UNIVERSITY OF TORONTO ANNUAL

Infectious Diseases and Microbiology Research Day

TRAINEE DAY - JUNE 19, 2023
MEDICAL SCIENCES BUILDING, ROOM 2170

9:15 AM — **Registration**

9:30 AM — **Welcome and opening remarks**

Jacqueline Watt, Maxine Ty

9:40 AM — **Systematic genetic analysis of *Candida albicans* filamentation in response to elevated temperature**

Emma Lash (PI: Leah Cowen)

10:00 AM — **Inhibition of coronavirus infection by ABO isoagglutinins**

Priyal Shah (PI: Donald Branch)

10:20 AM — **Recent sexual assault and genital biomarkers of increased HIV susceptibility**

James Pollock (PI: Rupert Kaul)

10:40 AM — **Coffee break**

11:10 AM — **Encapsulation of VAR6, a potential probiotic against dental caries**

Anqi Liu (PI: Siew-Ging Gong)

11:30 AM — **Gut microbiota and Nod2 signaling promote small intestinal restitution through the revival intestinal stem cell**

Derek Tsang (PI: Dana Philpott)

11:50 AM — **A multi-specific, multi-affinity antibody platform neutralizes sarbecoviruses and confers protection against SARS-CoV-2 in vivo**

Krithika Muthuraman (PI: Jean-Philippe Julien)

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Infectious Diseases and Microbiology Research Day

Time	Session
12:20 PM	**Lunch**
1:20 PM	**Detection of two alphacoronaviruses in bats in eastern Ontario, Canada** Jonathon Kotwa (PI: Samira Mubareka)
1:40 PM	**Micro and nanotopographies to reduce microbial surface attachment and transmission** Desmond van den Berg (PI: Benjamin Hatton)
2:00 PM	**Assessment of the protective potential of monoclonal antibodies against antigen candidate transferrin binding protein B** Natalie Au (PI: Trevor Moraes)
2:20 PM	**Coffee break**
2:50 PM	**Identification and characterization of a divergent Toxoplasmosis gondii associated virus for applications in therapeutics for apicomplexan parasites** Purav Gupta (PI: Artem Babaian)
3:20 PM	**Intestinal barrier disruption in malaria in pregnancy and risk of preterm birth: a cohort study** Julie Wright (PI: Kevin Kain)
3:40 PM	**TIFA-mediated regulation of innate immunity** Cynthia Guo (PI: Scott Gray-Owen)

UNIVERSITY OF TORONTO ANNUAL

Infectious Diseases and Microbiology Research Day

MAIN PROGRAM - JUNE 20, 2023
MEDICAL SCIENCES BUILDING, ROOM 3154

Time	Session
8:00 AM	**Registration** David Naylor Student Commons, MSB
8:30 AM	**Welcome and opening remarks** Scott Gray-Owen, Rupert Kaul
8:45 AM	**Clinical infectious diseases at U of T: A PRECISE vision for the next five years** Jennie Johnstone, Division of Infectious Diseases, U of T and Sinai Health
9:15 AM	**The platinum age of virus discovery: why the next five years will shape virology for the next five decades** Artem Babaian, Department of Molecular Genetics, U of T
9:45 AM	**Supporting healthy aging in people living with HIV** Alice Zhabokritsky, University Health Network
10:15 AM	**Coffee break**
10:45 AM	**Unlocking the secrets of measles and Nipah virus assembly: a path to antiviral development** Michael Norris, Department of Biochemistry, U of T
11:15 AM	**Arbovirus biology through the lens of miRNA interactions** Kathryn Rozen-Gagnon, Department of Molecular Genetics, U of T

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Infectious Diseases and Microbiology Research Day

11:45 AM	**Public health threats at the human-animal interface** Nelson Lee, Dalla Lana School of Public Health, U of T
12:15 PM	**Strengthening Canada's infectious disease research ecosystem to enhance pandemic preparedness: EPIC and HI3** Natasha Christie-Holmes, Patrycja Thompson
12:45 PM	**Closing remarks and awards presentation** Scott Gray-Owen, Rupert Kaul
1:00 PM	**Lunch** David Naylor Student Commons, MSB
2:00 PM	**Poster session A** (Even numbers) David Naylor Student Commons, MSB
3:00 PM	**Poster session B** (Odd numbers) David Naylor Student Commons, MSB

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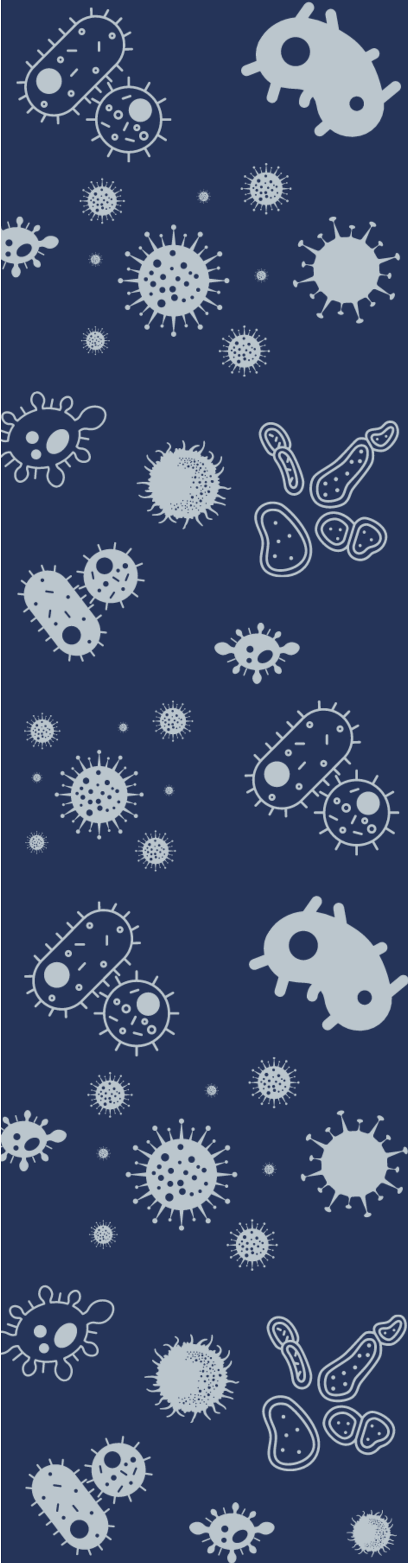

UNIVERSITY OF TORONTO ANNUAL

INFECTIOUS DISEASES AND MICROBIOLOGY RESEARCH DAY

JUNE 19 - 20, 2023

MEDICAL SCIENCES BUILDING, U OF T

ABSTRACT BOOKLET

PRESENTED BY

DIVISION OF INFECTIOUS DISEASES
DEPARTMENT OF MEDICINE

UNIVERSITY OF TORONTO

ORAL PRESENTATIONS

TRAINEE DAY - JUNE 19, 2023

MEDICAL SCIENCES BUILDING, ROOM 2170

Systematic Genetic Analysis of Candida albicans Filamentation in Response to Elevated Temperature

Emma Lash1, Victoria Prudent2, Corinne Maufrais3,4, Peter J. Stogios5, Alexei Savchenko5,6,7, Guilhem Janbon3, Suzanne M. Noble2, Nicole Robbins1, and Leah E. Cowen1

1 Department of Molecular Genetics, University of Toronto, Toronto, Ontario, M5G 1M1, Canada. 2 Department of Microbiology and Immunology, UCSF School of Medicine, San Francisco, CA 94143, USA 3 Institut Pasteur, Université Paris Cité, Unité Biologie des ARN des Pathogènes Fongiques, F-75015 Paris, France 4 Institut Pasteur, Université Paris Cité, USR 3756 IP CNRS, HUB Bioinformatique et Biostatistique, F-75015, Paris, France 5 Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, Ontario, M5S 3E5, Canada. 6 Department of Microbiology, Immunology and Infectious Diseases, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada. 7 Center for Structural Genomics of Infectious Diseases (CSGID)

The ability to transition between yeast and filamentous growth states is critical for virulence of the leading human fungal pathogen Candida albicans. Large-scale genetic screens have identified hundreds of genes required for this morphological switch, but the mechanisms by which many of these genes orchestrate this developmental transition remain elusive. We screened a mutant collection covering ~40% of the C. albicans genome and identified 39 genes required for filamentation at 39 °C, conditions relevant to febrile episodes in the host. In this study, we characterized the role of Ent2 in governing morphogenesis in C. albicans. We showed that Ent2 is required for hyphal morphogenesis under a wide range of inducing conditions and is also required for virulence in a mouse model of systemic candidiasis. We found that the epsin N-terminal homology (ENTH) domain of Ent2 enables morphogenesis and virulence and does so via a physical interaction with the Cdc42 GAP Rga2 and regulation of its localization. Further analyses revealed that overexpression of the Cdc42 effector protein Cla4 can overcome the requirement for the ENTH-Rga2 physical interaction, indicating that Ent2 functions at least in part to enable proper activation of the Cdc42-Cla4 signaling pathway in the presence of a filament-inducing cue. The functional genomic screen results also suggested a role for mRNA splicing in mediating morphogenesis and further work will elucidate how these processes are connected. Overall, this work illuminates genes important for morphogenesis in response to high temperature and advances our understanding of the factors important for C. albicans pathogenicity.

Inhibition of Coronavirus Infection by ABO Isoagglutinins

Priyal Shah(1,2), Beth Binnington(2), Martin L. Olsson(3), Jessica Lam(4), Donald R. Branch(1,2)

(1) Institute of Medical Science, University of Toronto, Toronto, ON, Canada (2) Centre of Innovation, Canadian Blood Services, Toronto, ON, Canada (3) Hematology & Transfusion Medicine, Dept. of Laboratory Medicine, Lund University, Lund, Sweden (4) CL3 and Viral Core, University of Toronto, Toronto, ON, Canada

Background: COVID-19, caused by SARS-CoV-2, requires coronavirus Spike(S)-protein, host receptor ACE2 and TMPRSS2 for infection. Emerging progeny virus use host plasma membrane, which may contain ABH(O) antigens, to form envelopes. Multiple studies reported that blood group O protects against severe COVID-19 disease, while group A patients show increased susceptibility. This suggests that anti-A from group O patients could provide natural protection against COVID-19.

Methodology: HEK293T/17 cells were transfected with CoV2-S lentivirus transferase and AB/FUT1(H)glycosyltransferases, to produce CoV2-S lentivirus that expresses ABH antigens. Vero cells that expressed TMPRSS2/Cathepsin-deletion (VeroTMP+/CTSL-), HT29 cells that expressed TMPRSS2 and human ACE2 (HT-29ACE2+TMP+), and 293T/17 cells that expressed human ACE2 (293T/17ACE2+) were generated and checked for permissiveness to CoV2-S lentivirus and/or SARS-CoV-2. Monoclonal anti-A/B were used to determine if ABO isoagglutinins could inhibit coronavirus infection. CoV2-S lentivirus infection was measured by luciferase expression and SARS-CoV-2 infection measured by RT-qPCR.

Results: VeroTMP+/CSTL-, HT29ACE2+TMP+ and 293T/17ACE2+ had a significantly greater coronavirus infection rate compared to their wildtype derivatives. Immunofluorescence data confirmed HT29 expresses A-antigen on their membrane; thus, producing A-expressing virus. A-expressing CoV2-S lentivirus infection in VeroTMP+/CSTL- was significantly inhibited by anti-A, but no inhibitory effects on wildtype, B-, or H-expressing virus. Similarly, anti-B only inhibited B-expressing CoV2-S lentivirus and showed no effects on other viruses. Anti-A also showed significant inhibition of A-expressing SARS-CoV-2 in HT29ACE2+TMP+. A post-SARS-CoV-2-vaccinated serum was used as a positive control which inhibited all infections.

Conclusions: Proof-of-concept was obtained with anti-A and anti-B inhibition of A- and B-expressing CoV2-S lentivirus, respectively. Inhibition by anti-A was also observed in SARS-CoV-2 infection. With so many reports about the importance of ABO for COVID-19, our study provides experimental evidence supporting these observations. Our results offer an underlying mechanism for ABO in the pathogenesis of SARS-CoV-2 and may aid in studies of coronavirus or other emerging virus epidemiology.

Recent sexual assault and genital biomarkers of increased HIV susceptibility

James Pollock1, Sanja Huibner2, Suji Udayakumar2, Erastus Irungu3, Peter Muthoga3, Wendy Adhiambo3, Joshua Kimani3, Janet Seeley4, Helen A. Weiss4, Tara S. Beattie4, and Rupert Kaul1,2

1 Department of Immunology, University of Toronto 2 Department of Medicine, University of Toronto 3 Partners for Health and Development in Africa (PHDA), UNITID, College of Health Sciences, Nairobi 4 Department of Global Health and Development, London School of Hygiene and Tropical Medicine

Objective: HIV risk is increased among victims of sexual violence, both in the short- and long-term. While the reasons for this are multifactorial, here we investigate inflammation and epithelial barrier disruption in the female genital tract of victims shortly after sexual assault occurs as potential biological mediators of HIV risk.

Methods: This cross-sectional study was nested within the Maisha Fiti cohort study, which investigates violence and HIV susceptibility among female sex workers in Nairobi, Kenya. Levels of proinflammatory cytokines and soluble E-cadherin (sE-cad), a marker of epithelial barrier disruption, were measured in self-collected cervicovaginal secretion samples from 746 HIV-uninfected Maisha Fiti participants using a multiplex electrochemiluminescent assay (MSD). Chi-square tests were conducted to define sociodemographic factors in participants who reported sexual assault during the 7 days preceding the study visit, and Welch's t-test was used to compare log-transformed immune factor concentrations between victims and non-victims. Associations between recent sexual assault and soluble immune factors were then further explored using simple linear regression.

Results: 44 (6%) of participants reported recent sexual assault victimization. Poverty ($p = 0.02$), adverse childhood experiences ($p = <0.001$), and mental health problems (depression, anxiety, or PTSD; $p = <0.001$) were associated with sexual assault exposure. Vaginal levels of IL-6 ($p = 0.04$), MCP-1 ($p = 0.02$), MIP-1α ($p = 0.04$), and MIP-3α ($p < 0.01$) were all significantly elevated in victims, and these associations remained significant when modelled using linear regression. sE-cad concentrations were not significantly different between groups ($p = 0.56$).

Conclusions: We found that several proinflammatory genital cytokines (IL-6, MCP-1, MIP-1α, MIP-3α) were elevated in victims of recent sexual assault, with no increase epithelial disruption. This has implications for enhanced HIV acquisition in victims of gender-based violence. Multivariate models are currently being built to control for potential confounders and further understand these results.

Encapsulation of VAR6, a potential probiotic against dental caries

Anqi Liu, Priyadarshani Choudhary, Celine Levesque, Siew-Ging Gong

Faculty of Dentistry

Probiotics are living microorganisms that are beneficial for the body. Our laboratory has identified VAR6, a novel Streptococcus salivarius strain from the mouth of a cavity-free child. We showed that VAR6 is effective at inhibiting the growth of cariogenic, or cavity-causing, bacteria such as Streptococcus mutans. We propose the use of VAR6 as a probiotic against dental caries. For VAR6 to be used intra-orally, it must be delivered in a form that can withstand the dynamic conditions of the oral cavity. The goal of our study is to develop and test the use of naturally occurring and non-toxic polysaccharide materials such as alginate (Alg) and chitosan (Ch) to encapsulate VAR6. We hypothesized that Alg-encapsulated VAR6 retained its antibacterial activity and maintained its stability at different temperatures. The aims of the study were to: i) test the cell survival and antibacterial activity of VAR6 upon encapsulation, ii) investigate the stability of the antibacterial activity and cell survival of encapsulated VAR6 at 4°C, 22°C and 37°C, and iii) investigate the stability of the antibacterial activity of encapsulated VAR6 at days 0, 7, 14, 21, 28, and 45. VAR6 was encapsulated with Alg (Alg-VAR6), some of which were coated with Ch (Alg-VAR6-Ch). Spot-on-lawn assays were utilized to evaluate the antibacterial activity of encapsulated VAR6. Results revealed that, compared to Alg-VAR6 capsules, Alg-VAR6-Ch capsules had higher antibacterial activity and were effective in inhibiting the growth of S. mutans planktonic cells. The antibacterial activity of Alg-VAR6-Ch capsules was stable for up to 45 days and maintained its highest antibacterial activity and cell survival at 4°C. In conclusion, Alg encapsulated VAR6 coated with Ch retains its antimicrobial activity over an extended period. Encapsulated VAR6, therefore, offers potential for use in the prevention of dental caries, one of the world's most common chronic diseases.

Gut microbiota and Nod2 signaling promote small intestinal restitution through the revival intestinal stem cell

Derek Tsang1, Charles Maisonneuve2, Arshad Ayyaz2, Catherine J. Streutker3,4, Daniel Trcka2, Jeffrey L. Wrana2,5, Stephen E. Girardi3, and Dana J. Philpott1

1Department of Immunology, University of Toronto, Toronto, ON Canada 2Centre for Systems Biology, Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON Canada 3Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON Canada 4Saint Michael's Hospital, Toronto, ON Canada 5Department of Molecular Genetics, University of Toronto, Toronto, ON Canada

Gut microbiota are critical mediators of inflammation and regeneration of the intestinal epithelium. Intestinal restitution is a coordinated response that involves the dedifferentiation of mature epithelial cell lineages, the proliferation of Lgr5+ intestinal stem cells, and the induction of Clu+ revival stem cells (RevSCs). How the gut microbiota directly impacts this regenerative process remains unclear. Using irradiation as a model for small intestinal epithelium restitution, we demonstrate that microbiota regulate the induction of RevSCs and the subsequent epithelial restitution response. Our results report that specific pathogen-free (SPF) mice induce greater RevSCs 3 days post-IR in comparison to germ-free (GF) mice. This microbiota-dependent increase in RevSCs was matched by an increase in BrdU+ proliferating cells, TUNEL+ apoptotic cells, and cumulative pathology score in SPF mice. Using intestinal organoids as an in vitro model of intestinal restitution, SPF and GF intestinal crypts demonstrated equal propensity to generate mature organoids suggesting the altered regeneration kinetics are driven by an epithelial-extrinsic factor. This finding is accompanied by a transcriptional increase in Tnfa, Ifnγ, and interferon stimulated genes at the onset of RevSC induction in SPF mice. Single-cell RNA sequencing of GF and SPF epithelial cells post-irradiation highlighted Nod2, a bacterial pattern recognition receptor, is induced specifically in RevSCs, suggesting bacterial detection through Nod2 may play a role orchestrating RevSC-mediated restitution. In support, intestinal epithelium specific Nod2-knockout mice have reduced BrdU+ proliferating cells following irradiation. Altogether, these findings suggest that microbiota-dependent expression of inflammatory cytokines and Nod2 signaling may be key regulators in facilitating the expansion and proliferation of RevSCs to efficiently repair the intestinal epithelium following damage.

A multi-specific, multi-affinity antibody platform neutralizes sarbecoviruses and confers protection against SARS-CoV-2 in vivo

Clare Burn Aschner*, Krithika Muthuraman*, Iga Kucharska, Hong Cui, Katherine Prieto, Manoj S. Nair, Maple Wang, Yaoxing Huang, Natasha Christie-Holmes, Betty Poon, Jessica Lam, Azmiri Sultana, Robert Kozak, Samira Mubareka, John L. Rubinstein, Edurne Rujas, Bebhinn Treanor, David D. Ho, Arif Jetha and Jean-Philippe Julien

University of Toronto and The Hospital for Sick Children

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19), has been responsible for a global pandemic. Monoclonal antibodies have been used as antiviral therapeutics; however, these therapeutics have been limited in efficacy by viral sequence variability in emerging variants of concern (VOCs) and in deployment by the need for high doses. In this study, we leveraged the multi-specific, multi-affinity antibody (Multabody, MB) platform, derived from the human apoferritin protomer, to enable the multimerization of antibody fragments. MBs were shown to be highly potent, neutralizing SARS-CoV-2 at lower concentrations than their corresponding monoclonal antibody (mAb) counterparts. In mice infected with SARS-CoV-2, a tri-specific MB targeting three regions within the SARS-CoV-2 receptor binding domain was protective at a 30-fold lower dose than a cocktail of the corresponding mAbs. Furthermore, we showed in vitro that mono-specific MBs potently neutralize SARS-CoV-2 VOCs by leveraging augmented avidity, even when corresponding mAbs lose their ability to neutralize potently, and that tri-specific MBs expanded the neutralization breadth beyond SARS-CoV-2 to other sarbecoviruses.

Detection of two alphacoronaviruses in bats in eastern Ontario, Canada

Jonathon D Kotwa1, Winfield Yim1, Finlay Maguire2,3,4, Arinjay Banerjee5,6,7,8, Juliette Blais-Savoie1, Hsien-Yao Chee1,9, Emily Chien1, Aaron Hou1, Oliver Lung10,11, Vikram Misra7, Lily Yip1, Valerie von Zuben12, Samira Mubareka1,8, Christina M Davy12,13,14

1 Sunnybrook Research Institute, Toronto, Ontario, Canada 2 Faculty of Computer Science, Dalhousie University, Halifax, Canada 3 Institute for Comparative Genomics, Dalhousie University, Halifax, Canada 4 Department of Community Health and Epidemiology, Faculty of Medicine, Dalhousie University, Halifax, Canada 5 Vaccine and Infectious Disease Organization, Department of Veterinary Microbiology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada 6 Department of Biology, University of Waterloo, Waterloo, Ontario, Canada 7 Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada 8 Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada 9 Duke Kunshan University, Kunshan, Jiangsu, China 10 National Centre for Foreign Animal Disease, Canadian Food Inspection Agency, Winnipeg, Manitoba, Canada 11 University of Manitoba Department of Biological Sciences, Winnipeg, Manitoba, Canada 12 Wildlife Research and Monitoring Section, Ontario Ministry of Northern Development, Mines, Natural Resources and Forestry, Peterborough, Ontario, Canada 13 Department of Biology, Trent University, Peterborough, Ontario, Canada 14 Department of Biology, Carleton University, Ottawa, Ontario, Canada

Introduction: Bats are key hosts for coronaviruses, some of which pose spillover risks to humans and other animals. Surveillance for bat coronaviruses in Canada remains limited, representing a critical blind spot for pandemic preparedness and bat conservation. Therefore, we began screening bats for endemic coronaviruses in Ontario, Canada.

Methods: Oral swabs were collected from 100 big brown bats (Eptesicus fuscus), 119 little brown myotis (Myotis lucifugus), 36 eastern small-footed myotis (Myotis leibii), and 35 tri-coloured bats (Perimyotis subflavus) in eastern Ontario, Canada from July to September in 2020 to 2022. RNA from sample pools of 2-4 individuals were extracted and analyzed using a nested pan-coronavirus RT-PCR targeting the highly conserved RNA-dependent RNA-polymerase (RDRP) of the coronavirus genome; samples from positive pools were re-analyzed individually for confirmation. Partial RDRP sequencing was conducted on positive samples and resultant consensus sequences were identified using BLASTn, limiting the search to North American coronavirus sequences. Phylogenetic analysis was performed including alphacoronavirus genomes, with SARS-CoV-2 as an outgroup representing betacoronaviruses.

