23rd Annual Infectious Diseases Research Day & 10th Annual Canadian Center for Vaccinology Symposium

DALHOUSIE UNIVERSITY
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Canadian Center for Vaccinology
Dalhousie University
IWK Health Centre
NS Health Authority

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Dalhousie Divisions of Infectious Diseases
of the Departments of Pediatrics and Medicine

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In keeping with CMA Guidelines, program content and selection of speakers are the responsibility of the planning committee. Support is directed toward the costs of the course and not to individual speakers through an unrestricted educational grant.

Thank you!

This program is supported in part by contributions-provided by:

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Glenn Patriquin
Natasha Squires
Karina Top
Allison Young

Welcome to the 23rd Annual Infectious Diseases Research Day and 10th Annual CCfV Symposium!

Lisa Barrett, MD, PhD, FRCPC
Division of Infectious Diseases, Department of Medicine, Department of Microbiology & Immunology, Department of Pathology

The Infectious Diseases Research Day and CCfV Symposium is a unique learning opportunity featuring experienced presenters and research trainees, and I am pleased to announce that the program for the 2018 Research Day features more presentations and posters than ever before.

We are excited to offer a variety of presentations and posters from national and local experts, addressing a range of topics from bench research to policy and programs.

Feedback and evaluation is extremely important, and you are the key to that process!! You will receive an email inviting you to take our post event survey and we urge you to give us your feedback so we can continue to improve this learning event.

Scott Halperin MD, FRCPC
Director
Canadian Center for Vaccinology

Last year we condensed the symposium into one full day, which made for a more streamlined and productive event. Due to last year's success, we have decided to extend the day by an hour and add a networking event with wine and cheese, giving research trainees and established researchers the chance to create new connections.

One of the great aspects of this event is that it gives researchers at different stages in their careers the opportunity to learn about the work of their colleagues, and I encourage everyone to make the most of this educational experience.

This is the 10th Annual CCfV Symposium, and it would not be possible without the hard work of our planning committee and the financial support from our corporate sponsors. I offer my sincere thanks to both for their continued commitment to this event.

Program

23rd Annual Infectious Diseases Research Day &
10th Annual Canadian Center for Vaccinology Symposium

Tuesday April 24, 2018

8:00-9:00am	TJ Marrie Lecture- Marcel Behr, *Genomic epidemiology of a TB outbreak (we need a TB vaccine)"*	Halifax Infirmary RB Theatre
9:30–12:30pm	Oral Presentations (10)	Dalhousie University McInnes Room
12:30–1:15pm	Lunch	
1:00–2:00pm	Poster judging (posters on display 1:00 – 5:30)	Dalhousie University McInnes Room Foyer
2:00–3:00pm	Presentation – Jeff Kwong, *Vaccine Research Using Large Linkable Databases*	Dalhousie University McInnes Room
3:00-3:15	Nutrition Break	
3:15–3:45pm	Presentation – David Kelvin, *China, Chickens and the Emergence of Pandemic Influenza Viruses*	Dalhousie University McInnes Room
3:45-4:15pm	Presentation – Jennifer Isenor, *Pharmacists as Immunizers "Improve ACCESS"*	Dalhousie University McInnes Room
4:15-4:30pm	Awards Presentations	Dalhousie University McInnes Room
4:30-5:30pm	Networking/Mentoring Wine and Cheese	Dalhousie University McInnes Room

Educationally co-sponsored by Dalhousie University Continuing Professional Development. This program is supported by educational grants from Sanofi Pasteur, Merck, Pfizer, Astellas, and Gilead

Speakers

Dr. Marcel Behr

Dr. Marcel Behr is Professor of Medicine at McGill University where he is Director of the McGill International TB Centre and Microbiologist-in-Chief of the McGill University Health Centre. His training included BSc (Biochemistry) from the University of Toronto, MD from Queen's University, Residency training in Infectious Diseases and Medical Microbiology at McGill, an MSc (Epidemiology) from McGill and then post-doctoral studies of Molecular Epidemiology and Bacterial Genomics at Stanford.

Dr. Behr's research interest is the application of bacterial genetics to study the epidemiology and pathogenesis of mycobacterial diseases, specifically, M. tuberculosis, the cause of TB, BCG, the vaccine used against TB, and non-tuberculous mycobacteria, including members of the M. avium-intracellular complex. This work has been recognized by numerous awards, in Quebec (Chercheur National of the Fonds de la Recherche en Sante du Quebec), Canada (Fellow of the Canadian Academy of Health Sciences, 2016, Fellow of the Royal Society of Canada 2017) and beyond (Election to the American Society for Clinical Investigation, 2010). The work of Dr. Behr is funded by a Foundation grant from the Canadian Institute for Health Research.

Dr. Jeff Kwong

Dr. Jeff Kwong is a senior scientist at the Institute for Clinical Evaluative Sciences (ICES), and at Public Health Ontario.

Dr. Kwong is also a family physician at the Toronto Western Family Health Team, and an Associate Professor in the Department of Family and Community Medicine and the Dalla Lana School of Public Health at the University of Toronto. His research interests include infectious diseases epidemiology and health services research using linkable data, influenza vaccine and vaccination program evaluation, and assessing the burden of infectious diseases.

Dr. David Kelvin

Dr. David Kelvin is known worldwide for his contributions to chemokine biology and host responses to infectious pathogens. He is a Professor in the Department of Microbiology and Immunology at Dalhousie University and is also a Scientific Director at the International Institute of Infection and Immunity Research, Shantou University Medical College, Shantou, China. His appointment in China allows him to integrate his research in the global problems of infectious disease giving a rich source of valuable clinical specimens and circulating viruses. Dr. Kelvin's research program is focused on understanding the pathogenic process and therapeutic intervention of emerging and re-emerging viral infection using immunological, virological and bioinformatic techniques.

Dr. Jennifer Isenor

Dr. Jennifer Isenor is an Assistant Professor at the College of Pharmacy and the Faculty of Medicine, Dalhousie University. She is an investigator with the Canadian Center for Vaccinology and a founding member of the Pharmacists as Immunizers (PAI) Research Team. Her research focuses on the role of pharmacists as immunizers.

Poster Presentations

(Presenter's name in **bold)**

Oral Presentations

(Presenter's name in **bold)**

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9:45-10:00	**S Davis**, R Warren, K Miller, J Moore, S Oldford, Y Lynch-Hill, I Davis, L Johnston, L Barrett. HCV CURE AND DEMOGRAPHIC PATTERNS OF HCV INFECTED INDIVIDUALS IN NOVA SCOTIA IN THE PRE- AND EARLY DAA ERA	41	57
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10:15-10:30	**M Jalink**, J Langley, L Dodds, P Andreou. RESPIRATORY SYNCYTIAL VIRUS (RSV) INFECTION IN PRETERM INFANTS AND LATER ONSET OF ASTHMA	43	60
10:30-10:45	**A Smith**, J McNair, J Bettinger, W Vaudry, OG Vanderkooi, S Desai, S Halperin, K Top. IMPACT OF PNEUMOCCOCAL CONJUGATE VACCINATION PROGRAMS ON *STREPTOCCOCUS PNEUMONIAE* DISEASE IN CHILDREN WITH CANCER: A REPORT FROM THE CANADIAN IMMUNIZATION MONITORING PROGRAM ACTIVE	44	61
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11:15-11:30	**P Slaine**, C MacRae, M Kleer, E Lamoureux, SMcAlpine, M Warhuus, A Comeau, C McCormick, T Hatchette, D Khaperskyy. IDENTIFICATION AND CHARACTERIZATION OF MUTATIONS RESPONSIBLE FOR MOUSE ADAPTATION OF INFLUENZA A VIRUS A/California/07/2009(H1N1)	46	65
11:30-11:45	**AM Di Castri**, H Ghandora, DM Halperin, J Isenor, BA Taylor, SA Halperin.KNOWLEDGE, ATTITUDES, BEHAVIOURS, AND BELIEFS OF HEALTHCARE PROVIDER STUDENTS REGARDING MANDATORY INFLUENZA VACCINATION	47	66
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12:00-12:15	**L Li**, S Halperin, S Lee. COMBATING STREP INFECTIONS: CHARACTERIZATION OF EXOTOXIN FOLDING PATHWAYS IN GROUP A STREPTOCOCCUS BACTERIUM	49	69
12:15	**LUNCH**		

Abstracts

(Presenter's name in **bold)**

1. CANADIAN OLDER ADULTS' EXPERIENCE AND KNOWLEDGE REGARDING INFLUENZA ILLNESS AND VACCINATION

Authors: **MK Andrew,** V Gilca, N Waite, JA Pereira

Affiliation: Dalhousie University Department of Medicine (Geriatrics), CCfV, Institut national de santé publique du Québec, School of Pharmacy, University of Waterloo, JRL Research & Consulting Inc.

Introduction: Older adults are at high risk for influenza-related complications, resulting in worsening frailty and function. We surveyed Canadian seniors to explore the impact of influenza and assess knowledge about this illness and its prevention.

Methods: A survey of Canadian adults aged 65+ was conducted through an online market research panel platform in March/April 2017. The survey included questions about the respondents' experiences during the 2016/17 influenza season, specifically, influenza vaccination practices and knowledge about influenza. Respondents were also asked to report their frailty and functional status prior to the season, during illness (if applicable), and following the season, using validated measures.

Results: 5014 older adults completed the survey; mean age 71.3 ± 5.17 years, 50% female, 42.6% had one or more chronic conditions, 7.8% vulnerable and 1.8% frail. 67.9% reported receiving last season's influenza vaccine. Those who rarely/never receive the influenza vaccine were less likely to correctly answer questions about influenza than those who receive the vaccine more consistently. Of the 21.5% who reported experiencing influenza or influenza-like illness (ILI) last season, one-fifth had health and function declines during this time. 40% indicated a recovery longer than two weeks and 3.1% "never fully recovered". Older age, memory loss, and having influenza/ILI were among the independent predictors of persistent declines in health and function.

Conclusions: Older adults' knowledge about influenza and influenza vaccine positively correlates with vaccine uptake, and knowledge about influenza and its prevention by vaccination remain suboptimal. Functional declines and worsening frailty are common during illness, and for some these declines can be persistent.

2. KEYS TO SUCCESS AND CONTINUED CHALLENGES: COMPARISON OF THE STRUCTURED, STEPWISE DEVELOPMENT OF TWO PROVINCIAL RESEARCH AND EVALUATION IMBEDDED HCV ELIMINATION STRATEGIES IN ATLANTIC CANADA

Authors: **L Barrett**

Affiliation: Dalhousie University, Halifax, NS; Nova Scotia Health Authority, Halifax, NS; Canadian Centre for Vaccinology, Halifax, NS

Introduction: In many provinces, HCV care is fragmented with little province-wide coordination, and limited real time evaluation. With the advent of curative but high cost HCV medications, it is critical to develop and implement a comprehensive HCV model of care with innovative cost containment solutions and improved access to patient care in a publicly funded system. The goal of this study was to compare the development of HCV elimination strategies in two Atlantic provinces and identify common processes, successes, challenges, and implementation strategies.

Methods: The process of concept development and implementation was described for each province through discussion with governmental, community, private, and academic partners, as well as review of relevant policy, public and contracted documents. A hybrid effectiveness-implementation type I mixed methods study design will be used to evaluate program implementation

Results: PEI is in Phase 2 while Nova Scotia is just beginning Phase 1. Key opinion leaders identify a single Health Authority, involved community members, political will, and provision for structured research and program evaluation as key to successful strategy development in both provinces. Public Health leadership occurred earlier in Nova Scotia than PEI, and was seen as an important part of the early Nova Scotia plan. Early integration of the correctional system and harm reduction providers, as well as a significantly novel model for drug payment, are important to the PEI success. Phase 2 implementation was delayed in PEI through a lack of formal structure within the Health Authority. Both strategies have deferred a formal HCV screening plan or enhanced public media awareness campaign.

