19th Annual Infectious Diseases Research Day

&

6th Annual Canadian Center for Vaccinology Symposium

April 14, 15, 2014

Halifax

Sponsored by

Canadian Center for Vaccinology

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

Dalhousie Infectious Diseases Research Alliance

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Welcome

Lynn Johnston MD,FRCPC
Chief, Div of Infectious Diseases,
Dept of Medicine, Dalhousie U

Scott Halperin MD,FRCPC
Director
Canadian Center for Vaccinology

Welcome to the 19th Annual Infectious Diseases Research Day and 6th Annual CCfV Symposium.

Again this year, we have combined Infectious Diseases Research Day and CCfV Symposium to bring a unique learning opportunity with experienced presenters and colleagues. Our guest presenters come from far away and right here in Halifax, to share knowledge and insights from their research.

The poster and oral abstracts will demonstrate the variety of research conducted by local faculty and trainees from different disciplines, confirming the need for innovative approaches to address pressing health issues.

As evidenced by recent outbreaks of preventable diseases, despite our best efforts to control them, our knowledge of vaccines and viruses continually evolves. In addition to microscopic examination, we must also look at the human behavior that leads to acceptance, or not, of evidenced based prevention methods. The knowledge translation of our research is as important as the initial design. As participants in this educational event, we encourage you to discuss what you have learned with others, and translate the evidence into your own front line practice.

This event is made possible by a dedicated planning committee, together with financial support from our corporate partners. We thank them all, as well as study participants who help us recognize and advance our necessary health research.

With thanks to….

This program is supported in part by educational grants provided by:

The Planning Committee

Joanne Langley, Chair
Mary Appleton
Susan Brushett
Glenn Campbell
Michael Fleming
Shelly McNeil
Audrey Steenbeek

Program

Monday April 14

1:00 – 2:00pm	Presentation – Mark Steinhoff MD *Maternal immunization: a 3-for-1 strategy*	IWK Health Centre O.E. Smith auditorium
2:00 – 2:30pm	Presentation – Courtney Ward PhD *Influenza Vaccination Programming in Canada*	
2:30 – 3:00pm	Presentation – Todd Hatchette MD *Towards Heterosubtypic immunity; the holy grail of influenza vaccines*	
3:00 – 4:30pm	Poster judging (posters on display 1:00 – 5:30)	IWK Health Centre Gallery
4:30 – 5:30pm	Public presentation – Mark Steinhoff MD *Vaccine trials and serendipity*	IWK Health Centre O.E. Smith auditorium
5:45 – 7:00pm	Reception hosted by Tom Marrie MD, Dean of Medicine Presentation – Gerald Johnston PhD *Dalhousie's Year of the Microbe; emerging trends in health research*	Tupper Link Theatre B

Tuesday April 15

8:00 – 9:00am	TJ Marrie Lecture (Grand Rounds) Didier Raoult MD PhD *Rebirth of culture in microbiology*	Halifax Infirmary RB Theatre
9:20 – 12:30pm	Oral Presentations (10)	1613 A-B Veterans' Hospital
12:30 – 2:00pm	Buffet lunch & presentation by Didier Raoult MD PhD *Emerging Rickettsioses*	1613 A-B Veterans' Hospital

Speakers

Mark Steinhoff MD

Dr. Steinhoff is a pediatrician with infectious disease sub-specialization. His undergraduate degree and MD was from the University of Chicago. He was a pediatric resident, chief resident, and pediatric infectious diseases fellow at University of Rochester, NY. He served on the faculty of the Department of Child Health, CMC Hospital, Vellore, India from 1980-1985. He has held faculty positions in the Departments of Public Health and Pediatrics at the University of Michigan, and since 1986 has been faculty at the School of Medicine, and the Bloomberg School of Public Health at Johns Hopkins University in Baltimore. He is currently Professor of Pediatrics and Director of the Children's Global Health Center at Cincinnati Children's Hospital in Ohio.

He has carried out research projects in a variety of regions including South and East Asia, Africa, South America, and Europe. He has authored over 160 peer-reviewed research papers, and over 20 textbook chapters in pediatric and tropical medicine textbooks.

His major research interest is in assessing the burden of preventable infectious diseases, and the effectiveness of vaccines in low resource settings. He has served as a consultant to CDC, NIH, FDA, WHO, the Rockefeller Foundation, the Ford Foundation, and as an advisor to the Bill and Melinda Gates Foundation. With colleagues he is currently conducting a large antenatal influenza vaccine trial in Nepal, with Gates Foundation support, and a NIH-funded post-partum influenza vaccine trial in the United States.

Didier Raoult MD PhD

Didier Raoult is a French biology researcher. He holds MD and PhD degrees, and specializes in infectious diseases. He is "classified among the first ten French researchers by the journal Nature, for the number of his publications (a credit of more than one thousand) and for his citations number.

In 1984, he created the Rickettsia Unit at Aix-Marseille University. He also teaches infectious diseases in the Faculty of Medicine of the University of the Mediterranean, and since 1982 has managed 74 M.D. theses and since 1989, 38 PhD theses. As of 2010, he has 1,531 indexed publications, with a sum of the times cited of 35,526 and an H-index of 83 (source: SCI, Web of Science), including 7 papers in *Science* and 2 in *Nature*, (source: PubMed) the two most representative reviews according to the academic ranking of world universities.

Since 2008, professor Raoult has been the director the "URMITE" i.e. the Research Unit in Infectious and Tropical Emergent Diseases, collaborating with CNRS (National Center for the Scientific Research), IRD (Research for the Development Institute), INSERM (National Institute of Health and Medical Research) and the Aix Marseille University, in Marseille. His laboratory employs 140 people, including 45 very active researchers who publish between 150 and 200 papers per year, and had produced 29 patents to date.

Courtnay Ward PhD

Courtney J. Ward is an Assistant Professor of Economics at Dalhousie University. She received her Ph.D. from the University of Toronto and her M.A. from Queens University. Her research focuses on the implications of externality and selection effects in health production, and her work has particular emphasis on how these aspects interact with policy in intended or unintended ways. Her work has been published in journals such as the American Economic Journal: Applied and the Canadian Journal of Economics. A list of her current working papers can found here: http://myweb.dal.ca/cr723224/.

Todd Hatchette MD

Associate Professor, Department of Microbiology and Immunology. Dr. Hatchette earned his MD from Memorial University of Newfoundland in 1995. He completed Internal Medicine training at Memorial University in 1997, followed by a Fellowship in Infectious Diseases and Medical Microbiology at Dalhousie University. Dr. Hatchette completed postdoctoral research training in virology at St. Jude Children's Research Hospital in Memphis, Tennessee, under the supervision of Dr. Robert G. Webster, a world authority on influenza A. He returned to Dalhousie in January 2004 to take over as Director of Virology and Immunology in the Division of Microbiology. In addition, he is cross-appointed with the Department of Medicine where he is an Infectious Diseases consultant.

Dr. Hatchette's main area of interest is Influenza A. Dr. Hatchette is also a collaborator in the newly created, CFI-funded, Canadian Center for Vaccinology, Halifax, (CCVH) that will bring together basic and clinical researchers investigating vaccine approaches to control infectious diseases.

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Abstracts

1. SYNERGISTIC EFFECT OF NOVEL ACYL CARRIER PROTEIN SYNTHASE (ACPS) INHIBITORS WITH POLYMYXIN B ON BACTERIAL GROWTH

Robert Boudreau, C. Barden, S. Brown, J. Browne, D. Byers, L. Fisher, A. Henneberry, E. Lu, C. McMaster, A. Meek, K. Sullivan, S. Sun, M. Taylor, D. Weaver, F. Wu

Affiliation: IWK Health Centre, Chemoinformatics and Drug Discovery Laboratory (CDDL)

Introduction: The decline in the discovery of new antibiotics, the misuse of current antibiotics, and the emergence of multi-drug resistant (MDR) bacteria have all led to antibiotic resistance being one of the top threats to human health. Alternative antibiotic targets represent the most direct way to reverse this crisis. Acyl carrier protein synthase (AcpS) is an essential bacterial enzyme, widely conserved, with no known mammalian homologue. AcpS small molecule inhibitors have been developed at the IWK Health Centre's Chemoinformatics and Drug Discovery Laboratory (CDDL), through a continuous and overlapping pipeline of computational modeling, rational drug design, synthetic organic chemistry, enzymology, and microbiology. A number of novel chemical entities (NCEs) have been shown to inhibit the growth of a variety of Gram positive and Gram negative bacterial strains, with numerous possibilities for use in combination with existing antibacterials. A decades-old antibiotic targeting Gram negative bacteria, polymyxin B, has been relegated to a last line of defense against MDR bacteria due to its toxicity levels, and so it would be beneficial if a newly-discovered antibiotic class was able to potentiate its effect, necessitating a lesser concentration of polymyxin B to achieve the same therapeutic effect.

Methods: AcpS in vitro enzyme assays were carried out in the presence of inhibitor compound or vehicle alone (dimethylsulphoxide; DMSO), to provide IC_{50} data. In vivo minimum inhibitory concentration (MIC) data employing a standardized 96-well plate assay were also obtained for growth inhibition effect on relevant bacteria, including against both Gram positive and Gram negative strains as indicated. Synergistic effects were determined by a slight modification of the MIC assay employing matrix-style concentration layouts. Toxicity was determined through an Alamar Blue cell viability assay.

Results: A number of compounds tested were able to inhibit AcpS in a standard in vitro enzyme assay, with corresponding growth inhibition of a panel of Gram positive bacteria. Interestingly, a sub-group of candidates, with no effect on Gram negative growth at concentrations at least as high as 500 uM when used alone, are able to synergize with polymyxin B even when they are delivered at low micromolar concentrations, to reduce its MIC on those Gram negatives. Furthermore, one of these drug candidates (DNM 0774) is non-toxic to mammalian cells up to 25 uM – greater than ten-fold more than the concentration needed for efficacy against *K. pneumonia* when combined with polymyxin B.

