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LAND ACKNOWLEDGEMENT

The Prairie Infection and Immunity Network (PIIN) is a collection of health researchers from across Saskatchewan and Manitoba (Treaties 1, 2, 3, 4, 5, 6, 8 and 10) and we acknowledge that we are meeting on Treaty 6 Territory, the traditional territory of the Cree, Saulteaux and Nakota Peoples as well as the homeland of the Métis Nation. We respect the principles of Treaties and Treaty Making and are unwavering in our commitment to collaborating with the First Nations, Inuit, and Métis people in their pursuits of the truth, reconciliation, and cooperation.

GENERAL CONFERENCE INFORMATION

Overview

The annual meeting of the Prairie Infection and Immunology Network is a gathering of researchers and trainees interested in immunity and infectious diseases. Since 2006, this event has brought together this research community from Manitoba and Saskatchewan along with invited speakers from across Canada and internationally to create a unique experience.

Funding and grant support

We are grateful to our academic and industrial sponsors for their generous support, enabling us to host the PIIN 2025 meeting! Be sure to visit our sponsor booths at the meeting and participate in our PIIN2025 Bingo prize! More information will be provided at the conference.

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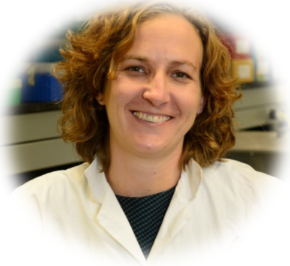
University of Saskatchewan

Thomas Murooka, PhD
PIIN 2025 Co-Chair
University of Manitoba



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Vaccine and Infectious Disease Organization

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WELCOME FROM THE ORGANIZING COMMITTEE

Welcome to PIIN 2025!

We are delighted to welcome you to the 2025 Prairie Infection and Immunity Network (PIIN) Conference, taking place in the vibrant city of Saskatoon, on Treaty 6 Territory and Homeland of the Métis! It is a privilege to gather on this land and we respectfully acknowledge the longstanding relationships that the First Nations Peoples have with this territory. Whether you've traveled across the prairies or live in the city, we are grateful for your participation in what promises to be a dynamic, engaging, and inspiring gathering!

Founded in 2006, PIIN brings together researchers and trainees in infectious diseases and immunology from across Manitoba and Saskatchewan, including the University of Saskatchewan, University of Manitoba, VIDO, CancerCare Manitoba, Cadham Provincial Lab, Public Health Agency of Canada, Canadian Food Inspection Agency, University of Winnipeg, University of Regina, and Brandon University. This year's conference reflects the continued growth of our network and the strength of emerging leadership across our institutions.

The PIIN 2025 scientific program includes five sessions, two poster sessions, and a trainee career development workshop. As our fifth session, we are honored to include Indigenous Engagement in Infection and Immunity Research, a session dedicated to recognizing the essential contributions of First Nations Peoples to health research and community well-being. This session will reflect on culturally grounded approaches to infectious disease prevention, the role of traditional knowledge in health and healing, and the importance of equitable research partnerships.

Our keynote speakers this year are two trailblazing scientists whose work spans cutting-edge discoveries in immunology, infectious disease, and translational medicine. Dr. Anna Blakney from the University of British Columbia will give the first keynote on September 28 followed by Dr. Nathalie Grandvaux from the Université de Montréal on September 30.

We extend sincere thanks to our sponsors for their generous support, to our volunteers for their tireless work, and to you, our attendees, whose curiosity and commitment make this conference such a vibrant and essential gathering.

We look forward to connecting, learning, and building together.

Warmest regards,

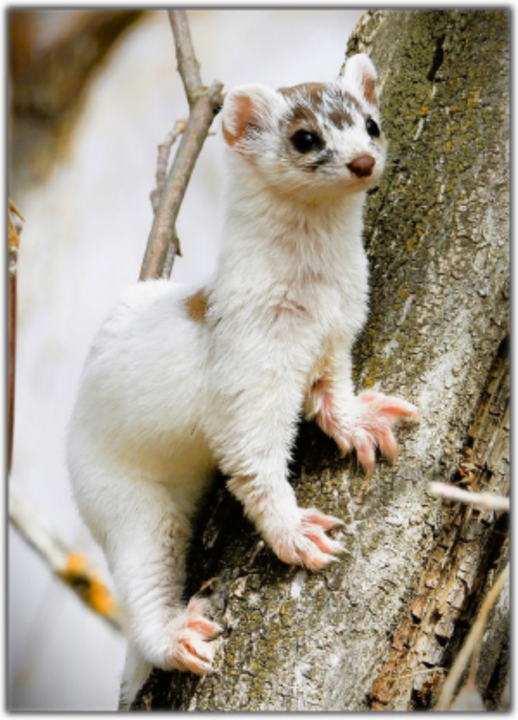
The PIIN 2025 Organizing Committee

PIIN 2025 welcomes André Letendre and thanks him for generously sharing his time and knowledge with us.

André Letendre is a Métis knowledge and ceremonial keeper from Batoche, Sk. He is a dedicated father and grandfather. In his present employment as Cultural System Advisor, André is committed to system advancement, relationship building and sharing his cultural knowledge within Saskatchewan Health Authority and its partners.

PIIN 2025 seeks to remind us that we have been given the privilege of living and working on Treaty Lands and that our research should seek to benefit all people throughout Saskatchewan and Manitoba. Please join us in remembering our place on these lands and our commitment to bettering all lives as well as pledging to understand how to respectfully engage Indigenous culture and people into our work.

PIIN Photographer



Sourabh Suran, Postdoctoral Fellow

Vaccine and Infectious Disease Organization (VIDO)
University of Saskatchewan, SK, Canada

About Me

I am a Postdoctoral Fellow at VIDO-USask, dedicated to advancing vaccine research to combat infectious diseases. Alongside my scientific pursuits, I am an avid photographer with a passion for capturing the world through a creative lens. My photography often intersects with science, helping to visually communicate research and fieldwork in engaging and accessible ways.

Photography & Science: Bridging Two Worlds

- Field Documentation: Capturing on-site research and biological phenomena.

- Visual Storytelling for Outreach: Translating complex scientific work into relatable visuals for public engagement.

- Knowledge Translation: Providing high-quality imagery for scientific reports, presentations, and publications.

Let's Collaborate

I welcome collaborations with researchers, communicators, and organizations, especially in the following fields:

- Entomology, Ornithology & Field Biology

- Agriculture & Crop Sciences

- Outreach & Public Engagement Initiatives

Stoat

Wetland Nature Trail,

National Wildlife Area- Last Mountain Lake, SK, Canada.

If you are looking to visually amplify your research or scientific story, let's connect!

Contact & Portfolio (Click below**) or scan** ➡

DR.PHOTOGRAPHERYXE

Conference Etiquette

Please ensure all mobile devices are set on SILENT or turned off during speaker's presentations. In consideration of the work of all presenters, attendees may not photograph posters, record parts of talks or post data without the presenter's consent. Be courteous to fellow attendees and presenters by keeping conversations to a minimum during sessions and allow everyone an equal chance to ask questions. Harassment, bullying, or discriminatory behavior of any kind will not be tolerated. Please treat all participants with kindness, respect, and professionalism. Thank you for helping make the conference a safe, enjoyable, and respectful experience for all.

Conference Venue and things to do

The conference centre is located in downtown Saskatoon, close to many great attractions. Unfortunately, the Sheraton Hotel pool and gym will be closed during our stay. However, the hotel will provide complimentary passes to the city's Leisure Centres and the YMCA, which include gym and pool facilities, along with transportation for conference attendees. Below is a list of the Leisure Centres, the YMCA, and nearby attractions, including their distance from the hotel.

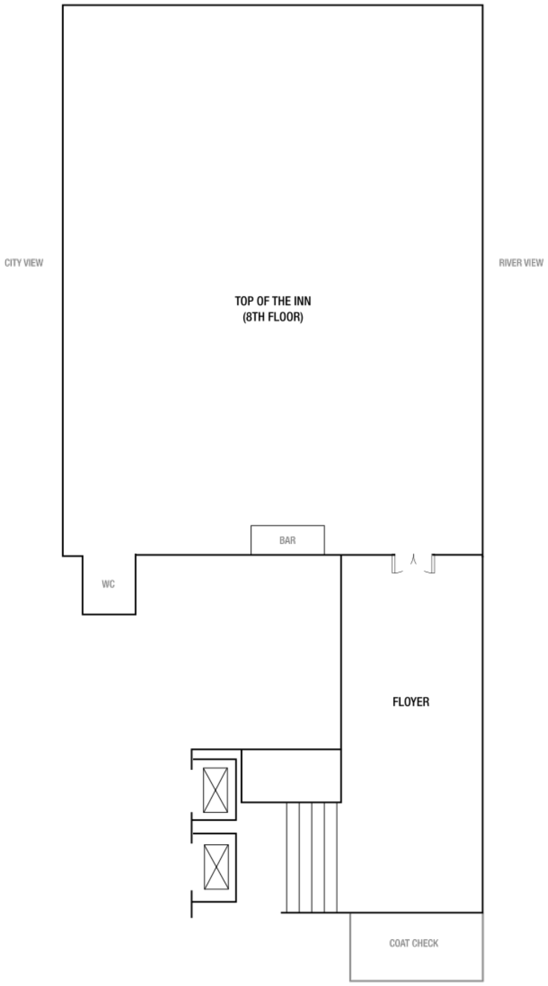
Pool and fitness centres	Amenities	Distance from hotel
Cosmo Civic Centre 3130 Laurier Dr. 306-975-3344	Gym, Fitness and weight room, indoor skating rink	5.3 km
Lakewood Civic Centre 1635 McKercher Dr. 306-975-3944	Pool, waterslide, hot tub, sauna, gym, fitness and weight rooms	8.1 km
Lawson Civic Centre 225 Primrose Dr. 306-975-7873	Wave pool, hot tub, sauna, fitness and weight room	6.9 km
YMCA Saskatoon 25 22nd St 306-652-7515	https://ymcasaskatoon.org/	850 m

Other attractions:

Recreation	Website	Distance from hotel
Remai Modern Art Museum and Persephone Theatre 102 Spadina Crescent E	https://remaimodern.org/	1 km
Saskatoon Farmer's Market 2600 Koyl Avenue	https://www.saskatoonfarmersmarket.com/	7.4 km
Western Development Museum 2610 Lorne Avenue	https://wdm.ca/saskatoon/	4.5 km
Wanuskewin Heritage Park RR #4 Penner Road	https://wanuskewin.com/	15.6 km
Beaver Creek Conservation Area	https://meewasin.com/beavercreek/	19.4 km
Saskatchewan Aviation Museum 5 Hangar Road Saskatoon	https://saskaviation.ca/	6.5 km
Stoked Centre 303 Owen Manor	https://stoked.splitsvillebowl.ca/	9.9 km
Nutrien Playland at Kinsmen Park 222 3rd Ave North	https://www.saskatoon.ca/parks-recreation-attractions/events-attractions/nutrien-playland-kinsmen-park (rides are closed, but it has a nice playground)	1.3 km
Nutrien Wonderhub 950 Spadina Crescent E	https://wonderhub.ca/	1.2 km
Clip'n Climb 97-127 McLeod Ave	https://cncyxe.ca/	5.9 km
Prairie Jurassic Laser Tag 618 Cynthia St	https://www.prairiejurassic.ca/	5.2 km

MAIN LEVEL CONFERENCE CENTER

TOP OF THE INN 8TH FLOOR



KEYNOTE SPEAKERS

Dr. Anna Blakney, The University of British Columbia

Dr. Anna Blakney, an Assistant Professor in the Michael Smith Laboratories and School of Biomedical Engineering at UBC. She received her Bachelor of Science in Chemical & Biological Engineering from the University of Colorado at Boulder, and her PhD in Bioengineering from the University of Washington. She completed a postdoctoral fellowship at Imperial College London on the development of molecular and biomaterial engineering strategies for delivery of self-amplifying RNA. Her lab uses bioengineering, molecular biology and immunology approaches to develop the next generation of RNA vaccines and therapies. She is also a passionate science communicator and runs a TikTok channel dedicated to educating the public about RNA biotechnology. She was one of MIT Review's 35 innovators under 35.

Dr. Nathalie Grandvaux, Université de Montréal

Dr. Nathalie Granvaux, a Professor in the Department of Biochemistry and co-founder and first president of the Canadian Society for Virology (CSV) and originator of the "Citizen Scientist." After earning an engineering degree and a master's in biochemistry from the National Institute of Applied Sciences in Lyon, she completed her doctoral studies at the University of Grenoble, investigating enzyme complexes involved in eliminating pathogenic bacteria. Her postdoctoral studies advanced research on the body's antiviral innate response. At the University of Montreal and the CRCHUM, her research focuses on host defense mechanisms against respiratory viruses. Over the past decade, her team has shown that oxygen derivatives at the respiratory mucosa are essential for effective antiviral defense.

For more information, please visit: https://www.piin2025.com/keynote-speakers

PROGRAM AT A GLANCE

Sunday, September 28

3:00PM-4:00PM
Bus arrival
Sheraton Cavalier Saskatoon

4:00PM-5:00PM
Registration and poster set up (Group #1; odd numbers)
Conference Foyer and East Ballroom

5:00PM-5:45PM
Welcome address: Drs. Lavender and Murooka, PIIN 2025 Co-chairs.
Blessing: André Letendre, Metis knowledge and ceremonial keeper
Centre Ballroom *Please no photography during the blessing.*

5:45PM-6:45PM
Keynote Lecture I: Dr. Anna Blakney, University of British Columbia
Centre Ballroom

7:00PM-8:30PM
Welcome Dinner
Centre Ballroom

8:30PM-10:30PM
Poster session #1 (odd numbers) and Sponsor exhibits
East Ballroom

10:00PM-1:00AM
Socializing Time
Centre and East Ballrooms

Monday, September 29

7:00AM-8:20AM

Buffet breakfast

Centre Ballroom

8:30AM-10:15AM

Session 1: Vaccines and Immunotherapeutics

Co-chairs: Dr. Heather Wilson and Courtney Marshall

Speakers: Drs. Zahed Khatooni and Logan Banadyga

Selected Abstracts: Naga Suresh Kola, Donaldson Magloire, and Courtney Marshall

Centre Ballroom

10:15AM-10:30AM

Coffee Break

Conference Foyer

10:30AM-12:15PM

Session 2: Chronic and Inflammatory Diseases

Co-chairs: Dr. Janilyn Arsenio and Dr. Aaron White

Speakers: Drs. Liam O'Neil and Neeraj Dhar

Selected Abstracts: Sina Taefehshokr, Reza Javan, and Fatemeh Hesampour

Centre Ballroom

12:15PM-2:30PM

Buffet Lunch and Poster #2 (even numbers) set up 1:30-2:30

Centre and East Ballrooms

12:45PM-1:30PM

Platinum sponsor presentations

Centre Ballroom

12:45-1:05 (5 min Q&A)

Dr. Kyle Brown, Associate Director, Field Medical Advisor Moderna

Dr. Brown is the Associate Director, Field Medical Advisor for Moderna Canada, and brings his expertise in Medical Affairs & Medical Information to our conversation today. Kyle obtained his B.Sc and Ph.D in Medical Microbiology from the University of Manitoba as well as completed a post-doctoral research role with the Vaccine & Infectious Disease Organization (VIDO) at the University of Saskatchewan. He has a broad history of working in the pharmaceutical industry with both US and Canadian-based healthcare providers. Kyle's experience focuses on infectious disease, human & animal vaccine development, molecular biology, and mRNA technology.

1:10 – 1:30 (5 min Q&A)
Kayla Clark, Technical Sales Consultant
Miltenyi Biotec

Kayla has been supporting researchers in Western Canada for over 2 years with Miltenyi Biotec. Before entering industry, Kayla did her undergraduate in Biology at the University of Victoria and has 4 years of experience manufacturing clinical patient cell therapies in an academic/hospital environment.

2:30PM-4:00PM
Trainee Career Development Workshop

Centre Ballroom

- **Industry (Scientific suppliers)**
  Kayla Clark, BSc
  Technical Sales Consultant, Miltenyi Biotec

- **Industry (Pharmaceutical)**
  Kyle Brown, PhD
  Associate Director, Field Medical Advisor, Moderna Canada

- **Commercialization**
  Erin Kulhawy, PhD
  Tech Transfer Manager, Research Excellence and Innovation
  University of Saskatchewan

- **Granting Agencies**
  Chelsea Cunningham, PhD
  Programs and Engagement Manager
  Saskatchewan Health Research Foundation (SHRF)

- **Clinical Microbiology**
  Ninad Mehta, PhD
  Clinical Microbiologist
  Saskatchewan Health Authority

- **Safety Management**
  Tara Donovan, MSc
  Biosafety and Environmental Officer, Safety Resources
  University of Saskatchewan

For more information, please visit: https://www.piin2025.com/piin2025-trainee-development-workshop

4:00PM-4:15PM

Coffee Break

Conference Foyer

4:15PM-6:00PM

Session 3: One Health including Livestock and Wildlife species

Co-chairs: Drs. Angela Rasmussen and Kaushal Baid

Speakers: Dr. Angela Rasmussen and Dr. Kaushal Baid

Selected Abstracts: Taylor Volappi, Ethan Done, and Kira Mudrey

Centre Ballroom

6:00PM-7:30PM

Buffet dinner

Centre Ballroom

7:30PM-9:50PM

Poster Session #2 (even numbers) and sponsor exhibits

East Ballroom

10:00PM-1:00AM

Gala Event

Top of the Inn

Tuesday, September 30

7:00AM-8:10AM
Buffet breakfast
Centre Ballroom

8:15AM-9:15AM
Keynote Lecture II: Dr. Natalie Grandvaux, Université de Montréal
Centre Ballroom

9:15AM-11:00AM
Session 4: Host-Pathogen Interactions
Co-chairs: Drs. Joyce Wilson and Zulma Rueda
Speakers: Dr. Anil Kumar and Dr. Zulma Rueda
Selected Abstracts: Olabisi Akinlabi, Madeline Stewart, and Riley Greenslade
Centre Ballroom

11:00AM-11:15AM
Coffee Break
Conference Foyer

11:15AM-12:45PM
Session 5: Indigenous Engagement in Infection and Immunity Research
Co-chairs: Drs. Wendie Marks and Jessica Sheldon
Smudging Ceremony: André Letendre ***(Please, no photography).***
Speakers: Dr. Ayush Kumar and Dr. Omar El-Halfawy
Centre Ballroom

12:45PM-2:00PM
Lunch, Awards, and Closing remarks
Centre Ballroom

2:00PM
Farewell

INSTRUCTION FOR PRESENTERS

Poster presenters:

1. All poster presenters were emailed information about their abstract number and presentation date. Abstract book in PDF format is available for download from the PIIN2025 website (https://www.piin2025.com/meeting-program)

2. Posters should be 4' x 3' (width x height) and all text legible for viewers. Clips will be available on site to put them up on poster boards.

3. All poster boards will be labeled with an abstract number (odd or even). Please put your poster up during the designated time and ensure that you are presenting within your designated group, as indicated below:

   Poster presentation Group #1 (odd numbers)
   Poster setup time: Sunday, Sept 28 from 4-5pm (East Ballroom)
   Poster presentation time: Sunday, Sept 28 from 8:30-10:30pm
   Please remove your posters by noon, Monday Sept 29

   Poster presentation Group #2 (even numbers)
   Poster setup time: Monday, Sept 29 from 1:30-2:30pm (East Ballroom)
   Poster presentation time: Monday, Sept 29 from 7:30-9:50pm
   Please remove your posters by noon, Tuesday Sept 30

4. Poster judging details will be announced at the conference. Awardees will be announced during lunch on Tuesday, Sept 30.

Oral presentations by trainees:

1. Please submit your PPT presentation to be uploaded to Saurav (pictured here) the <u>night before</u> your talk. He will be at the AV station in the Centre Ballroom from 8-9pm to upload talks for the following day. His cell number is: 306-270-2341 if you have any last-minute changes to make or need to contact him outside of this time.

2. Instructions regarding presentation length has been emailed to you by your Session Chair. If there are any questions, please reach out to them.

3. A round table located at the front of Centre Ballroom will be reserved for speakers and chairs of each session, as well as our invited Elder. Please make your way to this table immediately prior to your session.

4. There is a mic wired to the speaker podium and two screens in the room. We ask you remain at the podium and use the mouse pointer to refer to material on your slides so that people on both sides of the room can see what is being pointed at.

5. Session Chairs will introduce each talk and moderate the Q&A session. They will wave to you when your allotted time for speaking is nearly complete to help you keep to time. Question periods will be modified if needed to keep us on schedule.

6. Judges will award 1 oral abstract presentation award per session, based on presentation quality, research advance and the Q&A period. Awardees will be announced during lunch on Tuesday, Sept 30.

7. Trainees selected for oral presentations have the option to also present during the poster session but will not be judged in this category.

Abstracts

1. Regulation of Intestinal Mucosal Immunity to Johne's disease in Cattle Through Parenteral Vaccination

Itzel Guadalupe Aguilar-Lopez[1,2], Laura Sofia Gomez-Diaz[1,2], Travis Blimkie[3], Amy H. Lee[3], Antonio Facciuolo[1,2].
[1]*Vaccine and Infectious Disease Organization (VIDO), University of Saskatchewan, Saskatoon, SK, Canada*
[2]*Department of Veterinary Microbiology, University of Saskatchewan, Saskatoon, SK, Canada*
[3]*Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, BC, Canada*

Johne's disease (JD), caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), is endemic in all dairy and beef cattle-producing countries. Economic losses are attributed to decreased milk production and reduced slaughter value. Calves are most susceptible to enteric infection and remain asymptomatic for years while intermittently shedding MAP in feces. MAP targets macrophages within Peyer's patches (PPs) in the small intestine. Ruminants contain two distinct types of PPs: discrete PP, distributed throughout the jejunum, and a single continuous PP in the ileum. Vaccine development has been hampered by knowledge gaps surrounding which vaccine administration routes can induce durable immunity in PPs and the types of mucosal immune responses associated with protection. Previously, we demonstrated that calves given a parenteral MAP bacterin vaccine (Silirum®) showed a significant reduction in MAP burden in the continuous PP, but not in the discrete PP, at one month post infection (pi) compared to unvaccinated controls. Recently, we found that at 6 months pi, Silirum®-vaccinated calves showed significant ($p<0.01$) control of MAP infection in both discrete and continuous PP compared to unvaccinated controls. These findings reveal a delayed onset of immunity in the discrete PP at one month pi suggesting that parenteral vaccines may not rapidly induce protective mucosal immunity at this site, unlike the continuous PPs, where rapid and sustained protection is observed. MAP antigen-specific recall responses in intestinal leukocytes isolated from continuous PP revealed *IFNG, IL12B, IL21, IL122, IL27, and TNFA,* responses associated with protection. Ongoing flow cytometric and RNA-seq analyses are being used to further characterize the cellular responses and gene expression signatures associated with this protective immune response. Collectively, this advances our knowledge surrounding ruminant intestinal immunity to inform rational vaccine design for enteric MAP infection.