Conclusions: There are commonalities between the development of two provincial HCV strategies that highlight the need for a high degree of inter-departmental, as well as public private collaboration and investment for successful programing. While there are differences and unique needs in each group, public health involvement and the development of clear organizational structure is important to prevent programs from becoming stalled. These lessons will be used to guide and revise the programs in each province, and may provide some best practices for other similarly sized and structured provinces in Canada.

3. DEVELOPMENT OF AN EVIDENCE- BASED ANTIMICROBIAL STEWARDSHIP SMARTPHONE APPLICATION IN A TERTIARY ACADEMIC PEDIATRIC AND WOMEN'S HEALTH CENTRE

Authors: K Slayter, J Turple, **JL Comeau**, KA Top, JM Langley, T Mailman, SA Halperin

Affiliation: IWK Health Centre, Canadian Center for Vaccinology, Dalhousie University, Halifax, NS

Introduction: Smart phone use by medical professionals is ubiquitous. In a recent survey, > 90% of health care providers were interested in locally developed antimicrobial stewardship (AMS) and infectious diseases applications ("apps"). We describe the process by which our antimicrobial stewardship program (ASP) developed an app to provide guidance regarding empiric antimicrobial choice, and education about antimicrobials and pathogens, integrating local laboratory data. We also describe early app uptake.

Methods: The IWK Health Centre is a 271-bed tertiary care Pediatric and Women's health centre serving the Maritime Provinces in eastern Canada. Using the Spectrum Mobile Health platform, our ASP developed an app in consultation with pediatric and women's health clinical divisions. Through collaboration with the microbiology laboratory, the app was integrated with our laboratory information system (LIS) allowing real-time access to local antibiogram results.

The iPhone- and Android- compatible app was introduced to health care providers through presentations, hospital intranet, email, and word of mouth. Following the official launch, uptake was monitored both in number of app downloads and number of hits. Adherence to empiric treatment guidelines included in the app will be assessed utilizing our existing ASP prospective audit and feedback service.

Results: From December 2015 - March 2017, the ASP created content for the IWK AMS App. Three sections were developed. 1/ Syndromes: evidence-based empiric treatment guidelines for common syndromes. 2/ Antimicrobials: spectrum of activity, dosing regimens, drug monitoring, common usage, adverse effects, drug interactions and pharmacology. 3/ Pathogens: information on precautions, local susceptibilities through linkage with our recently developed virtual antibiogram, associated syndromes, and epidemiology.
In May 2017, the app was launched. Within the first 24 hours, it was downloaded 157 times and accessed 1193 times.

Conclusions: We describe the process and early uptake of a locally developed AMS app to complement our ASP, which includes a virtual antibiogram through interfacing with our LIS. This is the first AMS app available in a Pediatric and Women's Health Care Centre in Canada. Further analysis of the app's impact on antimicrobial usage is planned.

4. PCR-BASED DISCRIMINATION OF EMERGING STREPTOCOCCUS PNEUMONIAE SEROTYPES 22F AND 33F

Authors: **JJ LeBlanc [1,2]**, HD Gillis [1,2], WHB Demczuk[3], A Griffith[3], I Martin[3], M Warhuus[1], ALS Lang[1,2], M ElSherif [1,2], SA McNeil[1,2]

Affiliation: [1]Canadian Center for Vaccinology (CCfV), Dalhousie University, Halifax, NS, [2]Nova Scotia Health Authority (NSHA), Halifax, NS, [3]National Microbiology Laboratory, Winnipeg, MB

Introduction: Serotyping of Streptococcus pneumoniae is important to monitor disease epidemiology and assess the impact of pneumococcal vaccines. Traditionally, the Quellung reaction used serotype-specific antibodies to classify S. pneumoniae based on differences in capsular antigens. More recently, PCR-based serotype deduction relying on serotype-specific capsule biosynthesis genes has been broadly applied for pneumococcal surveillance. However, PCR-based serotyping lacks discrimination for certain S. pneumoniae serotypes, including the differentiation of serotype 22F from 22A, and serotype 33F from 33A and 37. Serotypes 22F and 33F are emerging serotypes that are absent in the currently licensed 13-valent pneumococcal conjugate vaccine, but present in the new candidate 15-valent formulation. This study validated novel PCR reactions to detect and discriminate S. pneumoniae serotypes 22F and 33F.

Methods: In order to differentiate S. pneumoniae serotypes 22F or 33F from genetically similar serotypes, two novel PCR reactions were designed and validated. The specificity of all PCR targets was evaluated using all 92 different S. pneumoniae serotypes, as well as 32 other streptococci. Reproducibility was evaluated using geographically and genetically diverse strains of S. pneumoniae serotypes 22F and 22A, or serotypes 33F, 33A, and 37 that were previously characterized by reputable reference laboratories.

Results: Overall, S. pneumoniae serotypes 22F and 33F could be accurately and reproducibly detected and discriminated.

Conclusions: Such a molecular serotyping approach provides a valuable diagnostic tool that is feasible in any molecular laboratory, to enable pneumococcal serotype surveillance and subsequent assessment of the impact of the new 15-valent candidate pneumococcal vaccine.

5. *STREPTOCOCCUS PNEUMONIAE* SEROTYPE 3 IS MASKING PCV13-MEDIATED HERD IMMUNITY IN CANADIAN ADULTS HOSPITALIZED WITH COMMUNITY ACQUIRED PNEUMONIA: A STUDY FROM THE SERIOUS OUTCOMES SURVEILLANCE (SOS) NETWORK OF THE CANADIAN IMMUNIZATION RESEARCH NETWORK (CIRN)

Authors: S McNeil[1], M Andrew[1], L Ye[1], T Hatchette[1], M Elsherif[1], H Gillis[1], A Lang[1], D Webster[2], J LeBlanc[1]

Affiliation: [1]Canadian Center for Vaccinology, IWK Health Centre and Nova Scotia Health Authority, Dalhousie University, Halifax, NS, [2]Horizon Health, Dalhousie University, Saint John, NB

Introduction: The 13-valent pneumococcal conjugate vaccine (PCV13) was recently shown to be effective against vaccine-type invasive pneumococcal disease (IPD) and pneumococcal community acquire pneumonia (CAP_{Spn}) in healthy adults, prompting many countries to re-evaluate vaccine recommendations. In Canada, the benefits of PCV13 in adults were unclear given anticipated herd immunity from childhood immunization. This study describes the clinical outcomes and serotype distribution in Canadian adults hospitalized with CAP_{Spn} and IPD from 2010 to 2015.

Methods: Active surveillance for CAP and IPD was performed in adult hospitals across five Canadian provinces, and patient demographics, outcomes, and specimens were collected. Bacteremic CAP_{Spn} was identified using blood culture. Non-bacteremic CAP_{Spn} using sputum culture or a PCV13-specific urine antigen detection (UAD_{PCV13}). IPD was defined by isolation of *Streptococcus pneumoniae* from sterile sites. Serotype was assigned using Quellung reaction, PCR, or UAD_{PCV13}.

Results: Of 6687 CAP cases where a test was performed, *S. pneumoniae* positivity decreased from 22.1% in 2011 to 10.2% in 2014, but increased to 14.3% in 2015. The proportion of CAP attributed to PCV13 serotypes followed a similar trend, dropping from 17.7% in 2010 to 6.2% in 2014, but increasing to 8.5% in 2015. The decline was attributed to serotypes 7F and 19A, and the increase to serotype 3. Similar trends were noted for bacteremic and non-bacteremic CAP_{Spn}. Intensive care unit admissions and requirement for mechanical ventilation remained unchanged over the years for CAP_{Spn} case, but changes were noted for 30-day mortality and length of hospital stay.

Conclusions: Herd immunity afforded by serotypes 7F and 19A appears to be partly masked by a concomitant increase in serotype 3. Herd immunity was evident in adults three to five years

following PCV13 in childhood immunization. Despite this, vaccine-preventable CAP_{Spn} and IPD remain prominent causes of morbidity and mortality in hospitalized Canadian adults.

6. RE-IMMUNIZATION OF PATIENTS WITH ADVERSE EVENTS FOLLOWING IMMUNIZATION IN THE CANADIAN SPECIAL IMMUNIZATION CLINIC NETWORK (2015-2017)

Authors: KA Top[1], FD Boucher, A McConnell, JM Pernica, A Pham-Huy[5] W Vaudry, S Deeks, F Noya, C Quach, D Tran, SK Morris, S Dobson, M Sadarangani, SA McNeil[1], D MacKinnon-Cameron[1], L Ye[1], SA Halperin[1], G De Serres

Affiliation: [1]Canadian Center for Vaccinology, IWK Health Centre, Nova Scotia Health Authority, and Dalhousie University, Halifax, NS

Introduction: The experience of an adverse event following immunization (AEFI) can increase vaccine hesitancy among patients and health professionals concerned about the risk of a more severe recurrent event following revaccination. Infectious disease physicians and allergists in the Canadian Special Immunization Clinic (SIC) Network developed standard protocols for evaluation and revaccination of patients with prior AEFIs. We analyzed the outcomes of patients evaluated for AEFIs from 2015 to 2017.

Methods: Patients are referred to one of 11 SICs in Canada by a physician or Public Health. Inclusion criteria are: patients of any age with injection-site reaction (ISR) ≥10 cm, allergic-like events (ALE) <24h post-immunization, neurological symptoms, and other AEFI of concern. SIC physicians evaluate eligible patients and make immunization recommendations according to network protocols. Patients are followed up after revaccination to capture AEFI recurrence. Outcomes of the consultation and revaccination(s) are transmitted to referring providers and public health. Following individual consent, data are transferred to a central database. For patients with more than one AEFI, the most severe event was included in the analysis.

Results: From June 2015 to May 2017, 172 patients with prior AEFIs were enrolled across the network. Most participants (90%) were <18 years of age. The most common types of AEFI were ALEs (35%), followed by ISRs (21%), neurologic events (13%), and other systemic events (e.g., high fever) (29%). The most commonly temporally associated vaccines were diphtheria-tetanus-pertussis, pneumococcal conjugate and influenza. Revaccination was recommended for 145 (84%) patients. AEFI recurrences occurred in 7/87 (8%) patients who were revaccinated and followed up: 1/38 ALEs, 5/21 ISR, 1/8 neurologic events. None were more severe than the first AEFI.

Conclusions: Patients with AEFIs benefit from clinical assessment by physicians with expertise in vaccines. The risk of an AEFI recurrence is low, except for ISRs, which are generally mild. Specialized immunization services can support health professionals managing patients with prior AEFIs.

7. BACTERIVOROUS PROTOZOA AS A SCREENING MODEL FOR SALMONELLA VIRULENCE

Authors: A Herod[1], J Rohde[1], R Garduno[2]

Affiliation: [1]Department of Microbiology and Immunology, Dalhousie University, Halifax, NS, [2]Microbiology Research Section, Canadian Food Inspection Agency Nova Scotia, Halifax, NS

Introduction: Predation by protozoa has a significant effect on bacterial populations in the environment; however, some bacteria have acquired factors to evade predation. These factors are believed to have evolved in the context of continuous bacterial interactions with bacterivorous protozoa, before animal forms of life emerged. Factors that inhibit or impair protozoa predation also play an important role in the survival and persistence of foodborne pathogens outside the host cell, and in the environment. A portion of these factors may also contribute to survival within human hosts due to similarities in bacteriocidal processes used by both protozoa and macrophages. The foodborne human pathogen *Salmonella* encodes a variety of virulence factors that may have evolved as survival tools against bacterivorous protozoa. Therefore, protozoa may represent potential screening models to distinguish between high- and low-virulence *Salmonella* strains. Four protozoan screening models were evaluated for their ability to discriminate between *Salmonella* strains that are virulent and avirulent in human cells.