Conclusions: A library of NCEs has been generated, capable of potent growth inhibition of Gram positive and Gram negative bacteria. An effect of a subset of these compounds on select Gram negatives such as *K. pneumonia* is a synergism with polymyxin B, with a potentially safe therapeutic index as determined by mammalian cell viability assays. Further experiments are required to more specifically determine the pharmacokinetics in animal models of administration.

2. THE ROLE OF *SHIGELLA FLEXNERI* IN AN ORAL MURINE INFECTION MODEL

Angela Daurie, L. Zhu, S. Gruenheid, J. Rohde

Affiliation: Department of Microbiology and Immunology, Dalhousie University

Introduction: *Shigella flexneri* is the causative agent of dysentery that kills thousands of people each year. It mainly affects children in the developing world where there is poor sanitation and lack of access to clean drinking water. An oral murine infection model, that uses BALB/c mice treated with the broad-spectrum antibiotic streptomycin, has been established and displays several hallmarks of *Shigella* infection in humans. Importantly, the streptomycin treated model of infection using wild-type *Shigella* does not result in mortality.

Methods: We have used this model to investigate the role of several mutant *Shigella* strains during infection and we have infected several different strains of mice with wild-type *Shigella*. We have collected weight loss, bacterial burden, and survival data from these experiments.

Results: We have identified a *Shigella* mutant (*ΔospG)* that causes 30% mortality in BALB/c mice. There is no significant change in bacterial burden and the histology of the infected colon does not reveal differences in the severity of gut pathology. The mechanism behind this increase in mortality is under investigation. Infecting various mouse strains with wild-type *Shigella* results in a range of disease, from severe with incidence of mortality to colonization with no signs of clinical illness. We have identified a susceptible strain of mouse that has increased mortality compared to the published BALB/c model, providing a possible system to identify genes that control susceptibility to infection.

Conclusions: The streptomycin treated mouse model provides an oral model of infection that can be used to examine genetic factors of *Shigella* pathogenesis in both the host and pathogen.

3. THE STREPTOCOCCUS GORDONII DISULFIDE OXIDASE SDBA EXHIBITS ACTIVITY USING A SINGLE C-TERMINAL CYSTEINE OF THE CXXC MOTIF

Lauren E. Davey, Scott A. Halperin, Song F. Lee

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

Introduction: Disulfide oxidases form protein disulfide bonds using a CXXC catalytic motif. In enzymes such as *Escherichia coli* DsbA, the paradigm for disulfide bond formation, the distinct properties of the active site cysteines are well established. The N-terminal cysteine interacts with substrates, while the buried C-terminal cysteine reacts solely with the first cysteine of the CXXC motif, forming an intramolecular bond. Previously, we discovered a disulfide oxidase, SdbA, in the bacterium *Streptococcus gordonii*, a potential live vaccine vector. Here we investigate the SdbA $C^{86}P^{87}D^{88}C^{89}$ catalytic motif.

Methods: Site directed mutagenesis was used to replace one or both SdbA active site cysteines in *S. gordonii* SecCR1, a strain that secretes a test protein that requires two disulfide bonds for folding, anti-CR1. The point mutants were tested for the ability to complement a *ΔsdbA* mutant.

Results: Western blots showed that the serine protease DegP fully degraded anti-CR1 in a *ΔsdbA* mutant, however, complementation with SdbA C86P (PXXC) restored production up to ~25% of the parent, signifying that the single C-terminal cysteine generated correctly folded disulfide bonded protein. The C86P mutant also complemented other *ΔsdbA* phenotypes, including biofilm formation, autolysis, bacteriocin production. In contrast, C89A (CXXA) and C86P/C89A (PXXA) mutants showed no indication of activity. Unexpectedly, RNase A folding assays showed that both mutants were active in vitro, suggesting that the difference in activity involved factors in the cell. Non-reducing blots showed that a C89A *ΔdegP* mutant formed disulfide-linked complexes when grown under aerobic conditions, suggesting a role for H_2O_2, which *Streptococcus* produces in the presence of oxygen. Conversely, eliminating H_2O_2 production through mutation of *spxB* restored anti-CR1 production to the C89A mutant.

Conclusion: The SdbA C-terminal cysteine shows oxidase activity, whereas the N-terminal cysteine is inactive due to inhibition by H_2O_2. This suggests that SdbA uses a novel mechanism for disulfide bond formation, possibly as an adaptation to endogenous H_2O_2 production. Understanding basic mechanisms in protein production will contribute to the development of *S. gordonii* live vaccines.

4. COMPARISON OF TWO MOLECULAR TYPING METHODS FOR *STREPTOCOCCUS PNEUMONIAE*

May ElSherif[1], A. Lang[1], S. McNeil[1,2], T. Hatchette[1,3], and J. Leblanc[1,3]_on behalf of the Public Health Agency of Canada/Canadian Institutes of Health Research Influenza Research Network (PCIRN) Serious Outcomes Surveillance Network

Affiliation: [1]Canadian Center for Vaccinology, IWK Health Centre and Capital Health, Dalhousie University, [2]Division of Infectious Diseases, Deptartment of Medicine, Capital Health, [3]Department of Pathology and Laboratory Medicine, Capital Health

Introduction: Two molecular methods for the typing of S. pneumoniae (Spn) have been developed by the Centers for Disease Control and Prevention (CDC) based on multiplexed conventional PCRs (MP-PCR) or triplex real-time PCRs (TP-PCR). To date, no studies have compared these two molecular typing methods.

Methods: The performance of MP-PCR and TP-PCR were verified using previously characterized Spn isolates (n = 45) and a variety of other organisms. The limit of detection (LoD) for each assay was determined using dilutions of quantified Spn. For clinical evaluation, 1770 nasopharyngeal (NP) swabs were obtained from patients with or without invasive pneumococcal disease (IPD) that were screened for Spn using two real-time PCRs targeting lytA and cpsA. Typing was performed on specimens that were lytA- and cpsA-positive (considered Spn-positive).

Results: MP-PCR and TP-PCR were highly specific, but their LoDs (~6700 and ~4800 copies/ml, respectively) were less sensitive than the screening PCR assays (lytA ~950; cpsA ~550 copies/ml). Of the 132 NP swabs identified as Spn-positive, 15 were assigned a type by MP-PCR alone since it has the ability to identify a larger number of different Spn types compared to TP-PCR. However, TP-PCR identified an additional 19 types in specimens that had low bacterial loads due to its increased sensitivity. Using an algorithm-based approach with both typing methods, a type could be assigned for 69 (52.3%) Spn-positive specimens. A large proportion of Spn-positive specimens remained untypeable: 33 had DNA concentrations that fell below the LoD for both assays and 30 displayed strong screening results suggesting types that were not included in either typing method.

Conclusions: While the molecular methods for typing of Spn are valuable tools for epidemiologic studies, further optimization is required to achieve sensitivities equivalent to molecular detection methods and typing methods should be expanded to identify and discriminate between all Spn types.

5. EPIDEMIOLOGY OF *MYCOBACTERIUM TUBERCULOSIS* WITHIN NEW BRUNSWICK

Brandyn Chase, Stephanie Purcell[ab], Stefanie Materniak[c], Hope MacKenzie[d], Duncan Webster[a,c,d,e]

Affiliation: [a]Dalhousie Medicine New Brunswick, Saint John, New Brunswick, Canada [b]*Horizon Health Network, New Brunswick, Canada*[c] *Infectious Diseases Research Unit, Saint John Regional Hospital, Saint John, New Brunswick, Canada*[d] *Level 3 Laboratory, Division of Microbiology in the Department of Laboratory Medicine, Saint John, New Brunswick, Canada.*[e] *Division of Infectious Diseases in the Department of Medicine, Saint John Regional Hospital, Saint John, New Brunswick, Canada*

Introduction: *Mycobacterium tuberculosis* (MTB) is classically viewed as a pulmonary disease, but can affect almost any organ system. Globally, the burden of the disease has actually fallen since 2006, however, in 2010 there were still 8.8 million active cases of tuberculosis worldwide, 1322 of which were in Canada. The laboratory at the Saint John Regional Hospital (SJRH) has maintained complete provincial records dating back to 1988.

Methods: This study retrospectively examined all new and resurgent cases of MTB in New Brunswick between 1988 and 2012 found in the Mycobacterium database in Microbiology department at the SJRH. Demographics tracked included: gender, age, body site source, health region, and antibiotic sensitivities. Population data for the province was obtained using table 051-0001 from Statistics Canada.

Results: A total of 285 MTB isolates were identified in the province within the timeline of the study, 6 of which were excluded as treatment for bladder cancer. Of the remaining 279, 76.5% were pulmonary specimens and lymphatic (7.7%) and genitourinary (8.1%) were the next most common sites of infection. The mean incidence rate was 1.50 MTB cases/100,000 population (1.13-1.86, 95% CI). Antibiotic resistance to at least one agent was seen in 7.9% of cases, with isoniazid resistance (5.7%) being most common.

Conclusions: *M. tuberculosis* infections are an ongoing healthcare problem in New Brunswick, although the incidence rate has decrease since the late 1980s. Both the sites of infection and the antibiotic resistance patterns were in congruence with national averages reported in the literature.

6. LYME DISEASE IN NOVA SCOTIA: HOW MANY HUMANS HAVE BEEN INFECTED?

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Background: Over the last 20 years, *Ixodes scapularis* ticks have been identified with increasing frequency in Nova Scotia (NS) and are now identified as endemic in at least six regions. As a result, the number of human cases of Lyme disease (LD) in NS has increased, with most cases occurring in localities where *I. scapularis* populations are endemic.

Objective: To understand the risk of LD for people living throughout NS.