2. DEVELOPMENT OF MULTIVALENT ANTI-UROGENITAL CHLAMYDIA VACCINE

Stephen Amos, Zhujun Ao, and Xaiojian Yao
Department of Medical Microbiology and Infectious Disease, University of Manitoba, Canada

Introduction: Over 130 million cases of urogenital chlamydial infection are recorded globally each year, making it the most common sexually transmitted disease caused by *Chlamydia trachomatis* (*C.t.*) in the 15-45 years age group. This infection can lead to severe conditions such as ectopic pregnancy, infertility, and pelvic inflammatory disease if left untreated. Despite antibiotic treatment, reinfections are frequently observed. Therefore, developing a vaccine against *C.t.* is urgently needed. Due to the antigenic variation of the *C.t.* major outer membrane protein (MOMP), a key factor in its pathogenicity, it has many serovars, which complicates vaccine development. Previous studies using some MOMP-based vaccine platforms have produced inconsistent results. Currently, the FDA has not approved any *C.t.* vaccine, and the main challenge remains creating an effective vaccine that covers all serovars. This study aims to develop a multivalent vaccine capable of combating all urogenital *C.t.* serovars.

Methods: Our previously developed dendritic cell (DC)-targeting and immuno-repeat vaccine technology will be employed to create a new vaccine targeting the conserved variable domains of MOMP. It is known that the variable domains VD1 and VD4 are shared by all urogenital *C.t.* serovars (D, E, F, G), with these sequences being surface-exposed, thus making them accessible for neutralizing antibodies. Consequently, the $VD4^{D\text{-}G}$ sequence was generated and fused with the DC-targeting domain of the Ebola glycoprotein to produce the Ebo-$VD4^{D\text{-}G}$ immunogen. Additionally, an immunogen combining VD1-$4^{D\text{-}G}$-$VD4^{D\text{-}G}$ was constructed. The expression of both immunogens was confirmed in mammalian 293T cells. Subsequently, these immunogens were cloned into a recombinant vesicular stomatitis virus (rVSV) vector.

Results: Following the successful rescue of rVSV vaccine vectors, we verified the expression of both immunogens in Vero-E6 cells. Currently, these vaccine candidates have been used to immunize mouse models via intramuscular injection. The immune responses induced by the vaccines against the conserved epitopes (VD1-4^{D-G} and VD4^{D-G}) and *C.t.* MOMP will be detailed.

Conclusion: We have developed multivalent *C.t.* vaccine candidates, which we hope will generate a high titer of anti-MOMP immune responses *in vivo*. Ultimately, we aim to demonstrate the vaccine-induced strong immune responses. Moreover, we will measure the neutralizing antibody levels and ADCC activity induced by the vaccines *in vitro*.

3. High-plex spatial analysis of T cell exhaustion using Imaging Mass Cytometry for immuno-oncology studies

Toby Astill and Qanber Raza
Standard BioTools, Ontario

Deciphering the spatial biology of the tumor microenvironment (TME) offers critical insights into the likelihood of therapeutic success. Of particular importance is the spatial organization of immune cells—especially T cells—which can become functionally exhausted through the expression of inhibitory markers such as PD-1, leading to diminished effector functions. Imaging Mass Cytometry™ (IMC™) is a highly multiplexed, quantitative spatial imaging technology that enables the simultaneous detection of over 40 biomarkers on a single tissue section, without signal amplification or autofluorescence interference.

We employed IMC technology to investigate the spatial distribution and phenotypic profiles of immune cells, with a focus on exhausted T cells within the TMEs of various cancers. Multiple IMC imaging modalities revealed striking heterogeneity in the TME, including clusters of lymphocytes at tumor margins—suggestive of active immune surveillance. Pixel-level clustering and single-cell analysis enabled the delineation of distinct tumor regions based on T cell exhaustion, immune cell density, proliferative activity, and stromal composition. These findings underscore the power of IMC to resolve the complex spatial landscape of the TME and highlight its potential in informing prognostic assessments and guiding personalized cancer therapies.

For Research Use Only. Not for use in diagnostic procedures.

5. Bioengineered yeast to provide antimicrobial peptides and EAA to improve gut health in piglets

Carolina Pantuzza-Ramos[1]; Siew H. Ng[1]; Zahed Khatooni[1]; John Husnik[2]; Ye Wang[2]; Partho Adhikary[2]; Erin Gilchrist[2]; Heather L. Wilson[1]
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Post-weaning stress in piglets has traditionally been managed with antibiotic growth promoters, supporting performance and health under intensive farming conditions. However, rising concerns over antimicrobial resistance have led to restrictions on their use, creating an urgent need for effective alternatives. This study aimed to evaluate antimicrobial peptides (AMPs) as potential substitutes, with the long-term goal of engineering *Saccharomyces cerevisiae* yeast as a cost-effective platform to deliver AMPs and essential amino acids (EAAs) in creep feed. The antimicrobial potential of the selected peptides was investigated: AcrAP2, Alloferon 1, HeL46-61, AMP1, and AMP2. To mimic the surface display on yeast, peptides were attached to Nickel beads at concentrations non-toxic to bacteria. Their stability against enzymatic degradation was also assessed by testing antimicrobial activity after trypsin digestion (peptide:trypsin ratios of 1:10 and 1:100). Minimum inhibitory concentrations (MICs) were determined using two-fold serial dilutions against relevant pathogens (*Escherichia coli* ATCC 25922, porcine enterotoxigenic *E. coli* K88, multidrug-resistant *Salmonella* Typhimurium, *Klebsiella pneumoniae* ATCC 43816, and *Staphylococcus aureus* ATCC 25923). Bacterial growth was evaluated both visually and by optical density at 630 nm. While AcrAP2, Alloferon 1, and HeL46-61 did not exhibit measurable antimicrobial activity under CLSI guidelines, AMP1 and AMP2 demonstrated notable inhibitory effects. AMP2 suppressed bacterial growth from 16 µg/mL, with inhibition of multidrug-resistant *S*. Typhimurium from 64 µg/mL. When conjugated to Nickel beads, AMP2 inhibited growth starting at 128 µg/mL. Nickel-conjugated AMP1 also displayed activity against *S*. Typhimurium above 256 µg/mL. Importantly, AMP1 maintained antimicrobial activity against *S. aureus* even after trypsin digestion for 2 h at 500 µg/mL. AMP1 and AMP2 emerged as the most promising antimicrobial candidates. The next phase of this work will assess whether feeding post-weaning piglets with bioengineered yeasts expressing these peptides enhances their resilience against experimental *S*. Typhimurium challenge.

6. Optimization of an inactivated vaccine against Porcine Epidemic Diarrhea Virus (PEDV)

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Porcine Epidemic Diarrhea Virus (PEDV) is a reemerging alphacoronavirus causing significant economic losses in global swine production. PEDV induces intestinal villous atrophy, resulting in severe diarrhea, vomiting, and dehydration, with up to 100% mortality in suckling piglets. Vaccination-induced maternal antibodies transferred through colostrum and milk represent the primary protection strategy for neonatal pigs. This project aims to develop a second-generation inactivated PEDV vaccine with improved formulation and reduced production costs. Building on VIDO's first-generation prototype vaccine, which demonstrated efficacy under emergency authorization in Manitoba (2016-2018), we optimized PEDV Colorado-2013 strain propagation in Vero 76 cells. This optimization involved testing different protease concentrations in cell media and utilizing the 10-layer Cell Factory System to achieve high viral titers. Such approach ensures homogeneous viral infection through improved consistency and simplified infection protocols. Following beta-propiolactone inactivation, novel adjuvants will be evaluated to enhance lactogenic immunity in pregnant sows. Humoral immune responses will be assessed by measuring serum IgG levels, colostral and milk IgA concentrations, and neutralizing antibody titers, with protective efficacy determined through controlled piglet viral challenge studies. This comprehensive optimization addresses current PEDV vaccine limitations through enhanced viral propagation protocols, advanced adjuvant formulations that improve immunogenicity, and cost-effective production procedures.

8. Development of novel microfluidic devices for delineating natural killer (NK) cell migration and cytotoxicity in defined microenvironments

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Natural killer (NK) cells are innate lymphocytes with high therapeutic capacity to detect and eliminate cancer cells. To effectively kill cancer cells, NK cells must migrate to and infiltrate the solid tumour mass. According to literature, there are lower densities of infiltrating NK cells in patient samples of solid tumours and increasing the number of infiltrating NK cells has been shown to positively correlate to better prognostic outcomes. However, mechanisms leading to high NK cell infiltration in solid tumours have not been elucidated. Here, I will describe my recent progress in creating a novel microfluidic device to examine NK cell migration and cytotoxicity toward 3D MDA-MB-231 breast cancer spheroids to test our hypothesis that 3D cellular organization of cancer cells negatively influences NK cell migratory and cytotoxic properties. Microfluidic devices manipulate small volumes of fluids using micron sized channels and are amendable to innovative designs supporting cellular processes under well-defined experimental conditions. Using a previously established D3 microfluidic device, we generated a mouse recombinant interferon inducible factor 10 (IP-10) chemical gradient to investigate its effect on primary NK cells (C57BL/6) using time lapse optical microscopy. 3D MDA-MB-231 spheroids were generated using the liquid overlay technique and characterized using brightfield microscopy for 14 days followed by an end-point apoptosis assay using Annexin-V APC via flow cytometry. Cytotoxicity of NK-92 cell line against MDA-MB-231 cells grown in 2D monolayer cultures vs. 3D spheroid cultures within 24-hour incubation were assessed for cell death via flow cytometry using labels 7-AAD for viability and CD45 FITC. Preliminary results indicate that MDA-MB-231 spheroids retain necrotic cells in its 3D structure after 14 days, and robust cytotoxicity of NK-92 can be seen both in 2D and 3D. Future work will focus on designing the “organ-on-chip” device, incorporation of fibroblasts into the 3D spheroid to investigate NK-spheroid interactions.

9. Stabilizing mRNA-Lipid Nanoparticles with Carbohydrates for Inhalation

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Purpose: Pulmonary delivery of messenger ribonucleic acid (mRNA) vaccines via inhalation holds promise for eliciting comprehensive mucosal and systemic immunity, which is pivotal for providing frontline protection against respiratory pathogens. However, delivering mRNA to the lungs presents challenges, particularly with conventional lipid nanoparticles (LNPs) that are prone to instability during nebulization owing to high shear stress. To address this challenge, we aimed to test carbohydrates as excipients in LNPs to improve their stability during nebulization. We hypothesized that incorporating saccharides into LNP formulation would prevent nanoparticle aggregation and protect the encapsulated mRNA from degradation during nebulization.

Methods: We formulated firefly luciferase (FLuc) encoding mRNA-loaded LNPs by microfluidic mixing and incorporated carbohydrates as excipients during dialysis. Nanoparticles were aerosolized by a vibrating mesh nebulizer. mRNA-LNPs were characterized for size, polydispersity, morphology, and mRNA encapsulation. To assess the efficacy of nebulized LNPs for mRNA transfection, we performed *in vivo* bioluminescent imaging in mice following intranasal administration.

Results: FLuc mRNA-LNPs exhibited monodispersed, spherical oligolamellar structures with a size<150 nm. Nebulization differentially impacted LNPs stability: conventional LNPs without excipients, *i.e.*, salt-based formulations underwent significant physicochemical alterations with almost 50% of encapsulated mRNA degradation. Formulations incorporating mono- and disaccharide excipients effectively preserved LNPs integrity. *In vivo,* luciferase expression studies in mice demonstrated sustained protein expression for 7 days. Notably, the lactose-based mRNA-LNPs resulted in higher expression ($p<0.001$) within the nasal cavity, while the sucrose-based formulation predominantly yielded a 3-fold higher expression in the lungs compared to unmodified mRNA-LNPs.

Conclusion: Our findings demonstrate that incorporating non-reducing disaccharides as excipients is a promising strategy to mitigate nebulization-induced mRNA-LNPs degradation and promote higher protein expression in the mice lungs. This suggests that the strategic selection of excipients can significantly improve the efficacy of inhaled LNP-based mRNA therapies by promoting both LNP stability and efficient mRNA transfection across the lungs.

10. Identification of T cell-specific immunogens among all *Streptococcus zooepidemicus* proteins

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Streptococcus equi subsp. zooepidemicus (Szoo) is a Gram-positive, opportunistic bacterium that can cause sepsis in pigs. To control outbreaks of Szoo infection, a safe, effective vaccine is urgently needed that contains immunogens capable of eliciting robust cell-mediated immunity. This study aimed to develop a high-throughput approach to screen for Szoo immunogens among its 2087 proteins that induce strong memory IFNγ responses, a surrogate for protective cellular immunity. This task required an innovative integration of existing protein separation techniques and in-vitro immunogen screening.

Splenocytes were isolated from three naïve pigs and nineteen pigs immunized with a live-attenuated or inactivated Szoo strain and subsequently challenged with virulent Szoo. These splenocytes were stimulated *in vitro* with heat-killed Szoo in the presence of IL-18 to enhance memory IFNγ responses, quantified by ELISA. While S.zoo did not trigger non-specific IFNγ responses in naïve pig splenocytes, it triggered robust Szoo-specific IFNγ secretions from sixteen immunized pigs, which were classified as Szoo-responsive pigs.

Szoo proteome was fractionated into water-soluble and water-insoluble fractions. Splenocyte restimulation with each fraction was repeated. The water-insoluble proteins induced a 59.4-fold higher median IFNγ secretion from Szoo-responsive splenocytes compared to the water-soluble fraction. SDS-PAGE electroelution of the water-insoluble fraction yielded seven size-based fractions (10–250 kDa). Among these, the 55–70 kDa fraction elicited the highest IFNγ response, exceeding responses to other fractions by 1.7- to 30.4-fold. Complementary native

electroelution of the water-insoluble fraction enabled us to identify a weakly anionic fraction at pH 8.9, which induced 1.6- to 12-fold greater median IFNγ secretions compared to other four fractions.
Mass spectrometry analysis of the most immunogenic fractions (water-insoluble, 55–70 kDa, and weakly anionic) revealed 80 shared proteins. We prioritized evaluations of 9 extracellular proteins to determine whether our approach worked in immunogen identification.

11. PEDVS1-specific Induction of T-bet expression

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Uterine immune cells are regulated by hormonal fluctuations that occur throughout the estrus cycle. Research from our lab has demonstrated that administering intrauterine vaccinations during estrus elicits both local and systemic immune responses in rabbits and pigs. Notably, peripheral blood mononuclear cells (PBMCs) exhibit increased expression of TH1 cytokines IL-2 and IFNγ. However, the precise mechanism through which intrauterine vaccination stimulates IFNγ production remains unclear.
Here, we studied the induction of immune transcriptional factors Foxp3, GATA3 and T-bet since these transcriptional factors drive differentiation of different T cell subsets. Using a nanoparticle-based vaccine to encapsulate a coronavirus spike antigen (PEDVS1), we examined the mechanism by which intrauterine mucosal vaccination induces different transcriptional factors response systemically and compared it to intramuscular vaccination in pigs. PBMCs were obtained from pigs vaccinated via different routes and restimulated in vitro.
Upon stimulation of PBMCs in vitro, the antigen-induced expression of GATA-3 and FoxP3 were low for the CD4+, CD4+CD8+ and CD8+ T cell populations. PBMCs showed PEDVS1-specific induction of T-bet expression for the 3 T cell populations. Inclusion of IL-18 plus PEDVS1 in the stimulation suggest an augmentation in the CD8+ cellular response but it did not impact the CD4+ T cell-mediated immune responses. Collectively, these result show that intrauterine vaccination may promote a systemic TH1 response through induction of the transcriptional factor T-bet expansion.

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12. Different roads, same destination: Shared and sex-specific effects of modulation of airway inflammation by Innate defence regulator (IDR) peptides

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Nearly 15% of asthma patients do not respond to therapies like corticosteroids, which also increases the risk of lung infections. Thus, new strategies are needed for treatment-unresponsive asthma that will not impair the ability to resolve infections. We have shown that an Innate Defence Regulator (IDR) peptide, IDR-1022, suppresses airway inflammation in a house dust mite (HDM)-challenged, Th2-high mouse model. As sex-related differences in asthma and HDM-challenged murine models are now established, here we examined sex-related differences in IDR-1002's function to modulate airway inflammation.
Female and male BALB/c mice were intranasally challenged with 25 μg HDM, 5 days a week for 2 weeks, with and without subcutaneous administration of IDR-1002 (6 mg/kg). We examined the abundance of IL-33 in lung tissue (by ELISA), a panel of 44 cytokines in bronchoalveolar lavage fluid (BALF) and lung tissue (by Luminex platform), leukocyte profile in BALF (by flow cytometry), and serum IgE levels (by ELISA). We identified differential expression of several key cytokine in females compared to males in response to HDM. Cytokines that were significantly increased in both sexes (lung tissue and BALF) were IL-4 and IL-5 (>4 Log2 fold change). We also showed specific sex-bias in the function of IDR-1002. For examples, decrease in the abundance of IL-33, TNFα and IL-1β by the

administration of IDR-1002 was higher in females compared to males. Whereas IDR-1002 significantly reduced Th2-responses e.g. IL-4 and IL-5 (between 40-50% in lung tissues and BALF), CD4+T cells and eosinophils (by 80% in BALF), and HDM-specific serum IgE, in both sexes. These results demonstrate that while modulation of certain cytokines is sex-specific, administration of IDR-1002 can regulate downstream allergic asthma-related Th2-high responses in both sexes.

13. Design and delivery of nanoparticle-based STING agonist for adjuvanting mucosal subunit vaccines

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Developing effective mucosal subunit vaccines is a challenge due to the inherent poor immunogenicity of subunit vaccine antigens and the complex barriers of mucosal tissues. The nose, mouth, and lungs are key entry points and targets for respiratory pathogens; however, no mucosal subunit vaccines exist to induce protection at these sites, primarily due to the non-availability of adjuvants that can induce respiratory mucosal immunity. The Stimulator of Interferon Genes (STING) pathway agonists are immunostimulatory molecules capable of eliciting strong type 1 interferon responses and both innate and adaptive immunity. Nevertheless, their clinical use is limited by poor stability and rapid host clearance. Therefore, we aimed to design lipid-based nanoparticles to safely deliver STING agonists and subunit antigens to the lungs, enhancing vaccine immunogenicity while minimizing off-target effects. We encapsulated STING agonist 2',3'-cyclic GMP-AMP (2’,3’-cGAMP) in cationic lipid didodecyldimethylammonium bromide and immunomodulator trehalose 6,6’-dibehenate liposomes using microfluidic mixing. Flow Rate Ratio (FRR) and Total Flow Rate (TFR) parameters were manipulated to produce 2’,3’-cGAMP-based DDAB/TDB liposomes with desired lung delivery properties, including particle size <200 nm and surface charge <-20 or >+20 mV. 2’,3’-cGAMP charge and dose in the liposomes was optimized for inducing Interferon Regulatory Factor 3 (IRF3) and Nuclear Factor-κβ (NF-κβ) responses in a STING reporter cell line (THP1-Dual KI-hSTING-R232 Reporter Monocytes). FRR 5:1 at TFR 15 mL/minute produced liposomes with desired sizes (67.44 ± 3.17 nm) and surface charges (25.46 ± 2.197 mV). *In vitro* screening identified 2’,3’-cGAMP concentrations ~50 µg/mL induced a 11.69-fold increase in IRF3 and ~30 µg/mL induced a 5.65-fold increase in NF-κβ, compared to non-2’,3’-cGAMP-containing liposomes. Neutral-charged 2’,3’-cGAMP performed best compared to negative-charged 2’,3’-cGAMP. Our results demonstrate 2’,3’-cGAMP-based DDAB/TDB liposomes mediated activation of IRF3 and NF-κβ responses. Future *in vivo* studies will test the safety, immunogenicity, and efficacy of these formulations following respiratory mucosal immunization.

14. Establishing how changes in glycosylation pattern in maternal immunoglobulin G and/or changes in antigen-presenting cell function impacts maternal immunity.

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OBJECTIVE: A recent publication in mice indicated that the deacetylation of sialic acid on maternal serum and colostrum IgGs can alter the cell-mediated immune function of B cells in neonatal pups. It was elucidated that the overexpression of sialic acid acetyl esterase (SIAE), that removes acetyl groups from sialic acids on Igs, was induced in later, but not early, pregnancy. Deacetylated IgG may then bind to the B cells in the pups and possibly impact the function of these cells. It is currently unknown whether species undergo similar changes. Therefore, Pig will be used as model to investigate this.
METHODS: To establish that the SIAE is present in serum, Western blot analysis performed on serum and PBMCs obtained at day 0, day 30 and day 100 gestation. SIAE enzyme changes in expression over pregnancy, was checked

(PBMCs) by RTqPCR. The changes in glycosylation patterning in pregnancy, was analyse by HPLC and Lectins Array Assay. Different functional assays to check impact of fucose change are currently being performed.
RESULTS: A western blot data showed that SIAE is expressed in peripheral blood mononuclear cells (PBMCs) but not in serum, there was no seen change along with pregnancy. RTqPCR data showed no change in expression level of SIAE in PBMCs across pregnancy. HPLC analysis data showed that Pig has Neu5A and Neu5B sialic acid derivatives on IgG. Lectin Assay showed increase of 50 % in fucose at 30 and 100 gestation point compared to Day 0. RTqPCR data for FUT8 showed no expression change in PBMCs.
CONCLUSIONS: Pig IgG sialic acid (Neu5Gc or Neu5Ac) is present as a deacetylated form in non-pregnant and pregnant animals, so SIAE expression changes are not of interest in the pig. Enriched IgG from pregnant gilts showed increased fucosylation suggesting that it impact IgG effector function.

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15. Molecular Engineering of *Borrelia burgdorferi* for Tailored OMV Composition in Lyme Disease Vaccine Design

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Lyme disease is the most common vector borne disease caused by spirochaete *Borrelia burgdorferi* and transmitted to vertebrate host by *Ixodes scapularis* ticks. As the incidence rate of disease rises, it creates serious threats to the public. Different approaches have been explored using molecular biology and advanced immunology to develop long lasting, safe, and efficacious Lyme disease vaccines. However, no approved vaccine currently exists for human use against this spirochete, highlighting the urgent need for an effective vaccine. Host immune responses to *Borrelia* infection are highly strain-specific, making it difficult to develop broadly protective vaccines that target specific proteins. The purpose of this study is to engineer *B. burgdorferi* to produce desired outer membrane proteins for use in an outer membrane vesicle (OMV)-based vaccine candidate. To achieve this, conserved protein sequences shared among Lyme disease *Borrelia* will be identified, and genes encoding proteins involved in autoimmune pathologies will be strategically deleted. This will be accomplished through plasmid displacement, or by replacing target genes with antibiotic resistance cassettes, generating an optimized strain suitable for OMV vaccine development. The resulting OMVs will be tested in mice to evaluate safety, efficacy and immunogenicity of the vaccine. OMVs derived from genetically engineered *B. burgdorferi* are naturally non replicating, spherical nanoparticles with intrinsic immunostimulatory properties. These vesicles can easily express heterologous outer surface protein (OSP) antigen in their native conformation targeting specific immune response. Decorating OMVs with heterologous OSP antigen targeting vertebrate specific proteins draws promising vaccine platform for the prevention of Lyme Borreliosis.

Key words: *Borrelia burgdorferi*, Lyme disease, OMVs, Vaccine

16. Generation and Characterization of Chimeric Lassa Virus Glycoproteins for Broadly Protective Vaccine Development

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Lassa virus (LASV), a highly diverse arenavirus with seven recognized lineages, presents significant challenges for effective vaccine development due to its extensive antigenic variation. To address this issue, we generated a chimeric antigen library derived from native glycoprotein precursor (GPC) sequences, specifically targeting Nigerian lineages 1, 2, 3, and 6. The chimeric GPC antigens have been successfully created, though further optimization for library scalability is currently underway. Concurrently, we developed a panel of 15 LASV-specific antibodies using a mammalian expression system, which are now ready for detailed characterization. These antibodies will facilitate flow cytometry-based sorting (FACS) to select chimeric antigens exhibiting robust antibody binding and stable expression in mammalian cells. Selected antigens will undergo further analysis through bio-layer interferometry (Octet) and ELISA to evaluate binding affinity, specificity, and lineage coverage. Selected antigens will subsequently be characterized and tested in animal models specific for LASV, facilitating the development of an mRNA-based

vaccine candidate. Additionally, selected antigens will be evaluated using a Vesicular Stomatitis Virus (VSV)-based vaccine platform to further assess their immunogenicity and potential for vaccine delivery. This systematic approach aims to identify broadly reactive immunogens that could serve as the foundation for a universally protective LASV vaccine, significantly advancing efforts against this critical public health threat.

