMRF** (Theatre B) **Sylvain Moineau** (U. Laval) "CRISPR-Cas Systems and Phages: The Ongoing Battle"
19:00 - 22:00	**Poster Session #1 – posters #1 to 72** (CHEB 170) Wine and Cheese, seafood and antipasto served during poster session

Thursday, June 14th

6:30	**Student-led Fun Run** Departing from LeMarchant Place and Lord Nelson Hotel, touring Point Pleasant Park
8:00 - 9:00	**Meet the PIs - Breakfast** (CHEB 170)
9:00 - 10:35	**Topic #1: Emerging Viruses** (Theatre B) **Session Chair: Darryl Falzarano (VIDO-InterVac)**
9:00	Vikram Misra (U. of Saskatchewan) "Bats: The source of the next pandemic or cure for deadly viral diseases"
9:25	Michael Drebot (Public Health Agency of Canada) "From the field to the lab and back again: On-going Lassa virus research at NML"
9:50	Md Niaz Rahim (U. of Manitoba) "Pan-filovirus T-cell vaccine provided complete protection against Ebola and Marburg virus lethal challenges"
10:05	Corina Warkentin (U. of Ottawa) "Inhibition of receptor tyrosine kinase signaling blocks entry of filoviruses"
10:20	Neda Barjesteh (McMaster U.) "Elucidating the role of viral infection on the pathogenesis of Amyotrophic Lateral Sclerosis"
10:35	Health Break (Tupper Link)
11:00 - 12:35	**Topic #2: Viral Subversion of Host Cell Processes** (Theatre B) **Session Chair: Nelly Pante (UBC)**
11:00	Bruce Banfield (Queen's U.) "Early Stages in the Morphogenesis of Herpes Simplex Virus"
11:25	Maya Shmulevitz (U. of Alberta) "Niche-to-niche variations in reovirus-host interactions"
11:50	Nichole McMullen (Dalhousie U.) "Unconventional release of fusogenic nonenveloped reoviruses"
12:05	Justine Sitz (U. Laval) "Interaction of human papillomavirus E7 with DNA repair-specific E3 ligase RNF168"

12:20	Quentin Osseman (U. de Montréal) "Regulation of Respiratory Syncytial Virus infection by the autophagy receptor SQSTM1/p62 in airway epithelial cells"
12:45 - 13:45	**CSV Business Meeting** (Theatre B) ***Or*** **Writing Workshop** (Theatre A) **Co-sponsored by: *PLoS Pathogens* and *PLoS ONE*** **Coordinated by: Eileen Clancy, *PLoS ONE* Senior Editor** **Karen Mossman, *PLoS Pathogens* Editor** **Contributor: Alan Dove, Science writer** Box lunches will be available
14:00 - 15:10	**Topic #3: Viruses of Microbes** (Theatre B) **Session Chair: Karen Maxwell (U. of Toronto)**
14:00	Karen Maxwell (U. of Toronto) "A chemical defence against phage infection"
14:25	Alexander Hynes (McMaster U.) "Following Phages through Fecal Transplants"
14:40	Casey Jones (Dalhousie U.) "The gut virome of pediatric Crohn's Disease following exclusive enteral nutrition"
14:55	Jaclyn McCutcheon (U. of Alberta) "Type IV pili-specific bacteriophages of Stenotrophomonas maltophilia"
15:10	Nikhil George (U. of Waterloo) "Examining viral populations, CRISPR-resolved virus-host interactions, and CRISPR-Cas system diversity in a municipal landfill"
15:50	**Health Break (Tupper Link)**
16:15 - 17:25	**Topic #4: Antivirals and Vaccines** (Theatre B) **Session Chair: Matthias Götte (U. of Alberta)**
16:15	3-Minute Thesis presentations from trainees: 1. Ali Zhang (McMaster U.) "Neuraminidase Inhibitors Enhance Fc-Dependent Effector Functions Elicited by Hemagglutinin Stalk-Binding Broadly-Neutralizing Antibodies" 2. Briti Saha (U of Ottawa) "Identification of pharmacological targets and small molecules inhibiting adenovirus replication" 3. Mariel Kleer (Dalhousie U.) "Characterization of a Novel Host-Targeted Antiviral Molecule" 4. Andrea Monjo (Dalhousie U.) "Photodynamic Inactivation of Herpes Simplex Viruses"
16:30	Panel Discussion on the future of vaccines and antiviral drugs (Theatre B) Panel Members: Lisa Barrett (Nova Scotia Health Authority), Marianne Stanford (VP Research, Immunovaccine), Marceline Côté (U. of Ottawa), Alyson Kelvin (Dalhousie U.), David Willer (Global Scientific Affairs Lead - Zoster, GSK)

17:25	**Soapbox Science Launch Event** (Tupper Courtyard) Dr Maya Shmulevitz, University of Alberta "Refurbishing viruses to seek, reveal, and destroy cancer cells" Dr Sarah M. Wells (@sarahwellsDal), Dalhousie University "Engineering replacements for the body" Dr Krysta Coyle (@microbialkrysta), Dalhousie University "Stacking the Deck for Cancer Treatment" Dr Alyson A. Kelvin (@KelvinAly), IWK Health Centre and Dalhousie University "Virus Hunter – Tracking the Origin of Disease"
18:00 - 18:40	**Keynote Lecture sponsored by CIHR** (Theatre B) **Kate O'Brien (Johns Hopkins U.)** "Chasing the causes of serious pneumonia among children in Africa and Asia: PERCH Study Results"
19:00 - 22:00	**Poster Session #2 – posters #73 to 127** Wine and Cheese, seafood and antipasto served during poster session

Friday, June 15th

8:00 - 9:00	**Meet the PIs - Breakfast** (CHEB 170)
9:00 - 10:35	**Topic #5: Emerging Methods in Virology** **Session Chair: Marceline Côté (U. of Ottawa)**
9:00	Roger Lippé (U. de Montréal) Flow virometry: A novel and powerful approach to decipher viral egress
9:25	Ryan Noyce (U. of Alberta) "SynViro: Building the next generation of therapeutic poxvirus vectors"
9:50	Vanessa Meier-Stephenson (U. of Calgary; U. of Lethbridge) "Targeting Persistent Hepatitis B Virus: Identification of a G4-Quadruplex Structure Motif in a Key Region of HBV's Genome"
10:05	Yumiko Komatsu (Kyoto U.) "Development of iPSC-derived neural stem cells transduced with Borna disease virus vector expressing thymidine kinase for cancer gene therapy"
10:20	Brennan Dirk (Western U.) "Seeing is believing: Visualizing multi-protein complexes mediating HIV-1 immune evasion"
10:35	**Health Break** (Tupper Link)
11:00 - 12:35	**Topic #6: RNA in Virus Infection** (Theatre B) **Session Chair: Andrew White (York U.)**
11:00	Selena Sagan (McGill U.) "miR-122-mediated protection of the HCV genome from pyrophosphatase activity"
11:25	Martin Pelchat (U. of Ottawa) "Insight into influenza A virus cap-snatching"
11:50	Carolyn Robinson (Dalhousie U.) "Viral Modulation of RNA Granules is Reliant on Autophagic Flux"
12:05	Eric Pringle (Dalhousie U.) "Alternative translation initiation during KSHV lytic replication"
12:20	Tyler Mrozowich (U. of Lethbridge) "Towards Obtaining a Nanoscale Structure of the 3' Terminal Region of Japanese Encephalitis Virus Genome"

12:45 - 13:45	**Live Taping of This Week in Virology** (Theatre B) **with Vincent Racaniello (Planet Earth's Virology Professor) and Science Writer Alan Dove, sponsored by CIHR and DMRF** Guests: Nathalie Grandvaux (U. de Montréal) & Craig McCormick (Dalhousie U.) discussing CSV Kate O'Brien (Johns Hopkins U.) discussing vaccine research Ryan Noyce (U. of Alberta) discussing horsepox virus engineering Lunch boxes will be available
14:00 - 14:35	**Topic #7: Viruses of Flora and Fauna** (Theatre B) **Session Chair: Eric Jan (UBC)**
14:00	Eric Jan (UBC) "Translation initiation of alternative reading frames by viral internal ribosome entry sites"
14:25	Hélène Sanfaçon (Agriculture Canada) "Expanding repertoire of plant (+)-strand RNA virus proteases: beyond the cysteine and serine proteases"
14:50	Shuang Yang (UBC) "The underlying mechanism of a unique nuclear entry pathway used by the parvovirus minute virus of mice"
15:05	Gerard Gaspard (Dalhousie U.) "Atypical Transmembrane Domain of Reptilian Reovirus p14 Fusion-Associated Small Transmembrane (FAST) Protein Mediates it's Trafficking"
15:25	Jared Rowell (U. of Calgary) "Porcine circovirus 2 (PCV2) activated apoptosis of non-infected cells"
15:40	**Health Break** (Tupper Link)
16:05	**Topic #8: Antiviral Innate Immunity** (Theatre B) **Session Chair: Karen Mossman (McMaster U.)**
16:05	Stephanie DeWitte-Orr (Wilfrid Laurier U.) "Class A scavenger receptors: their role in innate antiviral immunity in lower vertebrates"
16:30	Katharine Magor (U. of Alberta) IFN and cytokine responses in ducks to genetically similar H5N1 influenza a viruses of varying pathogenicity
16:55	Jiangyi He (Memorial U.) "Murine Cytomegalovirus Immune Evasion Targeting the MEK-IRF1 Connection"
17:10	Dacquin Kasumba (U. de Montréal) "The NADPH oxidase DUOX2 differentially modulates the production of respiratory virus-induced cytokines by airway epithelial cells"
17:25	Hannah Stacey (McMaster U.) "IGA immune complexes stimulate netosis during viral infection and autoimmunity"
17:45	**Closing Remarks and Awarding of Prizes** **Nathalie Grandvaux (U. de Montréal)** CSV co-founder CSV2018 co-organizer
18:30 - 19:30	**Tour of Halifax Citadel National Historic Site** Citadel tours by 78th Highlanders
20:00 - 23:00	**Reception at Alexander Keith's Brewery**

Saturday, June 16th

10:00 - 13:00	**Soapbox Science Event at Halifax Seaport Farmers' Market**
	Departure

KEYNOTE LECTURE / Biography -1

Dr. David Kelvin
Department of Microbiology & Immunology
Dalhousie University
Canadian Centre for Vaccinology
Shantou University Medical College

Biography

Dr. David Kelvin's research interests are focussed on emerging infectious diseases. He conducts research on the emergence of novel influenza strains in China. He has worked on H7N9, H5N1, SARS CoV, West Nile Virus, and Nipah Virus infections. He also has a research project on the impact of infectious disease on human immune gene evolution in Sardinia, Italy. Dr. Kelvin is Professor in the Department of Microbiology and Immunology at Dalhousie University in Halifax. He is the Canada Research Chair in Translational Vaccinology and Inflammation. Dr. Kelvin is Director of Immunity at the International Institute of Infection and Immunity, Shantou, China.

"1918 to 2018, one hundred years on the pandemic road"

Abstract:

In the modern era of medicine at least 6 well documented influenza pandemics have been identified. Even though 1898, 1918, 1957, 1968, 1977, and 2009 are well recognized as pandemic years, often a pandemic occurs in waves lasting years leading to significant human mortality and morbidity. The origins of influenza viruses and environmental factors often impact the emergence of pandemic influenza viruses. The course of an influenza pandemic and evolution of pandemic viruses is unpredictable and can enter into circulation as an endemic virus, or as in the case of the 1957 H2N2 pandemic, suddenly disappear. We will explore origins, mysteries, and anecdotes of influenza pandemics in "1918 to 2018, One Hundred Years on the Pandemic Road."

KEYNOTE LECTURE / Biography -2

Dr. Sylvain Moineau
Department of Biochemistry, Microbiology and Biology
Faculty of Science and Engineering, Université Laval

Biography

Sylvain Moineau is a Professor in the Department of Biochemistry, Microbiology and Bioinformatics at the Université Laval. He also holds the Canada Research Chair in Bacteriophages and is the Curator of the Félix d'Hérelle Reference Center for Bacterial Viruses, the world largest collection of reference phages (www.phage.ulaval.ca). His research group uses an integrative approach to study phage biology as well as to better understand the phage-bacteria interactions. He has characterized mechanisms used by bacteria to resist phage infections, including his landmark discoveries on CRISPR-Cas systems that is at the heart of the current widely acclaimed genome-editing technology. Prof. Moineau has won several teaching and research awards. In 2017, he was awarded the NSERC John C. Polanyi Award, the Canadian Society of Microbiologists Murray Award for Career Achievement and the DuPont Excellence Medal. Last Fall, he was elected to the Academy of Sciences of the Royal Society of Canada and appointed an Officer of the Order of Canada. His research work has also received the attention of Thomson Reuters and Clarivate Analytics who ranked him amongst the most cited and influential microbiologists on the planet for the past four years (2014-2017).

"CRISPR-Cas systems and phages: the ongoing battle"

Abstract:

Fighting viruses is no easy task. Bacterial cells have survived phage attacks by evolving sophisticated defence strategies that enable them to thrive even in virus-rich ecosystems. Phages have also evolved counter-tactics to thwart such mechanisms, leading to a biological arms race. Four decades after the discovery of restriction enzymes, another bacterial anti-phage system that cleaves foreign DNA was identified—one that acts as an adaptive immune system. Clustered regularly interspaced short palindromic repeats (CRISPR) and their associated cas genes protect microbial cells against infection by foreign nucleic acids, including phage genomes and plasmids. Bacterial CRISPR-Cas type II systems function by first incorporating short DNA 'spacers', derived from invading phage genomes or plasmid sequences, into a CRISPR array located in their genome. The CRISPR array is then transcribed and matured into short RNAs, which, by recruiting a Cas endonuclease, act as surveillance complexes that recognize and cleave invading matching sequences. The cleavage occurs near a short motif, called the PAM, adjacent to the sequence targeted by the spacer. Exploiting this system has also resulted in the development of the much-publicized CRISPR-Cas9 technology for precise genome manipulation of various organisms. However, phages can bypass the protection provided by the CRISPR-Cas system through point mutations or deletion of the CRISPR target in their genome or by the production of anti-CRISPR proteins (Acrs). These Acrs can also be used to fine-tune the activity of CRISPR-based genome editing tools. This lecture will recall the roles played by phages in the understanding of CRISPR-Cas systems. I will also highlight the recent discovery of new families of anti-CRISPR proteins and the of use of CRISPR-Cas9 technology for viral genome editing.

KEYNOTE LECTURE / Biography -3

Dr. Katherine O'Brien
Johns Hopkins University
On behalf of PERCH Study Team

Biography

Kate O'Brien is Professor of International Health and Epidemiology at the Johns Hopkins Bloomberg School of Public Health in Baltimore, Maryland. She is the Executive Director of the International Vaccine Access Center at the Johns Hopkins Bloomberg School of Public Health. Her scientific and policy work domestically and globally has focused on vaccine preventable illnesses, among both children and adults. The work has included surveillance, epidemiology, and vaccine clinical trials of pneumococcal disease; rotavirus; Haemophilus influenzae type b; respiratory syncytial virus and influenza vaccines. She has worked extensively with American Indian populations and in Africa and Asia, partnering with local scientists to develop rigorous scientific evidence and bring it into the vaccine policy arena, thereby accelerating the use and access to life-saving vaccines for children living in low resource countries and settings. Dr. O'Brien trained as a pediatric infectious disease physician, epidemiologist and vaccinologist with a BSc in chemistry from University of Toronto (Canada), an MD from McGill University (Canada), and an MPH from Johns Hopkins Bloomberg School of Public Health (US) before completing her training at the US Centers for Disease Control and Prevention as an Epidemic Intelligence Officer, in the Respiratory Diseases Branch. She has worked in close partnerships with the Gavi, the Vaccine Alliance, with the World Health Organization (WHO) and with countries to advance programs and policies on child health. She has been honored with the U.S. Department of Health and Human Services Secretary's Distinguished Service Award, the Sabin Vaccine Institute Young Investigator Award, the U.S. Presidential Early Career Award in Science and Engineering (PECASE). She serves on the WHO Strategic Advisory Group of Experts (SAGE) on Immunization, which develops global policy on vaccines and immunizations and on the Gavi Board.

"Chasing the causes of serious pneumonia among children in Africa and Asia: PERCH study results"

Abstract:

Understanding the aetiology of pneumonia is critical for accelerating interventions to treat and prevent this leading cause of child mortality. The Pneumonia Etiology Research for Child Health (PERCH) study is a 7 country study in Africa and Asia designed to determine the aetiology of pneumonia in this era of widespread conjugate Hib and pneumococcal vaccination. PERCH is a highly standardized case-control study of World Health Organization (WHO)-defined severe and very severe pneumonia among over 4000 hospitalized children 1 to 59 months of age and over 5000 age-matched controls. Cases and controls had multiple clinical specimens collected which were analysed by molecular and culture methods; cases had chest x-rays obtained and read using standardized WHO criteria. Important limitations of standard approaches to descriptive aetiology analysis were addressed using a Bayesian, partial latent class analysis approach to combine the results from the pathogen testing and quantify the etiologic probability in individual cases and in the population. The description of the cases and controls, the primary etiology results, and a clinical and etiologic description of mortality in PERCH will be presented. Further, the presentation will highlight the value of post-mortem minimal invasive tissue sampling to better characterise the complexity in attributing pathogen specific causes of death in children dying from pneumonia.

Topic #1: Emerging Viruses

Bats: The source of the next pandemic or cure for deadly viral diseases

Vikram Misra[1], Arinjay Banerjee[1], Sonu Subudhi[1], Noreen Rapin[1], Darryl Falzarano[1,2]
[1]Department of Microbiology, Western College of Veterinary Medicine, Vaccine and Infectious Diseases Organization/International Vaccine Centre, University of Saskatchewan, Saskatoon, Saskatchewan, S7N5B4

Several viruses, that appear to cause no overt disease in their natural bat hosts, have spilled over into other mammals causing serious and often fatal disease. These include viruses that cause: Severe acute respiratory syndrome (SARS) and Middle east respiratory syndrome (MERS); Marburg and Ebola haemorrhagic diseases; Hendra and Nipah respiratory and neurological diseases; and porcine epidemic diarrhea and swine acute diarrhoea. An overblown inflammatory response exacerbates the pathology in these diseases. While there are many studies on the interaction of these viruses with their spillover hosts or surrogate laboratory animals, there is little information on the relationship of these or similar viruses with their natural bat hosts. Our objective is to examine the apparently benign relationship between viruses and their natural bat hosts as well as the factors that upset this relationship leading to increased virus replication and, potentially, to spillover to other species. We have identified unique Corona and Herpes viruses that persistently infect most members of two Canadian bat species with no overt signs of illness or pathology. Secondary fungal infections, however, cause an increase in virus replication. We have also discovered that while both bat and human cells react to viral infection with an antiviral response, pro-inflammatory pathways are actively suppressed in bat cells. Bat cells are also resistant to the ability of viruses to subvert host antiviral responses. Our studies will not only provide fundamental information about virus-host relationships in these specialized mammals but will also give clues about mitigating the serious consequences that often accompany viral spill-over from bats to humans and other species.

From the field to the lab and back again: On-going Lassa virus research at NML

Michael Drebot[1]
[1]National Microbiology Laboratory, Public Health Agency of Canada

The recent deployment of the rVSV-Ebola vaccine has renewed interest in the VSV vaccine platform for the prevention of viral hemorrhagic fevers as well as many other infectious diseases. At the same time that the Ebola vaccine was created and characterized at the National Microbiology Laboratory in Winnipeg, a second VSV-based vaccine against Lassa fever was also created. Lassa fever is caused by the Lassa virus which is a rodent-borne pathogen endemic to many Western African countries including Guinea, Liberia, Nigeria, and Sierra Leone. The virus has an estimated incidence rate of 200,000-500,000 infections each year. In addition, Lassa virus has been recently associated with prolonged outbreaks in Nigerian where mortality rates have exceeded 20% in confirmed cases. Despite this, to date no approved treatment or vaccine exists for Lassa fever. This talk will summarize the pre-clinical studies conducted to date on the "made-in Canada" Lassa fever vaccine and highlight the important next steps towards clinical trials.

Pan-filovirus T-cell vaccine provided complete protection against Ebola and Marburg virus lethal challenges

Md Niaz Rahim[1,2], Edmund G. Wee[3], Shihua He[1], Jonathan Audet[1,2], Kevin Tierney[1], Tomáš Hanke[3,4], Xiangguo Qiu[1,2]
[1]National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, MB R3E 3R2 Canada, [2]Department of Medical Microbiology, University of Manitoba, Winnipeg, MB R3E 0J9, Canada, [3]The Jenner Institute, Nuffield Department of Medicine, University of Oxford, Oxford OX3 7DQ, United Kingdom, [4]International Research Center for Medical Sciences, Kumamoto University, Kumamoto, Japan

Filoviruses such as Ebola (EBOV) and Marburg (MARV) viruses cause deadly viral hemorrhagic fever in humans with high case fatality rates. To date, no licenced therapeutic or vaccine has been clinically approved to prevent infection. Several vaccine candidates are under development against the few most common filoviruses targeting the virus glycoprotein (GP). However, protective antibodies induced by such GP vaccines are usually limited to the same species. In contrast, T-cell vaccines offer an opportunity to design a single pan-filovirus vaccine protecting against all members of the Filoviridae family. Four most conserved regions were identified among filoviruses by epigraph analysis in previous study. In this study FILOcepX vaccines were constructed targeting the four most conserved regions among the viral proteomes with the aim to induce protective T-cell responses against different filoviruses. BALB/c mice were immunized with FILOcep 1 and 2 vaccines vectored by non-replicating engineered simian adenovirus and poxvirus MVA. Vaccinated mouse splenocytes were stimulated with 12 pools of peptides derived from the FILOcepX epigraphs, and Filocep X specific T-cell responses were determined by IFN-ϒ ELISPOT assay. In the next step, groups of 20 BALB/c mice were primed and boosted with either the FILOcep1 and FILOcep2 vaccines or control ChAdOx1- and MVA-vectored vaccines. Four animals in each group were sacrificed after 1 week of boosting to detect T-cell response for the FILOcepX antigen. High frequency T cells specific responses were detected in mice receiving the test vaccines by IFN-γ ELISPOT kits. Of the remaining 16 animals in each group, 8 were challenged with mouse-adapted EBOV and 8 were challenged with mouse adapted MARV in Containment Level 4 (CL4) laboratory. All the mice in the control group either died or had to be euthanized between 4 and 6 days post challenge. On the other hand all the FILOcepX vaccinated mice maintained their normal body mass and survived till the end of the scheduled protocol on day 29 post challenge. These FILOcepX vaccines provided 100% protection against the lethal challenges with filoviruses of two different genera. Currently we are assessing the efficacy of this vaccine in non-human primate (NHP).

Inhibition of receptor tyrosine kinase signaling blocks entry of filoviruses

Corina Warkentin[1,3], Marceline Côté[1,3]
[1]University of Ottawa, [2]University of Ottawa, [3]Ottawa Institute of Systems Biology

Ebola virus (EBOV) is an enveloped, negative stranded RNA virus of the Filoviridae family that causes severe outbreaks of hemorrhagic fever. Currently, there are no FDA-approved antiviral therapies against EBOV disease. EBOV entry involves internalization and trafficking within host cells for the virus to reach its intracellular receptor, Niemann-Pick C1 (NPC1), which is localized in late endosomes/lysosomes. Given the requirements for internalization and endolysosomal trafficking, we hypothesized that EBOV activates signaling pathways to induce its uptake and regulate trafficking to facilitate its delivery to NPC1+ cellular compartments. To determine if signaling events induced by the virus play a role in infection, we screened a library of kinase inhibitors for their effect on infection by MLV pseudotypes bearing EBOV or vesicular stomatitis virus (VSV) glycoproteins. We identified compounds that either specifically inhibited EBOV infection, or blocked infection by both viruses. However, results revealed that no compound exclusively inhibited VSV infection, suggesting that EBOV GP-mediated infection is dependent on specific signaling pathways. We additionally screened Marburg virus (MARV) and Lassa fever virus (LFV) and obtained similar results. One class of inhibitors that was over-represented in our hits for the filoviruses (EBOV and MARV) were inhibitors of receptor tyrosine kinases (RTKs). We further characterized specific inhibitors of epidermal growth factor receptor (EGFR) and tyrosine protein kinase Met in both Vero cells and bone-marrow derived macrophages, and found that they inhibited infection of all pathogenic filoviruses. Interestingly, these inhibitors also act synergistically, suggesting that EBOV utilizes multiple RTKs to promote entry. Furthermore, experiments on mode of action suggested that EGFR signaling occurs early during infection, likely during attachment or internalization, while Met signaling occurs at a post-internalization stage. Our studies highlight the important role of signaling pathways in EBOV infection, in addition to the identification of RTK activation as a potential mechanism through which signaling is initiated.

Elucidating the role of viral infection on the parthenogenesis of Amyotrophic Lateral Sclerosis

Neda Barjesteh[1,2], Jonathan Mapletoft[1,2], Braeden Cowbrough[1,2], Daniel Celeste[1,2], Matthew Miller[1,2]
[1]McMaster University, [2]Michael G. DeGroote Institute of Infectious Diseases Research, Department of Biochemistry and Biomedical Sciences, McMaster Immunology Research Centre, McMaster University, , [3]McMaster University

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease for which there are currently no effective treatments. While several genes have been associated with ALS, environmental triggers have long been thought to contribute to the onset and progression of ALS. Viruses have received longstanding attention as potential environmental triggers of ALS. However, no conclusive causative relationship between a specific pathogen and onset or progression of ALS have been identified to-date. We hypothesized that viral infection triggers the onset and progression of ALS by perturbing pathophysiological pathways associated with the disease. To assess the role of viral infection on ALS we utilized the $SOD1^{G93A}$ murine model of ALS and tested the effect of influenza A virus (IAV) infection on disease onset and progression. We demonstrated that IAV exacerbated ALS symptoms and accelerated mortality in $SOD1^{G93A}$ mice. Using mouse embryonic fibroblasts derived from either $SOD1^{G93A}$ or wild-type mice, we assessed the accumulation of misfolded SOD1following IAV infection. We have shown that infection caused accumulation of misfolded SOD1. We have also demonstrated that $SOD1^{G93A}$ MEFs produce elevated levels of inflammatory cytokines/chemokines upon infection. Our future work will focus on characterizing neuroinflammation within the CNS following viral infection. Additionally, to determine the role of the immune system in ALS, we will identify the source(s) of virus-induced neuroinflammation. We will also define the direct effects of viral replication on pathogenic mechanisms associated with ALS. This study demonstrates that common viral infections may accelerate disease onset and progression in the $SOD1^{G93A}$ murine model of ALS.

Topic #2: Viral Subversion of Host Cell Processes

Early Stages in the Morphogenesis of Herpes Simplex Virus

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Herpesvirus assembly starts in the nucleus where the viral DNA genome is packaged into a preformed capsid. As herpes capsids are too large to pass through nuclear pores, capsids must pass through both the inner and outer nuclear membranes (INM, ONM) to access the cytoplasm where final virus assembly occurs. This process, termed nuclear egress, is conventionally divided into 3 phases: 1) recruitment of capsids to the viral nuclear egress complex (NEC) residing in the INM; 2) primary envelopment of capsids at the INM; and 3) fusion of enveloped capsids in the perinuclear space with the ONM to release capsids into the cytoplasm. The HSV-encoded proteins UL31 and UL34 are conserved NEC components that play a central role in the primary envelopment of DNA containing capsids at the INM. Others have shown that expression of UL31 and UL34 in the absence of other viral proteins leads to vesiculation of the INM at the nuclear periphery and the interaction of purified UL31 and UL34 with membranes in vitro can induce vesiculation. While the NEC constitutes the minimal requirement for membrane vesiculation, this activity must be regulated in infected cells to prevent unproductive primary envelopment, thus other host/viral proteins likely play regulatory roles in nuclear egress. We investigated nuclear membrane remodeling by HSV-2 UL31 and UL34 and found that, in addition to INM vesiculation, cells expressing both proteins form long membranous tubules that extend inwards from the nuclear periphery deep into the nucleoplasm. These tubules are dynamic, contain cellular INM components, are devoid of nuclear pores, lack nuclear lamina on their nucleoplasmic face and form at times post infection consistent with a role in virus assembly. Extending the INM into the nucleoplasm would evade barriers to primary envelopment, such as the nuclear lamina and marginalized cellular chromatin, and thus might present an attractive surface for primary envelopment of HSV-2 capsids located in the nucleoplasm. We hypothesize that, rather than capsid recruitment to a peripheral INM, the INM is directed to the nucleoplasm where nuclear egress can be initiated. Our recent studies on the HSV-2 UL16 protein provide support for this hypothesis. HSV-2 lacking UL16 show defects in primary envelopment of capsids and severely reduced virus replication in the absence of complementation. Replication of this virus was markedly improved in HeLa cells stably expressing a version of UL31 with enhanced ability to form tubules compared to its replication in parental HeLa cells. If artificially evoking INM tubule formation can compensate for the lack of UL16, this may suggest a role for UL16 in directing the INM to capsids located in the nucleoplasm. We are currently testing predictions stemming from this alternate view of nuclear egress as well defining roles for other HSV-2 proteins in nuclear egress.

Niche-to-niche variations in reovirus-host interactions

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Mammalian orthoreovirus (reovirus) naturally causes benign infection of the intestinal tract. It was previously established that when administered to tumor-bearing animals or humans, reovirus specifically replicates in tumors with limited harm to non-transformed cells. Reovirus is therefore a candidate oncolytic therapy. In an effort to improve the oncolytic potency of reovirus, we asked whether specific virus characteristics that evolved towards the enteric niche could be modified to promote virus replication and dose-escalation in tumor environments. Our findings show that modifications to reovirus can promote both entry and post-entry steps of replication in tumor cells, without jeopardizing restriction by non-transformed cells. For example, reovirus variants with fewer cell attachment σ1 trimers relative to wild-type reovirus (which typically has twelve σ1 trimers) exhibit four-fold better uncoating on a panel of cancer cells in-vitro and demonstrate improved oncolytic potency in an in-vivo melanoma model. Specifically, we found that three σ1 trimers are sufficient for reovirus binding to tumor cells, and that progressively increasing levels of σ1 trimers results in decreasing proportions of virions that successfully shed σ1 during entry (a prerequisite to establish infection). Conversely, having fewer than wild type levels of σ1 trimers makes reovirions adversely unstable under enteric conditions, suggesting that optimal reovirus structure may depend on the niche of infection. As a second example, reovirus capsid cleavage mediated by intestinal and intracellular proteases has been well defined. However, the role of proteases in the extracellular tumor environment during reovirus oncolysis is less understood. We discovered that breast tumor proteases can lower reovirus infectivity by 100-fold. Specifically, metalloproteases secreted by a panel of breast cancer cells, and present in extracellular environments of in-vivo mouse breast tumors, were found to cleave reovirus σ1 proteins and reduce virus binding to tumor cells. Through mutagenesis of the σ1 protease sensitive site, we were able to overcome cleavage and inactivation of reovirus σ1 by breast tumor metalloproteases. Our findings not only suggest that virus therapies would benefit from full repurposing towards their target niche, but also exemplify how distinct cellular and environmental factors in different niches can impact the outcome of reovirus-host interactions.

Unconventional release of fusogenic nonenveloped reoviruses

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Fusogenic reoviruses induce cell-cell fusion and syncytium formation mediated by a virus-encoded fusion-associated small transmembrane (FAST) protein. FAST proteins enhance localized direct cell-cell spread of the infection via cell-cell fusion, followed by syncytial lysis and release of infectious virus particles for systemic dissemination. As with most nonenveloped viruses, virus-induced cell lysis is the primary mechanism for reovirus release from cells. Recent findings suggest various nonenveloped viruses, including hepatitis A virus (HAV) and poliovirus, can also be released non-lytically from cells inside extracellular vesicles (ECVs). Western blotting, transmission and cryo-electron microscopy revealed significant levels of non-enveloped virions and FAST protein-containing ECVs in cell culture supernatants. Kinetic analysis of virus release was coupled with live-cell imaging using Sytox to assess plasma membrane integrity (a membrane impermeable dye and only fluoresces when bound to nucleic acid inside cells). Results indicate release of substantial quantities of infectious virus particles from cells prior to any evidence of reduced plasma membrane integrity, indicative of non-lytic virus release. Unlike non-lytic HAV release, silencing of ESCRT proteins Tsg101 and Alix did not decrease FAST induced syncytium formation nor non-lytic reovirus release. Previous results, using yeast 2-hybrid analysis, indicated an interaction between FAST proteins and autophagy related 5 (Atg5) protein. Given recent evidence that shows involvement of Avian reovirus and Muscovy duck reovirus proteins in inducing autophagy and the release of autophagosome like structures, genetic and pharmacologic approaches are currently being used to assess the potential involvement of autophagy pathways in the mechanism underlying this non-lytic virus release.