Methods: A seroprevalence survey for antibodies to *Borrelia burgdorferi* was conducted from June - August 2012. To provide a representative provincial estimate, a convenience sample of residual sera submitted for diagnostic testing was used. Serum samples were selected from all regional hospitals in each health district proportional to the population age and sex distribution. Sera were screened using a commercially available EIA (*B. burgdorferi* ELISA II, Wampole Laboratories, Princeton, NJ) and positive and equivocal results were sent to the NML for testing using C6 ELISA and Western blot testing. A positive IgG Western blot was considered conclusive evidence of previous infection.

Results: A total of 1855 sera were submitted and screened. Of these, 215 (11.6%) were sent to the NML for further testing. Only 17 of these were positive or equivocal on the C6 EIA and only 2 had indeterminate IgG Western Blots, resulting in a population seroprevalence of 0.11% (95% confidence interval 0.01 – 0.43%). One of the indeterminate results came from an area not know to be endemic for Lyme disease.

Conclusion: While LD is an emerging infection in NS, the estimated number of Nova Scotians who have evidence of infection is low.

7. APPLICATION OF GENETIC BARCODES IN THE INVESTIGATION OF SHIGELLA VIRULENCE

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Introduction: *Shigella spp.* continues to be a major health concern with the emergence of antibiotic resistance. Vaccination may represent the best option as a preventative measure; however no vaccine is currently available. A plasmid required for virulence by all four species of *Shigella* may hold the key to developing a vaccine with species-wide protection. This plasmid encodes several genes that are essential to pathogenesis, while a large number remain without a defined function. Defining how these genes contribute to virulence may assist in the development of a live attenuated vaccine strain.

Methods: A collection of 100 *Shigella flexneri* mutants have been generated, each with a single deletion within the pwR100 virulence plasmid. A unique DNA barcode was also inserted into the plasmid of each mutant. These barcodes form the basis to distinguish the mutants from each other using a PCR-based detection assay. To validate the barcode detection assay, pools of mutants were assessed in a gentamicin protection assay. This *in vitro* assay distinguishes between bacteria that can enter epithelial cells and those that do not. This ability to evaluate internalization was used as a measurement of virulence for each mutant.

Results: All *Shigella* mutants from the deletion collection could be indentified using the PCR barcode detection assay. Mutants were distinguished after being recovered from the gentamicin protection assay. Those mutants lacking a gene encoding for a component of the type three secretion system were undetected in bacteria recovered from internalization.
Conclusions: The barcode detection assay successfully distinguishes between mutants that can enter cultured epithelial cells and those that do not. This extremely sensitive and quantitative method paves the way for new approaches utilizing the pwR100 deletion collection as a *Shigella* virulence discovery tool.

8. PROPERDIN PLAYS A PROTECTIVE ROLE IN INFECTIOUS COLITIS

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Introduction: Properdin is a positive regulator of the complement system and functions to directly eliminate microbes and apoptotic cells. Whether properdin plays any role in intestinal inflammation is unknown. Consequently, we aimed to determine the contribution of properdin in a murine model of infectious colitis.

Methods: Colitis was compared between wildtype (WT) and properdin deficient (P^{KO}) mice infected with *Citrobacter rodentium* and evaluated by histological observations of the colon, immunohistochemistry and mediator levels in the colon, and TUNEL staining for apoptosis. Complement activation was documented using C3a and C5a levels. Properdin sufficient serum was transferred to deficient mice to restore properdin levels

Results: Properdin expression increased in infected WT colons as compared to uninfected controls. Furthermore, infected P^{KO} mice had lower complement activation but exacerbated pathology, particularly characterised by increased ulceration. Interestingly, bacterial colonization was not significantly different between the two strains. Further looking for a potential mechanism, we found properdin co-localized with apoptotic cells in the mucosa of WT mouse colons. During the course of inflammation P^{KO} presented with a significantly higher number of apoptotic epithelial cells and a subsequent greater rise in inflammatory markers in comparison to the WT controls, implicating an upset in the balance between the trigger and removal of apoptotic cells underlying the exacerbation in colitis. Lastly, properdin sufficient serum restored C5a levels, reduced apoptotic cells and rescued P^{KO} mice from exacerbated inflammation

Conclusions: Properdin contributes to host defense during infectious colitis, possibly by reducing the number of apoptotic cells.

9. CHARACTERIZATION OF A MUTANT DEFECTIVE IN CCDA2 A POTENTIAL REDOX PARTNER OF THE DISULFIDE BOND-FORMING PROTEIN SDBA IN *STREPTOCOCCUS GORDONII*

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Introduction: The formation of disulfide bonds via thiol disulfide oxidoreductases (TDORs) is crucial for the proper folding and activity of many extracellular proteins, including a range of virulence factors. We recently discovered a novel TDOR named *Streptococcus* disulfide bond protein A (SdbA) in *Streptococcus gordonii* that contributes to multiple phenotypes. *S. gordonii* is one of the pioneer organisms colonizing the oral cavity of infants and a potential live oral vaccine vector. In this study, we constructed a *ccdA*$_2$ mutant as a first step towards testing CcdA2 as a redox-partner of SdbA.

Methods: A *ccdA*$_2$ mutant was constructed by allelic replacement strategy using an erythromycin-resistant cassette *ermAM*. The inactivation of *ccdA*$_2$ was confirmed by PCR and reverse transcription (RT)-PCR. The production of a disulfide bonded-protein anti-CR1 scFv antibody was examined by western blotting.

Results: The inactivation of *ccdA*$_2$ resulted in an erythromycin-resistant mutant. PCR analysis showed that the *ccdA*$_2$ mutant had an expected 2.1 kb fragment using primers specific to *ccdA2* compared to 1.3 kb in the parent strain. In addition, the mutant, but not the parent strain, showed a 1.5 kb PCR product indicating that the *ermAM* cassette was inserted in *ccdA*$_2$. RT-PCR showed the absence of *ccdA*$_2$ transcript in the mutant confirming the inactivation of *ccdA*$_2$. In broth cultures, the mutant formed clumps and settled to the bottom, a feature also displayed by the s*dbA* mutant but not the parent strain. Western blotting revealed that the *ccdA*$_2$ mutant produced 50% less anti-CR1 scFv compared to the parent strain presumably due to misfolding leading to degradation by cellular proteases.

Conclusions: A *ccdA*$_2$ mutant was successfully constructed and confirmed by PCR and RT-PCR. This mutant showed a defect in the production of anti-CR1 scFv antibody suggesting that CcdA$_2$ may involve in disulfide bond formation by acting as a redox-partner of SdbA in *S. gordonii*.

10. KSHV MODULATES THE IRE1-XBP1 AXIS OF THE UNFOLDED PROTEIN RESPONSE DURING LYTIC REPLICATION

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The author requests that this abstract not be published

11. MAST CELLS PROTECT AGAINST *P. AERUGINOSA* INDUCED LUNG INJURY

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Introduction:
Lung damage caused by bacterial pneumonia is a significant concern among immune compromised and mechanically ventilated patients. *P. aeruginosa* is an opportunistic bacterium which causes both acute and chronic lung infections in these patient groups. A hallmark of lung damage during these infections is bacteria induced disruption of the respiratory epithelium. Mast cells regulate host immune responses to invading pathogens and are closely associated with epithelial cells in the airways. However the impact of mast cells on *P. aeruginosa* induced lung damage remains unclear.

Methods:
An acute *P. aeruginosa* lung infection model was employed in Kitw-sh/KitW-sh (W-sh) mast cell-deficient mice, W-sh mice reconstituted with wild-type mast cells and wild-type C57BL/6 animals in order to elucidate the contribution of mast cells to *P. aeruginosa* induced lung injury *in vivo*. An *in vitro* 16HBE14o- and HMC-1 transwell co-culture model was used for further mechanistic studies.

Results:
Mast cell-deficient mice displayed increased epithelial permeability, bacterial dissemination and neutrophil accumulation following *P. aeruiginosa* lung infection compared to wild-type controls indicating increased bacteria-induced lung damage. This phenotype was fully reversed following reconstitution of W-sh mice with mast cells. *In vitro* studies demonstrated that a yet unidentified mast cell secreted factor opposed *P. aeruginosa*-induced changes in epithelial permeability through preventing caspase-3 dependent epithelial cell death.

Conclusions:
Mast cells play a previously unrecognized protective role against *P. aeruginosa* induced lung injury.

12. INFLUENZA A VIRUS BLOCKS ANTIVIRAL STRESS-INDUCED TRANSLATION ARREST

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13. INTERIM ESTIMATES OF 2013/14 INFLUENZA CLINICAL SEVERITY AND VACCINE EFFECTIVENESS IN THE PREVENTION OF LABORATORY-CONFIRMED INFLUENZA-RELATED HOSPITALIZATION IN CANADIAN ADULTS FROM THE PUBLIC HEALTH AGENCY OF CANADA/CANADIAN INSTITUTES OF HEALTH RESEARCH (PCIRN) SERIOUS OUTCOMES SURVEILLANCE NETWORK, CANADA, FEBRUARY, 2014

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Introduction: Ongoing assessment of effectiveness of influenza vaccines is critical to inform public health decision making regarding publicly funded immunization programs. The goal of the Canadian influenza immunization program is prevention of influenza-associated hospitalization and death but little data is available to assess the effectiveness of seasonal influenza vaccination in the prevention of serious outcomes. The PCIRN Serious Outcomes Surveillance (SOS) Network is a national hospital-based surveillance network which provides ongoing assessment of influenza vaccination effectiveness (VE) in the prevention of laboratory-confirmed influenza-related hospitalization in Canadian adults.