Results: Overall, 2% (2/100) E. fuscus and 0.8% (1/119) M. lucifugus samples were RT-PCR-positive. Two coronaviruses from the alphacoronavirus genus were identified, with 70% similarity between the two. The sequences from the E. fuscus samples had 95% similarity to an Eptesicus bat coronavirus (EbCov) previously identified from E. fuscus in South Dakota, USA. The sequence from the M. lucifugus sample had 97% similarity to a Myotis lucifugus coronavirus (MylCov) previously identified in M. lucifugus in Manitoba, Canada. Notably, EbCov is related to swine acute diarrhea syndrome coronavirus.

Conclusion: Our study provides preliminary insights into coronavirus diversity in bats in a previously under-sampled region. This work provides a baseline for more rigorous surveillance, and the opportunity to understand transmission dynamics of endemic coronaviruses in a natural setting.

Micro and nanotopographies to reduce microbial surface attachment and transmission

Desmond van den Berg[1], Dalal Asker[2], Benjamin D. Hatton[1,2]

[1] Institute of Biomedical Engineering, 160 College St., Toronto ON Canada. [2] Department of Materials Science & Engineering, 184 College St., Suite 140, Toronto ON Canada

Hospital acquired infections (HAIs) remain a serious problem within our healthcare systems, stretching healthcare resources and drastically affecting patient health. These infections can be caused by a wide variety of microorganisms, including the continuing rise of antimicrobial (or multidrug) resistant species (AROs/MDRs). Pathogenic transmission within hospitals occurs primarily through contact between infected and non-infected persons, and surfaces within their environments. A common mechanism is by touch contact, and the contamination of fomite surfaces by direct droplet contact. Our research explores the microbial contamination of fomite surfaces over short time scales (15 s – 30 min) using superhydrophobic micropost arrays. Through lithography techniques, several sizes of post topographies (0.3 – 40 micrometer diameter) were successfully molded. Pathogen adhesion experiments were performed with Staphylococcus aureus, Eschercia coli, Pseudomonas aeruginosa, and Candida albicans, for droplets pipetted onto the micropost surfaces. Our results show that there is an important relationship between the size of a topography and the size of the target microbe. Overall, a reduction of 3-4 log can be achieved when reducing the diameter of a topography from significantly larger than the cell (> 20 micrometer) to one smaller than the cell (0.3 micrometer). Further experiments aimed at testing the durability of these topographies to applied pressure revealed that the wettability interface can remain stable as confirmed through confocal microscopy under low values of pressure (~1N) comparable to resting touch contact. Brief theories on the mechanism of bacterial interaction with these topographies will be presented in terms of the surface wettability. These preliminary results highlight the potential impact that topographical modifications can have in reducing the transmission of pathogens and overall prevalence of HAIs.

Assessment of the protective potential of monoclonal antibodies against antigen candidate transferrin binding protein B

Natalie Y.T. Au 1, Dixon Ng1, Epshita Islam1, Isaac S. Lee2, Gursonika Binepal2, David Curran1, Jamie Fegan2, Anthony B. Schryvers3, Scott Gray-Owen2, Trevor Moraes1

1 Department of Biochemistry, University of Toronto 2 Department of Molecular Genetics, University of Toronto 3 Department of Microbiology, Immunology and Infectious Diseases, University of Calgary

Pathogenic neisserial species, which cause meningococcal disease and gonorrhoea, utilize nutrient acquisition proteins to thrive and establish infection in humans. One example is transferrin binding protein B (TbpB), a surface lipoprotein that acquires iron from human transferrin (hTf). As TbpB is surface-exposed and essential for colonization, it has been regarded as a gonococcal vaccine antigen candidate. Previously, we observed reactivity to both gonococcal and meningococcal strains upon immunization with two gonococcal TbpB variants.

To investigate the highly cross-reactive antibody response elicited by the gonococcal TbpB variants, we generated and identified reactive monoclonal antibodies (mAbs) and assessed their protective potential. Rabbits were immunized with each of the two variants to generate over 400 rabbit mAbs for characterization of epitope and affinity with immunoassays and biolayer interferometry (BLI) respectively. Using x-ray crystallography, we are currently characterizing the structure of each antigen binding fragment (Fab) of the mAbs. Aside from biophysical characterization, we are exploring the utility of these mAbs through serum bactericidal assays (SBA) and passive immunization.

Informed by immunoassays, we selected 8 mAbs that recognized conformational and highly cross-reactive epitopes present in both gonococcal and meningococcal TbpB variants. Selected mAbs were determined to bind to the homologous TbpB with nanomolar affinity. For a select mAb, we obtained a 2.3Å crystal structure of Fab in complex with the full-length homologous gonococcal TbpB. As it binds the hTf binding interface, we are presently assessing the mAb’s ability to neutralize hTf binding. Presently, we have verified the bactericidal activity of at least one selected mAb through SBAs.

We identified mAbs that react to surface-exposed epitopes on both pathogenic Neisseria species, supporting TbpB as a pan-neisserial antigen candidate. To inform both vaccine and potential therapeutic design, we will continue to determine immunologically relevant epitopes on TbpB and assess the bactericidal activity of these cross-reactive mAbs.

Identification and characterization of a divergent Toxoplasmosis gondii associated virus for applications in therapeutics for apicomplexan parasites

Purav Gupta, Artem Babaian

The Laboratory for RNA-Based Lifeforms (RNAlab) @ University of Toronto

Data-driven virus discovery is reshaping our understanding of the repertoire of infectious agents across Earth's biosphere. Since 2021, the known biodiversity of RNA viruses has increased by over an order of magnitude, yet the vast majority of these viruses remain entirely uncharacterized. In a screen of over 86,000 human neuro-inflammatory sequencing datasets, we uncovered a highly divergent RNA virus present in a sample co-infected by Toxoplasma gondii, which we termed T. gondii-associated virus (TgAV). Toxoplasma gondii is an intracellular eukaryotic parasite (apicomplexa) with the capacity to infect over 350 mammalian and avian species worldwide.

Cross-referencing 7.5 million sequencing datasets, we further identified a TgAV homologous RNA virus (79% amino acid identity) in 10+ ovine muscle tissue (market meat) samples from geographically disparate regions, suggesting TgAV as a potential marker for T. gondii. Additionally, we identified a 59% sequence match between TgAV and another apicomplexa parasite, Eimeria necatrix-associated virus. In reference databases, TgAV's polymerase sequence is 53% to an unclassified Riboviria sp virus in the phylum Lenarviricota, establishing TgAV as possibly an exemplar of a highly divergent and novel family of apicomplexa viruses.

The primary objective of this study is to characterize the TgAV genome, and the related apicomplexa viruses and lay the foundations for its future therapeutic applications. This work furthers the concept of TgAV as a "pathogen to a pathogen," which could serve as viral vectors for studying these non-model organisms, or even a new class of therapeutic agents for combating parasitic infections.

Intestinal Barrier Disruption in malaria in pregnancy and risk of preterm birth: a cohort study

Julie K. Wright [1,2,3], Andrea M. Weckman [1,3], Michelle Ngai [1], Veselina Stefanova [1,3], Kathleen Zhong [1], Chloe R. McDonald [1], Robyn E. Elphinstone [1], Andrea L. Conroy [4], Bryan A. Coburn [2,3], Mwayi Madanitsa [5], Steve M. Taylor [6,7], Feiko O. ter Kuile [8], Kevin C. Kain [1,2,3]

[1] Sandra Rotman Centre for Global Health, Department of Medicine, University Health Network-Toronto General Hospital, University of Toronto, Toronto, ON, Canada; [2] Division of Infectious Diseases, University Health Network, Toronto, ON, Canada; [3] Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada; [4] Ryan White Center for Pediatric Infectious Diseases and Global Health, Indiana University School of Medicine, Indianapolis, USA; [5] Malawi University of Science and Technology, Thyolo, Malawi; [6] Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA, [7] Division of Infectious Diseases and Duke Global Health Institute, Duke University, Durham, North Carolina, USA; [8] Liverpool School of Tropical Medicine, Liverpool L35QA, UK

BACKGROUND: Malaria infection reduces bioavailable L-arginine, which is a risk factor for impaired placental development and adverse pregnancy outcomes following malaria in pregnancy (MiP).

HYPOTHESIS: We hypothesized that MiP-induced disruption of nitric oxide biogenesis via L-arginine depletion may also impact intestinal permeability and contribute to poor birth outcomes. To test this hypothesis, we evaluated circulating markers of ‘gut leak’ (CD14, LPS binding protein (LBP)) in pregnant women at risk of MiP and defined their association with preterm birth (PTB), and L-arginine bioavailability.

METHODS: We conducted a secondary analysis of a randomized trial in HIV-negative pregnant Malawian women (n=1378). We evaluated the association of gut-leak marker plasma concentrations (Luminex multiplex) with malaria status (PCR) and PTB. In a subset of 331 samples, bioavailable L-arginine (defined as the ratio of serum L-arginine:asymmetric dimethyl arginine concentrations) was quantified by HPLC.

RESULTS: Mean gestational age at sample collection was 20 weeks (SD 2.3). Plasma concentrations of sCD14 and LBP were significantly higher in participants with malaria and were associated with parasite burden ($p<0·0001$, both analyses and analytes). The odds ratio for preterm birth associated with one log sCD14 was 2·67 (95% C.I. 1·33 to 5·35) $p=0·006$, and 1·63 (1·07 to 2·47) $p=0·023$ for LBP. Both gut leak analytes were positively associated with increases in proinflammatory cytokines CRP, sTNFR2, IL18-BP, CHI3L1 and Angptl3 ($p<0·05$, all analytes) and sCD14 was significantly associated with angiogenic proteins Angpt-2, sENG and the sFLT:PlGF ratio ($p<0·05$, all analytes). Both adjusted and unadjusted linear regression models found significant negative associations between L-arginine bioavailability and CD14 concentrations (unadjusted B=-0.153 (-0.241 to -0.065) $p<0.005$; adjusted model: B=-0.121 (-0.207 to -0.036) $p=0.001$).

CONCLUSIONS: Malaria infection in pregnancy may contribute to intestinal epithelial disruption and gut-leak, which can increase the risk of preterm birth. Improving L-arginine bioavailability by nutritional supplementation may improve outcomes in MIP.

TIFA-mediated regulation of innate immunity

Cynthia Guo, Victoria Gillmore, Nelly Leung, Scott Gray-Owen

Department of Molecular Genetics, Temerty Faculty of Medicine

Host detection of microbial products is an essential step in eliminating pathogens and maintaining homeostasis with commensal microbes. Heptose phosphates are a class of bacterial metabolites that are required for the synthesis of lipopolysaccharide, and is thus conserved in the vast majority of Gram-negative bacteria. Detection of heptose phosphates requires the host protein TIFA and results in activation of NF-κB and cytokine production. Although this pathway has been extensively characterized in cell lines, it's unknown how this pathway contributes to bacterial clearance and immunopathogenesis in vivo. To address this question, we generated a TIFA knockout mouse and tested whether loss of TIFA leads to increased susceptibility to infection by Gram-negative bacteria. We found that TIFA knockout mice were more susceptible to infection with Helicobacter hepaticus, a pathobiont that colonizes the mouse cecum and colon. Intestinal inflammation and pathology were significantly higher in TIFA knockout mice compared to wildtype littermates. However, H. hepaticus burden did not differ significantly between wildtype and TIFA knockout mice, suggesting that the increased susceptibility to intestinal inflammation is driven by an aberrant immune response rather than a failure to control bacterial spread. Consistent with this hypothesis, we observed that TIFA knockout macrophages produced higher amounts of proinflammatory cytokines compared to wildtype macrophages following H. hepaticus infection. Furthermore, adoptive transfer of TIFA knockout macrophages exacerbated H. hepaticus-induced intestinal inflammation. Lastly, although our study was focused primarily on H. hepaticus, TIFA knockout macrophages also produced high amounts of cytokines in response Neisseria gonorrhoeae and adherent-invasive E. coli, suggesting that our findings may be more widely applicable in other contexts as well. Overall, this study demonstrates that TIFA has a crucial role in regulating intestinal immunity, and suggests that targeted regulation of TIFA may reduce immunopathogenesis without impacting pathogen clearance.

POSTER PRESENTATIONS

MAIN PROGRAM - JUNE 20, 2023

MEDICAL SCIENCES BUILDING,
DAVID NAYLOR STUDENT COMMONS

EVEN NUMBERS ARE IN SECTION A.
ODD NUMBERS ARE IN SECTION B.

1

Identifying and characterizing genes important for Candida albicans fitness in diverse environmental conditions

Emily Xiong(1), Xiang Zhang(2), Huijuan Yan(3), Ci Fu(1), Nicole Robbins(1), Suzanne M. Noble(3), Chad L. Myers(2), and Leah E. Cowen(1)

(1)Department of Molecular Genetics, University of Toronto, ON., Canada (2)Department of Computer Science and Engineering, University of Minnesota, MN., USA (3)Department of Microbiology and Immunology, University of California San Francisco, CA., USA

Candida albicans is a leading cause of fungal infections in humans. Its impact on global human health is devastating, with mortality rates as high as 40% despite treatment. As the limited arsenal of effective antifungals in clinical use is threatened by emerging resistance, the demand for new therapeutic strategies to combat invasive fungi is urgent. A promising strategy to expand the therapeutic target space is to identify genes important for pathogen growth in environments relevant to the human host. This project aims to identify and characterize genes important for C. albicans fitness within host-relevant environments. This will be accomplished by leveraging a collection of barcoded C. albicans conditional-expression mutants. Pooled screening of this large-scale mutant library in minimal nutrient medium at 30 °C identified a novel essential gene with no known S. cerevisiae homolog, C1_09670C. Follow-up computational, genetic, and phenotypic analyses predicted this open reading frame (ORF) to encode subunit 3 of replication factor A (RFA). Further screens performed in additional conditions, including at physiological temperature, low iron, and serum-supplemented medium, generated a rich dataset of genes important for fitness under specific conditions. This approach identified C3_06880W, an uncharacterized gene required for fitness in physiological temperatures with no functional predictions to date. Future work will explore mechanisms through which C3_06880W, and additional genes with condition-specific growth phenotypes, regulate C. albicans fitness. This work will reveal novel insights into vulnerabilities of C. albicans, which could be exploited to enable the development of new therapeutic strategies.

2

PEP-in-Pocket (PIP): Long-term Follow-up of On Demand HIV Post-Exposure Prophylaxis

Maxime J. Billick [1], Karla N. Fisher [2], Samantha Myers [3], Darrell H. S. Tan [1,3], Isaac I. Bogoch [1,2,4]

1. Division of Infectious Diseases, Department of Medicine, University of Toronto, Toronto, Ontario, Canada; 2. HIV Prevention Clinic, Toronto General Hospital, Toronto, Ontario, Canada; 3. Division of Infectious Diseases, St. Michael's Hospital, Toronto, Ontario, Canada; 4. Division of Infectious Diseases, Toronto General Hospital, University Health Network, Toronto, Ontario, Canada

Objective: Pre-exposure prophylaxis (PrEP) and post-exposure prophylaxis (PEP) are established methods of HIV prevention through use of antiretroviral medications. The suitability of these tools for individuals with infrequent, higher-risk HIV exposures might be limited due to cost, high pill burden and/or barriers to care. PEP-in-pocket (PIP) involves prospectively identifying such individuals and proactively prescribing 28 days of PEP medication, then sharing instructions for when to initiate medications and how to access care. We present long-term follow-up of a cohort provided with PIP for HIV prevention.

Methods: We conducted a retrospective evaluation of the clinical characteristics and outcomes of patients using PIP as their primary HIV prevention modality. Patients referred for PrEP or PEP were offered PIP if they reported a low frequency (0-4 per year) of high-risk HIV exposures of any type. HIV prevention method was chosen by shared decision-making between patients and clinicians, and was outside the realm of this study. Patients were followed at regular 4-6 months intervals.

Results: We followed 112 patients aged 20-69 for a total of 184 patient-years. 108 (96.4%) patients were assigned male at birth. Thirty-five (31.3%) patients self-initiated 69 courses of PIP during the observation period. Patients transitioned between HIV prevention modalities as circumstances warranted: 34 (30.6%) changed from PIP to PrEP, and 33 (29.7%) changed from PrEP to PIP. There were 22 episodes of bacterial sexually transmitted infections in 13 individuals (14.4%) using PIP. No HIV seroconversions were detected.

Conclusions: PIP is an innovative and useful HIV prevention strategy for people with a low frequency of higher-risk exposures, and provides patients with autonomy and agency over their care. Patients may transition between PIP and PrEP based on evolving risk and shared decision-making. PIP should be included with PEP and PrEP as a biomedical HIV prevention option for individuals at risk for infection.

3

Functional Analysis Pipeline for Microbiome Metatranscriptomics

Ryan Chieu, John Parkinson, Scott Gray-Owen

SickKids, Parkinson Lab, Gray-Owen Lab

With increasing capabilities of sequencing technology, novel techniques for measuring gene expression have become more feasible. Metatranscriptomics entails RNA sequencing the microbiome to determine how a condition may correlate with changes in function. This allows for a more accurate analysis of function over other approaches such as metagenomics, measuring transcription directly. However, there are few analytical techniques designed for microbiome analysis, and fewer for metatranscriptomics specifically. Popular differential expression techniques such as edgeR or DESeq2 have been shown to perform poorly in benchmarking tests on both microbiome and metatranscriptomics data, often suffering from inflated false positive rates. To apply more statistically valid analysis techniques to metatranscriptomics data, I sought to benchmark and assemble a metatranscriptomics analysis pipeline. Along with a pre-processing pipeline developed in the Parkinson lab, this series of analytical methods is meant to address shortcomings of typical metatranscriptomics research, which makes use of methods developed for single-cell RNA seq or 16S analysis. To test this, I made use of both an infant gut dataset that examined the impact of gestational diabetes mellitus (GDM) on the gut microbiome, and simulated false positive data created from the GDM dataset. Benchmarking was centred around optimizing a differential expression technique, testing various techniques and variations on popular methods. Using DESeq2 poscounts, a variation on DESeq2 that includes better controls for zero-inflated data, and corncob, which additionally tests for differential variability, I found that the consensus between these two techniques produced significant results when validating with a hypergeometric test. Additionally, downstream analyses of these consensus set of results found several enriched Gene Ontology terms associated with mode of delivery and sex, but none for GDM. In order to strengthen the validity of this pipeline, I intend to apply these methods to different microbiome datasets.

4

Human transferrin and lactoferrin cooperatively support colonization by Neisseria meningitidis in the murine nasopharynx

Isaac Lee, Elissa Currie, Muhamed-Kheir Taha, Anthony Schryvers, Scott Gray-Owen

Molecular Genetics, University of Toronto

Neisseria meningitidis (Nm) is a regular colonizer of the human nasopharynx, whose human-restricted nature makes in vivo studies of host-pathogen interaction difficult. Expression of human CEACAM1 (hCCM1) is required for Nm colonization of mouse nasal passages. Nm also has an exquisite specificity for the human forms of transferrin (hTf) and lactoferrin (hLf), being unable to utilize iron from mouse homologues of these proteins when infecting the murine nasopharynx. To overcome this host restriction, we have used transgenic mice expressing various combinations of hCCM1, hTf and/or hLf to uncover the role of iron sources during Nm colonization.

In vitro studies revealed that serum from hTf- or hLf-transgenic mice supported Nm growth, but serum from their wild type littermates did not. Notably, growth in the transgenic mice serum was comparable to that seen in human sera.

Nasal infection of hCCM1 mice with iron-starved Nm resulted in no recoverable meningococci at 7 days post-infection. Mice co-expressing hTf and/or hLf along with hCCM1 allowed for Nm recovery at both 7 and 10 days post-infection. Notably, the expression of all 3 transgenes resulted in the highest median burden and colonization rates, suggesting that the two iron sources may have different contributions to Nm infection. The contribution of the two bacterial receptor systems was further explored by a competitive infection with a mixture of ΔtbpB and ΔlbpB strains of Nm in the triple transgenic (hCCM1/hTf/hLf) mice. At 5 days post-infection, 75% of mice were colonized by higher proportion of ΔlbpB Nm, suggesting that transferrin may be more useful during early colonization.

Collectively, this work establishes critical contributions of the meningococcal transferrin and lactoferrin receptors during nasal colonization and provides a novel in vivo model to better study Nm host-pathogen interaction at the mucosa.

5

Identifying and characterizing antifungals with novel activity against the emerging fungal pathogen Candida auris

Lina Lim (1), John A Porco (2), Lauren E Brown (2), Nicole Robbins (1), Leah Cowen (1)

(1) Department of Molecular Genetics, University of Toronto. (2) Center for Molecular Discovery, Boston University

Billions of people are infected by fungal pathogens every year, resulting in at least 1.5 million deaths worldwide. An emerging pathogenic yeast, Candida auris, causes invasive candidiasis with a mortality rate of 29-53%. Treatment is complicated by the emergence of drug-resistant isolates, of which 87-100% of C. auris clinical isolates are resistant to the most widely deployed antifungal class the azoles. Thus, novel treatment strategies to combat C. auris infections are urgently needed. The aim of the study is to identify and characterize molecules that enhance the efficacy of azoles against C. auris. The Boston University Center for Molecular Discovery (BU-CMD) chemical library, consisting of 3,066 structurally-diverse compounds, was screened against an azole-resistant C. auris strain to identify molecules with both single agent and azole potentiating activity. The screen identified thirty-six single agents and eight azole potentiators. Of those hit compounds, five were prioritized for further characterization. Some of the prioritized compounds displayed broad-spectrum activity against different C. auris clades while some were effective against other evolutionary diverse fungal pathogens including Candida albicans, Cryptococcus neoformans, and Aspergillus fumigatus. To identify mechanism of action, haploinsufficiency profiling (HIP) will be used to identify heterozygous deletion mutants hypersensitive to compound treatment. Resistant mutants will also be selected for and candidate resistance-conferring mutations will be identified by whole genome sequencing (WGS) and validated using allele-swap assays. This project has the potential to identify novel therapeutics to treat C. auris infections.

6

Single-cell RNA sequencing of PBMCs treated with ADP-heptose reveals a type-II interferon driven immune response

Furkan Guvenc, Scott Gray-Owen

University of Toronto

Neisseria gonorrhoeae (Ngo) elicits a potent immunopathogenic inflammatory response in infected tissues. Neisseria are unique among bacteria in that they shed heptose phosphates, which are metabolic intermediates of lipopolysaccharide synthesis. These were recently discovered to be recognized by mammalian cells as a microbe associated molecular pattern (MAMP) that elicits an innate inflammatory response through the ALPK1/TIFA/NF-κB signalling axis. While this effect was originally identified using the immortalized Jurkat CD4+ T-cells, their effect on primary innate and adaptive immune cells remains unclear. Here, human peripheral blood mononuclear cells (PBMCs) were exposed to the ADP-heptose and their transcriptional response was then characterized by single-cell RNA sequence analysis. While ADP-heptose did not activate naïve T cells, it does activate memory CD4 T-cell subsets, presumably through direct NF-κB engagement. Beyond this, exposure to ADP-heptose drives a predominant type-II interferon (interferon-gamma, IFNγ) response within this heterogenous population. Rather than being a T cell-driven effect, the IFNγ is primarily produced by a subset of NK-cells. This appears to drive a secondary defence response in monocytes, which displayed the greatest transcriptional response among cells in the heterogenous population, highlighted by interferon-stimulated gene upregulation. Of these, IL-15 is especially interesting as this interferon-stimulated cytokine has been shown to promote and stimulate the activation and proliferation of NK cells. However, our subsequent in vitro analyses revealed that isolated NK cells do not respond directly to ADP-heptose, suggesting that the monocyte-derived IL-15 supports the ADP-heptose-dependent NK cell activation and IFNγ expression. Together, this study suggests that heptose phosphates broadly stimulate memory T cells in an antigen-independent manner and initiate an immunological cross-talk between NK cells and monocytes that stimulates an IFNγ-dependent inflammatory response and upregulation of anti-bacterial effector functions.