Methods: Wild-type *Salmonella* (SL1344) and a double mutant defective for bacterial entry and intracellular survival (Δ*invA*Δ*sseB*) were used as virulent and avirulent *Salmonella* strains, respectively. *Tetrahymena*, *Acanthamoeba*, *Vermamoeba*, and *Dictyostelium* were all co-incubated with SL1344 and Δ*invA*Δ*sseB* for 24 hours and assessed for bacterial burdens at 1 and 24 hours. *Tetrahymena* and *Acanthamoeba* were counted at 1 and 24 hours to measure cell death in response to *Salmonella* co-incubation.

Results: Bacterial burdens and protozoa counts from grazing assays in *Tetrahymena*, *Acanthamoeba*, and *Dictyostelium* showed no difference between SL1344 and Δ*invA*Δ*sseB*. However, differences in bacterial burdens between SL1344 and Δ*invA*Δ*sseB* were observed during gentamicin protection assays in *Acanthamoeba* at 0 hours post infection. Additional *Salmonella* strains were examined using gentamicin protection assays in *Acanthamoeba*.

Conclusions: Gentamicin protection assays in *Acanthamoeba* represent the lone model to successfully discriminate between *Salmonella* strains that are virulent and avirulent in human cells. As a result, screening of additional *Salmonella* isolates will continue through this model.

8. IDENTIFICATION AND CHARACTERIZATION OF THE DISULFIDE BOND ISOMERASE SDBB IN *STREPTOCOCCUS GORDONII*

Authors: **NA Jalal**, SA Halperin, SF Lee

Affiliation: Department of Microbiology and Immunology, Dalhousie University; Canadian Center for Vaccinology, IWK Health Center

Introduction: The formation of disulfide bonds by thiol-disulfide oxidoreductases (TDOR) is crucial for the proper folding and activity of many extracytoplasmic proteins. Disulfide isomerases play important roles in oxidative stress protection and oxidative protein folding of proteins with more than two cysteines. *S. gordonii* is a normal inhabitant of the human oral cavity and a candidate live oral vaccine vehicle.

Methods: Mutants of TDOR genes were created by an allelic replacement strategy. Mutants were analyzed for sensitivity to oxidative stress and production of disulfide-bonded proteins. Isomerase activities and disulfide exchange reactions were assessed for the recombinant TDOR proteins.

Results: From *in silico* analysis, an operon carrying two TDOR genes, *sdbB* and *ccdA2*, were identified and inactivated creating single- and double-gene mutants. The results showed that the *sdbB* mutant was sensitive to H_2O_2, $CuSO_4$, and methionine sulfoxide. The *sdbBccdA2* double-gene mutant was even more sensitive to these agents. *sdbBccdA2* complementation restored the H_2O_2 resistance phenotypes. SdbB exhibited both oxidase and reductase activity and was able to isomerize and refold scrambled RNase A. The *sdbBccdA2* mutant also showed extensive degradation of the anti-CR1 single chain antibody, which contains 4 cysteines. The anti-CR1 antibody produced by the *sdbBccdA2* mutant in the *degP* (serine protease-negative) background was fully oxidized suggesting that anti-CR1 scFv was mis-disulfide bonded. In addition, disulfide exchange reactions showed that CcdA2 efficiently reduced SdbB and that SdbB was able to reduce MsrAB (methionine sulfoxide reductase) suggesting that SdbB-CcdA2 function in a reducing pathway.

Conclusions: The results suggest that SdbB is a disulfide bond isomerase in *S. gordonii* that is required for protection against oxidative stress, involved in the reduction of MsrAB, and proper folding of proteins with multiple cysteines.

9. TARGET SPECIFICITY AND PROTEIN-PROTEIN INTERACTIONS OF INFLUENZA A VIRUS ENDONUCLEASE PA-X

Authors: **D Khaperskyy[1,2]**, B Porter[1], C McCormick[1,2]

Affiliation: [1]Department of Microbiology and Immunology, Dalhousie University, Halifax, NS, [2]Canadian Center for Vaccinology, IWK Health Centre, Halifax, NS

Introduction: Influenza A viruses (IAVs) inhibit host gene expression by a process known as host shutoff. Several IAV proteins participate in host shutoff, including recently discovered ribonuclease PA-X. 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. The nuclear recruitment is dependent on the c-terminal X-ORF, which alone is sufficient to mediate nuclear recruitment of a reporter GFP fusion protein.

Methods: In human embryonic kidney HEK293T cells, BioID proximity labelling technique and mass spectrometry was used to identify host proteins that interact with X-ORF to create a list of potential factors that mediate nuclear localization and/or target recruitment of PA-X. Contribution of the selected proteins to the PA-X activity was examined using luciferase assay in cells in which individual candidate proteins were knocked down using shRNAs.

Results: Using the above BioID approach we identified 29 proteins as potential X-ORF interacting partners. Consistent with X-ORF known function in mediating nuclear localization of PA-X protein, all but one of these targets were either nuclear or nucleo-cytoplasmic shuttling proteins. Remarkably, the dataset was significantly enriched in proteins involved in splicing regulation. In the luciferase reporter assay, shRNA knockdown of 10 target proteins affected PA-X mediated inhibition of reporter expression.

Conclusions: BioID method of detecting protein-protein interaction in living cells allowed us to successfully identify interacting partners of influenza virus host shutoff endonuclease PA-X. Further characterization of the contribution of these proteins to PA-X activity will allow us to elucidate molecular mechanisms of IAV host shutoff and its contribution to IAV pathogenesis.

10. ALTERNATIVE TRANSLATION INITIATION DURING KSHV INFECTION

Authors: ES Pringle[1, 2], A Monjo[1], C McCormick[1,2,3]

Affiliation: [1]Microbiology and Immunology, Dalhousie University, Halifax, NS, [2]Beatrice Hunter Cancer Research Institute, Halifax, NS, [3]Canadian Center for Vaccinology, Halifax, NS

Not published by request.

11. NKR-P1B IS REQUIRED FOR RESIDENT ALVEOLAR MACROPHAGE DEVELOPMENT AND FUNCTION

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Introduction NKR-P1B is an inhibitory C-type lectin-like NK cell receptor. Its ligand, Clr-b, is a member of the C-type lectin-related ligand family. Expression and function of NKR-P1B:Clr-b axis in the context of myeloid derived immune cells has not been previously explored.

Methods: Using flow cytometric and confocal analysis we determine the effect of NKR-P1B ablation on alveolar macrophages (AMs) and dendritic cells (DCs). Histology and electron microscopy techniques are used to analyze AM lipid metabolic function and morphology. *In vivo* models utilizing *S. pneumoniae* and RSV will be used to determine AM function in response to infection. Finally, *in vitro* assays and RNAseq are going to be utilized to determine the extent of AM differentiation and functional impairment due to NKR-P1B ablation.

Results: Analysis of lungs from *nkrp1b*$^{-/-}$ mice has revealed a collapse of AMs starting at 4 weeks of age. Lungs of healthy 6-week-old *nkrp1b*$^{-/-}$ mice are almost completely devoid of AMs while exhibiting an increase in CD103^{+} DCs. AM population reconstitutes at 8 weeks of age and is present in significant numbers at 12 and 21 weeks of age. DC numbers also return to normal WT levels in older mice. Surprisingly, AM and DC numbers were found to be similar to WT controls in *Clr-b*$^{-/-}$ mice. Both the resident AM population prior to collapse, and reconstituted AMs in older *nkrp1b*$^{-/-}$ mice show a dysregulated CD11b^{hi} and F4/80^{lo} phenotype, suggesting a developmental arrest prior to terminal differentiation. Microscopic analysis of NKR-P1B-deficient lungs and isolated AMs shows a time-dependent presence of large, lipid filled cells suggesting an inability of AMs to degrade phagocytized pulmonary surfactant. Moreover, *nkrp1b*$^{-/-}$ mice are highly susceptible to pneumococcal infection, which could be related to AM disruption in these mice.

Conclusions: Overall, the work presented here sheds light on a potential new role of NKR-P1B in the development and function of the lung AM population.

12. IDENTIFICATION AND CHARACTERIZATION OF MUTATIONS RESPONSIBLE FOR ENHANCED INFLUENZA A VIRUS VIRAL POLYMERASE FUNCTIONALITY WITHIN THE MURINE MODEL

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Introduction: Influenza A viruses (IAV) are rapidly evolving viruses that exist as dynamic populations known as quasispecies. While the natural reservoir of IAV is water fowl, IAV has adapted to have an extensive host range and frequently crosses species barriers. Recently, a new IAV entered the human population causing the 2009 pandemic, subsequently causing thousands of deaths world-wide. The murine model is an attractive platform to study interspecies transmission and subsequent host adaptation due to their low cost, available genetic models, and available reagents. Mice are not naturally infected by IAV therefore, infection with most seasonal IAV isolates generates an asymptomatic infection with little viral replication. Adapting IAV to mice requires manual passaging of the virus from infected lungs to naive hosts, bypassing aerosol transmission. Understanding how influenza adapts to the murine model will provide insight into how new IAV strains will enter the human population and initiate the next pandemic.

Methods: Influenza A/California/07/2009 was serial passaged in Swiss-Webster mice for 10 passages to allow for adaptation mutations to arise within the population. We then used next generation sequencing to capture the entire genetic repertoire of the viral quasispecies allowing the identification of mutations responsible for murine adaptation. Mutations identified were genetically engineered into the wild-type virus to allow analysis between the parental strain and the virus harboring the mutations.

Results: Mutations were identified within the viral RNA dependent RNA polymerase (RdRP). The viral polymerase has been recognized previously to be responsible for virulence between hosts, and host restriction. Here we see the mutations within the viral polymerase increased function and replication of the viral genome. Recombinant virus containing the polymerase mutations replicated to a higher titer in mouse cell lines, while replicating to similar levels in human cell lines.

Conclusions: Taken together, these observations support the theory that the polymerase is critical in host adaptation. Understanding the mutations required for host adaptation is critical in surveillance of zoonotic influenza, highlighting key markers of host adaptation post zoonotic events.

13. B1 CELL SUBSETS DIFFER BETWEEN THE SEXES AND ARE INVOLVED IN RESPONSES TO CHLAMYDIA INFECTION

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Introduction: Human Immunodeficiency virus (HIV) establishes chronic infection leading to persistent immune changes even with antiretroviral disease control. While less effective in providing immunity, the influenza vaccine is still recommended in this population. We recently demonstrated that B cell subsets had a less efficacious subtype in influenza vaccine non-responders with uncontrolled viral load. It is still unclear if there are other clinical (HIV viral load, T cell counts), infectious (eg. CMV infection) or immune exhaustion factors that predict influenza vaccine response in HIV^+ individuals. Here, we identify clinical, functional, phenotypic, and transcriptomic markers associated with poor influenza vaccine response in HIV^+ individuals.

Methods: Peripheral blood mononuclear cells from 15 HIV^+ individuals were collected pre-influenza vaccination and 6 months post, after obtaining informed consent. Hemagglutinin inhibition assays for the 3 components of the 2015-2016 trivalent influenza vaccine were performed. B (CD10, CD19, CD20, CD21, CD27) and T cell (CD3, CD4, CD8, CD27, CD28, Tim-3, PD-1, CTLA-4, CD57, and perforin) immunophenotyping was performed by flow cytometry. Functional B and T cell responses to CMV, HIV, and influenza were examined by ELISPOT. Anti-CMV and anti-influenza antibody levels were determined by in-house serologic assay.

Results: Vaccine titers were limited and only 60% (9/15) of individuals responded to flu vaccine, regardless of previous vaccination. Influenza vaccine response was more common in those with controlled HIV viral load (63% (7/11) vs 25% (1/4)). Flu responders had higher IFN-□ producing CMV reactive T cells and higher anti-CMV antibody titers than non-responders. Transcriptomic analysis demonstrated distinct pre-vaccine B cell and CD4+ T cell gene expression in flu responders and non-responders.