Methods: In 2013/14, the PCIRN SOS Network conducted active surveillance for influenza among hospitalized adults from 15Nov to 30April in 45 acute care facilities in 7 Provinces, encompassing ~18,000 beds. A nasopharyngeal swab for influenza polymerase chain reaction (PCR) testing was obtained from all patients admitted with community-acquired pneumonia, exacerbation of chronic obstructive pulmonary disease or asthma, unexplained sepsis, any respiratory diagnosis or symptom, and, on one day per week, acute coronary syndrome, stroke, or other cardiac diagnoses with triage temperature ≥37.5°C. Cases were PCR-positive for influenza; controls were patients with negative influenza PCR. Interim unmatched VE was estimated as (1-odds ratio of influenza in vaccinated versus unvaccinated patients) X 100 for cases and controls enrolled up to Feb. 8, 2014.

Results: The 2013/14 influenza season in Canada has been dominated by influenza A(H1N1); 631/654 (96.5%) of admissions were due to Influenza A; of those with a known subtype, influenza A(H1N1) accounted for 357/375 (95.2%). Overall, 62.1% of patients admitted to hospital with influenza were under 65 years old and 20.6% had severe, life-threatening disease requiring admission to an intensive care unit, mechanical ventilation, or resulting in death; 84.7% of those with severe disease and completed medical records had one or more comorbidities and only 33.3% had received 2013/14 influenza vaccine. Interim unmatched VE adjusted for age and presence of one or more medical comorbidities was 58.5% (90%CI: 43.9; 69.3%) overall and 57.9% (90%CI: 37.7; 71.5) for confirmed A(H1N1) assessed on Feb. 8, 2014.

Conclusions: The 2013/14 influenza vaccines used in Canada offered substantial public health benefit, preventing approximately 60% of influenza-associated hospitalizations. Overall immunization rates among adults with comorbidities was low, highlighting the need for enhanced effort to ensure protection of this vulnerable group.

14. NOVEL *BORDETELLA PERTUSSIS* AND INFLUENZA VACCINES FORMULATED USING THE DEPOVAX™ PLATFORM CONFER LONG-LASTING IMMUNITY AND CROSS-PROTECTION IN MICE

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Introduction: *Bordetella pertussis* (Bp) and influenza A virus (IAV) cause morbidity and mortality, particularly in the young and elderly. While current vaccines protect against these pathogens, high IAV mutation rates prompting re-formulation each year and waning immunity to Bp highlight the need for more effective vaccines. DepoVax™, can enhance antigen uptake and drive Th1 immunity, and is currently in clinical trials as a cancer vaccine. We aim to understand how DepoVax™ may protect from Bp and IAV.

Methods: Mice were vaccinated i.m. with DepoVax™ vaccine containing either heat-inactivated IAV strain PR8 or Bp antigens, or with saline or standard IAV or Bp vaccines. Mice were challenged with the appropriate pathogen (PR8 or Bp) and monitored for clinical score and survival. Cellular immunity was assessed by antigen restimulation of splenocytes followed by analysis of the production of cytokines by ELISA and multiplex.

Results: Mice vaccinated with a single dose of DepoVax™-Bp and challenged five months later with Bp showed little to no bacterial load by 15 days post-infection. Antigen restimulation of splenocytes from mice vaccinated with DepoVax™-Bp demonstrated strong IFN-γ production on day 28 with little IL-13 compared to splenocytes from mice vaccinated with currently-licensed DTaP. By day 170, IFN-γ production was elevated in mice vaccinated with DepoVax™-Bp compared to DTaP. In the IAV model, changing the amount of antigen in standard IAV vaccines resulted in models that examine morbidity and mortality separately. A single dose of DepoVax™-IAV vaccines protected mice from lethal homosubtypic and heterosubtypic challenge.

Conclusions: Vaccines formulated with the DepoVax™ platform can induce improved immunity to IAV and Bp that result in balanced Th1 and Th1 immune responses in mouse models of lung infection. These preliminary findings may ultimately lead to the introduction of new vaccines in the clinic that prevent influenza and pertussis.

15. IMPACT OF REPEATED ANNUAL INFLUENZA IMMUNIZATION ON VACCINE EFFECTIVENESS

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Introduction: Influenza vaccination is recommended annually to protect the public from circulating strains of the virus. Over the last few decades however, there has been conflicting evidence surrounding the impact of prior influenza immunization on the utility and efficacy of current season influenza vaccination and as such further research is warranted.

Methods: Impact of prior vaccination on current season (VE) was assessed by stratifying participants into one of three groups: those vaccinated in both 2011/12 and 2010/11, those vaccinated only in 2011/12, and a referent group of those who had received neither vaccination. Multivariate logistic regression was used and VE was estimated as (1-odds ratio of current season vaccination in cases versus controls) X 100.

Results: A total of 1162 participants were included in the analysis by vaccination status; 651 had received vaccines in both 2011/12 and 2010/11, 70 had received only in the current season 2011/12, and 441 had received neither. After adjustment, VE was 48.9% among those who received both the current and previous season vaccines in 2011/12 and 2010/11, compared to 42.1% among those who had only received the current season influenza vaccine in 2011/12.

Conclusions: While the finding of increased VE with repeated influenza vaccination was not statistically significant it demonstrates, contrary to the concern raised by some experts, that prior season influenza vaccination does not impair the effectiveness of the current season vaccine in the prevention of hospitalization.

16. SAFETY AND IMMUNOGENICITY OF INACTIVATED VARICELLA-ZOSTER VIRUS VACCINE IN ADULTS WITH HEMATOLOGIC MALIGNANCIES RECEIVING TREATMENT WITH ANTI-CD20 MONOCLONAL ANTIBODIES

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Introduction: Herpes zoster (HZ) incidence is higher in patients with hematologic malignancies (HM) (25-100 cases/1000 person-years) than in the general population (3-5 cases/1000 person-years). This immunocompromised population can experience significant morbidity and occasional mortality from complications associated with reactivation of the varicella-zoster virus (VZV). In general, there is limited data in the literature regarding the effect of anti-CD20 monoclonal antibodies, used in treatment of HM patients, on vaccine-related cell-mediated immune response. Due to the potential negative impact of anti-CD20 monoclonal antibodies on vaccine immunogenicity and efficacy, HM patients receiving anti-CD20 monoclonal antibodies have been excluded from prior inactivated VZV vaccine (inactivated-ZV) studies. This study evaluated the safety and immunogenicity of inactivated-ZV in HM patients receiving anti-CD20 monoclonal antibody therapy

Methods: This was an open label, single arm, multicenter Phase I study of a 4-dose inactivated-ZV regimen (~30 days between each dose) in patients ≥18 years old with HM receiving anti-CD20 monoclonal antibodies either as a single agent or in a combination chemotherapy regimen and not likely to undergo HCT (n=80). Blood samples were collected at baseline prior to dose 1 and 28 days postdose 4 to measure VZV-specific T-cell responses using interferon-gamma enzyme-linked immunospot (IFN-γ ELISPOT). The primary hypothesis was that inactivated-ZV would elicit significant VZV-specific immune responses at ~28 days postdose 4, with the statistical criterion being that the lower bound of the two-sided 90% confidence interval (CI) on the geometric fold rise (GMFR) be >1.0. All vaccinated patients were evaluated for adverse events (AE), including VZV-like rashes, through 28 days postdose 4.

Results: The 4-dose inactivated-ZV regimen elicited a statistically significant VZV-specific immune response measured by IFN-γ ELISPOT at 28 days postdose 4 in the per-protocol population (GMFR = 4.34 [90% CI: 3.01, 6.24], p-value <0.001). As the lower bound of the 2-sided 90% CI for GMFR was >1.0, the pre-specified primary immunogenicity success criterion was met. Overall, 85% (68/80) of patients reported ≥1 AEs, 44% (35/80) reported ≥1 injection-site AEs, and 74% (59/80) reported ≥1 systemic AEs. The most common injection-site AEs were pain (32%), erythema (31%), and swelling (26%). The most common systemic AEs were pyrexia (25%) and diarrhea (14%). Twelve patients (15%) experienced serious AEs, including one event determined by the investigator to be vaccine-related (convulsion: day 8 postdose 1). One patient experienced a fatal serious AE (Richter's transformation to Hodgkin's disease; day 34 postdose 1) assessed as not vaccine-related by the investigator. In general, the frequencies of AEs did not increase with subsequent doses of vaccine. No inactivated-ZV recipient had a rash that was PCR positive for VZV vaccine strain.

Conclusions: In adults with HM receiving anti-CD20 monoclonal antibodies, inactivated-ZV was well-tolerated and elicited statistically significant VZV-specific T-cell responses ~28 days postdose 4

17. RESPIRATORY VIRAL INFECTIONS AMONG HOSPITALIZED ADULTS: NOT ALL ILI IS INFLUENZA

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Introduction: The aim of this study was to determine the viral etiology of hospitalized adults with acute respiratory disease using multiplex-PCR and determine the presenting features and clinical outcomes. In addition we assessed the ability of the ILI case definition to act as a predictive tool for non-influenza viral infections.

Methods: From November 2011to May 25 2012, the PCIRN SOS Network conducted active surveillance for influenza among hospitalized adults in 40 acute care facilities in 5 provinces across Canada. A nasopharyngeal swab for influenza polymerase chain reaction (PCR) testing was obtained from all admitted patients meeting study criteria. Specimens testing negative for influenza were tested for the presence of parainfluenza virus, adenovirus, respiratory syncytial virus (RSV), human metapneumovirus (hMPV), coronavirus and rhinovirus using the Seeplex®eRV15 assay. Clinical features and outcomes were compared between those with influenza and other respiratory viruses.

Results: 1213 patients were tested for influenza. 621 (51%) had negative influenza results and of these143 (23%) had another respiratory virus detected. hMPV was the most common virus detected. Equal proportions of patients with influenza and non-influenza viruses required ICU admission (12.7% and 13.3% respectively). Nine percent and 31% of non-influenza viruses fit the PHAC and CDC case definition for ILI respectively. Exacerbation of COPD was the admitting diagnosis in 36% and 39% of RSV and rhinovirus cases respectively. Compared to patients with influenza, rhinoviruses tended to be younger with a lower BMI but presented more often with unexplained sepsis (12% vs 3.5%), and more required ICU admission (19% vs 13%).