17. Regulation of IL-17-mediated airway inflammation by Innate Defence Regulator (IDR) peptides

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Asthma affects more than 4 million Canadians and costs over $2 billion annually. Nearly 15% of asthmatics develop steroid-resistant (SR) disease, representing 50% of asthma-related healthcare costs. SR asthma is characterized by a Th17/IL-17-high airway inflammation and these individuals do not respond to the mainstay-treatment of corticosteroids (inhaled or systemic). Corticosteroids also increase the risk of lung infections which in turn can exacerbate asthma. Therefore, new strategies that can reduce airway inflammation without compromising immune response to resolve infections are urgently needed. Innate Defence Regulator (IDR) peptides can regulate inflammation and resolve infection, thus making these attractive therapeutic candidates. The goal of this study is to examine the potential of IDR peptides to modulate IL-17-high airway inflammation and elucidate related molecular mechanisms. We propose to use selected IDR peptide with and without inhaled corticosteroid (ICS) fluticasone propionate (FP) to define overlapping, synergistic, and unique effects of the peptide compared to ICS. We have optimized an allergen-recall murine model that elicits a Th2-low and Th17-high lung inflammation. We demonstrated that this model is resistant to a clinically relevant dose of ICS. However, introduction of an IDR peptide, IDR-1002, results in the suppression of airway inflammation, specifically neutrophilia (determined by accumulation of neutrophils and abundance of neutrophil elastase) as well as levels of IL-17-family of cytokines, in the lungs of both female and male mice, where ICS alone fails. We also demonstrated that the combination of IDR-1002 and low dose FP suppresses neutrophil chemoattractants under inflammatory conditions relevant to SR asthma, in human bronchial epithelial cells. Based on this, in future direction we aim to delineate molecular processes related to modulation of airway inflammation in SR asthma, which will facilitate the development of IDR peptide-based immunomodulatory therapy.

18. HIV-1 disease progression and differential effects of IFN-α2 and IFN-α14 on HIV-1 specific T cell responses

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Interferon-alpha (IFN-α) is known to induce strong antiviral responses. Humans express 12 IFN-α subtypes that signal through the same receptor, yet they induce differential effects on immunity. Studies have shown that not all IFN-ɑ subtypes induce an adequate antiviral response to every viral infection. During HIV-1 infection, IFN-ɑ has been implicated as a factor in immune dysregulation. However, we have shown that subtype IFN-α14 controls HIV-1 more effectively and reduces T cell dysfunction than clinically approved IFN-α2. We mapped HIV-1 disease progression and HIV-1 related dysfunction in three donor cohorts of humanized mice. HIV-1 infection progressed differently between individual cohorts with differential onset of increased frequencies of T cells expressing dysfunction markers and lost responsiveness to T cell proliferative stimuli *ex vivo*. Peak HIV-1 specific T cell responses were observed at 8 weeks post infection (wpi), 11wpi and 5wpi for donor cohorts 1, 2 and 3 respectively. By 14wpi, HIV-1 specific responses had contracted in all three donor cohorts. Whether reduced HIV-1 specific responses were due to T cell dysfunction or viral escape remains to be elucidated. After 3 weeks of IFN-ɑ treatment, HIV-1 specific T cell responses were suppressed. However, IFN-ɑ14 treatment resulted in a greater breadth of response than IFN-ɑ2 despite a significantly suppressed viral load. In contrast, three weeks post cessation of treatment, a greater breadth of response was observed in IFN-ɑ2 treated mice than IFN-ɑ14 treated or untreated animals, suggesting a hyperactive response or greater viral evolution during IFN-ɑ2 treatment. Viral RNA sequencing will be used to elucidate the role of IFN-ɑ in viral evolution. Overall, T-cell responses and the development of HIV-1 related dysfunction evolves in a distinct manner between donor cohort of humanized mice. Additionally, IFN-α2 and 14 treatment had differential impacts on HIV-1 specific T cell responses.

20. Dual Defense - Developing a Subunit Vaccine against PEDV variants

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Porcine epidemic diarrhea virus (PEDV) is a member of the family *Coronaviridae* and genus *Alphacoronaviru*s in the order *Nidovirales*. PEDV causes diarrhea, vomiting, and dehydration in swine and is fatal in neonatal piglets with a mortality rate of up to 100%. It is highly contagious, thus resulting in mass epidemics that can have a significant impact on the swine industry and the overall economy. The emerging strains of PEDV are majorly divided into two genogroups based on the spike (S) protein, and their virulence in young pigs are, G1b, “S INDEL”, and G2b “non-S INDEL. Immunization of pregnant sows is an effective approach to protect neonatal suckling piglets against PEDV. The current vaccines available target only one genogroup at a time. Our ongoing research focuses on developing a subunit vaccine that can provide protection against both genogroups of PEDV at the same time in sows, which in turn will provide immune protection in newborn piglets against PEDV infection. The subunit vaccine will also allow us to differentiate the vaccinated from infected animals (DIVA vaccine). Our study includes recombinant protein production, expression, purification and, evaluating the immunogenicity of PEDV subunit vaccine formulated with varying adjuvants in mice. In future, the candidate with stronger immune responses will be then used to vaccinate pregnant sows to test the protective efficacy against virus challenge in newborn piglets. Our goal is to develop a bivalent subunit vaccine that is amenable to manufacture production and ready to be deployed to pork producers.

21. Hantavirus Glycoprotein Complex: A Computational Pipeline for Identifying Vaccine Targets

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Objectives: The study aimed to identify B and T cell epitopes from the spike glycoprotein of ANDV and design a structurally validated, multi-epitope vaccine candidate using a systematic computational approach.
Methods: A stepwise immunoinformatic pipeline where the complete glycoprotein 3D conformation was simulated and evaluated. The potential B-cell and T-cell epitopes were evaluated using BediPred and Discotope tools. Over 1,000 overlapping nine-mer peptides from the predicted conformation were generated to cover possible T-cell epitopes comprehensively. The conformations of nine-mer peptides were individually modelled and compared to ensure structural integrity. Globally prevalent HLA class I and II allele sets were generated. To simulate the interaction of each peptide with each HLA allele, a large-scale cross-docking analysis was performed, and broad population coverage and immunodominant candidates were predicted. The top peptides, selected based on docking scores and binding energies, were incorporated into a multi-epitope construct integrating both B and T cell targets. Molecular dynamics simulations were carried out to evaluate the construct's structural stability, flexibility, and physicochemical properties.
Results and conclusion: Several conserved and highly immunogenic epitopes were identified throughout the ANDV glycoprotein complex. Out of over 1000 modelled peptides, many demonstrated strong binding affinities with multiple HLA alleles, supported by stable and complementary docking poses. This study demonstrates a high-throughput, structure-based computational pipeline for rational epitope vaccine design. The approach effectively identified multiple promising B and T cell epitopes from the Andes virus glycoprotein, assembled them into a stable vaccine construct and contributed to the broader effort to develop targeted vaccines for hantavirus infections.

22. Extended Vaccine Dose Intervals Impact B Cell Maturation and Antibody Neutralization

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Introduction: Early in the COVID-19 vaccine rollout, Canada implemented a dose-sparing strategy by delaying second doses to increase first-dose coverage. A longer interval between doses was associated with improved antibody titers and variant breadth, possibly reflecting enhanced B cell maturation during the extended interval. This study investigates the impact of an extended dose interval on B cell maturation following primary vaccination and after a booster dose in mRNA vaccine recipients from Manitoba.
Methods: Blood samples were collected at multiple timepoints from participants who received either a short or extended dose interval. Peripheral blood mononuclear cells (PBMCs) were isolated and stained with fluorescent probes to detect and phenotype antigen-specific B cells using flow cytometry. PBMCs from various post-vaccination timepoints were stimulated in vitro to differentiate memory B cells into antibody-secreting plasma cells. Collected antibodies were assessed using a multiplex competitive binding assay.
Results: An extended interval led to a greater increase in SARS-CoV-2-specific B cells and antigen-specific memory B cells 1-2 weeks after the second dose compared to a standard dose interval. SARS-CoV-2-specific B cells in the standard dose interval participants increased to match the levels in extended interval participants 6 months after the second dose. Antibodies from plasma cells in extended interval participants had a wider breadth of inhibition for SARS-CoV-2 RBD 10-14 days following the second dose compared to standard dose interval participants. The third dose did not result in differences in the proportions of SARS-CoV-2-specific B cells or inhibition between standard or extended interval participants.
Conclusion: Dosing intervals affect B cell phenotypes and antibody responses. Extended intervals transiently increase SARS-CoV-2-specific B cell frequencies and antibody breadth, suggesting enhanced maturation, but shorter intervals eventually yield comparable responses. The study will determine whether the establishment of long-term memory B cells is also affected, with implications for future vaccine strategies.

23. SHORT-CHAIN FATTY ACID REGULATION OF TYPE III INTERFERON RESPONSIVENESS OF HUMAN INTESTINAL EPITHELIAL CELLS AND MACROPHAGES

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Introduction: Type III interferons (IFN-λs) are highly produced in the gut, where they can promote mucosal healing. In humans, they signal in epithelial cells and specific immune cells, such as macrophages with selective IFN-λR1 receptor expression. Prior studies have shown that commensal microbes can induce IFN-λ production, but there is less known for how microbe-derived metabolites regulate IFN-λ responsiveness. Here, we aimed to uncover if short-chain fatty acids (SCFAs), one type of microbe-derived metabolite that is essential for promoting gut health, alter IFN-λ activity. We *hypothesized* that SCFAs will upregulate IFN-λ activity in human intestinal epithelial cells (IECs) and macrophages.
Methods: IECs (Caco-2 cell line) and human monocyte-derived macrophages were pre-treated for 1 hour with microbial-derived metabolites, including SCFAs (acetate, butyrate, propionate), bile acids (cholic acid, lithocholic acid, chenodeoxycholic acid), or a tryptophan metabolite (kynurenine). IECs and macrophages were then treated with or without IFN-λ3 (50ng/ml) for 24 hours (n=3). IFN-stimulated genes (ISGs; *MX1* or *IFITI*) and *IFNLR1* expression were quantified by RT-qPCR. Surface IFN-λR1 levels were also quantified by flow cytometry. Cell viability was measured by PrestoBlue assay.
Results: All SCFAs tested had a concentration-dependent inhibitory effect on IFN-λ3-mediated induction of ISGs ($p<0.05$) in both cell types, without a decrease in cell viability. Other metabolites tested did not affect ISG induction by IFN-λ3 at any concentration tested. No metabolites significantly altered *IFNLR1* mRNA expression. However, acetate upregulated IFN-λR1 levels modestly, butyrate significantly downregulated IFN-λR1 levels, while propionate did not affect IFN-λR1 surface levels on Caco-2 cells.
Conclusions: Our findings demonstrate that SCFAs uniquely downregulate IFN-λ responses and surface IFN-λR1 levels, which was contrary to our hypothesis. This study adds to our fundamental knowledge of how IFN-λ biology could be regulated in the human gut, and further work is needed to uncover the mechanism of action.

25. Unraveling Novel Factors that regulate Semaphorin 3E-dependent Natural Killer Cell Proliferation and Cytotoxicity

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Introduction: Natural killer (NK) cells are vital innate cells in tumor and viral immunity. Semaphorins (Sema), originally discovered as axon guidance molecules, are now known to regulate immune functions. We previously reported that Sema3E from immature DCs modulates IL-2 activated NK-cell migration, and that Sema3E and its receptor PlexinD1 are tightly regulated in NK cells. Our recent work showed that Sema3E deficiency led to a modest but significant decrease in splenic NK cells (manuscript in preparation). We hypothesized further that the Sema3E/PlexinD1 signaling axis is a novel regulator of NK cell-effector functions.
Methods: Spleens were harvested from BALB/c or Sema-3E -/- mice, and NK cells (purity >90%) were isolated using a commercial kit. IL-2 (1000 U/ml) was used to activate NK cells in vitro. After three days of IL-2 activation, NK cell function was evaluated with a CD107a degranulation assay, intracellular IFNγ staining. Proliferation was assessed by EdU proliferation assay. Some IL-2 activated NK cells were preserved and sent for bulk RNA sequencing and kinome screening.
Results: The IL-2 activated Sema3E-/- NK have an impairment in cytotoxicity when co-cultured with either PMA+Iono cocktail or target cells, while the IFNg level remains comparable between Sema3E-/- and the WT control. Sema3E-/- NK also shows a proliferation defect which could be rescued by co-culturing with wildtype iDC. The screening analyses revealed multiple genes and signaling pathways related with cell cycle and cytoskeleton reorganization that were differentially de-regulated Sema3E-/- NK cells.
Conclusion: Proliferation and cytotoxicity, but not cytokine production, of the NK cells was impaired by the Sema3E deficiency when exposed to stimulants. My results further established the importance of Sema3E in the regulation of NK cell biology. A better understanding of this signaling axis will reveal novel therapeutic target(s) that allow one to manipulate NK cell cytotoxic function specifically.
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26. Factors associated with low childhood immunization coverage among Rohingya refugee parents in Cox's Bazar, Bangladesh

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Introduction: Immunization campaigns and Expanded Program on Immunization (EPI) were launched by Government of Bangladesh (GoB) in collaboration with WHO and other Non-governmental Organizations (NGOs) to tackle the increased risk of vaccine preventable disease outbreak in the Rohingya refugee camps. Immunization coverage was found to be lower than expected. However, a few studies explored the factors behind low vaccine uptake among Refugee children.
Methods: A cross-sectional study was carried out among Rohingya parents living Cox's Bazar, Bangladesh. A total of 224 Rohingya parents were conveniently selected. Data was collected using a pretested interviewer-administered semi-structured questionnaire with the help of bilingual volunteers who understand Rohingya dialect. All statistical analyses were carried out in IBM SPSS Version 26 (New York, USA).
Results: Total 63.1% of Rohingya parents had good practice regarding childhood immunization. Of all, 74.6% had good knowledge and 94.7% had positive attitude towards EPI vaccination. Good practice regarding vaccination was significantly more common among parents living in registered camps (77%) than those living in makeshift

settlements (49.2%, p<0.001). Multivariable logistic regression analysis revealed that living in registered camps (Adjusted Odds Ratio [aOR]: 2.99; 95% Confidence Interval [CI]: 1.41–6.32) and good knowledge level (aOR: 2.88; 95%CI: 1.32–15.82) were independent determinants of good practice. A separate analysis in both type of camps revealed that in registered camps, good knowledge level (aOR: 3.62; 95%CI: 1.45–9.04) and having >2 children (aOR: 3.71; 95%CI: 1.34–10.27), and in makeshift settlements, father's employment (aOR: 2.33; 95%CI: 1.34–6.72), father's education (aOR: 3.00; 95%CI: 1.34–6.72) and presence of any electronic device (aOR: 4.01; 95%CI: 0.96–16.84) were significant determinants of good childhood immunization practice.
Conclusion: Health education and promotion strategies should be implemented to increase knowledge and awareness about EPI immunization benefits among Rohingya parents to ensure greater coverage.

27. Effects of Innate Defence Regulator (IDR) peptides on Histone Deacetylase 2 (HDAC2) in steroid-unresponsive airway inflammation.

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Inhaled corticosteroids (ICS) and β2-agonists are the mainstay treatment for asthma, yet ~15% of asthmatics suffer from uncontrolled steroid-resistant (SR) asthma. Neutrophilic airway inflammation, increased levels of IL-17 cytokines, and reduced Histone Deacetylase 2 (HDAC2) expression and activity are associated with impaired response to ICS and SR asthma. Cationic Host Defence Peptides (HDP) and their synthetic derivatives, Innate Defence Regulator (IDR) peptides, have immunomodulatory properties to control inflammation and facilitate immune homeostasis in the lungs. Thus, the aim of this study was to examine the effect of an IDR peptide, IDR-1002, on HDAC2 under inflammatory conditions related to SR asthma, in an animal model and using human bronchial epithelial cells (HBEC).
HBECs were stimulated with a combination of IL-17A/F and TNFα, as we have shown that this combination uniquely facilitates neutrophilia. We demonstrated that the abundance of HDAC2 decreases in response to concurrent stimulation with IL-17A/F and TNFα by 24 hours. We also optimized an IL-17-driven mouse model that mimics severe, Th17-mediated, steroid-resistant airway inflammation. Lung tissue lysates were analysed for HDAC2 levels with and without treatment with IDR-1002, in the presence and absence of a clinically relevant dose of ICS, fluticasone propionate. We showed that HDAC2 levels were significantly decreased in the lungs, and introduction of IDR-1002 restored HDAC2 abundance, where ICS alone failed. Taken together, these findings emphasize the role of HDAC2 as a regulator of neutrophilic inflammation in SR asthma, and the ability of IDR peptides to restore impaired HDAC2 levels within inflammatory phenotypes of SR disease. This study expands our understanding of the molecular drivers of neutrophilic airway inflammation, opening a new line of investigation of potential histone modification and epigenetic regulation by IDR peptides to alleviate ICS-resistant severe asthma, a disease for which there are no available treatments.

28. Spatial modulation of immune cell distribution by Transcutaneous auricular vagus nerve stimulation (taVNS) in a preclinical model of ulcerative colitis

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Introduction: Ulcerative colitis (UC) features reduced vagal tone and heightened pro-inflammatory immune cell infiltration. Given the vagus nerve's immunomodulatory role, vagus nerve stimulation (VNS) is a promising therapeutic strategy. We previously showed that non-invasive transcutaneous auricular VNS (taVNS) protects against UC-like colitis in mice; however, the underlying immune targets remain unclear. Objective: To assess the impact of taVNS on granulocytes, macrophages, NK cells, and $CD4^+$/$CD8^+$ T cells in gut-associated and peripheral immune compartments during experimental colitis. Methods: Male C57BL/6 mice received daily 10-minute taVNS starting one day before colitis induction with 5% dextran sulfate sodium (DSS) in drinking water for five days; controls received water only. Stimulation continued throughout. A sham group received no stimulation. Disease activity index (DAI) and distal colon damage were evaluated. Flow cytometry quantified $Ly6G^+$ granulocytes, $F4/80^+$ macrophages, NK1.1$^+$ NK cells, and CD3$^+$CD4$^+$/CD8$^+$ T cells in the cecum, spleen, mesenteric lymph nodes (MLNs), and proximal, mid, and distal colon. Results: TaVNS significantly improved DAI and macroscopic outcomes in DSS-treated mice, with no impact in controls. In colitic mice, taVNS selectively decreased Ly6G$^+$ cells in the proximal colon and spleen, and F4/80$^+$ cells in the mid-colon. NK1.1$^+$ cell frequency was increased in the spleen following taVNS, with no changes observed in other tissues or in non-colitic mice. CD4$^+$ and CD8$^+$ T cell distributions remained unaffected across all groups. Conclusion: taVNS modulates innate immune subsets in colitic mice by reducing intestinal and systemic granulocytes and macrophages, while increasing splenic NK cells, supporting its anti-inflammatory potential in UC.

29. 3D Tumoroid Model for Studying Macrophage Polarization and Therapeutic Strategies

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Breast cancer affects millions of women worldwide, but treatment often fails due to the complexity of the tumour microenvironment (TME). Immune cells within the TME, particularly monocytes, play a crucial role in cancer progression and drug resistance. After recruitment, monocytes can differentiate into M2-like tumour-associated macrophages (TAMs), which support tumour growth and contribute to therapeutic failure. Traditional 2D culture systems fail to replicate the complexity of the TME, prompting the development of more physiologically relevant models. Our lab has established a novel 3D collagen-based microtissue model that better mimics the natural extracellular matrix and tumour structure. This model enables investigation of monocyte infiltration and polarization into M2-like macrophages. Recent data show that microtissues release higher levels of key cytokines involved in monocyte recruitment and TAM development, such as MCP-1, IL-10, IL-13, and VEGF, compared to 2D cultures. These findings highlight the model's potential to study immune–tumour interactions more accurately. Our research aims to understand monocyte-to-macrophage polarization and to explore macrophage repolarization strategies using dsRNA-loaded nanoparticles. By activating macrophages within the microtissue model, we aim to counteract immune suppression and enhance anti-tumour immunity. This approach may offer a novel immunotherapeutic strategy to overcome drug resistance in breast cancer.

30. Uncovering how STAT5 drives chemotherapy resistance in T-cell Acute Lymphoblastic Leukemia

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Background: Acute Lymphoblastic Leukemia (ALL) is the most common cancer in children, with T-cell ALL (T-ALL) representing 15-20% of pediatric ALL cases. Despite initial response to chemotherapy, T-ALL relapse occurs in up to 20% of pediatric and 60% of adult cases. Mutations leading to aberrant activation of Signal Transducer and Activator of Transcription 5 (STAT5) are common in T-ALL and associated with increased risk of relapse. Previous pre-clinical studies have shown that STAT5 promotes resistance to treatment, making STAT5 a promising therapeutic target. However, current STAT5 inhibitors cannot be used in the clinic due to toxicity. Therefore, my project aims to identify transcriptional partners and targets of STAT5 to inform novel therapeutic strategies.
Methods: We will utilize patient-derived xenograft (PDX) models to define STAT5-mediated transcriptional programs across different subgroups of T-ALL. We will perform in vitro stimulation assays with Interleukin-7 (IL-7) to activate

STAT5 in human T-ALL cells, as measured by phospho-flow cytometry. Inhibitors of different signaling pathways will be used as controls: Ruxolitinib (JAK), Dactolisib (AKT/mTOR) and Pimozide (direct STAT5 inhibition). Bulk analyses of gene expression (RNA-seq) and chromatin remodeling (ATAC-seq) will reveal STAT5-mediated programs. Finally, Immunoprecipitation (IP) assays will be used to identify STAT5 partners.

Results: Stimulation with IL-7 induced STAT5 phosphorylation in all T-ALL samples analyzed, irrespective of the presence of mutations known to activate STAT5. Inhibition of JAK by Ruxolitinib completely abrogated STAT5 phosphorylation by IL-7. Targeting of the AKT/mTOR pathway using Dactolisib decreased STAT5 activation, similar to STAT5 inhibitor Pimozide.

Expected Outcomes and Conclusions: Our research will deliver new knowledge on how STAT5 regulates proliferation and survival of T-ALL cells from genetically distinct subgroups. Identifying STAT5 transcriptional partners could inform the development of novel STAT5-targeting therapies for improving clinical response to initial treatment.

31. B-cell receptor signaling promotes IFN-λ responsiveness by healthy and chronic lymphocytic leukemia lymphocytes

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Introduction: The role of type-III interferons (IFN-λs) in regulating mucosal immune responses with less inflammation compared to other IFNs is partially due to the limited expression of IFN-λ receptor 1 (IFN-λR1) on epithelial cells and specific immune cells. We previously found that B-cell receptor (BCR)-stimulation upregulated IFN-λ3-binding to human B lymphocytes but lacked the tools to quantify IFN-λR1 levels until recently. Dysfunctional chronic lymphocytic leukemia (CLL) lymphocytes have overactive BCR-signaling. While the other IFN-λ receptor subunit IL-10RB is expressed ubiquitously, little is known about pathways regulating IFN-λR1 expression. We hypothesized that BCR activation in healthy or CLL conditions would promote IFN-λR1 surface levels leading to heightened IFN-λ responsiveness.