Interaction of human papillomavirus E7 with DNA repair-specific E3 ligase RNF168

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Human papillomaviruses are small DNA viruses that replicate in differentiating epithelium of the skin and the mucosa, and they are classified as low-risk or high-risk according to their respective ability to cause condylomas or cancers. Although high-risk HPVs contribute to the development of cervical cancer and a subset of head and neck cancers, only a small proportion of infected patients develops the disease, underlining the requirement of additional genetic alterations for the progression of the infected cells to carcinoma. Currently, HPV is known to interfere with at least two of the pathways that guard genomic stability in host cells by: 1) sequestering specific factors of the DSB signaling and repair pathways to replicate its genome, and 2) by actively blocking cell cycle checkpoints. In HPV+ cancer cells, two factors that plays central role in cellular response to DNA double-strand breaks (DSBs), the E3-ligase RNF168 and 53BP1, accumulate in atypical large nuclear bodies. How HPV interferes with DSB signaling pathways and how it affects the ability of the cell to deal with genomic instability is still unknown. Here, we report our progress in delineating the interplay between the virus and DSB responses. First, we found that the large nuclear bodies observed in HPV+ cancer cells are 53BP1 G1 bodies, a structure that assemble around DNA damage that occurred in the previous S-G2 phase of the cell cycle. These findings suggest that HPV+ cancer cells undergo, and adapted to live with high levels of replication stress. Consistently, we found that they are resistant to treatment with PARPi and etoposide, two molecules that enhance replication stress by inducing damage in S-phase. To determine whether the virus provides cells with a better ability to face specific types of DNA damage, we investigated whether viral proteins impact DSB signalling. Using a system based on the lac operator (lacO)/lac repressor (LacI), we found that two LacI-HPV fusion proteins promote the recruitment of RNF168 to a LacO array, independently of upstream DSB signalling factors such as γ-H2AX and RNF8. These results raise the possibility that HPV proteins interfere with DNA repair processes by interacting with the E3-ligase. Altogether, our results show that HPV proteins can rewire the cellular localization of DSB signalling proteins and interfere with the ability of cells to cope with DNA damaging agents. Further studies will aim at defining the interaction of the viral oncogenes with RNF168 and how it impacts their respective functions. This will contribute to elucidate the mechanism by which HPV factors perturb DSB repair pathways. As HPV extensively relies on the activation of DSB signaling to replicate its genome in infected epithelium, our studies may reveal important insight on the molecular events underlying these processes.

Regulation of Respiratory Syncytial Virus infection by the autophagy receptor SQSTM1/p62 in airway epithelial cells.

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Respiratory syncytial virus (RSV) causes a high rate of morbidity and mortality in infants, children and elderly, but also adults of all ages with a compromised immune system, cardiopulmonary diseases or who have undergone transplantation, worldwide. RSV vaccine development faces numerous challenges and current very costly prophylactics are restricted to high-risk children. Antivirals targeting host factors required for virus replication are an emerging alternative to traditional direct-acting antivirals. RSV primarily replicates in airway epithelial cells (AECs). Therefore, understanding RSV: host interaction in human AECs is crucial for future development of drugs targeting RSV replication. Autophagy is critical for intracellular homeostasis in response to stress, including virus infection. However, compelling evidence demonstrate that a number of viruses have evolved mechanisms to exploit and modulate the function of autophagy components to use them for their benefit. Selectivity in autophagy is conferred by cargo receptors, including p62/SQSTM1, which tether ubiquitylated (Ub) cargo to a autophagosomes for degradation into autophagolysosomes. Previous data have highlighted an important role for p62-dependent autophagy in the bacteria and virus clearance, although p62 was also shown to promote replication of some viruses. Very little information is available regarding the interplay between RSV and autophagy. Importantly, the role of autophagy in RSV infection in human AECs has not yet been addressed. Our goal is to decipher the role of p62-mediated autophagy in the regulation of RSV infectious cycle in human AECs. We observed that RSV triggers autophagy in A549 cells, a model of AECs. Replication of RSV was significantly decreased in cells with increased autophagic activity supporting an anti-RSV activity of autophagy. We also accumulated data supporting activation of the cargo receptor function of p62 during RSV infection. Phosphorylation of p62 on Ser403, a modification known to enhance binding of p62 to its cargo, was induced in a TBK1-dependent manner. Accordingly, p62 binding to poly-Ub proteins was increased during RSV infection. Inhibition of lysosomal enzymes using Bafilomycin confirmed that autophagy was responsible for p62 degradation during RSV infection. The finding of RSV proteins amongst pulled down polyUb proteins suggests either direct polyUb of RSV protein(s) or their interaction with polyUb host protein(s). Moreover, we observed colocalization between RSV F and LC3. Current proteomics-based studies are aimed at identifying p62 poly-Ub cargo that are targeted to autophagosomes and have a role in the regulation of RSV replication cycle. Altogether, our results imply that selective autophagy involving the p62 receptor exhibits antiviral activity against RSV in human AECs.

Topic #3: Viruses of Microbes

A chemical defence against phage infection

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The battle for survival between bacteria and the viruses that infect them, known as phages, has resulted in the evolution of a wide variety of anti-phage defences. These include cell surface modifications, restriction enzymes, abortive infection systems, and the CRISPR-Cas adaptive immune system. While these defence systems are highly varied mechanistically, they all rely on proteins or protein-RNA complexes to mediate their functions. We recently discovered a new chemically based anti-phage defence system in the soil dwelling bacteria Streptomyces. These bacteria are renowned for producing specialized metabolites that kill bacteria and other competitors that share their environment. We show that the production of anti-phage specialized metabolites is widespread in Streptomyces and that these compounds target a single step early in the phage infection process that would broadly inhibit the majority of phages. As bacteria are outnumbered by phages in all environments and the production of specialized metabolites by bacteria is ubiquitous, this mechanism of anti-phage defence likely plays a major evolutionary role in shaping bacterial communities.

Following Phages through Fecal Transplants

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Fecal microbiota transplants/transfers (FMTs) have been used to treat human intestinal diseases ranging from Clostridium difficile infections to ulcerative colitis. The compositional changes of the gut microbiome following FMT are of considerable interest. Surprisingly, despite the successful outcomes of this therapy, evidence for transplantation of the donor's microbiome remains inconclusive. An often-overlooked component of this transfer is the bacteriophage (phage) population, which could impact the recipient's microbiome. We tracked the highly prevalent gut phage, crAssphage, in samples collected from donors and recipients of FMTs. This phage, originally identified by metagenomics, is present in ~ 40% of humans, although its bacterial host is not yet known. Combining PCR and metagenomic analysis, we were able to observe temporal variability in crAssphage prevalence as well as correlate transfer of the phage with FMTs from phage-containing donors. To assess causality, we also demonstrated engraftment of crAssphage into germ-free mice receiving human fecal matter. We confirmed the presence of an established, replicating phage population. Identifying species transferred into mice alongside crAssphage has enabled us to embark on a targeted approach to culture the elusive host of this remarkable phage.

The gut virome of pediatric Crohn's Disease following exclusive enteral nutrition

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Background: Inflammatory bowel disease (IBD), is an increasingly common disorder that causes inflammation in the gastrointestinal tract. Although causes are elusive, the gut microbiome is known to play a prominent role in IBD pathogenesis. Additionally, the viral component of the gut microbiome, the gut virome, has been shown to be abnormal in IBD patients. Exclusive enteral nutrition (EEN) is used as a first-line induction therapy for pediatric IBD patients, but how it affects the gut microbiota is still not fully understood. In this study, we aimed to optimize characterization of the gut virome in response to EEN treatment and to identify longitudinal viral-bacterial interactions. Methods: Metagenomic shotgun sequencing (MGS) was carried out on 40 longitudinally collected stool samples enriched for free virions from 6 pediatric patients with CD. Viral taxonomy of each sample was determined by aligning sequencing reads against the IMG/VR protein database. Bacterial taxonomic profiles were generated using 16S rRNA data and were integrated with viral profiles to analyze interactions with treatment progression. Results: An average of 5,024,873 reads were sequenced in each sample, with an average of 8.0% of the reads (SD: 8.2%) having significant hits to the reference database. We identified 661 viral taxa, with the majority belonging to bacteriophages. Annotated reads were also collapsed into 44531 unique viral clusters. Additionally, presence of Enterococcus phage EF62phi was moderately correlated with relative abundance of its bacterial host during the two-year study period in one patient (Spearman r: 0.54; p=0.039). Significance: Our study is the first attempt at integrating viral MGS and 16S microbiome data in pediatric CD patients. This preliminary analysis warrants further work to gain insight into the bacteriophage-bacteria interaction during treatment.

Type IV pili-specific bacteriophages of Stenotrophomonas maltophilia

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The emerging bacterial pathogen Stenotrophomonas maltophilia is rapidly increasing in prevalence in nosocomial and community-acquired infections. This bacterium is ubiquitous in the environment and is easily transmitted between immunocompromised patients and health care providers. Treatment of S. maltophilia infections is difficult, however, due to its innate resistance to a broad range of antibiotics and assortment of virulence factors. A possible alternative treatment is phage therapy, the clinical application of bacteriophages to eradicate target bacteria. The specificity of phages to their hosts relies on the presence of the correct cell surface receptor. Identifying these phage receptors and characterizing the mechanism of phage-host interaction will help create effective phage cocktails containing multiple phages with different receptors for therapeutic use. Our lab has isolated a number of broad host range bacteriophages from soil samples obtained across Alberta, Canada that are capable of infecting 24 out of 27 S. maltophilia strains collectively. Previous study of the bacteriophage DLP1, capable of infecting two strains of Pseudomonas aeruginosa in addition to eight S. maltophilia strains, identified the type IV pilus as the primary receptor for infection across its host range. The type IV pilus is a virulence factor on the surface of the bacterium that plays an important role in attachment to hosts as well as biofilm formation and twitching motility in many bacterial pathogens. DLP1 along with five other bacteriophages isolated in our lab infect the S. maltophilia strain D1585 as their main host. Clean deletion of the major pilin subunit, encoded by pilA, in D1585 prevents infection by all six phage and subsequent complementation with the endogenous pilA gene restores infection. This indicates that these six unique bacteriophage share the same receptor for infection of their major host, however their host ranges are vastly different. Deletion of pilA in a second host, 280, infected by four of the six bacteriophage produced identical results, supporting the identity of the type IV pilus as the receptor for these phages. Examination of genome sequencing data of the strain D1571 that is susceptible to only a single bacteriophage of the six revealed that this strain does not encode the PilA major subunit for type IVa pilus assembly. Interestingly, expressing both D1585 and 280 pilA genes in D1571 broadens the phage susceptibility of this strain, allowing infection by all six phages. These results identify the type IV pilus in S. maltophilia as the receptor for six unique bacteriophages and suggest that phages may adopt evolutionarily conserved bacterial surface structures as primary receptors for initial host cell interaction. Future research will examine differing phage binding sites on the major pilin subunit as well as differences and similarities between phage receptor binding proteins to explain the different host ranges of these phages. This research further characterizes these phages as candidates for phage therapy cocktails in the treatment of multidrug resistant S. maltophilia infections.

Examining viral populations, CRISPR-resolved virus-host interactions, and CRISPR-Cas system diversity in a municipal landfill

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Recent culture-independent approaches have unveiled a staggering diversity of viruses from a wide variety of environments. The comparatively poor representation of viruses within reference databases, despite their numerical abundance in ecosystems, clearly illustrates how characterizing the virosphere has historically been a methods-limited endeavor. Understudied environments, like municipal waste sites, are likely to house novel, diverse viral populations, whose host interactions may impact nutrient cycling and contaminant degradation. Using metagenomics, we probed the viral diversity within a landfill and adjacent aquifer in Southern Ontario. Metagenomic sequences were generated from samples collected from three leachate wells, a leachate collection cistern, and from an aquifer adjacent to the landfill. DNA was extracted from filtered biomass, and sequenced by the Joint Genome Institute (JGI). Using FastViromeExplorer, a virus and phage identification pipeline, along with NCBI's RefSeq database and the JGI's Earth Virome Project database, we identified the viral signatures in all six metagenomes. The different sites varied in their viral presence, with the cistern samples containing the highest viral abundances and diversities, and the aquifer sample showing the lowest. To establish virus-prokaryotic host interactions within the landfill, we used Crass to first identify CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) within our metagenomes. The spacers within the CRISPR arrays were extracted, and searched against NCBI's Refseq database and our metagenomes using BLASTn, in order identify the viruses from which the spacers originated. The vast majority of identified spacers had no hits in Refseq, suggesting that many of the ecologically important viruses within our system are not present within this reference database. The few spacers that did have hits to Refseq had homology predominantly to Pseudomonad-infecting phage genomes. Spacer hits to our metagenomes were identified as spacer-viral element matches or spacer-host CRISPR array matches, allowing connections based on prior infections to be determined. CRISPR-Cas systems are useful in elucidating virus-prokaryotic host interactions, and are of huge value in the agricultural and health industries. Current biotechnology applications utilize CRISPR-Cas systems adapted from isolated bacteria, while novel Cas proteins may offer improvements or alternatives to current gene-editing tools. We sought to identify the Cas protein diversity within our metagenomes. Using profile Hidden Markov Models (HMMs) generated from specific Cas protein alignments, we screened our metagenomes for the presence of Cas proteins. Numerous Cas proteins types and subtypes were detected, aligned, and homology modeled in order to identify novel Cas proteins within the landfill microbial community with potential biotechnology applications.

Topic #4: Antivirals and Vaccines

Neuraminidase Inhibitors Enhance Fc-Dependent Effector Functions Elicited by Hemagglutinin Stalk-Binding Broadly-Neutralizing Antibodies

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Influenza A viruses (IAV) cause 3-5 million serious illnesses and half a million deaths each year. Vaccination is the best way to prevent infection, but protection provided is narrow and ineffective against pandemic strains due to antigenic drift in the immunodominant hemagglutinin (HA) head domain. Recently discovered broadly-neutralizing antibodies (bNAbs) that target the conserved HA stalk domain provide great promise towards development of a "universal" influenza vaccine. These bNAbs require Fc receptor binding and effector cell function to confer maximal protection. We now show that enzymatic inhibition of neuraminidase (NA) enhances Fc-dependent effector functions elicited by bNAbs and may reduce the development of therapeutic resistance. Using an in vitro antibody-dependent cell cytotoxicity (ADCC) assay, we demonstrate that oseltamivir, an NA inhibitor, causes a dose-dependent increase in bNAb-mediated ADCC of IAV infected cells. Using mouse models, we also show that oseltamivir/bNAb combination therapy is superior at protecting against lethal IAV infection compared to using either treatment alone in vivo. Furthermore, we are performing experiments to determine if oseltamivir/bNAb combination therapy is effective at preventing or delaying therapeutic resistance. The mechanism by which bNAbs protect against IAV propagation is incompletely understood. Our findings may explain the variable efficacy of oseltamivir in patients, and also guide design of universal influenza vaccines and other bNAb-based anti-viral therapeutics.

Identification of pharmacological targets and small molecules inhibiting adenovirus replication

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The human adenovirus (Ad) causes minor respiratory illnesses in most patients, but can lead to severe disease and death in pediatric, geriatric and immunocompromised patients. No approved antiviral therapy currently exists for the treatment of severe Ad-induced diseases. Within the first few hours of infection, the Ad DNA enters the host cell nuclei and associates with cellular proteins including histones, adopting a nucleoprotein structure similar to the cellular DNA. Assembly of the viral genome into this repeating nucleosome-like structure is required for efficient expression of virus-encoded genes. Consequently, one approach to treating Ad-induced disease may be to prevent the viral DNA from transitioning to this transcriptionally active state. Thus, our objective is to identify novel small-molecule inhibitors of Ad replication and to investigate the molecular mechanism underlying the inhibition, including effects on epigenetic regulation of the viral genome. We have generated a wild-type-like Ad construct encoding the red fluorescent protein (RFP) within the viral genome. RFP from this construct is only expressed following Ad DNA replication, which allows us to effectively monitor virus replication by fluorescence microscopy. Using this construct, we designed an efficient method for screening small-molecule libraries. We have screened two small molecule libraries consisting of over 1300 compounds to identify drugs that exhibit anti-Ad activity. Several positive hits have been validated to either inhibit or considerably delay Ad gene expression, and reduce virus yield. In particular, the pan-histone deacetylase (HDAC) inhibitor vorinostat was found to significantly reduce RFP expression. Follow-up studies revealed that vorinostat delays the onset of viral gene expression and replication, and decreases virus yield from infected cells. Vorinostat was also effective against more virulent and clinically relevant Ad serotypes. The drug's inhibitory effects were found to be mediated through the inhibition of HDAC2 activity. Further elucidation of the underlying mechanism and in vivo studies are underway. The costs associated with Ad-induced disease are significant in terms of medical expenses, lost work hours and loss of life in some populations. Identification of novel Ad inhibitors will allow the design and development of more effective antivirals, ultimately leading to decreased disease pathogenesis and higher survival rates in severe infections. Investigation of the mechanism by which these compounds impact Ad replication will provide useful insights on virus-cell interactions, and allow us to identify new pharmacological targets for therapeutic intervention.

Characterization of a Novel Host-Targeted Antiviral Molecule

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Introduction: Current front-line influenza antivirals rely on targeting specific viral proteins to limit replication. These drugs are known as direct-acting antivirals (DAAs). However, rapid viral evolution allows for the emergence of mutations that confer resistance to these types of antivirals. Because influenza viruses depend on many host processes for viral replication, host-targeted antivirals (HTAs), provide an attractive alternative to limit the emergence of drug-resistant viruses. Here, we characterize a novel host-directed antiviral, SG3, that effectively limits influenza A virus (IAV) replication in vitro. Methods: A549 lung epithelial cells were infected with IAV and treated with SG3 at 4hpi. Release of progeny virions was analyzed by plaque assays and protein expression was assessed via immunoblotting. Results: SG3-treated A549 cells exhibited a 2-log reduction in infectious virions compared to control cells. SG3 treatment caused activation of both the PERK and IRE1α arms of the unfolded protein response (UPR), and selectively reduced accumulation of viral HA and NA glycoproteins while having minimal effects on NP and M1 expression. Conclusions: SG3 treatment reduces IAV replication and accumulation of viral glycoproteins HA and NA, while activating the unfolded protein response. Our findings demonstrate that SG3 is a promising candidate HTA for influenza. These in vitro findings will have to be corroborated in an appropriate pre-clinical in vivo model.

Photodynamic Inactivation of Herpes Simplex Viruses

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Introduction: Photodynamic inactivation (PDI) employs a photosensitizer, light, and oxygen to inactivate microorganisms through the production of reactive oxygen species. PDI's mechanism of action is nonspecific but its effect is immediate and confined to regions where a photosensitizer, light, and oxygen overlap in space and time. Orthoquin™, a botanical plant extract developed by PhotoDynamic Inc., is a powerful photosensitizer with antimicrobial properties that are amplified by exposure to visible light. Orthoquin™-mediated PDI has been shown to kill bacteria and destroy biofilm without causing inflammation, making it an attractive candidate for treating oral bacterial diseases. Herpes simplex virus infections are common globally (affecting 67% of people under age 50) and symptoms include painful oral (HSV-1) or genital/anal (HSV-2) lesions. Orthoquin™-mediated PDI was investigated as a potential treatment for symptoms associated with these two viral infections. Methods: Orthoquin™-mediated PDI was tested against HSV-1 and HSV-2 infection of HeLa cells using plaque reduction assays and single cycle infection assays (via flow cytometry), where virus inoculum was incubated with different concentrations of Orthoquin™ and exposed to visible light before inoculating cells. Results: Upon photoactivation, Orthoquin™-mediated PDI inhibited HSV-1 and HSV-2 infections in a dose-dependent manner, though with different efficacies against each virus. Conclusions: PDI is a promising alternative to traditional antibiotics and antivirals due to its immediate and selective mechanism of action. We showed that Orthoquin™ inhibits herpes simplex virus infection.

Topic #5: Emerging Methods in Virology

Flow virometry: A novel and powerful approach to decipher viral egress

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Flow cytometry has been instrumental to characterize normal and infected cells. However, it has until recently not been possible to use such approach to analyze small entities such as viruses owing to the 0.5 µm resolution of most instruments. To circumvent this limitation, some laboratories decorate viruses with antibodies or nanoparticles. Our laboratory instead exploits an alternative approach that relies on the tagging of internal viral constituents with permeable syto dies or the fluorescent tagging of individual viral proteinaceous components, whether capsid, tegument or glycoproteins. This opens up a range of new research avenues and, for example, enabled us to characterize individual HSV-1 particles, discern and characterize their different nuclear subpopulations, measure the heterogeneity of mature virions in terms of protein content, sort these viral particles with >90% purity and, for the first time, directly address the impact of this heterogeneity on viral fitness. This approach, coined flow virometry by others, allows the study of a wide variety of pathogens with high statistical significance and the potential discovery of novel virulence factors.

SynViro: Building the next generation of therapeutic poxvirus vectors

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The power of synthetic biology is revolutionizing the way we build better therapeutics. Given the importance of modifying vectors to express immunodominant antigens against a particular pathogen or tumour antigens, it is vital to establish a platform to do this in a systematic and rapid manner. Poxviruses are well suited as biotherapeutic vectors to deliver foreign antigens, although manipulating their genomes can become time-consuming when one would like to manipulate a number of different areas of the genome simultaneously. Here, we will discuss a novel platform that we have developed to reactivate poxviruses from overlapping synthetic DNA. As a proof of principle, we chose to reactivate horsepox virus (HPXV) since molecular analyses have shown that modern vaccinia virus (VACV) vaccine strains share common ancestry with this virus. Fragments of DNA were synthesized based on a published HPXV sequence along with VACV terminal sequences. These fragments were recombined into a live synthetic chimeric HPXV (scHPXV) in cells infected with a helper virus. Sequencing of the 212kbp scHPXV confirmed it encoded a faithful copy of the input DNA. We believe this is the first complete synthesis of a poxvirus. This scHPXV produced smaller plaques in vitro and exhibited less virulence in mice compared to VACV, but still provided vaccine protection against a lethal VACV challenge. Our lab is further investigating this methodology for the manufacture of other poxvirus-based therapeutics, with applications for rapid synthesis of synthetic chimeric vaccines and oncolytics. We will also discuss some of the technical challenges associated with using synthetic biology as a tool to develop these next-generation of poxvirus-based therapeutics.

Targeting Persistent Hepatitis B Virus: Identification of a G4-Quadruplex Structure Motif in a Key Region of HBV's Genome

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Background: Approximately 240 million people worldwide are chronic carriers of hepatitis B virus (HBV), and are at increased risk of developing cirrhosis and hepatocellular carcinoma. The virus persists in the nucleus in a covalently closed circular DNA (cccDNA), which acts as the template for all HBV mRNA transcripts. While current antiviral therapies are effective at viral suppression, they do not target HBV cccDNA and cannot eradicate infection. Recently, we have discovered and provide evidence for a unique structural motif in HBV's pre-core promoter region—a G4-quadruplex—a distinct, stacked, four-guanosine folding arrangement of DNA. Such quadruplexes are being found at key transcription and translation sites of numerous organisms and are thought to regulate these processes. Methods: The wild-type and single-nucleotide mutation oligomers of the HBV pre-core promoter region were purified through size-exclusion chromatography. Fractions were analysed using circular dichroism (CD), electrophoretic mobility shift assay (EMSA), and small-angle X-ray scattering (SAXS) analyses. Next, a known quadruplex-binding protein, DHX36 was expressed via an E.coli expression system and purified using histidine-tag affinity chromatography. Finally, cccDNA was extracted from an HBV-infected liver explant and pull-down assays using DHX36 were performed on whole and fragments of cccDNA. Results: Through CD, EMSA and SAXS data, we demonstrate that the wild-type oligomer forms a parallel quadruplex structure, while the mutant oligomer remains linear. As well, we show the quadruplex-binding protein, DHX36, to bind the wild-type oligomer, as well as pull down cccDNA from clinically-relevant samples. Conclusions: This novel finding of a quadruplex in the HBV pre-core region provides a unique opportunity to study a critical host-protein interaction site in HBV cccDNA transcription. Through the pursuit of high-resolution structural data, we will be creating the framework for designing a novel inhibitor of the resilient HBV cccDNA, the master template for HBV replication.

Development of iPSC-derived neural stem cells transduced with Borna disease virus vector expressing thymidine kinase for cancer gene therapy

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Borna disease virus (BoDV) is an RNA virus that infects a wide range of vertebrates. An intriguing characteristic of BoDV is its ability to establish a persistent infection in the nucleus without requiring to integrate into the host genome. In our previous study, we exploited biological characteristics of BoDV and developed a non-integrating vector system capable of sustained transgene expression in human pluripotent stem cells (hiPSCs). Recently, we found that neuroblastoma cell lines are highly susceptible to infection by BoDV vector, which prompted us to explore potential applications of BoDV vector for cancer gene therapy. In this study, we developed a BoDV vector encoding thymidine kinase (TK) and evaluated anti-tumor effects of BoDV-TK vector in vitro. Herpes simplex virus TK was inserted between the viral phosphoprotein (P) and matrix (M) genes, and the recombinant vector was rescued using reverse genetics system of BoDV. To develop a system to target BoDV vector to cancer cells, we used neural stem cells (NSCs) as carrier cells, which have been demonstrated to possess inherent tumor tropic properties. hiPSCs transduced with BoDV-TK vector were differentiated into NSCs by culturing in neural induction medium. Expression levels of NSC markers Nestin and Pax6 were similar in both mock transduced and BoDV-TK vector transduced cells, indicating that BoDV vector does not interfere with differentiation of iPSCs into NSCs. Migration of NSCs loaded with BoDV-TK vector to cancer cells was analyzed by modified boyden chamber assay. Ongoing experiments evaluating anti-tumor effects of BoDV-TK NSCs and ganciclovir (GCV) against neuroblastoma will be presented.

Seeing is believing: Visualizing multi-protein complexes mediating HIV-1 immune evasion

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A major factor preventing the elimination of HIV-1 lies within the ability of the virus to hide from the immune system. This inherent ability of HIV-1 to confuse the immune system is accomplished by the small accessory protein Nef. Specifically, MHC-I downregulation by the HIV-1 Nef is of critical importance in preventing infected cells from cytotoxic T-cell mediated killing. Nef downregulates MHC-I by modulating the host membrane trafficking machinery, resulting in the endocytosis and eventual sequestration of MHC-I within the cell. Here, we expand on our previous work which identified a molecular map underlying the subcellular compartments mediating viral immune evasion. Utilized the intracellular protein-protein interaction reporter system, bimolecular fluorescence complementation (BiFC), in combination with super-resolution microscopy, we identified the membrane trafficking regulator PACS-1 to be critical in MHC-I sequestration. The interaction between PACS-1 and Nef was found to be critical in the recruitment of the clathrin adaptor AP-1 to the Nef:MHC-I complex. Through super-resolution microscopy, we were able to determine that the mutation of the Nef:PACS-1 interface doubled the molecular distance between AP-1 and MHC-I (30nm to 75nm), highlighting the role of PACS-1 as a critical mediator of Nef-mediated MHC-I downregulation. Furthermore, we demonstrate that mutation of the Nef:PACS-1 interaction drastically changes the localization of MHC-I to the early endosomes. Overall, we highlight the ability of super-resolution microscopy and fluorescent imaging to identify and track multi-protein complexes mediating HIV-1 immune evasion. Understanding these complex molecular pathways will aid in the development of new inhibitors targeted at crippling HIV-1's ability to evade our own immune surveillance system.

Topic #6: RNA in Virus Infection

miR-122-mediated protection of the HCV genome from pyrophosphatase activity

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Hepatitis C virus (HCV) recruits two molecules of the liver-specific microRNA-122 (miR-122) to the 5' end of its genome. In contrast to the canonical activity of miRNAs, the interaction of miR-122 with the viral genome promotes viral RNA accumulation in cultured cells and animal models of HCV infection. Although this interaction has been the subject of several studies, the precise mechanism of miR-122-mediated viral RNA accumulation remains incomplete. Previous studies suggest that miR-122 is able to protect the HCV genome from 5' exonucleases (Xrn1/2), but this protection is not sufficient to account for the effect of miR-122 on HCV RNA accumulation. We hypothesized that miR-122 interactions with the 5' terminus of the HCV genome prevents recognition of the viral 5' triphosphate by innate sensors of RNA or cellular pyrophosphatases. We thus explored miR-122-dependent and miR-122-independent viral RNA accumulation during knockdown or overexpression of several innate sensors of RNA or pyrophosphatases. We found that miR-122 does not play a protective role against recognition by protein kinase R (PKR), Retinoic acid inducible I (RIG-I)-like receptors, or IFN-induced protein with tetratricopeptide repeats (IFITs) 1 and 5. However, we found that knockdown of two cellular pyrophosphatases, DOM3Z and DUSP11, was able to restore viral RNA accumulation of subgenomic replicons in the absence of miR-122. However, pyrophosphatase knockdown was not able to rescue viral RNA replication in miR-122 knockout cells. Thus, we provide a model whereby miR-122 mediates protection of the viral 5' terminus from cellular pyrophosphatase activity and subsequent turnover by Xrn1/2. However, miR-122 must play an additional role(s) in the HCV life cycle beyond genome stability since pyrophosphatase knockdown was not able to rescue viral RNA accumulation in the absence of miR-122. In addition, we have used Selective 2' Hydroxyl Acylation analyzed by Primer Extension (SHAPE) to investigate how miR-122 binding alters the structure of the 5' non-coding region of the HCV genome. We also provide preliminary data suggesting that mutations in the 5' non-coding region that relieve HCV's dependence on miR-122 alter the structure of the 5' non-coding region in a manner that promotes HCV genome stability.

Insight into influenza A virus cap-snatching

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Influenza A Virus (IAV) causes yearly epidemics and significant worldwide mortality. IAV has a segmented genome composed of eight single-stranded negative sense viral RNAs (vRNAs). At the earliest step of IAV replication, a complex formed by the viral RNA-dependent RNA polymerase (RdRp) and any of the vRNAs interacts with host RNAP II, cleaves the 5' end of host pre-mRNAs, and uses these capped RNA fragments as primers for viral mRNA synthesis, using a process called cap-snatching. Because the capped RNA fragments also contain 10-15 nucleotides downstream of the cap, sequence heterogeneity is thus found at the host-derived 5' ends of viral mRNAs. Profiling of the host primers used by several IAV strains indicates that the eight RdRp: vRNA complexes cap-snatch different subsets of host RNAs, and that vRNA UTRs might provide target specificity during cap-snatching. This suggests that features on vRNA UTRs are responsible for target specificity, and/or that the availability of host capped primers might be dictated by the dynamics of both IAV transcription and the host response. More importantly, our results indicate that host RNAs are cap-snatched based on their abundance, and that primers associated with genes linked to the cellular response to IAV infection are overrepresented in the cap-snatched population. By targeting these RNAs early during infection, IAV could modulate the antiviral response early after infection, which could lead to enhanced replication and pathogenesis. Further studies are underway to elucidate the molecular mechanism of IAV cap-snatching, and to determine the role of cap-snatching as a molecular mechanism used by the virus to inhibit the cellular response early after infection.