Conclusions: Non-influenza respiratory viruses can cause significant morbidity in hospitalized adults. Approximately one third can present with ILI suggesting that patients with respiratory symptoms who test negative for influenza should remain on droplet precautions.

18. A NOVEL POLYBASIC MOTIF IN THE REOVIRUS P14 FAST PROTEIN IS NECESSARY FOR PLASMA MEMBRANE TRAFFICKING AND INVOLVES RAB11 AND AP1-DEPENDENT VESICLE TRANSPORT

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Introduction: Efficient transport of proteins to the plasma membrane has important implications on human disease and virus replication, however, the pathways and signals involved remain unclear. The reovirus fusion-associated small transmembrane (FAST) proteins traffic through the ER-Golgi pathway to the plasma membrane, where they cause cell-cell membrane fusion. In this study, we investigated the functional role of the polybasic motif (PBM) of p14 FAST protein. We report that the PBM is a novel tri-basic autonomus Golgi export signal that functions via the Rab11 and AP1 adaptor protein transport vesicle pathway.

Methods: Various p14 polybasic mutant proteins were created by site-directed mutagenesis and PCR based overlap-extension. The fusion activity of all constructs was analyzed by syncytial indexing assays, and cell surface expression was determined by flow cytometry. Subcellular localization of the constructs was determined by confocal microscopy and endoglycosidase assays. Co-immunoprecipation (co-IP) was performed to analyze interaction of Rab11 with p14.

Results: Alanine substitution of the p14 PBM led to a loss of plasma membrane localization and p14 accumulation in the Golgi, indicating the PBM acts as a Golgi export signal. Furthermore, extensive mutagenesis of the PBM indicated that a minimum of three basic residues are required for efficient Golgi export. Moreover, insertion of the PBM into a Golgi resident protein mediated protein export from the Golgi to the plasma membrane, suggesting a universal role of the PBM. Activated Rab11 interacted with p14, but not with a polybasic mutant, in co-IP experiments, which suggests a specific interaction of Rab11 with p14 via PBM. siRNA knock down of Rab11 and AP1 significantly reduced p14 surface expression indicating roles of Rab11 and AP1 coated vesicles in p14 trafficking to plasma membrane.

Conclusions: The p14 PBM is a novel, sequence independent, tri-basic autonomous Golgi export signal that interacts with activated Rab11 during trafficking to the plasma membrane. Additionally, p14 uses AP1 coated vesicles for its transport to the plasma membrane. This study adds to our current understanding of protein trafficking pathways and will provide useful insights into the transport systems of viral plasma membrane proteins.

19. JAMESTOWN CANYON VIRUS IN NOVA SCOTIA: A SEROPREVALENCE STUDY

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Introduction: Jamestown Canyon Virus (JCV), belonging to the Bunyaviridae family is an emerging pathogen in parts of North America, with the white-tailed deer as its natural host. Transmitted by mosquitoes to humans, JCV infection can manifest as meningitis, encephalitis, a mild febrile illness, or can be subclinical. As JCV is neither notifiable nor routinely tested for in Nova Scotia (NS), its prevalence is difficult to ascertain. In 2009, 6 individuals in NS had a positive JCV serology. One specimen was from someone requiring a pre-deployment screen for mosquito borne viruses, suggesting that JCV infection may go unrecognized. We wanted to determine the seroprevalence of JCV in deer and humans in NS.

Design and Methods: Seroprevalence in the deer population was defined from game sera collected in 2009. Randomly selected residual human sera collected in 2012 as part of a NS zoonotic infection seroprevalence project were also tested. The presence of JCV antibodies was determined using plaque reduction neutralization assay. A titre of >1/20 was considered positive.

Results: Eighty-eight percent (72/82) of deer had antibodies to JCV. Immature animals were more likely to be seronegative compared to adult deer (23% vs 6%). Of the 25 sera tested from humans living in the same region as the killed deer, 48% (12/25) were positive for JCV antibodies compared to 16% (4/25) of sera from humans living in a different region.

Conclusions: The seroprevalence of JCV is high in both deer and humans in NS, although there appears to be regional variation. The fact that very few cases of JCV infection have been documented in NS suggests that most human infections of JCV in NS are mild or asymptomatic.

20. A GENETIC APPROACH TOWARD UNDERSTANDING BACTERIAL VIRULENCE IN *SHIGELLA FLEXNERI*

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Introduction: *Shigella flexneri* is a Gram-negative bacilli and is the causative agent of shigellosis. Upon infection, *Shigella* invades the host colonic epithelium using a type III secretion system (T3SS) that is encoded by a large virulence plasmid (pWR100). This secretion system injects protein virulence determinants, or "effectors" directly into the cytosol of infected cells where they interfere with host cell functions that are required for an appropriate immune response. There are thought to be upwards of 50 effector proteins encoded on the virulence plasmid, many of which do not have an assigned function. The Rohde laboratory has constructed a library of single gene knockouts each deleted for a different gene on the pWR100 plasmid. Mutations in structural components of the T3SS render the bacteria avirulent but the phenotypes for most effectors are subtle. These results suggest that there is significant functional redundancy in the repertoire of factors encoded by pWR100.

Methods: The goal of this research project is to determine a strategy for generating double gene deletions in a high throughput manner using specialized suicide plasmids. These plasmids are capable of replicating in an *E. coli* donor strain but not in *Shigella.* My suicide plasmids target a specific gene on pWR100, undergo allelic exchange, and inactivate the targeted gene. Through conjugation these suicide plasmids can be introduced to the *Shigella* deletion collection to efficiently generate a new collection of mutants with their original mutation and an additional mutation from the suicide plasmid, thereby giving rise to double deletion collections.

Results: I have constructed one such suicide plasmid to inactivate *ipaD* that encodes a regulator of T3SS function. Results will be presented for the initial use of *ipaD* mutants constructed with this approach.

Conclusions: This approach will be applied to identify genetic interactions and ascribe gene function to genes encoded by the virulence plasmid. Further characterization of *Shigella* virulence may prove useful in the search for a vaccine against shigellosis.

21. VIRUS-INFECTED HUMAN MAST CELLS ENHANCE NATURAL KILLER CELL FUNCTIONS

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The author requests that this abstract not be published

22. REGULATION OF TRANSLATION DURING KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS (KSHV) LYTIC REPLICATION

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The author requests that this abstract not be published

23. INVESTIGATING THE ROLE OF KSHV MIRNAS IN THE BYPASS OF ONCOGENE-INDUCED SENESCENCE

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The author requests that this abstract not be published

24. INFLUENZA AND OTHER VIRAL AGENTS ASSOCIATED WITH RESPIRATORY OUTBREAKS IN NOVA SCOTIA

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Introduction: Many viral pathogens can cause respiratory outbreaks. In Nova Scotia, the Provincial Public Health Laboratory Network (PPHLN) provides testing for respiratory viruses for the province. To inform public health intervention, it is important to determine whether an outbreak is caused by influenza. Depending on the season, the PPHLN uses a test for influenza A, B, and RSV or a test for 15 viral pathogens, including influenza, for this purpose. Since many outbreaks are not caused by influenza A, B or RSV, pathogens associated with outbreaks often remain unknown when the former assay is used alone. Furthermore, testing for mixed infections is minimal. In January 2011, the PPHLN began testing for 11 additional respiratory viruses.

Objective: To identify respiratory viruses associated with reported respiratory outbreaks in Nova Scotia.

Methods: Data for influenza seasons 2010/2011, 2011/2012 and 2012/2013 were included. All specimens associated with a respiratory outbreak submitted to the PPHLN were tested following an established algorithm. Once influenza was detected in the province, a multiplexed RT-PCR for influenza A, B and RSV was performed as the initial test. Starting in January 2011, any specimens that were tested for influenza A, B and RSV without positive results were reflexively tested for 15 respiratory viruses via the Seeplex® RV15 OneStep ACE Detection kit, another multiplexed RT-PCR. Shoulder season testing was restricted to the RV15 detection kit only.

Results: A total of 815 specimens were submitted to the PPHLN for respiratory outbreak investigation during influenza seasons 2010/2011, 2011/2012 and 2012/2013. These specimens were related to 229 reported outbreaks. A respiratory pathogen was detected in 205 (89.5%) of these outbreaks. Only 122 (53.3%) outbreaks had associated specimens that were influenza A, B or RSV positive. Outbreaks were also associated with human metapneumovirus, coronavirus, parainfluenza virus, rhinovirus, adenovirus and enterovirus. More than one respiratory pathogen was detected in 44 (19.2%) of outbreaks.

Conclusion: With the addition of reflexive testing with the Seeplex® RV15 Detection kit, one or more respiratory pathogens were detected for 90% of outbreaks under investigation. A variety of respiratory viruses were associated with outbreaks, and mixed infections were common.

25. CHARACTERIZATION OF ANTIGEN-TARGETING FUSION PROTEINS FROM *STREPTOCOCCUS GORDONII* AND *ESCHERICHIA COLI*

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Introduction: *Streptococcus gordonii* is an attractive live mucosal vaccine vector. An obstacle to *S. gordonii* based vaccine development is the sub-optimal immune response in the oral cavity. Antigens delivered by coupled molecules specific for receptors on antigen-presenting cells can enhance immune responses. We have fused a pertussis antigen (type I domain of FHA) to anti-DEC205 scFv antibody and CD40 ligand and transformed the genetic constructs into *S. gordonii*. In this study, the ability of FHA and FHA fusion proteins to bind to immune cells was examined.

Design and Methods: Protein production was detected by Western blotting with anti-FHA antibody and proteins were quantified and standardized by densitometry. FHA and FHA fusion proteins were tested for binding to mouse mixed lymphocytes and splenocytes by Western blotting and ELISA.