Methodology: We analyzed whole peripheral blood mononuclear cells (PBMCs) or purified B cells of healthy donors (n=7) and PBMCs of CLL patients (n=7) before and after BTK-inhibitor (BTKi) treatment. Cell surface IFN-λR1 and IL-10RB levels were measured through flow cytometry. The mRNA levels of *IFNLR1* and ISGs induced by IFN-λ3 after *ex vivo* culture were quantified by qRT-PCR.

Results: IFN-λR1 mRNA and protein levels were significantly upregulated after anti-IgM (BCR agonist) stimulation in normal B cells in a dose-dependent manner and as early as 19 hours post-stimulation. Abnormally activated CLL B cells had significantly higher levels of surface IFN-λR1 at baseline compared with controls. Inhibition of BCR activation through BTKi treatment significantly reduced IFN-λR1 levels on normal and CLL B cells. The induction of ISGs (*MX1*, *IFIT1)* by IFN-λ3 was 2~5-fold higher in activated normal or CLL B cells than in resting B cells or CLL-post BTKi cells.

Conclusions: BCR activation is a key regulator in IFN-λR1 expression and IFN-λ responsiveness. IFN-λR1 is significantly upregulated in CLL, indicating its potential as therapeutic target. The influence of IFN-λs on B cells beyond antiviral immunity including CLL pathogenesis or therapy requires further investigation.

32. Altered B cell populations and antibody production within the inflamed colon and gut-associated lymphoid tissues

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Background: Gut-associated lymphoid tissues (GALTs) are central in generating IgA-producing B cells essential for gut homeostasis and controlling commensal bacteria and pathogens. However, little is known about altering B cell characteristics and antibody production within inflamed colon and GALTs.

Methods: B cell-related transcriptional programs were assessed in pan-colitic patients. In preclinical model experiments, male CD-1 mice were treated for 6 days with 2.5% dextran sulfate sodium, followed by 8 days of recovery. B cell subsets in colon and GALTs were analysed using flow cytometry and immunofluorescence microscopy. QRT-PCR evaluated expression of cytokines and immunoglobulin class switch genes. ELISA measured levels of antibody subclasses in colon tissue and serum.
Results: In pan-colitic patients, there was an enrichment for B cell-related genes and upregulation in IgG antibody subclasses. In colitic mice, TNF-α, Baff, and CXCL-13 mRNA expressions were significantly upregulated. Immunostaining demonstrated aggregation of CD19+B220+ B cells with the colon lamina propria that were largely IgD+ but negative for IgA and IgG expression. Flow cytometry analyses revealed significant increases in B cells within colon and mesenteric lymph nodes (MLNs), as well as increased expression of CD86 in colon, Peyers' patches, and MLN. In the colon of colitic mice, free fecal IgM antibody levels were increased, while total and autoantibody levels of free fecal IgA and IgG remained unchanged.
Conclusions: Acute intestinal inflammation leads to expression of factors driving B cell recruitment and activation, resulting in accumulation of aggregated B cells in the lamina propria, B cell activation in GALTs, and altered profile of systemic and colon antibodies.

33. Development and validation of a novel saliva-based rapid point-of-care device for early detection of periodontal disease

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Introduction: Periodontal Disease (PD) is a chronic infection of gum tissues that anchor teeth. PD is caused by specific anaerobic bacteria that produce unique metabolic by-products (peroxides, organic acids, lactoferrins, and thiols) as compared to healthy saliva.
Our hypothesis: colorimetric dyes with sensitivity for biological markers of PD bacteria, could be used to produce a prototype saliva-based device for earlier detection of PD compared to current physical examination methods.
Methods: Test cartridge was designed using Fusion360 and PrusaSlicer 2.7.1 software and 3D-printed with biodegradable plastic using Elegoo Neptune4 printer. Colourimetric dye strips were prepared then assembled into the 3D-printed cartridge. Assembled devices were vacuum sealed in bags for storage. Prototypes were tested by spiking relevant concentrations of metabolic by-products in artificial saliva pipetted onto the test device. Colour changes of the strips were imaged after 5 minutes for densitometric analysis and RedGreenBlue (RGB) intensity quantified with ImageJ software. Proof-of-concept validation for the study is currently underway using human salivary samples from the UofM and participating Winnipeg dental clinics in a blinded study (REB ethics#HS26411). PD staging of participants as reported by the clinic, followed by 16S rDNA sequencing will be used to validate the presence and abundance of pathogenic PD bacteria.
Results: Preliminary saliva samples ($n<50$) have demonstrated a 95% device detection rate of those with clinical PD versus healthy. More samples are required for in-depth statistical analyses and to correlate our device outcomes, PD staging, participant survey metadata, and the presence and abundance of PD-causing bacteria from ongoing sequencing data.
Conclusions: Our study results will provide valuable insights into how diet, lifestyle and behavior impact oral health and PD with quantitative techniques and clinical data. Our rapid test device demonstrates potential as a novel and cost-effective method for earlier detection and intervention of PD, improving overall health of Canadians.

34. Role of Pentraxin 3 (PTX3) in a chronic neutrophilic mouse model of severe asthma

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Introduction: Neutrophilic asthma is resistant to current therapies like inhaled corticosteroids, leading to substantial economic and social burdens. Pentraxin 3 (PTX3), a pattern recognition receptor, has been implicated in exacerbating airway inflammation and hyperresponsiveness (AHR) in ovalbumin models of allergic inflammation. This study aimed to investigate the role of PTX3 in modulating inflammation and its effects on AHR and airway remodeling in a chronic neutrophilic mouse model of severe asthma.

Methodology: PTX3$^{-/-}$ and wild-type (WT) mice were subjected to HDM+c-di-GMP to induce chronic severe asthma. Recombinant PTX3 (rPTX3) was administered to WT mice intranasally. Lung tissue and BALF were analyzed using flow cytometry, while cytokines and serum immunoglobulins were assessed via mesoscale and ELISA. AHR parameters were measured with a FlexiVent ventilator. Collagen deposition and mucus production were visualized with Sirius-red and Periodic acid-Schiff staining. Neutrophil extracellular traps (NETs) were studied with myeloperoxidase immunofluorescence.

Results: PTX3$^{-/-}$ mice exhibited significantly higher numbers and percentages of inflammatory cells, such as neutrophils and macrophages, in both BALF and lung tissue, indicating an exacerbated inflammatory response. In a chronic neutrophilic severe asthma model, these mice demonstrated increased AHR, characterized by elevated lung resistance, tissue resistance, and elastance, reflecting heightened airway sensitivity to stimuli. In contrast, female mice treated with rPTX3 showed reduced tissue elastance and resistance, suggesting a protective effect against airway constriction and inflammation. Additionally, PTX3$^{-/-}$ mice exposed to HDM+c-di-GMP had increased levels of total and HDM-specific IgA and IgE. These mice also exhibited increased mucus and collagen production, contributing to airway remodeling. Notably, PTX3-deficient mice displayed distinct morphological features of NETs compared to WT mice.

Conclusion: PTX3 deficiency increases neutrophil-dominant inflammation and AHR in a chronic severe asthma model. Also, rPTX3-treated mice exhibited reduced tissue elastance and resistance, suggesting a promising initial improvement in specific aspects of AHR.

36. Development of Peripheral-Tissue Biomarkers to Predict Disease Trajectory of Patients with Human Transmissible Spongiform Encephalopathies (TSEs)

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Transmissible Spongiform Encephalopathies (TSEs) are a group of fatal, rapidly progressive neurodegenerative disorders caused by the misfolding of prion proteins into an infectious form (PrPSc). Human TSEs are classified into three categories: sporadic, genetic, and acquired. In Canada, the most common form is sporadic Creutzfeldt-Jakob disease (sCJD), which can only be definitively diagnosed post-mortem through brain biopsy. Currently, cerebrospinal fluid (CSF) obtained via lumbar puncture is the primary pre-mortem diagnostic method. Although invasive, this procedure enables real-time quaking-induced conversion (RT-QuIC) assays, which offer high sensitivity for prion detection. This study aims to evaluate less invasive pre-mortem peripheral tissue samples—such as tears, saliva, and nasopharyngeal mucosa—as potential biomarkers for TSEs using RT-QuIC. The primary objective is to assess the diagnostic utility of these sample types while maintaining sensitivity and specificity comparable to CSF-based assays. Participant recruitment is ongoing and includes symptomatic individuals with probable sCJD, asymptomatic carriers of genetic TSE mutations, and family members of diagnosed patients, who will serve as non-CJD controls. Although no effective treatments currently exist for CJD, the ability to monitor disease progression through less invasive methods may support the development of future therapeutic interventions and the potential for earlier diagnoses.

37. Chemotherapy Induced Stress Reduces Protein Synthesis in T-cell Acute Lymphoblastic Leukemia Cells

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Background: Treatment for T-cell acute lymphoblastic leukemia (T-ALL) is limited to an aggressive chemotherapy regimen of vincristine, dexamethasone and L-asparaginase (VXL) that causes long-term side effects. Improvements to T-ALL treatment are needed but challenging due to the limited knowledge on how T-ALL cells respond and adapt to chemotherapy. Protein synthesis has emerged as a promising targetable pathway given its importance for cell function in homeostatic and stress conditions.

Aim: To define the effects of chemotherapy on T-ALL cell function, informing novel therapeutic strategies.

Methods: I determined the lethal concentration (LC_{50}) of VXL by assessing viability of T-ALL cell lines by flow cytometry. I also assessed cell cycle distribution and changes in phosphorylation of the ribosomal protein S6 (pS6), which is an established marker of protein synthesis. In addition, I measured pS6 in T-ALL samples harvested from patient-derived xenograft (PDX) models at diagnosis and 24 hours after VXL treatment. I will validate effects on protein synthesis using OP-Puromycin incorporation into nascent peptides in T-ALL cell lines as well as the human samples from PDX models of T-ALL.

Results: Treatment of the T-ALL cell lines with LC_{50} of VXL caused a significant reduction in pS6 after only 1 hour of exposure, prior to any measurable changes in cell cycle distribution. Treatment of PDX models with a single dose of VXL also causes a reduction in pS6 in human T-ALL samples, suggesting decreased protein synthesis in response to chemotherapy-induced stress.

Conclusion: My results suggest that protein synthesis is impaired in response to chemotherapy-induced stress rather than decreased proliferation which occurs later in T-ALL cells. Given these effects, targeting key components of this stress response might limit the capacity of T-ALL cells to adapt, thereby preventing resistance to chemotherapy and improving outcomes.

38. Characterizing the role of LSM7 in CD8+ T cell differentiation *in vitro*

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Introduction: The RNA-binding protein LSM7 plays crucial roles in the *LSM1-7* and *LSM2-8* complexes, regulating alternative splicing and mRNA decay to maintain gene expression. It is important in immune function, where it influences RNA splicing, stress granule formation, and autophagy to support cell survival. This study aims to elucidate the role of LSM7 in CD8+ T cell differentiation and survival, expanding on the field of how RNA-binding proteins influence immune function. While LSM7 is well-known for its roles in RNA metabolism, alternative splicing, and mRNA decay, its impact on immune cell regulation, particularly in CD8+ T cell differentiation, remains largely unexplored. Given the preliminary findings that: (a) LSM7 is expressed in CD8+ T cells early after T cell activation (after the first cell division in "pre-effector" cells, Kakaradov, Arsenio *et al., Nat Immunology* 2017), in exhausted CD8+ T cells, and in central memory CD8+ T cells, and (b) *LSM7* knockdown decreases the frequency of effector CD8+ T cells, this study aims to answer whether LSM7 plays a direct role in T cell fate, i.e. differentiation into effector and memory subsets.

Methods: Primary murine CD8+ T cells were isolated from the spleens of C57BL/6 mice using magnetic bead sorting. To evaluate differentiation and survival, cells were activated *in vitro* with anti-CD3 and anti-CD28 antibodies in the presence of IL-2. To investigate the role of LSM7 in CD8+ T cell differentiation, CRISPR-Cas9-mediated *LSM7* knockdown will be performed by transfecting ribonucleoprotein (RNP) complexes via electroporation. Flow cytometry will be used to assess the impact of *LSM7* deficiency on CD8+ T cell function, including cytokine production (IFN-γ, TNF), activation markers (IL-2Rα, CD62L), and cell viability. Data analysis will be conducted using FlowJo software, with appropriate gating strategies to compare control vs. *LSM7*-deficient CD8+ T cells.

Expected Impact: Understanding how LSM7 contributes to CD8+ T cell responses could provide valuable insights into enhancing immune memory formation and optimizing T cell-based therapies. Given the critical role of CD8+ T cells in clearing infections and targeting tumor cells, these findings may have implications for improving T cell persistence in chronic infections, enhancing vaccine efficacy, and improving immunotherapeutic strategies for cancer.

39. Interplay Between Butyrate and Chromogranin A in Regulating Intestinal Permeability and Systemic Immunity

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Short-chain fatty acids (SCFAs) like acetate, propionate, and butyrate are important microbial metabolites produced during the fermentation of dietary fibre. They play a key role in maintaining intestinal balance by enhancing permeability and the epithelial barrier through the upregulation of tight junction proteins, such as Zonula Occludens-1 (ZO-1), and by supporting mucosal immune functions, including the production of immunoglobulin A (IgA). Disruption in SCFA production or signalling is associated with increased intestinal permeability, microbial imbalance, and chronic inflammation, all of which are hallmarks of inflammatory bowel disease (IBD). IBD, which includes Crohn's disease and ulcerative colitis, is characterized by cycles of mucosal inflammation, epithelial barrier dysfunction, and dysregulated immune responses. Chromogranin A (ChgA), a peptide secreted by enterochromaffin cells, has also been linked to gut barrier regulation and immune modulation. Emerging evidence indicates that ChgA may interact with or respond to SCFA signalling. However, the nature of this interaction and its significance in intestinal inflammation remain poorly understood. This study aims to examine how SCFAs and ChgA together influence gut permeability and systemic immune responses during colitis. Wild-type (WT) and ChgA knockout (KO) mice were given 150 mM butyrate in their drinking water for 30 days. Colitis was then induced with 5% dextran sulfate sodium (DSS) for five days, and the mice were sacrificed on the sixth day. Intestinal permeability was assessed in proximal and distal colon segments using Ussing chambers with FITC-dextran. ZO-1 expression was evaluated through immunofluorescence. Serum IgG1 and IgG2 levels were measured by ELISA; analysis of IgA is currently ongoing. We hypothesize that butyrate will protect barrier integrity and maintain ZO-1 expression in WT but not in ChgA KO mice after DSS exposure, highlighting a crucial role for ChgA in mediating the beneficial effects of SCFAs. Understanding this SCFA/ChgA axis could lead to new therapeutic strategies for inflammatory bowel diseases involving epithelial barrier dysfunction.

40. Establishing an *in vitro* culture system which allows for the long-term maintenance and expansion of latent HIV-infected CD4⁺ T Cells

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Antiretroviral therapy (ART) has helped HIV become a manageable chronic infection by repressing viremia to undetectable levels in People living with HIV (PLWH). However, this treatment is not curative, as treatment interruption causes viremia to rebound to pre-treatment levels. This rebound is driven by *viral latency*, a reversible, transcriptionally silent state of infection predominantly established in CD4$^+$ T cells during acute infection. Since no viral proteins are produced, latently infected cells evade antiviral immune response and are not impacted by antiretroviral therapy. As a result, viral latency poses a significant barrier to achieving an HIV cure and requires a better understanding of latency maintenance to develop effective eradication measures.

The study of transcriptionally silent latently infected cells *in vivo* is highly challenging due to their rarity, with only about 1 in 10^5-10^7 CD4$^+$ T cells being latently infected. Therefore, the development of techniques and tools that enable the identification, isolation and enrichment of these latent cells is crucial for advancing research in the field. To address this, I generated a fluorescent protein-based HIV reporter system that allows for the characterization of the phenotypic and migratory characteristics of latent HIV-infected CD4$^+$ T cells *in vitro* and *in vivo*. However, a key limitation of this model is the low frequency of latently infected cells generated, which prevents in-depth studies of these cells. To circumvent this limitation, I will be developing an *in vitro* culture system that supports the long-term maintenance and expansion of latent HIV-infected CD4$^+$ T cells. This will enable us to do more in-depth studies to better understand the molecular and cellular mechanism that allows for the maintenance of latent infected CD4$^+$ T cells in PLWH.

42. Role of variant-specific SARS-CoV-2 non-structural accessory proteins in host-pathogen interactions

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes coronavirus disease 2019 (COVID-19). Viral adaptation can modulate pathogenicity, viral fitness, immune evasion, and transmissibility. Although much research on the functional significance has focused on mutations in the spike protein, adaptation is also dependent upon changes in other proteins. Many SARS-CoV-2 nonstructural and accessory proteins are involved in antiviral response inhibition or invasion. Variant-specific differences in interferon (IFN) induction and signaling mediated by nonstructural accessory proteins may reveal insights into the mechanisms regulating host antiviral response evasion. First, we identified the distinct non-synonymous mutations among different variants of SARS-CoV-2 (alpha, beta, gamma, delta, and Omicron BA.1, BA.2, BA.4, BA.5). We generated plasmids expressing ORF3a, ORF6, ORF7a, ORF7b, ORF8, and ORF9b from the ancestral B.1 virus or variant-specific mutations that have emerged since 2020. Protein expression was confirmed by immunoblotting. We used a luciferase reporter system to assess the impact of variant-specific mutations on IFN or IFN-stimulated gene (ISG) expression in HEK293T cells stimulated with IFN treatment or Sendai virus infection. Reporter assay data indicates that ORF D61L mutation (Omicron) inhibits ISG expression less than wild type up to 40%. On the other hand, mutations in ORF3a S26L (Delta), Q57H (Beta), T223I (Omicron) and S253P(Gamma) suppress ISG expression more than wild type ORF3a. IFIT1 and ISG54 expression were quantified using qPCR to confirm ISG induction. Data showed that these mutations do not impact IFIT1 and ISG54 expression inhibition compared to wild type proteins. The results highlight the functional role of variant-specific mutations in non-structural accessory proteins play in suppressing the innate immune response. Further research and work are needed to specify and address these accessory proteins' role in pathogenesis and transmission.

43. EXPLORING INTERFERON-LAMBDA3 RESPONSIVENESS OF HUMAN MACROPHAGE SUBSETS

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Introduction: IFN-λs are cytokines that play a crucial role in our innate immune defence signaling through a heterodimeric IFN-λR1/IL-10RB receptor to promote antiviral immunity. Novel IFN-λ immunomodulatory effects are just now being appreciated. We previously found that human monocytes are unable to respond to IFN-λ, but conflicting reports exist for macrophages. Human macrophages have been reported to express functional IFN-λR, but mouse macrophages are non-responsive. We aimed to profile various human macrophage subsets in their expression of *IFNLR1* and IFN-λ responses. We hypothesized that differentiation of monocytes to macrophages would promote *IFNLR* expression and IFN-λ3 could especially signal in pro-inflammatory macrophages.
Methodology: Monocytes were isolated from healthy donor peripheral blood mononuclear cells (PBMCs) using positive isolation(n=6). Monocytes were incubated for 6 days with macrophage colony-stimulating factor (M-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF) (M0). On day 6, cells were cultured with/without IFN-λ3 for 22 hours. RT-qPCR was performed to profile IFN-λ3 transcriptome changes. In a subset of experiments, macrophages were differentiated into M1 and M2a/M2c subsets for 1-3 days.
Results: We found that macrophages had >400-fold upregulation of *IFNLR1* expression compared to monocytes. GM-CSF promoted greater *IFNLR1* expression and higher IFN-λ3 responses compared to M-CSF macrophages. IFN-λ3 was more inflammatory in GM-CSF macrophages in the induction of *IL1B* and *TNF*, whereas these genes were not induced in M-CSF macrophages. Upon differentiation to M1 or M2 subsets, *IFNLR1* expression remained similar to the expression in M0 macrophages.
Conclusion: Our results demonstrated that macrophages grown in an inflammatory background (GM-CSF) demonstrate a stronger response to IFN-λ3 due to higher levels of the *IFNLR1* but may also have differential inflammatory potential compared to macrophages grown in a more homeostatic environment (M-CSF, M2).

44. CHARACTERIZATION OF THE EFFECT OF SEX STEROIDS ON CD8+ T CELL DIFFERENTIATION *IN VITRO* AND *IN VIVO*

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Introduction: CD8+ T cells play a crucial role in combating infections and chronic inflammation. Upon acute infection, naïve CD8+ T cells differentiate into short-lived effector and long-lived memory cells, whereas chronic infection can lead to T cell exhaustion. Biological sex differences exist in the immune system, however the impact of sex hormones on CD8+ T cell differentiation during chronic antigen exposure remains less understood.
Methodology: We utilized male and female transgenic P14 CD8+ T cells which have TCRs that recognize the LCMVGP33-41 peptide to investigate sex differences in antigen-specific T cell responses. *In vitro*, cells were activated with GP33-41 peptide and cultured with sex steroids (estradiol, dihydrotestosterone, and progesterone) and IL-2. Flow cytometry was performed for analyses of effector differentiation on day 3 and exhaustion differentiation on 7-days post-activation with continuous peptide stimulation to mimic the chronic antigen exposure. Apoptosis and proliferation assays were performed on day 7.
For *in vivo* studies, P14 T cells will be adoptively transferred into wild-type and gonadectomized male and female mice, followed by LCMV clone 13 infection. Spleens and peripheral blood will be harvested on days 4, 7, and 28 for flow cytometry characterization of exhaustion-associated markers, as well as for single-cell RNA-sequencing analysis.
Results and Conclusion: *In vitro*, female CD8+ T cells treated with estrogen and progesterone exhibited a higher frequency of effector T cells. IFN-γ production was significantly reduced in hormone-treated male cells, with no significant change in TNF-α production on day 3. On day 7, estrogen-treated female cells showed increased expression of exhaustion markers TIM-3 and LAG-3 (gated on CD44hi&PD-1hi), along with a higher frequency of terminally exhausted-like cells (TIM-3hiLY108lo). However, no significant differences were observed in apoptosis or proliferation, suggesting that hormone treatment differentially affects exhaustion-associated marker expression but not T cell survival and proliferation.

45. Quantification of the HIV reservoir in people living with HIV using a nested qPCR assay

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The HIV reservoir persists even after antiretroviral therapy (ART) is initiated, which can contribute to chronic inflammation observed in people living with HIV (PLHIV). Chronic inflammation can lead to co-morbidities unrelated to HIV, such as neurocognitive disorders and pulmonary diseases. Identification and quantification of the HIV reservoir is important to evaluate disease progression and to improve diagnostic algorithms for complex cases, such as individuals on pre-exposure prophylaxis (PrEP) and those presenting as spontaneous HIV controllers, with undetectable viral loads at the time of testing. Monitoring the size of the HIV reservoir will also be of importance as reservoir eradication strategies become used as therapies in clinical settings. Typically, a large number of cells is necessary to perform HIV reservoir quantification assays, which is not always possible. ACH-2 cells, which contain a single copy of integrated HIV DNA, were used to create a standard curve for this nested qPCR assay. Using previously published primers and probes, HIV DNA was quantified in samples from PLHIV obtained without leukapheresis. First, genomic DNA was isolated from biological samples and total HIV DNA or integrated HIV DNA was amplified along with CD3 DNA in a PCR reaction. Next, the product was added to two qPCR assays, one to quantify HIV DNA and the other to quantify CD3 DNA. From this, the number of copies of HIV DNA per one million cells was determined. In conclusion, this assay was able to estimate the frequency of HIV DNA in clinical samples by amplifying and quantifying both HIV DNA and CD3 DNA. This nested qPCR assay will be further evaluated for use in various biological sample types to estimate the size of the HIV reservoir without the need for a large number of cells.