7

Exploring the phenotype of alveolar and long bone loss in Borrelia burgdorferi infected mice

Imran Farooq (1), Anna Boczula (2), Tara J. Moriarty (3)

Faculty of Dentistry (1,2,3) and Faculty of Medicine (3), University of Toronto, Toronto, ON, Canada

Objective: To characterize alveolar and long bone loss phenotype in Bb-infected male and female mice at acute and chronic stages of infection.

Methods: To determine the phenotype of bone loss at different infection stages, we mock-infected (-Bb) and infected (+Bb) 120 male and female mice and collected femora, tibiae, and skulls at 1, 4, and 8 weeks post-infection. Three-point bending test was performed to determine the cortical bone's biomechanical properties. To recognize osteoblasts (OBs), bone sections were stained with Goldner's trichrome, whereas for osteoclast (OC) recognition, staining with Tartrate-resistant acid phosphatase (TRAP) was accomplished. DNA (to confirm bacterial burden) was extracted from the pulverized distal tibiae of mice using DNA extraction kits. Trabecular bone loss (in long bones) was determined using micro-computed tomography (µCT) to analyze various parameters (BMD, BV/TV, BS/BV, trabecular number, trabecular pattern factor, and trabecular separation). Alveolar bone loss will be measured in methylene blue-stained maxillae (pending).

Results: We observed that cortical bone's biomechanical properties and OC numbers were not significantly different between -Bb and +Bb mice. However, OBs in the +Bb mice were significantly reduced 1W post-infection compared to the mocks. We also detected that a significant Bb burden was only established at the 4W time point in +Bb male and female mice. Our current findings revealed that various µCT parameters (including BMD) significantly differed between -Bb and +Bb mice. Examination of mean T-scores for bones from +Bb mice indicated that significant osteopenia and osteoporosis were present in the femora of female and male infected mice at 4W time point, respectively. No sex related differences were observed for any of the experiments of this study.

Conclusions: Bb induces significant trabecular long bone loss in male and female infected mice 4W post-infection. Bone loss or change in the T-scores at 4W time point could be best explained by the significant reduction in OBs 1W post-infection. From these results, we predict that the OB reduction in the acute infection stage (1W) and consequent bone loss in the sub-acute infection stage (4W) is driven by the systemic cytokines and factors.

8

HSV-2 infection and epithelial barrier disruption in the female genital tract

Suji Udayakumar 1, James Pollock 2, Sanja Huibner 1, Erastus Irungu 3, Pauline Ngurukiri 3, Peter Muthoga 3, Wendy Adhiambo 3, Joshua Kimani 3, Tara S. Beattie 4, and Rupert Kaul 1,2

1 Department of Medicine, University of Toronto, Toronto, Canada 2 Department of Immunology, University of Toronto, Toronto, Canada 3 Partners for Health and Development in Africa (PHDA), UNITID, College of Health Sciences, Nairobi, Kenya 4 Department of Global Health and Development, London School of Hygiene and Tropical Medicine, London, UK

Objective: Herpes simplex virus type-2 (HSV-2) infection is usually asymptomatic, but increases genital CD4+ T cell activation and HIV acquisition risk. We examine whether HSV-2 infection is also associated with elevated vaginal levels of soluble E-cadherin (sE-cad), a novel biomarker of epithelial barrier disruption, a mechanism that can facilitate HIV acquisition.

Methods: This cross-sectional study was nested within the Maisha Fiti study, which investigates HIV-violence interactions among female sex workers in Nairobi, Kenya. In 731 HIV-negative participants, HSV-2 sero-status was assessed using the Kalon HSV-2 IgG assay. Cervicovaginal secretions were assayed for sE-cad using a multiplex chemiluminescent ELISA platform (MSD). Demographic and clinical characteristics and levels of sE-cad were compared between HSV-2 sero-positive and sero-negative participants. Multivariable regression models to control for potential confounders are currently being constructed.

Results: 381 (52%) participants were HSV-2 sero-positive. HSV-2 sero-positive participants in this study were older than HSV-2 sero-negative participants (average age = 34 vs 28 years, respectively, $p < 0.01$), more engaged in intravaginal washing practises (64 vs 55%, $p = 0.02$) and used less hormonal contraception (85 vs 91%, $p = 0.01$). There was no difference in vaginal levels of sE-cad between HSV-2 sero-positive and sero-negative participants (mean concentration = 95,847 vs 90,711 pg/mL, $p = 0.21$).

Conclusions: Our results demonstrate that subclinical epithelial barrier disruption is not likely to be a major mechanism underpinning increased HIV acquisition among HSV-2 sero-positive individuals. However, we will continue to investigate this association with potential confounding factors to gain further insight into HSV-2 pathophysiology.

9

A novel phage defense system block phage assembly without self-targeting

Pramalkumar H. Patel, Veronique L. Taylor, Landon J. Getz and Karen L. Maxwell

Department of Biochemistry, University of Toronto, MaRS West Tower, 661 University Avenue, Toronto, ON M5G 1M1, Canada

Bacteria and their phages (viruses) are in a perpetual struggle, giving rise to an impressive collection of defense and offensive weapons. Temperate phages insert their genome into the host chromosome as a prophage and form a symbiotic relationship with their host by expressing genes that increase bacterial fitness. Since invading phages are a serious threat to bacterial survival, prophages employ their own defenses to protect host cells from further phage infections. As prophages are pervasive, understanding these defenses and how they guard their host cells will provide insights into the phage-host evolutionary arms race. Here, we characterize a novel anti-phage system, Gp31, found in Pseudomonas phage JBD26. Biochemical assays showed that Gp31 does not inhibit phage adsorption, genome injection, or DNA replication but inhibits a step-in phage assembly process. Considering that Gp31 is a small protein with no known enzymatic activities, we propose a simple mechanism for Gp31 where it binds and interferes with a protein required for phage virion assembly. To our knowledge, this is the first known anti-phage defense mechanism mediated via inhibition of virion assembly.

Since Gp31 provides resistance to phages closely related to JBD26 (but not JBD26), a question arises: how does JBD26 escape Gp31 activity? Strikingly, we noticed that gp31 is always accompanied by another gene, gp30, suggesting a functional link. We showed that Gp30 is a counter-defense protein that is both necessary and sufficient to inhibit Gp31's function. We propose that JBD26 prophages constitutively express Gp31 which blocks invading phages. When JBD26 enter the lytic cycle, Gp30 is expressed along with virion assembly proteins, binds to Gp31, and shuts its inhibitory activity. Our work demonstrates that phages protect themselves from similar phages but contain a safeguard mechanisms to prevent self-targeting. Additionally, through bioinformatics, I identified several novel genes that also contribute to phage defense.

10

SARS-CoV-2 temporal dynamics, genomic surveillance, and disease severity of COVID-19 patients from Toronto, Canada

Kuganya Nirmalarajah1, Jonathon D. Kotwa PhD1, Patryk Aftanas BSc1, Xi Zoe Zhong PhD2, Natalie G. Bell MSc1, Shiva Barati MD2, Emily Chien MSc1, Gloria Crowl MS2, Amna Faheem MPH2, Lubna Farooqi MD2, Ryan Hiebert1, Alainna J. Jamal PhD2, Kevin Katz MD3, Saman Khan MPH2, Robert Kozak PhD1, Angel X. Li MSc2, Reena Lovinsky MD4, David Rose MD4, Finlay Maguire PhD5, Mohammad Mozafarihashjin MD2, Sheridan J.C. Baker PhD6,7, Hooman Derakhshani PhD7,8,10, Laura Rossi8,10, Jalees A. Nasir6,7, Emily M. Panousis6,7, Ahmed N. Draia6,7, Aimee Paterson MSc2, Jeff Powis MD9, Christopher Kandel MD9, Renée Schryer1, Altynay Shigayeva PhD2, Maureen Taylor PA9, Natalie Wilson1, Winfield Yim1, Lily Yip1, Michael G. Surette PhD6,10, Andrew G. McArthur PhD6,7, Allison J. McGeer MD2,11 and Samira Mubareka MD1,11

1Sunnybrook Research Institute, Toronto, Ontario, Canada 2Sinai Health System, Toronto, Ontario, Canada 3North York General Hospital, Toronto, Ontario, Canada 4Scarborough Health Network, Toronto, Ontario, Canada 5Faculty of Computer Science, Dalhousie University, Halifax, Nova Scotia, Canada 6Michael G. DeGroote Institute for Infectious Disease Research, McMaster University, Hamilton, Ontario, Canada 7Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada 8Farncombe Family Digestive Health Research Institute, McMaster University, Hamilton, Ontario, Canada 9Michael Garron Hospital, Toronto, Ontario, Canada 10Department of Medicine, McMaster University, Hamilton, Ontario, Canada 11Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada

Background: Genomic surveillance and monitoring disease severity are critical as SARS-CoV-2 variants emerge. We sought to temporally track variant dynamics, mutational diversity, and estimate genomic and clinical risk factors for hospitalization in COVID-19 patients.

Methods: Patients from 7 hospitals in the greater Toronto area were recruited from March 2020 to April 2022. Nasal swabs were subjected to whole genome sequencing for lineage assignment and mutation analyses. Multivariable backward-stepwise logistic regression models were constructed to investigate associations between lineages, patient factors, and disease severity.

Results: In 908 patients with available genomic data, we observed 89 unique lineages (including sublineages). Forty-one percent (372/908) of cases were variants of concern. SARS-CoV-2 mutation profiles from 629 patients were further investigated. Forty-eight percent (301/629) of patients were hospitalized. A total of 4222 amino acid substitutions were detected in inpatients and 5042 in outpatients. Transition/transversion ratios were compared; 2.5 for outpatients and 2.69 for inpatients. In a subset of 426 patients, multivariable logistic regression was performed. The median age was 59 (interquartile range 46-72) and sixty-six percent (267/426) were hospitalized. The B.1 lineage was most prevalent at 17% (72/426), followed by 12% (53/426) Delta and 11% (47/426) Alpha. Multivariate analysis indicated that vomiting (OR 27.90, 95%CI 2.77-281, P=0.005), shortness of breath (OR 26.58, 95%CI 9.86-71.63, P=0.000), cough (OR 4.00, 95%CI 1.94-8.69, P=0.000), and infection with Alpha (OR 6.84, P=0.013) significantly increased odds of hospitalization. Two vaccine doses (OR 0.03, P=0.000), loss of smell (OR 0.05, 95% CI, P=0.000), and muscle ache (OR 0.43, 95% CI 0.19-0.96, P=0.04) significantly reduced odds of hospitalization.

Conclusions: There were minimal mutational differences in hospitalized patients versus outpatients. Infection with alpha, shortness of breath, vomiting, and cough were associated with increased risk of hospitalization. Our work emphasizes the importance of ongoing combined clinical and genomic SARS-CoV-2 surveillance as new variants arise.

11

Body Mass Index for-age but not Height for-age z-scores are Associated with Altered Gut Microbial Functions in 13-Year-Old Brazilian Children

Arooj Asrar1,2, Lorena Lopez-Dominguez1,2, Celine Bourdon2, Jill Hamilton3, Maria Carolina Borges4,5, Luciana Tovo-Rodrigues6, Iná S Santos6, Alicia Matijasevich6,7, Aluísio JD Barros6, Robert HJ Bandsma1,2, Elena M Comelli1,8

1Department of Nutritional Sciences, Faculty of Medicine, University of Toronto, Toronto, Canada; 2Translational Medicine Program, Hospital for Sick Children, Toronto, Canada; 3Department of Pediatrics, Faculty of Medicine, University of Toronto, Toronto, Canada; 4MRC Integrative Epidemiology Unit, University of Bristol, Bristol, United Kingdom; 5Population Health Sciences, Bristol Medical School, University of Bristol, Bristol, United Kingdom; 6Post-Graduate Program in Epidemiology, Federal University of Pelotas, Pelotas, Brazil; 7Departamento de Medicina Preventiva, Faculdade de Medicina FMUSP, Universidade de São Paulo, São Paulo, Brasil; 8Joannah and Brian Lawson Centre for Child Nutrition, University of Toronto, Toronto, Canada

Background. Under- and over-nutrition are increasingly present in low- and middle-income settings, leading to a double burden of malnutrition at population or individual level. The gut microbiota may affect host’s health via dietary component degradation for energy provision and maintenance of a homeostatic gut environment. This relationship may reshape during adolescence, corresponding to the last step of gut microbiota maturation, yet remains under-investigated. Objective. To assess if gut microbial functions vary with body mass index-for-age and height-for-age z-scores (BMIZ, HAZ) in adolescents and are influenced by sex and pubertal stage. Methods. 340 13-year-olds (53% males) with no antibiotic use within the last 6 weeks from the 2004 Pelotas Birth Cohort, Brazil, were stratified using WHO BMIZ (wasting (n=11); normal (n=251); overweight/obese (n=78)) and HAZ (stunting (n=10); normal (n=330)) cutpoints. Tanner staging assessed pubertal development (1 (n=12); 2-4 (n=212); 5 (n=116)). Fecal microbiota was assessed via 16S rRNA sequencing and functions inferred with Piphillin and KEGG. Data were analyzed via DESeq2 in R. Results.170 prokaryotic pathways were identified. There was no differential representation according to HAZ and in overweight/obese vs normal. Using the wasting category as a comparator, 36 pathways were differently represented (q-value<.05) in the normal category and 16 pathway subset in the overweight/obese. Sex and pubertal stage accounted for variation observed in 2/36 and 0/16 pathways, respectively. Higher representation of Escherichia coli and Mycobacterium pathogenicity in the wasting category, aligns with increased susceptibility to intestinal infection in undernourished individuals and higher representation of Enterobacteriaceae pathways in the overweight/obese, aligning with dysbiosis and metabolic syndrome. Antibiotics synthesis and xenobiotics degradation were more highly represented in the wasting and overweight/obese category, respectively. Conclusion. These findings suggest that the functional potential of the adolescent gut microbiota is altered with BMIZ and may be a target of interventions aiming at sustaining adolescent health.

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Ultrasound-guided transfection of claudin-5 improves lung endothelial barrier function in lung injury without impairing innate immunity

R Sanwal1,2, V Mintsopoulos2, M Ditmans2, A Lang2, E Latreille1,2, S Ghaffari2, N Khosraviani1,2, R Karshafian3,4, H Leong-Poi2, DM Hwang5, L Brochard2,6, A Goffi2,6, AS Slutsky2,6, WL Lee1,2,4,6

1. Department of Laboratory Medicine and Pathobiology, University of Toronto 2. Keenan Research Center for Biomedical Science, St. Michael's Hospital 3. Department of Physics, Toronto Metropolitan University 4. Institute for Biomedical Engineering, Science and Technology (iBEST), Toronto 5. Department of Laboratory Medicine and Molecular Diagnostics, Sunnybrook Health Sciences Centre, Toronto 6. Interdepartmental Division of Critical Care Medicine, University of Toronto

In acute lung injury, the lung endothelial barrier is compromised. Loss of endothelial barrier integrity occurs in association with decreased levels of the tight junction protein claudin-5. Restoration of their levels by gene transfection may improve the vascular barrier but how to limit transfection solely to regions of the lung that are injured is unknown. We hypothesized that thoracic ultrasound in combination with intravenous microbubbles (USMB) could be used to achieve regional gene transfection in injured lung regions and improve endothelial barrier function. Since air blocks ultrasound energy, insonation of the lung is only achieved at areas of lung injury (edema, atelectasis); healthy lung is spared. Cavitation of the microbubbles achieves local tissue transfection. Here we demonstrate successful ultrasound-microbubble (USMB)-mediated gene transfection in the injured lungs of mice. After thoracic insonation, transfection was confined to the lung and only occurred in the setting of injured (but not healthy) lung. In a mouse model of acute lung injury, we observed down-regulation of endogenous claudin-5 and an acute improvement in lung vascular leakage and in oxygenation after claudin-5-over-expression by transfection. The improvement occurred without any impairment of the immune response as measured by pathogen clearance, alveolar cytokines and lung histology. In conclusion, USMB-mediated transfection targets injured lung regions and is a novel approach in the treatment of lung injury.

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Pangenome insights into diversification and disease specificity in plant pathogenic Xanthomonads

Viplav Agarwal, Rachel Stubits, Zain Nasrullah, Marcus Dillon

EEB Department, University of Toronto and Biology Department, UTM

The bacterial genus Xanthomonas is responsible for disease outbreaks in several hundred plant species, many of them economically important crops. In the last few decades, thousands of Xanthomonas genomes have been sequenced as part of isolated studies that focus on outbreak characterization, host range, diversity, and virulence factor identification. These data present a unique opportunity to study the evolutionary history of this large and diverse pathogenic bacterial genus, providing valuable insight into the factors underlying adaptation to different hosts. We present a pangenome analysis of 1,910 diverse Xanthomonas strains, highlighting the extensive diversity within the genus, the evolutionary relationships between classified species, and the distribution of virulence-associated genes across strains. We find a high degree of genetic cohesion between species that results from frequent inter-species horizontal gene transfer and identify a number of broadly conserved classes of virulence factors, including several families of type III secreted effectors. We also use these data to reassign a number of incorrectly classified strains to phylogenetically informed species designations and find evidence of both monophyletic host-specificity and convergent evolution of distantly related strains to the same host. Understanding the mechanisms by which these pathogens shift between hosts will enable us to develop more robust resistance strategies to protect economically important crops from these devastating diseases.

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Ex vivo replication kinetics and in vivo transmission of SARS-CoV-2 Alpha, Gamma, Delta, and Omicron variants

Andra Banete 1*, Bryan D. Griffin 1*, Juan Corredor 1, Lily Yip 1, Patryk Aftanas 3, Kuganya Nirmalarajah 1, Jady Liang 2, Hunsang Lee 2, Tarini Gunawardena 4, Hong Ouyang 4, Mikko Taipale 2, Natasha Christie-Holmes 2, Scott D. Gray-Owen 2, Finlay Maguire 3, Rob Kozak 1,2, Allison McGeer 2,5, Haibo Zhang 2, Theo Moraes 4,2, Samira Mubareka 1,2

1 Sunnybrook Research Institute; 2 University of Toronto; 3 Scarborough Health Network (SHL); 4 The Hospital for Sick Children (SickKids); 5 Mount Sinai Hospital

As a result of the widespread circulation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in humans since its emergence in 2019, several novel variants of concern (VOC) have been identified that spread more easily, cause greater disease severity, and are capable of partial escape of host immune responses against prior variants, monoclonal antibodies, or vaccines. As additional VOCs will presumably continue to be identified for the foreseeable future, it is crucial that we understand the molecular basis for the differences in disease severity and spread that have been observed.

Here, we investigate replication kinetics of SARS-CoV-2 VOCs in primary human nasal epithelial (HNE) cells and induced pluripotent stem cell derived-lung organoids (iPSC-LOs), as well as the pathogenesis and transmission in the Syrian golden hamster model of SARS-CoV-2 infection. HNEs and LBOs were infected with SARS-CoV-2 VOC (Alpha, Gamma, Delta, Omicron BA.1 and BA.2) and infectious virus production was quantified. Omicron isolates rapidly replicate in HNE compared to all other VOCs, yielding ~100-1000-fold higher viral titres.

Male and female 5-7 week old hamsters were infected with VOCs and co-housed in a transmission cage with naïve animals in groups of two. Oropharyngeal swabs were taken every other day until day 10 to quantify infectious virus shedding. Infection with SARS-CoV-2 VOCs resulted in ~10-15% reduction in body weight by 7dpi, with P.1 and Delta causing the greatest weight loss. We observed sex differences in pathogenesis, with males being more affected. A comparison in infectious viral shedding in oropharyngeal swabs at 3dpi shows no difference between VOCs.

This work aims to unravel some of the differences observed in spread, disease severity, and immunity for SARS-CoV-2 VOCs. Overall, infection with Omicron isolates demonstrate attenuated replication in lungs in rodent models and primary human cell models of SARS-CoV-2 infection.

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UnderstandIdentifying new prophage encoded anti-phage defence systems in P. aeruginosa

Véronique L. Taylor, Pramalkumar H. Patel, Karen L. Maxwell

University of Toronto Department of Biochemistry

Antimicrobial resistance is the silent pandemic and the need for new therapies against bacteria infection is essential. Bacteriophages (phages) viruses that specifically infect and kill bacteria are an attractive alternative. However, a greater understanding of phage-bacteria dynamics is required to maximize the usefulness of phages as therapeutics. Bacteria encode a plethora of accessory/mobile elements, including phage genomes from previous infections. The integrated phage, known as a prophage, ensures its' survival by protecting the bacteria from future infection. A common way prophages defend their host is by altering receptors which incoming phages use to bind and infect. I have identified a highly-conserved prophage-encoded anti-phage defence protein, Zip, in Pseudomonas aeruginosa that inhibits the type-iv pilus, a structure used for phage infection. The expression of Zip is driven by a host transcriptional regulator, LasR. The expression of this protein confounds the identification of novel anti-phage defence systems due to its high prevalence within phage genomes. However, creating genomic knockouts in each phage in our collection is time consuming. By creating prophages in a P. aeruginosa lasR delete strain, where Zip is no longer expressed, I have identified 3 new anti-phage systems in 3 different prophage genomes. I anticipate this high-throughput method will further identify additional anti-phage defence systems in our collection. Understanding what anti-phage defence systems are present within prophages will ensure selected therapeutic phages encode inhibitors to all defence systems.

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Detection of bacterial ADP-heptose by a rare intestinal stem cell population drives epithelial regeneration from Paneth cells dedifferentiation

Shawn Goyal [1], Scott Gray-Owen [2], Stephen Girardin [1,3]

[1] Department of Laboratory Medicine and Pathobiology, [2] Department of Molecular Genetics, [3] Department of Immunology

Background: The intestinal stem cell (ISC) niche is located in intestinal crypts and rarely exposed to microbial products because microbes are excluded from this area by both chemical and physical barriers. The presence of microbial products in the intestinal crypt indicates epithelial barrier disruption, which may arise from defective ISCs. ADP-heptose is detected in host cells by its receptor ALPK1 (α-protein kinase 1). We hypothesise activation of the receptor ALPK1 by its bacterial derived ligand, ADP-heptose (AH), serves as a signal of barrier disruption. We further hypothesise killing of defective ISCs is a required signal for the induction of the revival stem cell (revSC) response.

Purpose: My research seeks to characterize microbial dependent regulation of intestinal epithelium function and architecture, to identify disrupted pathways in inflammatory bowel disease (IBD) and establish novel targets for therapy.

Method: Single-cell RNA-seq analysis of ileal epithelium cells was used to identify localized expression of ALPK1 and TIFA (the down-stream target of ALPK1). ALPK1 stimulation by AH was assessed in intestinal organoids. Proliferation was assessed by immunofluorescence (IF), assessment of labelled nucleotide incorporation and brightfield crypt branching quantification. Induction of apoptosis was assessed ex vivo by western blot and IF. Organoids derived from transgenic mice were used for lineage tracing to characterize epithelial restoration by revSC.