Results: Western blotting showed that proteins of the expected size of FHA (44kDa), FHA-anti-DEC205 scFv (62kDa), and FHA-CD40L (55kDa) were present in the culture supernatants of *S. gordonii* confirming the proteins were produced. In an enzyme immunoassay (ELISA), FHA strongly bound to mixed lymphocytes and splenocytes while the FHA fusion proteins bound weakly. In Western blots, none of the fusion proteins showed a distinct binding profile to cell lysates compared to that of FHA. The constructs were subcloned into plasmid pCom3X and transformed into *E. coli*. The presence of these proteins in *E. coli* periplasmic extracts was confirmed by Western blotting. FHA and FHA fusion proteins prepared from *E. coli* were able to bind to the two cell types with similar reactivity.

Conclusions: The results suggest that the type I domain of FHA has the ability to bind to immune cells. FHA fusion proteins produced by *E. coli* showed excellent binding suggesting that these proteins were folded properly in this bacterium. However, whether the binding displayed by the fusion proteins was due to FHA or the targeting molecules remains to be determined.

26. THE ROLE OF IL-17RA- AND IL-17RC-MEDIATED SIGNALING PATHWAYS ON REGULATION OF *CHLAMYDIA*-INDUCED CELL DEATH PROGRAM *IN-VITRO*

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Affiliation: Microbiology and Immunology, Dalhousie University, and Canadian Centre for Vaccinology

Introduction: *Chlamydia* is the most common bacterial sexual infection worldwide and it causes a wide spectrum of diseases, including trachoma, ectopic pregnancy and many other chronic inflammatory conditions. Although antibiotics treatments are available, the disease is often asymptomatic and left untreated. As obligate intracellular bacteria, *Chlamydia* regulates host cellular responses including cell death program to facilitate its replication inside the host. Host innate and adaptive immunity work together to clear the infection, one of which involves the IL-17 signaling pathway that regulates inflammation and apoptosis. However, how IL-17 and its receptor axis is involved in modulating host immune responses during *Chlamydia* infection is still unclear. In this study, we characterized the direct impact of IL-17RA and IL-17RC on proinflammatory cytokine production and cell death program in hematopoietic cells upon *Chlamydia* infection *in vitro.*

Methods: We determined (1) the kinetics of apoptosis and necrosis by apoptosis assay and (2) proinflammatory responses by ELISA in bone marrow-derived dendritic cells from wild-type mouse and how these parameters are modified in IL-17RA- and IL-17RC-deficient mice upon *C. muridarum* infection. The amount of bacteria in the culture was subsequently measured using qPCR to determine how *Chlamydia* replication was affected in altered IL-17 signaling.

Results: Proinflammatory cytokines production is significantly upregulated in the absence of IL-17RA or IL-17RC during *Chlamydia* infection compared to wild-type. The ability of *Chlamydia* to suppress apoptosis and necrosis early in infection is reduced in the absence of IL-17RA while its replication is reduced in the absence of IL-17RC.

Conclusions: These results indicated the differential roles of IL-17RA and IL-17RC in *Chlamydia*-induced host cellular responses. Deeper understanding of *Chlamydia*-host interactions will subsequently contribute to developing effective vaccine against *Chlamydia.*

27. THE ROLE OF EARLY IL-17 PRODUCTION IN HOST RESPONSES TO *CHLAMYDIA* INFECTION

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Affiliation: Canadian Centre for Vaccinology, Dalhousie University Department of Microbiology & Immunology and Pediatrics

Introduction: *Chlamydia trachomatis* infects the epithelial lining of the genital, ocular, and respiratory tract causing a broad spectrum of diseases. The $CD4^+$ T helper IFN-γ-dependent immunity is a major host protective response for controlling *Chlamydia* infection. However, the role of the Th17 cell and its signature cytokine IL-17 in controlling *Chlamydia* infection are not fully understood. The early IL-17 production promotes Th1 responses during respiratory *Chlamydia* infection by modulating IL-12 production from bone marrow-derived dendritic cells. Our preliminary data showed that the early IL-17 appeared to suppress the production of proinflammatory cytokines, indicating a potential role of IL-17 in directly impacting chlamydial replication at the infection site. The objective of this research is to dissect the role of the IL-17/IL-17R signaling in controlling host responses and *Chlamydia* replication *in vitro* and *in vivo.*

Methods: The *Chlamydia muridarum* respiratory infection model was established in wild-type (C57BL/6) and IL-17 receptor A and C knockout mice. The percentage of body weight loss was measured daily and the mice were sacrificed during various time points to characterize the kinetics and magnitude of *Chlamydia*-induced IL-17 responses. The lung tissue was collected to measure the bacterial burden as well as the splenocytes for *in vitro* cytokine memory response.

Results: Preliminary data showed a trend where IL-17RAKO mice had greater bacterial burden in the lungs during early and late *Chlamydia* infection and had a greater percentage of body weight loss compared to wild-type and IL-17RCKO mice. Preliminary *in vitro* cytokine memory response data showed that splenocytes from IL-17RA and RC knockout mice had reduced IFN-γ responses compared to wild-type mice.

Conclusions: IL-17RA and IL-17RC signaling plays an important role in controlling early and late *Chlamydia* infection but IL-17RA may have a larger direct impact on controlling chlamydial replication.

28. IDENTIFICATION OF PUTATIVE KEY AMINO ACIDS FOR HTPB PROTEIN-FOLDING INDEPENDENT FUNCTIONS

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Introduction: Chaperonins are highly conserved housekeeping proteins that help other proteins to fold. The GroEL/GroES folding machinery of *E.coli* is formed by two GroEL heptameric rings that constitute the folding chamber and a GroES heptameric cap that keeps unfolded proteins inside the chamber. However, bacterial chaperonins also possess protein-folding independent functions acting as proteases or toxins and some bacterial species export their chaperonins to the outer membrane where play a role in adhesion. The *Legionella pneumophila* chaperonin (HtpB) has been implicated in host cell invasion, mitochondria recruitment, and cell signaling. We hypothesize that these unique HtpB functions are due to substitutions in key amino acid positions.

Methods: HtpB orthologs were downloaded from the NCBI protein database (E-value: 10E-6) and then aligned using ClustalOmega. The HtpB othologs from Eukaryota and Archaea were eliminated and the multiple sequence alignment was used as input for the evolutionary trace (ET) calculation which ranks amino acids by their relative evolutionary importance and then displays them in the protein 3D structure. Active sites or residues presumably linked to significant functional changes can be identified using this approach. Finally, the BLOSUM62 substitution matrix was use to score amino acids according to their substitution probability, where negative scores indicate the less likely to occur substitutions.

Results: After elimination of the most divergent orthologs from the alignment, 1374 sequences were analyzed using the ET method**.** As was expected, the most conserved amino acids were located in the HtpB area that faces the inside of the chamber, the apical domain (which interacts with the unfolded substrate) and the ATP binding pocket. Since GroEL is the most studied chaperonin, it was used as a reference to compare amino acids variations with HtpB, 142 substitutions were found. Finally, from the intersection between residues with low ET rank and negative BLOSUM62 score, 5 residues were selected: methionine 68 and 212, serine236, lysine 298 and asparagine 507.

Conclusions: Using bioinformatics tools we were able to predict the amino acids that could be involved in HtpB folding-independent functions. These data provide a foundation for testable models by which HtpB family members have acquired chaperonin-independent functions.

29. COMPARATIVE GENOMIC FINGERPRINTING OF *CAMPYLOBACTER JEJUNI* STRAINS ISOLATED FROM POULTRY AND CLINICAL PATIENTS IN ATLANTIC CANADA.

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Introduction: *Campylobacter* spp. are currently the leading cause of food-borne gastroenteritis in Canada, the US and many regions of Europe. Raw, retail poultry is a significant source of *Campylobacter*. However in Canada, there is limited information about whether strains carried on poultry actually cause disease in humans. The purpose of this study was to strain type *Campylobacter jejuni* and *C. coli* isolates recovered from raw, retail poultry, as well as clinical isolates collected from hospitals in Atlantic Canada and to determine whether certain *Campylobacter* types are found concurrently in both retail poultry and clinical patients.

Methods: Thirty packages of raw, retail poultry sold in Halifax, NS were tested for *Campylobacter* weekly between July and October (n=480) using a modified Bolton broth method. Clinical isolates were obtained retrospectively and prospectively from hospital laboratories in the Atlantic region. *C. jejuni* and *C. coli* isolates were typed using comparative genomics fingerprinting (CGF) and 34 isolates were further compared using whole genome sequencing.

Results: We isolated *Campylobacter* spp. from 65% of retail packages with 285 of the total 312 isolates (91%) identified as *C. jejuni*. The type of meat cut, presence of skin/bone or time of sampling did not significantly affect the frequency of recovery. Currently, 123 strain types have been identified. Of these, 47 types formed clusters (i.e. identified in more than one isolate). Of these clusters, 32 (26% of the total types) present in clinical patients and retail poultry, 3 (1.6% of the total types) present in only clinical patients and 14 (11.4% of the total types) present in only retail poultry. The remaining 76 (61.7 % of the total) did not form clusters. We obtained genome sequences with significant coverage for 32 of the isolates selected. Using *in silico* typing we found that the expected CGF profiles correlated closely with the CGF profiles obtained experimentally.

Conclusions: These results demonstrate that raw, retail poultry can be a source of *Campylobacter* strains carried in clinical patients. The results also suggest that not all poultry-associated *Campylobacter* strains are implicated in human disease.