Viral Modulation of RNA Granules is Reliant on Autophagic Flux

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Kaposi's Sarcoma-associated herpesvirus (KSHV) is the etiological agent of Kaposi's Sarcoma. Kaposin B (KapB) is a hallmark viral gene expressed in both the latent and lytic life cycles of the virus. KapB can activate the MK2-RhoA axis to promote the formation of actin stress fibres and the disassembly of processing bodies (PBs). PBs are small, cytoplasmic, ribonucleoprotein granules found in all cells that regulate gene expression via decay or translational suppression of mRNA. PBs play a central role in the constitutive decay of a subset of human transcripts that contain AU-rich elements (AREs) and typically code for molecules such as proinflammatory cytokines and angiogenic factors. The dispersal of PBs by KapB requires MK2 activation; however, the precise details of this mechanism remain unclear. MK2 activation has been shown to upregulate autophagy through the phosphorylation of Beclin. We hypothesize that KapB is engaging autophagy to disassemble PBs and prevent the decay of ARE-mRNAs, thereby increasing the expression of inflammatory molecules. Primary endothelial cells were transduced with lentiviruses containing KapB or a vector control; cells were treated with various inhibitors and activators of autophagy; LC3 punctae were visualized by immunofluorescence, p62 and resident PB protein expression levels were assessed by immunoblot. KapB-expressing cells showed increased LC3 punctae and decreased steady-state levels of p62 protein, indicating that KapB expression increases autophagic flux. KapB-expressing cells also showed a decrease in the expression of several resident PB proteins compared to controls, and droplet digital PCR revealed increased steady state levels of ARE-containing inflammatory cytokine mRNAs. When autophagic flux was blocked, PB levels increased, while decreased levels of PBs occurred during induction of autophagy. Furthermore, a luciferase assay for ARE-mRNA stability showed an increase in ARE-mRNA stability during induction of autophagy and a decrease during inhibition of autophagy. Collectively, these data show that the mechanism of KapB-induced PB dispersal and concomitant increases in inflammatory gene expression rely on engaging the autophagic pathway. We believe that through this mechanism, KapB creates a pro-inflammatory and pro-angiogenic environment that benefits KSHV infection and KS tumour progression.

Alternative translation initation during KSHV lytic replication

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Kaposi's sarcoma-associated herpesvirus (KSHV) the causative agent of Kaposi's Sarcoma and primary effusion lymphoma. As a herpesvirus, KSHV replicates in the nucleus where it has access to host mRNA processing machinery. However, we have found that translation of capped, poly-adenylated KSHV mRNAs does not require the canonical eIF4F translation-initiation machinery. Here the examine the role of stress-responsive, alternative initiation systems in KSHV replication and assess the composition of translating mRNA-ribonucleoprotein complexes (mRNPs) in viral latency and lytic replication. We isolated translating mRNPs using ultracentrifugation and sucrose-gradient fractionation. Protein components of translating mRNPs were identified by quantitative LC-MS/MS and confirmed with immunoblotting. We used RNA silencing to limit activity of different translation initiation systems and assess their role in virus replication. In lytic replication, eIF4F is functional but not required for virus replication, while methyl-6-adenosine-dependent translation is required. LC-MS/MS studies identify three viral proteins that specifically associate with actively translating mRNAs. KSHV usurps distinct host translation initiation systems during latent and lytic phases of infection. These different systems likely have important roles in altering the translatome to support viral latency or promote virion production.

Towards Obtaining a Nanoscale Structure of the 3' Terminal Region of Japanese Encephalitis Virus Genome

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Background: Japanese encephalitis virus (JEV) elongs to the Flaviviridae family of RNA viruses – a family that includes other pathogenic viruses such as Dengue, West Nile, and Zika. These viruses have a conserved 3' terminal region that plays a significant role in viral replication by mediating long-range interactions with the 5' terminal region of the genome. The 3' terminal region also interacts with host proteins aiding viral replication. Despite the significance these terminal regions play in human infection, their structural details are largely unknown. Methods: Plasmids were designed that encode for the entire ~600 kb long 3' terminal region of JEV, as well as numerous fragments therein. The resulting RNA was purified using size-exclusion chromatography (SEC). Structural studies of the constructs were performed using small angle X-ray scattering (SAXS) to generate low-resolution models of the RNA and selective 2' hydroxyl acetylation analyzed by primer extension (SHAPE). Results: Using in vitro transcription, full-length and fragments of the JEV 3' terminal region RNA have been generated. Preliminary SAXS data indicate that one of the segments of the terminal region adopts multiple conformations in solution, whereas other regions have elongated shapes. The data is being combined with that of SHAPE in an iterative process to obtain high-resolution models of these constructs. Conclusion: Future studies will focus on the structural analysis of the 3' terminal region in complex with the 5' terminal region as well as various host proteins, including DEAD box helicases, to give further insight into viral replication and thus give rise to potential new antiviral targets.

Topic #7: Viruses of Flora and Fauna

Translation initiation of alternative reading frames by viral internal ribosome entry sites

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RNA structures can interact with the ribosome to alter translational reading frame maintenance and promote recoding that result in alternative protein products. We showed previously that a subset of internal ribosome entry sites (IRESs) from the honey bee dicistroviruses can direct translation in the 0 and +1 frames. Here, we show that the IRES from the related dicistrovirus Cricket paralysis virus also drives translation of the 0-frame viral polyprotein and an overlapping +1 open reading frame, called ORFx. Using an unprecedented mechanism, the CrPV IRES utilizes a mechanism whereby a subset of ribosomes recruited to the IRES bypasses downstream to resume translation at the +1-frame 13th non-AUG codon. A mutant of CrPV containing a stop codon in the +1 frame ORFx sequence, yet synonymous in the 0-frame, is attenuated compared to wild-type virus in a Drosophila infection model, indicating the importance of +1 ORFx expression in promoting viral pathogenesis. Collectively, this work demonstrates a novel programmed IRES-mediated recoding strategy to increase viral coding capacity and impact virus infection, highlighting the diversity of RNA-driven translation initiation mechanisms in eukaryotes.

Expanding repertoire of plant (+)-strand RNA virus proteases: beyond the cysteine and serine proteases

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Many viruses encode one or several proteases that regulate the release of mature proteins from viral polyproteins. In addition, viral proteases have been shown to cleave specific host proteins to facilitate virus infection. So far, characterized proteases from plant viruses correspond to three classes: cysteine, serine or aspartic proteases. Cysteine and serine proteases are encoded by (+)-strand RNA viruses, while aspartic proteases are encoded by retroviruses and pararetroviruses. Members of the family Secoviridae (secovirids) constitute a large group of plant picorna-like viruses. Like picornaviruses, they encode a 3C-like cysteine protease. So far, no other viral proteases have been characterized from secovirids. Strawberry mottle virus (SMoV, family Secoviridae) is one of several viruses found in association with strawberry decline disease in Eastern Canada. It has a bipartite (+)-strand RNA genome, with each RNA encoding one large polyprotein. RNA1 encodes the 3C-like cysteine protease that cleaves at five sites in the RNA1 polyprotein and at a single site between the predicted movement protein and capsid protein (CP) domains in the RNA2 polyprotein. We detected two additional cleavage events in the RNA2 polyprotein downstream of the CP domain. Cleavage at these sites is orchestrated by a second protease domain present in the C-terminal region of the RNA2 polyprotein. Sequence alignments of SMoV isolates with related viruses (black raspberry necrosis virus and lettuce secovirus 1) revealed several highly conserved amino acids, which were mutated to alanine. Surprisingly, mutations of conserved serine, cysteine, histidine or aspartic acid residues did not prevent the proteolytic cleavage. Instead, we show that two glutamic acid residues are strictly required for polyprotein processing. N-terminal sequencing of the cleaved product using Edman degradation mapped the predominant cleavage site to a P/AFP sequence immediately downstream of the CP domain and systematic mutagenesis of amino acids around the mapped cleavage site confirmed a requirement for the two prolines at the -1 and +3 positions of the cleavage site. A secondary cleavage site was detected at a related P/KFP sequence 46 kDa downstream of the primary cleavage and was also confirmed by mutagenesis. We conclude that processing of SMoV polyproteins requires two viral proteases, the RNA1-encoded cysteine 3C-like protease and a second RNA2-encoded protease, which represents a novel type of viral protease, with putative glutamic acid catalytic residues.

The underlying mechanism of a unique nuclear entry pathway used by the parvovirus minute virus of mice

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Parvoviruses are small, non-enveloped, single-stranded DNA viruses that replicate in the nucleus of the host cell. Rodent parvoviruses are not only able to preferentially target rapidly dividing cancer cells, but also possess oncolytic properties. They have thus been evaluated as potential anti-cancer agents, either as unmodified infectious virus, attenuated mutants, or vectors. For the development of such parvoviruses-based therapy, we need to better characterize their life cycle, particularly their nuclear entry pathway. Our laboratory has previously found that the parvovirus minute virus of mice (MVM) and other parvoviruses enter the nucleus by a unique mechanism that involves small, transient disruptions of the nuclear envelope (NE) and partial dismantling of the underlying nuclear lamina. We are now investigating the molecular mechanism by which MVM disrupts the NE. Since N-myristoylation of different viral proteins has been reported to play roles in virus-cellular membranes interaction for many other non-enveloped viruses, and capsid proteins of MVM have putative N-myristoylation sites, we have been investigating the involvement of protein N-myristoylation in the nuclear entry mechanism of MVM. We found that the protein N-myristoylation inhibitor 2-Hydroxymyristic acid (HMA) significantly prevented viral gene expression in MVM-infected mouse fibroblast cells. More importantly, when added at different time point post-infection, the inhibitory effects of HMA on viral gene expression coincided with the time of the nuclear entry of MVM. To further illustrate the role of protein N-myristoylation during this process, we used semi-permeablized HeLa cells in which the influx of TRITC-dextran into the nucleus is an indicator for MVM-induced NE disruption. With this system we further found that when protein N-myristoylation was blocked by HMA, MVM-induced NE disruption was also inhibited. Therefore, we propose that protein N-myristoylation plays an important role in the mechanism of MVM-induced NE disruption during the nuclear entry of MVM.

Atypical Transmembrane Domain of Reptilian Reovirus p14 Fusion-Associated Small Transmembrane (FAST) Protein Mediates it's Trafficking.

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Reovirus fusion-associated small transmembrane (FAST) proteins are solely re-sponsible for the syncytiogenic phenotype of the fusogenic reoviruses. FAST pro-teins comprise very small N-terminal ectodomains and equal-sized or considera-bly larger C-terminal cytosolic endodomains flanking a single transmembrane (TM) domain. TM domains of different FAST proteins do not share sequence similarity but are interchangeable with each other, but not with TM domains from heterologous viral fusion proteins. These findings suggest the FAST protein TM domains are a fusion module that plays a more direct role in the fusion process, but insights into its structural and functional features are not known. To address this deficit, the atomic resolution structure of a FAST protein TM domain was determined using solution state nuclear magnetic resonance (NMR) spectroscopy in the presence of dodecylphosphocholine micelles. The 25-residue -helical TM domain of the reptilian reovirus p14 FAST protein consists of a funnel shaped he-lix owing to a skewed distribution of bulky aromatic residues towards its C-terminus. We also noted a preponderance of hydrophobic β-branched residues at the N-terminal interfacial region, and what could be a conserved solvent-accessible cleft in the concave face of the curved helical structure. Functional and biochemical analysis (cell fusion efficiency, protein abundance and cell surface expression) of individual point mutations affecting these atypical TM domain characteristics supported a role for all of these features in protein trafficking.

Porcine circovirus 2 (PCV2) activated apoptosis of non-infected cells

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Porcine circovirus 2 (PCV2) is a pathogen of major importance for swine production around the world. Despite development of several vaccines and robust vaccination programs, PCV2 continues to persistently transmit within and between swine herds causing infections marked by immunosuppression via lymphocyte depletion. This study aims to improve the understanding of the pathogenesis of porcine circovirus diseases (PCVD) caused by PCV2 capsid cytotoxicity by revealing a cytotoxic motif which activates apoptosis in non-infected bystander cells. The capsid protein is the only structural protein of PCV2. Preliminary results show that monomeric PCV2 Cap and 1% formaldehyde inactivated and dialyzed PCV2 are both able to activate apoptosis in porcine kidney (PK15) cells at a rate of ~%30 after a 24-hour exposure; which is similar to apoptosis caused by 50 μM etoposide, a strong chemical inducer of apoptosis. These results were obtained through flow cytometric analysis of apoptosis using Annexin V-FITC and 7-AAD to measure markers of apoptosis and cell death. The results support the conclusion that both monomeric PCV2 Cap and 1% formaldehyde inactivated PCV2 induce apoptosis in PK15 cells after external exposure without infection, lending credence to the idea of PCV2 Cap as a primary virulence factor during infections. Future experiments will utilize the above apoptosis assay flow cytometry experiment using non-pathogenic PCV1 and PCV2 Cap virus-like particles (VLPs) produced in insect cells through a baculovirus expression system (BES) to investigate the cytotoxicity of PCV2 Cap. This study may also shed some light on the difference in pathogenesis between PCV2 and the non-pathogenic PCV1 which is also poorly understood.

Topic #8: Antiviral Innate Immunity

Class A scavenger receptors: their role in innate antiviral immunity in lower vertebrates

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Class A scavenger receptors (SR-As) are a family of five surface expressed receptors who in mammals play roles in macrophage-mediated clearance of pathogens and apoptotic cells, atherosclerosis, and as surface receptors for innate immune stimulants and for virus entry. Very little is known of SR-As, including their sequence, expression profile and function in lower vertebrates. Our group has focused in the last few years on elucidating the role of SR-As in mediating innate antiviral immune responses in lower vertebrates, specifically using teleost fish and frog cell lines. Of particular interest is whether SR-As in lower vertebrates are similar to their mammalian counterparts in their ability to bind viral double-stranded (ds)RNA, a potent innate immune stimulant produced by viruses during replication. We have successfully identified the full coding sequence for MARCO, SCARA3, SCARA4 and SCARA5 from rainbow trout and have found that cells expressing functional SR-As are able to bind dsRNA. As opposed to mammalian SR-As where any member of the family appears to bind dsRNA, rainbow trout MARCO when over-expressed did not bind dsRNA, but did bind bacteria. We have also characterized CHSE-214, a Chinook salmon cell lines, as a new model for studying SR-As and dsRNA binding, as this cell line is unable to bind dsRNA unless engineered to over-express exogenous SR-As. Finally, we have begun work identifying SR-A sequences in newly developed frog cell lines. We have found our frog cell lines all express SR-As at the transcript level, albeit with different expression patterns, and those expressing SR-AI are able to bind AcLDL, an SR-AI ligand. We have also found that all of our frog cell lines are exquisitely sensitive to dsRNA-induced cell death compared to fish cell lines. Finally we have evidence that SR-As may be functioning as surface receptors for the ranavirus, frog virus 3. These data provide evidence for the significance of SR-As in innate antiviral immunity in lower vertebrates and the importance of understanding this complex family of receptors in fish and frogs.

IFN and cytokine responses in ducks to genetically similar H5N1 influenza a viruses of varying pathogenicity

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Ducks, the reservoir host, are generally permissive to influenza A virus infection without disease symptoms. This natural ecology was upset by the emergence of H5N1 strains, which can kill ducks. To better understand the host-virus interactions in the reservoir host, and influenza strain-specific molecular contributions to virulence, we infected White Pekin ducks with three similar H5N1 viruses, with known differences in pathogenicity and replication rate. We quantified viral replication and innate immune gene activation by qPCR, in lung and spleen tissues, isolated on each of three days of infection. All three viruses replicated well, as measured by accumulation of matrix gene transcript, and viral load declined over time in the spleen. The ducks produced rapid, but temporally limited, interferon and cytokine responses, peaking on the first day post-infection. Interferon and proinflammatory cytokine gene induction were greater in response to infection with the more lethal viruses, compared to an attenuated strain. We conclude that a well-regulated interferon response, with the ability to overcome early viral immune inhibition, without hyperinflammation, contributes to the ability of ducks to survive H5N1 influenza replication in their airways, and yet clear systemic infection and limit disease.

Murine Cytomegalovirus Immune Evasion Targeting the MEK-IRF1 Connection

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Murine cytomegalovirus (MCMV) modulates host immune system for their propagation. Although MCMV immune evasion against NK cells or CD8 T cells has been extensively studied, less is known about modulation of host innate immunity by MCMV. Recently, we found that activation of mitogen-activated protein kinase kinase (MEK) suppresses antiviral functions of interferon regulatory factor 1 (IRF1) (ref.1). As MCMV infection is known to activate MEK (ref.2), we hypothesized that MCMV suppresses antiviral functions of IRF1 by promoting MEK activation during infection. When NIH3T3 cells or mouse embryonic fibroblasts (MEF), were infected with MCMV, we observed increased phosphorylation of extracellular signal-regulated kinases (ERKs), downstream elements of MEK, at 1, 2, 3 and 4 days after infection, and decreased expression of IRF1 at 2, 3 and 4 days after infection. Treatment of the MEK inhibitor U0126 restored IRF1 expression and further inhibited MCMV infection. We are currently investigating whether MEK activation regulates IRF1 functions by modulating one of its posttranslational modifications, SUMOylation, during MCMV infection. This study will identify a novel strategy of MCMV immune evasion that targets the potent host antiviral protein IRF1.

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The NADPH oxidase DUOX2 differentially modulates the production of respiratory virus-induced cytokines by airway epithelial cells

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Airway Epithelial Cells (AECs) constitute the first line of defense against respiratory viruses. AECs autonomous antiviral response relies on the secretion of mucus, peptides and a wide range of soluble mediators, called cytokines. As part of the antiviral response, efficient production of cytokines by AECs is an important determinant of the outcome of infection through the shaping of the antiviral (innate and adaptive) and inflammatory response. However, an excessive inflammatory response leading to an exaggerated recruitment and activation of inflammatory cells often contributes to virus-associated pathogenesis and disease severity. We and others have previously documented the induction of the epithelial H_2O_2-generating NADPH oxidase DUOX2 expression and activity at late time of infection by respiratory viruses, including Sendai virus (SeV), Respiratory Syncytial Virus, and influenza virus. DUOX2 activity was found to be essential for the establishment of an antiviral response through the sustained production of type I and III Interferons. Considering the role of various cytokines in shaping the antiviral and inflammatory response against respiratory viruses, we sought to define the contribution of DUOX2 in the regulation of a panel of cytokines produced by AEC during respiratory virus infections. DUOX2 expression in A549 cells (a human alveolar epithelial cell lineage) was silenced by siRNA transfection. Cells were then infected with Sendai virus (SeV) as model of respiratory virus infection. Using multiplex luminex-based assays, we profiled the levels of a panel of 48 cytokines. Amongst those, 30 cytokines are induced by SeV independently of DUOX2. Interestingly, DUOX2 modulates, either positively or negatively, a limited subset of twelve SeV-induced cytokines. Our results propose a role of DUOX2 in the modulation of the intensity or quality of antiviral and inflammatory responses orchestrated by AECs. Current studies are aimed at validating these results in AECs deficient in DUOX2 obtained via genome editing and at studying the impact on the recruitment and activation of immune cells. The proposed DUOX2-linked immune-modulation of cytokine production suggests a previously unsuspected involvement of this H_2O_2-producing protein in inflammatory responses such as cytokine-driven recruitment and activation of inflammatory cells.

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IGA immune complexes stimulate netosis during viral infection and autoimmunity

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Neutrophil extracellular traps (NETs) are extracellular chromatin structures that play a role in trapping and killing a variety of pathogens. NETs are released in response to a variety of stimuli such as bacteria, fungi and viruses. Immunoglobulin A (IgA) is enriched at mucosal sites and interacts with FcαRI on the surface of neutrophils. We have found that IgA-opsonized antigens stimulate NETosis using a pathway that is distinct from that of immunoglobulin G (IgG). Human neutrophils were first isolated from peripheral blood and stimulated with immune complexes (ICs) containing immunoglobulins and Influenza A virus (IAV) or human immunodeficiency virus (HIV). IgA-containing ICs potentiated NETosis when compared to IgG containing complexes, and this effect was dependent on FcαRI. We have also shown that IgA antibodies purified from patients with rheumatoid arthritis (RA) potently induced autoantigen-dependent NETosis relative to IgA from healthy controls. Our data suggests that IgA-stimulated NETosis may occur through a previously uncharacterized signaling pathway, which we are currently elucidating. Since IgA stimulates NET release, mucosal vaccinations could enhance NET production, thereby reducing the severity of viral infections. In contrast, during autoimmunity the production of NETs may exacerbate disease through the release of self-antigens. Therefore, inhibiting this pathway could be of therapeutic benefit.

Posters 1 – 72: poster Session #1

1 - Proteomics of Herpes simplex virus type 1 nuclear capsids

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Herpes simplex virus has a complex life cycle that includes the production of distinct particles in the nucleus, where new capsids are assembled. Among these nuclear capsids, procapsids are devoid of nucleic acid but contain large amounts of the preVP22a scaffold protein. These thermo-unstable particles then mature into A-, B- or C-nuclear icosahedral capsids, depending on their ability to shed the proteolytically processed scaffold and incorporate the viral genome. Interestingly, C-capsids are preferentially exported to the cytoplasm and ultimately give rise to infectious virions. While A-, B- and C-capsids share several components, their distinct fate hints at meaningful differences. To probe them, we performed proteomics studies of highly enriched nuclear capsids, relying in part on flow virometry to purify C-nuclear capsids. We found that while many proteins are indeed common among these nuclear capsids, they exhibit unique sets of proteins. These naturally include viral proteins but also many cellular proteins.

3 - Development of pre-pandemic influenza vaccine against highly pathogenic H5 strains

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The emergence of highly pathogenic avian influenza A viruses (HPAI) and their spillover into human population poses substantial economic burden and public health threats. Among HPAI, H5 viruses are of particular concern given their global spread and pandemic potential. In this study, we aimed at developing a novel pre-pandemic H5 influenza vaccine that is of potential importance in improving pandemic preparedness. The first North American HPAI H5N1 strain (A/Alberta/01/2014) was selected for recombinant H5 hemagglutinin (HA) design.

Recombinant H5 HA is expressed in both mammalian and bacterial system for comparison of H5 antigenicity from distinct expression hosts. The oligomeric status and functional characterization of purified H5 immunogens from different expression systems were determined by native PAGE and hemagglutination assay in vitro. In the meantime, we established the mouse diseases model for the HPAI H5N1 virus (A/Alberta/01/2014) with 3 different doses. In this model, viral replication and innate immune gene upregulation were quantified by $TCID_{50}$ and qPCR, respectively, in mouse lung and spleen tissues. Immunogenicity and protective efficacy of H5 immunogens will later be determined by vaccination and challenge studies in vivo.

4 - Elucidating the Role of Senataxin During Class Switch Recombination and Somatic Hypermutation

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Senataxin (SETX) is a large, 303kDa, ubiquitously expressed DNA: RNA helicase that has been shown to play a role in meiotic recombination, the DNA damage response and the antiviral response. There is significant overlap between the known functions of SETX and the pathways required for both class switch recombination (CSR) and somatic hypermutation (SHM). For example, SETX is known to resolve R-loops and both CSR and SHM require R-loop formation to generate a ssDNA substrate for AID. Furthermore, SETX co-localizes with proteins involved in the DNA damage response that are known to play a central role in CSR, including 53BP1 and γ-H2AX. To elucidate the role of SETX in CSR and SHM we are utilizing a SETX knockout mouse model. We hypothesized that SETX would play an important role in both CSR and SHM via cell intrinsic and extrinsic mechanisms. Currently, we have demonstrated that SETX knockout mice exhibit a prolonged antigen-specific IgM response, coupled with a delay in the production of antigen specific IgA antibodies, following vaccination with influenza virus. Additionally, we have demonstrated that SETX deficiency results in an increased mutation rate in the V(D)J region. Our future work will focus on defining the precise mechanisms through which SETX affects both CSR and SHM. These studies will expand our knowledge of the processes that regulate antibody generation and may inform rational vaccine design strategies.

6 - Neuraminidase Inhibitors Enhance Fc-Dependent Effector Functions Elicited by Hemagglutinin Stalk-Binding Broadly-Neutralizing Antibodies

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Influenza A viruses (IAV) cause 3-5 million serious illnesses and half a million deaths each year. Vaccination is the best way to prevent infection, but protection provided is narrow and ineffective against pandemic strains due to antigenic drift in the immunodominant hemagglutinin (HA) head domain. Recently discovered broadly-neutralizing antibodies (bNAbs) that target the conserved HA stalk domain provide great promise towards development of a "universal" influenza vaccine. These bNAbs require Fc receptor binding and effector cell function to confer maximal protection. We now show that enzymatic inhibition of neuraminidase (NA) enhances Fc-dependent effector functions elicited by bNAbs and may reduce the development of therapeutic resistance. Using an in vitro antibody-dependent cell cytotoxicity (ADCC) assay, we demonstrate that oseltamivir, an NA inhibitor, causes a dose-dependent increase in bNAb-mediated ADCC of IAV infected cells. Using mouse models, we also show that oseltamivir/bNAb combination therapy is superior at protecting against lethal IAV infection compared to using either treatment alone in vivo. Furthermore, we are performing experiments to determine if oseltamivir/bNAb combination therapy is effective at preventing or delaying therapeutic resistance. The mechanism by which bNAbs protect against IAV propagation is incompletely understood. Our findings may explain the variable efficacy of oseltamivir in patients, and also guide design of universal influenza vaccines and other bNAb-based anti-viral therapeutics.

7 - Regulation of Respiratory Syncytial Virus infection by the autophagy receptor SQSTM1/p62 in airway epithelial cells.

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Respiratory syncytial virus (RSV) causes a high rate of morbidity and mortality in infants, children and elderly, but also adults of all ages with a compromised immune system, cardiopulmonary diseases or who have undergone transplantation, worldwide. RSV vaccine development faces numerous challenges and current very costly prophylactics are restricted to high-risk children. Antivirals targeting host factors required for virus replication are an emerging alternative to traditional direct-acting antivirals. RSV primarily replicates in airway epithelial cells (AECs). Therefore, understanding RSV: host interaction in human AECs is crucial for future development of drugs targeting RSV replication. Autophagy is critical for intracellular homeostasis in response to stress, including virus infection. However, compelling evidence demonstrate that a number of viruses have evolved mechanisms to exploit and modulate the function of autophagy components to use them for their benefit. Selectivity in autophagy is conferred by cargo receptors, including p62/SQSTM1, which tether ubiquitylated (Ub) cargo to a autophagosomes for degradation into autophagolysosomes. Previous data have highlighted an important role for p62-dependent autophagy in the bacteria and virus clearance, although p62 was also shown to promote replication of some viruses. Very little information is available regarding the interplay between RSV and autophagy. Importantly, the role of autophagy in RSV infection in human AECs has not yet been addressed. Our goal is to decipher the role of p62-mediated autophagy in the regulation of RSV infectious cycle in human AECs. We observed that RSV triggers autophagy in A549 cells, a model of AECs. Replication of RSV was significantly decreased in cells with increased autophagic activity supporting an anti-RSV activity of autophagy. We also accumulated data supporting activation of the cargo receptor function of p62 during RSV infection. Phosphorylation of p62 on Ser403, a modification known to enhance binding of p62 to its cargo, was induced in a TBK1-dependent manner. Accordingly, p62 binding to poly-Ub proteins was increased during RSV infection. Inhibition of lysosomal enzymes using Bafilomycin confirmed that autophagy was responsible for p62 degradation during RSV infection. The finding of RSV proteins amongst pulled down polyUb proteins suggests either direct polyUb of RSV protein(s) or their interaction with polyUb host protein(s). Moreover, we observed colocalization between RSV F and LC3. Current proteomics-based studies are aimed at identifying p62 poly-Ub cargo that are targeted to autophagosomes and have a role in the regulation of RSV replication cycle. Altogether, our results imply that selective autophagy involving the p62 receptor exhibits antiviral activity against RSV in human AECs.

8 - The NADPH Oxidase DUOX2 Differentially Modulates the Production Of Respiratory Virus-Induced Cytokines By Airway Epithelial Cells

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Airway Epithelial Cells (AECs) constitute the first line of defense against respiratory viruses. AECs autonomous antiviral response relies on the secretion of mucus, peptides and a wide range of soluble mediators, called cytokines. As part of the antiviral response, efficient production of cytokines by AECs is an important determinant of the outcome of infection through the shaping of the antiviral (innate and adaptive) and inflammatory response. However, an excessive inflammatory response leading to an exaggerated recruitment and activation of inflammatory cells often contributes to virus-associated pathogenesis and disease severity. We and others have previously documented the induction of the epithelial H_2O_2-generating NADPH oxidase DUOX2 expression and activity at late time of infection by respiratory viruses, including Sendai virus (SeV), Respiratory Syncytial Virus, and influenza virus. DUOX2 activity was found to be essential for the establishment of an antiviral response through the sustained production of type I and III Interferons. Considering the role of various cytokines in shaping the antiviral and inflammatory response against respiratory viruses, we sought to define the contribution of DUOX2 in the regulation of a panel of cytokines produced by AEC during respiratory virus infections. DUOX2 expression in A549 cells (a human alveolar epithelial cell lineage) was silenced by siRNA transfection. Cells were then infected with Sendai virus (SeV) as model of respiratory virus infection. Using multiplex luminex-based assays, we profiled the levels of a panel of 48 cytokines. Amongst those, 30 cytokines are induced by SeV independently of DUOX2. Interestingly, DUOX2 modulates, either positively or negatively, a limited subset of twelve SeV-induced cytokines. Our results propose a role of DUOX2 in the modulation of the intensity or quality of antiviral and inflammatory responses orchestrated by AECs. Current studies are aimed at validating these results in AECs deficient in DUOX2 obtained via genome editing and at studying the impact on the recruitment and activation of immune cells. The proposed DUOX2-linked immune-modulation of cytokine production suggests a previously unsuspected involvement of this H_2O_2-producing protein in inflammatory responses such as cytokine-driven recruitment and activation of inflammatory cells.
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9 - Epinephrine and norepinephrine augment Ebolavirus replication

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Ebolavirus (EBOV) causes multisystem failure mimicking sepsis in humans, with a fatality rate of up to 90%. Although there are a number of experimental and prophylactic treatments for EBOV, there are currently no approved therapeutic agents. As such patients diagnosed with EBOV are treated with supportive care therapy, including the use of fluids and vasoactive substances (epinephrine /and norepinephrine) to stabilize blood pressure in an attempt to maintain adequate organ function. Preliminary evidence from our laboratory suggests that these vasoactive agents can augment replication of EBOV in cell culture. This implicates these hormones, both from supportive care therapy (exogenous) and endogenous secretions, in playing a role in the pathogenicity of the disease. Although this is preliminary data in regards to EBOV, other viral infections, such as Enterovirus 71 (EV71) have been shown to have increased viral replication and enhanced disease severity in response to epinephrine and norepinephrine. The aim of the current study is to explore the effects of vasoactive agents on EBOV replication. Experiments will be first completed in an enhanced level 2 biosafety laboratory using a cell culture model with various cell lines infected with Pseudotyped Vesicular stomatitis virus-Ebola (VSV-EBOV), which expresses EBOV transmembrane glycoproteins exposed to varying concentrations of epinephrine and norepinephrine. Later studies will perform infections of the same cell lines under similar conditions with wild EBOV in a containment level 4 (CL4) environment. If vasoconstrictors are shown to augment the viral replication as hypothesized, this could lead to future reconsiderations of blood pressure management techniques for infected patients.

12 - The inhibitory effect of proprotein convertase subtilisin/kexin type 9 on hepatitis C virus replication

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Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a serine protease that plays an important role in lipid homeostasis through facilitating the degradation of low-density lipoprotein receptor (LDLR). Hepatitis C virus (HCV) is a positive-sense single-stranded RNA virus that can result in chronic hepatitis. No HCV vaccine has been developed and the current antiviral treatments have some limitations. Although PCSK9 has been shown to inhibit HCV replication, the underlying mechanism has not been thoroughly characterized. In this study, we first confirmed the inhibitory effect of PCSK9 on HCV replication by over-expressing or knocking down PCSK9 in HCV genomic replicon cells. Then we demonstrated that PCSK9-induced LDLR degradation was not involved in HCV replication regulation using gain-of-function (D374Y) or loss-of-function (Δaa. 31-52) PCSK9 mutants for LDLR degradation. Moreover, the auto-cleavage of PCSK9 affected HCV replication since only uncleaved proPCSK9 suppressed HCV replication and cleaved PCSK9 did not have effect on HCV replication. Next, we found that PCSK9 could interact with several HCV proteins including NS5A. The PCSK9 interacting region of NS5A was aa. 95-215 in Domain I. The interaction between PCSK9 and NS5A inhibited NS5A dimerization and HCV RNA binding to NS5A. Considering that NS5A dimerization, RNA binding activity of NS5A is required for HCV replication, the interaction between PCSK9 and NS5A could be a mechanism of how PCSK9 inhibits HCV replication. This study may improve the understanding of the antiviral effect of PCSK9 on HCV and help optimize anti-HCV regimens.