46. A Human Immune System Mouse Model to Study Neurological Infection and Disease

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Neurological infection and disease such as Human Immunodeficiency Virus (HIV) associated neurocognitive disorder (HAND) and Multiple Sclerosis are among the most challenging diseases to study. Brain resident immune cells called microglia have been identified as critical in the development and resolution of HAND and MS. Despite the importance of microglia, few animal models have been established to study human microglia. We aimed to develop a human immune system (HIS) mouse model with an enhanced systemic myeloid population and human microglia-like cells in the brain to fulfil this niche. To accomplish our goal, we utilized two mouse strains: Balb/c $CSF1^{h/h}$ $Rag2^{-/-}$ $\gamma c^{-/-}$ (MRG) and C57BL/6 $Rag2^{-/-}$ $\gamma c^{-/-}$ $CD47^{-/-}$ Triple Knock-out (TKO). Both mouse strains have genomic modifications to stop murine immune cell development and are thus permissive to human stem cell implantation following preconditioning. In the Lavender lab we use the bone marrow, liver and thymus (BLT) humanization protocol which results in extensive and long-term systemic human immune cell reconstitution. To boost the myeloid and microglia-like cell population we focused on the expression of human colony stimulating factor 1 (CSF1). The MRG mouse strain transgenically expresses CSF1 whereas we employed an adeno associated viral vector (AAV) to induce the expression of CSF1 in TKO mice. We found that compared to saline controls, TKO-BLT animals administered $2x10^{10}$ genome copies of AAV-hu-CSF1 at 8 weeks post humanization surgery (wps) developed similar overall numbers of human immune cells but more myeloid cells by 12wps. MRG-BLT animals preconditioned with either 400cGy of X-ray irradiation or 50mg/kg chemotherapy busulfan matched TKO-BLT systemic humanization at 16wps and had higher numbers of human myeloid cells in the blood. In conclusion, we developed two HIS mouse models with enhanced systemic myeloid cell populations and are now optimizing the development of human microglia-like cells in the mouse brain.

48. Uncovering the Role of Interferon Signaling in T-ALL Chemotherapy Resistance

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Introduction: T-cell Acute Lymphoblastic Leukemia (T-ALL) is a heterogeneous and aggressive blood cancer that is more common in children, adolescents and young adults (AYAs). Advances in treatment have significantly improved overall survival and outcomes for patients. However, survival rates for recurrent disease remains less than 25%, and relatively few new treatments have been developed for children and AYAs with therapy-resistant T-ALL. Thus, there is an urgent need to better understand the mechanisms of therapy-resistance in T-ALL for developing more effective treatments. Genetic profiling of patient samples has found that an inflammatory signature characterized by aberrant interferon (IFN) signaling is associated with poor outcomes, linking IFN with chemotherapy-resistance in T-ALL. My project is exploring the role of IFN signaling in chemotherapy-resistance, with the goal of informing the development of novel therapeutic strategies to prevent relapse and improve outcomes for patients living with T-ALL.
Methods: Front-line chemotherapy for pediatric T-ALL is a combination of Vincristine, Dexamethasone, and L-Asparaginase (VXL). I assessed the sensitivity of inflammatory (LOUCY, PEER, CUTLL3) and non-inflammatory (P12-Ichikawa, CCRF-CEM, MOLT-4) T-ALL cell lines to VXL chemotherapy, with or without IFN. Lethal concentration 50 (LC_{50}) values were determined to quantify drug sensitivity. Additionally, we conducted time-course analyses of signaling mediators of IFN, to define how inflammatory response affects resistance to VXL chemotherapy.
Results: My data show that T-ALL cells are more resistant to VXL in presence of IFN. Stimulation of T-ALL cells with IFN induced rapid phosphorylation of STAT1, followed by later upregulation of IFN Regulatory Factors (IRF5, 7, 9).
Conclusion: Our findings reveal that IFN has a chemoprotective effect on T-ALL cells, suggesting a role of IFN signaling in chemotherapy-resistance and T-ALL relapse. Future work will combine transcriptomic and proteomic approaches to define how IFN signaling promotes chemotherapy-resistance and identify novel targets for preventing relapse in T-ALL.

49. PATHOGENIC POST-TRANSLATIONAL MODIFICATIONS OF NEUTROPHIL PROTEASES DRIVE INFLAMMATION IN RHEUMATOID ARTHRITIS

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Introduction: Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by persistent joint inflammation, driven by immune cell infiltration and fibroblast-like synoviocytes (FLS) activation. Neutrophils contribute to disease progression through serine proteases- Proteinase-3 (PR3), Cathepsin-G (Cat-G), and Neutrophil Elastase (NE), which regulate immune responses but, when dysregulated, promote inflammatory pathology. During RA's pre-clinical phase, genetic and environmental factors trigger post-translational modifications (PTMs) such as citrullination and carbamylation. While these PTMs alter protease activity and immunogenicity, their direct role in RA pathogenesis remains unclear. This study investigates how PTMs enhance protease function, promote inflammatory signaling, and drive autoantibody responses- highlighting novel therapeutic targets.
Methods: Purified neutrophil proteases and cell supernatants were modified via PAD2/PAD4-mediated citrullination or potassium thiocyanate (KOCN)-mediated carbamylation. Protease activity was measured using fluorogenic assays. Rhodamine-PG probes and Western blotting verified modifications. FLS were stimulated with modified proteases, and cytokine production was assessed via ELISA and flow cytometry. PAR signaling was evaluated using specific inhibitors. Autoantibody reactivity to PAD-modified PR3 was examined in RA patients and at-risk individuals.
Results: Citrullination increased PR3 and NE enzymatic activity by 2-fold and 4-fold, respectively. Carbamylation further enhanced PR3 function in a concentration-dependent manner. Modified PR3 triggered elevated IL-6 and TNF-α secretion in FLS, with PAR2 inhibition reducing this response, confirming its involvement in PR3-driven signaling. Autoantibody profiling revealed increased reactivity to citrullinated-PR3 in at-risk patients.
Conclusion: Our findings establish PTM-modified neutrophil proteases as potent mediators of inflammation and autoimmunity in RA. Targeting these modifications offers promising therapeutic interventions for modulating immune dysregulation in early and established RA.

52. Using a Transposon Knock-Out Library to Determine Genes Essential for *Salmonella* Survival in Water

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Salmonella spp. are a major causative agent of human diarrheal disease worldwide, with up to a 30% mortality rate in developing countries. Despite this, researchers still do not understand the specifics of how *Salmonella* spp. are able to survive outside of a host. It is hypothesized that *Salmonella* spp. are able to spread through irrigation practices in which water contaminated with *Salmonella* is spread onto produce meant for human consumption. The survival in water and subsequent transmission are known to be important for pathogenic success, but there is a gap in knowledge on how *Salmonella* can do this. *Salmonella* uses biofilms to survive desiccation, resist antimicrobials and increase its survivability in general, therefore it has been hypothesized that *Salmonella* would use its biofilm capabilities to survive in water. To answer the question of what genes *Salmonella* utilizes to survive in water, we developed a transposon (Tn) library consisting of strains where individual genes are disrupted by insertion of a Tn; the entire collection of strains represents every gene in the *Salmonella* genome. By inoculating the Tn library of Salmonella serovar Typhimurium into water and incubating for 42 days, we are able to determine what genes are most important for water survival. Comparison of the output Tn-library (after 42 days in water) from multiple experiments to the starting input Tn-library (i.e., what was inoculated into water) allows us to differentiate between genes that are consistently identified versus those that appear randomly. I will describe the building and validation of the library and present data from our water survival experiments. Using a gene knock-out library is an efficient way to characterize what genes are essential for water survival in an unbiased fashion. By characterizing the mechanisms used by *Salmonella* to survive in water, we will be able to further understand *Salmonella* transmission.

53. Identification and characterization of genes that differentiate the two described genotypes of *Mannheimia haemolytica*

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Mannheimia haemolytica is an opportunistic gram-negative bacterial pathogen associated with bovine respiratory disease (BRD), an economically costly disease that primarily impacts the beef industry. Two genotypes of *M.*

haemolytica have been described, with genotype 2 (G2) being more frequently isolated from clinical cases of BRD than genotype 1 (G1). This study expands on previous investigations into the intraspecies genetic variation of *M. haemolytica* to identify genes that can differentiate the two genotypes.

A collection of 206 unique whole genome sequences was generated using genomes from NCBI and a previous collection of our labs. Genomes were genotyped using an *in silico* PCR assay resulting in 61 G1 and 145 G2 genomes. Pangenome wide analysis was conducted to identify genotype differentiating genes. Genomes were also examined for general genomic differences and the prevalence of integrative and conjugative elements (ICEs).

A total of 422 genotype differentiating genes were identified. Differentiating genes of note included genotype variants of a TonB dependent siderophore receptor homologue, transferrin binding protein B (TbpB), leukotoxin A (LktA) and IgA1 proteases. We also identified a G1 associated lytic transglycosylase and G2 specific highly immunogenic outer membrane protein. G2 genomes were found to carry more genes and had a greater prevalence of ICEs than G1 genomes.

Identified genes may contribute to observed differences in disease association between the two genotypes by influencing fitness (iron acquisition proteins such as TbpB, TonB dependent receptor) and/or virulence (IgA1, LktA) of *M. haemolytica*. The greater number of genes in G2 suggests G2 may have a wider repertoire of tools to survive in a wider array of environments than G1. The identification of 422 genotype differentiating genes provides a useful starting point for future work investigating the phenotypic differences between the two currently characterized genotypes of *M. haemolytica*.

54. On-Site Detection Where It Matters: A Cold Chain-Independent Point of Collection System for One Health Pathogen Detection

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Point-of-collection testing (POCT) is an emerging technique that aims to identify pathogens in situ. It aims to reduce the cost and time required to identify pathogens, thus enabling enhanced pathogen management. POCT has been primarily applied in healthcare, despite being desperately needed across all domains of one-health, including agriculture and veterinary medicine, where rapid on-site diagnosis can critically impact case management. As such, we present progress on the development of a novel POCT system that utilizes Loop-Mediated Isothermal Amplification (LAMP): a well-established nucleic acid amplification test (NAAT). Our system successfully detects a variety of pathogens from across all domains of life, including *Claviceps purpurea*, avian influenza virus, and *Salmonella* sp., using diverse sample types such as beverages and plant leaves. We demonstrate the potential for POCT deployment in localities without access to traditional cold chains. Additionally, we present metrics provided by people in need of POCT on what such a system must include to be widely adopted. Finally, we demonstrate the real impact of our system by detecting asymptomatic *Botrytis cinerea* infections in situ, which enabled early intervention and reduced yield losses by $240,000.

55. Emerging Infectious Disease Threats to Canada: Risk Assessment and Diagnostic Preparedness

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Emerging infectious diseases continue to remain a persistent threat to Canadians and their public health security. This project, conducted at the Vaccine and Infectious Disease Organization (VIDO), supports the Serology and Diagnostics High-Throughput Facility (SD-HTF) at the University of Ottawa by providing up-to-date risk assessments and diagnostic information on emerging pathogens. Focusing on priority pathogens identified by the WHO, CEPI, and PHAC, we aim to fill potential critical gaps in Canada's diagnostic preparedness. This is done by categorizing pathogens into three tiers (i.e., pathogens to monitor, pathogens of concern, or pathogens requiring immediate

action). All this work is conducted to help ensure that Canada's diagnostic capacity stays effective, as pathogens evolve and potentially reduce the accuracy of existing assays.The objective of this project is to evaluate the risk of priority pathogens and support the continuous improvement of molecular and serological diagnostics. This includes assessing whether mutations could impact assay performance and recommending updates to primers, probes, and antigen targets as needed. We systematically monitor international outbreak platforms, including ProMED Mail, BlueDot Global Insights, CIDRAP, WHO Disease Outbreak News, ECDC Threat Reports, HealthMap, and CDC outbreak listings. For each pathogen, we assess its likelihood of affecting Canadians, the reliability of current diagnostic tools, and the availability of vaccines or treatments. Currently, we have reviewed SARS-CoV-2, Lassa virus, Highly Pathogenic Avian Influenza (HPAI) (H5N1), Hantavirus, Measles, and West Nile virus as examples of pathogens that could impact the health of Canadians. We have categorized Lassa Virus, HPAI (H5N1), and Measles as pathogens of concern, requiring close monitoring. Notably, HPAI (H5N1) is a highly mutable virus, capable of frequent reassortment (antigenic shift) and antigenic drift. The ongoing situation with HPAI globally requires ongoing monitoring given the frequency of outbreaks in domestic and wild animals. Overall, this work aims to strengthen Canada's preparedness for emerging threats and directly supports diagnostic research facilities like the ST-HTF in maintaining accuracy as pathogens evolve.

56. Defining the role of histamine in the biology of Morganella morganii, an opportunistic superbug

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In bacteria, histamine plays a key role in both inter- and intracellular communication. At elevated concentrations, it can trigger immunomodulatory effects and cause allergic responses in the host. In bacteria, it can induce physiological effects including chemotaxis and virulence gene expression. Morganella morganii is a known histamine-producing bacterium and a causative agent of scombroid poisoning—an allergic-like reaction characterized by skin rashes and other symptoms associated with allergy, following the consumption of histamine-rich fish. Beyond this, M. morganii is increasingly being recognized for its involvement in various human infections, including those of the urinary tract, bloodstream, and postoperative wounds. However, the overall response of M. morganii to histamine remains uncharacterized. To investigate this, we performed RNA sequencing (RNA-seq) to assess the transcriptional response of M. morganii exposed to different concentrations of histamine (0 mM, 2 mM, and 20 mM) for 20 minutes and 4 hours. While transcriptional changes were noted under all conditions, those assessed when the bacteria were exposed to 20 mM histamine for 4 hours, revealed to have the most robust transcriptional changes, and were indicative of a response to toxicity. The hdc locus, putatively associated with histamine biosynthesis and transport was downregulated—which is congruent with an effort to detoxify histamine. Additionally, several amino acid transporter genes were downregulated, while additional stress response genes were upregulated, suggesting an adaptive change to environmental stress. To further characterize these genes, we required a chemically defined medium (CDM), which has not yet been developed for this bacterium. We formulated a CDM with individual amino acid dropouts alongside a complete amino acid (positive control) to assess amino acid auxotrophies in our laboratory strain. Our experiments revealed that the omission of L-cysteine and L-leucine significantly impaired bacterial growth relative to optimum growth with all the amino acid. Further investigation is needed to determine whether these growth phenotypes are conserved across clinical strains.

57. Molecular Diagnostic Methods for *Brucella abortus* Detection in Wood Bison

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Wood bison, a species at risk globally, are threatened by a high prevalence of bovine brucellosis, a zoonotic bacterial infection caused by *Brucella abortus* that colonizes the reproductive tract, resulting in infertility and abortions. An estimated 25-37% of the Wood Buffalo National Park population, which is the largest and most genetically diverse in the world, are affected. To support wood bison conservation efforts, *B. abortus* infection must be controlled and prevented from spreading to healthy herds. However, current diagnostic methods are time-consuming, require specialized equipment, or lack specificity. In this study, we have leveraged newer molecular diagnostic technologies to develop a rapid, portable diagnostic assay for *Brucella* species. We designed quantitative polymerase chain reaction (qPCR) and loop-mediated isothermal amplification (LAMP) assays and compared their performance characteristics (analytical and diagnostic sensitivity and specificity), speed, and cost with previously published LAMP and recombinase polymerase amplification (RPA) assays. We also developed a simplified DNA extraction protocol for bison semen and assessed the performance of our DNA extraction and amplification method outside of a traditional laboratory. Our results demonstrate almost perfect agreement (Cohen's kappa > 0.9) compared to gold-standard culture results for all assays except the *bcsp31* LAMP. qPCR showed the best analytical sensitivity (lowest limit of detection), followed by the *cpn60* LAMP, the RPA assay, and then the *bcsp31* LAMP. However, the RPA assay, when converted to a lateral flow detection format, is the fastest and most suitable for point-of-care applications, while still maintaining a low limit of detection and excellent diagnostic sensitivity and specificity. Further, using a short proteinase K treatment and syringe-based DNA extraction, we were able to extract amplifiable DNA in under ten minutes. Our proof-of-concept field trials indicate that this method can feasibly be applied at the point-of-care to rapidly detect and help prevent the transmission of *Brucella abortus*.

58. Characterization of iron acquisition in the emerging opportunistic One Health pathogen *Morganella morganii*

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Morganella morganii is an emerging multidrug-resistant (MDR) pathogen, however vanishingly little is known about the basic biology of this bacterium. Iron is an essential micronutrient for almost all organisms and can markedly impact the fitness of bacteria when homeostatic concentrations are perturbed. As such, bacteria have finely tuned transcriptional responses to iron availability that mediate its uptake when limited, and its efflux and/or sequestration when in excess. Although *M. morganii* possesses genes for the uptake of small iron-binding molecules, known as siderophores, sequencing performed by our lab on modern clinical isolates reveals no discernible loci for siderophore synthesis. This project thus aims to identify the key iron uptake pathways and preferred source(s) of metal for *M. morganii*. RNA sequencing of *M. morganii* exposed to iron -deplete and -replete conditions revealed genes that were highly upregulated under iron limitation. These genes were predicted to be involved in the uptake of ferric citrate, siderophores, and ferrous iron, suggesting that they may encode for the iron acquisition machinery of the pathogen. This analysis also revealed iron transport-related clusters that may enhance our understanding of how *M. morganii* maintains iron homeostasis. Growth curves are being used to assess the contribution of various iron sources to the iron-dependent growth of the bacterium under nutrient limitation. Due to the essentiality of iron and the surface-exposed nature of the receptors for its acquisition, these uptake pathways are commonly exploited as druggable targets in bacteria. Insights from this study may thus inform future therapeutic development for treatment of MDR *M. morganii* infections. For future directions, we aim to study these pathways in detail by generating mutants in key genes and assessing their individual effects on the iron-dependent growth of *M. morganii*.

59. Extracellular Porin Variability Characterized in Avian Pathogenic *Escherichia coli* Strains

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Avian pathogenic *Escherichia coli* (APEC) causes a wide variety of infections in poultry, collectively termed 'colibacillosis'. Colibacillosis is the bacterial disease with the greatest economic impact to poultry producers, because it affects birds at all stages of production. Due to colibacillosis infection less eggs are laid, less eggs hatch, meat quality decreases, and chicken death increases. Investigation of *E. coli* and other closely related bacterial species led to the discovery that several conserved outer membrane proteins (OMPs) have evidence of mosaic evolution, where the individual protein has a different evolutionary path than the overall bacterial strain. Specifically,

portions of the loop regions of the OMPs which face the external environment evolve differently than the rest of the protein in question. We hypothesize that these hypervariable regions (HVRs) are the result of environmental pressures associated with evading the host immune system and bacteriophage infections. We are investigating the HVR patterns in distinct sets of *E. coli* strains obtained from Saskatchewan broiler producers and are trying to determine if there are unique patterns in virulent APEC strains.

60. Conducting Field Studies to Estimate the Prevalence of Hantavirus Infection in Wild Deer Mouse Populations in Saskatchewan

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Sin Nombre virus (SNV) is a New World hantavirus responsible for Hantavirus Cardiopulmonary Syndrome (HCPS), a severe respiratory illness. The most prevalent rodent in North America, the western deer mouse (*Peromyscus sonoriensis;* previously *Peromyscus maniculatus*), is the main reservoir host for SNV in Canada. The case-fatality rate of HCPS is 30% to 35%, underscoring the critical need for virus surveillance. Thus far, no study has looked at the prevalence of SNV in wild rodent populations in Saskatchewan, despite the province reporting a high number of HCPS cases. This study seeks to close that gap by examining the prevalence of SNV and attempting the first isolation of SNV from the Saskatchewan deer mouse populations, contributing novel viral material for future research.

For this study, trapping sessions were conducted at the Vaccine and Infectious Disease Organization research farm, located outside of Saskatoon. Sherman live traps were deployed in the evening and checked for deer mice the following morning. Captured deer mice were anesthetised using isoflurane, and blood samples were taken by cardiac puncture before euthanasia. Oral swabs and ear punches were taken along with data on body weight, length and sex.

RNA was extracted from field samples (mouse tissues, oral swabs and serum) and quantitative RT-qPCR was conducted using specific SNV primers and probe. ELISA was used to measure seroprevalence among trapped deer mice. In mice with positive detection of SNV RNA, follow-up work will include attempts to isolate SNV.

This study is the first to examine the prevalence of SNV in wild rodent populations in Saskatchewan. The results will help guide zoonotic risk assessments and disease surveillance, especially in rural and agricultural areas where human contact with deer mice is common. Our data provides a foundation to support future ecological and epidemiological modelling, trapping and testing that will occur in future seasons.

61. Wildlife as sentinels for One Health: surveillance in wild carnivores for zoonotic parasites such as *Echinococcus multilocularis*

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Wildlife plays a critical position in One Health, acting as both a sentinel for zoonotic pathogens and a source for disease crossover to humans. Wild carnivores, with their position at top of the food chain as predators and scavengers, are excellent sentinels for trophically transmitted pathogens and have a high level of interaction with both humans and companion animals. Therefore, in collaboration with wildlife stakeholders in Saskatchewan, we sampled over 500 carcasses from 10 host species representing the *Mustelidae*, *Felidae*, and *Canidae* families during the winter trapping seasons of 2021-2025. Diaphragm and tongue tissues were tested for the muscle nematode *Trichinella* spp., blood for the felid-borne protozoan *Toxoplasma gondii*, blood for antigen of the heartworm *Dirofilaria immitis*, and intestine/fecal samples were examined for taeniid eggs and DNA of zoonotic *Echinococcus* spp. cestodes. *Trichinella nativa* was found in lynx, fisher, marten, weasel, and wolverine, at relatively low prevalence compared to arctic carnivores. Seroprevalence of *Toxoplasma gondii* varied from 12.4-38.5% in the studied host species, with higher prevalence in specific carnivores like fishers, lynx, and mink compared to coyotes, marten, and weasels. Antigen of *Dirofilaria immitis* was detected in wolves, fox, coyote, and lynx. Taeniid eggs were detected in 37.2 % of wild canids, of which 40.9% were identified using PCR as *Echinococcus multilocularis*. Therefore, wild carnivores were useful sentinels for detection of circulating food borne zoonoses like *Trichinella* and *Toxoplasma,* and mosquito borne heartworm which may be newly present in Saskatchewan. This study also demonstrated that wild canids (specifically coyotes and foxes) are sources of environmental contamination with E*chinococcus multilocularis*, an emerging parasite in North America that requires further study to explore the interplay between native and introduce haplotypes, host specificity and pathogenesis, and consequently zoonotic transmission.