Results: This work demonstrates: [i] Active ALPK1-TIFA signalling in IECs. [ii] Activation of the ALPK1-TIFA pathway by AH inhibits epithelial proliferation. Finally, [iii] ALPK1-TIFA dependent killing of ISCs induces the generation of revSC through de-differentiation of Paneth Cells, to regenerate the intestinal epithelium.

Conclusion: This work, for the first time, characterises the ALPK1-TIFA signalling pathway in ISCs and demonstrates specific killing of ISCs by an innate immune receptor ligand. Additionally, this work provides the first characterisation of revival stem cell induction by a physiologically relevant stimulus (AH).

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Using a hierarchical machine learning approach to improve taxonomic classification of metagenomic short-reads

Bhavish Verma

Dr. John Parkinson Lab

The microbiome has a substantial influence on the health of its host environment. For instance, the human gut microbiome has been found to aid in nutrient metabolism and inhibit pathogen colonization, among many other functions. However, during periods of extreme stress in the host environment, the composition of the microbiome often undergoes modifications that have been implicated in the development of various human diseases. To gain a deeper understanding of the functional role of the microbiome, researchers have employed metagenomic and metatranscriptomic sequencing methodologies across diverse environments. To effectively utilize this data, it is imperative to map the resultant sequence reads to their respective taxonomic origins. Traditional approaches to taxonomic classification heavily rely on reference databases. A significant drawback to these traditional approaches is that their reference databases are heavily biased towards a small subset of microbes, leading to a higher likelihood of false positives. To address this limitation, researchers have turned to reference-free machine learning techniques, which have shown promising results. However, current machine learning approaches still face challenges in accurately classifying species compared to traditional algorithms. One promising avenue for enhancing taxonomic prediction is to adopt a hierarchy-informed machine learning methodology whereby higher-level taxa can assist in species identification. Here, we describe HiTaxon, a hierarchical machine learning approach for the taxonomic classification of short-reads. Using a novel hierarchical framework, HiTaxon generates predictions for individual reads at the most specific taxonomic level that can be confidently classified. Our results demonstrate that HiTaxon outperforms traditional approaches at the species level, using both real and simulated short-read datasets. Moreover, HiTaxon excels in characterizing novel species compared to the best species-level traditional classifier. To facilitate the adoption of HiTaxon, we have developed an automated pipeline that streamlines the collection and preprocessing of training data, as well as the model training process.

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Discovery and Characterization of the Elusive Zymophage

Daniel Anderson, Zeynep Cildir, Adam Rudner

University of Ottawa, Faculty of Medicine

Antimicrobial resistance is slowly becoming one of the greatest threats to human health as many antimicrobials are becoming obsolete when overused against bacterial and fungal “superbugs”. Bacteriophages (or phages), viruses which infect and kill bacteria, hold promise as a new anti-microbial by targeting specific bacteria. However, very few viruses that infect unicellular eukaryotic organisms are known, and none have been found that infect Saccharomyces cerevisae, or budding yeast, which serves as a model fungal system, crucial in the understanding of fundamental mechanisms of cell biology and genome-wide approaches in biomedical sciences. When genetic research in budding yeast began in the 1960s, researchers looked for viruses that infect yeast, named “zymophages”, to use in genetic engineering. None were found. However, by adapting modern methods used for bacteriophage discovery for use in yeast, testing both laboratory strains and wild isolates from bark, we hope to finally resolve this 60-year mystery. This discovery work also tests whether the endogenous 2-micron plasmid, a ~6kb selfish DNA element, may function as a host defence system. By curing this plasmid from any host strain used for zymophage hunting, we can test if it serves as a restriction system to protect yeast from zymophage infection. Any discovered zymophage will be amplified, its genome purified and sequenced, and its morphology determined by transmission electron microscopy. This project holds the potential to not only uncover a new class of viruses of unknown size, genomic organization, and morphology, but could open the door to combatting resistant fungal pathogens.

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Tissue Specific Differences in IL-18 Regulation in Human Monocytes

Lu Yi (Lina) Li, Jessica Bruce, Stephen E. Girardin, Dana J. Philpott

1) Department of Laboratory Medicine and Pathobiology, University of Toronto 2) Department of Immunology, University of Toronto 3) School of Biomedical Science and Pharmacy, University of Newcastle

Inflammasomes are vital cytosolic immune complexes that play a crucial role in protecting against infectious pathogens. Inflammasomes activate inflammatory caspases, which cleave and release cytokines interleukin-18 (IL-18) and interleukin-1β (IL-1β). Notably, dysregulation of inflammasomes is implicated in various auto-inflammatory and autoimmune diseases, making the understanding of inflammasomes increasingly important. Moreover, the development of therapeutics targeting inflammasomes has become one of the major topics in the field. Currently, inflammasome research predominantly focuses on murine macrophages, but there are distinct differences between mice and humans. In mice, caspase 1 cleaves IL-18 and IL-1β. Our lab has identified that in human epithelial cells, caspase 4 cleaves IL-18. However, it remains unknown whether this finding extends to broader cell types. Here, we aim to investigate how inflammatory cytokines are activated in human immune cells. We hypothesize that caspase 4 processes and activates IL-18 in human immune cells. To test our hypothesis, we pharmacologically inhibited caspase 1 activation in two ways, using dimethyl fumarate (DMF) and MCC950, in human monocytes and then activated inflammasomes. Through the blockade of caspase 1 activation, we observed a significant reduction in IL-18 production. This outcome remained consistent across both methods of inhibition and even when different inflammasomes are activated, all involving caspase 1 in their downstream pathway. Together, our results demonstrate that in human immune cells, IL-18 production is governed by caspase 1 and not caspase 4, revealing tissue-specific differences in inflammasome signalling. These findings highlight the complexity and the diversity of inflammasome regulation in different human tissues and emphasizes the need for further research in a relevant human cellular context. Furthermore, human inflammasome research will allow us to uncover not only species-specific differences, but also tissue-specific differences, ultimately paving the way for targeted therapy development for human inflammatory and autoimmune disorders.

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Neurofilament Light Chain as a Biomarker for Neurological Injury in African Children with Malaria

Caroline K. Francis, Nuria Balanza, Valerie M. Crowley, Andrea Weckman, Rosauro Varo, Quique Bassat, Kevin C. Kain

UHN, University of Toronto, ISGlobal

Background: Malaria is a leading cause of neuro-disability in African children. However, current tools to evaluate brain injury or sequelae are complex and inaccessible. Neurofilament light chain (NfL) is a neuron-specific protein, whose levels in blood increase when axonal injury occurs due to inflammatory, neurodegenerative, traumatic, or vascular injury. Here we test the hypothesis that plasma NfL levels function as a biomarker of neuronal damage in children with malaria.

Methods: We examined longitudinal plasma NfL levels in children aged 1-12 years with uncomplicated (UM; n=30) and severe malaria (SM; n=137) from Mozambique, using enzyme-linked lectin assay (ELLA) technology. NfL levels for all children were quantified at admission and at 12, 36, 60, and 84 hours. For those with SM, additional samples at 96 hours and day 14 were also included.

Results: We found that NfL levels were similar in all malaria cases at healthcare presentation, but levels increased over time. 59.9% of SM cases displayed neurological manifestations (i.e., coma, impaired consciousness, or repeated seizures). The increment in NfL levels was significantly higher in SM cases with neurological manifestations.

Conclusions: Our results support plasma NfL as a potential biomarker of neuronal damage in pediatric malaria. However, our findings indicate that NfL is not an early indicator, given its gradual increase after neurological manifestations appear. Measurement of NfL levels during malaria infection may enhance the identification and quantification of brain injury.

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Elucidating the Role of Leucine-Rich Repeat Kinase 2 in Crohn's Disease Pathogenesis and Neutrophil Biology

Bana Samman,(1) Elisabeth Foerster,(2) Lina Chen,(3) Stephen Girardin,(1,2) Dana Philpott(1,2)

1) Department of Laboratory Medicine and Pathobiology, Faculty of Medicine, University of Toronto; 2) Department of Immunology, Faculty of Medicine, University of Toronto; 3) Department of Laboratory Medicine and Molecular Diagnostics, Sunnybrook Health Sciences Centre

Background:
LRRK2 kinase domain gain-of-function variants, such as G2019S, have been linked to the development of Crohn's disease (CD), yet their exact roles in pathogenesis remain elusive. LRRK2 is most robustly expressed in immune cells, such as neutrophils, lymphocytes, and macrophages. LRRK2-deficient macrophages exhibit defective antimicrobial responses. As an enteric colitis-inducing extracellular pathogen, Citrobacter rodentium can help us better understand the consequences of LRRK2 kinase hyperactivity on intestinal inflammation by correlating pathogen burden with key host response parameters over the course of infection. Additionally, models of neutrophil activation and murine peritonitis can help us understand the role of LRRK2 in mediating neutrophil effector functions.

Purpose: To investigate the effects of the CD-associated Lrrk2 G2019S hyper-kinase mutation on pathogen burden and colonic inflammation in the context of C. rodentium-induced infectious colitis, and to investigate the effects of Lrrk2 deficiency and hyper-kinase mutations on neutrophil function.

Methods: Wild-type and G2019S mutant mice were infected with 10^8 CFU of C. rodentium. Various markers of inflammation as well as pathogen burden were measured over the next 14 days to assess differences in the magnitude and kinetics of colonisation and inflammation between genotypes. Additionally, bone marrow-derived neutrophils from WT, Lrrk2 KO, and Lrrk2 G2019S mice were stimulated with PMA, and their activation-induced degranulation response was assessed via flow cytometry (CD11b externalisation) and ELISA (lipocalin-2 secretion). Lastly, a model of MDP-induced peritonitis was used to assess neutrophil migration capacity in WT, KO, and G2019S mice.

Results and Conclusions: No significant differences were found in overall pathogen burden or pathogen clearance rates between genotypes over the first 14 days of infection. No significant differences were observed in the secretion and expression of inflammatory and antimicrobial factors of interest between WT and G2019S mutant mice at the various infection timepoints assessed. Additionally, neither the deficiency of Lrrk2 nor mutation of its kinase domain altered neutrophils' capacity to undergo degranulation in vitro or migration in vivo. Further research will aim to investigate the role of Lrrk2 in neutrophils using other models of inflammation.

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Population genomics of Neisseria gonorrhoeae

Duncan Carruthers-Lay, Emil Jurga, Scott Gray-Owen, John Parkinson

University of Toronto, Faculty of Medicine, Department of Molecular Genetics; Hospital for Sick Children, Molecular Medicine

Neisseria gonorrhoeae is an emerging pathogen of global concern due to the emergence of widespread antimicrobial resistance (AMR). A better understanding of underlying population structures and its diverse collection of infection-associated genomic factors will aid in combatting the spread of this rapidly developing public health threat. We began by collecting a set of ~7400 genomic sequences from the NCBI Sequence Read Archive and aligning them to the reference genome of N. gonorrhoeae strain FA1090 to identify and define a reference set of maximally diverse isolates. This set encompasses 767 isolates from around the world and forms the basis for the following analyses. Using the population genomics tool PopNet developed by the Parkinson lab, I interrogated population structures using a whole genome approach to compare and contrast with the commonly used MLST scheme for N. gonorrhoeae typing. We also sought to compare the pangenome of N. gonorrhoeae across the 767 isolates which may include relevant virulence-associated genes or genetic elements absent in the core genome. We identified a core genome of 1526 genes, and 8105 accessory genes for a total of 9631 unique genes shared among the isolates. We began by identifying mobile genetic elements, prophage genes and phase variable genes. We then used the Comprehensive Antibiotic Resistance Database Resistance Gene Identifier to identify AMR associated genes and the Scoary tool to analyze genes overrepresented in isolates with multiple determinants of AMR. We found strong associations between the presence or absence of accessory genes and PopNet groups, indicating genomic and evolutionary relationships. We also analyzed connections between mobile elements, AMR and phase variable genes, indicating possible linkage of specific virulence-associated traits. This analysis will provide a powerful resource for researchers in the gonococcal field and will reveal important insights into gonococcal population structures around the world.

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Gasdermin-D modulates inflammasome activity and IL-18 production

Jessica K. Bruce, Lina Li, Sabrina Tang, Nate Winsor, Dana J. Philpott, Stephen E. Girardin

University of Toronto

Introduction: Inflammasomes are cytosolic complexes that activate inflammatory caspases. Inflammatory caspases activate the cytokine IL-18 and lead to the formation of Gasdermin-D (GSDMD) plasma membrane pores to release mature IL-18. Inflammasome dysregulation underlies multiple autoinflammatory disorders and disease states and large research efforts are underway to design therapies that block inflammasome signalling. However, our current understanding of inflammasomes is derived primarily from mouse models and there are important differences between inflammasome in humans and mice. This has implications for designing therapies for human disease.

Aim: We sought to characterise inflammasome signalling in human cells and identify novel mechanisms to regulate inflammasome activation.

Methods: We developed systems to activate inflammasomes in human epithelial cells. Next, we used CRISPR/Cas9 to knock out key inflammasome components and compare the function of human inflammasomes to known murine pathways. We then assessed the effect of modulating key components of the pathway on proinflammatory caspase activation and cytokine production.

Results: Unexpectedly, we discovered that caspase-4 but not caspase-1 cleaves and activates IL-18 in human cells, whereas murine cells required caspase-1 for IL-18 production. Interestingly, pharmacological inhibition and knockdown of GSDMD dramatically increased pro-IL-18 processing in both human and mouse cells. The hyperproduction of IL-18 in GSDMD knockdown cells was linked to an increase in caspase-4 catalytic activity in humans and caspase-1 in mice.

Conclusion: Here we have uncovered that caspase-4 plays a major role in producing IL-18 in human epithelial cells. Moreover, we identified that GSDMD negatively regulates inflammatory caspase activation and IL-18 production, revealing a cell-intrinsic feedback system to modulate inflammasome signalling. Together these results define pro-IL-18 as a novel caspase-4 substrate and identify new potential therapeutic targets for inflammasome regulation.

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A Key Role for Rbpj-Dependent Notch Signaling in Memory CD8 T Cell Development

Siamak Haddadi, Juan Carlos Zúñiga-Pflücker

Sunnybrook Research Institute, Department of Immunology

Background: Memory CD8 T cells (CD8 TMEM) are a central component of host immunity against secondary viral infections. Although the role of Notch signaling during effector CD8 T cell differentiation is known, its requirement for the establishment of CD8 TMEM remains elusive.

Objectives: We asked whether RBPJ-dependent Notch-signaling has an impact during CD8 TMEM development upon acute viral infections.

Methods: Here, we used an Rbpj-inducible mouse model that enabled temporal and tissue specific regulation of the Notch activity in all hematopoietic cells.

Results: We found that Notch-RBPJ responsiveness during T cell activation results in enhanced effector CD8 T cell proliferation and survival, which consequently enlarges the magnitude of CD8 TMEM. While Notch activity skews differentiation programming towards an effector memory fate with augmented effector features, its deficiency promotes a central memory fate with enhanced stemness characteristics.

Conclusion: Our study demonstrated that Notch is a major determinant of CD8 TMEM fate and controls the quantity and quality of CD8 TMEM responses. Our findings will ultimately aid the development of efficacious immunotherapeutic strategies against intracellular infections and cancers.

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Characterizing the novel phage tail-like bacteriocins of Salmonella and Escherichia

C.M. Burk, W.W. Navarre, A.R. Davidson

Department of Molecular Genetics, University of Toronto

Tailocins are phage tail-like bacteriocins that have bactericidal activity against closely related bacteria. They are highly specific, have a narrow host range, and are evolutionarily related to the tails of phages. Using an HMM-based approach, we have identified a previously undescribed tailocin-like gene cluster in Salmonella and Escherichia. This 20 kb gene cluster encodes 24 genes, and is found at a conserved genomic location in Salmonella and Escherichia. We have found that this tailocin gene cluster is highly conserved at the sequence level, and is present in 40% and 5% of fully sequenced Salmonella and Escherichia, respectively. Most sequence divergence in the cluster occurs in the receptor binding protein, therefore we grouped the tailocins into bioinformatic subtypes based on tail fiber and chaperone sequences. We identified 11 Salmonella and 4 Escherichia tailocin subtypes, and these subtypes were found to correlate with Salmonella serovars and Escherichia species. Heterologous expression of the S. Typhimurium LT2 tailocin in E. coli resulted in contractile particle formation. When tested against a panel of Salmonella strains, Salmonella tailocins demonstrated non-self serovar killing. This work sheds light on a previously overlooked aspect of Salmonella and Escherichia biology, and adds to the arsenal of described tailocins, which have been shown to have potential as therapeutic agents.

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Mitigating stress in Lactiplantibacillus plantarum using the bacterial unsaturated fatty acid pathway

Jhenielle Campbell, Jiabao Liu, Henry Krause and William Navarre

University of Toronto, Molecular Genetics

The enzymatic bacterial unsaturated fatty acid pathway oxidizes the unsaturated C-C bonds in dietary lipids such as oleic and linoleic acid. Many lactic acid bacteria utilize this pathway to produce the oleic acid metabolites, 10-hydroxysteric acid (10-HSA) and 10-oxostearic acid. Both metabolites are high affinity ligands for the PPARα nuclear receptor; demonstrated by nuclear receptor pulldowns on human fecal samples. Experiments investigating the role of the unsaturated fatty acid pathway in Lactiplantibacillus plantarum have identified this bacterial species as a robust producer of 10-HSA in the presence of oleic acid, and the production of 10-HSA is dependent on the activity of the oleate hydratase enzyme. In addition to showing that the loss of oleate hydratase activity in L. plantarum significantly reduces the production 10-HSA, results also indicate that the oleate hydratase enzyme allows L. plantarum to detoxify growth-inhibiting polyunsaturated lipids. Furthermore, oleate hydratase activity mitigates non-lipid stressors, such as heat stress. Together, our data identify the bacterial unsaturated fatty acid pathway as a stress response pathway in L. plantarum that also leads to the production of bioactive metabolites.

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A Primed Neutrophil Subset Predicts the Risk of Bloodstream Infections in Allo-HSCT Patients: A Prospective Study

Omnia Elebyary, Noah Fine, Chunxiang Sun, Sourav T Saha, Shawn Robinson, Zahra Dorna Mojdami, Nicole Khoury, Erin Watson, Bryan Coburn, Jeffrey H Lipton, Michael Glogauer

1 Faculty of Dentistry, University of Toronto,Toronto, Ontario, Canada 2 Dental Oncology, Princess Margaret Cancer Centre,Toronto, Ontario, Canada 3 Department of Medicine, Division of Infectious Diseases, University Health Network,Toronto, Ontario, Canada 4 Hans Messner Allogenic Transplant Program, Division of Medical Oncologyand Hematology, Princess Margaret Cancer Centre, University Health Network,Toronto, Ontario, Canada

Background: Bloodstream infections (BSI) are the most common infectious complications in patients receiving allogeneic hematopoietic stem-cell transplants (allo-HSCT). Polymorphonuclear neutrophils (PMN) are quantified to monitor the susceptibility to BSIs, however, their degree of activation is not. We previously identified a population of primed PMNs (pPMN) with distinct markers of activation representing ∼10% of PMNs in the circulation. In this study, we investigate whether susceptibility to BSIs is related to the proportion of pPMN rather than strictly PMN counts. Methods: In this prospective observational study, we used flow cytometry to assess pPMNs in blood and oral rinse samples collected from patients receiving an allo-HSCT over the course of their treatment. We used the proportion of pPMNs in the blood on day five post-transplant to categorize patients into a high- or a low-pPMN group (> or <10% pPMNs). These groups were then used as a predictor of BSIs. Results: A total of 76 patients were enrolled in the study with 36 in the high-pPMN group and 40 in the low-pPMN group. Patients in the low-pPMN group had lower expression of PMN activation and recruitment markers and displayed a delay in PMN repopulation of the oral cavity after the transplant. These patients were more susceptible to BSI compared to patients in the high-pPMN group with an odds ratio of 6.5 (95% CI= 2.110-25.07, P= 0.002). Conclusion: In patients receiving an allo-HSCT, having less than 10% pPMNs early in the post-transplant phase can be an independent predictor of BSI in allo-HSCT patients.

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Tissue damage enhances the immune response to microbes: Synergy between ATP and Heptose phosphates

Victoria Gillmore, Dr. Cynthia Guo, Amit Weiner, Dr. Ryan Gaudet, Dr. Spencer Freeman, Dr. Scott Gray-Owen

Department of Molecular Genetics, Temerty Faculty of Medicine, University of Toronto

An effective immune response requires recognition of both foreign invaders such as microbes and host danger molecules such as those resulting from tissue damage. Specifically, our cells can mount this immune response through the recognition of microbe-associated molecular patterns (MAMPs) and danger-associated molecular patterns (DAMPs). My work focuses on a previously undiscovered MAMP-DAMP relationship between heptose phosphates and ATP. Heptose phosphates are a new class of MAMPs conserved across gram-negative bacteria. Heptose phosphates cause inflammation by binding directly to mammalian host proteins in the cytosol, leading to the activation of NF-kB and subsequent inflammation. ATP, classically thought of as the energy molecule of the cell, is also a well-established danger molecule and plays a crucial role in the persistence of chronic inflammation. My work uncovers a previously unidentified relationship between ATP and heptose phosphates. I show that ATP increases the cytosolic import of heptose phosphates, increasing the recognition and subsequent inflammatory response. Interestingly, during gram-negative bacterial infection, there is an abundance of ATP released by both bacteria and the host cells. My work shows that this ATP presence is crucial for the inflammatory response to gram-negative bacteria and for mounting an effective immune response. We hypothesize that this ATP mechanism of increased heptose phosphate import is an evolved mechanism to allow bacteria to increase the internalization of their MAMPs and amplify the immune response. This work reveals a novel MAMP-DAMP relationship, furthering our understanding of the requirements for an effective immune response.

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The NOD-like receptor protein NLRP6 regulates the colonic mucus layer during Tritrichomonas infection

Nathaniel J. Winsor1, Giuliano Bayer1, Elisabeth Foerster1, Heather Maughan3, Cathy Streutker4, George Birchenough5, Dana Philpott1 and Stephen E. Girardin1,2.

1University of Toronto, Department of Immunology 2University of Toronto, Department of Laboratory Medicine and Pathobiology 3Ronin Institute, Montclair NJ 4Saint Michael’s Hospital, Toronto 5University of Gothenburg, Sweden

Introduction: Pathogen detection often involves members of the NOD-like receptor (NLR) family of proteins. However, the role of these proteins in parasitic infection is poorly characterized. A key step in the intestinal anti-parasitic response is the growth of the mucus layer. NLRP6 is the most common NLR protein in intestinal epithelial cells and it has been suggested that NLRP6 regulates both goblet cell abundance and mucus secretion.

Methods: We developed a novel protist infection model utilizing Tritrichomonas (Tm) species. Protists were harvested and FACS-purified from the cecum of infected, specific pathogen free (SPF) mice and delivered by oral gavage into recipient animals.

Results: Upon infection, Nlrp6-/- mice displayed a thinner colonic mucus layer, and increased levels of tuft, goblet, and proliferating cells when compared to Nlrp6+/+ littermates. Mucus release was dependent on endocytosis, and the inflammasome components ASC and Caspase-1/11, but not IL-18. Tritrichomonas-induced mucus release required the microbiome, as germ free mice were unable to mount a mucus response during infection. Tritrichomonas infection caused a shift in the composition of the microbiota and of luminal metabolites in SPF animals, which is required to activate the NLRP6 inflammasome ex vivo.