13 - Principal component analysis as a tool to study the evolutionary history the viral AlkB domain

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AlkB proteins are ubiquitous among cellular organisms where they act to reverse damage in RNA and DNA resulting from methylation, such as 1-methyladenine, and 3-methylcytosine. Recently, it has been discovered that an AlkB domain is encoded as part of the replicase polyprotein by a small subset of single-stranded, positive sense RNA viruses that mostly infect woody perennial plants. However, the boundary of these viral AlkB domains as well as their function in viral replication and infection is unknown. Phylogenetic characterization has indicated that this AlkB domain was likely acquired relatively recently by an ancestor of the family Betaflexiviridae. Here, we utilize principal component analysis (PCA) to establish the boundaries of a functional viral AlkB domain at a primary structure level. Additionally, we apply PCA and other clustering techniques to analyze the relationship between viral AlkB domains from different viral families to examine the hypothesized single acquisition event. Using a similar approach, we compare and group AlkB proteins/domains from virruses, bacteria, plants, and animals into distinct substrate-specific clusters to hypothesize a broad origin of the viral AlkB domain. We hope this in silico analysis would allow us to make predictions of the structure and function of viral AlkB domains that can be tested through experimentation.

17 - Identification of virus-host receptors for Atlantic salmon bafinivirus, a coronavirus of fish

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Aquatic virology is a rapidly expanding field. This is due to the growing global demand for seafood, and subsequent intensification of aquaculture. This intensification can serve to facilitate diseases in fish due to the chronic stress caused by the high-density confinement. These conditions provide opportunities for the emergence of viral pathogens that may be harmless under natural conditions, such as Atlantic Salmon Bafinivirus (ASBV). ASBV is a recently discovered coronavirus, isolated from farmed Atlantic salmon. As ASBV is a recently isolated virus, its clinical significance is still unknown, and as such it is uncertain how it may threaten both wild, and farmed populations of Atlantic salmon. The primary aim of this research is to map the receptor-binding domain in the S1 subunit of the spike (S) protein of the (ASBV), and the corresponding host cell surface receptor(s) to which it binds. This will be elucidated using recombinant PCR techniques, and specific co-purification of receptor-binding subunit from lysates of susceptible cells. These studies will serve to elucidate the emergence, virulence and persistence of ASBV.

18 - Involvement of the 90kDa Ribosomal S6 Kinases (RSKs) in viral oncolysis

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Oncolytic viruses exploit tumor-specific cellular changes for their selective replication, including p53 deficiency, oncogenic Ras activation, insensitivity to type I interferon (IFN) and viral receptors uniquely expressed on cancer cells. Our previous research showed that activation of the Ras/MEK pathway suppresses the host antiviral response induced by IFN [1]. This is caused by suppression of the transcription of a group of IFN-inducible genes [2,3]. Our research further showed that this suppression is caused by downregulation of interferon regulatory factor 1 (IRF1), the transcriptional regulator of the IFN-inducible genes [4,5]. Together, we have demonstrated that the Ras/MEK downregulation of IRF1 is one of the major mechanisms underlying viral oncolysis. Activated MEK 1 and 2 phosphorylate ERK 1 and 2, which in turn activate their downstream signalling (MAPK-interacting Kinases (MNKs) 1-2, Mitogen- and Stress-activated Protein Kinases (MSKs) 1-2 and the 90kDa Ribosomal S6 Kinases (RSKs) 1-4) to regulate transcription and translation. While we previously demonstrated the role of ERK in IRF1 regulation, the elements further downstream of ERKs that are involved in this regulation still remain to be identified. In this study, we first conducted RNAi screening of MNK1-2, MSK1-2 and RSK1-4 using RasV12-transformed NIH3T3 cells. IRF1 expression was restored by knockdown of RSK3 and RSK4, but not by that of the other ERK downstream elements in the cells with activated Ras. Overexpression of RSK3 in NIH3T3 cells reduced IRF1 expression and inhibited transcription of IRF1-mediated antiviral genes. CRISPR knockdown of RSK3 also restored IRF1 expression in human cancer cells. These results suggest that RSKs are the ERK downstream elements involved in IRF1 downregulation in cancer cells with activated Ras and subsequently in viral oncolysis.

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20 - Bovine Viral Diarrhea Virus: Quantifying Antigenicity

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Bovine viral diarrhea virus (BVDV) is a globally distributed pathogen responsible for a broad spectrum of illnesses in cattle. The rapid evolution and substantial diversity of the virus have made broadly protective vaccination challenging. The variability of BVDV is well documented, however the impact of this diversity on the antigenicity of the virus remains to be fully characterized. We aim to investigate the antigenic components of the E2 surface protein of the virus that induce immune responses in cattle. To do so, an expression vector for the production of a wide array of variants of the immunodominant protein E2 was designed using enzyme-mediated restriction and ligation. Unique enzyme restriction sites flanking the E2 coding sequence were included to allow for replacement with alternate protein sequences. The resulting constructs were introduced into mammalian cell culture via electroporation in order to produce appreciable quantities of the protein for downstream antigenic analysis using the Bio-layer interferometry platform. This experimental framework will allow for the analysis of antigenic variants present in wild type viruses, and the evaluation of the affinities and avidities of antibodies induced through vaccination towards field viruses.

21 - Gene expression modulation within NPTr-CD163 infected cells which are resistant to cell death induced by porcine reproductive and respiratory syndrome virus (PRRSV)

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Porcine reproductive and respiratory syndrome (PRRS) is a disease widely spread in North America and is the source of huge economic losses in the porcine industry. The PRRSV is known to trigger the death of permissive infected cells through apoptosis, such as the porcine alveolar macrophages (PAM), and MARC-145 cells. Recently, a porcine cell line was genetically modified to study PRRSV co-infection pathogenesis with others swine pathogens. More precisely, newborn pig trachea cells (NPTr) were modified to express the CD163 receptor, to allow PRRSV infection. Interestingly, PRRSV can replicates into NPTr-CD163 cells at a similar rate than in MARC-145 infected cells but without inducing cell death. That observation lead to the following question: how does PRRSV affect the gene expression in MARC-145 cells and in NPTr-CD163 cells. Our hypothesis is that NPTr-CD163 cells response against PRRSV infection is better adapted which allow the survival of the cells compared to MARC-145 infected cells. Therefore, mRNA expression modulation of the MARC-145 cells and the NPTr-CD163 cells infected cells was determined by a RNA-Seq experiment using the Illumina technology. This experiment will give us important insights in regards to the different mechanisms involved into PRRSV pathogenicity.

22 - Genomic characterization of porcine reproductive and respiratory virus (PRRSV) Quebec strains within clinical samples using whole genome sequencing

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PRRSV is responsible of major economic losses in the swine industry worldwide. There are currently efforts to sequence the ORF5 of all circulating PRRSV strains in Quebec, with a current database containing over 4000 ORF5 sequences, in order to do molecular epidemiological surveillance but no data is available on the full-length genome of PRRSV strains in Quebec. We hypothesized that full-length genome sequencing of PRRSV would provide a better insight into the pathogenicity of different strains and would allow for a better epidemiological monitoring compared to ORF5 sequencing. Serum, saliva and lung tissue samples with PRRSV viral load evaluated by RT-qPCR were used. The RT-qPCR Ct values of the samples sequenced were varying between 10 and 32. Viral genomes were concentrated using poly(dt) magnetic beads and library were prepared using Nextera XT technology. They were sequenced on an Illumina Miseq sequencer. 24 full-length genomes were obtained. It was found that the whole PRRSV genome was more variable than the ORF5 (nucleotide identity between strains :78.17%-99.63% vs 83.06%-100%) which is of interest because the ORF5 is recognized as one of the hypervariable region of the PRRSV genome. It was found that all wild-type strains sequenced had 3 important deletions totalling 513 nucleotides in the ORF1a, more precisely in the NSP2 and NSP7 region, two different lineages of PRRSV in Quebec were defined based on the presence of these deletions. Interestingly, the presence of two different PRRSV strains (co-infection) were found in two lung tissue samples suggesting an 8.7% prevalence of PRRSV co-infection within clinical samples.The two strains in one of the samples shared 92.12% identity at the nucleotide level. Moreover, one recombinant PRRSV strain (i.e. recombination between the two lineages) was also found. While more full-length viral genomes are still required to improve our PRRSV genome databank and our analyses, it is expected that whole-genome sequencing of PRRSV will allow us to better understand the pathogenesis of the virus and control the disease it causes.

24 - Epidemiology and molecular characterization of novel parvoviruses in Canadian wildlife

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Viruses of the genus Amdoparvovirus, family Parvoviridae, cause an immune-associated and often lethal wasting syndrome in various carnivores. The Aleutian mink disease virus (AMDV) was the only member of this genus for many years but many novel amdoparvoviruses have been discovered more recently. Since these viruses can infect multiple hosts and most of the research so far has been focused on captive animals, there is limited understanding of their ecology, host- and geographic distributions. To fill this knowledge gap, we investigated the presence and molecular features of amdoparvoviruses in Canadian wildlife. Tissue samples (spleen, muscle or intestine) collected over the years for various studies were screened with a PCR assay designed to detect all known amdoparvoviruses. These efforts allowed us to identify amdoparvoviruses in skunk (43/50, 86%) and mink (1/2, 50%) from British Columbia (BC), in pine marten (1/58, 17.2%) and red fox (7/127, 5.5%) from Labrador, and in mink (8/23, 34.8%) and red fox (2/29, 6.9%) from Newfoundland. Samples collected from Arctic and red foxes from Quebec, Nunavut, Northwest Territories and Manitoba (46, 65, 12 and 105 samples, respectively) were negative. Complete or partial genomes were obtained from positive samples and viruses were molecularly characterized. Three potentially novel viruses were detected. Two of these viruses were identified in samples from BC, one in mink (Aleutian mink disease virus 2, AMDV-2) and one in skunks (skunk amdoparvovirus, SKAV). The non-structural protein sequences (NS1) of these viruses diverge by >15% from other amdoparvoviral species, fulfilling the ICTV molecular criterion for species demarcation. AMDV-2, phylogenetically close to AMDV (NS1 protein identity: 78-82%) and different from viruses of farmed mink in BC, has so far been identified in only one individual, and its distribution is currently under investigation. SKAV, now officially classified by the ICTV as Carnivore amdoparvovirus 4, was phylogenetically close to the mink viruses (NS1 protein identity: 77-83%) and analyses performed on 44 sequences revealed the presence of at least 3 circulating lineages. This virus was also identified in skunks and in one mink in other distant areas of North America (such as Ontario) and strains showed geographic segregation. The third divergent virus was identified in red foxes from Labrador and is tentatively named Labrador fox amdoparvovirus (LaFAV). We have only obtained capsid protein (VP2) sequences for this virus so far, a region that is highly conserved between species and phylogenetically uninformative, but preliminary data suggest that this virus is likely a novel species and among the 7 LaFAV strains we identified two variants with distinct geographic origins. Viruses identified in Newfoundland (from both mink and red fox) could be classified as AMDV and they were phylogenetically close to viruses previously identified in farmed mink. However, 5 of these viruses, all from mink, were grouped within a clade that contained only viruses found in the wild, providing evidence for viral perpetuation and maintenance in the wild. Overall, our work has proved that several different amdoparvoviruses are present in various wild carnivores across Canada. The individual viral species seem to affect predominantly one animal species, with only rare spillover events, and viruses show geographic segregation. Because several different (novel) viruses have been identified, we believe that amdoparvoviral diversity is higher than anticipated and many amdoparvoviruses could exist that are yet to be discovered. It is our priority to complete the genomic sequencing of the detected viruses and future screening efforts are likely to reveal additional diversity.

26 - Analysis of Programmed Cell Death Induced by HCV Infection

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Virus infection results in host cell death via various modes of programmed cell death. In our previous study, we showed that hepatitis C virus (HCV, JFH-1) infection induces apoptosis and pyroptosis in both infected and bystander cells. Pyroptosisis a caspase-1-dependentform of cell death that is considered an inflammatory form of cell death. Therefore, pyroptosis may play roles in the development of liver pathogenesis and hepatocellular carcinoma in HCV-infected individuals.The objective of this study is to identify cellular mechanisms by which HCV infection induces programmed cell death.When Huh-7.5 cells were infected with HCV in vitroat MOI=0.1, we observed activation of caspase-1 (pyroptosis) at 3 days after infection and activation of caspase-3 (apoptosis) at 4 days after infection. As the nucleotide-binding oligomerization domain-like receptor containing pyrin domain 3 (NLRP3) is one of the upstream modulators of caspase-1, we generatedNLRP3 knockout Huh-7.5 cells by CRISPR. HCV infection did not induce pyroptosis in NLRP3 knockout Huh-7.5 cells. Surprisingly, apoptosis was also inhibited in the absence of NLRP3. Furthermore, we observed lower levels of viral proteins and virus titer in NLRP3 knockout Huh-7.5 compared to control Huh-7.5 cells, suggestingthat programmed cell death affects HCV replication. As apoptosis is considered a host defense mechanism to inhibit HCVreplication, pyroptosis appearsto be a strategy used by HCV to enhance virus production. The findings from this study have the potential to identify mechanisms underlying chronic liver inflammation and viral spread in HCV patients.

27 - The Role of the NLRP3 Imflammasome in HCV-Induced Pyroptosis

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Pyroptosis, one of several described inflammatory types of programmed cell death, is induced after activation of the NLRP3 inflammasome, which ultimately results in pore formation and cell lysis. One of the main factors in the development of the liver cirrhosis associated with Hepatitis C virus (HCV) infection is caspase-3-mediated apoptosis (non-inflammatory). However, recently it was shown that both apoptosis and pyroptosis occur in cultured human hepatocyte-like cells infected with HCV as well as in uninfected bystander cells. It remains unclear whether pyroptosis is beneficial to the virus or is an innate antiviral response potentially benefitting the host. The current research has aimed to detect universal inflammasome components (cleaved caspase-1) in HCV-infected cells and to study the effect of the inhibition of these components on virus titer. Optimization of a protocol using FAM-FLICA probes has been used effectively to visualize caspase-1 activation in HCV-infected cells. Higher levels of caspase-1 activation were consistently observed in HCV-infected cells compared to uninfected cells, indicating more inflammasome activation occurs in infected cells. Interestingly, levels of NLRP3 activation were higher in HCV-infected cells than observed with the LPS/Nigericin priming typically used as a positive control. To further confirm the role of the NLRP3 inflammasome in HCV-induced pyroptosis, we interfered with inflammasome activation using a NLRP3 inhibitor as well as a NLRP3 knockout cell line. We found that virus titer was significantly decreased when NLRP3-mediated pyroptosis was inhibited by either method. In conclusion, these data confirm the presence of pyroptosis in HCV infected cells and specifically demonstrate the involvement of the NLRP3 inflammasome. These results suggest that pyroptosis is a mechanism used by HCV to cause pathogenesis.

29 - Phage moron JBD26-15 disrupts Pseudomonas aeruginosa flagella biosynthesis

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Bacteriophages (phages), viruses that infect bacteria, can integrate their genomes into bacterial chromosomes and exist as prophages. In this state, most phage genes are silenced; however, a small number of genes known as 'morons' are actively expressed from the prophage. These genes are not required for the life cycle of the phage itself, but provide some selective advantage to the phages that carry them. Some of these phage morons have been shown to increase bacterial virulence by mediating toxin production, increasing antibiotic resistance, and immune evasion. Our lab is studying morons isolated from phages that infect the opportunistic pathogen Pseudomonas aeruginosa. We discovered that the expression of moron JBD26-15 in P. aeruginosa strain PAO1 abrogates swimming and twitching motility, two key forms of bacterial motility. While twitching motility is abrogated, the bacteria remain susceptible to phages that require the type IV pilus for infection. This lack of phage resistance suggested the pili responsible for twitching motility are present on the surface of the cells but are defective, rendering the bacteria nonmotile. As swimming motility is driven by a single polar flagellum in liquid environments, and twitching motility is mediated by type IV pili upon adherence to solid surfaces, we examined the production of these bacterial structures in the presence of JBD26-15 expression. Bacterial surface protein-shearing assays showed that expression of JBD26-15 downregulates localization of FliC, an essential flagellar component, to the surface of the cell. In addition, we observed an increase in PilA, the type IV pilus monomer, relative to wild type PAO1, indicating a hyperpilliated phenotype. A hyperpiliated phenotype explains both the loss of twitching motility and the retained sensitivity to phage infection and is consistent with a fliC knockout strain of PAO1. To our knowledge this is the first instance of phage-mediated disruption of flagellar biosynthesis in P. aeruginosa. Additional experiments to determine the mechanism by which JBD26-15 impairs swimming and twitching motility are underway. JBD26-15 is a nonessential phage gene, but its conservation among many prophages in P. aeruginosa clinical isolates suggests it confers a fitness advantage. This fitness advantage is likely increased resistance to phages - the single biggest danger to Pseudomonas survival. However, since flagella are highly immunogenic, we believe that JBD26-15 may also play an important role in human disease by decreasing the immune reactivity of the strains expressing the moron, while allowing for persistence within the human host. Understanding the role of JBD26-15 in PAO1 will further our understanding of phage-host interactions and ultimately how these interactions affect the course of bacterial infections.

30 - Regulation of IRF1 antiviral functions by its SUMOylation

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Interferon regulatory factor 1 (IRF1) is a transcriptional factor that regulates expression of antiviral, antitumor and immunoregulatory genes. We recently demonstrated that oncogenic Ras/MEK activation suppresses antiviral functions of IRF1, which is one of the mechanisms underlying Ras-dependent viral oncolysis [1]. In this study, we sought to clarify how Ras/MEK activation downregulates antiviral functions of IRF1 in human cancer cells. First we determined whether Ras/MEK activation regulates IRF1 post-translational modifications such as acetylation, phosphorylation, SUMOylation and ubiquitination. SUMOylation, but not the other post-translational modifications, was suppressed in human cancer cells when the Ras/MEK pathway was inhibited. To determine whether IRF1 SUMOylation regulates its antiviral functions, we prepared SUMO-conjugated IRF1 constructs. Human cancer cells transfected with wild type IRF1 construct was protected from infection of oncolytic vesicular stomatitis virus (VSV) while those transfected with SUMO-conjugated IRF1 constructs were not. SUMO-conjugated IRF1 was not capable of activating transcription of antiviral genes. To identify an IRF1 SUMOylation site(s) targeted by Ras/MEK, we identified predicted IRF1 SUMOylation sites by in silico analysis and generated SUMO-resistant IRF1 mutants by mutating the predicted SUMOylation sites (78K, 254K, 275K, and 299K). Human cancer cells transfected with the wild type IRF1 or a SUMO-resistant IRF1 mutant were challenged with oncolytic VSV. The cells transfected with the 275K or 299K IRF1 mutants were more resistant to the infection than those transfected with the wild type IRF1, suggesting that 275K and 299K are the SUMOylation sites of IRF1 that is targeted by Ras/MEK activation.

1. Komatsu, Y. et al. Oncogenic Ras inhibits IRF1 to promote viral oncolysis. Oncogene 34, 3985–3993 (2015).

32 - T-cell exhaustion is a hallmark of immunological failure to control chronic viral infection and cancer.

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T-cell exhaustion is a hallmark of immunological failure to control chronic viral infection and cancer. Blocking immune inhibitory receptors such as programmed death-1 (PD-1) can re-invigorate exhausted T cells (T_{EX}), but many patients fail to achieve durable disease control. Thus, a deeper understanding of other molecular pathways underlying reversal of T-cell exhaustion is needed. Little is known about "reprogramming" of T_{EX} into recovered T cells (T_{RECOV}) with better functional and memory profiles following non-immunological cure of chronic disease. Here, we aim to determine the cellular profiles of T_{RECOV}, and molecular mechanisms underlying recovery. For this, we utilized the well-defined LCMV mouse model, that was initially used to define immune exhaustion and re-invigoration using checkpoint blockade. Experimentally, we adoptively transferred T_{EX} from mice chronically infected with LCMV-clone 13 into LCMV-immune mice to examine changes in T_{EX} following antigen elimination. We also investigated the recall capacity of T_{RECOV} upon antigen re-exposure compared to T_{EX} and memory T cells (T_{MEM}). Our data indicate that upon cure of chronic infection some markers of exhaustion are downregulated on T_{RECOV}, while some markers of T_{MEM} are recovered. For example, previously-exhausted T cells gradually downregulated the expression of the exhaustion marker PD-1 and increased expression of the memory marker CD127. Nevertheless, PD-1 expression was still higher and the percentage of CD127+ cells were lower on those recovering T cells when compared to bona fide memory T cells. These changes, were also accompanied by partial recovery from dysfunction, where T_{RECOV} partially restored the capacity of dual production of effector cytokines. However, not all aspects of T_{EX} biology are corrected simply by eliminating chronic infection. Transcriptional profiling using single-cell RNA sequencing (scRNA-seq) confirmed our cellular profiling, as it suggests that the gene-expression profile of T_{RECOV} resembles T_{MEM} in some respects, while other features are still remnants of T_{EX} cells. T_{RECOV} are heterogeneous and fall into 2 main clusters; one that is memory-like and the other that is more effector-like. Another main features of memory cells is their ability to generate an effective recall response upon re-encountering antigen. Results from rechallenge studies show that upon re-encountering antigen T_{RECOV} had an enhanced ability to expand compared to T_{EX}. However, these cells were still inferior on a per cell basis compared to true T_{MEM}. Together, these results suggest that following elimination of persistent antigenic stimulation and inflammation previously-exhausted T cells are able to recover some properties of T_{MEM}, while other aspects remained "scarred" from their history of exhaustion. We are currently performing deeper analysis of the scRNA-seq data to unravel mechanistic pathways underlying the remaining defects in T_{RECOV}. We are also examining the epigenetic landscape of T_{RECOV} to verify whether the cellular and transcriptional defects we observed are imprinted epigenetically. These studies will enhance our understanding of immunological and molecular mechanisms of T_{EX} recovery, and have the potential to identify novel therapeutic strategies for recovery of more functional T cells. This will have major implications on the treatment of cancer or chronic infection.

38 - TRIM5α sensing of HIV-1 counteracts transcriptional latency

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Elite controllers (ECs) are a rare subset of HIV-1 infected individuals that maintain undetectable viremia and normal $CD4^+$ T cell counts without antiretroviral therapy. They also display a relatively small reservoir of latent proviruses. In a previous study, we showed that HIV-1 capsid cores isolated from ECs were efficiently detected by human TRIM5α. In addition to blocking their replication, TRIM5α sensing of HIV-1 from ECs activated TAK1 as well as the NF-κB and AP-1 pro-inflammatory pathways, leading to an antiviral state. Both NF-κB and AP-1 are transcription factors that bind well-characterized DNA motifs in the HIV-1 promoter and activate transcription of the provirus. Here, we asked whether pro-inflammatory signals triggered by TRIM5α could 1) prevent latency establishment, 2) reactivate transcription of latent proviruses. To address the first question, we infected TRIM5α-knockout (KO) and control THP-1 cells with GFP-expressing chimeric HIV-1 vectors constructed with capsids derived from ECs or from normal progressors (NPs). Three days later, latent proviruses were reactivated by PMA (Phorbol 12-myristate 13-acetate) or SAHA (suberanilohydroxamic acid) treatments, and levels of latency establishment were then analyzed by FACS. We observed that TRIM5α-sensitive vectors derived from ECs were significantly less prone to become latent proviruses in cells that express TRIM5α. Furthermore, latency levels were independent of TRIM5α for HIV-1 vectors in which GFP was expressed from a promoter different from the viral LTR. Also, prevention of latency establishment by TRIM5α was counteracted by pre-treating the cells with NF-κB or TAK-1 pharmacological inhibitors. For our second objective, we used the Jurkat-derivative JLat6.3 cells, which contain a latent GFP-expressing HIV-1 provirus in their genome. We infected JLat6.3 cells that were TRIM5α-knockout or not with DsRed-expressing HIV-1 vectors in which the capsid was derived from ECs or NPs. Latent GFP^+ proviruses were significantly more reactivated by the EC-derived vectors and only in cells that express TRIM5α. Similar to what was observed in the first experimental setting, latent provirus reactivation was dependent on NF-κB or TAK-1. In conclusion, HIV-1 recognition by TRIM5α both prevents latency and reverts the transcriptional silencing of latent proviruses. This study proposes a new role for the restriction factor TRIM5α and provides a novel rationale for the small latent reservoir in elite controllers.

39 - Investigating the response of activated macrophages to LCMV infection

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Macrophages (Mϕ) are innate immune tissue sentinels with a variety of functional phenotypes depending on the cytokine microenvironment. Parallel to T helper cell polarization into Th1 and Th2 cells, Mϕ exhibit distinct activation patterns across a continuum from pro-inflammatory (M1), associated with the Th1 cytokine IFNγ, to anti-inflammatory (M2a), associated with the Th2 cytokine IL-4. Given the critical role of Mϕ in regulating innate and adaptive immunity, it is not surprising that Mϕ activation status directly influences the outcome of viral infections. Generally, M1 polarization restricts viral replication. However, viruses can exploit inflammation to bypass host barriers and support replication. Additionally, viruses such as lymphocytic choriomeningitis virus (LCMV) and Lassa Virus directly suppress Mϕ activation leading to viral chronicity and severe disease. Studies using HIV showed that certain activation states are able to prevent viral infection of cells, while others are able to stop viral replication inside the cells. However, the mechanism through which polarized macrophages are able to induce this resistance is unknown. Here, we investigate the response of activated spleen Mϕ (SpM) to LCMV infection to understand how polarization influences the initiation of cellular mechanisms that regulate an adequate immune response. Following infection, we discovered that polarized Mϕ show an altered resistance to LCMV infection, which was not due to modifications in viral entry. Protein expression of innate recognition molecules involved in the RLR pathway were evaluated in the activated Mϕ. *Research funded by NSERC and CIHR*.

40 - Characterization of the novel temperate Stenotrophomonas maltophilia bacteriophage DLP5

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Stenotrophomonas maltophilia is an aerobic, opportunistic Gram-negative bacterium ubiquitous in aqueous environments, soils, plant rhizospheres and hospital settings [1]. S. maltophilia is capable of causing a variety of infections and limited treatment options exist due to S. maltophilia's exquisite innate multidrug resistance to a broad array of antibiotics[1–3]. Alternative treatment options, such as the therapeutic use of bacteriophages, are being examined to try and stem antibiotic resistance related deaths. In order to use phages therapeutically, initial research must begin with the characterization of phages to ensure they do not contain harmful moron genes. The novel S. maltophilia bacteriophage DLP5 (vB_SmaS_DLP_5) was isolated from garden soil collected in Edmonton, Alberta, Canada using the clinical S. maltophilia strain D1614. Transmission electron micrographs identify DLP5 as a B1 morphotype Siphoviridae phage due to its icosahedral head and long noncontractile tail. DLP5 possesses a relatively narrow tropism, infecting only 5/27 clinical S. maltophilia isolates tested. Successful infection with DLP5 results in clear plaques with defined boarders averaging 0.5±0.2 mm and an average burst size of 36. Restriction fragment length polymorphism analysis of DLP5 genomic DNA (gDNA) suggests it is highly modified, with only 4/36 endonucleases tested capable of digesting the gDNA. A de novo SPAdes assembly of paired-end reads produced a 96,542 bp contig (295-fold coverage) with a GC content of 58.4 %. The contig is predicted to encode 149 ORFs including four tRNAs (Sup-CTA, Glu-TTC, Gly-TCC and Ser-GCT). Only 39 ORFs could be assigned a putative function based on BLASTp and CD-search analysis. Surprisingly, six of these ORFs are predicted to be virulence factors: serine protease XkdF, SAM methyltransferase, rhomboid membrane protein, PIG-L family deacetylase, WecE, and an SPFH domain-containing protein. Of the remaining 33 ORFs assigned putative functions, phage partitioning proteins were identified which suggested DLP5 is capable of lysogenizing its host as a phagemid. Experimental evidence confirms DLP5 forms as a stable phagemid during its lysogenic cycle. Studying the growth characteristics of the wildtype and lysogenic D1614 strains revealed differences in the lag phase of growth, with wildtype D1614 exhibiting a slower growth rate when compared to its lysogenic counterpart. This is the first S. maltophlia phage identified to date capable of lysogenizing its host as a phagemid. Future studies with DLP5 will attempt to investigate the potential role each moron gene plays in the virulence modulation of strain D1614. Although DLP5 is not suitable for therapeutic use due to its temperate lifestyle and the presence of moron genes, these results highlight the need for more research on Stenotrophomonas maltophilia temperate phages and their potential involvement in virulence modulation in their hosts.

42 - Using phylogenetics to reconstruct the integration dates of individual latent human immunodeficiency virus reservoir sequences

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Background: Human immunodeficiency virus (HIV) still contributes to a global pandemic. Though combination antiretroviral therapies (cART) can reduce HIV viremia in the blood down to undetectable levels, limiting transmission and disease progression, there is still no cure. A major barrier to cure development is the fact that HIV integrates into the host's DNA. These integrated proviruses can remain in host cells for years in the so-called latent reservoir, only to spontaneously reactivate and produce virions at a later date. However little is known about the dynamics of the latent HIV reservoir including how often new latent reservoirs are seeded, and how long latently-infected cells persist. To address this, we developed a phylogenetic method to estimate the integration dates of HIV sequences in the latent reservoir. Methods: The framework first involves inference, and optimal rooting, of a maximum-likelihood phylogeny relating longitudinal within-host plasma HIV RNA and putative latent sequences. Phylogenies were inferred with RAxML and optimally rooted via root-to-tip regression using the R package ape. A linear model relating the root-to-tip distances of plasma HIV RNA sequences to their sampling dates was used to predict the establishment (integration) dates of putative latent lineages from their root-to-tip distances. Three validations were performed. In the first, we attempted to reconstruct the collection dates of the plasma HIV RNA sequences by removing the putative reservoir sequences from the dataset of one individual and then designating sequences from select time points as censored sequences. We then used the used the remaining sequences to train the linear model and estimate the collection dates of the censored sequences. In the second validation, we removed whole time points of plasma HIV RNA sequences from the data set of one individual and attempted to fit the linear model in order to estimate the integration dates of the putative reservoir sequences. We performed both of these validations on 1096 different sets. For our final validation, we compared our rooting method (root-to-tip regression) to using outgroup rooting with HX-B2 as the outgroup in our framework instead. Results: Model performance was first validated on simulated and published longitudinal HIV env sequences. The model was subsequently applied to single-genome-amplified HIV nef sequences sampled in-depth over a ~20 year timeframe (including ~10 years on suppressive cART) in two individuals with long-term viremia suppression. Results revealed a genetically heterogeneous reservoir that recapitulated HIV's within-host evolutionary history, where putative reservoir sequences interspersed throughout multiple within-host lineages with the oldest dating to >20 years prior to sampling. Notably, analysis of plasma HIV RNA sequences isolated from a viremia blip on suppressive cART revealed a genetically diverse viral pool spanning a 20-year age range, consistent with spontaneous within-host HIV reactivation from a large pool of latently-infected cells. The validations showed that linear models could be calibrated from as few as two time points and that model predictions were robust to rooting method. Conclusion: Our reservoir dating method provides a novel and powerful tool that can reveal key insights into latent HIV reservoir establishment and longevity. Our results show that the latent reservoir is comprised of a genetically heterogeneous population with long persistence. This heterogeneity and longevity of the latent HIV reservoir poses a challenge to immune-based HIV elimination strategies, which aim to reactive the latent reservoir and assist the host's immune system to clear the infection.