62. Elucidating the interactions between *Acinetobacter baumannii* and host cells

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Acinetobacter baumannii is notorious for causing drug-resistant nosocomial infections. Articles published in the last few years have suggested that modern clinical isolates of *A. baumannii* possess facultative intracellular capabilities within host cells. We hypothesized that, compared to laboratory-adapted type strains, modern *A. baumannii* clinical isolates persist and replicate inside of both phagocytes and nonphagocytic cells. Gentamicin protection assays were first carried out with type strain 17978, against RAW 264.7 murine macrophages, to set a benchmark for future screens with our clinical isolate collection. Here, 17978 was readily phagocytosed and killed by the macrophages. Subsequent fluorescence-based proliferation assays to visualize intracellular localization of 17978 in macrophages, and to assess for any intracellular replication, were performed. Here, 17978 was nonreplicative within RAW264.7 cells, and localised to LAMP-1-positive vacuoles after phagocytosis, indicating successful phagolysosome maturation and bacterial killing. Finally, we sought alternatives to gentamicin, as over half of the isolates in our collection are gentamicin resistant. The aminoglycoside, apramycin, was tested against a gentamicin-resistant isolate, HUMC1. HUMC1 treated with apramycin at concentrations and exposure times relevant for use in protection assays experienced >4 log fold decreases in CFU/mL, thus making apramycin a suitable alternative to gentamicin in protection assays. Future work will involve screens of clinical isolates using gentamicin, or apramycin, where necessary. Screens will be expanded to the A549 cell line, a relevant cell line to study the pathogenesis of *A. baumannii*. The long-term goal of this project is to identify and characterize potential intracellular niches of *A. baumannii*.

63. The role of bacterial load in vaginal microbiota and HPV outcomes in women from Kenya

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Human papillomavirus (HPV) is the most common sexually transmitted infection worldwide and a key cause of cervical cancer. Emerging evidence suggests that the vaginal microbiota, including total bacterial load (TBL), may influence HPV persistence and disease progression. This study aimed to evaluate differences in TBL between Lactobacillus-dominant and non-Lactobacillus-dominant community state types (CSTs) and their association with high-risk HPV (hrHPV) status.

We screened 1,901 participants from the Sex Workers Outreach Program (SWOP) in Nairobi for hrHPV using GeneXpert and randomly selected 394 for further analysis. Cervicovaginal swabs were collected, and DNA was extracted. TBL was quantified via 16S rRNA qPCR using the Vaginal Microbiome Genomic Mix (MSA-1007™) as a standard. Bacterial community profiling was performed via 16S rRNA sequencing (Illumina MiSeq), and CSTs were assigned using the VALENCIA.

HPV positivity was detected in 166 (42.1%) of the participants. The most prevalent CSTs were IV-B (141; 35.8%) and III-A (126; 32%), followed by III-B (59; 15%). Fewer participants exhibited CSTs I-A, I-B, II, IV-C, and V. Higher vaginal TBL was observed in Non-Lactobacillus-dominant samples ($p < 2.2e{-}16$). TBL varied by CST, with BV-associated CSTs generally exhibiting higher TBL alongside relative depletion of protective *Lactobacillus* species. The vaginal TBL for hrHPV-positive and negative had a median of 5.58 logcopies/µL [IQR: 5.12–5.88] and it was higher in hrHPV-positive participants but not statistically significant ($p = 0.065$), while HPV prevalence was associated with CST I ($p < 0.05$).

Vaginal TBL does not directly correlate with hrHPV infection status but varies significantly by CSTs and is elevated in BV-associated, non-*Lactobacillus*-dominant communities. Given the known association between these CSTs and genital inflammation, our results also suggest that TBL may be a useful marker of the genital immune environment, possibly more informative than current BV diagnostics. Further studies with immune markers are needed to confirm this.

64. Establishing an insectivorous bat model to study MERS-CoV infection dynamics, immune responses and viral persistence

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Background: Middle East respiratory syndrome coronavirus (MERS-CoV) is a zoonotic virus first identified in Saudi Arabia in 2012 and is associated with severe respiratory disease in humans. Vesper bats, which harbor betacoronaviruses, are hypothesized to be ancestral reservoirs of MERS-CoV. Despite the identification of MERS-CoV-related viruses in vesper bats, the infection dynamics of MERS-CoV in these species remain poorly understood. In this study, we experimentally infected *Eptesicus fuscus*, an insectivorous vesper bat, with MERS-CoV to investigate viral replication, shedding, clinical symptoms, pathology, and the development of antiviral immune responses.

Methods: *E. fuscus* bats were infected or mock-infected with 10^5 $TCID_{50}$/mL of MERS-CoV. Bats were sampled at 2-, 4-, 7-, and 14-days post-infection. Clinical parameters, including body weight and temperature, were monitored throughout the study. Tissues were examined for gross pathology. We evaluated antiviral gene expression using qPCR and bulk RNA sequencing. Virus-specific neutralizing antibody titers were assessed by micro-neutralization assays.

Results: Infected bats showed no signs of weight loss or elevated body temperature during the study period. MERS-CoV RNA was detected in samples from infected bats, confirming viral replication. Gene expression analyses revealed upregulation of interferon-related genes, indicating activation of an antiviral response. No gross lung pathology or overt tissue damage was observed in infected animals. Infected bats also developed virus-specific B cell responses, including the production of neutralizing antibodies. Further investigations are ongoing.

Conclusions: MERS-CoV can productively infect *E. fuscus* bats without inducing clinical disease or lung pathology, despite evidence of viral replication and antiviral immune activation. These findings support the use of *E. fuscus* as a viable experimental model for studying MERS-CoV and MERS-CoV-related viruses infection dynamics, tissue tropism, and host immune responses in bats. This model may provide critical insights into mechanisms of viral tolerance and persistence in reservoir hosts.

65. Infection of bovine mammary gland cells with highly pathogenic avian influenza virus (HPAI) H5N1

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Influenza A Virus (IAV) infects a variety of animals, including birds, pigs, and humans. While cattle are susceptible to infection with influenza C and D viruses, they have been resistant to IAV. However, the HPAI H5N1 clade 2.3.4.4b infection was confirmed in dairy cattle in Texas in March 2024. Since then, over 1000 confirmed cases in dairy farms have been reported across USA. The infected cattle experienced a dramatic drop in milk quality and production. Additionally, several farm workers who had close contact with infected dairy cows tested positive for the H5N1 virus. Bovine respiratory and mammary gland epithelial cells are rich in sialic acid α2,3-galactose and α2,6-galactose which are used as receptors for avian and human influenza virus, respectively. The presence of these receptors in mammary glands suggests a potential susceptibility to IAVs of diverse origins. To investigate the tropism of IAV in bovine mammary gland, we evaluate the susceptibility of bovine mammary gland epithelial cells (MAC-T) to infection with HPAI H5N1 and human pandemic H1N1 virus. We used bovine tracheal epithelial cells (EBTr) as a representative control for the respiratory tract, the primary target of IAV. We observed that both H5N1 and H1N1 bind to bovine mammary gland tissue, and they are capable to attach and enter both MAC-T and EBTr cells. However, their replication outcomes differed, while H5N1 replicates efficiently in both cell lines, H1N1 does not replicate in MAC-T cells. Our study provided an in vitro model to understand by which HPAI H5N1 specifically targets and efficiently replicates in bovine mammary gland cells.

67. AAV-mediated human IL-15 enhances NK cell development, function in humanized mice: A platform for HIV investigation

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The multifaceted functions of natural killer (NK) cells in controlling human immunodeficiency virus (HIV-1) and various cancers remain actively investigated. To fully elucidate host-pathogen interactions and NK cell contributions, humanized mouse models mimicking human immune systems are indispensable. Our research leverages the C57BL/6 $Rag2^{-/-}\ \gamma c^{-/-} CD47^{-/-}$ triple knockout (TKO) mouse model humanized using the bone marrow, liver, and thymus (BLT) method. The TKO-BLT model resists graft-versus-host disease (GVHD), making it highly suitable for protracted HIV-1 cure studies. We aimed to improve NK cell development in the model to make it more useful for HIV-1 cure studies involving NK cells. Human interleukin 15 (hIL-15), essential for NK cell development, was delivered via an adeno-associated virus (AAV) vector, creating the TKO-BLT$_{15}$ model. The hIL-15 normalized human NK cell development and subset distribution in TKO-BLT$_{15}$ mice to human-like levels, without impacting other major immune subsets. The mice also had high survival rates and resistance to GVHD for over seven months, consistent with the foundational TKO-BLT model. Following HIV infection, viremia in TKO-BLT$_{15}$ mice was comparable to TKO-BLT mice over 4.5 months. However, their NK cells exhibited enhanced degranulation and proinflammatory cytokine production ex vivo. Interestingly, replication competent provirus in spleen, quantified by tat/rev induced limiting dilution assay, was relatively lower in TKO-BLT$_{15}$ mice compared to TKO-BLT mice. Additionally, TKO-BLT$_{15}$-derived NK cells demonstrated human-like levels of cytotoxicity against cancer and HIV-1-infected CD4+ T cells ex vivo. Antibody-dependent cellular cytotoxicity against cancer cells and HIV-1-infected CD4+ T cells was also enhanced to human-like levels ex vivo. In conclusion, AAV-mediated hIL-15 gene delivery allows the versatile enhancement of human NK cell development and function in humanized mice without the need to maintain a hIL-15 transgenic mouse strain. The TKO-BLT$_{15}$ humanized mouse model will provides an improved platform for our long-term studies aiming to harness NK cells for HIV-1 cure.

68. Targeting B-Raf kinase protects airway epithelial cells from RSV infection and modulates interferon responses

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Introduction: Respiratory syncytial virus (RSV) is the leading cause of hospitalization due to acute bronchiolitis in children under 2 years old. It is responsible for 200,000 deaths annually worldwide. There are no effective antiviral therapies or clinically approved RSV vaccines for children. Dabrafenib is an FDA-approved anticancer drug that inhibits B-Raf kinase. Dabrafenib impairs SARS-CoV-2 and Zika virus replication and ameliorates tissue injury in different inflammatory disease models. Thus, we hypothesized that dabrafenib could be repurposed to treat RSV infection. Methodology: A549 and HBEC3-KT cell viability was measured using MTT assay. A549, HBEC3-KT and HNECs cells were infected with RSV-GFP (0.5 MOI) and treated with dabrafenib at different timepoints. Infection rate, IC_{50}, and fluorescence intensity were quantified by immunofluorescence. Lytic cell death was assessed by measuring LDH release. IFNs secretion was measured using ELISA. RT-qPCR was used to measure ISGs and RSV mRNA expression. The effect of dabrafenib on virus infectivity was determined by plaque assay. Results: Dabrafenib evokes a dose-dependent inhibition (IC_{50} 40.17μM) of RSV infection in A549 cells. Moreover, the drug also decreases infection after different treatments: prophylactic, simultaneous, and therapeutic. In addition, cell death was reduced by 23.4% after dabrafenib treatment and no effect on cell viability was observed. Virus infectivity and the release of infectious progeny virions were decreased by dabrafenib. B-Raf kinase blockade with dabrafenib also diminished RSV infection in immortalized and primary airway epithelial cells (HBEC3-KT and HNECs). The treatment decreased viral mRNA expression by 72% in HNECs. Furthermore, IFIT1 and ISG15 mRNA expression, as well as IFN-β and INF-λ release, were significantly reduced after dabrafenib treatment in both A549 and HBEC3-KT cells, which is consistent with the observed reduction in viral loads. Conclusions: Dabrafenib treatment significantly impairs RSV replication *in vitro*, demonstrating its potential as an antiviral drug against RSV infection.

69. REGULATION OF ANTIGEN-SPECIFIC CD4+ T CELL RESPONSES IN CUTANEOUS LEISHMANIASIS

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Background: Leishmaniasis is a neglected zoonotic disease caused by protozoan parasites transmitted through the bite of an infected female sandfly. Despite its public health burden, no approved vaccine is currently available due to poor understanding of mechanisms in development of memory $CD4^+$ T cell which are key mediators of long-term protective immunity.

Aim: This study determines how antigen dose and T cell receptor (TCR) affinity influence the activation, differentiation, function, and persistence of *Leishmania*-specific $CD4^+$ T cells.

Methodology: We used transgenic (PEG) mice whose CD4+ T cells were engineered to express TCR with distinct affinities and MHC-peptide contact times (TCR16, TCR31, TCR62) for a peptide derived from *Leishmania* phosphenol pyruvate carboxylkinase (PEPCK) antigen. PEG $CD4^+$ T cells were isolated, CFSE-labeled, and co-cultured with dendritic cells in the presence of varying concentrations of $PEPCK_{335-351}$ peptide to assess proliferation and cytokine production. For *in vivo* studies, naive PEG $CD4^+$ T cells were adoptively transferred into congenic recipient mice, followed by immunization with PEPCK peptide and CpG adjuvant. Donor T cells were tracked using congenic markers, and memory responses were assessed by rechallenging mice 50 days later.

Results: TCR16, characterized by the highest affinity and shortest contact time, showed robust proliferation and cytokine production both *in vitro* and *in vivo* following peptide challenge. Under limited antigen availability, TCR62, which has the longest contact time, demonstrated higher cytokine production capacity.

Conclusion: This study provides novel insight into how TCR affinity, contact time and antigen dose collectively influence CD4+ T cell response including memory development. These findings provide important guidance manipulating effective T cell response and designing effective T cell vaccine strategies to elicit durable immunity against cutaneous leishmaniasis.

71. Small Ubiquitin-like MOdifier (SUMO) Accumulates in Equid Alphaherpesvirus 1 (EHV1) Replication Compartments

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EHV1 is prevalent, highly infectious, and causes severe disease outcomes within equids. Primary lytic infection within epithelial cells of the upper respiratory tract leads to infection of recruited monocytes, which then spread EHV1 throughout the body in a cell-associated viremia. Cell-to-cell contacts between infected monocytes and endothelial cells within the vasculature initiates a secondary lytic infection. Associated disease outcomes consequent to endothelial cell infection reflect the infecting strain pathotype. Neurotropic strains infect cells within the vasculature of the central nervous system to cause neurological diseases, including Equine Herpesvirus Myeloencephalopathy (EHM). Conversely, non-neurotropic strains can infect endothelial cells within the vasculature of reproductive organs to cause negative reproductive outcomes, including abortion or neonatal foal death. Molecular mechanisms underlying EHV1 pathotype are not yet understood. However, neurotropic EHV1 spreads more efficiently within epithelial cells, has greater infection of monocytes, and has greater transmission to endothelial cells. Neurotropic strains may thus be more efficient at establishing infection within host cells. We thus hypothesize that neurotropic EHV1 more efficiently antagonizes host intrinsic antiviral defences. To test our hypothesis, we aim to characterize the equine intrinsic antiviral response. As post-translational modification with SUMO (Small Ubiquitin-like MOdifier) plays key roles in intrinsic antiviral responses to herpesviruses, we first evaluated SUMO during infection with neurotropic or non-neurotropic EHV1. Infection with either strain did not notably alter expression levels of SUMO1 or SUMO2/3 isoforms. Interestingly, SUMO1 and SUMO2/3 relocalized to, and accumulated within, EHV1 replication compartments. Thus, we show that there are no broad differences in SUMO expression levels or localization during infection with neurotropic or non-neurotropic EHV1. The accumulation of SUMO within EHV1 replication compartments suggests that EHV1 may less efficiently antagonize SUMO-mediated intrinsic antiviral responses. Alternatively, SUMO may have proviral roles during EHV1 infection.

72. Neutrophil extracellular traps impair vaginal barrier function and increase HIV acquisition in response to non-optimal bacteria species

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The mucosal surface of a healthy female genital tract (FGT) is comprised of physicochemical, immunological and microbial components that serve as a rapid, first line of defense against infections. Alterations of these components have been associated with higher HIV acquisition risk. Analysis from >700 women from the CAPRISA004 cohort show that women with a non-*Lactobacillus* dominant vaginal microbiome were at significantly higher risk of sexual HIV acquisition, and that this strongly correlated with barrier dysfunction, inflammation and neutrophil accumulation. However, how FGT barrier function is impacted by changes in the vaginal microbiome, and a mechanistic understanding of neutrophils in this process, remain unclear. Here, we utilized microscopy and proteomic approaches to better define the interplay between vaginal microbial species, epithelial barrier function and neutrophils *in vivo*. Balb/c mice intravaginally inoculated with *Lactobacillus crispatus* (optimal bacteria species) had little impact on FGT biology, whereas inoculation with *Mobiluncus mulieris* or *Gardnerella vaginalis* (non-optimal bacteria species) induced inflammation, high neutrophil accumulation and epithelial barrier dysfunction *in vivo*. Interestingly, we observe an increase in the release of neutrophil extracellular traps (NETs) shortly after challenge. Degradation of NETs with Dnase I treatment substantially improved vaginal barrier function, implicating NETs as an

important driver of FGT barrier breakdown. Finally, to assess the biological impact of vaginal barrier dysfunction on HIV transmission *in vivo*, humanized mice were intravaginally inoculated with *M. mulieris* and subsequently exposed to weekly HIV challenges. We show that HIV acquisition rates were significantly higher in the presence of *M. mulieris* compared to control groups, indicating that increased HIV translocation across the FGT barrier contribute to systemic viremia. Together, our work provides a mechanistic understanding of how the composition of the vaginal microbiome can alter epithelial barrier function and innate immune responses to modulate HIV risk.

73. Exploring the role of phospholipid influence on antimicrobial resistance phenotypes of *E. coli*

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Introduction: *Escherichia coli* is a World Health Organization critical priority antimicrobial resistance (AMR) Gram-negative bacterial pathogen, which possess a dual membrane cell wall. Despite nearly a century of antimicrobial drug development and study in *E. coli*, drug permeability and membrane entry mechanisms are still largely uncertain. *E. coli* membranes have 3 major types of phospholipids (PL): 70% phosphatidylethanolamine (PE), 20% phosphatidylglycerol (PG) and 5% cardiolipin (CL). Here, I examine how alterations in PL composition influence drug permeability and AMR phenotypes of *E. coli*. I hypothesize that the PL composition, specifically PE, influences AMR phenotypes and permeability in *E. coli*.
Methods: To assess the impact of PL deficits on AMR profiles, *E. coli* K-12 mutants with knockout mutations of key PL biosynthesis genes were examined: AL95 (lacking PE; Δ*pssA*), UE54 (Lacking CL*;* Δ*clsABC*) and BKT12 (Reduced CL and PG; Δ*pgsA*). Antimicrobial susceptibility testing (AST) was conducted on the mutants using a standard 96-well broth microdilution AST method[12] across all classes of antimicrobials. Ongoing experiments are separating inner and outer membranes using gradient ultracentrifugation to verify the lipid and proteomic contents of these ΔPL mutants.
Results: The AL95 had a unique susceptibility profile with a 2048-fold decreased susceptibility to colistin while also exhibiting an 8-fold increased susceptibility to vancomycin. We observed no significant changes (>2-fold) with the other ΔPL mutants. AST profiles of the AL95 mutant shifts the expected *E. coli* antimicrobial susceptibilities of colistin and vancomycin to resemble a Gram-positive. This suggests that PE is an important determining factor for the enhanced Gram-negative susceptibility profiles of *E. coli*.
Conclusion: This study offers the first comprehensive AST analysis and profiling for ΔPL *E. coli* mutants, making this study's findings valuable for future studies exploring novel antimicrobials and AMR mechanisms associated with bacterial lipids and their roles in antimicrobial permeability.

74. The role of T-cell activation in the natural clearance of Human papillomavirus

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Background: Human papillomavirus (HPV) is the most common sexually transmitted infection. In most HPV infections, the immune system can clear the virus within 1-2 years. However, in some individuals, the infection persists. In women, a persistent HPV infection is the cause of 100% of cervical cancer cases. Although HPV vaccination has drastically decreased the development of cervical cancer, vaccine uptake remains suboptimal. To inform therapeutic vaccine development, this study investigates the role of T-cell activation in HPV natural clearance. Specifically, this study will investigate Th17-cell and γδT-cell activation as these pro-inflammatory T cells have been hypothesized to hinder HPV clearance.
Methods: This study will utilize cryopreserved peripheral blood mononuclear cells (PBMCs) from HPV infected female sex workers recruited through the SWOP-HPV Gates cohort in Nairobi, Kenya. This study enrolled 701 individuals (mainly cis-women) who had high-risk HPV at baseline and had HPV testing results and PBMCs cryopreserved at baseline and every 2 months for 1 year. Flow cytometry will analyze the activation and exhaustion

markers on Th17 cells and γδT-cells at baseline and the 12-month follow-up visit (no clearance) or at a clearance timepoint for individuals that clear a high-risk HPV infection during the 12-month period. The hypothesis to be tested is that women who clear HPV infection within 12 months will display less activated Th17 cells and γδT-cells. T-cell profiles of those who were able to clear the HPV infection and those who were not able to clear the infection will be compared.
Results (Current Progress): Study samples have been collected and shipped to Winnipeg for downstream analysis. Flow cytometry methods are being optimized.
Conclusion: By investigating the role of T-cell activation on HPV natural clearance, this study will increase understanding of the immunological response responsible for HPV infection clearance, which can aid in developing a therapeutic HPV vaccine.

75. Antigen stimulation drives clonal expansion the HIV reservoir under ART suppression

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Antiretroviral therapy (ART) successfully reduces mortality rates in people living with HIV (PLWH) by suppressing Human Immunodeficiency Virus-1 (HIV) replication to undetectable levels. However, ART is not curative due to the establishment of the HIV reservoir that continues to persist despite prolonged ART suppression. It has been established that long-lived memory T cells represent the most important HIV reservoir population which is predominantly composed of CD4+ T cell clones that are established early after infection and increase under ART treatment. While factors such as integration site effects have been proposed to induce clonal expansion of HIV-infected T cells, our data points to cognate dendritic cell (DC):T cell interactions as the main driver of clonal expansion of latent T cell subsets through T cell receptor (TCR)-dependent signaling and cytokines that support a pro-survival state in these cells. Depending on the nature of the TCR stimulus, latent T cell proliferation can occur in the absence of virus reactivation. However, a remaining gap in knowledge is how antigen stimulation regulates opposing biological processes of (i) proliferation leading to clonal expansion or (ii) viral production leading to cell death. Here, we utilized a dual-fluorescent HIV latency reporter and antigen-specific human CD4+ T cell clones to modulate TCR signaling magnitude using a panel of altered peptide ligands and directly examined the relationship between TCR signals and proliferative responses by latent T cells. Our preliminary data suggests that low avidity antigen stimulation drives proliferation without viral reactivation in latent T cell subsets while high avidity stimulation promotes virus reactivation and cell death. Our data argue that a critical balance between stimulatory and inhibitory pathways dictate which T cell subsets clonally expand under ART suppression. These studies have implications on stimulatory signals that can be therapeutically targeted to reduce the HIV reservoir size in PLWH.

76. The impact of sex in determining tolerance to pathogenic coronavirus infection

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Middle East respiratory syndrome coronavirus (MERS-CoV), severe acute respiratory syndrome coronavirus-1 (SARS-CoV-1), and -2 (SARS-CoV-2) are highly pathogenic coronaviruses capable of causing lethal illness in humans; however, some individuals exhibit tolerance to infection, displaying mild or asymptomatic disease. Although sex-specific MERS-CoV, SARS-CoV-1 and -2 disease outcomes have been observed, sex is a poorly understood determinant of pathogenicity. Sex bias during infection can result from distinct responses to infection by sex-linked gene expression, sex hormones, or anatomical and physiological variations; however, the specific mechanisms underlying sex biases in infection and pathogenesis are unknown. This project will investigate the mechanistic basis of two primary sex hormones, 17β-estradiol (E_2) and dihydrotestosterone (DHT), in determining tolerance to highly pathogenic human coronavirus infection. We will analyze existing transcriptomic data collected from tolerant and severe models of MERS-CoV infection to interrogate distinct responses driven by sex hormones *in vivo*. To further investigate the impact of sex hormones *in vitro*, we will treat sex hormone-responsive T47D, LNCaP and MCF-7 cells

with E_2 or DHT then infect with SARS-CoV-1, -2 or MERS-CoV to observe effects on viral entry, viral replication and induction of host inflammatory signalling. This study will provide insight into the sex hormone dependent mechanisms and will assess whether these different coronaviruses display a common sex-biased response. By examining the role of sex in determining viral fitness and disease severity, this study will advance our understanding of sex hormones as a biological variable in coronavirus pathogenesis research.