Conclusion: An NLRP6 inflammasome regulates mucus secretion and epithelial proliferation in response to Tritrichomonas infection. Future work aims to determine which ligands trigger the NLRP6 inflammasome in the context of parasitic infection.

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Defining the associations microbial binding antibodies with the female genital microbiota and immune parameters

Rachel Liu, James Pollock, Bryan Coburn, Rupert Kaul

University of Toronto and UHN

Mucosal immunoglobulins in the gut maintain homeostasis between the host and the local microbiome through clearance of pathogenic bacteria and the development of immune tolerance to inflammatory bacteria. However, little is known regarding the role of immunoglobulins in the vaginal microbiome, and whether they can account for the heterogeneity in genital inflammation observed amongst women with bacterial vaginosis (BV)-type microbiomes.

We optimized a flow cytometry-based assay to quantify antibodies (IgG and IgA) from clinical samples capable binding to specific bacteria taxa. We analyzed microbial binding antibodies from cervicovaginal secretions of 200 HIV uninfected women from Nairobi, Kenya and assessed their associations with the vaginal microbiome composition and soluble immune factors.

Women with BV had significantly lower levels of IgG and IgA binding to key vaginal bacterial taxa (Gardnerella vaginalis, Lactobacillus iners, and Lactobacillus crispatus), and overall lower absolute abundance of IgA and IgG. Both absolute and microbial specific IgA and IgG negatively correlated with the overall bacteria abundance, but only L. iners-binding IgA negatively correlated with L. iners colonization. Amongst women with BV, absolute bacterial abundance and overall abundance of IgA and IgG were the strongest correlates of inflammatory cytokines and chemokines.

This is the first study to investigate the relationship between vaginal immunoglobulins, inflammatory cytokines, and bacterial colonization in the female genital tract. Future studies will investigate the role of microbe binding antibodies in determining clinical outcomes after microbial focused interventions (antibiotics and probiotics).

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Myocarditis and Pericarditis as a Complication of Mpox: An International Case Series and Literature Review

Sheliza Halani MD1, Sean Cai MD1, Juan Carlos Monge MD1,2, Philippe Brouillard MD3,4, Cécile Tremblay MD3,4,6, Jose Luis Blanco MD PhD7, Ana Isabel Pinho MD8, Guillermo Rodriguez Nava MD9, Supriya Narasimhan MD MS10, Joseph David Cooper MD10, Peter Kadlecik MD11, Darrell H. S. Tan MD PhD1,12,13

1Department of Medicine, University of Toronto, Toronto, Canada 2Division of Cardiology, St. Michael's Hospital, Toronto, Canada 3Centre Hospitalier de l'Université de Montréal (CHUM), Montréal, QC, Canada 4Département de médecine, Université de Montréal, Montréal, QC, Canada 5University of Montreal Hospital Centre (CRCHUM)-Research Centre, Montréal, QC, Canada 6Departement de Microbiologie, Infectiologie et Immunologie, Faculté de Médecine, Université de Montréal, Montréal, QC, Canada, 7University of Barcelona, Infectious Diseases Service, Hospital Clinic-IDIBAPS, Barcelona, Spain 8Department of Cardiology, São João University Hospital Centre, Porto, Portugal 9Stanford University School of Medicine, Stanford, California, USA 10Santa Clara Valley Medical Center, San Jose, California, USA 11Mid-Atlantic Permanente Medical Group, Rockville, Maryland, USA 12Division of Infectious Diseases, St. Michael's Hospital, Toronto, Canada 13MAP Centre for Urban Health Solutions, St. Michael's Hospital, Toronto, Canada

Background: Mpox virus was declared a public health emergency of international concern by WHO in 2022. The full range of clinical manifestations of this emerging infectious disease continues to be elucidated.

Methods: We present a case series and literature review of patients with mpox myocarditis and pericarditis, including demographics, clinical symptoms, diagnostic and management strategies, and outcomes.

Results: We identified 13 patients aged 21-51 (median 32) years with PCR-confirmed mpox and myopericarditis (n=3), pericarditis (n=1), or myocarditis (n=9), from 6 countries. All but one were men. One was HIV-positive (viral load undetectable) and 4 were on HIV PrEP. None had prior cardiac disease and 3 used tobacco. Most acquired mpox via sexual contact; one heterosexual patient reported non-sexual close contact. Cutaneous/mucosal lesions occurred in 11/13 patients, and fever in 11/13. Where reported, cardiac symptom onset was 2-8 (median 5.5) days after mpox illness onset. C-reactive protein ranged from 9.3-154.5 (median 52.6) mg/L. Diagnosis of myocarditis/myopericarditis was based on symptoms (chest discomfort 11/12, dyspnea 3/4), elevated troponin (range 165-21200 ng/L, peaking 1-2 days after cardiac symptom onset), supportive ECG findings (diffuse/territorial ST elevation, T-wave inversions, and/or non-specific ECG changes 9/12), and/or cardiac imaging findings (pericardial effusion 1/12, left ventricle [LV] abnormalities on echocardiogram 4/12, abnormal cardiac MRI in 7/7 done acutely). In the pericarditis case, ECG showed widespread ST elevation and echocardiogram showed hyperdynamic LV. Treatments included ASA or NSAIDs (n=7), tecovirimat (n=5), colchicine (n=4), ACE-inhibitors (n=3) and bisoprolol (n=3). All were hospitalized, with lengths of stay of 4-10 days, and at least 3 patients required intensive care. Cardiac symptom recovery occurred within 1-3 days of admission; in at least 1 patient symptoms continued beyond 1 month.

Conclusions: Mpox is rarely associated with myocarditis and/or pericarditis, with cardiac symptoms beginning on day 2-8 after illness onset. Long-term outcomes require further study.

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Characterizing the role of a bacterial heme acquisition system in macrophage persistence

Mahrukh Fatima, Trevor F. Moraes

Department of Biochemistry, University of Toronto

Pathogenic bacteria and the host's immune system are in a constant evolutionary arms race. Homeostasis and the acquisition of essential micronutrients, such as heme and iron, is an important area of coevolution and potential antimicrobial therapeutic targets. The host sequesters these essential micronutrients to starve bacterial pathogens and limit their pathogenicity. However, bacteria have many specialized nutrient acquisition systems to scavenge these micronutrients from the host environment. Our lab discovered a high-affinity heme acquisition system in Acinetobacter baumannii consisting of HphA, a secreted protein, that steals heme from host hemoglobin for delivery to the bacteria via its cognate receptor, HphR. Through bioinformatic analysis, our lab discovered this system is conserved across diverse Gram-negative bacterial species, including the opportunistic, human pathogen Stenotrophomonas maltophilia. Recently, S. maltophilia's HphA was shown to allow bacterial persistence in macrophages by inducing upregulation of anti-inflammatory cytokine, IL-10. Under anti-inflammatory conditions, macrophages also play an important role in heme recycling. Therefore, we hypothesize that the HphA heme acquisition system may be an adaptive mechanism for survival in host by hijacking and hiding in heme-rich macrophages. For my project, I am testing if this anti-inflammatory, immune evasion role is unique to S. maltophilia's HphA or conserved across HphAs from diverse species. I have reconstituted the HphA heme acquisition system from five different species in a lab Escherichia coli strain, which does not persist in macrophages. Next, I will infect murine macrophage cells with either wild-type E. coli strains or these recombinant strains. Then I will assess the cytokine profiles of the infected macrophages to determine if the reconstituted HphA system from different species triggers an anti-inflammatory response (IL-10 upregulation). I would also perform microscopy experiments to determine if the recombinant E. coli strains are able to persist in macrophages. Overall, it will provide new insights into host-pathogen interactions.

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Assessing the Limit of Detection of Candida auris Screening Methods using Direct-to-Agar, Broth-Enriched-Culture, Direct-Polymerase Chain Reaction, and Broth-Enriched-Polymerase Chain Reaction

Christina Wong HBSc (1,2), Shaista Anwer MSc (1), Ceylon Simon MSc (1), Susan M Poutanen MD (1,2,3)

(1) Department of Microbiology, University Health Network/Sinai Health, (2) Department of Laboratory Medicine and Pathobiology, University of Toronto, (3) Department of Medicine, University of Toronto, Toronto, Canada

Background: A sensitive and reliable screening procedure is necessary for the effective management of Candida auris infections and nosocomial outbreaks. This study aimed to determine the optimal screening procedure for C. auris by assessing and comparing the limit of detection (LOD) of C. auris for culture-based screening procedures using CHROMagarTM Candida Plus and Auris Enrichment Broth (AEB), and PCR-based screening methods using the BioGX C. auris research-use-only PCR assay.

Methods: Amies transport media from fresh nasal-axillary-groin-perineum-rectal swabs (n=11) were each spiked with a unique C. auris isolate, and six 5-fold dilutions (from 250 CFU/mL to 0.08 CFU/mL) of each spiked sample were prepared. Each diluted sample was: 1) directly inoculated onto CHROMagarTM Candida Plus; 2) inoculated and incubated in AEB before subculturing and incubating on CHROMagarTM Candida Plus; 3) extracted and tested directly by PCR; and 4) inoculated and incubated in AEB then tested by PCR. The LODs of each screening procedure was calculated using two probit analysis tools.

Results: The culture-based AEB-enriched procedure and the PCR from AEB-enriched specimen procedure had the lowest LODs (3 CFU/mL and 3 CFU/mL respectively), which were significantly lower than the direct-from-specimen procedures. The direct PCR from specimen procedure had the next lowest LOD, followed by the culture-based direct-to-agar procedure (275 CFU/mL and 484 CFU/mL respectively, not significantly different).

Conclusion: AEB-enrichment of specimens increased the sensitivities of both culture-based and PCR screening procedures compared to direct-from-specimen procedures. Direct culture-based and PCR screening procedures showed similar LOD. These LOD data, considered alongside the cost, workload, and turn-around time of each screening procedure, can be used to determine the optimal procedure to implement within clinical laboratories.

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Probiotic-derived bacterial membrane vesicles for Crohn's disease management: engineering vesicle production and cargo encapsulation

Yilan Liu, Jinjin Chen, Kaushik Raj, Lauren Baerg, Nayanan Nathan, Dana Philpott, Radhakrishnan Mahadevan

University of Toronto

Bacterial membrane vesicles (BMVs) are bilipid nanoparticles released as a conserved method of intercellular communication for the delivery of diverse bacterial products. These biological nanoparticles have potential to be leveraged to mimic microbe-microbe and host-microbe interactions for significant biomedical applications. Specifically, for the management of Crohn's disease (CD), a chronic inflammatory bowel disease. In CD, the nucleotide binding oligomerization domain 2 (NOD2) signalling pathway is suppressed by genetic and environmental factors. Overcoming NOD2 hypoactivity presents a novel therapeutic target for CD. However, muramyl dipeptide (MDP), the biological NOD2 agonist, is a poor drug candidate due to its large molecular weight and hydrophilicity. MDP is a motif of bacterial peptidoglycan (PG) and is a potential cargo of BMVs. We propose the utilization of probiotic-derived BMVs as a MDP delivery system in the gut for the management of CD. Here we demonstrate that isolated BMVs from B. subtilis carry PG fragments capable of inducing IL-8 secretion and CXCL1 expression in HCT116 cells in a NOD2-dependent manner. In addition, we applied metabolic engineering approaches to optimize BMV production and MDP packaging through modulation of PG degradation and recycling pathways. Overexpression of PG hydrolases, induced during the late-exponential phase, resulted in increased production of BMVs and coincided with increased NOD2 activity of bacterial supernatants, as observed in biological assays. Future work aims to implement these engineering strategies in the probiotic Lactiplantibacillus plantarum. Current CD therapeutics target immune suppression, yet surgical intervention remains necessary for over 75% of CD patients. Facilitated delivery of MDP by probiotic-derived BMVs represents an innovative strategy to enhance NOD2 signalling activity. This work contributes to the advancement of CD management strategies, towards the goal of reducing rates of surgical intervention and improving quality of life of CD patients.

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Inflammatory gene expression profile of bronchoalveolar lavage CD4+CD57+ PD1+ T cells suggests a functional role in the pathogenesis of lung allograft dysfunction

Sajad Moshkelgosha, Stephen Juvet

UHN, Toronto, ON, Canada

Purpose: Our analysis on longitudinal bronchoalveolar lavage (BAL) cells obtained from 50 lung transplant recipients (LTRs) using mass cytometry revealed a strong association between the frequency of CD4+CD57+PD1+ T cells and subsequent lung allograft dysfunction (LAD).However, the function of this T cell subset and its potential role in LAD pathogenesis is not clear. The purpose of this study is to explore the function of this cell subset using cellular indexing of transcriptomes and epitopes (CITEseq).

Methods: CITEseq is a sequencing-based method that simultaneously quantifies cell surface proteins and transcriptomic data within a single cell readout. CITEseq was performed using a panel of 15 oligo-tagged antibodies against surface markers on magnetically enriched T cells from 4BAL samples from patients with LAD. Cells then underwent gene and oligo library preparation before sequencing. Single cell data for individual BAL samples were analyzed before integration. CD4+CD57+PD1+ T cells were identified based on protein expression. We then examined the DEG between this cell subset and other CD4+ T cells.

Results: The integrated CD4+ T cell data were clustered into ten distinct subsets. Our data show that CD4+CD57+PD1+ cells were distributed in three different clusters. We then tried to identify the functional properties of each cluster based on differentially expressed genes. The unique gene signature of cluster 1 suggested that these cells might be an exhausted or senescent subset. Cluster 4 has a gene expression pattern suggesting cytotoxic function. Finally, we identified cluster 2 as mucosal-associated invariant T cells (MAIT)-like cells.

Conclusions: These data suggest that BAL CD4+CD57+PD1+ T cells have phenotypic properties that contribute to lung allograft injury through different mechanisms. Functional studies on this cell population are underway and may lead to improved prediction of LAD and a better understanding of lung transplant immunobiology.

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Genomic epidemiology of extended clusters of invasive Streptococcus pneumoniae in long-term care homes

Nicholas Waglechner1, Allison McGeer1✉, Wallis Rudnick1,2, Walter Demzcuk5, Wayne L. Gold2,6, Ian Kitai2,8, Sigmund Krajden2,7, Reena Lovinsky9, Irene Martin5, Matthew Muller2,10, Jeff Powis11, Neil Rau2,12, Gregory Tyrrell13, Andrew Simor2,13

For the Toronto Invasive Bacterial Diseases Network

1Mount Sinai Hospital, Toronto, Canada; 2University of Toronto, Toronto, Canada; 3Mackenzie Health, Richmond Hill, Canada; 4Southlake Regional Health Centre, Newmarket, Canada; 5National Microbiology Laboratory, Winnipeg, Canada; 6University Health Network, Toronto, Canada; 7 St. Joseph's Health Centre, Toronto, Canada ; 8Rouge Valley Health System, Toronto, Canada; 9The Scarborough Hospital, Toronto, Canada; 10St. Michael's Hospital, Toronto, Canada; 11Toronto East General Hospital, Toronto, Canada; 12Halton Healthcare Services, Oakville, Canada; 13Sunnybrook Health Science Centre, Toronto, Canada; 14Alberta Provincial Public Health Laboratory, Edmonton, Canada

Background: Streptococcus pneumoniae is an important cause of pneumonia and invasive pneumococcal disease (IPD). Reductions in IPD during the pandemic have emphasized the degree to which PD may be a result of person-to-person transmission. We investigated the extent to which PD in long-term care (LTC) homes might be a result of transmission of pneumococci.

Methods: Population-based surveillance of LTC home pneumococcal disease (PD) requiring hospitalization was used to identify clusters of same-serotype PD occurring in LTC homes. Epidemiologic clusters were defined as ≥3 cases of the same serotype in one home over with a rate of disease in that home higher (at P≤0.0005) than the rate of disease for the same serotype during the same time period in all other homes. Isolates were sequenced to establish whether disease in epidemiologic clusters was caused by genetically highly related isolates; <38 SNVs different was defined as highly related. Genetic relatedness of case isolates was also compared with temporally, geographically and serotype matched isolates from community-dwelling adults.

Results: Overall, 631 episodes of PD with isolates available for serotyping occurred in residents of 103 LTC homes between 1995 and 2018. 144/631 (22.8%) isolates occurred in 29 epidemiologic clusters in 20 homes (3 – 13 isolates/cluster; clusters spanning 6 months to 22 years). Overall, 135/144 case isolates and 254/288 matched control isolates were successfully sequenced; 26 of the 29 clusters had ≥3 case isolates sequenced. Case isolates were more highly related than controls (P<0.0001). Using genomics alone, in 11/29 epidemiologic clusters all isolates were highly related, in 12/29 clusters all isolates but one were highly related, and in 6 clusters isolates were not closely related.

Conclusions: WGS confirmed that most epidemiologically-defined clusters of PD in LTC homes were caused by genetically highly-related isolates, suggesting that undetected transmission in homes may be associated with significant burden of PD.

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Improved Test Sensitivity of Direct Gram stain and Calcofluor in the Diagnosis of Blastomycosis after repeated microscopic examination

Mohammed A. Sarhan, Ceylon Simon and Susan M. Poutanen

Department of Laboratory Medicine and Pathobiology, Division of Microbiology. Department of Medicine, Faculty of Medicine, University of Toronto, Canada

Objective: Blastomyces spp. is a dimorphic fungi that is endemic around the Great Lakes, Mississippi and Ohio rivers. Early diagnosis of blastomycosis is fundamental in starting empiric therapy. Fungal culture is the gold standard and the most sensitive and specific diagnostic method, but it requires days to weeks to get a definitive diagnosis. Direct examination of clinical specimens using Gram stain or Calcofluor White stain are simple and rapid techniques to provide presumptive diagnosis. However, the yield of direct specimen examination is reported to be low (36-46%). The objective of this study was to document the sensitivity of direct examination compared to culture.

Methods: A retrospective study was conducted on all confirmed cases with positive Blastomyces cultures at a tertiary-care centre in the Toronto region between 2010 and 2021 inclusive. The laboratory information information system record was reviewed to document the yield of Gram stain and Calcofluor White stain. The specimen type, region, and year of each case was documented.

Results: 82 fungal cultures were positive for Blastomyces spp. from 33 cases (7 females and 26 males) distributed mainly in the Greater Toronto Area (55%). Increased numbers were identified in recent years. Positive specimens included BAL (70%), sputum (10%), and other (20%). The sensitivity of Gram stain and Calcofluor White stain direct examination was 30% and 61% respectively. Repeated examination of direct Gram and Calcofluor white smears, after culture results were available or after re-review by a senior technologist, increased the yield to 52% and 70%, respectively.

Conclusions: The sensitivity of direct examination with Calcofluor White stain and Gram stain is increased with repeated examination with sensitivities as high as 70% and 52%, respectively. Laboratories should recognize the importance of thorough direct examination and staff training in this regard given the value added to the early diagnosis of blastomycosis.

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The human pathogen Streptococcus mutans encodes a conserved DpnII restriction-modification system involved in biofilm development

Haowei Zhao, Céline M. Lévesque

Oral Microbiology, Faculty of Dentistry, University of Toronto, Toronto, Ontario, Canada

Background: Biofilms represent the predominant form of bacterial life. When growing in biofilms, bacteria use quorum sensing to coordinate bacterial behavior at the whole population level using signaling pheromones. Using the dental pathogen Streptococcus mutans as model organism, we showed that a locus encoding a restriction-modification (R-M) system, DpnII, was strongly upregulated by the quorum sensing pheromone. DpnII R-M is usually composed of an adenine methylase (DpnA) and a restriction endonuclease (DpnB).

Objectives: To perform the genetic analysis of the DpnII R-M system and evaluate the impact of the absence of adenine methylation on biofilm development. Methods: Knockout mutants were generated by PCR ligation mutagenesis. Biofilms were developed under sucrose condition in microtiter plates. Adenine methylation screening was performed with methylation sensitive restriction enzymes and dot blot assay for m6A modification. The activity of DpnII restriction on incoming foreign DNA was confirmed by quantitative transformation assay.

Results: S. mutans species encodes a conserved chromosomal DnpII R-M system. DpnII R-M is functional as it methylates target sites to form 5'-GmeATC-3' and can cut DNA lacking the epigenetic marks. The DpnII R-M of S. mutans is atypical as it contains an additional adenine methylase, DpnM, that was found highly expressed in biofilms. A null mutant for adenine methylase forms fragile biofilms that appeared very weakly attached to the substratum contrary to those produced by the WT strain.

Discussion: Our study suggests that bacterial adenine methylases may have important functions beyond their traditional role in R-M. Since lack of adenine methylation strongly reduced the ability of S. mutans to develop robust biofilms, we can hypothesize that a subset of genes may be governed by the presence of methylation. Summary: Inhibition of adenine methylation could represent an attractive strategy to develop new treatments that target the biofilm mode of growth.

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Diversification in microbial communities through trade-offs

Ga Ching Lui, Sidhartha Goyal

University of Toronto, Department of Physics

One of the important questions in ecology is the paradox of plankton. In gist, it refers to the rich diversity observed in nature in contradictions to the theoretical upper bound set by competitive exclusion principle. More generally, how does a small system with only few species diversify against selective pressure, and gives rise to a richer system that is relatively more stable? There is a hypothesis that in order to support such diversity in an environment, where microbes with trade-offs in their strategies for competing over substitutable resources, the community can self-organize and flatten the fitness landscape. This idea was explored theoretically both in small systems with only few growth-limiting abiotic factors such as types of nutrients, and large systems where the interactions through the environment are randomized. The commonality in these models is that due to the symmetries introduced by the trade-offs, these diverse communities can shield off external asymmetry such that every species have the same fitness. Using a coarse-grained model of metabolism, we extend this idea to other modes of interactions such as cross-feeding, toxins, and a combination of both essential and substitutable resources. Naturally comes out of this approach is the interpretability of the required trade-offs that gives rise to self-organization, and the coarse graining of the variables which allows a description of highly diversified systems in a low dimensional space, which suggests they would still satisfy competitive exclusion principle by measuring diversity differently. This provides implications to fine-scale diversity, and a connection between early microbial system that only consists of few species and more stabilized large systems with non-trivial structures in their interactions. We presented some simulations in a chemostat setting, and propose several tests on the abundance time series data to detect the effective diversity through coarse graining in a seemingly ecologically rich system.