44 - Cellular protein kinase D modulators play a role upstream and downstream of the TGN during herpes simplex virus type 1 egress

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The assembly of novel herpes simplex virus type 1 particles takes place in the nucleus. These particles then travel across the two nuclear membranes and acquire a final envelope from a cellular compartment, likely the TGN based on studies from several laboratories. The molecular actors involved in the release of the virus from this compartment are however little known. We previously demonstrated that the host protein kinase D plays a crucial role in releasing virus from the TGN to the plasma membrane and that diacylglycerol, a lipid that facilitates the recruitment of the kinase to the TGN, is also implicated. Given the role of these molecules in the herpes simplex virus type 1 life cycle, we aimed to determine to what extent this virus utilizes the protein kinase D pathway. Thus, both upstream and downstream molecular players of this pathway were targeted by RNA interference and viral production quantified. Surprisingly, several of these modulators negatively impacted the extracellular release of the virus. The use of mutant and pharmacological reagents targeting CERT, a lipid transfer protein, suggested that these effects were independent of total intracellular diacylglycerol levels. Electron microscopy revealed that the host proteins Nir2 and protein kinase D may also modulate viral nuclear egress. Altogether, this suggested a complex scenario whereby upstream or downstream modulators of the protein kinase D pathway can impact the virus at different stages of its egress.

47 - A Genome-wide Screen of Epstein-Barr Virus Proteins that Modulate Host SUMOylation Identifies a SUMO E3 Ligase conserved in Herpesviruses

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Epstein-Barr virus (EBV) infects most people worldwide and is a causative agent in several kinds of lymphoma, nasopharyngeal carcinoma and 10% of gastric carcinoma. EBV encodes ~80 proteins many of which are poorly or completely uncharacterized, but are thought to modulate cellular processes. Modification by the small ubiquitin-like modifier (SUMO) controls the activity of many proteins central to antiviral responses and oncogenesis, and therefore the SUMO pathway is a likely target of EBV proteins. We generated an expression library of most of the encoded EBV proteins and screened them for the ability to globally modulate SUMOylation. We screened for cellular SUMO1 and SUMO2 modifications in three different cellular systems, including nasopharyngeal carcinoma cells relevant for EBV infection. One EBV protein (BRLF1; Rta) globally induced the loss of SUMOylated proteins, in a proteasome-dependent manner, as well as the loss of promeylocytic leukemia nuclear bodies, suggesting that, like its homologue (Rta) in Kaposi's sarcoma associated herpesvirus, it is a SUMO-targeted ubiquitin ligase. Four EBV proteins consistently upregulated the levels of SUMO-conjugated proteins, including the EBV SM (EB2) protein. SM homologues exist in herpes simplex virus (UL54 or ICP27) and cytomegalovirus (UL69), prompting us to examine the effects of these proteins on cellular SUMOylation. Like SM, HSV-1 UL54 and CMV UL69 induced global SUMOylation. All three viral proteins were purified and tested in an in vitro assay for SUMOylation using p53 as a substrate, revealing that all three proteins had titratable SUMO E3 ligase activity. Consistent with this activity, all three viral homologues bound SUMO and the SUMO E2, Ubc9. These are the first SUMO E3 ligases discovered for EBV, HSV-1 and CMV. Interestingly the homologues had different specificities for SUMO1 and SUMO2, with SM and UL69 preferentially binding SUMO1 and inducing SUMO1 modifications, and UL54 preferentially binding SUMO2 and inducing SUMO2 modifications. The results provide new insights into the function of this family of conserved herpesvirus proteins. The conservation of this SUMO E3 ligase activity across diverse herpesviruses suggests the importance of this activity for herpesvirus infections.

48 - Isolation and characterization of two novel cyanophages from a subarctic Canadian lake

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Cyanobacteria are key components of arctic aquatic ecosystems, as both essential primary producers, and as significant contributors to the biogeochemical cycling of essential nutrients such as carbon and nitrogen. The two primary mechanisms of top-down control of cyanobacterial populations are grazing and viral lysis. Viruses are the most diverse and abundant biological entities on Earth with estimates of 10^{31} viral particles globally and 10^{4} virotypes per mL of water. It is now widely recognized that viruses are important drivers of host evolution and influence nutrients cycles and microbial dynamics through viral lysis. Cyanophages, viruses that infect cyanobacteria, infect keystone primary producers in marine and freshwater environments, and have been shown to harbour host-like genes that are involved in essential host metabolic processes. In order to better understand cyanophages dynamics, genomics and ecology, we focused our research on the isolation and characterization of cyanophages that infect polar cyanobacteria. Water from a Canadian subarctic lake located in the vicinity of Whapmagoostui-Kuujjuarapik (Nunavik, Canada) was sampled in August 2015 and was filtered through 0.45µm and 0.22µm pore size filters to remove large particles (cells, clay, etc.). The filtrate containing viruses was used to inoculate different cyanobacterial cultures for viral amplification. Cultures presenting signs of lysis relative to an untreated control were serially diluted to isolate a single virus. Clonal isolates were subsequently sequenced on a NGS sequencing platform (MiSeq, Illumina). We succeeded in isolating two novel cyanophages (B3 and B23). Electron micrographs revealed that both isolates possessed icosahedral capsid and a long contractile tail, typical features of viruses in the order Caudovirales, family Myoviridae. B3 and B23 genome lengths were 244,930 bp and 243,633 bp respectively. Genomic analysis and ORFs annotation indicated the presence of two host-related metabolic genes (hli and phoH) in both genomes. However, the function of only 25% of the putative ORFs was identified using RAST based on homology with sequences in the SEED database. Phylogenetic analysis based on alignments of the B3 and B23 primase gene and those from phages from a representative number of taxa showed that both viruses clustered with other cyanophages. These results suggest that B3 and B23 are highly divergent from previously described cyanophages and that this study will result in new insight into the world of arctic cyanophages, of which little is known.

49 - Persistence of enteric virus surrogates in static bacterial biofilms

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Background: Norovirus and other enteric viruses are recognized as leading cause of foodborne infections. Fresh produce, meats and ready to eat foods provide an ideal route of transmission for those viruses. Unlike most microbiological agents, viruses cannot replicate on food, therefore contamination levels do not increase during processing or storage and very few viral infectious particles are necessary to induce disease. Enteric viruses are known for their stability in various environments and on surfaces. Also, food processing environments are prime locations for the development of surface-associated microbial communities known as biofilms. It is well known that biofilms contribute to contamination, persistence and hygiene failure in the food industry. Still, relatively little is known about the behavior of foodborne viruses dwelling within these complex communities. The aim of this study was to evaluate the association between enteric viruses and biofilms. Methods: Murine norovirus MNV-1 and rotavirus Wa were used as surrogate and biofilm formation (48h) of mono-culture and mix-cultures (L. plantarum; L. rhamnosus; L. pseudomesenteroides; P. fluoresences) were prepared on 3 surfaces: glass, stainless steel and polystyrene coupons. Viruses were added at final concentration of 10^{5} pfu/ml with each formed biofilm and incubated for 15, 90 min and 24 hrs. Infectious viral particles were detected in biofilms attached at surfaces by plaque assays. Results: An average persistence of 2.4 log/ml of infectious particles of MNV on the 3 surfaces tested without biofilm at all incubation times was observed. No significant association or persistence has been noted between MNV and the mixed biofilm. However, a significant increase of its persistence was detected in presence of simple formed biofilms from the two Lactobacillus used in this study. For rotavirus, the association between the virus and the surfaces without biofilm at 15 min, 90 min, 24 h were shown with a recovery of 2.5, 2.4, and 0.1 log/ml, respectively. However, significant amounts of infectious particles were recovered after 24 hours with some simple and mixed biofilms. These results showed that biofilms can influence the persistence of the rotavirus over time on the glass, stainless steel and polystyrene surfaces. Conclusion: Biofilms on surfaces used in the food industry can influence the presence and persistence of enteric viruses in the environment. However, various factors surrounding this association are unknown and appear to vary depending on viruses and biofilm composition.

50 - Adaptive mutations in influenza A/California/07/2009 enhance polymerase activity and infectious virion production

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Mice are not natural hosts for influenza A viruses (IAVs), but they are useful models for studying antiviral immune responses and pathogenesis. Serial passage of IAV in mice invariably causes the emergence of adaptive mutations and increased virulence. Typically, mouse-adaptation studies are conducted in inbred laboratory strains BALB/c and C57BL/6, which have defects in the antiviral Mx1 gene that results in increased susceptibility to infection and disease severity. Here, we report the adaptation of IAV reference strain A/California/07/2009(H1N1) (a.k.a. CA/07) in outbred Swiss Webster mice. Serial passage led to increased virulence and lung titers, and dissemination of the virus to brains. We adapted a deep-sequencing protocol to identify and enumerate adaptive mutations across all genome segments. Among mutations that emerged during mouse-adaptation, we focused on amino acid substitutions in polymerase subunits: polymerase basic-1 (PB1) T156A and F740L, and polymerase acidic (PA) E349G. These mutations were evaluated singly and in combination in minigenome replicon assays, which revealed that PA E349G increased polymerase activity. By selectively engineering these three adaptive PB1 and PA mutations into the parental CA/07 strain, we demonstrated that adaptive mutations in polymerase subunits decreased the production of defective viral genome segments with internal deletions, and dramatically increased the release of infectious virions from mouse cells. Together, these findings increase our understanding of the contribution of polymerase subunits to successful host adaptation.

51 - Construction of a Synthetic KSHV Genome by Transformation-Associated Recombination

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Kaposi's sarcoma-associated herpesvirus (KSHV) is the infectious cause of Kaposi's sarcoma (KS), primary effusion lymphoma (PEL) and multicentric Castleman's disease (MCD). There is no KSHV vaccine. Rational attenuation of viruses for vaccine development requires intimate understanding of viral antigens and viral gene function, and efficient methods for viral genome engineering. Despite recent advances in en passant mutagenesis and CRISPR/Cas9-based genome editing, genetic manipulation of KSHV remains cumbersome. Developments in synthetic biology has made it feasible to synthesize, assemble, and re-code large viral genomes. Here, we describe our approach to assemble a synthetic KSHV genome in yeast via transformation-associated recombination (TAR). The widely-used KSHV strain JSC1 (~165 kbp) is the reference genome for this study. The TAR cloning vector contains bacmid and yeast centromere cassettes to facilitate growth in E. coli and S. cerevisiae, respectively. The KSHV genome will be sheared and oligonucleotide primers will be used to clone ~14 kbp fragments into the TAR Vector pCC1-BAC. Genome fragments will be cleaved from the TAR vector and co-transformed into yeast; 40 base pairs of conserved overlapping sequence at the end of each fragment ("TAR hooks") will facilitate homologous recombination in yeast. This 'parental' recombinant KSHV genome will be sequenced via Illumina MiSeq to ensure faithful assembly. In cell culture, KSHV establishes a non-productive latent infection. To recover infectious virus, the KSHV-TAR genome will be transfected into a widely used epithelial cell line expressing the KSHV lytic switch protein RTA on a tetracycline-regulated promoter, allowing inducible replication of virus. Virus stocks will be amplified and plaque-purified on a cell line that constitutively expresses low levels of RTA to support KSHV lytic replication. Establishment of TAR methods for KSHV will facilitate rapid mutagenesis in yeast, allowing the creation of re-coded viruses that lack immune evasion genes, or have other defects leading to attenuation. This technology will likely advance fundamental studies of virus gene function, while accelerating discovery and development of candidate attenuated vaccines with desirable properties.

52 - Modulation and recruitment of TRF2 at viral telomeres during HHV-6A/B infections

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Human herpesvirus-6A (HHV-6A) and HHV-6B are two distinct beta herpesviruses with different epidemiological and biological characteristics. HHV-6B is a ubiquitous virus that infects nearly 100% of world population and is the etiological agent of roseola infantum, an infantile febrile illness characterized by high fever with occasional skin rash. HHV-6B is also a concern in hematopoietic stem cell and solid organ transplant recipients with frequent reactivation and medical complications. Pathological and epidemiological data on HHV-6A remain scarce. The viral genomes of HHV-6A/B are composed of a unique segment of approximately 143 kbp flanked at both extremities with identical and directly repeated (DR) termini of approximately 9 kbp each. Each DR contains two regions with repeated TTAGGG telomeric sequence that play a role in the ability of these viruses to integrate their genomes into human chromosomes. Although HHV-6 integration can occur in several distinct chromosomes, it invariably takes place in the telomeric/sub-telomeric regions of chromosomes. To prevent activation of the damage recognition pathways at inappropriate times, telomeres are protected by the shelterin complex. The shelterin complex folds the telomere in a structure called the T-Loop, preventing the recognition of the telomere extremity as a double-strand break (DSB). The shelterin complex is made of six proteins: TRF1, TRF2, TPP1, RAP1, TPP2 and POT1. TRF1 and TRF2 both form homodimers that bind directly to the double-strand TTAGGG repeats in a sequence-specific manner. TRF2 represses the ATM pathway and cell cycle arrest and plays a role in the repression of telomere repair by non-homologous end-joining (NHEJ) and homologous recombination (HR). POT1 binds to the single-strand section of the telomeres and protects the telomeres against recognition by the ATR pathway. During infection by DNA viruses such as herpesviruses, the dsDNA viral genomes are likely to be recognized as broken DNA molecules and trigger a DNA-damage response. In the present study, we analyzed whether sheltherin proteins would associate with viral telomeric sequences during infection and if these could prevent a DNA damage response. HHV-6A infection of HSB-2 cells led to increased expression of TRF1, TRF2, RAP1 and TPP1 mRNAs. Similarly, HHV-6B infection of MOLT-3 cells caused a significant increase in TRF2 and TPP1 mRNAs levels relative to uninfected control cells. Variations in POT1, TIN2 and TERT mRNA levels were minimal. Increase in TRF2 mRNA levels were validated at the protein level. Our results also indicate that HHV-6A IE2 colocalizes with sheltherin complex proteins at telomeres during infection. We could also document that TRF2 colocalizes with viral replication compartment (VRC) during infection, suggesting that telomeric sequences within HHV-6 genome are likely recognized and bound by TRF2 during infection. Such hypothesis was confirmed by ELISA and ChIP. In cells transiently knockdown for TRF2, a robust DDR was observed at VCR during infection. In summary, our results indicate that HHV-6A/B infection modulate sheltherin protein expression levels. TRF2 is recruited and binds to viral DNA during infection and protects against the development of DDR at VRC.

54 - Viral manipulation of host cholesterol homeostasis is required for viral packaging and egress

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Kaposi's sarcoma-associated herpesvirus (KSHV) is the etiological agent of Kaposi's sarcoma (KS) and primary effusion lymphoma (PEL). Like all herpesviruses, newly-assembled KSHV particles acquire a host-derived lipid envelope during egress. To acquire this envelope, KSHV must reorganize host cellular compartments and manipulate cholesterol homeostasis. The liver X receptor α (LXRα) transcription factor is a central regulator of lipid homeostasis. LXRα activation by oxysterols stimulates transcription of cholesterol efflux and fatty acid synthesis and storage genes, thereby altering the bioavailability of host-derived cholesterol. We employed a specific endogenous LXRα agonist, 22R-hydroxycholesterol (22OH) to investigate the impact of lipid homeostasis perturbations on KSHV replication. KSHV infection was monitored in iSLK.219 cells that are latently infected with a GFP-expressing recombinant KSHV which can be reactivated from latency by addition of doxycycline. These cells were also monitored for cholesterol distribution, storage, and efflux during latency and throughout lytic replication. Treatment of iSLK.219 cells with 22OH caused increased reactivation from latency compared to controls. Strikingly, LXRα activation significantly decreased the production of infectious virions from these cells by impeding the egress of viral capsids from the host cell nucleus. Furthermore, 22OH treatment depleted both free and stored host cell cholesterol, thereby decreasing its bioavailability for viral envelopes. Taken together, these findings suggest that KSHV replication requires perturbations in host lipid homeostasis, and that LXRα activation disrupts KSHV-mediated lipid changes to block the production of infectious virions.

58 - Extensive host immune adaptation in the Saskatchewan HIV epidemic

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Background: Since 2003, a concentrated HIV epidemic has been expanding in the Canadian province of Saskatchewan (SK), where injection drug use represents the primary mode of transmission (60%) and where nearly 80% of infected persons self-identify as Indigenous. While the overall HIV diagnosis rate in SK (14.5 per 100,000 in 2016) is already more than twice the national average, regional rates are up to fourfold higher (e.g. 67.8 per 100,000 in Prince Albert). The SK HIV epidemic may also have unique clinical characteristics. Anecdotal reports of more rapid than expected progression to HIV-induced immunodeficiency are emerging from the province, and the 2016 SK HIV Prevention and Control report estimated that fewer than 50% of persons diagnosed since 2007 remained alive in 2016. Furthermore, studies in neighbouring Manitoba have reported accelerated HIV progression among individuals expressing certain Human Leukocyte Antigen (HLA) class I alleles, including the typically protective B*51. This is notable, as a study investigating HLA diversity among Indigenous Saskatchewan residents identified B*51 as the most common HLA-B allele in this group. Given that acquisition of HIV pre-adapted to host HLA can significantly accelerate clinical progression, we hypothesized that elevated HIV adaptation to host HLA may explain the reports emerging from the region. Specifically, given the established link between population-level spread of the B*51-associated RT-I135X escape mutation and erosion of HIV control by this allele in other global regions, notabaly Japan where B*51 prevalence is high, we further reasoned that HIV adaptation to B*51 might be particularly elevated in SK. We investigated our hypotheses using comprehensive longitudinal HIV Pol sequence datasets collected between 2000-2016 from Saskatchewan and elsewhere in Canada and the USA. Methods: We analyzed 1,144 HIV subtype B Pol sequences from unique Saskachewan residents spanning 2000-2016 for the presence of 70 known HLA-associated Pol mutations. These sequences were originally collected for the purposes of HIV drug resistance genotyping and represent ~65% of cumulative HIV cases diagnosed in Saskatchewan to date. HIV Pol sequences from British Columbia (N=6,525) and elsewhere in Canada/USA (N=6,517), spanning the same date ranges, were used for comparison. Overall HIV adaptation levels to 34 HLA alleles were also quantified. Putative HIV transmission clusters, and the distribution of HLA-associated adaptations within and external to these clusters, was also investigated. Results: The molecular epidemiology of HIV in Saskatchewan is highly unique, with 78% of circulating sequences residing in large phylogenetic clusters (compared to 48% in the densely-sampled BC epidemic and only 15% in the North America dataset). Analyses confirmed significantly elevated levels of HIV adaptation to numerous HLA alleles commonly expressed in Indigenous populations in Saskatchewan, in particular B*51. The prevalence of most of these HLA-adapted viral polymorphisms was also increasing over time. Notably, HIV strains harboring HLA-adapted polymorphisms were significantly overrepresented within large phylogenetic clusters in SK, indicating the preferential and widespread transmission of these polymorphisms within the province. Conclusions: High levels of circulating HIV adaptation to HLA in Saskatchewan provide a plausible explanation for reports of accelerated progression in the province, and identify Saskatchewan as the first example of a North American HIV epidemic characterized by significant circulating viral adaptation to host immunity. Results highlight the utility of Pol sequences, routinely collected for drug resistance monitoring, for surveillance of HIV-host adaptation, and underscore the urgent need to expand HIV testing, treatment and harm reduction programmes in the region.

59 - Type I Interferon-Associated Impairment of the Humoral Immune Response against LCMV

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Type I interferon (IFN-I) has been characterized to enhance cell-mediated immune responses against acute viral infections whilst impair immune activation in chronic viral settings as would be in the case of HCV and HIV. Here, we show that in addition to its effect on T cells, IFN-I drives impairment of effective humoral immune responses through direct interaction with B cells upon chronic viral infection. Using the classic LCMV murine infection model, we co-administered 4-hydroxy-3-nitrophenyl (NP) at the time of infection whereby flow cytometry analysis of B cell proportions and ELISPOT data revealed that compared to a normal humoral immune response in the VSV (acute) infection, LCMV-infected mice developed non-specific hypergammaglobulinemia along with diminished NP-specific responses shortly after infection. Notably, during persistent viral infections, infected hosts also exhibit aberrancies to the humoral response such as polyclonal activation and hypergammaglobulinemia. Accompanying this dysregulation, the emergence of neutralizing antibodies (nAbs) is delayed and bears negligible impact on the progression of the disease by the time they are successfully elicited. Altogether, these perturbations result in a diminished antigen-specific Ab response and an enhanced non-specific polyclonal response. These are hallmarks of disruption in the humoral immune response during a chronic infection. Our results also demonstrated that this impairment was limited to the T-cell dependent B-cell response and function was restored by ablation of IFN signaling through antibody-dependent IFN receptor blockade as well as B-cell specific IFN receptor knockouts. In addition, disrupted lymphoid architecture observed following immunofluorescent microscopy was also restored upon elimination of B-cell specific IFN signaling. Importantly, restoration of effective B-cell responses in transgenic mice also featured increased neutralizing antibody titers in ELISA assays, which were absent in the wildtype model with functional IFN signaling. Our findings illustrate the role played by IFN in limiting effective antibody responses by action on B-cells. Whereas complete blockade of IFN signaling would be deleterious, targeted B-cell specific restriction could improve humoral responses towards effective therapeutic and prophylactic measures against chronic infections such as HCV.

62 - Oncolytic virus-induced expression of an alternative leader drives translation of an mRNA encoding an inositol phosphatase that limits infection.

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Innate cellular immunity protects from invading pathogens through a network of molecular sensors that activate antiviral effectors and dampen pro-viral cellular activities. The protein synthesis machinery constitutes a valuable arm of innate immunity yet becomes deregulated in the contexts of viral infection and cancer. The inability to mount an appropriate innate immune response served as the original basis for the cancer cell tropism of oncolytic viral therapies. However, residual innate immunity represents a significant barrier to therapeutic efficacy. We sought to identify new innate immune effectors that are modulated at the level of mRNA translation during infection of murine breast cancer cells with three different oncolytic viruses (HSV-1, Reovirus, Vaccinia). Using ribosome profiling, we found that the response of cancer cells to all three viruses at the level of mRNA translation was distinct from the transcriptional response. We identified a translation de-repression phenotype associated with the upregulated mRNA set common to all three viruses and found that a candidate mRNA encoding an inositol phosphatase (Inpp5e) harbours an alternatively spliced 5' leader, with the shorter isoform favourably expressed during infection. Critically, this shorter 5' leader leads to a higher translational efficiency of the Inpp5e ORF. Moreover, interruption of INPP5E expression by RNAi or CRISPR-Cas9 led to enhanced infection by various oncolytic viruses and appears to be mediated by pleiotropic mechanisms. Thus, using a ribosome profiling approach, we have uncovered an innate immunity effector that could be exploited to engineer oncolytic viruses with increased clinical efficacy.

63 - Poxvirus genome editing in the development of a safe and efficient oncolytic platform

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Vaccinia virus has a large and still incompletely understood genome although several strains of this virus are already in clinical development. For the most part, clinical candidates have been attenuated from their wild type vaccine strains through deletion of metabolic genes like the viral thymidine kinase gene. We decided to carry out a more in depth understanding of the genetic elements of vaccinia which could be modulated to improve the oncolytic/therapeutic characteristics of the virus. Using a variety of cancer cell lines and primary tumor explants, we performed a fitness assay that compares head to head five wild-type Vaccinia strains to identify the genetic elements that together create an optimal "oncolytic engine". Using a transposon insertion strategy and deep sequencing of viral populations we systematically examined vaccinia genes that do or do not play a role in the therapeutic activity of the virus. Our studies allowed us to identify large areas of the vaccinia genome that when deleted, augment the oncolytic activity of a newly created recombinant virus (35 genes deleted). This novel virus was compared to five vaccinia strains currently in the clinic and in a variety of assays, the deleted virus displayed superior therapeutic activity and an enhanced safety profile. Studies in a variety of in vitro and in vivo models will be presented illustrating the strategy we have used to create this optimized oncolytic virus platform. We are currently carrying out further pre-clinical studies to accelerate the translation of this new virus into the clinic.

64 - Influenza A virus host shutoff nuclease PA-X: target specificity and protein-protein interactions

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Influenza A viruses (IAVs) inhibit host gene expression by a process known as host shutoff. The host shutoff facilitates virus replication primarily by blocking induction of antiviral factors. Several IAV proteins participate in host shutoff, including the endoribonuclease PA-X. In in vivo models of infection, PA-X deficiency exacerbates lung inflammation and increases morbidity and mortality. However, a mechanistic understanding of PA-X effects on the cell transcriptome is lacking. We previously demonstrated that PA-X specifically degrades transcripts produced by host RNA polymerase II, yet how PA-X distinguishes between different host transcripts as well as between viral and host mRNAs is currently unknown. Our recent findings suggest that the target discrimination is linked to nuclear mRNA biogenesis pathways. Indeed, upon ectopic expression PA-X localized to the cell nucleus and we hypothesize that PA-X is recruited to its target transcripts through interaction with mRNA processing factors, and that the differential sensitivity of RNAs reflects differential engagement of this machinery. We employed two complementary approaches to test this hypothesis: the transcriptome-wide profiling of host PA-X targets and the proteomic analysis of PA-X-interacting proteins using BioID. Using these approaches, we discovered that the RNA splicing is a determinant of susceptibility to PA-X and that the PA-X C-terminal domain interacts with a number of nuclear, nucleolar, and nucleocytoplasmic shuttling proteins involved in RNA processing. Taken together, our data support a model in which PA-X interacts with cellular mRNA processing pathways to target subsets of host RNA polymerase II transcripts. This target discrimination mechanism sets PA-X apart from other viral host shutoff nucleases, which preferentially target actively translated messenger ribonucleoproteins in the cytoplasm.

65 - Characterization of a Novel Host-Targeted Antiviral Molecule

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Introduction: Current front-line influenza antivirals rely on targeting specific viral proteins to limit replication. These drugs are known as direct-acting antivirals (DAAs). However, rapid viral evolution allows for the emergence of mutations that confer resistance to these types of antivirals. Because influenza viruses depend on many host processes for viral replication, host-targeted antivirals (HTAs), provide an attractive alternative to limit the emergence of drug-resistant viruses. Here, we characterize a novel host-directed antiviral, SG3, that effectively limits influenza A virus (IAV) replication in vitro. Methods: A549 lung epithelial cells were infected with IAV and treated with SG3 at 4hpi. Release of progeny virions was analyzed by plaque assays and protein expression was assessed via immunoblotting. Results: SG3-treated A549 cells exhibited a 2-log reduction in infectious virions compared to control cells. SG3 treatment caused activation of both the PERK and IRE1α arms of the unfolded protein response (UPR), and selectively reduced accumulation of viral HA and NA glycoproteins while having minimal effects on NP and M1 expression. Conclusions: SG3 treatment reduces IAV replication and accumulation of viral glycoproteins HA and NA, while activating the unfolded protein response. Our findings demonstrate that SG3 is a promising candidate HTA for influenza. These in vitro findings will have to be corroborated in an appropriate pre-clinical in vivo model.

66 - Photodynamic Inactivation of Herpes Simplex Viruses

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Introduction: Photodynamic inactivation (PDI) employs a photosensitizer, light, and oxygen to inactivate microorganisms through the production of reactive oxygen species. PDI's mechanism of action is nonspecific but its effect is immediate and confined to regions where a photosensitizer, light, and oxygen overlap in space and time. Orthoquin™, a botanical plant extract developed by PhotoDynamic Inc., is a powerful photosensitizer with antimicrobial properties that are amplified by exposure to visible light. Orthoquin™-mediated PDI has been shown to kill bacteria and destroy biofilm without causing inflammation, making it an attractive candidate for treating oral bacterial diseases. Herpes simplex virus infections are common globally (affecting 67% of people under age 50) and symptoms include painful oral (HSV-1) or genital/anal (HSV-2) lesions. Orthoquin™-mediated PDI was investigated as a potential treatment for symptoms associated with these two viral infections. Methods: Orthoquin™-mediated PDI was tested against HSV-1 and HSV-2 infection of HeLa cells using plaque reduction assays and single cycle infection assays (via flow cytometry), where virus inoculum was incubated with different concentrations of Orthoquin™ and exposed to visible light before inoculating cells. Results: Upon photoactivation, Orthoquin™-mediated PDI inhibited HSV-1 and HSV-2 infections in a dose-dependent manner, though with different efficacies against each virus. Conclusions: PDI is a promising alternative to traditional antibiotics and antivirals due to its immediate and selective mechanism of action. We showed that Orthoquin™ inhibits herpes simplex virus infection.

68 - KSHV hijacks the unfolded protein response to promote lytic replication

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Kaposi's sarcoma-associated herpesvirus (KSHV) is the infectious cause of the complex endothelial neoplasm Kaposi's sarcoma, and two rare lymphoproliferative disorders, primary effusion lymphoma (PEL) and multicentric Castleman's disease. KSHV activates multiple cellular stress responses during infection, but our understanding of their role in viral replication and tumorigenesis is lacking. One such stress management pathway is the unfolded protein response (UPR), which is activated by endoplasmic reticulum (ER) stress. The three sensors of ER stress that initiate UPR signaling are PERK, IRE1, and ATF6, which induce a translational and transcriptional response to restore homeostasis. Failure to restore homeostasis results in the UPR shifting from an adaptive response to pro-apoptosis. IRE1 plays a key role in this life-or-death decision. During ER stress IRE1 splices out a 26-nucleotide region of xbp1 (X-box binding protein 1) mRNA, shifting the reading frame to translate the active transcription factor XBP1s. In latently infected cells, chemical induction of ER stress initiates lytic reactivation through XBP1-mediated transcription of the KSHV latent-lytic switch gene, K-RTA. Thus, it appears that KSHV has evolved a mechanism to respond to ER stress. IRE1 also cleaves and degrades a subset of ER-bound mRNAs in a process known as regulated IRE1-dependent decay (RIDD), which reduces the protein load within the ER, and is also thought to be important for inducing apoptosis. In this study, we investigated the role of the UPR during lytic replication. We found that lytic replication activates all three sensors of the UPR but the downstream signaling events, except for RIDD, are inhibited. Knockdown or chemical inhibition of any of the UPR sensor inhibited virus replication. Surprisingly, ectopic expression of XBP1s also inhibited viral production. Therefore, while XBP1s plays an important role in reactivation from latency, it inhibits later steps in lytic replication. These data also indicate that RIDD may be important for KSHV replication. Overall our findings suggest that KSHV hijacks each of the UPR sensors to promote virus production instead of resolving ER stress. These findings identify a key signaling node in KSHV replication, which may lead to the development of new therapeutic strategies to inhibit KSHV replication and tumorigenesis.

69 - Understanding viral dsRNA-mediated innate immune sensing and cellular responses in rainbow trout cells

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Viruses across genome types produce long dsRNA (viral (v-) dsRNA) molecules during their replicative cycle. These dsRNA molecules are potent immunostimulatory molecules and induce type I interferon (IFN), that in turn induces the production of a series of genes known as interferon-stimulated genes (ISGs), the accumulation of ISGs within a cell can result in a protective antiviral state. Current research on dsRNA-induced immune responses often uses polyinosinic: polycytidylic acid (poly I:C), a commercially available product with limited biological relevancy. An alternative synthetic dsRNA can be produced by in vitro transcription, (ivt-) dsRNA, with a defined sequence and length. We hypothesized that ivt-dsRNA, containing a viral sequence and length, would be a better proxy for v-dsRNA, compared to poly I:C. This is the first study to investigate the effects of v-dsRNA on the innate antiviral response and to compare v-dsRNA to ivt-dsRNA induced responses in rainbow trout cells. In rainbow trout gut cells class A scavenger receptors (SR-As) were found to be a surface receptor for poly I:C. In the current study, ivt-dsRNA binding was blocked by poly I:C and v-dsRNA, as well as by poly I, a SR-A competitive ligand. These data suggest all three dsRNA molecules are recognised by the same receptor, likely an SR-A. To determine the ability for dsRNA molecules to stimulate the innate immune response, IFN1 and the ISG, vig-4, transcript levels were measured using qRT-PCR, and a protective antiviral state against two aquatic viruses was observed in rainbow trout gonadal cells. V-dsRNA was able to induce IFN and ISG transcript production at 3h and 24h, and its effects increased in a length-dependent manner, as has been previously seen in rainbow trout cells with ivt-dsRNA and poly I:C. V-dsRNA and ivt-dsRNA molecules that were length and sequence matched both induced similar IFN and ISG transcript levels, and subsequently similar antiviral states. Two novel putative dsRNA-receptors were also identified, DHX9 and DDX3. These sensors have similar domains to their mammalian counterparts and both were found to bind to ivt-dsRNA. This study is the first of its kind to look at the effects v-dsRNA in fish cells; and the first to compare matched ivt-dsRNA to v-dsRNA molecules. Taken together these results suggest that ivt-dsRNA may be a good surrogate for v-dsRNA in the study of dsRNA-induced responses and may be a candidate molecule for dsRNA-based antiviral therapies.