30. METRONOMIC CYCLOPHOSPHAMIDE ENHANCES THE IMMUNOGENICITY AND ANTI-TUMOUR ACTIVITY OF A DEPOVAX BASED VACCINE AND MAY BE FURTHER ENHANCED WITH INHIBITORS OF CTLA-4 OR PD1

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Introduction:

To counteract tumor-induced immune suppression, cancer vaccines are increasingly being combined with immune modulators that can not only reverse immune suppression but also enhance vaccine induced immune responses. We found that metronomic cyclophosphamide (mCPA; 50 mg BID) enhanced the immunogenicity of a DepoVax^{TM} (DPX) based cancer vaccine (DPX-Survivac) in ovarian cancer patients in a phase I clinical study. We emulated these results using transplantable tumor models which allowed us to study the underlying mechanisms of mCPA induced immune modulation.

Methods:

Mice were implanted with C3 tumor cells subcutaneously (expressing HPV16E7). Once tumors were palpable, they were treated with mCPA, 20 mg/kg/day for 7 days, PO. At the end of one week, mice were vaccinated with DPX containing HPV16E7$_{49-57}$ peptide. Eight days later, mice were terminated and tumors, spleens and lymph nodes collected for analysis.

Results:

mCPA had a pronounced lymphodepletive effect on the vaccine draining lymph node, yet did not reduce the development of antigen-specific CD8^{+} T cells induced by vaccination as detected by MHC-multimer flow cytometry. Combination treatment also increased cytotoxic T cell activity in the spleen measured by IFN-γ ELISPOT and in vivo cytotoxic T cell assay. Analysis of immune gene signatures in the tumor microenvironment by RT-qPCR detected elevated levels of cytotoxic markers, such as IFN-γ and granzyme B, as well as co-inhibitory markers, such as PD-1 and CTLA-4, in mice treated with combination therapy, the latter providing a strong rational for modulating these pathways with recently available monoclonal antibodies. Analysis of spleen cell populations by flow cytometry indicated that mCPA induced transient lymphodepletion that was marked by a selective expansion of myeloid-derived suppressor cells in the absence of vaccination. Selective depletion of regulatory T cells was not observed, in contrast to other regimens of low dose CPA.

Conclusions:

These results demonstrate that mCPA provides a complex form of immune modulation that is most effective when combined with active immunization. Using these models we can evaluate other immune modulator agents that may enhance vaccine activity, including checkpoint inhibitors or other immune based therapies.

31. DETECTION OF CIRCULATING NOROVIRUS GENOTYPES: HITTING A MOVING TARGET

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Introduction: Like influenza virus, epidemic strains of norovirus emerge through antigenic drift and immune escape. Although national surveillance programs are in place to monitor norovirus epidemiology, the emergence of a new pandemic strain (GII.4-2012 Sydney) and the genetic diversity among other genotypes can be challenging for laboratories. In this study, we evaluated the analytical and clinical performance characteristics of one real-time assay and two end-point RT-PCR assays commonly used in microbiology laboratories.

Methods: The analytical specificity and sensitivity for each assay was determined using a panel of enteric organisms and 10-fold serial dilutions of various circulating norovirus genotypes. Clinical specimens (n=186 stool specimens) were tested concurrently and clinical performance of the real-time method was compared to the two conventional methods.

Results: The real-time RT-PCR was highly sensitive and specific for the detection of all norovirus genotypes currently circulating in Canada. In contrast, the two end-point RT-PCRs displayed poor analytical sensitivity or complete failure to detect certain norovirus genotypes, which was correlated to sequence mismatches in the primer-binding sites. In an attempt to improve detection norovirus for the end-point RT-PCRs and cover a broader range of genotypes, both end-point assays were processed concurrently and detection from either assay was considered a positive result. Concurrent testing resulted in only a modest increase in clinical sensitivity (75.0%) compared to each assay alone (62.5% and 71.9%). However, the false positivity rate increased from 1.98% and 3.36% for the end-point assays alone to 5.47% with concurrent testing.

Conclusions: With the dynamic nature of norovirus epidemiology and challenges faced for norovirus detection, this study highlights the importance of routine surveillance and ongoing proficiency testing for circulating norovirus genotypes. When sequence mismatches are identified in RT-PCR target regions or when new norovirus variants emerges, proficiency panels should be promptly disseminated to clinical laboratories to ensure accurate detection.

32. EVALUATION OF CROSS-REACTIVITY OF SYPHILIS AND LYME DISEASE SEROLOGIC TESTING

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Introduction: The Canadian guidelines for sexually transmitted infections state that false positive syphilis serologic testing may be due to Lyme disease (LD). Conversely, a recent case series has shown that syphilis-positive patients may have false positive LD serology. Given the concurrent emergence of LD and resurgence of syphilis in Nova Scotia, we aimed to quantify cross-reactivity of the diagnostic tests used for each disease.

Methods: Serum from syphilis patients underwent LD enzyme immunoassay (EIA) testing (*Borrelia burgdorferi* IgM/IgGELISA (Zeus Scientific) or C6 Lyme ELISA (Immunetics)), and reactive samples underwent Western Blot (WB), with confirmation defined as positive IgG or IgM. LD-positive sera underwent syphilis testing by Abbott Architect Syphilis EIA, followed by confirmatory rapid plasma regain (RPR) and *Treponema Pallidum* Particle Agglutination (TPPA). A positive result was defined RPR- and TPPA-reactive. Cross-reactivity was reported as a percentage.

Results: Of 50 LD samples, one was reactive with the Architect EIA but was not confirmed, suggesting an initial false positive screen and a cross-reactivity rate of 2%. Of 60 LD-negative samples, one was reactive with the Architect EIA and was positive by RPR and TPPA, suggesting a baseline reactivity rate of 1.7%. Of the 30 syphilis-positive sera, three samples were either positive (1/30) or indeterminate (2/30) for LD using the C6 assay, for a cross-reactivity rate of 10%. When using the Zeus assay, 21/30 required further confirmatory testing due to positive (19/30) or indeterminate (3/30) results, for a cross-reactivity rate of 73.3%.

Conclusions: When an increase in syphilis rates coincides with the emergence of LD, the rates of diagnostic cross-reactivity is an important consideration for the clinician. Our data suggest that syphilis infection can lead to false positive LD serology, but fail to support the converse claim made by Health Canada.

33. SEASONAL INFLUENZA VACCINE IN PREGNANCY: RATES, DETERMINANTS, AND OUTCOMES

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Introduction: We determined influenza vaccination rates among pregnant women during the two non-pandemic influenza seasons (2010-2012) following the 2009 H1N1 pandemic. We explored various maternal factors as predictors of influenza vaccination status. We also evaluated the relationship between maternal influenza vaccination and neonatal outcomes

Methods: We used a population-based perinatal database in Nova Scotia, Canada to examine maternal vaccination rates, determinants of vaccination status and neonatal outcomes. Our cohort included women delivering between November 1, 2010 and March 31, 2012. Logistic regression was used to compare various demographic variables and neonatal outcomes between vaccinated and unvaccinated women.

Results: Overall, 1958/12223 (16.0%) women in our cohort received the influenza vaccine during their pregnancy. Marital status, parity, rural vs. urban residency, smoking status during pregnancy, and maternal influenza risk status were all important determinants of maternal vaccine receipt. Preterm birth (< 37 weeks gestation) was less likely among infants of vaccinated mothers (OR = 0.76; 95% CI 0.61-0.95) when compared to infants whose mothers did not receive the vaccine. The rate of low birth weight infants was also lower among vaccinated women in our cohort (OR = 0.74; 95% CI 0.57-0.95).

Conclusions: Current guidelines advise that all pregnant women should receive the seasonal influenza vaccine. Despite these recommendations, influenza vaccination rates among pregnant women in Nova Scotia remain disappointingly low in the aftermath of the 2009 H1N1 pandemic. In light of mounting evidence suggesting an association between maternal influenza vaccination and improved neonatal outcomes, stronger initiatives promoting vaccination during pregnancy are essential

34. UNDERSTANDING STI TESTING RATES AMONG HIGH-RISK MARITIME CANADIAN UNDERGRADUATE STUDENTS

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Introduction: In Canada and worldwide, individuals aged 15 to 29 have the highest rates of diagnosed sexually transmitted infection (STI). STI testing services are free and readily available to most university students in Canada, however, only some individuals choose to access them. Little is known about the correlates of STI testing in Canadian undergraduate populations. This study aims to characterize those individuals most at risk of STI and least likely to be tested. This information can be used to refine the targets for STI testing health promotion at Maritime universities.

Methods: The data for this study comes from the Maritime University Health Survey, an internet-based survey of the undergraduate student populations at eight different universities in Atlantic Canada. This dataset contains information on the sexual health and use of health services of 10,361 university students weighted to be representative of the basic demographics of the participating universities and imputed for missing values. To analyze this data, we stratified by biological sex and STI risk based on reported sexual behaviors and performed a descriptive analysis of the characteristics of each subpopulation and both simple and multiple logistic regression analyses of the factors associated with being tested for STI in each subpopulation.

Results: Preliminary results indicate that 62% of sexually active students and 44.3% of the 303 students most at-risk of STI based on their sexual behavior have never had an STI test. In both males and females at high risk for STI, younger students with less sexual health knowledge were significantly more likely to have never been tested for STI; these students were also significantly more likely to have never been tested in those students less at-risk of STI.

Conclusions: Health promotion may be most effective at increasing lifetime STI testing when targeting younger students with campaigns designed to increase general sexual health knowledge related to contraception, STI signs and symptoms and healthy sexual behavior; these interventions would likely be effective means to increase STI testing rates in those most at-risk of undiagnosed STI while still benefiting the remaining sexually active student population.