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A Structure-Guided Approach to Identify Fungal-Selective Yck2 Inhibitors

Bonnie Yiu(1), Emily Puumala(1), Pascal Marchand(2), Luke Whitesell(1), Nicole Robbins(1), Leah E. Cowen(1)

(1)Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada. (2)Nantes Université, Cibles et Médicaments des Infections et de l'immunité, IICiMed, Nantes, France

On a global scale, fungal pathogens are responsible for ~13 million infections annually and 1.5 million deaths per year. With the widespread emergence of antimicrobial resistance and the limited arsenal of antifungal therapeutics, there is a need to identify novel antifungals. Yck2 is a fungal member of the casein kinase 1 (CK1) family that governs Candida albicans pathogenesis. Chemical screens revealed a 2,3-arylpyrazolopyridine compound, as inhibitor of Yck2, resulting in fungal-selective growth impairment in host-relevant conditions and enhancement of antifungal efficacy. However, this compound suffers from poor pharmacological properties. To determine the potential of Yck2 inhibitors as a novel class of antifungal, I utilized genetic and biochemical approaches to characterize nine newly-synthesized imidazo[1,2-x]azine derivatives. Using a kinase assay to measure casein kinase inhibition, I showed several derivatives displayed fungal-selective activity against the fungal isoform Yck2 compared to mammalian CK1alpha. In a standard dose-response assay, I identified CTN1756 and CTN1844 as the most bioactive molecules against C. albicans. CTN1756 and CTN1844 demonstrated on-target whole cell activity as compound treatment resulted in polarized growth in C. albicans, a phenotype consistent with Yck2 inhibition. Furthermore, I showed that CTN1756 and CTN1844 enhanced caspofungin efficacy in an echinocandin-resistant strain. Unfortunately, all compounds demonstrated low metabolic stability in a mouse liver microsome assay. Future work will focus on the generation of additional imidazo[1,2-x]azine derivatives to further optimize the potency, selectivity, and metabolic stability of the scaffold to optimize Yck2 inhibitors as a novel class of antifungal.

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Characterizing the Immune Kinetics driving Neisseria gonorrhoeae-induced Pelvic Inflammatory Disease in Female Mice

Anna Bojagora, Genevieve Mailhot, Epshita Islam, Scott Gray-Owen

Department of Molecular Genetics

Ngo infections in women are a significant public health concern as they are often asymptomatic and the lack of treatment can lead to the spread of Ngo from the lower genital tract to the upper genital tract (UGT) in 10-25% of cases. While the recruitment of neutrophils to the site of infection is a hallmark of symptomatic gonorrhea, the mechanisms driving the overexuberant neutrophilic response to Ngo infection remains poorly understood. Using a transcervical infection model in female mice, we established uterine infection and characterized the immune kinetics using a combination of immunofluorescence microscopy, ELISAs, and flow cytometry. Our research aims to characterize the effector cells that drive inflammation and to target this response to suppress the Ngo-driven immunopathogenesis.

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Defining the zinc uptake systems of Klebsiella pneumoniae

Matthew Giles1,2, Gregory Cole1, Eve A. Maunders2, Dixon Ng1, Christine Lai1, Aimee Tan2, Christopher A. McDevitt2, and Trevor Moraes1

1 Department of Biochemistry, University of Toronto, Toronto, Ontario, M5S 1A4, Canada and 2 Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Melbourne, Victoria, 3000, Australia

Multidrug resistant pathogens are becoming globally ubiquitous and represent a substantial threat to human health. The World Health Organisation priority pathogen Klebsiella pneumoniae is predominantly associated with hospital acquired infections of the immunocompromised, but hypervirulent strains are increasingly observed as a causative agent of community-acquired infections in healthy individuals. Treatment of K. pneumoniae infections is frequently dependent upon last line antibiotics, but emerging resistance has diminished the efficacy of these drugs. Accordingly, new antimicrobial therapeutic strategies are urgently required.

Bacterial pathogens, including K. pneumoniae, are dependent upon the acquisition of essential micronutrients, such as zinc, from the host to maintain cellular processes. Zinc is essential to all forms of life and is estimated to be associated with up to 7% of the proteome in bacteria. The presence of excess zinc can mediate substantial toxicity and in K. pneumoniae its cellular abundance is tightly regulated by the P-type ATPase ZntA. However during infection, the host environment modulates the abundance of zinc imposing both restriction, also known nutritional immunity, and intoxication at distinct time points. Thus K. pneumoniae has dedicated transporters to maintain zinc homeostasis.

Here, we begin to define the role of the zinc uptake transporters within K. pneumoniae zinc homeostasis and report recent advances in the structure determination, interaction with metal ligands, and complex formation. I will present structural data, including the x-ray crystal structures of the periplasmic components of the transport systems. In addition, biochemical and biophysical characterisation of the transport proteins using nano Differential Scanning Fluorimetry and Isothermal Titration Calorimetry tools provide an understanding of affinities and specificities for particular metal transporters. Collectively, these findings provide new insight into the molecular basis of zinc homeostasis in K. pneumoniae. This knowledge will provide a foundation for future development of antimicrobials targeting K. pneumoniae infections.

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Factors associated with re-infection in adults with SARS-CoV-2 infection early during the pandemic in Toronto, Canada

Lubna Farooqi1, Zoe Zhong1, Altynay Shigayeva1, Shiva Barati1, Karen Colwill4, Gloria Crowl1,2, Roya M Dayam4, Melanie Delgado-Brand4, Nazrana Haq1, Kaniz Jannat1, Philip Kim1, Moe Kyaw6, Maxime Lefebvre1, Angel Xinliu Li1, Genevieve Mailhot4, Maria Major7, Nadia Malik1, Catherine Martin6, John Michael McLaughlin6, Mohammad Mozafarihashjin1, Mare Pejkovska1, Freda Qi4, Asra Quadri1, Susmitha Rallabandi1, Sameera Rizvi1, Asfia Sultana1, Tulunay Tursun4, Tamara Vikulova1, Jelena Vojicic7, Jingyan Yang6, Pingping Zhang6, Brenda Coleman1, Christopher Kandel2, Kevin Katz5, Rob Kozak3, Samira Mubareka3, Anne-Claude Gingras4, Allison McGeer1

1Sinai Health System, Toronto, Ontario, Canada 2Toronto East Health Network, Toronto, Ontario, Canada 3Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada 4Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, Ontario, Canada 5North York General Hospital, Toronto, Ontario, Canada 6Pfizer Inc. USA 7Pfizer Canada

BACKGROUND: As the COVID-19 pandemic evolves, understanding risk of SARS-CoV-2 re-infection is increasingly important. We identified factors associated with re-infection in adults with COVID-19 in the first months of the pandemic.

METHODS: We enrolled SARS-CoV-2 infected adults admitted to 7 hospitals between February and September 2020 and those managed as outpatients between February and July, 2020. Consenting participants provided demographic, clinical, and COVID-19 vaccination information and were followed until May 2022. Serum or dried blood spot samples at enrolment, 2-4 weeks after any vaccine dose received, and 3-5 weeks after infection were obtained to assess antibody responses to nucleoprotein (NP), spike trimer (S), and receptor binding domain (RBD). Mutivariable logistic regression was performed to determine risk factors for re-infection.

RESULTS: The cohort included 249 inpatients and 300 outpatients. Median age at first infection was 54 years (IQR 41-64); 288 (53%) were female. Seven re-infections occurred between Nov 2020 and Dec 15, 2021 (before Omicron predominated); 82 re-infections occurred between Dec 13, 2021 and June 1, 2022 (Omicron wave). In multivariable analysis, older age and receipt of ≥2 vaccine doses were significantly associated with reduced risk of re-infection. Among individuals with convalescent serum 21-180 days after their first infection, a higher anti-NP antibody level was associated with reduced risk of re-infection (ratio normalized concentration 075 [IQR 0.39, 0.96] versus 0.91 [IQR 0.75, 0.99] in those re-infected versus not; P=.003). Re-infection with Omicron was significantly less common in persons with detectable anti-NP antibody in Oct-Dec 2021 (9/92 versus 23/94; P=0.01).

DISCUSSION: In persons with SARS-CoV-2 infection before October 2020, re-infection in subsequent 18-24 months was more common in younger persons, those with <2 doses of vaccine, and those with lower concentrations of convalescent anti-NP antibody.

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Burden of severe illness associated with laboratory-confirmed influenza in adults aged 50-64 years, 2010/11 to 2016/17

Philip Kim1, Brenda Coleman1,2, Jeff Kwong2,3,4, Agron Plevneshi1, Kazi Hassan1 Karen Green1, Shelly McNeil5, Irene Armstrong6, Hannah Chung4, Wayne Gold7, Jonathan Gubbay8,9, Kevin Katz9,10, Stefan P. Kuster11, Reena Lovinsky12, Larissa Matukas9,13, Krystyna Ostrowska14, David Richardson15, Allison McGeer1,2,9

1Sinai Health System, Toronto, Canada; 2Dalla School of Public Health, University of Toronto, Toronto, Canada; 3Department of Family and Community Medicine; 4ICES, Toronto, Canada; 5 Department of Medicine, Dalhousie University, Halifax, Canada; 6Toronto Public Health, Toronto, Canada; 7 Department of Medicine, University Health Network, University of Toronto, Toronto, Canada; 8Public Health Ontario Laboratories, Toronto, Canada; 9Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada; 10Sunnybrook Health Sciences, Toronto, Canada; 11Cantonal Hospital St Gallen, Division of Infectious Diseases and Hospital Epidemiology, St Gallen, Switzerland; 12Scarborough Health Network, Toronto, Canada; 13Division of Microbiology, Unity Health, Toronto, Canada; 14Trillium Health Partners, Mississauga, Canada; 15William Osler Health System, Brampton, Canada

Summary: Despite a universal influenza vaccination program, average annual rates of influenza-associated hospitalization and mortality in 50-64-year-old adults were 22.4 and 0.9/100,000/year in our population. Incidence increased with age, and to a greater extent, in the presence of underlying co-morbidities.

Background: Understanding the burden of influenza is necessary to optimize recommendations for influenza vaccination. We describe the epidemiology of severe influenza in 50-64-year-old residents of metropolitan Toronto and Peel region, Canada, over seven influenza seasons.

Methods: Prospective population-based surveillance for hospitalization associated with laboratory-confirmed influenza was conducted from 9/2010-8/2017. Conditions increasing risk of influenza complications were as defined by Canada's National Advisory Committee on Immunization. Age-specific prevalence of medical conditions was estimated using Ontario administrative data (ICES); population rates were estimated using Statistics Canada data.

Results: Over 7 seasons, 1,228 hospitalizations occurred in patients aged 50-64 years: 40% due to A(H3N2), 30% A(H1N1), and 22% influenza B. The average annual hospitalization rate was 15.6, 20.9 and 33.2/100,000 in 50-54, 55-59, and 60-64-year-olds; average annual mortality was 0.9/100,000. Overall, 33% of patients had received current season influenza vaccine; 963 (86%) had ≥1 underlying condition increasing influenza complication risk. The most common underlying medical conditions were chronic lung disease (38%) and diabetes mellitus (31%); 25% of patients were immunocompromised. The average annual hospitalization rate was 6.1/100,000 in those without and 41/100,000 in those with any underlying condition, and highest in those with renal disease or HIV infection/organ transplant (138 and 281/100,000 respectively). The case fatality rate in hospitalized patients was 4.4%; median length of stay was 4 days (IQR 2-8).

Conclusions: The burden of severe influenza in 50-64-year-olds remains significant despite our universal publicly funded vaccination program. These data may assist in improving estimates of the cost-effectiveness of new strategies to reduce this burden.

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Defining the allosteric activation path for ClpP

Marim M. Barghash # (1), Mark F. Mabanglo # (1), Dmytro Brozdnychenko # (3), Samuel E. Hoff # (4), Siavash Vahidi* (3), Massimiliano Bonomi* (4), Walid A. Houry* (1,2). #Co-first authors, *co-corresponding authors.

1. Department of Biochemistry, University of Toronto, Toronto, Canada. 2. Department of Chemistry, University of Toronto, Toronto, Canada. 3. Department of Molecular and Cellular Biology, University of Guelph, Guelph, Canada. 4. Department of Structural Biology and Chemistry, Institut Pasteur, Paris, France

Molecular chaperones and proteases exist in all organisms where they play a critical role in maintaining cellular protein homeostasis. ClpP is one such protease present in both bacteria and eukaryotes. It is composed of fourteen identical subunits that typically assemble as stacked heptameric rings to form a hollow barrel-like structure with 7-fold symmetry. Chemical interference may be used to activate ClpP and dysregulate its function, resulting in the unregulated proteolysis of non-substrate proteins, causing cell death. As such, targeting ClpP has recently emerged as a promising avenue for the development of novel antimicrobial drugs. Classical activators bind in the hydrophobic sites of ClpP, while more recently, other activators have been seen to bind in the catalytic sites. Here, we identified synthetic compounds that are able to bind in both sites by utilizing protease degradation assays and X-ray crystallography. We also solved the first structure of a fungal ClpP, both bound and unbound to Dioctatin, a small molecule activator produced in Streptomyces. Dioctatin binds both hydrophobic and catalytic sites of ClpP. Inspired by this phenomenon, we defined the allosteric pathway for ClpP activation by using hydrogen deuterium exchange mass spectrometry (HDX-MS) and molecular dynamics (MD) simulations. Taken together, this work advances our understanding of ClpP allostery, which can aid in drug design and development efforts in the future.

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Isolation and characterization of SARS-CoV-2 Omicron Sublineages

Yaejin Lee[1,2], Juan Corredor[1], Winfield Yim[1], Lily Yip[1], Xinliu Angel Li[3], Maxime Lefebvre[3], Aaron Campigotto[4], Allison McGeer[2,3], Samira Mubareka[1,2]

1 Sunnybrook Research Institute, 2 Department of Laboratory Medicine and Pathobiology, University of Toronto, 3 Lunenfeld-Tanenbaum Research Institute, Mt Sinai Hospital, 4 Hospital for Sick Children

Background: SARS-CoV-2 variant of concern (VOC), Omicron emerged in late 2021 and rapidly became the dominant circulating strain globally. Further mutations gave rise to Omicron sub-lineages some of which may have enhanced transmissibility. As of April 2023, in Ontario, BA.2 recombinant subvariant, XBB.1.5 is the most prevalent lineage followed by BA.5 subvariant, BQ.1.1. The descendants carry signature amino acid substitutions particularly on spike protein which have shown to evade immunity permitting re-infection and vaccine breakthroughs. However, it's not clear if enhance transmissibility is due to immune evasion or enhanced intrinsic replicative property.

Objective: Characterize replicative properties of Omicron subvariants in vitro through a) Isolation of key Omicron sublineages, b) Viral replication kinetic study and competition assay to ultimately identify determinants of transmission.

Methods: Viral isolation was carried out using recombinant Vero-E6 cells overexpressing TMPRSS2 (VTNX) and were whole-genome sequenced to ensure no adaptive mutations occur. Replication kinetics of BA.2, BA.5, BQ,1.1 and XBB.1.5 were assessed by infection of VTNX and Calu-3. Cell supernatants were harvested for 5 days to measure viral titre by TCID50 endpoint assay. Competition assays were carried out using two omicron sublineages, 1:9 ratio of viral isolates was inoculated onto Calu-3. Daily harvests were whole-genome sequenced to determine the sublineages proportions.

Results: SNP analysis showed no significant mutational changes occurred in Omicron subvariants isolates throughout the genome. Replicative properties study showed that by 1dpi, BQ.1.1 growth was significantly greater than other sublineages in VNTX and Calu-3 though peak infectious viral titres were comparable in VNTX. XBB.1.5 showed delayed growth towards peak titre in Calu-3 by 24h. Competition assay highlighted the great replicative ability of BQ.1.1 when infected with XBB.1.5.

Conclusion: Omicron sublineages gained additional mutations or have undergone recombination which have led to increased transmission capacity. It's important to rapidly characterize newly emerged lineages to limit the viral spread.

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Biochemical characterization of a Streptococcus anti-CRISPR protein

Jolie Hamel, Dr. Karen Maxwell

University of Toronto

In their natural environments, bacteria are constantly under attack by viruses known as phages. To defend themselves, bacteria use CRISPR-Cas, a complex of RNA and proteins, to target incoming phage genomes for cleavage, thereby allowing the bacterial host to survive the infection. Phages, in turn, have evolved anti-CRISPR proteins that inhibit CRISPR-Cas systems to allow successful infection, usually through direct binding to the CRISPR-Cas complex. Anti-CRISPRs are highly diverse and vary widely in their structure and sequence, with over 90 families described to date. Through bioinformatics, a novel anti-CRISPR termed AcrIIA33 was identified in a Streptococcus phage. Using in vivo phage targeting assays we were able to show that it has activity against the type II-A Cas9 proteins from Streptococcus pyogenes (SpyCas9), Staphylococcus aureus (SauCas9), and one system in Streptococcus thermophilus (Sth1Cas9). When co-expressed with SpyCas9, AcrIIA33 does not form a direct interaction. Guide RNA remains present and intact, indicating that AcrIIA33 does not interfere with RNA loading or stability. AcrIIA33 also interacts with fluorescent nucleic acid dyes, including SYBR-Gold and acridine orange. The stained band is resistant to nuclease treatment, but susceptible to protease, suggesting this property is due to the AcrIIA33 protein itself and not a co-purifying nucleic acid. We have generated a high confidence predicted structure of AcrIIA33 using AlphaFold 2, which reveals a compact globular protein with a highly negatively charged surface. As many previously characterized anti-CRISPR proteins have negatively charged surfaces and act as DNA mimics, this suggests a mechanism through which AcrIIA33 may function.

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Rapid host adaptation of a bacterial plant pathogen to a resistant host is driven by genomic island loss

Jacy Newfeld, Erka Shata, Marcus M. Dillon

Department of Ecology and Evolutionary Biology, University of Toronto (JN, MMD); Department of Biology, University of Toronto Mississauga (JN, ES, MMD)

Plant pathogens pose a serious threat to worldwide crop yields and cause widespread food insecurity. Insertion of host resistance genes is a common strategy deployed to protect crops; however, this resistance can be overcome by pathogen evolution. To combat pathogen evolution in the field, it is necessary to understand the evolutionary pathways pathogens take to overcome host resistance barriers. Our research aims to characterize the evolutionary trajectories that enable the bacterial plant pathogen Pseudomonas syringae to overcome host resistance barriers to cause disease. Specifically, we applied in planta experimental evolution to adapt initially non-pathogenic strains of P. syringae to the model host Arabidopsis thaliana. While A. thaliana can resist these strains initially, we predicted that pathogens would evolve to overcome host resistance. During each infection cycle, we quantified in planta bacterial concentration as a measure of bacterial fitness, and when in planta concentrations were elevated, we conducted a targeted PCR reaction to determine whether genomic island loss was driving the adaptation. We found that, on average, P. syringae emerged as a strong pathogen within 5 passages and that multiple lineages had lost a genomic island harbouring a known immune-eliciting effector. These findings will facilitate the development of new sequence-based diagnostics for tracking emerging pathogens in the field and enable us to engineer more robust resistance in crops.

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Systematic Discovery of Anti-Phage Systems Functioning in Pseudomonas aeruginosa

Chi Zhang, Pramalkumar Patel, Tatiana Lenskaia, Jaden Bhogel, Wenjia Hao, Karen Maxwell, Alan Davidson

Department of Molecular Genetics, University of Toronto

Most anti-phage defence systems have been discovered bioinformatically and then functionally characterized through plasmid-based expression in E. coli or B. subtilis. To investigate the mechanisms of anti-phage systems operating within their normal genomic contexts, we have systematically identified active systems within strains of Pseudomonas aeruginosa. We first used established bioinformatic methods to identify genomic regions within P. aeruginosa strains that encode multiple known defence systems. Once these “defence islands” were identified within strains of interest, we targeted them with a plasmid-encoded a type I-C CRISPR-Cas system. This system generates deletions (~10 to ~100 kbp) that remove large portions of the defence island. Strains with different deletions were subsequently challenged with a variety of phages to identify mutant strains that had become sensitive to one or several phages. These strains were sequenced and analyzed through bioinformatics and targeted deletions to determine which gene(s) was responsible for preventing phage replication in the wild-type strain. Using this approach, we have discovered two completely novel anti-phage system and a diverged variant of the anti-viral STAND (Avs) systems described by Gao et al., which we refer to as Avs6.

Avs6 is comprised of an N-terminal Cap4 nuclease domain followed by STAND ATPase and TPR repeats. There is no detectable sequence similarity between Avs6 and previously characterized Avs systems. Through mutagenesis studies, we have found that all domains of Avs6 are essential for anti-phage activity in P. aeruginosa except for a C-terminal SEC-C domain, uniquely found in Avs6. Previous work suggested that Avs systems protect bacteria from phages via abortive infection. However, Avs6 blocks phage DNA replication without inducing abortive infection, which indicates a distinct immune mechanism. Closely related homologues of Avs6 are found in diverse bacterial clades include alpha-, beta- and gamma-Proteobacteria, Myxococci, and Actinomycetes.

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Potent transmission-blocking monoclonal antibodies from naturally exposed individuals target a conserved epitope on Plasmodium falciparum Pfs230

Danton Ivanochko 1 , Amanda Fabra-García 2 , Karina Teelen 2 , Marga van de Vegte-Bolmer 2 , Geert-Jan van Gemert 2 , Jocelyn Newton 1 , Anthony Semesi 1 , Marloes de Bruijni 3 , Judith Bolscher 3 , Jordache Ramjith 4 , Marta Szabat 5 , Stefanie Vogt 5 , Lucas Kraft 5 , Sherie Duncan 5 , Shwu-Maan Lee 6 , Moses R Kamya 7 , Margaret E Feeney 8 , Prasanna Jagannathan 9 , Bryan Greenhouse 10 , Robert W Sauerwein 3 , C Richter King 6 , Randall S MacGill 6 , Teun Bousema 11 , Matthijs M Jore 12 , Jean-Philippe Julien 13

1 Program in Molecular Medicine, the Hospital for Sick Children Research Institute, Toronto, ON, Canada. 2 Department of Medical Microbiology, Radboudumc, Nijmegen, the Netherlands. 3 TropIQ Health Sciences, Nijmegen, the Netherlands. 4 Radboud Institute for Health Sciences, Department for Health Evidence, Biostatistics Section, Radboudumc, Nijmegen, the Netherlands. 5 AbCellera Biologics Inc., Vancouver, BC, Canada. 6 PATH's Malaria Vaccine Initiative, Washington, DC 20001, USA. 7 Infectious Disease Research Collaboration, Kampala, Uganda. 8 Department of Medicine, University of California, San Francisco, San Francisco, CA, USA; Department of Pediatrics, University of California, San Francisco, San Francisco, CA, USA. 9 Department of Microbiology and Immunology, Stanford University, Stanford, CA, USA. 10 Department of Medicine, University of California, San Francisco, San Francisco, CA, USA. 11 Department of Medical Microbiology, Radboudumc, Nijmegen, the Netherlands. Electronic address: teun.bousema@radboudumc.nl. 12 Department of Medical Microbiology, Radboudumc, Nijmegen, the Netherlands. Electronic address: matthijs.jore@radboudumc.nl. 13 Program in Molecular Medicine, the Hospital for Sick Children Research Institute, Toronto, ON, Canada; Departments of Biochemistry and Immunology, University of Toronto, Toronto, ON, Canada. Electronic address: jean-philippe.julien@sickkids.ca.

Pfs230 is essential for Plasmodium falciparum transmission to mosquitoes and is the protein targeted by the most advanced malaria-transmission-blocking vaccine candidate. Prior understanding of functional epitopes on Pfs230 is based on two monoclonal antibodies (mAbs) with moderate transmission-reducing activity (TRA), elicited from subunit immunization. Here, we screened the B cell repertoire of two naturally exposed individuals possessing serum TRA and identified five potent mAbs from sixteen Pfs230 domain-1-specific mAbs. Structures of three potent and three low-activity antibodies bound to Pfs230 domain 1 revealed four distinct epitopes. Highly potent mAbs from natural infection recognized a common conformational epitope that is highly conserved across P. falciparum field isolates, while antibodies with negligible TRA derived from natural infection or immunization recognized three distinct sites. Our study provides molecular blueprints describing P. falciparum TRA, informed by contrasting potent and non-functional epitopes elicited by natural exposure and vaccination.