Conclusions: HIV+ individuals that respond to influenza vaccine have immunologic differences marked by unique transcriptomic signatures, as well as skewed B cell subsets, and higher immune reactivity to another viral antigen, CMV. Together these data suggest that genetic factors in addition to just HIV immune dysfunction may be important for flu responses in HIV + individuals. Ongoing work will assess the contribution of specific genes and CMV immunity.

14. ASSESSING HIV CURE FOLLOWING SUCCESSFUL CCR5DELTA32 HOMOZYGOUS STEM CELL TRANSPLANT FOR HEMATOLOGIC MALIGNANCY

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Introduction: Functional HIV cure by stem cell transplant (SCT) has been attempted multiple times but only durably achieved once in the so-called 'Berlin patient'. This HIV positive person received a SCT from a CCR5delta32 homozygous donor as treatment for a hematologic malignancy with discontinuation of antiretroviral therapy (ART) without subsequent HIV viral rebound. Here, we Describe the clinical course, immunologic assessment and quantification of inducible latent HIV reservoir of a HIV positive person who underwent a CCR5delta32 SCT for chronic myelogenous leukemia.

Methods: The 58 year old HIV+CMV+ individual provided written informed consent, and is enrolled as part of the ICISTEM (International Collaboration to guide and investigate the potential for HIV cure by Stem Cell Transplantation). Peripheral blood cells were collected by leukapheresis, in addition to ileal biopsies and cerebrospinal fluid prior to transplant. ART was maintained pre- and post-transplant. Immune function and phenotype are assessed by ELISPOT and flow cytometry. Inducible latent HIV reservoir is quantified using the Tat/Rev Limiting Dilution Assay (TILDA).

Results: At submission, the individual is 568 days post-transplant with full engraftment, complete donor molecular chimerism, and CML remission. No HIV virologic rebound was noted and CD4+ T cell counts are near pre-treatment levels. B and T cells showed immunophenotypic profiles similar to other demographically matched HIV+ individuals. There are robust recall B cell responses to influenza, HIV gp41, CMV, and Candida pre-transplant. The latent inducible viral reservoir pre-transplant is in keeping with previously reported values of similar long term HIV positive people. Residual anti-HIV antibodies are detectable post-transplant by western blot. Viral outgrowth assays will assess the level of replication competent HIV reservoir remains post-transplant, with HIV deep sequencing and microchimerism analysis to detect host cells in the donor PBMC population.

Conclusions: This HIV+ person has been, to date, successfully treated from a hematologic perspective, but HIV status in the context of a potentially curative stem cell genotype is

unknown. Follow up studies of functional and phenotypic immune changes after interruption of ART will be necessary to determine HIV cure.

15. A CRITICAL ROLE FOR LY49 RECEPTORS IN ADAPTIVE NATURAL KILLER CELL MEMORY

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Introduction: Recent advances have identified adaptive immune memory in the classically 'innate' natural killer (NK) cell. Here, we provide the first evidence explaining how this adaptive memory arises in NK cells. We also demonstrate that adaptive NK memory can be used to mediate cancer vaccines in a proof-of-concept melanoma model, opening NK memory as a viable avenue of clinical study and vaccine development.

Methods: Mice lacking T and B lymphocytes (*Rag1*$^{-/-}$ mice) were analyzed using the classical mouse ear swelling test for immune memory, as well as using a model ovalbumin oil-in-water vaccine against B16 melanoma to test their ability to form memories without an adaptive immune system. Further genetic modifications were used to delete Ly49 receptors or other NK cell effector genes to test the significance of NK cell target recognition and effector functions in developing and acting on immune memories.

Results: Mice lacking T and B cells were found to have effective NK-mediated adaptive immune responses. These responses depended on Ly49 receptor expression and Ly49:MHC-I interactions, giving rise to a model for adaptive NK memory based on Ly49 acting as an antigen-specific receptor. NK memory was found to mediate measurable protection against B16 melanoma when properly vaccinated.

Conclusions: Adaptive NK cell memory requires Ly49 receptors acting in an antigen-specific role to function, and can be used to mediate cancer vaccines. The clinical relevance for human vaccine efforts of this finding is currently under investigation.

16. USING CELLPROFILER 2.3.0 TO ANALYZE PROCESSING BODY DYNAMICS IN RESPONSE TO VIRUS INFECTION

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Not published by request.

17. ENHANCING DETECTION AND RESPONSE FOR FUTURE INTERVENTIONS: BUILDING SUSTAINABLE COMMUNITY-BASED CAPACITY THROUGH THE EXPERIENCES OF MOBILE LAB AND CLINICAL TRIAL INTERVENTIONS

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Introduction: The gut houses thousands of bacteria, encoding millions of genes, that participate in various host processes. One such bacterial gene codes for high temperature protein G (HtpG). The copy number of HtpG varies between bacteria (0-3), the significance of which is uncertain. HtpG stabilizes proteins during cellular stress and can induce an inflammatory response in endothelial cells. *Bacteroides fragilis,* a commensal and sometimes opportunistic bacterium, has two copies of HtpG. Only *B. fragilis* HtpG2 has been shown to enter epithelial cells via outer membrane vesicles. We recently observed that following exclusive enteral nutrition (EEN), pediatric Crohn's disease patients able to sustain remission had greater abundances of HtpG than those patients who relapsed. Understanding how bacterial HtpG influences intestinal immune responses will provide important insight into how HtpG may promote sustained remission following EEN. The aim of my project is to establish the impact of *B. fragilis* HtpG on innate immune functions of intestinal epithelial cells.

Methods: HtpG protein evolution was used to identify HtpG proteins for use in *in vitro* experiments. HtpG amino acid sequences were collected from the KEGG database, and aligned using MAFFT G-INS-1. A neighbour-joining tree was constructed using the conserved sites. Next, HT-29 cells were treated with *B. fragilis* rHtpG2 (0.78-50 µg/ml), TNF (10/20 ng/ml), or TNF (10 ng/ml) and rHtpG2 (6.25-50 µg/ml) for 24 hours. CXCL8 cytokine expression was measured using an ELISA assay.

Results: When HT29 cells were treated with both TNF and a high concentration of HtpG (50 µg/ml), CXCL8 expression decreased below what was observed for TNF treatment alone. As the concentration of HtpG decreased, the expression of CXCL8 increased.

Conclusions: Lower concentrations of bacterial HtpG, as observed in pediatric Crohn's patients unable to sustain remission, resulted in increased expression of CXCL8 in intestinal epithelial cells.

18. TAKING THE KAP OUT OF KAPOSI'S SARCOMA: GENERATING TOOLS FOR THE STUDY OF A HERPESVIRUS PROTEIN

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Not published by request.

19. A *SHIGELLA* E3 UBIQUITIN LIGASE CO-OPTS THE UFM1 SYSTEM

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Introduction: Ubiquitination is a post translational modification that proceeds via a three enzyme cascade (E1 activating enzyme > E2 conjugating enzyme > E3 ligase) to control the fate of cellular proteins. Bacteria have evolved to co-opt the ubiquitination system by encoding proteins that mimic host E3 enzymes. Notably, *Shigella flexneri* encodes the E3 ubiquitin ligase IpaH9.8, which it delivers via its type III secretion system. However, IpaH9.8 substrates have proven difficult to elucidate. Recently, ubiquitin-like modifiers (UBLs) have emerged, including the poorly-understood UFM1. UBLs are conjugated to target proteins in a similar fashion, and have been shown to work in concert with traditional ubiquitination, which may help to explain why IpaH9.8 substrates have been elusive.

Methods: We used yeast two-hybrid screening, biochemical ubiquitination assays, co-immunoprecipitation, and mass spectrometry to look for proteins that interact with IpaH9.8.

Results: Yeast two-hybrid screening identified UFM1 as an interactor with IpaH9.8, and in vitro ubiquitination reactions demonstrate that IpaH9.8 functionally interacts with the UFM1 machinery. Co-immunoprecipation and subsequent mass spectrometry in human cells identified a suite of differentially UFMylated proteins directed by IpaH9.8, including Galectin-7, a carbohydrate-binding protein involved in recognizing bacterial infections.

Conclusions: Taken together, our data support a model where IpaH9.8 either a) acts as an E3 ligase for the UFM1 system, or b) has its E3 ubiquitin ligase activity regulated by UFMylation. Our data provides insight to how IpaH-class E3s function, and proposes a new target of IpaH9.8 and UFM1 alike: Galectin-7.

20. LINKING PHENOTYPE AND PATHOGEN GENOMICS: TNSEQ AND NEXT GENERATION DNA SEQUENCING

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Introduction: Studying bacterial phenotypes on a whole genome scale is not a trivial task. Currently, genome sequencing allows the identification of genes that bacteria encode. However, this identification is dependent on computational programs and annotations that link to putative cellular functions. An innovative approach, called Transposon-Sequencing (Tn-Seq), focuses on removing this uncertainty, by combining whole-genome sequencing with functional phenotypic data. Tn-Seq offers an unparalleled depth of analyses that serves to identify vital genes for distinct cellular processes. This allows probing of the entire genome to discover which genes are involved in antibiotic resistance, pathogenic programming, and environmental survival in a variety of pathogens.

Methods: Tn-Seq uses next-generation sequencing approaches to identify regions of the genome that confer a selective advantage in a set of conditions. Bacterial populations are mutagenized with a transposon – a mobilizable genetic element that randomly integrates into the genome – and this population is placed under selective growth conditions. The genomic DNA of the population is then isolated and prepared for Illumina sequencing by targeting the regions surrounding the transposon insertions. Alignment of these sequences to the genome then determines which mutations are present and can be enumerated to determine individual fitness. Through inference, we can determine which mutants did not survive (those that are not present) and, therefore, which genes are required for adequate fitness under selection.

Results: Currently, we are investigating pathogenomic aspects of sugar metabolism relating to *Vibrio* species in the environment and host intestine. We have constructed Tn-Seq libraries using both a previously published method as well as a novel approach, which will be compared post-sequencing.. These were confirmed using qPCR to identify the concentration of the transposon junction population to be sequenced.

Conclusions: Pathogenomics can be an extremely powerful tool in the discovery of genes and regulatory elements necessary for infection, environmental survival, or antibiotic resistance in pathogens. Identifying these elements will help researchers and physicians develop ways to prevent, treat, and diagnose infections.

21. ATTITUDES TOWARDS INFLUENZA VACCINATION IN THE EMERGENCY DEPARTMENT (WORK IN PROGRESS)

Authors: N Ozog, A Steenbeek, J Curran, N Kelly

Affiliation: Dalhousie University, Halifax, NS

Introduction: The yearly influenza (flu) season is an ongoing public health challenge in Canada. Complications of the flu can lead to pneumonia, hospitalization and even death. While a flu vaccine is available free of charge, the rate of uptake in Nova Scotia (NS) is below target rates. 26% of Canadians who did not get the flu vaccine reported that they "didn't get around to it"; this presents an opportunity to combine the task of flu prevention with the logistical issue of another health system challenge: escalating emergency department (ED) wait times. At the Queen Elizabeth II Health Sciences Centre (QEII) in Halifax, NS, average wait time is 4.6 hours. Offering the flu vaccine during this time could increase convenient access to health services.

Methods: The proposed study will involve an observational, cross-sectional design. Data will be collected via a short, anonymous, close-ended questionnaire distributed to emergency department clients and health care providers (HCPs). The Health Belief Model and Promoting Action on Research Implementation in Health Services framework will provide the conceptual basis for the client and HCP questionnaires respectively. Client participants will be sampled consecutively over a one month, from low-acuity, adult clients who use the QEII ED. The HCP group will be a quota sample recruited via email. Following data collection, descriptive and inferential statistics will be calculated to provide meaningful quantitative data that can be used by the QEII to inform future program planning.

Results: No results are available currently as data collection is planned for summer of 2018.