72 - Viral amyloid protein

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The aberrant assembly of host cell proteins into amyloid fibrils causes many fatal brain disorders, e.g. Creutzfeldt-Jacob, Alzheimer's, and Parkinson's disease. These conditions can arise spontaneously, be inherited, or in the case of prion diseases, occur through infection. Investigating the mosquito-borne Rift Valley fever virus, a neurotropic human pathogen, we found that the virus-encoded NSs protein forms massive bundles of twisted fibrillary tangles, reminiscent of the twists in amyloidogenic fibrils. We also identified NSs structures resembling amyloid oligomers and protofibrils. Accordingly, a large fraction of NSs multimers remained resistant to SDS and, the amyloid-marker thioflavin-T bound to NSs. Fibrillar aggregates grew in mammalian host cells until they die. Filamentous structures also grew in mosquito cells. In a starking contrast to the dynamics in mammalian cells, arthropod cells lost NSs fiber-bundles thereafter in a process involving RNA interference and phosphorylation but not proteasomal activity and, cells survived infection. Together our results confirmed experimentally the concept that viruses can encode amyloid proteins. Our study also highlights the existence of endogenous cellular mechanisms controlling amyloid fibrils and pave the way for future investigations into an array of neurodegenerative diseases.

Posters 73 – 127: poster Session #2

73 - Evaluation of the influenza A virus quasispecies populations shed by different avian reservoir hosts

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Influenza A viruses (IAVs) evolve quickly through mutation, due to an error-prone viral replicase enzyme, and through the process of segment reassortment during co-infections. Mutations generate new closely related virus variants that can have different capacities of infection and replication, and the pool of closely related variants that are produced during an infection is known as a quasispecies. Wild birds are the natural reservoir of IAVs and are known to carry the majority of IAV subtypes and overall genetic diversity. Most avian IAVs have low pathogenicity in their natural hosts and circulate without causing overt symptoms, but they are still of broader importance due to their involvement in the generation of highly pathogenic viruses in domestic poultry and viruses infecting humans. To better understand the evolutionary dynamics of IAVs in their natural hosts, we performed a comparative analysis of the quasispecies populations shed by wild birds. This was done by performing high-throughput sequencing of IAVs present in paired oropharyngeal/cloacal swab samples collected from two of each of three different groups of wild bird hosts: seabirds, gulls and ducks. One H2N3 and one H3N2 virus were analyzed from American Black Ducks, two H1N2 viruses were analyzed from Common Murres, and two H13N6 viruses were analyzed from American Herring Gulls. Our analysis shows that the numbers of overall variants were similar within the different bird hosts but varied among hosts. The amount of sequence diversity, as quantified by Shannon entropy, varied among the gene segments but showed similar patterns for the same segment in different viruses from the same host. The proportion of shared variants for any pair of segments for two viruses from the same host was correlated with the phylogenetic relationship of the segments such that higher proportions of identical variants were detected in more closely related segments. Close to 100% of the variants observed in the PA, HA, NA, NP and NS segments were found in both of the murre viruses, and these segments are all phylogenetically very close for the two viruses. The murre virus PB1 and PB2 segments were phylogenetically more distant and shared approximately 50% of variants between the two viruses. The M segments of these murre viruses have different phylogeographic origins (Eurasia versus North America) and shared few variants. The two gull viruses are phylogenetically close for all eight segments and a high proportion of identical variants were observed for all segments. Similar to the patterns for the other hosts, the duck viruses showed variant conservation levels that corresponded to the phylogenetic relatedness for each segment. Finally, a high proportion of non-synonymous substitutions was observed for the NS segment relative to the other segments for all viruses analyzed.

74 - Conserved microRNA Cluster's Role in Modulating the Innate Immune Response to Viral Infection

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Successful recognition of viral infections and consequential triggering of antiviral innate immune responses are crucial for host persistence and survival. Traditionally, to learn more about these interactions, regulatory networks at the transcriptional and translational levels have been extensively studied. Recently, with the discovery of microRNAs, there has been a shift in this dogma. microRNAs (miRNAs) are a class of highly conserved and abundant, small non-coding RNAs involved in post-transcriptional regulation of gene expression. miRNAs perform their function by binding to the 3'-untranslated region of target mRNAs to induce degradation and suppress translation. Herein we describe an interferon-regulated miRNA cluster that modulate interferon production and signaling during viral infection. The identified miRNA cluster appears to regulate key phosphorylation events which are crucial to the antiviral signaling cascade. We found that the cluster enhances the innate antiviral response by augmenting the phosphorylation of transcription factors, such as interferon regulatory factors (IRF) and signal transducer and activator of transcription (STAT), during vesicular stomatitis viral infections. We demonstrate that the cluster elicits its antiviral response by targeting negative regulators of the IFN signaling cascade and JAK-STAT signaling pathway. Overall, our work reveals a novel mode of cooperation between a miRNA cluster in the regulation of antiviral cellular responses and aids in uncovering fundamental roles for non-coding RNAs in infection and immunity.

75 - Kicking the Kap out of Kaposi's Sarcoma: Tools for Studying a Herpesvirus Protein

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Kaposi's sarcoma-associated herpesvirus (KSHV) is the cause of the endothelial cell (EC) cancer, Kaposi's sarcoma (KS). KSHV latent infection of ECs predominates in KS, and drives viral tumorigenesis. In latent infection, a subset of viral genes reprogram normal ECs and induce aberrant proliferation, altered morphology and inflammatory gene expression. Lytically infected cells are far less abundant in KS lesions; however, they serve as a source of new virus and promote tumorigenesis in a paracrine manner. KapB is expressed from a highly repetitive, GC-rich, polycistronic transcript that also encodes KapA and KapC. KapB has been shown to recapitulate latent phenotypes when ectopically expressed in ECs. We recently conducted shRNA-mediated knockdown of the kaposin transcript in the context of KSHV latent infection to study the contribution of this gene to latency phenotypes. However, the tools to investigate KapB-specific roles during either state of KSHV infection have been lacking in the field. To address this, we utilized the BAC16 bacterial artificial chromosome clone of KSHV to eliminate the expression of KapB. Because the KapB and KapC open reading frames (ORF) overlap, we synthesized a gene block with a recoded version of KapC, designed to eliminate the KapB ORF expression by insertion of several nonsense codons into the KapB reading frame. Using homologous recombination, this recoded KapC was inserted into a delKapBdelKapC backbone, generating a KapB knockout sequence. These sequences are being used to produce recombinant mutant viruses that will allow us to determine KapB-specific effects on normal cell function during KSHV infection that can now be differentiated from KapA and KapC phenotypic effects. With these tools, we intend to reveal, with greater precision, functions of KapB in both latent and lytic infection cycles.

76 - Expression of the p14 Fusion Associated Small Transmembrane Protein in an Oncolytic Adenovirus Improves Vector Efficiency

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The use of fusogenic proteins to arm Adenovirus (Ad) has enormous potential. These proteins function through cell to cell fusion within the tumor which leads to the generation of multinucleated syncytia, with the concomitant release of immunologically active syncytiosomes. However, the size of typical viral fusogenic proteins makes their inclusion into an oncolytic Ad genome difficult due to viral DNA packaging constraints. The p14 Fusion Associated Small Transmembrane (p14 FAST) protein is a non-structural viral fusogenic protein originating from reptilian reovirus, which is significantly smaller than typical fusogenic proteins. Our objective is to assess the anti-cancer potential of an Ad that undergoes cancer specific conditional replication (CRAd) in cancer cells, and expresses the p14 FAST fusogenic protein (CRAdFAST). In an immune-competent mouse model of cancer, CRAdFAST provided some anti-cancer effect, limited by the ability of Ad to replicate in mouse cells. In an A549 human lung adenocarcinoma xenograft model of cancer, p14 FAST expression significantly improved the oncolytic ability of CRAd in vitro and in vivo. We have also shown that p14 FAST expression and associated cell fusion induce a dramatic increase in the production of extracellular vesicles, which may have immuno-stimulatory properties. We are currently exploring the characteristics of fusion-derived extracellular vesicles to determine the extent of their ability to initiate adaptive immune targeting of tumor associated antigens. Taken together, our results suggest immunologically active cancer syncytiosomes released during CRAdFAST infection may possess potent anti-cancer activity initiated by the development of an anti-tumor adaptive immune response.

78 - Clinical role of enteric viruses in suckling piglets

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Neonatal diarrhea is a major concern for swine producers and causes significant economic losses to the industry each year due to the death of thousands of animals worldwide, or increased production costs associated with treatments and extended periods of growth. Some viruses are suspected, and probably underestimated, for their importance in neonatal diarrhea in animals. Indeed, enteric viruses such as caliciviruses (including norovirus and sapovirus), rotaviruses, astrovirus, kobuvirus and torque teno sus virus have been associated with episodes of diarrhea in piglets. However, viral excretion and diarrhea are not always concomitant. In order to monitor the excretion of enteric viruses in suckling piglets, a sampling scheme using diarrheic piglets faeces (yellow-brown soft to liquid) and normal faeces from healthy piglets was carried out. A total of 203 faecal samples, 70 of which were from diarrheic piglets, were collected from 11 farrowing farms in a farrow-to-finish integrated swine production network in the province of Quebec. Samples were transported on ice and stored at -80°C. Faecal samples were suspended in PBS 20% (w/v) and centrifuged 16000 x g for 5 minutes. Total nucleic acids (DNA/RNA) were extracted with a QIAamp Viral RNA Mini Kit (Qiagen). Rotavirus A and C, caliciviruses and kobuvirus were detected using a targeted RT-PCR, astrovirus genotypes 1 to 5 were detected using a multiplexed RT-qPCR and torque teno sus virus was detected using a qPCR. Positive bands of caliciviruses were excised from a 2% agarose gel, purified and cloned using a p-Gem-T vector system and JM-101 competent cells (Promega). A total of 4 colonies per samples were sequenced and consensus sequences were obtained with the BioEdit software version 7.2.6. Astroviruses (genotypes 1 to 5) were by far the most frequently detected viruses with 85% positive samples, followed by rotavirus C (42%), kobuvirus (41%), torque teno sus virus (25%), rotavirus A (20%) and the caliciviruses (16%). However, only the rotavirus C, the astrovirus genotype 3 and the caliciviruses were associated with symptoms of diarrhea, with chi-square p values of 0,001, 0,005 and 0,02 respectively. All sequenced caliciviruses were classified as sapoviruses. This study re-evaluated the presence of selected enteric viruses in piglets from the province of Quebec and detected for the first time the presence of kobuvirus in Canadian pigs. We were also able to demonstrate that caliciviruses, astrovirus genotype 3 and rotavirus C are possible causative agents in neonatal diarrhea in Canadian swine, which may be useful in future assessment of clinical portraits of diarrheic herds. Although present in a large proportion of samples, rotavirus A, kobuvirus, torque teno sus virus and the other astroviruses genotypes were not associated with diarrhea as reported elsewhere. Only sapovirus was found in the sequenced calicivirus-positive samples, as it is usually the case in younger pigs. Further work is needed to assess the prevalence of these viruses in coinfection with other pathogens such as bacteria and other viruses to provide an in-depth insight as to their role in the pathogenesis of piglet diarrhea.

79 - Sample pretreatments affect the outcome of viral metagenomics analyses

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High-throughput sequencing is more affordable than ever before and it is now the method of choice when studying microbiomes. The porcine enteric microbiome is composed of genetic material from bacteria, archaea and viruses. One of the many challenges when studying the virome (viral component of the microbiome) with high-throughput sequencing is the isolation of the viruses from the rest of the microbiota. This is usually accomplished using various sample pretreatments, incorporated into the procedure to remove as many non-viral components as possible. These steps are presumed to lead to increased sequencing depth and, therefore, facilitate downstream analysis. To determine if these upstream steps were useful for downstream analyses, we performed an evaluation of the differences between high throughput sequencing results from samples that underwent pretreatments from samples that did not. A maximum of 8 g of feces were collected from pigs at different periods, ranging from <1 week to 22 weeks old. All samples were submitted to a homogenization step at 20% in PBS, and centrifuged for 5 min at 16000 x g. The following steps were either performed or omitted: samples were passed through 0,45 µm and 0,22 µm filters followed by RNase and DNase treatments. A total of 32 treated and untreated samples (8 samples receiving 4 different series of treatments) underwent nucleic acid extraction, random reverse transcription and amplification as well as purification steps. Then, libraries were prepared and samples were sequenced using an Illumina MiSeq sequencer. Reads were trimmed for quality, adapters were removed and were classified using the Centrifuge microbial classification engine with a custom database based on the "nt" database from NCBI and visualized using Krona. When the samples went through the complete series of pretreatments, 6/8 samples had their highest viral read percentages of all 4 series tested (from 10% in a 22 weeks-old pig to 86% in a 1 week-old piglet, with a mean of 27%), the other 2 had their highest read percentages with the filtration steps only (30% in a 1 week-old piglet and 45% in a 4 weeks-old piglet). In all samples (8/8), the lowest viral read percentages were observed when the homogenate was used without pretreatment (from 0,06% in a 22 weeks-old pig to 18% in a 4 weeks-old piglet, with a mean of 7%). Most of the non-viral reads were bacteria in 27/32 samples (from 6% in a 4 weeks-old piglet to 86% in a 22 weeks-old pig, with a mean of 57%) and eukaryotes in the other 5 samples (from 26% in a 22 weeks-old pig to 86% in a 14 weeks-old pig, with a mean of 68%). With all pretreatments, the youngest pigs (1 and 4 weeks-old) had the higher percentage of viral reads, with an average of 49% compared to the oldest ones (14 and 22 weeks-old) with an average of 13%. Most notable viral family read count variations when enzymes were added were the astroviridae, caliciviridae and picornaviridae, with read counts varying decreasingly in some samples (70496 to 39, 17271 to 21 and 41000 to 22 reads, respectively). Using pig feces, we have shown that sample pretreatments profoundly affect the overall relative quantity of reads from each of the virus, bacteria and eukaryote classes in the pigs' microbiome. Pretreatments affect pigs' viromes depending on their age: older pigs' viromes benefit from the enzyme treatments, as there seems to be a lot more bacteria and eukaryote DNA in them compared to the younger ones. Also, RNA viruses such as the astroviridae, caliciviridae and picornaviridae appear to be more affected by the enzyme treatments than other viruses from the same samples. Using filtration treatment preceding genomic extraction is therefore paramount to improve downstream analyses of the pig's virome. However, the use of enzymes should be evaluated according to the nature of the sample tested.

80 - Ebola virus requires phosphatidylinositol (3,5) bisphosphate for viral entry

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Ebola virus (EBOV) is a highly pathogenic enveloped filovirus that causes sporadic outbreaks of severe hemorrhagic fever disease. EBOV entry requires internalization into host cells and trafficking to late endosomes/lysosomes where its receptor, NPC1, resides. How viral trafficking occurs, and whether it is regulated by the virus, remain unclear. Here, we show that the PIKfyve-ArPIKfyve-Sac3 cellular complex, which is involved in the metabolism of phosphatidylinositol (3,5) bisphosphate (PtdIns(3,5)P_2), is critical for EBOV infection. Although the expression of all subunits of the complex was required, PIKfyve kinase activity and PtdIns(3,5)P_2 production were found to be the critical determinants for efficient entry. Inhibition of PIKfyve reduced delivery of virions to NPC1 and resulted in virus accumulation in early endocytic compartments. Furthermore, phosphoinositide probes and live cell imaging revealed an increase in PtdIns(3,5)P_2-positive vesicles in cells during EBOV entry, indicating potential upregulation of this lipid by the virus. Taken together, our studies suggest that EBOV requires and potentially upregulates PtdIns(3,5)P_2 production in cells to promote efficient delivery to NPC1.

81 - Evaluating fidelity mutants in an alphavirus

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RNA viruses replicate with a degree of plasticity in their genome, creating a swarm of closely related sequences. This diversity must be maintained in the virus population to navigate bottlenecks and establish infection in new tissues and hosts. There is a precise mutation rate for optimal viral fitness, avoiding error catastrophe and lack of diversity. Altering the mutation rate of alphaviruses is a promising method to produce a range of commercially available attenuated vaccines. Venezuelan equine encephalitis virus (VEEV) fidelity mutants targeting the nsP4, which encodes the RNA-dependent RNA polymerase (RdRp), have recently been discovered. However, the tools available to characterize the mutation rate of altered fidelity mutants are unreliable and have provided inconsistent results. Optimizing an assay to evaluate mutation rate with reproducible results will be a priority for vaccine development of fidelity mutants. Ideally, only a single round of infection should be measured to determine the effect of a specific mutation. This would eliminate biased data from fitness imparted by random mutations that are selected during sequential infection of cells. For ease of processing, virus particles containing RNA should also be released into the media. To create a method to evaluate fidelity mutants, several previously characterized residues, E3 Δ56-59, E1 F253L, and E1 G91D, were mutated in the structural proteins of the VEEV vaccine strain, TC-83. Electroporation of the transcribed VEEV genome into Vero cells allowed replication of non-infectious viruses. RT-PCR was used to determine the presence of viral RNA in the media, and genomes containing a GFP reporter gene were used to confirm that resulting viruses were non-infectious. The optimal structural mutation was chosen for combination with VEEV nsP4 fidelity mutants, G14R, A106T, C488Y (previously characterized fidelity mutant from chikungunya virus), G14R+E37G+A106T, and G14R+E37G+A106T+C488Y. Sequence diversity will be calculated from next generation sequencing data and compared to diversity measured from infectious virus genomes. Using these non-infectious clones will allow characterization of all non-structural mutations and a majority of structural mutations that are suspected to alter fidelity. Implementation of this pipeline will be vital to accurately characterize mutations and evaluate their effects on mutation rate in efforts to expedite vaccine development.

82 - Protein-protein interactions of ME53 from Autographa californica nucleopolyhedrovirus

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ME53 is a 53 kDa protein from the baculovirus Autographa californica multiple nucleopoly-hedrovirus (AcMNPV) and is conserved in all sequenced alpha- and betabaculovirus genomes but its precise function remains unknown. ME53 is a structural protein incorporated into the nucleocapsid of both budded and occlusion-derived viruses. It is expressed at both early and late times of infection. While deletion of ME53 does not affect viral DNA synthesis lack of ME53 reduces virus production by 99% causing a restriction of the cell to cell spread of the virus. ME53 localizes to the cytoplasm at early times post-infection and forms foci on the cytoplasmic membrane with GP64, which is the major envelope glycoprotein. Despite the absence of a recognizable nuclear localization signal, ME53 also translocates to the nucleus mainly at the ring zone where occlusion-derived viruses form at late times post-infection. We hypothesize that the nuclear localization is accomplished through a viral or host chaperon protein using the ME53 "nuclear translocation sequence" located between amino acids 109 and 137. Therefore, we aimed to identify the viral and host proteins that bind to ME53 in order to be better understand the molecular mechanism of ME53 intracellular translocation and ME53 function. Yeast two-hybrid and mass spectrometry analyses were used to identify protein interacting partners of ME53. AcMNPV-infected or uninfected Sf9 cells were used to construct prey cDNA libraries to identify the potential protein-binding partners of ME53 using ME53 protein as bait. Cellular receptor for activated protein kinase c (RACK1) was identified as one putative ME53 cellular protein interacting partner from a cDNA library constructed from AcMNPV uninfected cells. Two AcMNPV proteins, VP80 (a nucleocapsid protein which interacts with nuclear actin) and LEF5 (late expression factor 5), were also determined as putative ME53 protein binding partners from a cDNA library constructed from AcMNPV-infected cells. These three genes (RACK1, VP80, and LEF5) and several targeted AcMNPV ORFs including p6.9, Exon0, VP39, GP64 and segments of ME53, were cloned as reciprocal baits and preys and tested for protein-protein interaction in the yeast two-hybrid system. Using N terminally fused GFP ME53 to pull down ME53 -binding partners followed by mass spectrometry analysis we identified Hsp60 (heat shock protein 60) as another potential ME53 binding protein. The interaction between VP80 and ME53 was confirmed by bimolecular fluorescence complementation. Since VP80 is a structural protein interacting with VP39 (major capsid protein), the interaction between VP80 and ME53 may enable the attachment of nucleocapsids to the cellular membrane allowing for nucleocapsid budding. It may also involve occlusion-derived virus formation in the nucleus at the ring zone or facilitate nucleocapsid transport from the nucleus to the cellular membrane.

83 - Decoding Intraviral and Virus-Host Protein Interaction Networks of Porcine Nidoviruses by Quantitative Proteomics

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The long-term goal of our research program is to better understand molecular mechanisms of interactions between animal nidoviruses and their hosts in order to develop new strategies for effective control of viral infections. The present research project is focused on emerging swine pathogens of unprecedented economic significance, such as the porcine reproductive and respiratory syndrome virus (PRRSV) and the porcine epidemic diarrhea virus (PEDV). The PEDV and PRRSV are responsible for severe economic losses and considered as the primary emerging livestock pathogens worldwide. Insufficient understanding of virus-host interactions impedes the development of effective animal vaccines against PRRSV and PEDV. Host-virus interactions are highly dynamic and may involve multiprotein complexes. Earlier, our group employed biochemical and proteomics approaches to identify virus-host multiprotein complexes, and showed that their composition is controlled by the virus either by direct recruitment of or by binding to host proteins. Growing evidence indicates that extracellular microvesicles play an important role in viral pathogenesis and modulation of host immune responses to infection. Consequently, the characterization of the composition of the extracellular microvesicles and identification of the host proteins that are specifically encapsidated into or bound to virions are important for our further understanding of virus-host interactions. To accomplish this objective, we produced and purified PRRSV and PEDV using both the simian cell cultures that are routinely used for virus production and PRRSV/PEDV natural target cells (of porcine origin). We hypothesized that the composition of PRRSV and PEDV virions and virus-host molecular complexes will reflect changes in environmental conditions (e.g., pH, activities of host proteases, and tissue-specificity). Furthermore, we hypothesized that the tight interactions between host and viral proteins defines the fate of infection and pathogenesis. Proteomics is the best method to directly characterize the multimolecular complexes important for virus entry and pathogenesis. We examined the composition of progeny virions in order to identify cellular proteins that are associated with or encapsidated into viral particles using state-of-the-art mass spectrometry (MS) strategies, including a high-resolution hybrid Quadrupole-Orbitrap MS. The present study has demonstrated the incorporation of cellular proteins in PRRSV and PEDV virions. We found that the PRRSV and PEDV infections affected the abundance levels of numerous host proteins associated with microvesicles. Our proteomic data showed that the abundance of proteins involved in immune responses and metabolic processes was dramatically affected by PRRSV infection. The abundance of proteins involved in immune responses was also changed in PEDV infected cells. Interestingly, in PEDV infected cells, host proteins involved in cell cycle regulation and cytoskeletal system were affected in abundance, which is not surprising because several investigators reported that cytoskeletal proteins are actively participating in moving the viral components to the assembly site, and that many viruses manipulate DNA repair and cell cycle in order to achieve a cellular environment favorable for their replication. Further investigations are needed to evaluate the role of individual cellular proteins in the nidoviral replication, assembly, and pathogenesis.

86 - Development of a Humanized Antibody for Prevention of Epstein-Barr Virus Infection and Associated Cancers

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Acute Epstein-Barr virus (EBV) infection in immunosuppressed transplant patients can give rise to malignant B-cell proliferation known as post-transplant lymphoproliferative disease (PTLD). The EBV major virion surface glycoprotein (gp)350 is a principal target of naturally occurring neutralizing antibodies, and is viewed as the best target to prevent acute infection and PTLD in at-risk transplant recipients. We have constructed a humanized (hu) version of the murine anti-gp350 neutralizing monoclonal antibody 72a1. The hu72a1 IgG1 antibody displays no significant anti-mouse activity, recognizes both gp350 and its splice variant gp220, as well as a gp350 peptide that was shown to constitute the principal EBV gp350 neutralizing epitope when tested in immunoassays. Further, hu72a1 antibody can block EBV infection of B cells in vitro at a level equal to that of a mouse-human chimeric 72a1 antibody construct. This work provides a further structural and immunological understanding of the 72a1 antibody interaction with EBV gp350, as well as a launch point for future anti-EBV therapeutic antibodies designed to block EBV infection and prevent PTLD while eliminating the deleterious antigenic murine features of the original 72a1 antibody.

87 - ViDiT-CACTUS: a fast, low-cost and sensitive wrapper-method for virus discovery and other microbiology applications

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As the per-base costs of high-throughput sequencing (HTS) technologies decrease it is becoming increasingly common in microbiology. In contrast, library preparation methods often carry a high per-sample cost, possess strict input concentration requirements and different methods are usually needed for different applications. These aspects make HTS difficult to implement in some niche applications and especially for research groups on a budget. Furthermore, the immense volume of HTS-generated data often requires bioinformatic expertise and high-end equipment for data analyses. To answer these needs, we developed ViDiT, a universal PCR-based library preparation method optimized for the Ion Torrent PGM sequencer, that includes 3 steps: DNA amplification with tailed random octamers; library generation, where adaptor sequences are introduced; and library enrichment to increase the library concentration. We further developed CACTUS, an open-source Python wrapper-script that handles sequence quality checks, filtering, PHRAP-based assembly and alignment of sequences to reference databases using BLAST and DIAMOND. CACTUS is designed to off-load intensive workloads to cloud platforms to generate high-quality data without the need for high-end servers. We validated our method within three applications: virus discovery, performed on 36 oropharyngeal-cloacal swabs collected from avian influenza virus (AIV)- negative wild birds; amplicon-based full-genome sequencing, implemented on products of the influenza virus whole genome RT-PCR completed on 3 AIV-positive cloacal swabs; and microbiome characterization of a deep-sea carnivorous sponge. ViDiT-CACTUS was successfully used as virus discovery tool as it allowed the identification of fragments of putative viruses from 25 viral families, including 10 vertebrate-infecting viruses, even from samples with undetectable nucleic acid (NA) concentrations. Most of the identified viruses shared low identity with reference sequences and could be considered novel viruses. The presence of viruses from 5 families (families Caliciviridae, Coronaviridae, Papillomaviridae, Parvoviridae, Pneumoviridae) were confirmed by PCR in 6 samples and complete genome sequencing and epidemiological investigations are ongoing. The successful characterization of 3 AIV near-complete genomes (99.0%, 90.4% and 99.6% coverage) showed our approach can be also useful as an amplicon-based complete genome sequencing system in a streamlined and multiplex-capable setting. Furthermore, CACTUS assemblies consistently produced long contigs spanning the entire length of each viral segment, demonstrating its strong performance in complete genome assembly. Lastly, ViDiT-based shotgun libraries were successfully used to profile microbial diversity in a deep-sea sponge. Phylum-level composition showed a diverse assemblage of bacteria, including Proteobacteria, Bacteroidetes, Cyanobacteria, Actinobacteria and Firmicutes, while the detected Archaea phyla represented the Euryarchaeota and Thaumarchaeota and we detected both double-stranded (Caudovirales, Mimiviridae and Phycodnaviridae) and single stranded (Microviridae) DNA viruses. Comparing our data to previously published 16S rDNA-based data of the same sponge showed ViDiT-CACTUS can capture all but the lowest abundance bacterial taxa. Furthermore, ViDiT based taxonomic profiles revealed additional taxa and longer contigs that could be used to assemble a preliminary functional metagenome of the sponge. In conclusion, ViDiT-CACTUS demonstrated its validity in a wide range of microbiology applications. Valuable information was produced at an extremely low cost (<$5 USD per library preparation) and the high-sensitivity of our method allowed for investigation of samples with low to unmeasurable NA concentrations. The simplicity and modularity of ViDIT-CACTUS make it easily implementable in any molecular biology laboratory, for various research goals and with different sequencing platforms.

88 - Molecular characterization and pathogenicity analysis of a fowl adenovirus 4 isolate

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Some fowl adenovirus 4 (FAdV-4) isolates cause severe hepatitis-hydropericardium syndrome (HHS) with mortality up to 80%, especially in 3-5 week-old broiler chickens worldwide. However little is known about virulence determinants of FAdV-4. Preliminary in ovo experiments showed that 10-day-old specific pathogen free (SPF) chicken embryos inoculated with $1x10^5$ of an Ontario FAdV-4 isolate, FAdV-4ON2, had 33% mortality (2 out of 6) post-inoculation on day 5 and showed typical HHS lesions, such as necrotic hepatitis. In contrast to the ON2 group, no clear lesions and mortality were observed at the same dose of inoculation with the nonpathogenic FAdv-4ON1 and the PBS mock groups. In this study, we sequenced the entire genome of the putative virulent strain, FAdV-4ON2. Compared to FAdV-4ON1, FAdV-4ON2 has a genome size of 45,577 bp which is 57 bp shorter than that of the ON1 isolate. Both the left end (7,088 bp) and center part (25,717 bp) of the ON2 genome are 99% identical to ON1. However, the right end sequence (12,772 bp) is only 96% identical to that of ON1. The significant differences are in TR-E and ORF19. The ON2 TR-E sequence is 144 bp shorter than the ON1 TR-E and there is a stop codon in the ON2 ORF19 that would make the ON2 lipase 209 amino acid shorter than the ON1 lipase. Our data indicate that the deletion of TR-E and the mutation of ORF19 are potentially associated with FAdV-4CAV pathogenicity.

90 - Blocking the RNA interference pathway improves oncolytic virotherapy

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Antiviral responses are barriers that must be overcome for efficacy of oncolytic virotherapy. In mammalian cells, antiviral responses involve the interferon pathway, a protein-signaling cascade that alerts the immune system and limits virus propagation. Tumour-specific defects in interferon signaling enhance viral infection and responses to oncolytic virotherapy, but many human cancers are still refractory to oncolytic viruses. Given that invertebrates, fungi and plants rely on RNA interference processes for antiviral protection, we investigated the potential involvement of this alternative antiviral mechanism in cancer cells. In our studies, we detected viral genome-derived small RNAs, indicative of RNA interference-mediated antiviral responses in human cancer cells. To combat RNA interference-mediated antiviral responses, only a few mammalian viruses encode viral suppressors of RNA silencing (VSR) that interact with cellular silencing machinery. As such, we engineered an oncolytic vesicular stomatitis virus variant to encode an ectopic VSR that is known to bind double-stranded RNA and inhibit processing by Dicer to prevent the production of anti-viral small interfering RNAs. Our virus showed enhanced viral replication and cytotoxicity, impaired viral genome cleavage and altered microRNA processing in cancer cells. Our data establish the improved therapeutic potential of our novel virus which targets the RNAi-mediated antiviral defense of cancer cells.