77. Understanding the Interaction Between the Vaginal Microbiome and Host Immune Response in the Progression of Papillomavirus-Mediated Cervical Cancer

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Background: The female genital tract (FGT) is comprised of a specialized immune system that maintains homeostasis, protects against infections and supports reproductive health. A healthy vaginal microbiome is dominated by *Lactobacillus* species but a shift to specific anaerobic bacterial species is associated with inflammation and increased acquisition risk for STBBIs. High risk human papillomavirus (HPV) infection is the leading cause of cervical cancer in women in Canada and across the globe. Current HPV vaccines are strictly prophylactic, indicating that people already infected are at risk of spreading the disease and developing cancer. Studies show strong associations between the presence of inflammation-causing vaginal bacteria and cervical cancer progression, but the mechanistic understanding of HPV+ cervical cancer development is incomplete.
Methodology: Using a mouse model of vaginal mouse papillomavirus (MmuPV1) infection, we aim to understand how specific anerobic bacteria may induce immune cell recruitment, immune activation and cancer development *in vivo*. We will evaluate mucosal T-cell responses in the lower FGT using MHC-I tetramers against MmuPV1 E6 and E7 oncogenes by flow cytometry. We will perform immunohistochemistry to look for cancer progression with markers associated with cervical cancer as p16INKA4a$^+$, suprabasal p63+ localization, Ki67+ in the FGT.
Results: Our studies show that the presence of anaerobic bacteria causes inflammation, epithelial barrier dysfunction *in vivo*. However, we show that the presence of inflammation-associated anaerobes does not enhance susceptibility to vaginal MmuPV1 infection. We are currently evaluating whether these bacteria accelerate MmuPV1-induced cervical cancer development *in vivo*.
Conclusions: Our study will demonstrate the role of vaginal microbiome in HPV mediated cervical cancer progression. It will address the critical role of mucosal T-cells in clearing the viral infection *in vivo*. These studies will provide a mechanistic understanding of how bacteria, and their metabolites, modulate vaginal immunity and affect mucosal T-cells contributing to papillomavirus-mediated cancer development.

78. Determining the impact of iron availability on the physiology of *Morganella morganii*, an emerging superbug

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Morganella morganii, a commensal of humans, fish, and other organisms, is prevalent in environmental, clinical, and agricultural settings. This opportunistic One Health pathogen is garnering attention for causing multidrug-resistant infections ranging from those of the urinary tract and post-operative wounds through meningitis and sepsis. *M. morganii* is a member of the SPICE organisms (*Serratia Providencia*, Indole-positive *Proteus* and others (e.g. *M. morganii*), *Citrobacter*, and *Enterobacter*)), a group of bacteria that initially appear drug-sensitive but express an inducible beta-lactamase which confers resistance to this class of antibiotics upon exposure. Despite its emergence as a potential superbug, vanishingly little is known about the factors contributing to *M. morganii* virulence. Iron availability is a key factor in the pathogenesis of most bacteria. Whilst iron starvation iis commonly investigated and typically results in upregulation of metal import systems and genes involved in the iron sparing response, iron intoxication is less well characterized. We observed that *M. morganii* forms a glucose-repressible unidentified black compound upon exposure to excess exogenous iron. Not only this, but robust transcriptional changes are induced upon iron exposure, including pathways putatively involved in iron detoxification and homeostatic regulation, as well as genes with no known function. Conversely, downregulated pathways include those associated with iron uptake. Given the phenotypic response to iron, transcriptional data, mass spectrometry, and targeted mutagenesis are being used to identify the black compound and elucidate mechanisms used to resist iron toxicity. Further, not all clinical isolates produce this cryptic compound, and thus comparative genomics is being used to help identify contributing loci. We are characterizing the iron-responsive transcriptional profile, reveal the identity of the black substrate, and determine its role in iron homeostasis. Not only will this provide insights into the basic biology of *M. morganii*, it may also inform targetable pathways for future drug development.

79. Role of Apolipoprotein B mRNA Editing Enzyme Catalytic Subunit 3 (APOBEC3) in clade IIb mpox virus evolution and characterizing host responses to mpox virus infection.

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Monkeypox virus (MPXV) clade IIb has caused an outbreak since at least 2016, initially in West Africa and subsequently leading to a global outbreak in 2022. Genomic sequences showed at least 46 SNPs linked to the activity of the cytidine deaminases in the apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3 (APOBEC3) family. This unusual MPXV mutation suggests human APOBEC3 activity, rather than zoonotic spillover. However, the role of APOBEC3s in the evolution of MPXV in humans and rodents, as well as their impacts on host cellular responses, remains unclear.

To assess differences in host responses across species, we investigated how MPXV clade IIb reprograms host transcriptional networks in human and mouse macrophage cell lines, THP-1 and RAW 264.7, respectively. We also examined whether the activity of endogenous APOBEC3 enzymes in these cell lines influences MPXV evolution.

We profiled nucleotide changes in the MPXV genome and transcriptional changes in THP-1 and RAW 264.7 MPXV-infected macrophages over a time course. Although MPXV replicated and produced infectious virus in both human and murine macrophage cell lines, we observed no APOBEC3-like mutations in the sequenced MPXV genomes.

Host transcriptome analysis shows that several interferon-stimulated genes (ISGs), APOBEC3B, and APOBEC3C are upregulated in THP-1-infected cells and are predicted to be negatively regulated by the transcription factor zinc finger protein 750 (ZNF750). Furthermore, upregulation of the vitamin D receptor suggests the role of vitamin D in modulating the innate immune response.

Our data indicate that the expression of APOBEC3s in human cells, but not in mouse cells, suggests that APOBEC3 enzymes may be involved in the evolution and sustained human-to-human transmission of MPXV, rather than in direct zoonotic transmission from rodents to humans. Understanding the specific roles individual APOBEC3s play in MPXV mutations will provide more insight into how the host restricts MPXV and transmission dynamics.

80. Exploring the role of exotoxins in Porcine ear-tip necrosis

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Porcine ear-tip necrosis results in loss of ear tissue in nursery and grower pigs. Lesions occur at the tip of the ear and when severe can result in complete ear loss. We have recently identified *Agent X* as the etiological cause of this disease an obligate anaerobic bacterium. *Agent X* secretes a toxin (>100 kDA) that is hypothesized to be associated with disease pathogenesis in other species. The aim of the experiment was to extract this toxin from a swine strain and evaluate its cytotoxicity to swine cells. *Agent X* was grown anaerobically until OD600 of 1.1. Culture supernatant was extracted by centrifugation followed by filtration through a 0.2 μm membrane-filter. The resulting extract was submitted to double filtration with 30K UltraFilters. Next, the collected filtrate was analyzed by SDS-PAGE. A major band was detected with the double filtrated toxins at the expected size. More work is needed to analyze cytotoxicity of this toxin in porcine cells to further confirm if this toxin plays a role in ETN disease progression.

81. Development of an *in vitro* assay to quantitate alphaherpesvirus transcription regulator binding to naked or chromatinized viral promoters.

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Alphaherpesviruses are prevalent double-stranded DNA viruses. During productive lytic infections, alphaherpesvirus genomes assemble with cellular histones into chromatin that controls viral gene expression. In contrast to cellular chromatin however, the chromatin assembled with herpes simplex virus 1 (HSV1) or equid alphaherpesvirus 1 (EHV1) genomes is highly unstable and dynamic. Only genomes in unstable chromatin are biologically relevant, as they are transcribed.

Alphaherpesvirus transcription is temporally regulated. Immediate early (IE) genes are expressed first, followed by early (E), then late (L) genes. The IE proteins ICP4 (HSV1) or IE1 (EHV1) are the only essential transcription regulators. These homologous proteins can repress or activate transcription and are therefore required for productive infection and progeny virion production. They repress IE gene transcription through sequence-specific promoter binding and activate E and L gene transcription through non-sequence-specific promoter binding. Promoter binding of ICP4 and IE1 was originally characterized using naked DNA fragments before it was understood that viral genomes are chromatinized during lytic infection. However, these proteins must function in the context of chromatin, which regulates access to viral DNA and affects promoter recognition. Therefore, it is important to test whether ICP4 or IE1 bind chromatinized templates directly and preferentially over non-chromatinized DNA to precisely determine how they regulate viral transcription.

We hypothesize that ICP4 or IE1 bind chromatinized viral DNA to regulate transcription. I will test binding of ICP4 or IE1 to chromatinized or non-chromatinized DNA using *in vitro* electromobility shift assays. To this end, I will assemble DNA fragments that contain representative HSV1 IE, E, or L promoters into chromatin with purified recombinant histone octamers. Determining whether ICP4 or IE1 preferentially bind chromatinized or naked DNA will reveal if they directly recognize viral chromatin or require prior chromatin remodeling. This will guide future studies to understand how these proteins regulate viral gene expression.

82. Understanding the role of human microproteins in Zika virus infection

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Zika virus (ZIKV) is an important virus of global public health concern, and its primary mode of transmission is through mosquito bites. ZIKV infection in pregnant women can lead to serious neurological and congenital complications including microcephaly in developing fetus. There are currently no approved drugs or vaccines available against ZIKV. Due to its limited genome coding ability, ZIKV hijacks several host factors during its life cycle. Recently, more than 7000 previously unknown non-canonical small open reading frames were discovered in the human genome, encoding for proteins that are usually <100 amino acids long, termed as “microproteins”. The functions of these microproteins in viral infections are currently unknown. We hypothesize that, human microproteins may serve as host dependency factors modulating ZIKV replication in cells. Our study aims to identify and characterize microproteins that facilitate ZIKV replication.

Using a CRISPR/Cas9 knockout screen in NCI-H23 cells, we identified several microproteins that support ZIKV infection. To validate the hits, two independent guide RNAs were used to knockout the top ten microprotein genes and the corresponding effect on virus infection was measured by viral titrations using plaque assays and viral RNA quantification using qRT-PCR. Knockout of microproteins encoded upstream of ZNF599 (Zinc Finger protein 599), and EMC6 (Endoplasmic Reticulum membrane protein complex subunit 6) as well as a microprotein encoded by a long non-coding RNA, LINC00662, reduced ZIKV titers and viral RNA load significantly, without affecting cell viability

of the knockout cells. We are now further investigating the roles of these microproteins in ZIKV infection, as well as with other flaviviruses, to test whether these microproteins have a pan-flaviviral phenotype.
Identifying the unexplored functions of microproteins in the context of viral infections can reveal novel roles of microproteins as well as provide novel insights into ZIKV biology. This may also lead to development of new host-directed antivirals.

83. Transformation of *Gardnerella vaginalis* with a *Bifidobacterium-Escherichia coli* shuttle vector plasmid

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Gardnerella spp. significantly influence female reproductive health, and are indicators of bacterial vaginosis, a common gynecological disorder. Lack of genetic tools for *Gardnerella* spp. is a hindrance in fully understanding their role in the vaginal microbiome and no naturally occurring plasmids have yet been identified in these organisms. The aim of this study was to transform *Gardnerella vaginalis* and characterize transformants carrying *Bifidobacterium-E. coli* shuttle vector pKO403-*lacZ*'-Sp. *G. vaginalis* ATCC 49145 was selected for protocol development based on its high growth rate, lack of restriction activity and susceptibility to spectinomycin. Low efficiency (~102 cfu/µg of plasmid DNA) but reproducible transformation was achieved. The expression of the spectinomycin resistance gene and the β-galactosidase gene of pKO403-*lacZ*'-Sp in *G. vaginalis* ATCC 49145 resulted in an increase in spectinomycin tolerance from 2 µg/ml (MIC) to >512 µg/ml, and an appreciable increase in β-galactosidase activity compared to the wild type. Plasmid copy number was determined to be ~3 per genome copy. Plasmid was lost rapidly in the absence of spectinomycin selection, with only ~13% of colony forming units retaining the resistant phenotype after 24 h of growth without selection. These results demonstrate that *G. vaginalis* can be transformed by electroporation and that pKO403-*lacZ*'-Sp can be maintained and its genes expressed in this host, offering a starting point for the development of genetic tools for mechanistic studies of this important member of the vaginal microbiome.

84. Investigating interferon lambda as a potential antiviral therapy against respiratory syncytial virus infection

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Introduction: Accounting for over 2% of all deaths of children under 5 years old, respiratory syncytial virus (RSV) poses a significant burden to global pediatric health. RSV infection has been linked to persistent lung disease in the form of recurrent wheeze and asthma. Despite extensive research efforts, there is still a lack of effective treatments against RSV specifically for children. Interferon lambdas (IFNLs) are secreted following viral infections in mucosal surfaces, and treatment with IFNLs is protective against other respiratory viral infections, such as SARS-CoV-2 and influenza A virus. This study aims to investigate the effect of IFNL3 as an antiviral agent against RSV infection in human bronchial epithelial cells.
Methods: Immortalized bronchial epithelial cells (HBEC3-KT) and monocyte-derived macrophages were cultured and infected with RSV-GFP, which allows for visualization of infection over time. Cells were treated with different concentrations of IFNL3 either prophylactically or therapeutically to investigate the protective effect of IFNL3 on RSV infection. Cells were also treated with IFNβ to compare the protective effect of type I and type III interferons on RSV infection. RSV infection was visualized using fluorescence microscopy for GFP expression and replication was quantified with a classical plaque assay. RSV fusion protein expression was assessed with western blot. The expression of interferon-stimulated genes (ISGs) was measured via qPCR.
Results: Prophylactic treatment with IFNL3 inhibited RSV infection HBEC3-KT and macrophages in a concentration-dependent manner, while therapeutic treatment with IFNL3 did not protect cells from RSV infection. Prophylactic IFNL3 treatment also greatly reduced RSV titre compared with untreated cells. Prophylactic IFNβ treatment confers greater inhibition of RSV infection compared to IFNL3 treatment. Additionally, IFNL3 potently induced baseline IFIT1

and ISG15 expression compared with untreated HBEC3-KT cells. IFNL3 also upregulated IFIT1 and ISG15 expressions during RSV infection compared with untreated infected cells.

85. Modulation of the RIG-I dependent IFNβ response by Highly Pathogenic Avian Influenza H5N1 virus NS1 protein

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In the spring of 2024, a highly pathogenic avian influenza (HPAI) H5N1 strain was reported in dairy cattle in Texas, USA. This strain is widely spread among aquatic birds. Moreover, the strain acquired the ability to spill over to mammals. The strain has shown a high infectious capacity among and between cattle herds affecting their milk production. The spillover has spread to other animals such as cats, goats and alpacas. Importantly, human cases have been reported with the majority presenting influenza-like symptoms and one death reported this year. The highly infectious capacity of HPAI H5N1 has raised concerns regarding its pathogenic and immune escape ability in mammals. The non-structural protein NS1 from influenza A virus (IAV) is a key factor for pathogenicity due to its multiple functions and plasticity to improve IAV replication and immune evasion. One of the primary functions of NS1 is the inhibition of type I IFN induction affecting IAV replication. Here, we described the potency of the H5N1 NS1 to inhibit the IFNβ response, in a RIG-I dependent manner, compared to NS1 derived from seasonal or pandemic strains. We also observed a profound downregulation of RIG-I protein by H5N1 NS1. Understanding the molecular mechanism by which NS1 regulates innate immune response will advance our understanding of the virus's spillover potential and its pathogenicity in mammalian hosts.

86. Characterizing polymicrobial sepsis induced early immune T cell activation and function.

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Introduction: Sepsis is a dysregulated host response to infections and can lead to organ failure. Sepsis has an early phase of hyperinflammatory and suppressive/weakened immune responses, and a late phase of immunosuppression, with higher susceptibility to infections and dysfunctional immune T cells. The immunobiology of how sepsis-induced immunosuppression develops is unclear. We aimed to examine polymicrobial induced T cell activation and function in a pre-clinical sepsis model.

Methods: In a fecal-induced peritonitis (FIP) sepsis model, 8–10-week-old C57BL/6N mice were injected with fecal slurry (50mg/mL) and compared to a vehicle control group. A 4-hour early sepsis (n=3 per sex) and 2- week late sepsis models were used. We assessed disease severity via a quantitative clinically relevant modified Murine Sepsis Score, body temperature, and ePOC analysis. T cells in the blood, spleen, mesenteric lymph nodes, kidneys, liver and muscle were examined using multiparameter flow cytometry. Bacterial loads were enumerated in blood and peritoneal lavage fluid. Sera cytokines were quantified using a multiplex cytokine assay. T cell depletion was performed in male mice (n=6) in the 4-hour model using monoclonal α-CD3 vs. isotype control antibodies (n=4). Unpaired t tests and Two-way ANOVA tests performed for significance. Spatial RNA-sequencing will be performed on tissue sections in the future.

Results: We found early T cell activation in multiple organs at 4-hours, with males displaying higher activated T cell counts compared to females and elevated levels of pro-inflammatory cytokines. At 2 weeks, septic animals showed features of immunosuppression. T cell depleted septic mice showed increased disease severity and lowered bacterial loads compared to T cell-competent mice.

Conclusion: In the FIP model, T cells are activated by bacterial sepsis as early as 4 hours post induction and show signs of immunosuppression at 2 weeks post induction. Absence of T cells exacerbates disease severity despite promoting bacterial clearance.

87. Exploring the Microbiota-Host Dynamic through the Lens of Membrane Vesicles

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Introduction: The vaginal microbiota (VMB) can drastically influence female reproductive health. Some bacteria, like *Lactobacillus* sp. have been shown to promote vaginal health and limit pathogen invasion, while other species, like *Gardnerella vaginalis*, can disrupt the stability of this microenvironment and increase susceptibility to infection. The project focus narrows in on lipid nanoparticles released by bacteria, called membrane vesicles (MVs), which are released by all bacteria and can shuttle biomolecules like DNA, RNA, proteins, and metabolites. These MVs have been implicated in bacteria-host communication, but little research has been done for VMB derived MVs. The project objective is to develop an MV isolation protocol for *Lactobacillus acidophilus, Limosilactobacillus reuteri,* and *Gardnerella vaginalis*, then characterise their RNA and protein content.

Methods: MVs were isolated using ultracentrifugation. MV size and concentration was characterised using a Nanosight particle tracker and images were acquired using cryo-TEM. RNA extraction from MVs and bacterial cells was done using the phenol to lyse the cells/MVs and purify RNA. RNA sequencing was conducted using the Illumina platform. Protein will be profiled using shot-gun LC-MS/MS.

Results: We continued to optimize the isolation of MVs from *G. vaginalis* and RNA from *L. acidophilus* MVs. Preliminary data of MVs isolated from *L. reuteri* demonstrated a significant amount rRNA (n=1). Ribosomal RNA will need to be depleted in future samples to better characterise lesser abundant transcripts in following RNA samples. Preliminary analysis showed that MVs had different transcriptomic profiles compared to the cell lysates and in turn contained a wide variety of transcripts that could have potential biological implications for bacteria-host communication.

Conclusion: Although work is still in progress to better isolate MVs from bacterial culture, these preliminary data will guide our future work.

88. Reovirus induces citrullination of host cell proteins which modify enzyme properties for a rapid inflammatory response.

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Citrullination is a post-translational modification that has the potential to alter how a protein folds, interact with ligands and other proteins. This post-translational modification renders proteins antigenic in the autoimmune disease rheumatoid arthritis, but it has also been detected as part of the inflammatory response to viral infections. Calcium-dependant peptidyl arginine deiminases (PADs) are the enzymes responsible for modifying an arginine residue into a citrulline residue, making these enzymes potential therapeutic targets, as it has been shown that citrullination is important for entry of several viruses. Although aberrant levels of citrullination occur in disease, the base levels of citrullination or if environmental factors can alter it remain unclear.

In our lab we have used reovirus infection model consisting of subtypes T1L and T3D in mouse fibroblast cell line L929. We found that serine hydrolase activity peaks coincide with high levels of citrullination, as enzymes belonging to the serine hydrolase family are involved in many cellular processes during viral infection, including cell death and virus assembly. We used specific enzyme assays, citrulline-specific probes, the OLINK cytokine and interleukin detection technology, and deep bidimensional HPLC coupled to mass spectrometry and found that enzyme activity peaks and citrullination accompanied inflammatory response. Citrullinated proteins including enzymes of different families including serine hydrolases and cysteine proteases were detected as early as 6 hours post infection.

These results show that in-situ modification rather than de-novo expression of enzymes is used to accelerate an inflammatory response to virus and that these responses were different for both subtypes. Enzyme activity was modulated by calcium availability which suggests that virus uses citrullination as another way to manipulate cellular responses that can be controlled by secondary messengers like calcium.

89. INVESTIGATING THE INTERPLAY BETWEEN THE VAGINAL MICROBIOTA AND PAPILLOMAVIRUS INFECTION IN VIVO

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Background: Human papillomavirus (HPV) infection is one of the most common sexually transmitted diseases worldwide and the causative factor for most cervical cancers. We and others have reported strong associations between the vaginal microbiome and HPV acquisition, persistence, and cervical neoplasm in women. Notably, a polymicrobial vaginal microbiota dominated by *Gardnerella*, *Prevotella* and *Mobiluncus* spp, among others, is common in bacterial vaginosis (BV) and is associated with high-risk HPV (HR-HPV) persistence and high-grade lesions. In contrast, *a Lactobacillus* spp. dominant vaginal microbiota is associated with protection against HR-HPV infection.

Purpose: Our project objective is to define a mechanistic role for cervical cancer-associated bacteria in enhancing papillomavirus replication and tumor development. We hypothesize that BV-associated bacteria directly enhance HPV infection and accelerate cervical cancer development by inducing a prolonged inflammatory state within the female reproductive tract (FRT).

Methods: We have previously described a BV-like mouse model that recapitulates key biological features of vaginal inflammation, epithelial barrier function and immune activation. We have overlayed the vaginal murine papillomavirus (MmuPV-1) infection challenge model to address how BV-associated bacteria impacts papillomavirus infection in the FRT. Our primary readouts include: (1) viral DNA/RNA measurements in vaginal lavage by qPCR and: (2) immunohistochemistry to localize infected cells with markers of heightened cell proliferation, abnormal epithelial differentiation, biomarkers of cervical cancer development.

Results and Conclusion: MmuPV-1 viral load and persistence dynamics was not altered in the presence of BV-associated bacteria species, and mice proceeded to cervical cancer development within 4-6 months. While *M. mulieris* inoculated challenge did not significantly increase viral replication in the lower FRT, the presence of *L. crispatus* showed a remarkable reduction in MmuPV1 replication. Current studies are focused on determining whether reduction in viral replication kinetics reduces cervical cancer progression. These studies represent efforts to better understand the mechanistic underpinnings of bacteria/viral co-infections during cervical cancer development.