Conclusions: An ED vaccination program could add value to the hours clients spend waiting in the ED and make the care we provide more cohesive. It essential that clients and ED staff are approached prior to any new initiative; this study is one way we can lay this groundwork for a program that would utilize patient "wait time" more effectively.

22. UNRAVELING THE SECRETS OF MK2 ACTIVATION BY KAPOSIN B

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Not published by request.

23. VANCOMYCIN PRESCRIBING AND THERAPEUTIC MONITORING IN CRITICALLY ILL PATIENTS ON CONTINUOUS RENAL REPLACEMENT THERAPY: RETROSPECTIVE EVALUATION AND PROSPECTIVE MULTI-MODAL PRACTICE CHANGE

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Introduction: Critically ill patients on continuous renal replacement therapy (CRRT) are at high risk of mortality. CRRT alters the pharmacokinetics of vancomycin and current literature concludes under-dosing of vancomycin is prevalent in critically ill patients on CRRT. The primary objective was to describe vancomycin prescribing and monitoring practices in critically ill patients on CRRT, and to implement an individualized approach to optimize vancomycin therapeutic target attainment.

Methods: A 2-year retrospective chart review was completed evaluating prescribing, monitoring and vancomycin concentrations in critically ill patients on CRRT. Subsequently, a practice change was initiated. A vancomycin 30 mg/kg intravenous loading dose was utilized. Peak and repeat serum concentrations were used to calculate the patient specific volume of distribution and clearance, which were used to calculate individualized dosing regimens. A 3-month prospective evaluation of the practice change was then completed.

Results: A 2-year retrospective chart review was completed evaluating prescribing, monitoring and vancomycin concentrations in critically ill patients on CRRT. Subsequently, a practice change was initiated. A vancomycin 30 mg/kg intravenous loading dose was utilized. Peak and repeat serum concentrations were used to calculate the patient specific volume of distribution and clearance, which were used to calculate individualized dosing regimens. A 3-month prospective evaluation of the practice change was then completed.

Conclusions: Our retrospective review suggests vancomycin prescribing and therapeutic drug monitoring practices resulted in sub-therapeutic vancomycin concentrations. The prospective case demonstrated application of the prescribing and monitoring strategy, however further prospective evaluation is required.

24. IMPLEMENTATION OF A MATERNAL PERTUSSIS IMMUNIZATION PROGRAM AND IMPROVING COVERAGE AMONG INUIT WOMEN: A LITERATURE REVIEW

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Introduction: Pertussis causes life-threatening disease in young children especially <6 months of age. To protect infants, pregnant mothers can be given a tetanus, diphtheria, acellular pertussis (Tdap) vaccine. Nunavut introduced a routine maternal Tdap program in May 2016 in response to an outbreak of 154 cases. We sought to determine existing knowledge on immunization and infectious disease research in preparation for a study of maternal Tdap immunization in Nunavut.

Methods: We systematically reviewed the literature using MeSH terms “epidemiology,” “pertussis vaccine,” “health knowledge,” “Inuit,” and the free text term “maternal immunization.” PubMed was used for the first search in English between July 31 and August 9, 2017. A second search of Google Scholar, Science Direct, the Cumulative Index of Nursing and Allied Health, Novanet, ProQuest, Wiley, the National Center for Biotechnology Information and SAGE Journal was done. We sought additional references after discussions with stakeholders and a perusal of Canadian First Nations, Métis, Inuit research organizations. Inclusion criteria were peer-reviewed articles available in English, published within the last 10 years about Canadian Inuit. Exclusion criteria were works not focused on Canadian Inuit and immunization.

Results: The most relevant literature was found in the second search which yielded 70 articles of which 61 were eligible; 7 reported on indigenous knowledge, 12 detailed the status and determinants of indigenous health, 5 provided historical and anthropological context, 19 were vaccinology studies, 6 were infectious disease studies, and 12 were grey literature documents. In general, the literature demonstrated that there is a high burden of infectious disease in the Canadian Inuit community. No literature on Inuit-specific determinants of immunization was found.

Conclusions: Data on the knowledge, attitudes, beliefs and behaviours of Canadian Inuit women about maternal immunization are lacking.

25. B1 CELLS MIGRATE TO THE LUNG AND PROMOTE DIFFERENTIAL IMMUNE RESPONSES TO *CHLAMYDIA* IN A SEX-DEPENDENT MANNER

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Introduction: *Chlamydia* infections of the lung and genital tract differ significantly between the sexes, with females being infected more often and having more severe outcomes. It is possible that these differences are due to sex-specific immune responses but this possibility has not yet been studied. Herein, we examined the possibility of sex-specific immune responses to *Chlamydia* infection with a focus on the role of innate-like B1 cells.

Methods: Male and female mice were infected intranasally with the mouse pathogen *Chlamydia muridarum* (*Cm*). Following infection body weight was measured daily. At the time of sacrifice, bacterial burden was measured by qPCR, memory responses were measured by antigen recall assay, antibody levels were measured by direct ELISA, and the immune cell populations were analyzed by flow cytometry. To further characterize B1 cells, *in vitro* stimulations were performed and naïve levels of B1 cells were measured by flow cytometry.

Results: Following intranasal infection, female mice lost significantly more weight compared to male mice and had a higher bacterial burden. Furthermore, female mice had higher antibody, IL-10, and IL-13, while males had protective IFNγ responses. Interestingly, 3 days post-infection, female mice had significantly more B1 cells in the lung compared to males and demonstrated that these cells migrate from the peritoneal cavity. When whole peritoneal exudate was stimulated with *Cm in vitro*, cultures from females produced more IL-10 and had more IL-10$^+$ B cells. To determine if B1 cells were producing/inducing IL-10 in these cultures, we performed co-cultures using B1 cells as antigen presenting cells and observed that B1a cells were able to induce strong IL-10 responses. Finally, we examined B1 cells at rest and found that females naturally have more B1 cells in the peritoneal cavity compared to males.

Conclusions: We have demonstrated that female mice have more B1 cells compared to males. Following infection, these B1 cells migrate to the lung where they induce IL-10 responses in females, which dampen protective IFNγ responses, and may be involved in promoting antibody production. Together, we have demonstrated that sex-specific immune responses occur

following *Chlamydia* infection and that these responses are mediated by innate differences in B1 cells.

26. CHARACTERIZATION OF A NOVEL HOST-TARGETED ANTIVIRAL MOLECULE

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Not published by request.

27. COMBINATION OF A T CELL ACTIVATING IMMUNOTHERAPY WITH IMMUNE MODULATORS ALTERS THE TUMOUR MICROENVIRONMENT AND PROMOTES MORE EFFECTIVE TUMOUR CONTROL IN PRECLINICAL MODELS

Authors: A MacKay[1], G Weir[1], H Koblish[2], A Vila- Leahey[1,3], V Kaliaperumal[1], C Tram[1], P Scherle[2], M Stanford[1,3]

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Introduction: Combinations of immune therapies for cancer treatment may improve clinical responses, and a treatment that stimulates a robust T cell response may be a key component of therapy in patients with poorly infiltrated tumours. DPX-Survivac is a T cell activating therapy targeting survivin, formulated in DepoVax™ (DPX), an oil based delivery platform. In clinical studies, DPX-Survivac induced strong and sustained T cell responses when used in combination with metronomic cyclophosphamide (mCPA) in ovarian cancer patients. Epacadostat is an indoleamine-2,3-dioxygenase 1 inhibitor which has been shown to reduce immune suppression in tumours and has demonstrated encouraging results in clinical trials. Using preclinical mouse tumour models, we evaluated the combination of these three immune therapies.

Methods: C57Bl/6 mice were implanted subcutaneously with murine pancreatic adenocarcinoma (Panc02) or HPV16 E7 expressing (C3) cells. Groups of mice were vaccinated with DPX vaccine by subcutaneous injection and treated with mCPA and epacadostat. In the C3 model, mice were terminated at defined endpoints to evaluate systemic immune responses in the spleen by IFN-g ELISPOT and profile tumour infiltration by flow cytometric analysis.

Results: The combination immunotherapy provided a significant delay in tumour progression and improvement in survival over untreated animals. Although antigen-specific immune responses in the spleen were not increased by the triple combination in comparison to the DPX-based vaccine, there was a significant impact on several immune subtypes found in the tumour. Notably, antigen-specific $CD8^+$ T cells were increased and regulatory T cells were decreased.

Conclusions: In preclinical tumour models, the combination immune therapies can significantly improve survival, tumour suppression and selectively alter the immune cell phenotype in the tumour microenvironment. Other immune modulating therapies, such as anti-PD-L1, may further enhance the tumour control induced by this treatment. The combination of DPX-based, T cell activating therapy with epacadostat, a drug that reduced tumour immune suppression is a

rational, synergistic combination that is currently being evaluated in advanced ovarian cancer patients in the DeCidE[1] clinical trial.

28. PHOTODYNAMIC INACTIVATION OF HERPES SIMPLEX VIRUSES

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Not published by request.

29. EVALUATION OF THE BD MAX™ ENTERIC BACTERIAL PANEL WITH THE EXTENDED ENTERIC BACTERIAL PANEL VERSUS STOOL CULTURE

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Introduction: Identification of the cause of enteric infection is useful to direct treatment, avoid further investigation, and can lead to public health measures to control spread. Culture, microscopy and other methods have been used to detect these pathogens, but availability of results is slow and some causes may be difficult to detect using conventional methods.

Methods: We compared conventional stool culture for *Campylobacter* spp., *Salmonella* spp., *Shigella* spp., *Yersinia enterocolitica*, *Plesiomonas shigelloides*, *Vibrio* spp. and *E. coli* O157 with BD Max™ (BD Diagnostics, Sparks MD, USA) Enteric Bacterial Panel and Extended Enteric Bacterial Panel which detects these pathogens and also STEC, ETEC, and EIEC (as *Shigella*/EIEC) to evaluate the sensitivity of detection, and determine the prevalence of pathogens for which we are not currently testing. Fresh stools (437) were tested prospectively and blinded. Discrepant and failed results were tested retrospectively by collaborators using molecular methods.

Results: The results of testing were (listing BD Max™/culture): *Campylobacter* spp. 23/20, of which 2 BD Max™+ were not confirmed on discrepant analysis, and 1 culture-positive failed by BD Max™; *Salmonella* spp. 11/9, where one BD Max™+ had *Salmonella* on re-culture; STEC 6/1; *Shigella*/EIEC 2/0; ETEC 1/0; BD Max™ detected 1 *Vibrio* and 1 *Yersinia* which were not confirmed. In total, BD Max™ detected 45 pathogens, 4 of which were not confirmed and missed one *Campylobacter* as a sample fail. Five STEC and 1 ETEC would not have been detected on our conventional culture, but 6 other pathogens went undetected by culture.

Conclusions: BD Max™ Enteric Panels were rapid and provided a sensitivity of detection of 98%. Conventional methods detected 83% of the pathogens with a further 14% yield of pathogens not detected by culture. The clinical and public health significance of the additional agents detected requires further investigation.

30. INFLUENZA VIRUSES EVADE NATURAL KILLER CELL RESPONSES VIA MODULATION OF THEIR LIGANDS ON INFECTED CELLS.

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Introduction: Pulmonary influenza A virus (IAV) infection is controlled through the concerted action of multiple immune cells. Natural killer (NK) cells play an important role early during infection to lower virus titers, reduce lung inflammation, and improve survival in mice. However, IAV has evolved mechanisms, including changes in NK cell ligand expression in infected cells, to evade detection by NK cells.

Methods: Using bioinformatic analysis, we determine expression levels of known ligands for NK cell receptors on infected primary and transformed human lung cells. Ligand expression is further analyzed by quantitative RT-PCR and flow cytometry. Mechanisms of NK cell ligand modulation, and their impact on immunity and IAV pathogenesis, will be evaluated *in vitro* and *in vivo* in genetically modified mouse models.