94 - The viral protein Kaposin B elicits processing body dispersal by activating the cytoskeletal kinase ROCK

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Kaposi's sarcoma herpesvirus (KSHV) is the etiologic cause of Kaposi's sarcoma, a highly inflammatory endothelial cell cancer that is most severe in immunosuppressed individuals. During latent KSHV infection, the virus promotes two notable phenotypes: cell elongation (spindling) and increased expression of inflammatory and angiogenic molecules. Ectopic expression of the latent viral protein Kaposin B (KapB) recapitulates these features by promoting the formation of actin stress fibers (SF) and the dispersal of processing bodies (PBs). PBs are ribonucleoprotein-containing granules that mediate degradation or translational suppression of cellular mRNA. Because PBs are a major site for the constitutive decay of a class of mRNA that encode cytokines and angiogenic factors, their size and number controls inflammatory gene expression. KapB induces both SF and PB dispersion by activating a stress-responsive signaling axis that links the kinase MK2 to the cytoskeletal regulator, RhoA. Previous work in our lab used knockdowns to demonstrate that each component of this signaling axis is required for both these phenotypes. Therefore, we have not yet been able to uncouple KapB-mediated effects on the actin cytoskeleton from KapB-mediated PB dispersal. This has led us to pose the following research question: Is actin SF formation required for the dispersal of PBs during KapB expression? To investigate this question, we investigated the effects of shRNA knockdown of downstream RhoA effectors known to coordinate SF formation (ROCK1, ROCK2, mDia1, mDia3) on KapB-induced actin SF formation and PB dispersal. If actin SF formation was eliminated using these knockdowns but PB dispersion was not affected, we would know that actin SF were not required for PB loss. Knockdown of ROCK1 and ROCK2 eliminated actin SF formation and prevented PB dispersal in cells expressing KapB. Notably, though knockdown of both isoforms increased the size and number of PBs, knockdown of ROCK1 restored PBs only in KapB-expressing cells while knockdown of ROCK2 increased PB numbers in both KapB and control cells. Knockdown of mDia proteins also eliminated SF formation but did not affect PB dispersal in KapB-expressing cells. These data suggest that KapB-mediated activation of the kinases ROCK1/2 is required for PB dispersion and that actin SF formation is not required for KapB-mediated PB dispersal. These results provide mechanistic insight into how KapB manipulates cellular stress responses in order to create an immunological niche that facilitates viral survival.

95 - Enhancing oncolytic adenovirus efficacy by improving viral spread

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Conditionally-replicating adenoviruses (CRAds) have shown promise in preclinical studies, based on their ability to selectively replicate in and kill cancer cells, but leave healthy cells unharmed. However, CRAds have demonstrated limited therapeutic efficacy in human clinical trials, in part due to their poor ability to spread throughout a tumor mass. Typically, CRAds only diffuse a few millimeters from the site of injection, leaving much of the tumor unaffected. Overexpressing the Ad death protein (ADP), which improves virus-mediated cell lysis and release of viral progeny, and incorporating the p14 FAST protein, which causes host cell-cell membrane fusion, may help overcome these physical barriers. We previously developed CRAdFAST, a fusogenic vector in which the early region 3 (E3) of the viral genome, which encodes ADP, was removed and replaced by a p14 FAST expression cassette. CRAdFAST efficiently fused cancer cells and improved oncolytic activity compared to a non-fusogenic CRAd in tissue culture and mouse models of cancer, but had only a modest effect on overall tumor growth and mouse survival. We hypothesize that expression of the Ad death protein and p14 FAST protein from a CRAd vector will further enhance viral replication, oncolysis, and spread in preclinical models of cancer. To evaluate the effect of including ADP in a fusogenic CRAd, we developed two vectors, CRAdRC109 (E3+) and CRAdRC111 (E3-). In these viruses, the p14 FAST expression cassette was relocated downstream of the viral late 5 (L5) region, allowing reinsertion of the entire E3 region including ADP. CRAdRC109 and CRAdRC111 replicate efficiently in A549 cancer cells yet have a drastic reduction in p14 FAST protein expression relative to CRAdFAST. Consequently, CRAdRC109 and CRAdRC111 have reduced fusogenic and oncolytic properties compared to CRAdFAST. However, inclusion of the E3 region in CRAdRC109 increased viral plaque size, suggesting ADP did improve viral replication and spread. We subsequently developed CRAdRC116, in which the E3 region of the viral genome was removed and replaced with a bicistronic expression cassette containing p14 FAST and ADP separated by a self-cleaving P2A peptide. Preliminary results suggest CRAdRC116 efficiently replicates in and fuses A549 cancer cells. We are currently evaluating this new vector in tissue culture and mouse models of cancer. In summary, we have developed a CRAd vector with improved viral replication and spread, which may improve therapeutic efficacy of oncolytic adenovirotherapy in cancer patients.

97 - Analysis and mapping of subgenomic RNAs of Grapevine rupestris stem pitting-associated virus

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Grapevine rupestris stem pitting-associated virus (GRSPaV) is a member of the genus Foveavirus in the newly established family Betaflexiviridae, which contains pathogenic viruses of important woody fruit crops. Due to difficulties working with woody plants and unavailability of effective experimental systems, the molecular biology of the genus Foveavirus and the family Betaflexiviridae is scarce. GRSPaV is prevalent among commercial grapevine cultivars with a worldwide distribution. GRSPaV comprises a family of sequence variants and has been associated with three distinct diseases: Rupestris Stem Pitting, Vein Necrosis and Syrah Decline, although its etiological role in these diseases remains to be established. The GRSPaV genome is a positive sense, ssRNA, 8,725 nucleotides in length and encodes 5-6 ORFs. ORF1 encodes the replicase polyprotein; ORFs2-4 constitute the triple gene block (TGB), encoding TGB proteins 1, 2 and 3; ORF5 encodes the capsid protein. However, how these proteins are expressed from the GRSPaV genome remains unknown. By default, ORF1 would be translated directly on the viral genomic RNA to produce the replicase polyprotein. ORFs2-5 are likely expressed from sub-genomic (sg) RNAs that share the 3'-terminal sequence with the genomic RNA. An additional ORF, ORF6, was identified via in silico analysis at the 3'-terminal end of the viral genome, with a putative translation product of 11 kDa. The present study aims to determine the expression profile of genomic and subgenomic RNAs of GRSPaV through northern blotting analysis, and to map the transcription start sites of these sgRNAs through RLM-RACE. We have confirmed the presence of three sgRNAs, corresponding to ORF2, ORF3 plus ORF4, and ORF5. We also have mapped the transcription start site of these three sgRNAs. We are further examining the presence of the fourth sgRNA for ORF6. Information generated from GRSPaV would help advance the study of other members of the Foveavirus. ACKNOWLEDGEMENTS: This research was funded by the Natural Science and Engineering Research Council of Canada, Discovery Grant program (RGPIN-2014-05306).

98 - Statistical and Functional Analyses Reveal Subtype-specific Constraints on HIV-1 Escape from Host Cellular Immunity

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Background: Pandemic HIV-1 group M strains, which comprise nine subtypes and dozens of circulating recombinant forms, are extraordinarily diverse both at the individual and population scales. Selection of viral variants harbouring mutations enabling the virus to evade an individual host's Human Leukocyte Antigen (HLA) class I-restricted CD8+ cytotoxic T-cell (CTL) response is reproducible based on their HLA profile. Termed HLA-associated polymorphisms or 'escape mutations', patterns of adaptation have been comprehensively mapped in different global populations, however the extent to which viral genetic context constrains HLA-mediated escape remains incompletely understood. We combine statistical analyses with in vitro functional assessments to investigate HLA-driven adaptation in a population where multiple HIV-1 subtypes co-circulate (Uganda). Methods: HLA-associated polymorphisms in HIV-1 Gag, Polymerase (Pol), and Nef were identified in 200 subtype A1- and 135 subtype D-infected individuals using published statistical association approaches that take into consideration the phylogenetic relationships between HIV-1 sequences. The strength of selection of each polymorphism was compared between subtypes using a phylogenetically-informed logistic regression approach. The functional impact of select HLA-associated polymorphisms in each subtype was assessed, in the Gag protein, by generating VsVg-pseudotyped virus stocks expressing parental/mutant subtype A1 or D gag-protease sequences in an NL4.3 reference strain backbone using Gibson enzymatic assembly, following which in vitro replication capacity was determined using a multi-cycle GFP-reporter assay. In the Nef protein, the abilities of mutant subtype A1 and D Nef sequences to downregulate host cell surface proteins CD4, HLA and SERINC5 were evaluated by flow cytometry. Results: A total of 83 Gag, 198 Pol and 105 Nef HLA-associated polymorphisms were identified in subtype A1 and/or D at $q<0.2$. Of these, 34% exhibited significant differential selection between subtypes ($q<0.1$; $p<0.05$). Moreover, experimental confirmation revealed that some instances of differential selection result from subtype-specific mutational constraints. For example, in the HIV-1 subtype D p24 capsid protein, HLA-B*57:03 strongly selected the Gag-T242N mutation (Odds Ratio [OR]=250). In contrast, HLA-B*57:03 did not select Gag-T242N in subtype A1 (OR=1.8, p=0.8). This intersubtype difference in the strength of selection of Gag-T242N by B*57:03 is highly statistically significant ($p=8x10^{-6}$). The lack of selection of B*57:03-T242N in subtype A1 is likely because in this subtype the consensus proline at the adjacent codon 243 is incompatible with T242N. Molecular modeling predicted marked conformational changes in p24 structure when the 242N escape variant was adjacent to proline-243, but not leucine-243, the subtype D consensus. Indeed, in vitro replication was abolished when these residues were engineered together (p=0.0009), while T242N minimally impacted replication when leucine was at the adjacent codon. Other viral polymorphisms that were differentially selected across HIV-1 subtypes only minimally impacted protein function. For example, the HLA-A*74:01-selected Nef 196K mutation, which represents one of the strongest examples of subtype-specific selection observed (subtype D OR=2.08, $p=3.8x10^{-6}$; subtype A1 OR=-0.4, p=0.7; inter-subtype comparison $p=3.6x10^{-5}$) had a negligible impact on Nef-mediated downregulation of CD4, HLA and SERINC5 in both subtypes A1 and D, suggesting other mechanisms besides mutational constraints contribute to subtype-specific HLA-mediated adaptation. Conclusions: Results highlight the influence of viral subtype on HLA-mediated selection of immune escape mutations in HIV-1. Mechanistic elucidation of differential immune escape pathways may help identify mutationally-constrained viral regions for the design of subtype-specific and/or universal HIV-1 vaccines.

99 - Identification of pharmacological targets and small molecules inhibiting adenovirus replication.

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The human adenovirus (Ad) causes minor respiratory illnesses in most patients, but can lead to severe disease and death in pediatric, geriatric and immunocompromised patients. No approved antiviral therapy currently exists for the treatment of severe Ad-induced diseases. Within the first few hours of infection, the Ad DNA enters the host cell nuclei and associates with cellular proteins including histones, adopting a nucleoprotein structure similar to the cellular DNA. Assembly of the viral genome into this repeating nucleosome-like structure is required for efficient expression of virus-encoded genes. Consequently, one approach to treating Ad-induced disease may be to prevent the viral DNA from transitioning to this transcriptionally active state. Thus, our objective is to identify novel small-molecule inhibitors of Ad replication and to investigate the molecular mechanism underlying the inhibition, including effects on epigenetic regulation of the viral genome. We have generated a wild-type-like Ad construct encoding the red fluorescent protein (RFP) within the viral genome. RFP from this construct is only expressed following Ad DNA replication, which allows us to effectively monitor virus replication by fluorescence microscopy. Using this construct, we designed an efficient method for screening small-molecule libraries. We have screened two small molecule libraries consisting of over 1300 compounds to identify drugs that exhibit anti-Ad activity. Several positive hits have been validated to either inhibit or considerably delay Ad gene expression, and reduce virus yield. In particular, the pan-histone deacetylase (HDAC) inhibitor vorinostat was found to significantly reduce RFP expression. Follow-up studies revealed that vorinostat delays the onset of viral gene expression and replication, and decreases virus yield from infected cells. Vorinostat was also effective against more virulent and clinically relevant Ad serotypes. The drug's inhibitory effects were found to be mediated through the inhibition of HDAC2 activity. Further elucidation of the underlying mechanism and in vivo studies are underway. The costs associated with Ad-induced disease are significant in terms of medical expenses, lost work hours and loss of life in some populations. Identification of novel Ad inhibitors will allow the design and development of more effective antivirals, ultimately leading to decreased disease pathogenesis and higher survival rates in severe infections. Investigation of the mechanism by which these compounds impact Ad replication will provide useful insights on virus-cell interactions, and allow us to identify new pharmacological targets for therapeutic intervention.

100 - Cardiotonic steroids suppress adenovirus replication by affecting early gene expression

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Human adenoviruses can cause severe, even life-threatening, infections yet there are no approved agents for treatment. We have shown that cardiotonic steroids, in particular, digoxin and digitoxin, reduce the yield of multiple adenovirus species by 2-4 logs in human lung epithelial cells (A549) by suppressing viral DNA replication at one or more steps beyond immediate early E1A expression. The current study aimed to characterize the nature of the antiviral block by analyzing the effect of digoxin and digitoxin on specific viral proteins normally expressed prior to DNA replication. A549 cells were infected with human adenovirus C5 under conditions sufficient to infect most of the cells. After one hour adsorption, virus inoculum was removed and replaced with fresh medium with and without 100 nM digoxin or digitoxin. Cells were harvested at different times post infection for analysis of specific viral proteins by immunoblot and by fluorescence microscopy. Immunoblot analysis at 14 hours post infection showed ~60% reduction in expression of E2 72K DNA binding protein (DBP), in contrast to E1B 55K protein which was barely detectable in treated cells. Despite appreciable levels of E2 72K DBP, its staining pattern was altered. Though still exclusively nuclear, the signal in most cells was diffuse rather than clustered at viral replication centres, consistent with a block in viral DNA replication. Analysis of transcripts by RT-qPCR showed a reduction in levels of E1B and E4 expression to ~20% of levels in untreated cells. E2B transcripts encoding the pre-terminal protein, pTp, however, were barely detectable. Overall, digoxin and digitoxin compromise transcription of early regions E1B, E2 and E4, after E1A expression. The block in genome replication likely reflects insufficient levels of the essential pTP and viral DNA polymerase. The antiviral effect of digoxin, but not digitoxin, was overcome by increasing the extracellular concentration of potassium. Overall, this work supports the idea that cardiotonic steroids could be developed for treatment of severe adenovirus infections.

101 - SUMO Wrestling with KSHV: Could Disruption of a Post-Translational Modification Contribute to KS?

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Background: To maintain balance, cells need ways to respond to stress, and use signaling pathways to cope with stress. One stress responsive pathway is the p38 mitogen-activated protein kinase (MAPK) cascade; in this cascade, p38 MAPK phosphorylates MAPK-activated protein kinase 2 (MK2). MK2 plays a role in actin dynamics and cytokine production, and is of interest to our lab because it can promote inflammation and tumorigenesis. Our lab studies Kaposi's sarcoma-associated herpesvirus (KSHV), which causes the endothelial cell (EC) cancer known as Kaposi's sarcoma (KS). KS lesions are characterized by inflammation, proliferation, angiogenesis and cytoskeletal dysfunctions such as cell spindling. The viral gene product Kaposin B (KapB) can recapitulate two of these phenotypes in vitro (cell spindling and stabilization of inflammatory mRNAs) by directly binding to and activating MK2. However, KapB does not act as a kinase, so the mechanism of MK2 activation remains unclear. Recently, it was discovered that the post-translational modification known as SUMOylation regulates MK2 activity. When one of the key SUMO-associated lysines in MK2 was mutated to an arginine, kinase activity and stress fibre induction increased, suggesting that SUMOylation dampens MK2 kinase activity. These phenotypes are similar to those observed during in vitro KapB expression; therefore, I hypothesize that KapB binds to MK2 and reduces its SUMOylation. Methodology: I will address this hypothesis using two approaches: 1) MK2 SUMOylation will first be reaffirmed in vitro. HEK293T cells will be co-transfected with myc-MK2, Ubc9-HA and HA-tagged SUMO proteins. Standard co-immunoprecipitation approaches will be used to pull-down myc-tagged protein complexes, and this precipitant will be immunoblotted for HA-tagged protein complexes. After successful reaffirmation, cells will then be co-transfected with and without KapB. 2) MK2 constructs will be created that lack predicted SUMO motifs, as determined using the online algorithm JASSA (Joined Advanced SUMOylation Site and Sim Analyser). Primers have been designed to mutate three predicted SUMO-associated lysines (K64, K188, and K353) to arginine. New England Biolab's Q5 site-directed mutagenesis protocol will be used to create the MK2 mutant constructs, which will be verified through sequencing. Expression and SUMOylation statuses of these constructs will be verified in HEK293T cells. These mutants will then be subcloned into lentivirus expression vectors to generate recombinant lentiviruses, which will be used to transduce ECs. Results: Expression of the KapB, myc-MK2, HA-SUMO (1 and 3) and Ubc9-HA constructs has been verified in HEK293T cells using Western blot. Creation of the K353R mutant MK2 construct has been initiated, with colonies being actively screened for the mutation by sequencing. Co-immunoprecipitation of controls has been performed. However, low expression of the HA-tagged SUMO 3 construct has limited success. I predict that the presence of KapB will reduce MK2 SUMOylation. I also predict that expression of the mutant MK2 constructs will recapitulate the KapB associated phenotypes of stress fibre induction and inflammatory cytokine stabilization. Impact: This research will provide insight into how KapB contributes to viral pathogenesis during KSHV infection by elucidating the precise details of the mechanism of MK2 activation by KapB. This research will also reveal the central importance of SUMOylation to normal cellular function, and how viruses can disrupt this post-translational modification to cause cellular dysfunction and disease.

102 - Viral Protein Engagement of Selective Autophagy Pathways Controls Inflammatory Gene Expression

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Kaposi's Sarcoma-associated herpesvirus (KSHV) is the etiological agent of the angiogenic and highly inflammatory endothelial cell (EC) cancer, Kaposi's Sarcoma (KS). Kaposin B (KapB) is a viral protein expressed during the latent lifecycle of KSHV that contributes to the establishment of the inflammatory character of the KS lesion. When expressed in primary ECs, KapB promotes the dispersal of cytoplasmic ribonucleoprotein (RNP) granules called processing bodies (PBs). PBs are the major site for the constitutive degradation of AU-rich element mRNAs (ARE-mRNAs), a class of cellular mRNAs that code for inflammatory molecules. PB dispersal by KapB correlates with increases in steady-state levels of endogenous ARE-mRNAs (IL-6, IL-8, COX-2) by droplet digital PCR(ddPCR) and qPCR. More recently, our lab has shown that KapB upregulates bulk autophagic flux, a process by which cells degrade cytoplasmic components such as organelles in vesicles called autophagosomes. Inhibiting autophagosome maturation (BafilomycinA) and knocking down the expression of essential autophagy genes (Atg 5) reverses KapB-mediated PB dispersal. Furthermore, treatment of KapB-expressing ECs with autophagy-inducing drugs (Torin, Thapsigargin) increases steady-state levels of endogenous ARE-containing transcripts via ddPCR and qPCR. These findings indicate that KapB-mediated PB dispersal requires upregulated autophagy; however, we do not understand precisely how autophagic flux modulates ARE-mRNA levels or how PBs are recruited to nascent autophagosomes for degradation. Recently, PBs and other RNP granules were shown to be targeted specifically to autophagosomes in a process termed selective autophagy. During selective autophagy, proteins such as NDP52 act as molecular bridges to bind and target cargo to growing autophagosomes. Given recent evidence implicating selective autophagy in RNA granule degradation and the role of PBs in regulating ARE-mRNA expression, I hypothesize that KapB upregulates selective autophagy to facilitate PB degradation and to promote the expression of inflammatory ARE-mRNAs that are typically degraded in PBs. My preliminary data suggests that knockdown of the selective autophagy receptor, NDP52 reverses both KapB-mediated PB dispersal (via immunofluorescence) and KapB-mediated increases in ARE-mRNAs (via qPCR). Collectively, these data suggest KapB is co-opting selective autophagy machinery to modulate PBs and ARE-mRNA expression thus promoting an inflammatory environment beneficial for tumourogenesis and viral infection.

103 - Differential Restriction of Paramyxoviruses by the Long Isoform of ZAP

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Paramyxoviruses (PVs) are negative sense, non-segmented single-strand RNA viruses that infect a wide range of vertebrate hosts, and are responsible for endemic human and animal diseases like measles and mumps, or distemper and Newcastle disease. Nipah and Hendra viruses are recently emerged zoonotic viruses, and surveillance has identified numerous other PVs in bats and rodents with unknown potential for zoonotic spillover. Understanding host factors that permit or restrict PVs in humans and domestic animals is a first step in determining the risks posed by both familiar and new PVs. ZAP (zinc-finger antiviral protein) exists as two splice-variant isoforms that have differential expression, localization, and antiviral activity. Both mediate antiviral activity primarily by binding viral RNAs through their common zinc-finger domain and directing the RNA to the exosome complex for degradation. The ZAP-S (short) isoform is an interferon-stimulated gene; it was identified first, and so is known to restrict a wide range of viruses, from negative- and positive-sense RNA viruses to some DNA viruses, retroviruses, and retroelements. ZAP-L (long) is much more recently discovered, but appears to have a more potent antiviral activity when compared to ZAP-S. ZAP-L is constitutively expressed, and bears a prenylation motif not found on ZAP-S; this localizes ZAP-L to endosomal membranes and is thought to enable better interaction with viral RNAs. Activity of either ZAP isoform has not been reported against Paramyxoviruses, so we generated inducible HEK-293 cells to overexpress ZAP-S or ZAP-L, as well as mutants of each isoform to add (ZAP-S+pren) or remove (ZAP-LΔpren) the prenylation motif. We then infected these cells with a panel of PVs: Human parainfluenza virus 3 (HPIV3), Measles (MeV), Mumps (MuV), Sendai (SeV), and Newcastle disease virus (NDV). Replication of HPIV3, MeV, and MuV was reduced over 100-fold by overexpression of ZAP-L, but this effect was abolished in the ZAP-LΔpren cells, indicating that prenylation of ZAP-L was key to its effect on these viruses. This can be seen early during infection, in both reporter gene expression and viral RNA levels. ZAP-S did not impact any PV, but addition of the prenylation motif in the ZAP-S+pren cells conferred intermediate antiviral activity against these three viruses. Interestingly, SeV and NDV were not strongly affected by overexpression of any ZAP protein. SeV and HPIV3 are closely-related viruses in the same genus, and so it is not clear precisely why ZAP-L restricts only HPIV3, and not SeV. Conversely, knockdown of endogenous ZAP-L positively impacts HPIV3 replication, without affecting SeV replication. Thus, the long isoform of ZAP bears antiviral activity against some, but not all paramyxoviruses; its prenylation motif is necessary for this activity, and can confer intermediate activity when added to ZAP-S. Additionally, of the viruses tested, human ZAP-L appears to only restrict the human pathogens (HPIV3, MeV, MuV), and does not affect the murine pathogen SeV, despite the fact that SeV and HPIV3 are so closely related, nor the avian pathogen NDV. We plan to expand our panel of PVs to further explore potential implications for the role of ZAP in tropism. Mechanistically, overexpression of ZAP-L impacts HPIV3 RNA levels early in infection, suggesting that its restriction occurs before virus assembly and budding. While previous work defining the effects of ZAP-S on a wide range of viruses showed that it targeted specific viral mRNAs for degradation, no common motif or structure has yet been defined. We are currently exploring whether ZAP-L activity can be similarly linked to degradation of specific HPIV3 mRNAs, and are in parallel passaging virus in the presence of overexpressed ZAP-L to identify potential escape mutants that may provide insight into the interactions between PVs and ZAP.

104 - From the Arctic to Antarctica: Probing the geographic distribution of marine RNA virus quasispecies

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RNA viruses, particularly the genetically diverse members of the order Picornavirales, are widespread and abundant in the ocean. Gene surveys suggest that there are spatial and temporal patterns in the composition of RNA virus assemblages, but there are limited data on their diversity and genetic variability in different oceanographic settings. Here, we show that specific RNA virus genomes have widespread geographic distributions and that the dominant genotypes are under purifying selection. Three previously undescribed picorna-like viruses (BC-1, -2 and -3) and three previously described marine RNA viruses (JP-A, -B and Heterosigma akashiwo RNA virus), all originally identified at the same location in coastal British Columbia, exhibited different biogeographical patterns, indicating that RNA virus distributions are affected by biotic factors such as host-specificity and viral lifecycle, and not just abiotic processes such as dispersal. Sequence differences relative to the reference genomes imply that virus quasispecies are under purifying selection, with geographically distinct genomes dominated by synonymous substitutions and conserved amino acid sequences. This is the first biogeographical analysis of marine RNA viruses across oceanographic provinces and evaluation of the selection pressures acting on them. These data add to the collection of known marine RNA viruses, show the importance of dispersal and purifying selection for marine RNA viruses, and demonstrate that closely related RNA viruses are pathogens of eukaryotic microbes across the global ocean.

105 - Examining viral populations, CRISPR-resolved virus-host interactions, and CRISPR-Cas system diversity in a municipal landfill

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Recent culture-independent approaches have unveiled a staggering diversity of viruses from a wide variety of environments. The comparatively poor representation of viruses within reference databases, despite their numerical abundance in ecosystems, clearly illustrates how characterizing the virosphere has historically been a methods-limited endeavor. Understudied environments, like municipal waste sites, are likely to house novel, diverse viral populations, whose host interactions may impact nutrient cycling and contaminant degradation. Using metagenomics, we probed the viral diversity within a landfill and adjacent aquifer in Southern Ontario. Metagenomic sequences were generated from samples collected from three leachate wells, a leachate collection cistern, and from an aquifer adjacent to the landfill. DNA was extracted from filtered biomass, and sequenced by the Joint Genome Institute (JGI). Using FastViromeExplorer, a virus and phage identification pipeline, along with NCBI's RefSeq database and the JGI's Earth Virome Project database, we identified the viral signatures in all six metagenomes. The different sites varied in their viral presence, with the cistern samples containing the highest viral abundances and diversities, and the aquifer sample showing the lowest. To establish virus-prokaryotic host interactions within the landfill, we used Crass to first identify CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) within our metagenomes. The spacers within the CRISPR arrays were extracted, and searched against NCBI's Refseq database and our metagenomes using BLASTn, in order identify the viruses from which the spacers originated. The vast majority of identified spacers had no hits in Refseq, suggesting that many of the ecologically important viruses within our system are not present within this reference database. The few spacers that did have hits to Refseq had homology predominantly to Pseudomonad-infecting phage genomes. Spacer hits to our metagenomes were identified as spacer-viral element matches or spacer-host CRISPR array matches, allowing connections based on prior infections to be determined. CRISPR-Cas systems are useful in elucidating virus-prokaryotic host interactions, and are of huge value in the agricultural and health industries. Current biotechnology applications utilize CRISPR-Cas systems adapted from isolated bacteria, while novel Cas proteins may offer improvements or alternatives to current gene-editing tools. We sought to identify the Cas protein diversity within our metagenomes. Using profile Hidden Markov Models (HMMs) generated from specific Cas protein alignments, we screened our metagenomes for the presence of Cas proteins. Numerous Cas proteins types and subtypes were detected, aligned, and homology modeled in order to identify novel Cas proteins within the landfill microbial community with potential biotechnology applications.

107 - PML Isoform Switching During KSHV Infection

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Promyelocytic leukaemia nuclear bodies (PML-NBs) are dynamic and heterogeneous protein complexes, involved in regulation of diverse cellular functions. The promyelocytic leukemia (PML) protein is the main component of PML-NBs and is essential for their formation. PML is alternatively spliced to generate six nuclear localized, functionally distinct protein isoforms. PML-NBs mediate an intrinsic antiviral defence, following virus entry into the cell, inhibiting viral genome replication and protein production. Accordingly, many viruses encode mechanisms to disrupt PML-NBs, PML mRNA and protein expression. One hypothesized mechanism involves modulation of PML pre-mRNA splicing, therefore influencing the production of PML protein isoforms to disrupt PML-mediated antiviral responses. Kaposi's sarcoma-associated herpesvirus (KSHV) has been shown to modulate host splicing and mRNA export during infection, therefore we hypothesized that KSHV lytic reactivation from latency would result in modulation of PML splicing, resulting in PML isoform switching. Using immunofluorescence and PCR based methods, we characterized PML expression and splicing during KSHV latency and lytic reactivation. PML-NBs were quantified by immunofluorescence. Following KSHV lytic reactivation, we observed a disruption in PML-NBs. PML2-to-PML5 isoform switching was detected using a fluorescent reporter construct specific for this isoform-switching event and qRT-PCR. We further observed PML isoform switching from the antiviral PML2 protein to PML5 and identified KSHV ORF57 as a mediator of PML splicing and subsequent PML isoform switching. Together, these findings advance our understanding of PML isoform function during viral replication and the mechanisms employed by viruses to antagonize host antiviral responses.

108 - Discovery and molecular characterization of three novel avian papillomaviruses

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Papillomaviruses (PVs) are small, non-enveloped viruses characterized by a circular dsDNA genome of ~7.5-8Kb. These vertebrate viruses infect epithelial cells and infection can be either asymptomatic or result in neoplasias, which vary in severity from benign lesions (warts) to invasive tumors and cancer. PVs belong to the family Papillomaviridae and are classified based on nucleotide sequence identities of the capsid protein gene, L1, into genera (>60% identity), species (>70% identity) and types (>90% identity). Currently, over 200 PV types (belonging to 52 genera) have been identified and more than 50% of these are human types. In birds, avian PVs (APVs) can cause cutaneous or mucosal proliferative lesions on the feet, legs and near the beak. Eight APV sequences (1 partial and 7 complete genomes) are present in public databases and have been obtained from various bird species from cutaneous lesions, healthy skin, fecal specimens/cloacal swabs, mesenchymal tissue and oral swabs. In this study, we applied an in-house virus discovery method (ViDiT-CACTUS) in search for known and unknown viruses in wild birds, and identified PV genetic fragments in paired oropharyngeal/cloacal swabs collected from a mallard (Anas platyrhynchos), an Atlantic puffin (Fratercula arctica) and an American herring gull (Larus smithsonianus). To obtain more genetic information a ViDiT-based genome walking technique (ViDiWa) was applied and the viruses were molecularly characterized and phylogenetically analyzed. The complete genome (7699 bp) of the Larus smithsonianus PV (LsPV1) was obtained and we could locate all of the typical PV open reading frames (ORFs) for the capsid (L1 and L2) and regulatory (E1, E2, E6 and E7) proteins. Only partial genomic sequences were obtained for the other two viruses: approximately 4000 bp of the Anas platyrhynchos PV (ApPV1) and 5000 bp of the Fratercula arctica PV (FaPV1). All three viruses contained an E9 ORF, found exclusively in APVs. The L1 nucleotide sequences of FaPV1 and LsPV1 were 69% identical to each other but <60% identical to their closest relatives in GenBank. Phylogenetic analyses revealed these two viruses were included within the APV clade, where they clustered together but separately from other genera. These results indicate that these two viruses constitute two different novel species within the same genus. The L1 sequence was not available for ApPV1 but preliminary sequence identity investigations involving partial E1 sequences indicated that ApPV1 could also represent a novel species. The closest relative to this virus, which shared approximately 68% nucleotide identity, was a partially sequenced virus identified in a cloacal swab collected from ducks in India. Phylogenetically, these two viruses formed a distinct clade, separate from other genera, within the APV clade, indicating they could be part of the same genus. In conclusion, we have identified and partially molecularly characterized three novel APV species, belonging to two novel genera within the Papillomaviridae. These viruses were identified in three birds from three different families, indicating that a great diversity of PVs is present among birds and that many more PV genera and species potentially remain to be discovered. It is our priority to complete the genome sequences of FaPV1 and ApPV1 and perform screening to evaluate the distribution of all three viruses among wild birds. Future investigations will also aim to reveal if these viruses are associated with cutaneous lesions in infected birds.

109 - A conserved role for a histidine triad in old world arenavirus glycoprotein during viral entry

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Old world arenaviruses, such as lassa fever virus (LFV), and lymphocytic choriomeningitis virus (LCMV), are enveloped viruses that can cause severe diseases in humans. Despite the extensive use of LCMV in studying immune responses to viral infection, very little is known about its entry pathway. A critical step for entry is interaction between the viral glycoprotein (GP) and a receptor. Previous studies have shown that LFV GP contains a triad of histidines (His) that, when protonated in the acidic environment of the endosomes, allows binding with its intracellular receptor, LAMP1. Interestingly, the His triad is also found in LCMV GP suggesting that protonation of GP might be required for interaction with an unknown intracellular receptor. Using site-directed mutagenesis, we found that while mutation of the His triad had no effect on GP expression and incorporation onto viral particles, viral entry was abrogated. To test the importance of the His triad in surface binding, we engineered soluble GPs and found that both proteins were able to attach to cells, suggesting that the His triad is critical for a step following cell attachment, presumably binding to an intracellular receptor. Taken together, my studies reveal a conserved role for a His triad in arenavirus entry.