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COVID-19 infection early in the pandemic and protection from infection during the Omicron BA.1/BA.2 waves in Ontario, Canada

Altynay Shigayeva[1], Lubna Farooqi[1], Christopher Kandel[2,3], Zoe Zhong[1], Brenda Coleman[1,4], Samira Mubareka[5,6], Shiva Barati[1], Lois Gilbert[1], Anne-Claude Gingras[7], Wayne Gold[3,8], Nazrana Haq[1], Kaniz Janat[1], Kevin Katz[5,6,9], Philip Kim[1], Maxime Lefebvre[1], Angel Li[1], Maria Major[11], Tony Mazzulli[1,6], Jeff Powis[2], Sameera Rizvi[1], Asra Quadri[1], Asfia Sultana[1], Jelena Vojicic[11], Jingyan Yang[10,12] , Pingping Zhang[10], Catherine Martin[10], Moe H. Kyaw[10], John M. McLaughlin[10] , Allison McGeer[1,4,6]

1Department of Microbiology, Sinai Health System 2Michael Garron Hospital, Toronto East Health Network 3Department of Medicine, University of Toronto 4Dalla Lana School of Public Health, University of Toronto 5Department of Microbiology, Sunnybrook Health Sciences Centre 6Department of Laboratory Medicine and Pathobiology, University of Toronto 7Lunenfeld Tannenbaum Research Institute, Sinai Health System 8Department of Medicine, University Health Network 9North York General Hospital 10Pfizer Inc. USA, 11Pfizer Canada 12Institution of Social and Economic Research and Policy, Columbia University

Background: Both vaccination and prior infection protect against COVID-19. However, the extent and duration of protection remain uncertain. We assessed whether prior COVID-19 provided protection against Omicron (BA.1/BA.2) infection.

Methods: Patients infected with SARS-CoV-2 between March and September of 2020 “early infection” [EI]) were enrolled along with a cohort who were matched on hospitalization status, age-group, and indication for and timing of SARS-CoV-2 testing who did not have an early infection (non-EI). Participants completed baseline questionnaires and were followed bi-weekly until May 31, 2022 to identify qPCR or RAT-confirmed SARS-CoV-2 infections and COVID-19 vaccine doses received.
The primary outcome was SARS-CoV-2 infection between Dec 15, 2021 and May 31, 2022 (BA.1/BA.2 period). Multivariable Cox regression was used to estimate the hazard ratio (HR) of infection comparing EI and non-EI groups, adjusted for age, sex, immunosuppression, household income. Vaccination was treated as a time-varying covariate.

Results: Data were available for 622 EI (280 inpatients and 342 outpatients) and 169 non-EI participants. Median age was 56y for EI and 57y for non-EI. 325 (52.3%) EI and 93 (55.0%) non-EI participants were female. 38 (6.1%) EI and 11(6.5%) non-EI participants were immunocompromised. 587/622 (94.4%) EI and 158/169(93.5%) non-EI participants received ≥2 doses of a COVID-19 vaccine prior to the onset of the Omicron wave.
During the BA.1/BA.2 period, 89 EI and 38 non-EI participants were infected, an incidence 0.86 per 1000 person-days in the EI cohort vs 1.35 per 1000 person-days in the non-EI cohort (IRR=0.64 , 95%CI: 0.44-0.93). In multivariable analysis, EI individuals had a lower risk of SARS-CoV-2 infection (HR=0.59 [95%CI: 0.40-0.89]; P=0.01). Older and vaccinated participants were less likely to develop COVID-19 (Table).

Conclusions: Prior SARS-CoV-2 WT/D614G infection was associated with a 40% reduction in the risk of infection during the Omicron BA.1/BA.2 wave. COVID-19 vaccination conferred additional protection.

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Employing a synthetic human gut microbial community to study host gene-microbe interactions in inflammatory bowel disease

Giuliano Bayer, Bana Samman, Boyan K Tsankov, Amy Cao, Grace V Visser, Tapas Mukherjee, Elisabeth G Foerster, Emma Allen-Vercoe, Dana J Philpott

University of Toronto, Department of Immunology, Toronto, ON, Canada (GB, BKT, GVV, EGF, DJP); University of Toronto, Department of Laboratory Medicine and Pathobiology, Toronto, ON, Canada (BS, TM); University of Toronto, Division of Comparative Medicine, Toronto, ON, Canada (AC); University of Guelph, Department of Molecular and Cellular Biology, Guelph, ON, Canada (EAV)

Gnotobiotic mouse models are important tools for studying the gut microbial ecosystem and its role in health and disease. We have generated a reductionist model microbiome, termed Human Defined Community 1 (HDC1), that recapitulates certain microbe-host interactions of the complex microbiome in vivo. We demonstrate that colonization of gnotobiotic mice with 8 human-derived bacterial species can give rise to normal proportions of colonic lamina propria immune cells. Furthermore, we show that HDC1 mice can be transplanted with human gut commensals and Crohn's disease (CD)-associated bacteria, allowing us to address the intricate interplay between genetic and environmental factors contributing to inflammatory bowel disease pathogenesis. We are employing the HDC1 mouse model to decipher the role of Nod2 – the strongest genetic risk factor for CD development – in regulating pathobiont-associated intestinal inflammation.

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Investigating the role of dl-endopeptidase in patient response to ICI therapy

Oliver De Sa, Charles Carr

University of Toronto Department of Immunology, Philpott Lab

Stimulation of nucleotide oligomerization domain 2 (NOD2) receptors by peptidoglycan (PG)-derived ligands is critical in regulating intestinal homeostasis and modulating the host response to immunotherapies. Mutations in the NOD2 gene are the strongest genetic risk factor for Crohn's disease development and impaired function has been linked to a variety of immune-associated diseases. DL-endopeptidases (DLEs), which are a class of bacterial PG hydrolases that cleave PG at specific sites and release NOD2-stimulating fragments, are limited within the gut microbiome. Here, we develop tools to quantify the presence of DLE-encoding genes within a microbiota, as well as downstream changes in NOD2 ligand abundance. We identify key bacterial families, such as Bacillaceae, that are enriched in DLE genes compared to previously reported top families. We annotate the functional domains and localization tags of our novel DLEs to identify key therapeutic targets. By analysis of meta-omic data from publicly available datasets, we make unique insights into changes in DLE distribution from inflammatory bowel disease and immune checkpoint inhibitor (ICI) patients and as a result of antibiotics. By supplementing multiple mouse models with DLE, we identify varying rates of efficacy dependent on microbiota complexity. Finally, we see that DLEs are enriched in the MET4 community and that an increase of these genes is associated with successful response to ICI therapy.

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Adapting the neisserial type XI translocon, SLAM, as a molecular multitool in antigen production and delivery

Dixon Ng, Natalie YT Au, Christine CC Lai, Sang M Huynh, Quynh Huong Nguyen, Clement KO Chan, Epshita A Islam, Jamie E Fegan, Anthony B Schryvers, Trevor F Moraes

University of Toronto, Department of Biochemistry

The type XI secretion system, also designed at the Surface Lipoprotein Assembly Modulator (SLAM), translates surface lipoproteins (SLPs) from the periplasm across the bacterial outer membrane. This translocon has a broad range of substrates including neisserial SLPs: TbpB, LbpB, fHbp, and HpuA. These SLPs are highly conserved and surface-exposed virulence factors, making them excellent targets for vaccine and therapeutic development. We have shown that heterologous expression of SLAM and its cognate SLP in Escherichia coli (E.coli) reconstitutes translocation and surface display. While the molecular mechanism of the translocation process is unclear, we identified key elements that enabled us to secrete normally anchored SLPs as untethered exoproteins in a SLAM-dependent manner. Our novel expression and purification strategy allows for full-length untethered SLPs to be secreted into the culture supernatant in a SLAM-mediated manner in E.coli. Expresion of SLPs natively in Neisseria is often not feasible at levels necesssary for structural or immunological studies. Alternatively, cytoplasmic expression in E.coli requires separation of the SLP from lysis debris. These considerations present a challenge in SLP-antigen production with substantial cost, safety and technical implications. Our SLAM-based system overcome these difficulties and offers a more efficient and robust method in SLP-antigen production. We demonstrated that our secretion system produces SLPs at scales and purity better than conventional cytoplasmic approaches while requiring far fewer downstream purification steps. As such, the secretion process is stable and efficient, yielding 25 to 50 mg/L of cell culture for select cognate substrates. We utilized this system to produce and crystallize a full length meningococcal HpuA, which we resolved to 2.1A by x-ray crystallography. To extend the utility of our system we secreted SLPs in fusion with other proteins and tested the limitations of the the secretion system to gain insights into the translocation process itself.

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Characterizing a novel prophage-encoded phage defense gene

Matthew McCarthy (1,2), Pramalkumar Patel (1), Karen Maxwell (1)

1.University of Toronto, 2. McMaster University

Temperate phages can integrate into the host genome as a prophage, replicating alongside the host cell. During this phase, the survival of the prophage is directly tied to the survival of the bacteria. Therefore, many prophages will encode genes to improve their host's fitness. These include phage defense genes, which provide defense against superinfection, allowing the prophage to monopolize host resources, and protect against cell death via diverse mechanisms. Therefore, prophages represent an underexplored reservoir of uncharacterized phage defense systems. Here, we identified a phage defense gene in the Escherichia coli phage HK446, Gp37, and demonstrated that Gp37 represents a family of phage-defense genes found across diverse bacteria. We showed that Gp37 is a transmembrane domain-containing phage defense gene, and that the membrane domain plays an important but not essential role in phage defense. We also found that, when overexpressed, Gp37 results in a short-term defect in cell growth, which cells can recover from and avoid when re-induced, while retaining the ability to provide phage defense. We also demonstrated that the transmembrane domain plays some role in mediating this toxicity, and that removal of this domain results in growth defect. Finally, we showed that while Gp37-expressing cells provide phage defense, the infected cells are not protected from lysis, demonstrating that Gp37 blocks phage intracellularly.

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Probiotics Administered Since Pre-Conception Accelerate Offspring Gut Microbial Function Maturation with Modest Composition Changes in Juvenile Mice

Céline Cuinat[1], Amel Taibi[1], Julien Tremblay[2], Giorgio Gargari[3], Simone Guglielmetti[3], Thomas Tompkins[4], Elena M. Comelli[1]

[1]Department of Nutritional Sciences, Faculty of Medicine, University of Toronto, Toronto, Canada; [2]Energy, Mining and Environment, National Research Council Canada, Montréal, Canada; [3]Department of Food, Environmental and Nutritional Sciences (DeFENS), University of Milan, Italy; [4]Rosell® Institute for Microbiome and Probiotics, Montreal, Canada

Background: The establishment of a health-compatible gut microbiome in early life is critical to life-long health. We previously found that maternal supplementation with a probiotic mixture containing Lacticaseibacillus rhamnosus R0011 and Lactobacillus helveticus R0052 accelerates the establishment of gut microbial metabolic functions in the offspring before weaning. Notably, 54 pathways involved in biosynthesis and degradation (amino acids, carbohydrates, secondary metabolites) matured earlier (before weaning) in the supplemented group. Here, our aim was to identify the bacterial taxa and enzyme-encoding genes driving these probiotic effects.

Methods: We used 16S rRNA gene sequencing data that we previously generated from cecal content microbiota of 14 days old pups (C57BL/6 specific pathogen-free mice) born to mothers who received or not probiotic-supplemented water starting one week before mating and during lactation. Bacterial genomic information and metabolic pathways were inferred with PICRUSt2 and the MetaCyc database. Taxa contributions were determined using stratified PICRUSt2 output. Data were analyzed with DESeq2-Wald test and taxa-gene-pathway networks were visualized using NAViGaTOR.

Results: 50 of the 220 microbial genes annotated to the altered pathways were affected by maternal probiotic administration (19 enriched and 31 depleted in response to probiotics). 67 and 47 bacterial taxa contributed to these genes in the control and probiotic groups, respectively, including shared (Parabacteroides, Lactobacillus, Intestinimonas, Anaerotruncus, Lachnoclostridium, Blautia, Lachnospiraceae) and specific (control: Bacillus, Tyzzerella; probiotic: Pseudogracilibacillus, Lactococcus) genera. Interestingly, of these, only Lactobacillus, Mucispirillum, Staphylococcus, Lachnospiraceae, Enterobacteriaceae, and Clostridiales were differentially abundant between groups at PND14.

Conclusion: During early colonization, microbial functions evolve in a manner that does not fully depend on the relative abundance of the contributing taxa. Probiotics partially modify the pattern of taxa contributing to these functions. Because pups were only exposed to probiotics through their mothers, these findings also suggest that maternal probiotic intake affects the offspring metabolic potential.

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Identifying inhibitors and elucidating their inhibitory mechanism in surface lipoprotein (SLP) translocation in gram-negative bacteria

Jiaming Xu, Esther Shin, Trevor Moraes

Biochemistry program, Univeristy of Toronto

Bacterial surface lipoproteins (SLPs) are peripheral outer membrane proteins, many of which are nonessential but are important in virulence-associated processes. Therefore, targeting proteins involved in SLP translocation is attractive as they do not impose a strong selection pressure that leads to the rise of multi-drug resistant strains. In 2016, our group discovered that a novel family of outer membrane proteins called Surface Lipoprotein Assembly Modulator (Slam) is required to translocate SLPs to the cell surface in Neisseria meningitidis. Yet, the mechanism of such translocation is yet to be deciphered. Past studies utilizing inhibitors have shed light on protein structures involved in lipoprotein biogenesis and provided insights on translocation mechanisms. To aid in screening for Slam dependent SLP translocation inhibitors, we have developed two translocation assays. First, we have successfully reconstituted Slam-dependent SLP translocation in E. coli. We observed that Slam reconstitutes the surface display of co-expressed TbpB whose surface amount can be quantified with biotinylated human transferrin and a streptavidin-conjugated fluorophore. Using this approach, we screened various chemical and natural product libraries and discovered an actinomycetes extract that reproducibly decreased surface TbpB display. We will further purify the crude extract to isolate the Slam inhibitor using purification methods including flash chromatography and high-performance liquid chromatography. A second in vitro assay comprised of only Slam-integrated liposomes and the substrate SLP, revealed that the inhibitor inhibits this last Slam-dependent outer membrane translocation step. After obtaining the pure inhibitor, we aim to use biomolecular interactions tools to directly probe what the inhibitor binds. As bacterial surface lipoproteins play critical roles during host invasion, inhibitors of Slam could be used to probe the structural basis of SLP translocation and provide a novel avenue for therapeutics to treat bacterial infections.

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Characterizing the NLRP1B inflammasome in the intestinal epithelium

Ryan Mazzone

Department of Laboratory Medicine and Pathobiology

The gastrointestinal tract is inhabited by trillions of microbes in constant contact with intestinal epithelium cells (IECs) that line the mucosa. It is well established that there is constant interplay between these microbes and the host immune system, with NOD-like receptors (NLRs) in IECs mediating anti-microbial responses. NLRs become activated by MAMPs and DAMPs in the cytosol of both immune and epithelial cells. Upon activation, they form multiprotein signalling complexes known as inflammasomes by NLR oligomerization and recruitment of downstream proteins apoptosis-associated speck-like protein (ASC/PYCARD), and pro-caspase 1. This leads to activation of caspase 1, which cleaves gasdermin-D (GSDMD), as well as pro-inflammatory cytokines pro-IL-18 and pro-IL-1β. The liberated N-terminus of GSDMD mediates pore formation at the plasma membrane causing a form of cell death known as pyroptosis and release of mature cytokines. NLRP1B, the mouse homolog of the human NLRP1, forms a canonical inflammasome in immune cell lines, but it has yet to be investigated in the intestinal epithelium.

Here we find more NLRP1B protein expression by western blot in wildtype small intestinal organoids compared to wildtype BMDMs that are derived from the same C57BL/6J mouse. However, despite this difference in expression, only BMDMs are responsive to the NLRP1B activator Val-boro-Pro (VbP), leading to downstream canonical inflammasome activation, as shown by the appearance of the p20 of caspase 1 and p30 of GSDMD, in an NLRP1B-dependent manner. To further understand this result, we are looking at VbP targets upstream of NLRP1B activation; specifically, dipeptidyl peptidases 8 and 9 (DPP8 and DPP9) which have been shown to inhibit NLRP1B activation in the absence of VbP.

Our findings suggest that if the NLRP1B inflammasome is functional in the intestinal epithelium, the mechanism by which it is regulated may differ from what has been reported in other cell lines.

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FIEBRE Substudy: Gut Epithelial Integrity Markers in Febrile Children in Mozambique

Kain T, Zhong K, Balanza N, Andelic SA, Kain KC

University of Toronto, University Health Network

Background: Many children in low-resource settings present to care with fever; most could be safely discharged, but some become critically ill. Current clinical prediction scores exist but are imprecise. Biomarkers of gut epithelial injury (sCD14, LBP) represent one potential mechanism to better identify children who should receive limited resources. Methods: Children presenting to healthcare in Mozambique were enrolled in the study. Plasma biomarkers were measured using the Luminex® Assay and clinical data was collected locally. Baseline levels of sCD14 and LBP were used as predictive variables for d28 mortality.

Results: 1042 children were enrolled, 10 had insufficient plasma and were excluded, leaving 1032 children. Baseline sCD14 was significantly higher in children who survived to d28 compared to those that died (2604 ng/mL vs 2005 ng/mL, p=0.04). There was no significant difference in baseline LBP (23,996.0 ng/mL vs 22,761.0 ng/mL, p=0.99). Logistic regression modelling showed baseline concentration of sCD14 was associated with significantly lower odds of death by d28 (OR=0.44, 95% CI 0.22-0.84, p=0.02), while baseline LBP did not show a significant association (OR=1.46, 95% CI 0.94-2.11, p=0.06). Secondary outcomes, including correlation with clinical scoring tools, respiratory failure, day 7 mortality, and length of stay showed similar trends.

Discussion: Gut epithelial integrity biomarkers, sCD14 and LBP, do not appear to be good predictors of mortality for children presenting to healthcare with fever in low resource settings.

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Elucidating the role of the intestinal epithelial NAIP-NLRC4 inflammasome in the innate immune response against enteric pathogens

Adrienne Ranger, Shawn Goyal, Marry Nissan, Stephen Girardin

University of Toronto

Acute gastroenteritis is caused by infection of the intestinal mucosa with enteric pathogens which damages the mucosal barrier, leading to malabsorption, diarrhea and consequent dehydration. Infectious gastroenteritis contributes significantly to the burden of illness from infectious diseases worldwide, where those living in developing countries and young children are most at risk. Intestinal epithelial cells (IECs) that line the mucosa possess cytosolic NAIP-NLRC4 inflammasomes that can detect bacterial flagellin and virulent type-III secretions systems to rapidly initiate inflammation to stop infectious spread. Activated NAIP-NLRC4 triggers the extrusion of infected cells, lytic cell death known as pyroptosis, and the release of active IL-18. Despite the highly inflammatory nature of inflammasomes, it remains unclear how NAIP-NLRC4 provides signalling mechanisms to ensure inflammation is not at the expense of mucosal barrier integrity. By challenging murine intestinal organoids with various bacterial ligands, we revealed a novel response whereby IECs internalize flagellin which triggers cytosolic NAIP-NLRC4 activation. Surprisingly, this mechanism was unique to flagellin and occurred independently of TLR-5. Activation of NAIP-NLRC4 by flagellin resulted in pyroptotic cell death and secretion of IL-18. Using inflammasome knockout organoids revealed dependence on the adaptor molecule ASC for IL-18 processing but not cell death. Furthermore, we took advantage of this physiological trigger to assess how NAIP-NLRC4 alters the transcriptome of IECs. Bulk RNA-seq analysis revealed a subset of genes that were fully dependent on NAIP-NLRC4 activation. Interestingly, these genes were involved actin cytoskeleton rearrangement, epidermal growth factor signalling, and regulation of cell-cell adhesion. This data suggests that NAIP-NLRC4 signalling not only governs effector mechanisms such as cell death and IL-18 release, but simultaneously contributes to transcriptional reprogramming of IECs that activates pathways involved in maintaining epithelial barrier integrity.

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Graph-based inferences for RNA virus interpretation

Luke Pereira, Artem Babaian

University of Toronto

The enormous societal cost of the current pandemic highlights the need for a pathogen surveillance strategy that improves its predictive power as virus-host interaction data amasses over time. This study aims to develop a data-driven pipeline to predict unobserved and anomalous virus-host interactions using evaluation criteria that prioritize computability, accuracy, and relevance. To map out the known virus-host interaction network while remaining computationally tractable, we employ the Serratus database, which has clustered the catalytic cores of the RNA viral hallmark gene, RNA-dependent RNA polymerase (RdRp), into a reduced subset of species-like operational taxonomic units (sOTUs). Similarities between sOTUs form a distance metric and can be represented as weighted edges in a mathematical graph. This data representation allows us to perform meaningful inferences at the planetary scale of exponentially growing collections of RNA viruses. With the projected growth of training data in mind, we evaluate various topological link prediction approaches, from traditional graph algorithms to embedding-based graph neural networks. Although including contextual information in a heterogeneous graph with multiple node types may pose computational challenges, for appropriate models it may reveal the hidden structures necessary for categorizing risk and relevance (i.e. zoonotic potential, impacts on wildlife, agriculture, etc.). We initially include host taxonomy and sampled tissue ontology with plans to extend to host ecology, disease ontology, and more. We also evaluate methods to reduce the effects of naturally occurring biases resulting from geographic and taxonomic sampling preferences which would otherwise skew our inferences over time. Finally, we briefly consider the benefits, risks, and safeguards that can be put in place to aid in the stewardship of potential information hazards uncovered by this research.

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An unusual response regulator with two kinases involved in antibiotic sensitivity and resistance in Staphylococcus aureus

Marina Suppi, Ian R. Monk, Stephanie Tan, Katharine Myler, Sheila Elardo, Jan V. T. Falguera, Timothy P. Stinear, Sacha J. Pidot & Justin R. Nodwell

Department of Biochemistry, University of Toronto, Toronto, Ontario, Canada; Department of Microbiology and Immunology, Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, Victoria, Australia

WalKR is one of the sixteen and the only essential two-component system in the human pathogen Staphylococcus aureus. Mutations within this system have been associated with the VISA phenotype, with several clinical VISA strains containing walKR mutations. We have previously reported a single mutation in walR gene at the phosphorylation site (T101) of the eukaryotic-like Serine/Threonine kinase, PknB, conferring a VISA-like phenotype (walR1 mutant). Interestingly, previous work has described other mutations in the same residue found in VISA clinical strains, suggesting a clear relationship between WalR and this resistant phenotype. Here we aim to understand the role of PknB kinase in WalR functionality taking advantage of the WalRT101M mutant. Understanding the mechanisms that potentially cause this antibiotic resistance is of crucial importance to assess and evaluate specific targets (known or novel) for drug discovery. Thus far, we have expressed and purified successfully full-length wildtype and mutant WalR, and truncated WalK and PknB kinases (cytoplasmic region). We have demonstrated a defect on the phosphorylation of mutant WalR by PknB-kinase domain (KD). Also, we show the dimeric nature of WalR in solution, which is partially compromised in the mutant protein, suggesting that dimerization could be crucial for optimal functionality of the protein. Lastly, we show that a deletion of pknB gene results in a similar phenotype compared to walR1 mutant (great sensitivity to the antibiotic tunicamycin and deficient autolytic activity).