Results: Expression of a number of NK cell ligands were found to be modulated in IAV-infected cells. Downregulated ligands included those recognized by the activating NKG2D (MICA and MICB) and DNAM-1 (PVR/CD155 and NECTIN2) receptors. By contrast, HLA (major histocompatibility complex, class I) molecules, which are recognized by inhibitory killer-cell immunoglobulin-like receptors (KIR), were upregulated. Analysis of cells infected with multiple strains showed a general trend towards HLA-A/B/C upregulation, with some strain-specific differences. HLA upregulation depended on infection since it was abrogated by UV-inactivation of IAV, and was distinct from that caused by extrinsic soluble factors released during infection.

Conclusions: IAV infection appears to cause cell-intrinsic changes in NK cell ligand presentation. We have previously shown that disruption of inhibitory NK cell receptors in IAV-infected mice resulted in better survival. Understanding the mechanisms by which ligands for NK cells are induced during IAV infection to disrupt NK cell responses is a first step towards finding ways to counteract them so that NK cell responses are restored.

31. VIRAL MANIPULATION OF HOST CHOLESTEROL HOMEOSTASIS IS REQUIRED FOR VIRAL PACKAGING AND EGRESS

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Not published by request.

32. CONSTRUCTION OF A SYNTHETIC KSHV GENOME BY TRANSFORMATION-ASSOCIATED RECOMBINATION

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Not published by request.

33. NUMBER NEEDED TO VACCINATE (NNV) WITH THE NONAVALENT VACCINE TO PREVENT ONE HPV-RELATED DISEASE IN CANADA.

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Introduction: Human papillomavirus (HPV) vaccination is recommended for females aged 9-45 and males up to 26 years. The HPV vaccine is provided in the school-based vaccination programs of all provinces and territories. Nonavalent HPV vaccine (HPV9) prevents HPV 6/11/16/18 and the five next most common cancer-causing HPV types. Here we aimed to estimate the NNV to prevent a case of HPV disease (within the approved indications) by vaccinating 12-year-old girls and boys in Canada with HPV9.

Methods: NNV was defined as NNV = N ÷ P, where N is the size of the vaccinated cohort, and P is the predicted number of HPV-related events prevented in the vaccinated cohort over its lifetime. We assume the current epidemiology of HPV-related diseases and cervical cancer screening practices remain stable over the time. We estimate 90 % vaccine coverage of a cohort of 12-years-old girls and boys and a lifetime vaccine protection. Vaccine efficacy, disease incidences, and proportion of cases attributed to the HPV-types included in the vaccine were retrieved from the literature.

Results: NNV estimates to prevent a case of cervical, vaginal, and vulvar cancer were 149, 3633 and 1975, respectively. Furthermore, NNV estimates were 4, and 8 respectively to avert a case of CIN2+ and genital warts in women. In men, the NNV to prevent one case of genital warts is 8 and to prevent anal cancer is 2061.

Conclusions: Overall, NNV with HPV9 are low, illustrating the important benefits expected from the prophylactic use of the vaccine against 9 prevalent HPV types to reduce the burden of genital warts, cervical intraepithelial neoplasia and HPV related cancers in Canada.

34. HEALTHCARE PROVIDERS' PERCEPTIONS OF ANTIMICROBIAL USE AND STEWARDSHIP AT ACUTE CARE HOSPITALS IN NOVA SCOTIA.

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Introduction: Antimicrobial stewardship (AMS) has been recommended as a strategy to improve antimicrobial use. The objective of this study was to determine healthcare provider perceptions of current antimicrobial use and stewardship including barriers and facilitators to improving antimicrobial use at acute care hospitals in Nova Scotia (NS).

Methods: This study was conducted using a series of focus groups and semi-structured interviews. Healthcare providers (nurses, nurse practitioners, pharmacists, physicians, and trainees) working at acute care hospitals in NS were invited to participate. Focus groups and interviews were conducted at the participants' place of employment. Discussions were facilitated using an interview guide, audio-recorded, and transcribed verbatim. Transcripts were independently coded by two investigators and analyzed using thematic analysis.

Results: A total of 9 focus groups and 3 individual interviews were conducted between June and August 2017. Fifty-four healthcare professionals and trainees (pharmacists/pharmacy students N = 24, physicians N = 14, and nurses/nurse practitioners N = 16) from 5 acute care hospitals participated. Participants discussed current practices, prescribing influences, access to information, collaboration and communication, resources, and antimicrobial stewardship. Within each theme, a number of barriers and facilitators to improving antimicrobial use were identified.

Conclusions: Participants recognized current barriers and suggested facilitators that may improve antimicrobial use. Results of this study may be used by antimicrobial stewardship teams and decision makers to improve antimicrobial use and AMS initiatives throughout NS.

35. LIMITED AWARENESS AND PROVIDER-ASSOCIATED INFORMATION OF HEPATITIS C VIRUS ARE COMMON IN NOVA SCOTIA

Authors: **J Boudreau[1]**, J Moore[2,3], SA Oldford[1,2], L Barrett[1,2,3]

Affiliation: [1]Dalhousie University, Halifax, NS, [2]Canadian Centre for Vaccinology, Halifax, NS, [3]Nova Scotia Health Authority, Halifax, NS

Introduction: Population level HCV treatment strategies will be necessary to garner most benefit from HCV treatment and affect elimination, and most countries will need to engage broad scale public payer systems to cover the costs of such plans. Public opinion may play a role in influencing healthcare policy in democratic systems, however it is unclear if HCV is a public priority or even a known health issue in Nova Scotia. Our goal was to assess public awareness and opinion around HCV treatment and elimination prior to introduction of a provincial HCV elimination strategy.

Methods: Health care associated opinions were assessed in a Nova Scotia. A questionnaire was developed and beta tested to assess knowledge and attitudes related to nationally relevant health care initiatives, including HCV treatment, care and elimination. 68 adults (>18 years of age) were approached in public areas, and those providing appropriate informed consent completed the anonymous questionnaire. Those with self-identified literacy issues completed the task with assistance from a trained study team member. A combination of Likert scale, binary responses, and free text answers were used to gauge opinions. Open ended questions were analyzed by Braun and Clarke's six phase analytic method.

Results: Only 7% of respondents viewed HCV treatment and care as more of a priority than the 4 other health care priorities (youth mental health, cancer care costs, hip replacement wait times, homecare for seniors), and this was associated with high media coverage of the other issues. Only 5% of individuals had lived experience with HCV. In contrast, 71% of respondents believe the government and public health care system should make HCV cure a priority. Thematic analysis identified proximity and prevalence as key factors in health prioritization, as well as a lack of knowledge. No individuals identified health care providers as a source for information on HCV.

Conclusions: While the majority of individuals feel HCV is an important public treatment priority for all persons, opinions surrounding HCV treatment and elimination are informed by lack of education and awareness in a public sample in Nova Scotia. HCV awareness and provider education will be important components of the proposed Nova Scotia HCV elimination strategy to engage both the public, policy makers, health providers and patients.

36. PML-ISOFORM SWITCHING DURING KSHV INFECTION

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37. FUNCTIONAL ANALYSIS OF PUTATIVE THIOL-DISULFIDE OXIDOREDUCTASES (SMU1070 & SMU1268) IN *STREPTOCOCCUS MUTANS* UA159

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Affiliation: Dalhousie University, Halifax, NS

Introduction: Disulfide bond formation is important for the proper folding and activity of many extracellular proteins and virulence factors of bacteria. Generally, these bonds are catalyzed by a class of enzymes known as thiol-disulfide oxidoreductases (TDORs), which typically have two signatures: a CXXC catalytic motif and a C-terminal proline residue. *Streptococcus mutans* is a Gram-positive oral bacterium, which is known to cause dental caries and infective endocarditis in humans. The secretion of extracellular proteins (e.g. bacteriocins and adhesins) in *S. mutans* plays an important role in inhibiting the growth of other bacteria and in colonization or biofilm formation, respectively. Both traits involve proteins that require disulfide bond formation to function properly; however, little is known about how disulfide bonds are formed in *S. mutans*.

Methods: To investigate disulfide bond formation in *S. mutans*, two knock-out mutants were created by inserting an erythromycin resistant cassette (*ermAM*) into the putative TDOR genes, *smu1070* and *smu1268*. The mutation in the knock-out mutants were confirmed by polymerase chain reaction. The mutants were tested for a number of phenotypes including bacteriocin production, disulfide-bonded single chain antibody production, biofilm formation and sensitivity to salts, acids, oxidative stress (hydrogen peroxide and paraquat induced), copper stress, and antibiotics.

Results: Both mutants (Δ*smu1070* & Δ*smu1268*) produced a lower amount of a disulfide bonded anti-CR1 single chain antibody and formed more biofilm than the parent strain. The mutants did not show any differences in bacteriocin production, sensitivity to salts, acidity, oxidative stress, copper stress, and cell wall- and cell membrane-acting antibiotics compared to the parent strain.

Conclusions: The findings showed that Smu1070 and Smu1268 plays a role in biofilm formation and anti-CR1 production suggesting that they are involved in disulfide bond formation in *S. mutans*. These phenotypes will be further analyzed by creating double mutant strains, as well as analyzing these phenotypes after gene complementation.

38. INVESTIGATION OF INFLUENZA A VIRUS NP PROTEIN-MEDIATED STRESS GRANULE INHIBITION VIA PROXIMITY-LABELLING PROTEOMICS

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Not published by request.

39. IDENTIFYING GAPS IN THE HEPATITIS C CASCADE OF CARE

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Introduction: Hepatitis C is the first human chronic viral infection with the potential for cure and elimination at the population level with the advent of new highly effective direct acting antiviral therapies. However, despite the relatively widespread availability of these medications through the public formulary, there is limited treatment uptake in the community. Others groups in Canada and the world have described gaps in the hepatitis C virus (HCV) cascade of care (limited referral after diagnosis, limited treatment after referral to specialist, limited followup after treatment). Our objective was to identify the main gaps in care for HCV before initiation of a provincial HCV elimination strategy.

Methods: Data from patients referred and/or seen at the QEII ID clinics was anonymized and analyzed from January 2017-February 2018.

Results: A total of 174 referrals were received over the time of interest, 67 (37%) of whom had been seen. It was not possible to assess how many of the diagnosed people in the province have been seen by an HCV provider or are lost to followup as that data linkage currently does not exist. Most are from the Halifax Regional Municipality however they are dispersed across the province. The main reasons patients were not booked for an appointment were inability to contact the patient, no blood work completed, and a challenge with getting clinical time in correctional settings. Only 39 (58%) patients started HCV therapy, with the number significantly increasing around times of dedicated HCV clinics in after hours times and the addition of a dedicated HCV nurse. Most common reasons for delayed treatment starts were loss to followup, and did not qualify for public medication coverage for HCV (disease too early for public coverage to pay). For example, while 23 people are being seen in February 2018, only 3 are on therapy. With the addition of a dedicated HCV clerk who can spend time finding more marginalized and underhoused patients, the number of HCV patients getting bloodwork and being booked for care has increased 125% in 3 weeks. Wait times are unequal across geographic distribution.

Conclusions: The main gaps in the HCV cascade of care are first in even assessing how many of the diagnosed individuals are not referred for care, followed by limited capacity for treatment

access in less severe liver disease, and difficulty in contacting the patient. A coordinated strategy with a prospective database, data linkage, centralized referral, less restriction on public HCV medication coverage would be key to understanding (and filling) the gaps in the HCV cascade of care during the forthcoming HCV elimination strategy.

40. KSHV HIJACKS THE UNFOLDED PROTEIN RESPONSE TO PROMOTE LYTIC REPLICATION

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Introduction: 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 propagation 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. Emerging evidence suggest that viruses may use the UPR to promote virus replication, but this has not been thoroughly investigated for KSHV. In this study, we investigated the interplay between the UPR and KSHV lytic replication.

Methods: We used multiple KSHV-infected cell lines to monitor UPR activation during lytic. Lentiviral vectors were used for ectopic expression and knockdown studies. Flow cytometry, qPCR, western blot, and immunofluorescence were used to monitor viral replication and UPR modulation.