110 - Host influenza history dictates vaccine responses through a memory B cell mechanism

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Influenza viruses are constantly changing. Antigenic shifted and drifted viruses re-emerge every year to infect the now naïve human population and cause disease. As people are susceptible to multiple infections over a lifetime, each influenza exposure shapes future immune responses to infections and vaccinations. Unfortunately, vaccines are neither developed or tested with the human influenza history in mind. We have developed a preimmune ferret model that incorporates influenza history as it is seen in humans with the purpose of understanding the mechanisms of vaccine responses in the previously infected host. Ferrets are the most accepted animal model for studying influenza pathogenesis and evaluating vaccines. Using ferrets, the immune responses of preimmune ferrets to influenza vaccination and challenge were investigated. To establish preimmunity, adult ferrets were infected with a sublethal dose of the historical seasonal H1N1 A/USSR/90/1977 (USSR/77). Following recovery (67 days), ferrets were vaccinated and boosted with the Sanofi QIV split virion vaccine (FluZone® Sanofi-Pasteur, PA, USA). To evaluate protection, the ferrets were challenge with a currently circulating 2009 H1N1 pandemic virus on D123. This virus, A/California/07/2009 (Cal/2009), is one of the components of the QIV vaccine. Following challenge, ferrets were monitored for signs of clinical disease including weight loss, fever, and lethargy. Preimmune-vaccinated ferrets lost minimal weight and did not experience a temperature increase. Interestingly, the control group, naïve-vaccinated ferrets that did not have an influenza history, developed the most severe disease with significant weight loss and high temperature increases. Hemagglutination inhibition (HAI) assays showed significant, sustained increases in antibody titers in preimmune ferrets post vaccination for antigens in the vaccine compared to titers from naïve-vaccinated ferrets. Furthermore, early time points post vaccination showed that Cal/09 HA antibodies were present at 7 days post vaccination in preimmune ferrets and absent in the control naïve-vaccinated group. This indicating that animals with an immune background were able to generate vaccine specific antibodies more quickly. Investigation of the immunoglobulin isotype profiles showed a higher level of virus specific antibodies of the IgG isotype in the serum of the preimmune-vaccinated ferrets suggesting maturity in antibody producing B cells. Taken together, my results showed that preimmune animals had greater responses to vaccination and were more protected during challenge. The early generation of antibodies toward an antigenically distinct virus and the predominant IgG virus specific antibodies in circulation suggest plasticity in an existing memory B cell clone. These results are important and should be applied to influenza vaccine development. By understanding the mechanisms of more effective vaccination responses, we can design the next generation of smarter vaccines that both incorporates influenza history and mechanisms of immunogenicity.

111 - Province-wide survey for major viral pathogens in commercial vineyards in Ontario

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Grapes rank as the second largest fruit crop in Ontario, with a farm gate value of $100 million annually. Ontario wines have garnered prestigious recognition in the international market, and the grape and wine industry together produces an economic impact estimated at $4.1 billion. Viruses are highly detrimental pathogens of grapes, causing devastating economic losses due to reductions in vigor, yield, quality, and productive lifespan of vineyards, as well as poor yield and quality of wine products. In recent years, the industry has experienced outbreaks of viral diseases across the province, resulting in severe and even total crop losses. The ultimate and most effective way to fight against viruses is the broad use of clean propagating stocks. However, little is known about the prevalence of viruses and viral diseases in Ontario. Since 2015, we have conducted large-scale surveys for major viruses in commercial wine grapes as well as in other types of grapes in order to obtain a comprehensive understanding of the prevalence and severity of viral diseases in Ontario. Over 657 composite grape leaf samples were collected from 3,285 vines in the 137 vine blocks of 33 vineyards from the three primary appellations: Niagara peninsula, Lake Erie North Shore and Prince Edward County. These samples covered 5 major red grape varieties (Cabernet Franc, Cabernet Sauvignon, Pinot noir, Merlot, Syrah) and 5 major white grape varieties (Chardonnay, Riesling, Pinot gris, Sauvignon blanc, Gewürztraminer). We have developed and used multiplex RT-PCR for the simultaneous detection of multiple viruses in a single assay. Composite sampling and the use of multiplex assays result in substantial time and cost-savings. We tested for 17 major viruses including all viruses involved in grapevine leafroll (GLRaV-1, 2, 3, 4, 7), Grapevine red blotch virus (GRBV), Grapevine Pinot gris virus (GPGV), Grapevine fleck virus (GFkV), Grapevine rupestris stem sitting-associated virus (GRSPaV), Grapevine virus A (GVA), Grapevine virus B (GVB), Arabis mosaic virus (ArMV), Tomato ringspot virus (TomRSV), Grapevine fanleaf virus (GFLV), among others. 14 of them have been detected, which include GLRaV-3, GLRaV-1, GLRaV-2, GRBaV, GPGV, GVA, GVB, GRSPaV and GFkV and their incidence were 47.9, 2.1, 4.4, 18.3, 21.6, 6.2, 3.0, 84.0 and 21.8% , respectively. Mixed infections with multiple viruses are common. 95.6% of the samples tested were infected with at least one virus; 67% of the samples with 2-4 viruses and 4.7% of the samples with 5-6 viruses. The major grape cultivars tested were all susceptible to the infection of GLRaV-3, GRBaV, GPGV, GRSPaV and GFkV. The results also suggested that planting of infected materials is one of the most important factors responsible for the recent outbreaks of viral diseases across the province. Findings from this survey provides a baseline for the grape and wine industry in developing strategies for managing grapevine viral diseases in Ontario vineyards. ACKNOWLEDGEMENTS: This project was funded by OMAFRA-UoG Partnerships EM program. We thank the wineries and grape growers who have participated in this project. We also thank R. Monteith, O. Thompson, S. Kim, G. Lees, J. Easlick, J. Goltz, S. Thomas, A. Kaur, V. Turon and L. Ishaky for technical help.

112 - Identification and Characterization of Immunodominant MHC Class I-restricted Epitopes of Oncolytic Reovirus

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Background: Reovirus, a benign human pathogen, selectively targets and kills cancer cells by direct oncolysis and induction of anti-tumor immunity. Unfortunately, anti-viral immune responses eliminate reovirus from the host before it can completely fulfill its anti-cancer effects. Thus, measuring the magnitude and specificity of anti-reoviral T cell responses is critical for understanding the generation and regulation of adaptive immunity during reovirus-based anti-cancer therapies. Methods: Computerized algorithms (SYFPEITHI) were used to predict MHC class I-restricted 8- or 9-mer epitope sequences. A subset of epitopes was synthesized and tested for the induction of CD8+ T cell interferon-gamma responses by intracellular cytokine staining using splenocytes harvested from reovirus-infected C57BL/6 mice and stimulated in vitro with the individual peptides. A panel of MHC class I tetramers loaded with the identified immunodominant peptides were generated and used to stain reovirus-specific CD8+ T cells by flow cytometry. Results: Based on the SYFPEITHI-predicted MHC binding affinity, we identified >1,500 MHC class I-restricted (H-2Kb, H-2Db) epitopes found within the 12-13 reoviral proteins. We synthesized 118 peptides with the highest MHC binding affinity, and observed that only 20% of the peptides were capable of inducing T cell responses. Interestingly, MHC affinity did not always correlate with immunogenicity, and majority of the repertoire of anti-viral CD8+ T cell response was directed against 8-10 peptides in a hierarchical fashion. Conclusion: The identification of the immunodominant epitope of reovirus provides a promising candidate that can be used for the selective inhibition of anti-viral immunity, thereby enhancing the efficacy of reovirus-based anti-cancer therapy.

113 - Influenza burden of disease and preliminary 2016/17 end-of-season influenza vaccine effectiveness estimates for preventing influenza-associated hospitalization among Canadian adults: an analysis from the Canadian immunization research network (CIRN) serious outcomes surveillance (SOS) network

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Background: The influenza virus contributes to thousands of hospitalizations and influenza-related serious outcomes in Canada each season. To inform public health decision making around influenza prevention and treatment, ongoing surveillance of the influenza burden of disease and assessment of influenza vaccine effectiveness (VE) is critical. The Canadian Immunization Research Network (CIRN) Serious Outcomes Surveillance (SOS) Network conducts active surveillance each influenza season to characterize the burden of influenza disease in Canada and to provide estimates of influenza VE to prevent influenza-related hospitalization in adults. Methods: The CIRN SOS Network conducted active surveillance for influenza infection in adults (≥16 years of age (y)) beginning on November 15th, 2016 and ending April 30th, 2017 at 13 adult academic and community hospital sites in four provinces (Ontario, Quebec, New Brunswick, Nova Scotia). Patients with an admitting diagnosis of community acquired pneumonia (CAP), exacerbation of chronic obstructive pulmonary disease (COPD)/asthma, unexplained sepsis, any respiratory diagnosis or symptom were eligible for enrolment. Nasopharyngeal swabs obtained from eligible patients were tested for influenza by polymerase chain reaction (PCR). Patients who tested positive for influenza were considered cases and enrolled; patients who tested negative for influenza were eligible to become controls. Detailed demographic and medical information, course of illness details, and outcomes were obtained from the patient's medical record. To calculate influenza VE, influenza positive cases were matched to influenza-test negative controls on admission date (within 14 days of date of admission), age stratum (≥65y or <65y) and site of enrolment. Using conditional logistic regression, overall influenza VE and influenza A VE was estimated as 1- odds ratio (OR) of influenza in vaccinated vs. unvaccinated patients x 100%, with corresponding 95% confidence intervals (CIs) for all patients, and patients ≥65y and <65y. Results: During the 2016/2017 influenza season there were 1431 influenza cases enrolled in the SOS Network. The majority of these cases were influenza A (n=1299), and among subtype-known influenza A cases, 100% (n=546) were influenza A/H3N2. Among influenza positive cases, 144 patients were admitted to the intensive care unit (ICU) and 91 patients died within 30d of discharge. Overall influenza VE for prevention of influenza-related hospitalization in all ages was 29.8% (95% CI: 12.0-43.9%), with slightly lower VE observed in patients ≥65y (VE: 21.3%; 95% CI: -1.1-38.8%) and higher VE observed in patients <65y (VE: 55.6%; 95% CI: 25.2-73.5). Influenza VE estimates for prevention of influenza A hospitalization were similar to overall VE estimates. Conclusions: The 2016/2017 influenza season was characterized by dominant circulation of influenza A/H3N2. A moderately effective influenza VE of 30% was demonstrated for the prevention of influenza-related hospitalization. Given the substantial burden of disease caused by influenza and the modest influenza VE observed, continued assessment of influenza VE is crucial to inform immunization policy in Canada, and to emphasize the importance of the development and utilization of improved influenza vaccines.

114 - A genetic approach to investigate the intrinsic antiviral properties of stress granules

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Protein synthesis is a tightly regulated and energy-intensive process. In response to diverse environmental stresses, including the stress of viral infection, sentinel kinases phosphorylate eukaryotic initiation factor 2-alpha (eIF2a) and prevent the formation of the eukaryotic initiation factor 2 (eIF2)–GTP–Met-tRNA^{Meti} ternary complex required for translation initiation. Stalled messenger ribonucleoprotein (mRNP) complexes are bound by aggregation-prone RNA-binding proteins that drive the formation of cytoplasmic stress granules (SGs). All viruses require host protein synthesis machinery to decode viral mRNAs, and diverse viruses have been shown to regulate SG formation, with SG inhibition being the predominant outcome. In many cases, viral suppression of SG formation has been shown to act downstream of stress-induced translation arrest, suggesting that SGs may have independent antiviral properties that reinforce the antiviral effects of protein synthesis arrest. Moreover, the observation of SG-localized pathogen recognition receptors (PRRs) has prompted some to suggest that SGs aid antiviral responses by providing a platform for detection of viral patterns. This hypothesis remains unproven. To uncouple the effects of SG formation from protein synthesis arrest, we created lentiviruses bearing doxycycline (dox)-inducible red fluorescent protein (RFP) fused to a 40 amino-acid fragment of the ubiquitin specific peptidase 10 (USP10) fusion protein. This RFP-USP10 fusion construct had previously been shown to act as a dominant negative protein that inhibits SG aggregation irrespective of protein synthesis arrest and without affecting levels of endogenous SG-nucleating proteins. U2OS osteosarcoma cells were transduced with lentiviruses and treated with dox to induce RFP-USP10 expression. Treatment of uninduced control cells with 500 mM sodium arsenite caused the rapid formation of SGs as expected, whereas the RFP-positive dox-treated cells failed to form SGs in response to treatment. This inducible cell line will be used to complement existing approaches to investigate potential antiviral properties of SGs against a wide variety of viruses.

115 - HIV latency is controlled by a novel histone deacetylase complex

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Human immunodeficiency virus (HIV) latency is a major obstacle for the development of an HIV cure or vaccine. The therapeutic control of HIV latency is therefore an important biomedical goal. Viral transcription is a rate-limiting step in the control of latency and occurs via the hijacking of the host cell RNA polymerase II (Pol II) machinery by the 5' HIV LTR. At the heart of the LTR of HIV lies a particularly crucial DNA region termed the core promoter that is specifically required for the reactivation of HIV by the viral trans-activating protein Tat. Tat controls HIV latency as part of a positive feed-forward loop that strongly activates HIV transcription. The recognition of the core promoter by host cell pre-initiation complexes of HIV (PICH) has been shown to be necessary for Tat trans-activation, yet the protein composition of PICH has remained obscure. We employed DNA-affinity chromatography to identify a new deacetylase complex as a component of PICH that selectively recognize the HIV core promoter. Our data reveal three new PICH proteins that act in a complex to reactivate latent HIV including one that directly recognizes the HIV core promoter, one that regulates chromatin, and a third that binds directly to the HIV Tat protein. Our data fill a significant and long-standing mechanistic gap in the understanding of latency and identify potential new drug targets for HIV cure strategies.

116 - Investigation of Influenza A Virus Nucleoprotein-Mediated Stress Granule Inhibition via Proximity-Labeling Proteomics

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Introduction: Each segment of the influenza A virus (IAV) genome is associated with many copies of the viral nucleoprotein (NP). During replication, NP association is essential for synthesis of new viral genomes. Recently, we discovered that IAV NP also participates in suppression of host innate immune responses by inhibiting the formation of cytoplasmic mRNP aggregates known as stress granules (SGs). SG inhibition requires NP oligomerization, but RNA-binding and nuclear localization are dispensable. We hypothesize that the interaction of oligomeric NP with specific host proteins is required for SG suppression. Methods: A proximity-labeling proteomic method known as BioID was used to identify host NP-interacting proteins. The BirA* biotin ligase enzyme was fused to NP proteins predicted to inhibit SGs (wild type (WT), nuclear localization mutant, RNA-binding mutant), as well as an oligomerization mutant protein that should not inhibit SGs. Effects of BirA* tag on subcellular localization and SG inhibition by NP was verified by transfection into either HEK293A or U2OS cells and immunofluorescence microscopy. Results: As predicted, the oligomerization mutant NP protein fused to BirA* failed to suppress SG formation, whereas other BirA*-NP fusion proteins suppressed SG formation. Biotinylated host proteins largely accumulated in the same cellular compartments as transfected BirA* fusion proteins, except that proteins labeled by proximity to the cytoplasmically-restricted oligomerization mutant NP fusion protein accumulated in the nucleus. Distinct biotinylated host proteins were observed by immunoblotting. Conclusions: BioID is a powerful method to discover transient and stable protein-protein interactions. This work sets the stage for identification of host proteins that selectively associate with NP isoforms that can suppress SG formation using mass spectrometry.

118 - Establishment of a novel animal model for a human tumor virus, Epstein-Barr virus

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More than 90% of the world's population is infected with Epstein-Barr Virus (EBV), a member of the gamma herpesvirus subfamily. EBV is the cause of 2% of all neoplasms worldwide, and responsible for the development of infectious mononucleosis also known as glandular fever, a disease affecting mainly young adults. Despite 50 years of research, there is no preventive measure available against EBV infection and EBV-cancer formation in humans. This deficiency can be attributed to the lack of a suitable animal model for studying EBV associated immune responses and disease manifestation. We intend to establish a novel pig model, in which transmission, prevention, immune response and treatment of a porcine virus, very similar to EBV, will be studied. This approach opens new dimensions for tackling EBV. Porcine lymphotropic herpesvirus (PLHV) is a virus that is closely related to human EBV. In pigs, PLHV causes a proliferative disease of lymphocytes that resembles EBV-lymphoma in EBV-infected humans. We started to characterize the novel pig virus, and identified several pig tissues and a porcine tumor cell line to be infected with PLHV. Based on previous data from our and other research groups, we hypothesize that PLHV is capable of infecting pigs, establishing an infection, inducing specific immunity, and cause cancer of white blood cells, like EBV. Before testing this hypothesis in vivo, we began to characterize the porcine gamma herpesvirus in vitro. Next generation sequencing is currently being employed to gather full sequence information on PLHV, and we added new information on 40000bp of PHLV genome. We are currently establishing a reverse genetic system for PLHV. A major goal of our proposed research is the manipulation of the viral genome by exchanging porcine gamma herpesvirus genes against homologous EBV genes. The chimeric viruses will be used in immunological and pathogenesis studies in pigs. Around 200.000 cases of EBV-associated malignancies are reported every year worldwide. Our work utilizes a new strategy for gathering valuable knowledge on EBV infection and disease manifestation. This will aid in the development of an EBV vaccine and/or antiviral therapy, and thus, having a substantial public health and economic impact.

119 - A VSV-Measles Chimeric Vrus Can Target Nectin-4 Positive Breast Cancer Tumours In An Immune Competent Mouse and Is Enhanced By Transient Inhibition of Anti-Viral Innate Immunity

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Our laboratory previously discovered that the tumor marker Nectin 4 (PVRL4) is the epithelial cell receptor for measles virus, and is expressed on many adenocarcinomas. Using a breast cancer cohort and analysis of tissue from 176 patients, we showed that Nectin 4 is upregulated on metastatic breast tumors. Breast cancer cells expressing Nectin 4 can be infected with measles virus or a vesicular stomatitis virus (VSV) hybrid virus containing the H and F proteins of measles virus. Tumors can be readily infected with measles and VSV-measles viruses that express either green fluorescent protein (eGFP) or firefly luciferase (Luc) reporter genes. Histological analysis of MD468 or patient derived triple negative tumors showed that they expressed eGFP reporter and viral proteins. Although the viruses replicate in the tumors engrafted onto immune deficient mice, the infectious agents were not oncolytic and only slowed tumor progression. To test the contribution of the host immune system, mouse 4T1 and 4T1-Nectin 4 tumors were produced in immune competent BALB/C mice. The highly conserved Nectin-4 protein increased growth and metastasis to produce aggressive 300 cubic mm mouse breast tumors within 6 days. 4T1 and 4T1-Nectin 4 tumors on xenographic (NIH III nude) and syngeneic (BALB/C) mice were injected with measles and VSV-measles viruses and monitored over 24 days. The growth of 4T1-Nectin 4 tumors in the immune competent BALB/C mice was slowed by intra-tumoral injection of measles virus or VSV-measles chimeric virus. Tumor regression with the VSV-measles chimeric virus was further enhanced using either BX795 or vanadate chemical inhibitors, which transiently inhibited anti-viral innate immunity. Infection of tumors was documented using confocal immune microscopy. We concluded that the anti-tumor properties of measles and VSV-chimeric viruses are dependent on both the innate and adaptive host immune systems.

120 - Phylodynamic insight into HIV epidemic dynamics within Canada

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Background: HIV is one of the most devastating infectious diseases to impact Canada in human history, with an estimated 75,500 people living with HIV in Canada. However, despite the national importance of HIV, the early transmission, geographic dissemination, and dynamics of the virus within populations and aggregate risk factors across Canada remain unclear. Epidemiological processes such as geographic spread and population growth stamp a measureable signature on HIV-1 genomes sampled from infected individuals at different places and times. Using statistical phylodynamic approaches applied to HIV-1 sequence data sampled from across Canada we test hypotheses concerning past and present HIV epidemic dynamics. Methods: We compiled a dataset of 51,493 doubly-anonymized HIV protease and reverse transcriptase sequences sampled from more than 20,000 patients annotated with clinical and socio-demographic parameters. Data were available from 6 Canadian provinces: British Columbia, Alberta, Saskatchewan, Ontario, and a limited amount from Quebec. To mitigate the influence of therapeutic interventions we removed codons associated with known drug resistance from the sequences prior to analysis. We performed phylogenetic clustering and diversification rate analyses on the entire "Canada-wide" dataset. Bayesian phylogeographic analyses utilized reduced datasets, down sampled from the full dataset, to avoid potential bias in spatial inference estimates that arise from over sampling one particular location over others. All datasets were analyzed using general time-reversible nucleotide substitution models specifying a gamma distribution as a prior on each substitution rate. We employed an uncorrelated lognormal relaxed molecular clock in order to infer the timescale of HIV evolution while accommodating among lineage rate variation. To reconstruct the spatial dynamics of HIV-1 we employed a Bayesian discrete phylogeographic approach using Markov chain Monte Carlo sampling. Results: We observed substantial variation among provinces in the proportion of non-subtype B infections ($P<0.05$) with the Prairie Provinces having relatively greater numbers of non-B infections. Phylogenetic clustering occurs predominantly within provinces through local transmission. Most provinces contain large province specific clusters dominated by transmission through intravenous drug use. Some between province clustering is observed dominated by transmission amongst men who have sex with men. Consistent with other sources of evidence Saskatchewan and Manitoba are the provinces observed to have the highest rates of HIV diversification in our dataset. We also observe variation in timing of the geographic spread of HIV within Canada with the Prairie Provinces experiencing later periods of epidemic ignition and growth. Conclusion: Secondary analysis of genotypic resistance data provides useful epidemiological inferences on a national scale. Different combinations of circumstances have permitted establishment, spatial dissemination, and epidemic growth of the HIV epidemic in Canada at different times within component subpopulations. Our results emphasize the varied challenges facing different regions of Canada in controlling the HIV epidemic going forward. Similar patterns may underlie past emergence of other blood-borne pathogens, particularly HCV.

121 - Cell-mediated immune responses directed against Zika virus (ZIKV) envelope peptides in a cohort of ZIKV-exposed women.

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Background: ZIKV infection during pregnancy has been associated with a dramatic increase in the incidence of cases of congenital microcephaly in Brazil since 2015. Little is known about cell-mediated immune responses to ZIKV, particularly in pregnant women. The aim of this study was to assess ZIKV-specific cell-mediated immunity in pregnant women who were exposed to the virus. Methods: Pregnant women suspected of ZIKV infection according to WHO criteria were enrolled at CHU Sainte-Justine (n=12). ZIKV infection was confirmed by serology and PCR. Screening for other endemic flaviviruses (e.g. Dengue) was also performed. Peripheral blood mononuclear cells (PBMC) were obtained at the time of enrolment. Newborns were screened for ZIKV infection by PCR in blood, CSF, urine, and throat. IFN-gamma ELISpot was performed on PBMC and cord blood mononuclear cells (CBMC) using pools of synthetic peptides based on the sequence of ZIKV core (C), matrix (M), envelope (E), and non-structural-1 (NS1) proteins. Results were expressed as spot-forming units (SFU) per 10^6 cells. Results: Three of 12 women (25%) were not infected. Eight of 12 (75%) exhibited no symptoms. ZIKV-specific IFN-g responses were higher in ZIKV-infected women as compared to uninfected control subjects ($p<0.05$). Responses directed against E protein peptides were the largest and most frequently observed. No reactivity was seen in CBMC (n=3) despite the presence of one confirmed case of mother-to-child ZIKV transmission (positive PCR in CSF). No impairment in growth and neurological development was observed in this child. Conclusion: To our knowledge, this report is the first description of cell-mediated immune responses against ZIKV in pregnant women. These results could improve our understanding of ZIKV-specific antiviral immunity and inform vaccine design.

122 - Quasispecies diversity and neutralizing antibody responses in vertical HCV transmission.

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Background: Vertical transmission is the major cause of pediatric HCV infection worldwide. To better understand the pathogenesis of hepatitis C during and after pregnancy and to provide insights into the risk factors and mechanisms involved in vertical HCV transmission, the evolutionary dynamics of HCV variant spectra and HCV-specific neutralizing antibody responses were examined using high throughput sequencing and pseudoparticle-based assays in pregnant women monoinfected with HCV (n = 17) or coinfected with HCV and HIV-1 (n = 15). Results: Overall, statistically significant associations were found between HCV quasispecies diversity, selective pressure exerted on the HCV E2 envelope protein, and neutralizing activity of maternal immunoglobulins. Women with low quasispecies diversity displayed significantly higher mean AST and ALT levels throughout pregnancy, but this difference was restricted to monoinfected participants. Low quasispecies diversity and inefficient neutralizing activity were also significantly associated with vertical transmission, but only in the monoinfected group. Discussion: These results indicate that maternal neutralizing antibody responses play an important role in the prevention of vertical HCV transmission, but not in presence of coinfection with HIV-1, and suggest that the mode/route of vertical HCV transmission differ between monoinfected and coinfected women. These findings could inform clinical management strategies for prevention of vertical HCV transmission.

125 - High Content Screening Implicates the Pi3k isoform p110α in FAST Protein-Mediated Cell-to-Cell Fusion.

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The reovirus fusion-associated small transmembrane (FAST) proteins are the smallest known viral membrane fusion proteins. Unlike enveloped virus fusion proteins, FAST proteins evolved specifically to mediate cell-cell fusion, not virus-cell fusion. As such, they represent a simple model system to dissect host cell machinery necessary for cell fusion. High-content siRNA and small molecule screens of FAST protein-mediated cellular fusion, quantified using an automated fluorescence microscopy assay, were employed to identify endogenous proteins and pathways involved in syncytium formation. In this fashion, phosphoinositide 3-kinase (PI3K) p110α subunit inhibitor Pik-75 and AKT kinase inhibitor A-443654 were identified as agents that dramatically inhibited syncytiogenesis. The necessity and isoform specificity of the p110apha/AKT signaling pathway to FAST protein-mediated cell fusion was validated using isoform-specific shRNAs, a transgenic $AKT1^{-/-}/AKT2^{-/-}$ knockout cell line, and pharmacologic inhibition using PI3K isoform-specific inhibitors. Interestingly, Pik-75 and A-443654 also inhibited syncytiogenesis mediated by the measles virus F+H fusion protein complex, implying p110α/AKT involvement in a common cell-cell fusion mechanism. Fluorescence videomicroscopy revealed colocalization of AKT1-PH-GFP (a biosensor of phosphatidylinositol 3,4,5-phosphate) and the reptilian reovirus p14 FAST protein at areas of cell-to-cell contact, and this colocalization increased immediately prior to the formation of a cell-cell fusion synapse. Treatment with Pik-75 disrupted p14/AKT-PH-GFP colocalization and altered actin dynamics at areas of cell-to-cell contact resulting in a failure to form a cell fusion synapse. These studies identify the PI3K-AKT pathway as critical player involved in virus-mediated cell-cell fusion.

126 - TH2 Cytokines Modulate Human Mast Cell Responses to Viruses

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Introduction: Mast cells are resident in mucosal and epithelial tissues, where they serve as sentinel cells against parasitic and bacterial infections. Mast cells can also respond to multiple viruses, including respiratory viruses such as influenza A virus (IAV), reovirus, and respiratory syncytial virus through the selective production of pro-inflammatory cytokines. Allergic asthma is associated with a Th2 driven inflammation and increased susceptibility to viral infections which are the main triggers of asthma exacerbations. Interferons (IFNs) have a key role in controlling virus replication and their production has been reported to be lower in asthmatic compared to healthy subjects. Th2 cytokines may be involved in this difference in response. Mast cells have the ability to respond to Th2 cytokines and are an important source of IFNs. The objective of this work is to analyze whether mast cell IFN responses to reovirus and IAV are impaired in the presence of Th2 cytokines. Methods: Cord blood-derived human mast cells (CBMC) were cultured in medium alone or stimulated with IL-4 (10 ng/ml), IL-5 (10 ng/ml), IL-9 (10 ng/ml), IL-13 (10 ng/ml), and IL-33 (1 ng/ml) for 48 h followed by infection with reovirus type 3 Dearing (5 MOI) or IAV (H1N1 A/CA/07/2009, 5 MOI). Supernatants were harvested 24 h p.i. for ELISA analysis. mRNA gene expression was analyzed by qPCR. Results: IFN production by reovirus infected CBMC (Reo-CBMC) was not impaired by pretreatment with Th2 cytokines. Strikingly, IL-4 selectively enhanced the production of type I and type III IFNs. Although IL-4 and IL-13 share a common receptor, these responses were specific for IL-4. Reo-CBMC stimulated with IL-4 only concurrently with viral infection did not enhance IFN production. In addition, IL-4 pretreated CBMC showed an increased production of IL-6, CCL-3, and CXCL10 in response to reovirus. Similar to Reo-CBMC, IL-4 pretreated CBMC showed an enhanced IFN production in response to IAV. Conclusions: Our data suggest that IL-4 modifies mast cell IFN responses when administered prior to reovirus or IAV infection. IL-4 actions on mast cells are likely to be induced through type I IL-4 receptors. We suggest that mast cells might be of importance in the control of viral infection in asthmatics by IL-4-enhanced production of IFNs, which are critical for viral clearance.

127 - Enteroviral Infection Inhibits Autophagic Flux by Disrupting SNARE Complex Formation and through Targeting Autophagy Receptors

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Background: Our recent evidence suggests that enteroviral infection is a putative causal/risk factor in the development of sporadic ALS (>90% of all cases). Enteroviruses, including poliovirus, coxsackievirus, EV-A71 and EV-D68, can target motor neurons and cause polio or polio-like flaccid paralysis. Analogous to other neurodegenerative diseases, ALS is characterized by the accumulation of misfolded proteins. We recently showed that EV-induced pathology resembles that of ALS, including TDP-43 proteinopathy and aberrant accumulation of misfolded/ubiquitinated proteins. However, the molecular mechanisms involved are unclear. Autophagy is a fundamental cellular process by which misfolded proteins/damaged organelles and invading microbes are sequestered within an autophagosome and degraded following fusion with lysosomes. Disruption of the autophagic pathway has been suggested to play key roles in neurodegeneration. We hypothesize that enterovirus infection contributes to ALS-like pathology by targeting autophagy receptors and fusion adaptor proteins, leading to enhanced viral growth and impaired clearance of protein aggregates/damaged organelles. Methods/Results: To delineate the possible mechanism involved, we focused on proteins previously reported to be involved in autophagosome fusion. Notably, we found that the autophagosomal SNARE protein SNAP29 and the tethering protein PLEKHM1, two critical proteins known to regulate autophagosome-lysosome fusion, were cleaved upon coxsackievirus B3 (CVB3) infection. Further in vivo (in cells transfected with protease constructs) and in vitro (using recombinant proteases) cleavage assays demonstrated that CVB3-encoded proteinase $3C^{pro}$, not $2A^{pro}$ or caspases, is responsible for these cleavages. We identified the cleavage sites on SNAP29 (Q161) and PLEKHM1 (Q668), respectively, leading to impaired SNARE complex formation. Moreover, we showed that gene-silencing of SNAP29/PLEKHM1 inhibited autophagic flux, resulting in a significant increase in viral growth, likely due to enhanced accumulation of autophagosomes that provide sites for viral RNA replication and assembly. Finally, we identified the xenophagic receptors NDP52 and TAX1BP1 as bona fide substrates of viral proteinases, complementing our previous finding that p62 and NBR1 are also targeted. Conclusion: EV infection inhibits cargo recruitment and autophagic flux by viral proteinase-mediated cleavage of autophagy receptors (p62, NBR1, NDP52, TAX1BP1) and fusion adaptor proteins (SNAP29 and PLEKHM1). Blockage of autophagic flux results in accumulation of misfolded protein and enhanced viral growth to promote ALS pathogenesis. Future studies will examine the significance of disrupting the virus –autophagy interface as a potential therapeutic strategy.

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