35. KAPOSIN B DISRUPTS PROCESSING (P)-BODIES IN A RHO A GTPASE- AND P115 GUANINE EXCHANGE FACTOR (GEF)-DEPENDENT MANNER DURING LATENT KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS INFECTION

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The author requests that this abstract not be published

36. EXAMINING THE ROLE OF IPAH9.8 DURING *SHIGELLA FLEXNERI* INFECTION

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The author requests that this abstract not be published

37. RISK FACTORS FOR CENTRAL LINE ASSOCIATED BLOOD STREAM INFECTION (CLABSI) IN CHILDREN 0 TO 18 YEARS OF AGE: A COHORT STUDY

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Introduction: Central Venous Access Devices (CVADs) deliver fluids and medications to main blood vessels, and are vital to the care of many pediatric patients, but carry the risk of Central Line Associated Blood Stream Infection (CLABSI). The incidence of and risk factors for CLABSI have not been investigated in a large cohort study in a Canadian population.

Methods: This is a prospective cohort study of children and youth with CVADs treated at the IWK Health Centre, Halifax between January 1995 and December 2013. Information on the CVADs and patient characteristics was captured from time of CVAD insertion to removal in the Central Venous Access Database, and cases of CLABSI were collected using standard Infection Control and Prevention (ICPS) methods and recorded in the ICPS Hospital Acquired Infection Database. The crude incidence of CLABSI in the overall population was determined via linkage of the two databases. Cox Proportional Hazard models will be used to identify risk factors and protective factors for CLABSI in the cohort.

Results: In the first step of the analysis the data has been successfully linked. The population includes 5,656 patients with 9,080 CVADs over 939,262 line-days over a 19 year period. The median number of CVADs per patient over time was 2 (range: 1 to 12). The crude CLABSI rate at the IWK was 0.71 per 1,000 line-days (95% CI: 0.66-0.77 per 1,000 line-days). Rates of CLABSI stratified by risk (e.g. neonatal intensive care, pediatric intensive care, oncology populations), frequency of mechanical populations, and Cox Proportional Hazards model will be presented.

Conclusions: A large prospective cohort of patients with CVADs has been assembled. The crude overall CLABSI rate is low, reflecting dilution by low risk patients and a large denominator, in comparison with IPCS reports which present rates by quarter. Stratification by underlying morbidity and assessment of CLABSI risk will be helpful in providing care to these vulnerable children.

38. IMPACT OF PHARMACISTS AS IMMUNIZERS ON VACCINATION RATES, VACCINE-PREVENTABLE MORBIDITY AND MORTALITY, SAFETY, AND COST EFFECTIVENESS: SYSTEMATIC REVIEW INTERIM RESULTS

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Introduction: Immunization is among the most cost effective health interventions on a global scale. However, underutilization of vaccination programs remains a significant public health concern. Among the strategies suggested to improve immunization rates is the utilization of non-traditional immunization providers, such as pharmacists, to administer vaccines safely and effectively in their practice settings. Though pharmacists have been able to provide immunization services in some countries for many years, there has yet to be a systematic review assessing the impact of pharmacists as immunizers.

Methods: PubMed, EMBASE, Cochrane Library, Cumulative Index to Nursing and Allied Health Literature (CINAHL), International Pharmaceutical Abstracts (IPA), and Google Scholar were searched for all relevant studies investigating immunization outcomes in the general population when pharmacists are involved in the vaccination process in addition to traditional providers. Grey literature was identified through use of the Canadian Agency for Drugs and Technology in Health (CADTH) “Grey Matters” search tool, searching various grey literature databases, as well as searching the table of contents of relevant journals.

Results: Titles and abstracts of 3,501 articles were searched and independently assessed by three investigators (SB, NE, JI), with 2,517 remaining following removal of duplications. Of the 2,517 articles, 255 were deemed appropriate for full article review, resulting in 2,262 articles being excluded based on title and abstract. Full studies will be included if (1) they involve only humans, (2) are clinical or epidemiologic studies, (3) evaluate the impact of pharmacists as immunizers, and (4) measure immunization outcomes (vaccination rates, vaccine-preventable morbidity and mortality, safety, and cost-effectiveness). Studies will be excluded if no comparator is reported. Additional studies will be identified through Web of Science and manual reference review of the remaining studies. Data extraction and qualitative synthesis will be done on all studies meeting the criteria for inclusion.

Conclusions: Research still in progress. Lessons learned will be shared.

39. PRIMARY HUMAN MAST CELLS' ANTIVIRAL AND PRO-INFLAMMATORY RESPONSE TO RESPIRATORY SYNCYTIAL VIRUS (RSV)

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Introduction: Respiratory syncytial virus (RSV) is a major cause of infant bronchiolitis and the leading cause of their hospitalization worldwide, and is also linked to airway hyper-responsiveness. Mast cells are essential in allergies and the immune response to pathogens, and can produce various mediators that influence vasodilation, bronchoconstriction, and immune cell recruitment to sites of infection. Since mast cells are abundant in the airways at the site of RSV infection, we examined the human mast cell response to RSV and the role type I interferons, major antiviral cytokines, play in such a response.

Methods: Cord blood-derived human mast cells (CBMC) were infected with RSV. In some experiments, RSV infection was blocked with palivizumab, and the type I interferon (IFN) receptor was blocked with a specific antibody. At various times post-infection, the supernatants and/ or RNA prepared from CBMC cultures were assayed for the expression of cytokines, chemokines, type I IFN, IFN-stimulated genes (ISGs), viral titers, RSV gene expression, and leukotriene C4.

Results: CBMC infection with RSV, at 24 hours, resulted in low levels of RSV protein and expressed RSV genes, but failed to successfully produce infectious viral particles. However, RSV inoculated mast cells selectively up-regulated the chemokines CXCL10, CCL4 and CCL5, type I IFNs, and ISGs, critical for the anti-viral response. They also produced VEGF-A, an important pro-angiogenic cytokine. Blockade of the type I IFN receptor during RSV infection of CBMC significantly reduced the induction of CXCL10, CCL4 and VEGF-A, but not CCL5. CBMC did not produce leukotriene C4 in response to RSV exposure.

Conclusions: Our data show that human mast cells respond to RSV by producing pro-inflammatory cytokines some of which are dependent on type I IFN response, which may enhance inflammation and effector cell recruitment during RSV disease.

40. AUTOPHAGY ENHANCES BACTERIAL CLEARANCE DURING *P. AERUGINOSA* LUNG INFECTION

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Introduction:
Autophagy is an evolutionarily conserved catabolic process which has emerged over the past decade as a central component of the immune response. Dysregulation of autophagy contributes to various disease states, including cystic fibrosis (CF), a disease characterized by colonization of the respiratory tract with bacterial pathogens including *P. aeruginosa*. However the contribution of autophagy to host defense against *P. aeruginosa* remains unclear.

Methods:
Autophagy was monitored in response to *P. aeruginosa* infection in mast cells and airway epithelial cells. Pharmacological and genetic manipulation of the autophagy pathway was used to elucidate the contribution of the process to bacterial clearance. The therapeutic potential of autophagy modulating therapies was further examined *in vivo* using an acute *P. aeruginosa* lung infection model.

Results:
P. aeruginosa infection induced significant autophagy in mast cells and airway epithelial cells which could be enhanced through treatment with autophagy inducing compounds. Pharmacological and genetic disruption of autophagy, and defective CFTR significantly increased numbers of intracellular bacteria, while pharmacological induction of autophagy accelerated bacterial clearance *in vitro*. Pharmacological inhibition and induction of autophagy significantly increased and decreased bacterial load respectively in an *in vivo* model of acute *P. aeruginosa* lung infection.

Conclusions:
Autophagy contributes to normal host defense against *P. aeruginosa*, and can be enhanced by pharmacological intervention. Defective autophagy may contribute to colonization of CF airways, and treatments aimed at restoring autophagy in these patients may have considerable therapeutic potential.

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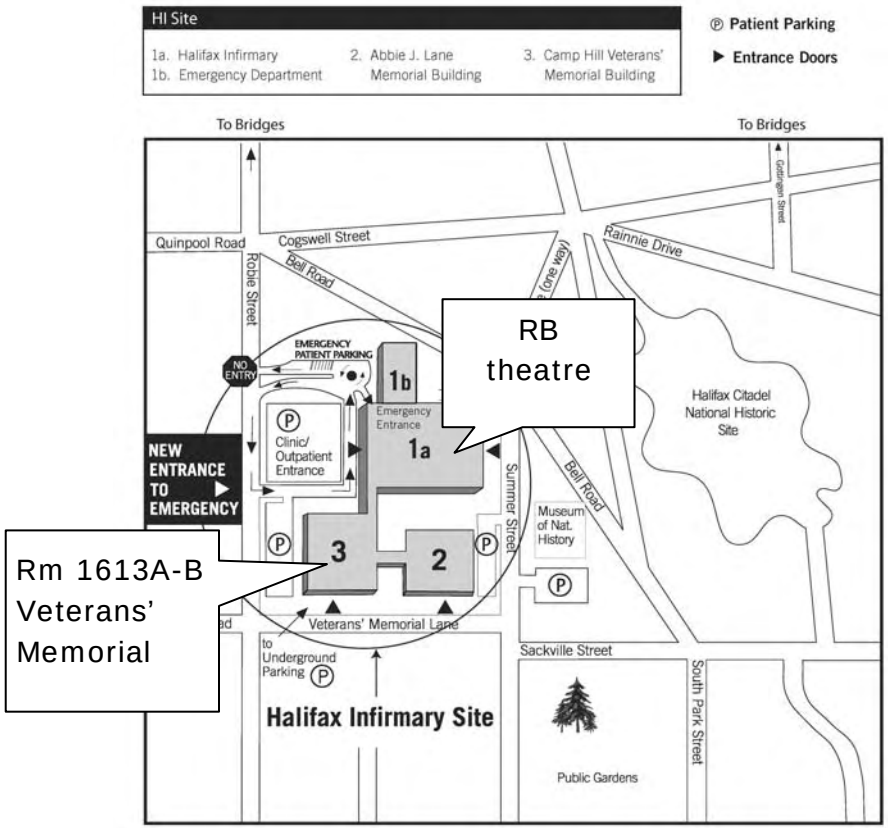

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