Results: We found that lytic replication activates all three sensors of the UPR but all the downstream signaling events are inhibited. Interestingly, inhibiting or knocking down either PERK, IRE1, or ATF6 inhibits virus replication.

Conclusions: We show a novel scenario where a cancer-causing herpesvirus activates a cellular stress response but inhibits its canonical downstream signaling pathways. Our findings suggest that KSHV likely 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 may, which may lead to the development of new therapeutic strategies that inhibit KSHV tumorigenesis.

41. HCV CURE AND DEMOGRAPHIC PATTERNS OF HCV INFECTED INDIVIDUALS IN NOVA SCOTIA IN THE PRE- AND EARLY DAA ERA

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Introduction: Direct-acting antiviral (DAA) medications cure hepatitis C virus (HCV) infection with high efficacy. However, these studies typically include highly adherent populations. Many of these trials excluded individuals with comorbidities (eg. HIV co-infection) and those in vulnerable populations (eg. people who use drugs - PWUD). Here, we determine the efficacy of DAA medications in Nova Scotia by examining the rates of sustained virologic response (SVR) following DAA treatment in subpopulations of different sub-groups of HCV-infected individuals.

Methods: Information was collected from the medical charts of 258 individuals seen at the infectious diseases clinic of the Queen Elizabeth II Health Sciences Centre in Nova Scotia from 2011 until 2017 for HCV care. An anonymized database was compiled to view SVR rates among subpopulations and summarize demographics of the HCV infected population.

Results: The most common age group was between 25 and 34 years, followed by 55 to 64 years, and the cohort was primarily male (69% vs. 31% female). HCV genotype 1 (1a in particular) was most common, followed by genotype 3a. Stage of liver fibrosis ranged from F0 to F4. HIV co-infection was present in 17% of the population. Overall, DAA treatments were more effective than previous interferon-based treatment regimens (SVR 87% vs. 78%), with fewer relapse or failed treatment outcomes. Of the PWUD, currently or in the past, 36% have received treatment compared to 47% of those who never used intravenous drugs. HIV coinfected individuals had a lower SVR compared to those without (79% vs. 98%), primarily driven by treatment failures in the pre-DAA, IFN era. Most individuals (71%) were previous PWUD, with only 9% currently injecting drugs.

Conclusions: The database of HCV patients demonstrates that the outcomes of HCV treatment in the post- interferon era are very successful for all groups of individuals including PWUD, those co-infected with HIV and people who had previous HCV treatment. Those actively injecting drugs were not treated as frequently, in juxtaposition to Canadian and international treatment guidelines. These data suggest that HCV treatment rates in some populations need

to be maximized to adhere to current standard of care, and may require a change in the HCV model of care.

42. SILENCING RACK1 INHIBITS *SHIGELLA FLEXNERI* MOTILITY WHITIN HELA CELLS

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Introduction: *Shigella flexneri* is an intracellular bacterium that infects human colonic epithelial cells causing inflammatory colitis. This pathogen replicates in the cytoplasm of cells and induces the polymerization of host actin to facilitate dissemination. The receptor for activated C kinase 1 (RACK1) is a scaffolding protein that provides a platform essential for protein-protein interactions, therefore playing pivotal roles in cell motility and protein synthesis. RACK1 has been reported to bind to components of the cell cytoskeleton. Since *S. flexneri* requires host cell cytoskeleton machinery that RACK1 interacts with, we hypothesise that *S. flexneri* requires RACK1 function to spread in HeLa cells.

Methods: RACK1 was knocked down in HeLa cells using lentiviral shRNA. RACK1 expression was assessed by western blotting. *S. flexneri* intracellular growth in wildtype (WT) and RACK1 knock down (KD) HeLa cells was assessed by quantification of bacterial colony forming units (CFU). Bacterial spreading was evaluated by plaque formation assay. Actin tail formation and bacterial intracellular motility was assessed by immunofluorescence (IF) and live microscopy.

Results: RACK1 expression was successfully knocked down using lentiviral shRNA. RACK1-KD cells infected with *S. flexneri* showed lower CFU compared to WT cells. Plaque formation induced by *S. flexneri* spreading was diminished in RACK1-KD HeLa cells compared with control. IF experiments showed that *S. flexneri* induced less actin tail formation in RACK1-KD HeLa cells versus WT cells. Live microscopy measurements of bacterial motility showed diminished velocity and distance travelled by *S. flexneri* in RACK1-KD HeLa cells. Directionality of *S. flexneri* motility was impaired in RACK1-KD HeLa cells.

Conclusions: RACK1 depletion is detrimental to *Shigella* growth in HeLa cells, suggesting that this pathogen requires RACK1 to successfully infect the host. RACK1 is required for efficient bacterial cell-to-cell spreading, actin tail formation and intracellular motility in HeLa cells. These data contribute to the understanding of the mechanisms by which *S. flexneri* exploits the host cellular machinery to sustain its intracellular life style.

43. RESPIRATORY SYNCYTIAL VIRUS (RSV) INFECTION IN PRETERM INFANTS AND LATER ONSET OF ASTHMA

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Introduction: Respiratory Syncytial Virus (RSV) is the leading cause of viral lower respiratory tract infections in infants. RSV infections have a substantial disease burden particularly among preterm infants who are at a high risk of hospitalization (RSV-H). Increased risk for asthma is hypothesized to result from RSV infection in early life. We estimated the incidence rates of RSV-H in the first year life, and asthma during a 5-year individual follow-up, and evaluated the association between asthma and preceding RSV-hospitalization.

Methods: A retrospective cohort of preterm infants aged 29 weeks 0 days gestational age to 35 weeks 6 days gestational age was constructed from Nova Scotia (NS) population-based databases (NS Atlee Perinatal Database, CIHI Discharge Abstract Database, NS Palivizumab Utilization Program Database and MSI Physician Billing Database). The incidence rates of RSV-H and asthma were calculated. RSV-H and potential confounding factors were entered into a Cox proportional hazards (CPH) model to estimate the hazards ratio (HR) and 95% confidence interval (CI) for the association with asthma development.

Results: In the final cohort of 3,916, the incidence rate of RSV-hospitalization was 25/1000 infants. The cumulative incidence rate of asthma at 5 years of age was 37.1/1000 person-years. No infant prophylaxed with Pz was hospitalized with RSV or developed asthma. The CPH model yielded a HR (and 95% CI) of 1.58 (1.03-2.41).

Conclusions: The incidence of RSV-H in our cohort was lower than expected. There is a moderate association between RSV-hospitalization and asthma in preterm infants. Further Evidence of an association is needed to establish causation and estimate the size of the effect, if present. These results will be provided to the NS Palivizumab Utilization Management Program for RSV to aid decision-making in RSV-prevention and intervention strategies.

44. IMPACT OF PNEUMOCCOCAL CONJUGATE VACCINATION PROGRAMS ON *STREPTOCCOCUS PNEUMONIAE* DISEASE IN CHILDREN WITH CANCER: A REPORT FROM THE CANADIAN IMMUNIZATION MONITORING PROGRAM ACTIVE

Authors: **A Smith**, J McNair, J Bettinger, W Vaudry, OG Vanderkooi, S Desai, S Halperin, K Top

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Introduction: Children with cancer are at increased risk of invasive pneumococcal disease (IPD). The Canadian Immunization Monitoring Program ACTive (IMPACT) conducts active surveillance for IPD at 12 pediatric referral centres. Canadian provinces and territories introduced 7-valent pneumococcal conjugate vaccine (PCV7) into infant immunization schedules from 2002 to 2006 and 13-valent PCV from 2010 to 2011. The objective was to describe the epidemiology of IPD in children with cancer in the pre-PCV and PCV eras.

Methods: We analyzed IMPACT reports of IPD among children with cancer 0–16 years of age receiving chemotherapy or post-hematopoietic stem cell transplant (1991–2014). Clinical data were extracted from IMPACT databases. IPD case counts were compared between the pre-PCV (1991–2002) and PCV eras (2003-2014). Immunization status and serotypes responsible for IPD were examined.

Results: From 1991-2014, 303 IPD cases were reported in children with cancer. Annual cases ranged from 7-20 each year. Mean annual IPD cases were similar in the pre-PCV and PCV eras (mean 13.17 vs 12.83, p=0.95). Manifestations of IPD were: bacteremia alone (67%), respiratory infection (11%), otitis media with bacteremia (8%), central nervous system infection (6%), septic shock (3%), and other (5%). Median hospital stay was 6 days, 23 patients (8%) were admitted to ICU and 4 patients (1%) died. Sixty-one percent of cases occurred before PCV was included in all provincial programs. Of cases who were eligible for infant PCV, 43% were age-appropriately immunized (≥3 PCV doses and 1 dose after 1 year of age), 19% were incompletely immunized (<3 PCV doses or 0 doses after 1 year of age), 37% had unknown immunization status. During the PCV era, 4 age-appropriately immunized cases developed IPD due to PCV7 serotypes and were considered vaccine failures. Cases immunized with ≥1 dose of PCV (n=65) were less likely to have IPD due to a serotype in PCV7 than cases that did not receive PCV (n=238) (22% versus 52%, p<0.001).

Conclusions: Mean cases of IPD in children with cancer remain unchanged in the PCV era. The

frequency of cases due to serotypes found in the PCV7 vaccine was lower among PCV-immunized patients, suggesting a strong benefit of vaccination in this population.

45. A PUBLIC HEALTH APPROACH TO ENHANCING ADULT IMMUNIZATION IN PEI

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Affiliation: Government of Prince Edward Island

Introduction: Despite the availability of publically funded vaccines, adults account for 73.9% of the vaccine preventable disease (VPD) deaths in Canada. Recognizing the need to improve adult immunization coverage, the National Immunization Strategy (NIS) has established a target of 80% coverage of pneumococcal immunization among adults aged 65 and older by 2025. Vaccine uptake can be influenced by vaccine hesitancy, defined by the World Health Organization (WHO) as "*the delay in acceptance or refusal of vaccines despite availability of vaccination services*". The factors contributing to vaccine hesitancy may include a lack of awareness, lack of vaccine access, and concerns about the safety and efficacy of vaccines. In an effort to improve adult immunization rates, the government of PEI was awarded funding from the Public Health Agency's Immunization Partnership Fund for an initiative to boost adult immunization rates across the province.

Methods: In the fall of 2017, Public Health Nurses (PHNs) screened adults at PHN Influenza Immunization Clinics using a question-and-answer based immunization assessment tool. Nurses were able to verify client vaccine histories using a newly designed electronic registry that consolidates immunization records from multiple health systems into a single searchable database. Social media, news releases, and print advertisement campaigns were used to raise the overall public awareness about the need for adult immunizations. In conjunction with CANImmunize, a web-based version of the immunization assessment tool was developed to allow adults to self-assess their vaccine needs. In addition, adults attending PHN influenza clinics were surveyed about their knowledge, attitudes, beliefs, and behaviours (KABB) about adult immunizations.

Results: Over 700 adults were screened using the Immunization Assessment Tool at PHN Influenza Immunization Clinics and over 900 adults completed the KABB survey. A follow up survey will be conducted in the spring of 2018. Of the adults who participated in the immunization assessment, 75% were in need of at least one additional vaccine. Approximately 1/3 of the under-immunized adults were identified as high risk for contracting or developing serious complications from VPDs.

Conclusions: Although vaccination is one of public health's most successful disease prevention strategies, the identification of under-immunized adults in a vaccine-ready population at the

influenza immunization clinics highlights that there are missed opportunities to discuss immune status with clients at various encounters with the healthcare system. The short- and long-term impacts of the adult immunization intervention as well as the feasibility of implementing screening opportunities into regular public health programming will continue to be evaluated leading up to the 2025 NICS deadline.