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The Genetic Determinants of Host Range Evolution in the Plant Pathogen Xanthomonas campestris

Bull, E.M., and Dillon, M.M.

Department of Ecology and Evolutionary Biology, University of Toronto & Department of Biology, University of Toronto Mississauga

The emergence of novel plant diseases has catastrophic effects on food security and the global economy. However, due to our limited understanding of the factors that contribute to host specificity in many pathogens, predicting when and where outbreaks will arise in agriculturally important hosts is challenging. The bacterial species Xanthomonas campestris is a devastating crop pathogen, yet little is known about the factors that have led to its broad species-level host range, or the evolutionary tradeoffs that limit the host range of individual strains. Our research aims to leverage the natural diversity of X. campestris strains to identify the key genomic loci associated with host specificity in this pathosystem. Specifically, we have collected a diverse group of 67 fully-sequenced strains from various hosts around the world and are now screening them on the model host Arabidopsis thaliana to quantify virulence. Two complementary approaches, genome wide association and machine learning, will then be used to identify loci that contribute to variation in the observed virulence phenotypes. We hypothesize that a relatively small number of loci will determine host specificity in X. campestris. Type III secreted effector genes are prime candidates, as they can both suppress and elicit host immunity, but phytotoxins and other secretion systems may also play a role. These results will enhance our understanding of host range evolution by identifying convergent and/or divergent virulence strategies across lineages, as well as tradeoffs that limit fitness across hosts. This data will ultimately be used to better protect crops from potentially destructive bacterial pathogens.

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Genetic diversity and Phylodynamics of 2022 Enterovirus D68 Outbreak in Ontario, Canada

Martin Grunnill1,3, Alireza Eshaghi1, Sandra Isabel1, Sandeep Nagra1, Paul Nelson1, Adriana Peci1, Aimin Li1, Alex Marchand-Austin1, Jianhong Wu3, Thomas Braukmann1, Shawn Clark1, Maan Hasso1, Jonathan Gubbay4, Samir N. Patel1,2 and Venkata R. Duvvuri1,2,3

1 Public Health Ontario, Toronto, Ontario, Canada 2 Department of Laboratory Medicine and Pathobiology, Faculty of Medicine, University of Toronto, Toronto, ON, Canada 3 Laboratory for Industrial and Applied Mathematics, Department of Mathematics and Statistics, York University, Toronto, ON, Canada 4 BC Children's Hospital and BC Women's Hospital & Health Centre, Vancouver, BC, Canada

Enterovirus D68 (EV-D68) has become a common cause of acute respiratory illness in children worldwide since its widespread outbreak in North America in 2014. The most recent outbreak of EV-D68 in North America occurred between July and October 2022 and was associated with a more severe respiratory illness. In Ontario, Canada, 238 of 396 enterovirus positive samples (specimen source: 329 respiratory, 12 cerebrospinal fluid, 11 stool and 44 other) analyzed between July 31 and October 22 2022 were genotyped as EV-D68 (60.1%). To understand the evolutionary history of the 2022 EV-D68 outbreak, we sequenced 88 samples (of 238, 37%), and conducted phylogenetic and phylodynamic analyses using currently available genomic sequences in GenBank. The phylogenetic analysis with global EV-D68 whole genome sequences (WGS) (n=1070, ~length=7170 bp) and VP1's hypervariable region sequences (n=3118, length=339 bp) available in GenBank revealed divergence of three distinct clades (A, B and C) from reference EV-D68 Fermon strain (AY426531). The mean time to the most recent common ancestor of the Ontario 2022 EV-D68 strains indicates a potential emergence between May and June 2021. The mean evolutionary rate of the Ontario 2022 EV-D68 for the WGS was 4.8 x 10-3 substitutions/site/year. The 2022 Ontario EV-D68 WGS cluster in the sub-clade B3, having close homology with WGS temporally related but geographically distant in the United States (Maryland, Virginia, and Washington D.C.) outbreak. This suggests genetic relatedness and a possible commonality of North American 2022 EV-D68 outbreaks. Coalescent and birth-death phylodynamic modelling is in progress to estimate the transmission potential (R0, basic reproduction number) of the 2022 EV-D68 outbreak.

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Investigating the role of nucleotide-binding oligomerization domain (NOD) proteins during bacterial insults in intestinal epithelial cells

Marry Nissan, Stephen Girardin

Lab Medicine and Pathobiology- University of Toronto

Nucleotide-binding oligomerization domain (NOD) 2 is integral to the innate immune system of mammals, and functions as a pattern recognition receptor (PRR) within the cytosol of cells. It detects conserved bacterial peptidoglycans (PGN) to elicit an anti-microbial response by activating the nuclear factor kappa B (NF-κB) pathway. Crohn's disease prevalence is increasing in Canada and is most often associated with polymorphisms in the NOD2 gene. A hypothesis as to why this polymorphism causes the Crohn's disease phenotype is defective barrier function in intestinal paneth cells allowing bacteria to colonize the intestine. Although NODs have been well characterized in immune cells, little research has been done to interrogate the role of NOD proteins during bacterial dysbiosis in intestinal epithelial cells. Ileal organoids, or enteroids, are a 3D cell culture model that recapitulates the different cell types seen within the intestine, making it an ideal model to use when researching the effects of our insults on various cell types at once. We hypothesize that the NOD proteins play a role in bacterial clearance in intestinal epithelial cells, and should they be absent, the organoids will succumb to bacterial invasions more readily. To test this hypothesis, intestinal crypts were harvested from Nlrc4 -/- C57BL/6 mice to generate primary ileal organoids. Organoids infected with Shigella flexneri were harvested for protein and RNA. Downstream western blot and qPCR analyses were performed to ensure efficacy of infection. It was found that transcription profiles and colony forming units (CFU) counts were only able to be recorded in a non-pyroptotic background (Nlrc4-/-). Future research involves Immunohistochemistry assays to assess paneth cell integrity in these organoids post-infection. Elucidating the link between the NOD proteins and the Crohn's disease phenotype in intestinal epithelial cells will give insight on possible targets for therapy in future research.

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Lung Epithelial Cells in Culture Develop Resistance to Inhibitory Effects of Digoxin on Cell Growth and Adenovirus Replication

Kathryn Lloyd-Smith, Martha Brown

Department of Molecular Genetics, University of Toronto

Adenovirus is a common cause of respiratory disease, conjunctivitis, and enteritis, typically causing a mild form of disease. However, in some cases it can cause severe disease and is a significant problem in transplant patients. Currently, there are no approved antiviral therapies for adenovirus. Digoxin is a drug that has been used to treat heart failure for centuries and can inhibit replication of several viruses, including adenovirus, in cell culture. As well as inhibiting virus replication, digoxin (100 nM) inhibits growth of A549 cells in culture. Extended incubation of A549 cells with 100 nM digoxin led to the isolation of two colonies that have continued to grow well with 100 nM digoxin. Virus replication in these A549dig cells is no longer inhibited by 100 nM digoxin. A549dig cells were able to grow in concentrations of digoxin up to 1.25μM though growth was inhibited at 2.5 uM. At concentrations that suppress growth of the A549dig cells, virus replication is also inhibited. Experiments are ongoing to determine whether decreased sensitivity of the A549dig cells reflects decreased binding of digoxin to the cells or some downstream effect. Digoxin typically blocks the sodium potassium ATPase (NKA) at the cell surface, leading to altered ionic concentrations within the cell, but it is not clear how altered ionic concentrations interfere with virus replication, either directly or indirectly due to effects on the host cell. Understanding the mechanism by which the A549dig cells have become less sensitive to digoxin should help us to understand the mechanism by which digoxin exerts its inhibitory effect on virus replication.

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Reversible Effects of Digoxin on Spread of Human Adenovirus Type 5 in Cell Culture

Naila Ahmad (1), Sarah Manianis (1), Ga Min Jeong (1), Filomena Grosso (2), Martha Brown (1,2)

Dept. of Molecular Genetics (1) and Dept. of Laboratory Medicine and Pathobiology (2), University of Toronto

Human adenoviruses cause considerable morbidity and mortality, even in people who are immunocompetent, yet there are no approved antiviral agents for treatment. Digoxin is a broad-spectrum antiviral agent with a long history of clinical use for treatment of heart failure. It inhibits replication of multiple human viruses, including adenovirus, directly and/or indirectly due to its inhibitory effect on growth of host cells in culture. Growth curves of human adenovirus type 5 (HAdV-C5) in A549 lung epithelial cell cultures, with most cells infected at the outset, showed a 2-3 log reduction in yield of infectious virus, with little detectable release of progeny virus up to 5 days pi. Reduced infectious yield per cell reflects a reduction in physical particles rather than production of particles with poor infectivity. Kinetic analysis of selected early gene expression showed delayed expression of E2B transcripts. Under conditions to mimic infection in vivo, with few of the A549 cells infected at the outset, digoxin compromised virus spread for up to 14 days. Without digoxin, cultures of primary nasal epithelial cells, grown at the air-liquid interface, supported spread of progeny virus to form clusters of infected cells but infection did not spread from isolated infected cells to form clusters when treated with digoxin. When treated cultures of A549 cells were fluid-changed to remove digoxin at 5 days pi, the rate of virus replication increased rapidly and, within a few days, yield was comparable to that in untreated cultures.

Compromised virus spread with digoxin reflects low virus yield per cell coupled with inefficient release of progeny virus. Although efficient virus replication resumes when the drug is removed, the ability of digoxin to slow adenovirus replication and spread could be helpful in vivo to limit spread of infection while allowing the host immune system to clear the infection.

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Mitochondrial Enhancers for Neuropsychiatric Diseases

Dana El Soufi El Sabbagh, Alencar Kolinski Machado, Lauren Pappis, Ana C Andreazza

Krembil Brain Institute, UHN; Dept. of Pharmacology & Toxicology, Temerty Faculty of Medicine, University of Toronto; Emerging & Pandemic Infectious Consortium (EPIC), University of Toronto

The long-term effects of SARS-CoV-2 include serious consequences, such as an increase in the diagnosis of psychiatric diseases after infection. An incidence of 34% is estimated in neurological diagnosis after 6 months of COVID-19, with 13% receiving a psychiatric diagnosis for the first time. To date, there is a vague understanding of the biological mechanism and pathophysiology contributing to the vulnerability of COVID-19 patients to develop persistent neurological or psychiatric illness after infection.

Studies have shown that SARS-CoV-2 can induce activation of NACHT, leucine-rich repeat, and pyrin domain-containing protein 3 (NLRP3)-inflammasome. This activation seems to be present in blood, lung autopsies, and post-mortem brain samples where the main product is interleukin (IL)-1β, responsible for a downstream inflammatory activation cascade known as “cytokine-storm”. More recently, the NLRP3 pathway has shown to have significant effects in the immune response of mice lungs after SARS-Cov-2 infection. Increased NLRP3 expression has also been noted in patients with psychiatric diseases such as major depressive disorder and bipolar disorder. In this regard, NLRP3 activation could potentially be the mechanism involved in the development of post-COVID-19 psychiatric disease and/or cognitive impairments.

Cerebral organoids (COs) are complex 3D structures derived from human embryonic stem cells (H9) or patient derived induced pluripotent stem cells (iPSCs) that recapitulate a human brain. Using well established CO generation protocols, COs are currently being used to develop high-level studies compared to patient samples tests and regular 2D cell culture. Recent advancements in using COs for SARS-CoV-2 studies include showing tropism for cortical astrocytes in brain COs infected by SARS-CoV-2.

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Scalable computational methods for identification of structural proteins in phage genomes

Tatiana Lenskaia, Alan Davidson

University of Toronto, Molecular Genetics

We have developed a computational framework for the identification of structural proteins in phage genomes using a comprehensive set of Hidden Markov Models (HMMs) and big genomic data. This cutting-edge framework empowers researchers to efficiently analyze vast amounts of genomic data and unravel the complex structures within phage genomes. By harnessing the power of custom and publicly available HMMs, we have established a robust and versatile computational tool. HMMs allow us to integrate prior knowledge of protein domains and structural characteristics, enabling the accurate identification of potential structural proteins. These models are constructed through the alignment of known protein sequences, revealing conserved patterns and motifs that serve as vital indicators for structural protein presence. One of the strengths of our framework lies in its scalability. As the volume of genomic data increases, our computational methods can handle these immense datasets. Our computational framework not only expedites the analysis process but also minimizes the need for manual annotation. By automating the identification of structural proteins, we eliminate time-consuming and error-prone manual tasks, allowing researchers to focus on higher-level analysis and interpretation. This pioneering framework has transformative implications for phage biology research. By unveiling the structural proteins hidden within phage genomes, we gain deeper insights into their biological properties and potential applications. This knowledge is invaluable in fields such as phage therapy and biotechnology, where understanding the structure and function of phage genomes is of paramount importance. In conclusion, our computational framework utilizing HMMs represents a paradigm shift in the identification of structural proteins in phage genomes. By harnessing the power of big genomic data and advanced statistical models, we empower researchers to delve into the intricacies of phage genomes and unlock their untapped potential.

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Exploring novel approaches to control fungal infections

Sergei Lenskii

University of Minnesota

Most infections in plants are caused by fungi. The growing challenge of antimicrobial resistance in fungi requires novel methods of plant disease treatment and control. Fungi have several natural mechanisms that allow them to outcompete other fungi. For example, killer yeast. The yeast strains that carry dsRNA viruses (L-A and M) are capable to produce toxins (and gain resistance to it) that destroy other yeast strains without the viruses. The main goal of this study is to explore the prevalence and conservative features of antiviral defense mechanisms among fungi. Exploring the defense mechanisms in fungal pathogens has been a challenging endeavor that has traditionally relied on experimental approaches. However, by utilizing computational techniques such as genome sequencing and multiomics, researchers have a powerful toolkit to unravel the intricate interactions between fungi and viral pathogens. Also, computational methods allow for the rapid and comprehensive screening of large datasets, enabling the identification of key genes and pathways involved in virus-host interactions. I have applied computational methods to screen for prospective candidates suitable for validation by molecular genetic experiments. I would like to demonstrate this approach using one of the identified candidates, Grosmannia clavigera, a fungus that infects conifers with a high economic impact on timber industry and forestry in Canada. The results of this study help deepen understanding of virus-host relationships for the purpose of developing new antifungal treatment strategies to control fungal infections.

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Microbial adhesion to silicone medical devices

D. van den Berg, D. Asker, T. Awad, B. Hatton

UofT

Microbial attachment to material surfaces is a complex phenomenon involving a complexity of electrostatic, biochemical and physical mechanisms. Despite the major problems associated with medical device-associated infection, infectious disease transmission within healthcare environments, and hospital acquired infections (HAIs), many aspects of microbial surface attachment are not well understood. Rates of HAI remain relatively high, and there is significant concern about the effects of antibiotic resistance, globally. Most approaches to antimicrobial material design still usually just rely on the release of biocidal chemical species (such as Ag ions).

Our work has focussed on strategies to disrupt the early stages of microbial surface attachment in antimicrobial material design, as an alternative to biocidal chemistries. In the context of medical device-associated infections, there is an important advantage to keeping bacteria in a planktonic (swimming) state, as they are much more vulnerable than in a biofilm community.

Recently we have discovered some effects of mechanical deformation of elastomer (silicone) medical devices, such as silicone catheter tubing, on the microbial surface adhesion. We have investigated this mechanism, to find a strong attraction to topographical surface defects such as microcracks and scratches, which can be easily formed through normal handling, wiping or manufacturing 1. This interdisciplinary work may shed light on how and where medical device-associated infections occur clinically.
(1) van den Berg, D.; Asker, D.; Awad, T.; Hatton, B. D. Mechanical deformation of elastomer medical devices can enable microbial surface colonization. Scientific Reports 2023, 13.

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The curious case of Vancomycin Dependent Enterococci

Dr Ruchika Bagga and Dr Vanessa Allen

University of Toronto

BACKGROUND: Enterococci are known for the plasticity of their genome. While Vancomycin Resistance in Enterococci is well known now, Vancomycin Dependence is a microbiological oddity which is not completely understood/ well known. During my presentation, I shall highlight the mechanism of Vancomycin dependence, present a case of orthotic liver transplant patient with VDE and present a retrospective review of 4 patients. I shall also highlight laboratory and clinical challenges in isolating and managing VDE.

CASE SUMMARY: 59 year old male status post orthotic liver transplant X 2 admitted with pancytopenia and disseminated varicella zoster infection. He lost his first liver transplant due to acute cellular rejection and biliary stricturing. Had a second liver transplant in March 2022 with post-transplant course complicated by probable Aspergillosis treated with voriconazole for 3 months, CMV viremia on valganciclovir and had recent admissions for C. difficile colitis as well. Currently he was on valganciclovir for CMV viremia, amoxicillin/ clavulanic acid for perianal abscess, vancomycin po BID (prophylaxis while on amox/clav) and on atovaquone prophylaxis.

His surveillance screening swabs grew Enterococcus faecium which did not grow on blood agar (BAP) but grew well on VRE agar. The isolate was then streaked on a BAP with a standard vancomycin disc and grew well around the disc which confirmed the diagnosis of Vancomycin Dependent Enterococcus (VDE). Retrospective data review revealed 4 additional isolates, all grew in patients who had prolonged exposure to Vancomycin, had renal impairments and were from same rehab.

CONCLUSION: VDE is the lesser known entity, which is neither reported routinely not tracked and hence the data on it is lacking. Its an ultimate heist were Enterococci rob Vancomycin of its efficacy and infact use it for survival. There are no standard Microbiological reporting/ tracking for VDE and given lack of clinical knowledge, management of these cases is often challenging,

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Impact of SARS-CoV-2 infection on the neuropathogenic potential of myelin-primed Th17 cells in animal models of MS

Baweleta Isho, Salma Sheikh-Mohamed, Catherine Chi, Jennifer L. Gommerman

Department of Immunology, University of Toronto, Toronto, ON, Canada

Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS). Due to sub-optimal responses to SARS-CoV-2 vaccines, MS patients are at high risk for COVID-19, a disease in which ~14% of convalescents experience neurological symptoms. Comparison of COVID-19 and MS brain autopsies showed similar neuropathology including axonal damage, blood-brain barrier leakage and microglial nodules. It is critical to learn whether SARS-CoV-2-induced brain pathology augments MS disease severity or accelerates progression. As such, we wish to determine whether SARS-CoV-2 infection augments the neuropathogenic potential of myelin-primed Th17 cells in animal models of MS. We have established a working model that combines experimental autoimmune encephalomyelitis (EAE) with SARS-CoV-2 infection. Following passive EAE induction, I infect mice with a non-lethal dose of SARS-CoV-2 and assess clinical presentation of both EAE and SARS-CoV-2 infection using pre-established scoring systems, CNS pathology, and immune cell activation using flow cytometry. In a pilot experiment, Syrian hamsters were infected with 10^5 TCID50 of the D614G SARS-CoV-2 strain. Infected hamsters exhibited meningeal inflammation, including the accumulation of myeloid cells, like observations in humans. Next, I adoptively transferred different doses of Th17 cells derived from mice immunized with either MOG35-55 peptide or ovalbumin peptide to induce EAE in male hACE2-KI mice. During the chronic phase of disease, mice were infected with the delta variant of SARS-CoV-2 (2.5x10^6 TCID50). Interestingly, hACE2-KI mice that received higher doses of MOG-primed Th17 cells experienced worsened symptoms of SARS-CoV-2 infection compared to lower doses of Th17 cells or OVA-primed Th17 cells. In the reciprocal experiment, where male hACE2-KI mice received encephalogenic Th17 cells after the clearance of a prior SARS-CoV-2 infection, animals with prior viral infections exhibited less severe symptoms during the chronic phase of EAE. With further work, we hope to understand the impact of SARS-CoV-2 infection on neuroinflammation.

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Harmonization of Quality Indicators used in Clinical Microbiology Laboratories Affiliated with the University of Toronto Microbiology Training Program

Ellen Avery[1], Ghulam Dhabaan[1]*, Mohammed Sarhan[1]*, Jennifer Tat[1], Adriano Airo[1], Shawn Clark[2], James Burns[1], Sandra Isabel[1], Ruchika Gupta[1],Manal Tadros[1,3], Gregory German[1,4], Robert Kozak[1,5], Antoine Corbeil[1,2], Susan M. Poutanen[1,6,7]*

**authors contributed equally to this work*

[1] Dept. of Laboratory Medicine and Pathobiology, Temerty Medicine, U. of Toronto, [2] Public Health Ontario, [3] The Hospital for Sick Children, [4] Unity Health Toronto, [5] Sunnybrook Health Sciences Centre, , [6] University Health Network/Sinai Health, [7]Dept. of Medicine, Temerty Medicine, U. of Toronto – all in Toronto, Ontario

Background: Quality indicators (QIs) are valuable tools to monitor lab performance and identify areas for improvement. Although all labs use QIs as a part of their quality management systems, there is a lack of consensus and standardization of QIs used amongst clinical microbiology laboratories in Ontario. Harmonization of QIs has the potential to improve consistency and enhance the quality of laboratory services.

Methods: The aim of our project was to create consensus statements comprised of key QIs relevant to hospital-based microbiology labs. Based on an earlier survey of University of Toronto-affiliated microbiology labs and a scoping review of the literature, nine high-impact, well-evidenced statements were drafted. The anonymized Delphi method of consensus building was used and input from microbiology lab quality team members from five University of Toronto-affiliated microbiology laboratories was sought. Respondents were asked to score statements using a scale and comments were invited. Iterative changes to the statements were made based on respondents' feedback. Additionally, respondents were asked to self-identify as microbiologists, trainees, managers, technologists/technicians or trainees.

Results: Survey respondents were generally supportive of the QI statements and at least 75% agreement was achieved for all nine statements. However, among the microbiologist subgroup, there was a lack of at least 75% agreement for some statements. Feedback was at times conflicting and could not fully be reconciled using the anonymized Delphi method.

Conclusion: At least 75% agreement was achieved on all nine QI consensus statements. However, the lack of at least 75% agreement on some statements amongst the microbiologist subgroup was notable. The inclusion of members with diverse skills and levels of training could be viewed as both a strength and limitation of our study. While the Delphi process provides a useful framework for developing consensus, the anonymized nature made it difficult to resolve differences of opinion.

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Jacqueline Watt
Maxine Ty

Trainee Scientific Selection Committee:
Anna Waldmann
Duncan Carruthers-Lay
Steve Lee
Dana El Soufi El Sabbagh
Ryan Chieu
Cynthia Guo
Mindy Lam
Ashley Rooney
Jessica Bruce
Dixon Ng
Bradley Laflamme
Kevin Champagne-Jorgensen
Huong Nguyen
Natalie Au
Grace Visser
Jady Liang

Judges for Trainee Oral Presentations:
Anna Waldmann
Amanda Norton
Minh Sang Huynh
Bradley Laflamme
Jady Liang
Jamie Fegan
Andra Banete
Véronique Taylor
Pramalkumar Patel
Naimeh Rafatian
Steve Lee

Judges for Trainee Poster Presentations:
Anna Waldmann
Amanda Norton
Minh Sang Huynh
Sophie Dyzenhaus
Cynthia Guo
Ashley Rooney
Jady Liang
Jamie Fegan
Andra Banete
Julien Couture-Senécal
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Chenyang Yue
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