46. IDENTIFICATION AND CHARACTERIZATION OF MUTATIONS RESPONSIBLE FOR MOUSE ADAPTATION OF INFLUENZA A VIRUS A/California/07/2009(H1N1)

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Introduction: Influenza A viruses (IAV) are rapidly evolving viruses that exist as dynamic populations known as quasispecies. Recently, a new IAV entered the human population causing the 2009-2010 pandemic. The murine model is an attractive platform to study interspecies transmission and subsequent host adaptation. Mice are not naturally infected by IAV, and adapting IAV to mice requires manual passaging of the virus from infected lungs to naive hosts. Characterising the genetic changes that are necessary for virus adaptation in a new mammalian host is necessary to inform our understanding of IAV transmission and pathogenesis.

Methods: Reference influenza A/California/07/2009(H1N1) virus strain was mouse-adapted by 10 serial lung passages in outbred Swiss-Webster mice. Next, the parental reference strain and the mouse adapted virus from passage 10 were sequenced using Illumina deep sequencing platform. Reverse genetics were utilized to introduce mutations found in the RNA polymerase genes into the parental strain and their contribution to viral replication efficiency in mouse cells was tested.

Results: Seven non-synonymous substitutions were found in mouse-adapted virus genome compared to the parental strain. Of these, the substitution E349G in the PA subunit of viral RNA polymerase was shown to increase RNA polymerase activity and contribute to increased replication of the recombinant virus in murine cells.

Conclusions: Together, these findings provide evidence that adaptive mutations can increase IAV polymerase activity, which correlates with increased viral replication and virulence in mice. Understanding the mutations required for host adaptation is critical in surveillance of zoonotic influenza.

47. KNOWLEDGE, ATTITUDES, BEHAVIOURS, AND BELIEFS OF HEALTHCARE PROVIDER STUDENTS REGARDING MANDATORY INFLUENZA VACCINATION

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Introduction: Influenza infection poses the same risk to healthcare students and to their patients as practicing clinicians. While there is substantial dialog about the benefits, risks, and ethics of mandatory influenza vaccination policies in Canada, there has been little engagement of healthcare students. Our objective in conducting this study was to explore the knowledge, attitudes, beliefs, and behaviours of Canadian medical, nursing, and pharmacy students towards mandatory policies related to influenza vaccination.

Methods: A web-based survey with 40 questions was administered to medical, nursing, and pharmacy students at Dalhousie University in Halifax, Nova Scotia.

Results: Influenza vaccination status varied by program type, with 86.3% of medical student respondents (n=124) self-reporting receipt of the influenza vaccine both in the previous and current seasons, 52.4% of nursing students (n=96) self-reporting receiving both, and pharmacy students' coverage falling between the two. Pharmacy students had higher mean knowledge scores (10.0 out of 13 questions) than medical (9.26) and nursing (8.88) students. Between 56.1 and 64.5% of students across disciplines were in support of a mandatory masking or vaccination policy, and between 72.6 and 82.3% of students would comply if such a policy were in place. A sense of duty to be immunized, desire to be taught more about influenza and influenza vaccine, belief that the hospital has a right to know vaccination status, support for declination policy, and willingness to accept consequences of noncompliance were all predictors of student support of mandatory vaccination policies.

Conclusions: Medical and pharmacy students tended to hold more pro-influenza vaccination attitudes, had higher knowledge scores, and better vaccine coverage than nursing students. Despite these differences, based on the overall vaccination behavior, knowledge, beliefs, and attitudes of students surveyed in this study, mandatory influenza vaccination is generally supported by the next generation of practitioners.

48. A COMPARATIVE EVALUATION OF THE BURDEN OF DISEASE CAUSED BY INFLUENZA A AND INFLUENZA B DURING THE 2011/2012, 2012/2013 AND 2013/2014 INFLUENZA SEASONS IN CANADA

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Introduction: When assessing burden of influenza disease, influenza B has typically been associated with infection in children and young adults, and is considered less prevalent and/or severe in older adults. We sought to assess the burden of influenza type A disease compared to influenza type B disease in Canadian adults (>16 years) admitted to hospital with laboratory-confirmed influenza.

Methods: The Serious Outcomes Surveillance (SOS) Network of the Canadian Immunization Research Network (CIRN) conducted active surveillance for laboratory-confirmed influenza in adults (≥16 years) hospitalized across Canada during the 2011-2014 influenza seasons. Each season, SOS Network monitors reviewed all daily admissions of adult patients (≥16 years) admitted to hospital with any acute respiratory illness or symptom. Eligible patients had a nasopharyngeal swab collected and tested for influenza virus using reverse transcriptase polymerase chain reaction. Demographic/clinical information, as well as in-hospital outcomes such as duration of hospitalization, admission to the ICU, and influenza-attributable mortality, was collected. Frailty Index scores were also recorded at baseline and 30-days after discharge, when possible, in patients ≥65 years. Patients with influenza A and B were compared using descriptive statistics; discrete outcomes were compared using Chi-squared (χ^2) and Cochran-Mantel-Haenszel methods; continuous outcomes were compared using student's t-tests.

Results: Overall, there were 3484 influenza A cases and 1375 influenza B cases enrolled in the SOS Network from 2011-2014. There was a significant difference in patient age between influenza A and influenza B cases (mean age of influenza A: 65.8, mean age of influenza B: 71.2, $p<0.01$). A significantly larger proportion of influenza B patients were admitted from long-term care (A: 5.5%, B: 12.1%, $p<0.01$). There was no significant difference with respect to length of hospitalization (influenza A: 11.1 days, influenza B: 10.27 days, $p=0.07$) or mortality (A: 9.01%, B: 9.45%, $p=0.63$) between influenza A and B. Patients with influenza B were significantly more frail prior to the onset of illness (A: 0.21, B: 0.22, $p<0.01$)

Conclusions: Current attitudes consider influenza A to be the more significant virus in terms of morbidity and mortality. However, influenza B is responsible for similar duration of hospitalization and similar mortality rates. In addition, influenza B is dominant in affecting the frail elderly and thus optimizing influenza B protection is important in this population.

49. COMBATING STREP INFECTIONS: CHARACTERIZATION OF EXOTOXIN FOLDING PATHWAYS IN GROUP A STREPTOCOCCUS BACTERIUM

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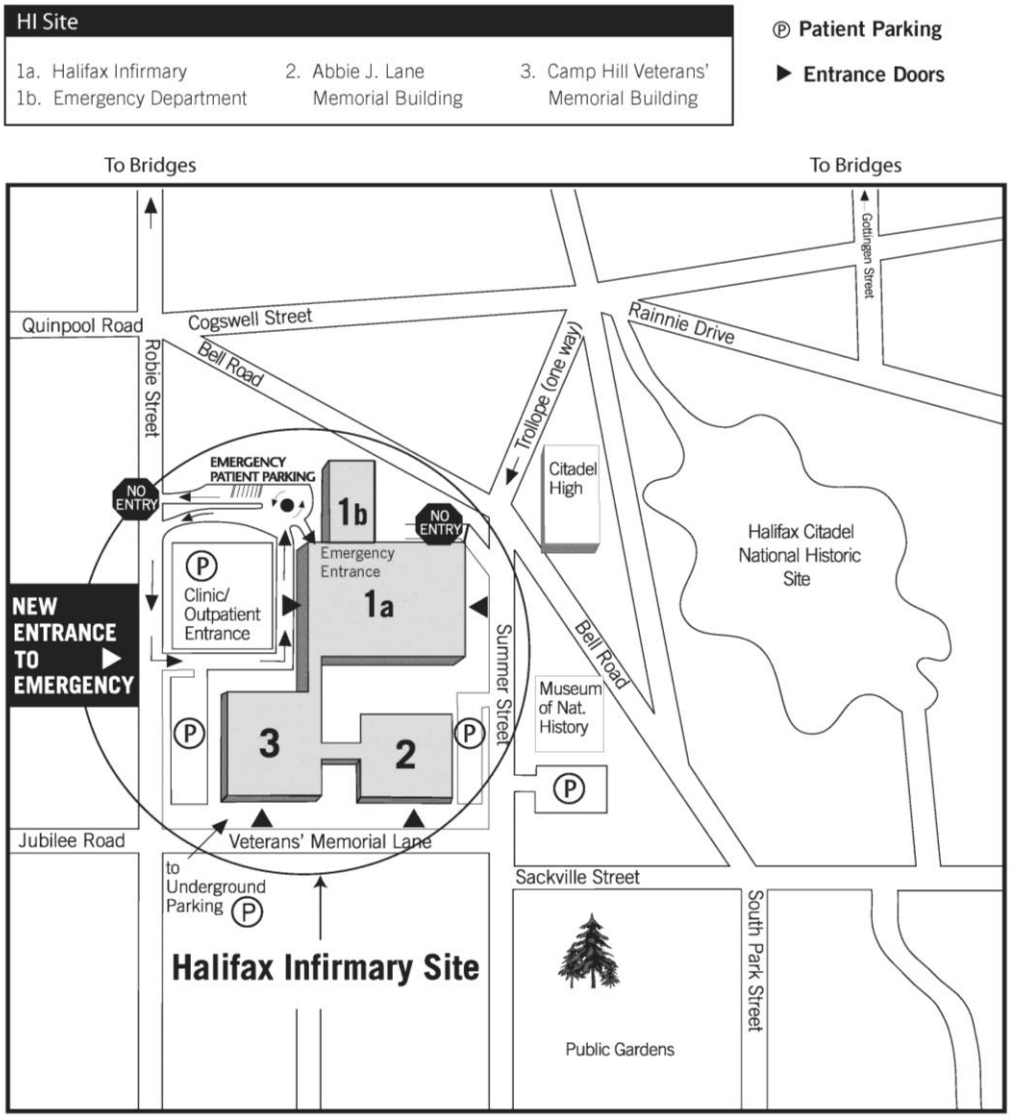
Introduction: Group A *Streptococcus* (GAS) is a pathogenic bacterium that strictly infects humans, causing a range of diseases from severe toxic shock syndrome to moderate strep throat infection, especially in children. The ability of most bacteria, including GAS, to secrete toxins is a strategy used to help promote their survival inside the human body. Structural bonds and linkages are important for the proper folding and stability of protein toxins, and any disruptions in the natural protein-making processes of bacteria lead to misfolded proteins, which are quickly degraded without further damaging the human host. To date, there is little known about the enzymes and molecular pathways involved in the proper folding of exotoxins produced by GAS.

Methods: An *in silico* approach was used to identify 5 candidate enzymes predicted to be involved in proper protein formation in GAS. Candidate enzymes were mutated one-by-one using gene insertional inactivation techniques and their subsequent biological roles characterized. Differences in phenotypic assays, including resistance to oxidative stress, exotoxin production, *in vivo* protein redox status and bacteria percent killing by macrophage cell lines, were assessed compared to the parent GAS strain.

Results: Our findings suggest that one enzyme in particular (2037) is needed for proper disulfide bond formation in an important GAS exotoxin: streptococcal pyrogenic exotoxin A (SpeA). Redox state analysis of SpeA secreted by the parent GAS culture showed the presence of a disulfide linkage (oxidized form), however this disulfide bond was broken (reduced form) in mutant 2037 cultures. Additionally, purified recombinant 2037 enzyme was able to reform the disulfide bond in reduced SpeA during an *in vitro* disulfide exchange reaction. Bacteria lacking the 2037 enzyme also showed significantly increased sensitivity to oxidative stress compounds and appeared to be more susceptible to phagocytic death.

Conclusions: This is the first report of an enzyme being directly involved in the proper folding of GAS exotoxin SpeA. Our novel findings highlight the importance of protein-folding-pathways in modifying virulence factors secreted by pathogenic bacteria and pave the way for new drug targets and vaccine development strategies that offer an alternative to antibiotics.

QEII Halifax Infirmary Site
(Lecture in Royal Bank Theatre)



Dalhousie University Site
(Presentations held in SUB)