90. Porcine β-defensin 5 (pBD-5) enhances mitochondrial activity and modulates TNF-α gene expression in immortalized swine B-cells

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Our team recently discovered the porcine β-defensin 5 (pBD-5), a host defense peptide with putative immunomodulatory properties. This study evaluated its effects on immortalized swine B-cells, focusing on cytotoxicity, mitochondrial activity, gene expression, and viability following an enterotoxigenic *Escherichia coli* (ETEC) F4 challenge. Cells were exposed to pBD-5 (0–1600 μM), and cytotoxicity was evaluated by quantifying lactate dehydrogenase (LDH) activity in the culture supernatant, indicative of cell membrane disruption. Mitochondrial function was assessed through the WST-1 assay, which measures the cleavage of tetrazolium salts by mitochondrial dehydrogenases in metabolically active cells. Transcriptional responses of TNF-α were determined using RT-qPCR. All assessments were done after one and 24 hours of exposure to the varying concentrations of pBD-5. B-cells were incubated for 24 hours with pBD-5 (1600 μM), LPS (40 ng/mL), ETEC F4 (4×10^4 CFU/mL), or their combinations, and contrasted to a control group. B-cell viability was assessed via trypan blue exclusion by a researcher blinded treatment identity. After 24 hours, pBD-5 significantly reduced LDH release at concentrations between 200 and 800 μM. A concentration-dependent increase in mitochondrial enzymatic activity was observed, plateauing at ≥200 μM (*P*<0.001), indicating increased bioenergetic capacity. TNF-α transcript levels were significantly downregulated at 100–200 μM (*P*<0.05), suggesting immunomodulatory signs. Following ETEC F4 exposure, pre-treatment with pBD-5 resulted in a numerical increase in B-cell viability (29.44% ±35.07) compared to untreated controls (9.37% ±13.68), though this difference was not statistically significant (*P*=0.1656). While these findings suggest that pBD-5 may influence B-cell metabolic activity and transcriptional regulation, no conclusive evidence was found to support a direct protective effect on cell viability under bacterial challenge. Further investigation is warranted to clarify these preliminary observations and explore underlying mechanisms.

91. Expansion of genital Tregs reduces neutrophil influx and maintains mucosal barrier integrity during inflammatory bacteria challenge.

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Introduction: Genital inflammation is associated with increased HIV risk. Expanding FGT Tregs is a potential strategy to reduce genital inflammation and HIV acquisition risk. In support of this, we recently showed that endocervical Tregs were associated with decreased genital inflammation and a lower abundance of HIV target cells. IL-2 conjugated to IL2mAb clone JES6-1 (IL-2C) has been shown to preferentially expand Tregs. Here we, hypothesized that by increasing the frequencies and functions of Tregs in the FGT using IL2C, the tissue would be better able to regulate genital inflammation induced by inflammatory stimuli and preserve mucosal barrier integrity.
Methods: The estrous synchronized female FoxP3^{GFP} mice were randomized into three groups: those that received eight vaginal (n=6) or intraperitoneal (n=7) doses of 6μg IL2C and controls that received vaginal PBS (n=6). Following dosing, mice were euthanized to obtain the lower FGT and spleen for immunohistochemistry and flow cytometry.
Results: we show that intraperitoneal (IP) administration of IL-2C in estrous-synchronized female FoxP3^{GFP} mice induced approximately 4.5- and 80-fold increases of CD4$^+$GFP$^+$Tregs in the lower FGT (frequencies and counts, respectively), with a similar trend being observed when Tregs were defined conventionally as CD25$^+$CD127^{lo}. This was confirmed by immunofluorescence analyses. In addition, IL-2C resulted in an approximately two-fold increase in the mean fluorescence intensity (MFI) of GITR expression on Tregs, with the majority adopting CD103$^+$CD69$^+$ phenotype. In contrast, IL-2C had limited effects on non-Treg populations such as NK1.1$^+$NK cells, CCR6$^+$Th17 cells, and CD4$^+$CXCR3$^+$T cells in the lower FGT. In mice exposed to N-9, pre-treatment with IL-2C reduced neutrophil infiltration into the vaginal tissue but had no impact on epithelial permeability. Conversely, in mice exposed to *Mobiluncus mulieris*, IL-2C pre-treatment maintained higher E-cadherin expression and prevented both neutrophil influx and mucosal barrier disruption. We further demonstrated that IL2C effects were mediated by Tregs by using mice, where depletion of Treg in FoxP3^{DTR} abrogated the beneficial effects of IL-2C, in particular the neutrophil influx. These data shows that, induction of Tregs using IL-2C could represent a potential strategy to regulate genital inflammation to both reduce HIV acquisition risk and improve reproductive health outcomes in women.

92. Characterization of Adaptive Immune Responses for HPV Clearance Among Female Sex Workers in Kenya

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Background: The inability to clear high-risk human papillomavirus (HR-HPV), particularly types 16 and 18, is a major driver of cervical cancer, which disproportionately affects women in low and middle-income countries (LMICs).

Although most HPV infections resolve spontaneously, the immune mechanisms underlying clearance remain poorly defined. Among female sex workers (FSWs) in Kenya, who often present with multiple HPV infections and a high prevalence of HIV, understanding adaptive immune responses is critical for informing HPV therapeutic vaccine development and cervical cancer prevention. We hypothesize that HPV clearance is mediated by stronger antigen-specific IgG, IgA and T cell responses, whereas persistence, especially in HIV positive individuals, is associated with impaired T-cell function.

Methods: A longitudinal cohort of 702 FSWs (18% HIV positive) was enrolled through the Sex Worker Outreach Program (SWOP) in Nairobi. Participants were followed bi-monthly over 12 months with concurrent HPV typing and STI testing. HPV-specific humoral responses (IgG to L1 and L2, antigens for HPV types 6/11/16/18) were assessed using a Luminex-based multiplex serology assay. Memory T cells and functional cell-mediated immunity will be evaluated on the samples collected at baseline, after clearance and after 12 months using flow cytometry in PBMCs stimulated with HPV 16/18/35/58 E6/E7 peptide pools.

Results: We have developed the Luminex-based multiplex serology assay and tested this on reference sera, negative controls and healthy controls. We have also developed and are currently optimizing the panel for the characterization of the cell-mediated immune responses to HPV 16/18/35 E6/E7 peptide pools. Next steps will be to apply these assays to participant samples which have been collected.

Conclusion: This study seeks to elucidate the adaptive immune responses associated with natural HPV clearance in a high-risk population. Findings will contribute insights for the rational design of HPV therapeutic vaccines, especially for immunocompromised or under-vaccinated populations in LMICs.

93. Investigation of Virus and Host Factors Contributing to Efficient Replication of Highly Pathogenic Avian Influenza (H5N1) Virus in Cattle

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Infection of Highly Pathogenic Avian Influenza (HPAI) viruses (H5N1) are usually limited to poultry and aquatic birds; however, recently HPAI infection spilled over to cattle in the USA and caused severe reduction in milk production in lactating cows. HPAI infection of bovine mammary glands elaborated the need for urgent studies on understanding the mechanisms of infection in this tissue, which is essential to develop strategies to control virus infection. Influenza A virus (IAV) mutates its genome and exploits host factor to facilitate the adaptation to a new host. The genome of IAV consists of 8 negative-sense single stranded RNA segments, which is replicated by viral RNA-dependent RNA polymerase (RdRp), a heterotrimer consisting of PB2, PB1 and PA. The RdRp requires the host factor Acidic Nuclear Phosphoproteins (ANP32) for efficient activity. There are three ANP32 proteins in bovine (ANP32A, ANP32B and ANP32E) and the role of the individual ANP32 proteins in supporting bovine HPAI H5N infection and host adaptation remains unknown. We generated knockouts of ANP32A, ANP32B and ANP32E in bovine mammary gland epithelial cells (MACT) using CRISPR genome editing. Combining with influenza polymerase minigenome assay, we showed that knock out ANP32A reduced RdRp activity, indicating it is required for bovine H5N1 replication. We also investigated the impact of specific mutations in PB2 protein on RdRp activity. We found that albeit lacking well-characterized mammalian adaptation mutation at amino acid 627 and 701, the bovine H5N1 virus utilizes amino 631L for efficient replication in MACT cells. Altogether, these results contribute to our understanding of the mechanisms of HPAI infection in cattle.

94. Interferon beta specificity in humans and bats contributes to differences in viral replication and tolerance.

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Background: Bats are ancestral hosts of multiple zoonotic viruses, including betacoronaviruses (βCoVs) that cause severe disease and death in humans and livestock. Conversely, bats infected with these viruses do not show clinical signs of disease, making them a fascinating model to study the evolution of virus-host interactions. The innate immune response is the first line of defense against viral infections. Type I interferons (IFNs) are among the first cytokines to be released when an infection is detected in vertebrates. IFNs bind to the interferon-α/β receptor (IFNAR1/2) in host cells and induce the production of antiviral Interferon Stimulated Genes (ISGs). βCoVs have evolved to impair type I IFN activity in humans, increasing our vulnerability to infections. Little is known about how type I IFNs signal in bat cells and whether bats have evolved more efficient processes to better tolerate viral infections.
Methods: We have identified that wildtype bat IFNβ do not protect human cells and vice versa, suggesting species-specific mechanisms. Based on computational modelling and positive selection analyses of IFNβ sequences from several mammals, we have produced mutant human and bat (*Eptesicus fuscus* and *Pteropus alecto*) recombinant IFNβ to test their antiviral potency against βCoVs.
Results: Infection studies show that IFNβ with two specific point mutations have lower antiviral capacity compared to wildtype IFNβ in species-matched cells for both humans and bat species. Mutated IFNβ cannot effectively phosphorylate STAT transcription factors, suggesting a failure to prime IFNAR1/2. These results are supported by structural modelling showing significant changes on binding interface properties for mutated IFNβ-IFNAR1/2.
Conclusions: The observed differential antiviral protection suggests that our identified amino acid residues are key determinants of IFNβ-mediated protection. Our study identifies remarkable species-specific adaptation of IFNβ and downstream antiviral processes which will inform basic and translational science for the development of IFNβ antiviral therapies for humans.

95. Unraveling the regulatory network of a crucial protein involved in Lyme Borreliosis

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Lyme disease, caused by the spirochetal bacterium *Borrelia burgdorferi*, is the most prevalent tick-borne illness in the Northern Hemisphere, with over 400,000 new cases annually in the United States. Despite its significant public health impact, no effective human vaccine is currently available. *B. burgdorferi* exists in an enzootic cycle involving a hard *Ixodes* tick vector and a susceptible vertebrate host. Transmission to vertebrates occurs through tick bites, requiring rapid and precise adaptation by the bacterium in response to varying environmental cues. This adaptation is governed by differential gene expression, with the alternative sigma factor RpoS essential for vertebrate infection. Tight regulation of RpoS is essential, as its overexpression is lethal to the spirochete. Our research aims to elucidate the regulatory network of BBD18, a protein in *B. burgdorferi* which acts as a negative regulator of *rpoS*/RpoS. Preliminary data suggests that BBD18 is a DNA or RNA binding protein which influences the expression of 15 other genes. We have further identified a 49 bp conserved motif located upstream of these 15 genes which we hypothesize is bound by BBD18 for gene regulation. Since RpoS is the critical component for *B. burgdorferi's* vertebrate adapted response, we aim to further define the BBD18 regulon. To determine the ability of BBD18 to bind nucleic acids and its impact on RpoS regulation, we are performing electromobility shift assays (EMSAs) using DNA/RNA probes corresponding to the conserved motif and the postulated regulatory regions of the 15 differentially regulated genes. The results of this study will advance our understanding of *B. burgdorferi*'s pathogenic mechanisms.

96. Variable Structure in HIV-1 Vif Amino Acid Sequences Impacts Degradation of Host APOBEC3F and APOBEC3G Enzymes

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APOBEC3 enzymes (named APOBEC3A-H, excluding E) are host restriction factors that can inhibit HIV-1 replication via deoxycytidine deamination of (-)DNA through formation of promutagenic deoxyuridine. As a countermeasure, HIV-1 Vif protein induces degradation of human APOBEC3 enzymes through the formation of an E3 ubiquitin ligase complex with host molecules to ubiquitylate APOBEC3s and induce their degradation. Vif also interacts with the co-transcription factor CBF-ß for thermodynamic stability. APOBEC3F and APOBEC3G restrict HIV-1 most strongly and have distinct interactions with Vif. APOBEC3F interacts with both CBF-ß and Vif, but APOBEC3G interacts only with Vif. HIV-1 Subtype B and Subtype C Vif proteins have different amino acid sequences, and we hypothesized that this would lead to different abilities to degrade APOBEC3 enzymes. To test the hypothesis, we used site-directed mutagenesis to introduce commonly found HIV-1 Subtype C-like Vif amino acids into HIV-1 Subtype B LAI Vif. Co-expression of the HIV-1 LAI Vif mutants with APOBEC3G, APOBEC3F or both APOBEC3F/APOBEC3G, to enable their hetero-oligomerization, allowed for detection of Vif-mediated degradation through subsequent western blotting. One particular mutation of methionine to leucine at position 8 to make HIV-1 LAI-Vif Subtype C-like decreased degradation of APOBEC3F when expressed with APOBEC3G. This amino acid is within the Vif/CBF-ß interface and supports a hypothesis that Subtype C virus Vifs may interact less strongly with CBF-ß resulting in less ability to induce degradation of APOBEC3F when it is bound to APOBEC3G.

97. Uncovering Diversity among Penicillin Binding Protein Genes in Canadian *Helicobacter pylori* Isolates

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Introduction: *Helicobacter pylori* is a Gram-negative, microaerophilic bacteria that can infect the gastric epithelium to cause gastritis, peptic ulcers, gastric cancer and mucosa associated lymphoid tissue lymphoma. In Canada, *H. pylori* colonization is estimated to occur in 38% of the population with a higher prevalence among immigrant, Indigenous and racialized Canadians. The rapid mutation rate and high prevalence of *H. pylori* has resulted in rising levels of antimicrobial resistance. Amoxicillin (AMX) is a -lactam antibiotic used in several multi-drug treatment regimens for *H. pylori*. Resistance to AMX is associated with mutations, including single nucleotide variants (SNVs), insertions and deletions (indels) in the *pbp1, pbp2* and *pbp3* genes. Here, we describe a genomics approach for characterization of *pbp* diversity among Canadian isolate of *H. pylori*.
Methods: Genomic DNA was extracted from Canadian clinical *H. pylori* isolates and sequenced using the Illumina MiSeq platform. A Galaxy pipeline was used for quality control and *de novo* genome assembly. Seven genes (*atpA, efp, mutY, ppa, trpC, urel, yphC*) were used for multilocus sequence typing and phylogenetic comparison of *H. pylori* lineages. Sequences for the *pbp1, pbp2 and pbp3* genes were extracted from genome assemblies and analysed for mutations using a custom R script.
Results: The analyzed *pbp1, pbp2 and pbp3* genes show a high degree of diversity between isolates with numerous SNVs and indels detected. Phylogenetic analysis of concatenated MLST genes reinforces the diversity observed among *H. pylori* isolates from Canada.
Conclusions: Canadian *H. pylori* isolates are diverse both in terms of their *pbp* and MLST genes. Variations and indels found in PBP1, PBP2 and PBP3 protein sequences could result in AMX resistance. Antibiotic susceptibility testing is required to evaluate the role of specific mutations on AMX resistance. Genomics-based analysis of *pbp* diversity among Canadian *H. pylori* isolates may potentially uncover the prevalence of AMX resistance and serve as the basis for implementing effective antibiotic treatments.

98. The antiviral breadth of bat TRIM5a-like restriction factors

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Background: Bats are being recognized as reservoir hosts of several viruses yet show minimal or no negative effects of viral infections. This study investigates the mechanisms behind the tolerance of bat cells to viruses by examining TRIM5a-like proteins in two divergent bat species-Eptesicus fuscus and Pteropus alecto.
Methods: We identified TRIM5a-like genes in the two bat species and compared with human TRIM5a gene expression levels and subcellular localization in respective cells. We tested the interactions of bat TRIM5a-like proteins with the capsids of various retroviruses -Hervey pteropid gammaretrovirus (HPG) -a Koala-Retrovirus (KoRV) related retrovirus, Human Immunodeficiency Virus (HIV), Simian Immunodeficiency Virus (SIV), Feline Immunodeficiency Virus (FIV), B- and N-tropic Murine Leukemia Virus (B-MLV and N-MLV). We also assessed the antiviral potency of bat TRIM5a-like proteins against flaviviruses -Tickborne encephalitis virus (TBEV), Yellow fever virus (YFV), and Rio bravo virus (RBV).
Results: The expression of bat TRIM5a-like gene is inducible by species-specific interferon-ß with cytoplasmic localization like human TRIM5a, indicating that endogenous bat TRIM5a-like proteins function as interferon-stimulated genes (ISGs) in bat cells. Bat TRIM5a-like proteins restricted SIV, FIV, HPG and N-MLV pseudotyped viruses but did not inhibit the replication of TBEV, YFV, and RBV wildtype viruses.
Conclusions: The study compares the efficacy of human TRIM5a with two bat-derived TRIM5a-like proteins against a panel of retroviral capsids and flaviviruses which enables the assessment of the breadth and specificity of the antiviral activities mediated by these proteins to seek insights into their evolutionary adaptations and capacity to block cross-species transmission. Significance - This study explores molecular factors that contribute to the occurrence of KoRV-related exogenous retroviruses in bat populations in Australia and provides insights into the role bats could play as reservoirs for KoRV-related gammaretroviruses during the ongoing KoRV epidemic in the Australian Koala population.

99. High Level of Short Chain Fatty Acids Disrupt Cervicovaginal Epithelial Integrity and Promote Inflammation

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Background: Short-chain fatty acids (SCFAs), such as butyrate and propionate, are recognized for their beneficial roles in gut health and immune regulation. However, their impact on mucosal surfaces outside the gut, particularly the cervicovaginal epithelium, remains poorly understood. Elevated SCFA levels in vaginal fluid have been linked to dysbiosis and a higher risk of sexually transmitted infections (STIs), including HIV-1 and HPV. This study investigates the impact of SCFAs on mucosal barrier integrity and immune activation in cervicovaginal epithelial cells.
Methods: Using air-liquid interface cultures of ectocervical (Ect1) and vaginal (Vk2) cell lines, we assessed the impact of SCFAs on epithelial barrier function and immune activation. Epithelial cells were pre-treated with SCFAs and then stimulated with Poly(I: C) to simulate viral infection. We measured trans-epithelial electrical resistance (TEER) and dextran permeability to evaluate barrier integrity. Additionally, we used confocal microscopy to examine tight junction integrity and cellular death, while gene expression was quantified using RT-qPCR. Additionally, the effects of SCFAs on epigenetic mechanisms were assessed by analyzing the acetylation of histone and NFκB(p65).

Results: We show that a high concentration of butyrate (20mM) significantly disrupted epithelial barrier integrity, as indicated by a decrease in TEER (twice lower, $p < 0.05$) and a four-fold increase in dextran transmigration ($p < 0.01$). This dysfunction correlated with impairment of E-cadherin membrane localization and increased apoptosis (>2-fold, $p < 0.01$). SCFAs treatment or pre-treatment followed by Poly(I:C) stimulation upregulated the level of *p21, Noxa*, and *TNF-α* transcripts (by >2-fold and $p < 0.05$, multiple comparison adjusted). Additionally, SCFAs increased histone-3 acetylation at lysine-9 and NFκB (p65), likely linked to increased *TNF-α, p21*, and *Noxa* transcripts.
Conclusion: While SCFAs are generally anti-inflammatory in the gut, our findings indicate a contrasting role in the cervicovaginal mucosa. Therefore, increasing the systemic concentration of SCFAs might have undesirable consequences for females.

100. Analysis of Eastern Equine Encephalitis Virus NSP1 protein's interaction with cellular vATPase complex

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Eastern equine encephalitis virus (EEEV) is a mosquito-transmitted pathogen endemic to eastern regions of Canada and the United States. The virus circulates in nature between birds and mosquitoes. During infection in humans, EEEV can cross the blood-brain barrier and cause fatal encephalitis. No antiviral therapies or vaccines are currently available to manage this disease in humans. Previously, the Kumar lab mapped the host protein interactome of all EEEV-encoded proteins using affinity purification and mass spectrometry. This study aims to investigate the biological relevance of one of those interactions between the viral-encoded NSP1 and the components of the cellular vATPase complex. NSP1 is required for efficient translation of viral proteins and in evasion of host immune responses by capping viral RNAs. Vacuolar ATPases have known roles in the acidification of intracellular vesicles, membrane traffic, protein processing and degradation. Therefore, we hypothesize that the interaction of NSP1 with vATPase machinery may enhance viral replication fitness through modulation of membrane traffic and protein metabolism in infected cells. Current work includes validation of this interaction via co-immunoprecipitation and western blot assays, along with subcellular localization through immunofluorescence. Further studies will examine the functional consequences of this interaction on viral replication and assess the impact of NSP1 expression on cellular pathways modulated by vATPases. This research will contribute to a deeper understanding of host-pathogen interactions by uncovering novel aspects of EEEV biology.

101. A humanized mouse model to study immature immune responses to Respiratory Syncytial Virus infection

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Respiratory syncytial virus (RSV) is the most common cause of lower respiratory tract disease in children aged 1-3 years old. Approximately 30-70% of infants and children infected with RSV develop bronchitis, resulting in hospitalization of 3% of these cases. Premature and immunocompromised infants are at a higher risk of developing severe RSV infection, which can lead to respiratory issues such as bronchiolitis and asthma. During the neonatal immune response to RSV there is reduced viral clearance and a Th2 skewed response that contributes to pathogenesis. Currently, there are limited options for approved treatment methods and no approved RSV vaccine for infants. The study of severe RSV disease has been hindered by difficulties in working with pediatric human cohorts. Neonatal mouse models of RSV have provided multiple insights into the potential mechanisms of severe RSV disease. However, no animal perfectly mimics a human immune response. Therefore, we require additional validation and study in human systems. We utilized our humanized mouse model with a highly complete and functional human immune system with subcutaneously implanted autologous human lung implants (TKO-BLT-L), for the study of the immune response to RSV. We found that RSV was cleared from the lungs by day-14 post viral challenge. There were significantly increased levels of both IgG2 and IgA in the lung organoids by 28 days-post challenge. Additionally, both type 1 and type 2 cytokines were produced with sustained type 2 cytokine production at day-28 post challenge. Overall, our TKO-BLT-L mouse model of RSV infection may recapitulate aspects of severe neonatal RSV disease. We aim to further utilize this model to study the immunopathology of RSV and for the development and pre-clinical testing of therapeutics.

102. Characterization of Host Response to Highly Pathogenic Avian Influenza (HPAI) A (H5N1) Infection in Bovine Mammary Gland

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Highly pathogenic avian influenza (HPAI) H5N1 clade 2.3.4.4b was introduced into North America in 2020 during wild bird migration. On March 25th, 2024, H5N1 was detected in Texas dairy cattle. As of June 2025, over 1,000 herds have been affected by H5N1 in the U.S. The infected cows showed fever and drop of milk production with poor milk quality. Since April 2024, there have been 70 confirmed human cases in the U.S., including one death, which raises concern that HPAI H5N1 may pose a threat to public health. To characterize dairy cattle host response to HPAI H5N1 infection in mammary tissues, RNA was extracted from experimentally H5N1 virus infected cows using a panel of milk proteins, interleukins, interferons and host factors associated with viral polymerase activity by RT-qPCR. Milk related genes were found downregulated 4 days post infection and do not recover by the end of the animal experiment (day 41), which agrees with observations from herd outbreaks. On day 4 post infection, pro-inflammatory, immune modulating cytokines, and interferons were upregulated, indicating a strong activation of innate immune response upon H5N1 virus infection. ANP32A and B host factors were upregulated as well, indicating their roles in H5N1 replication in cows. Investigating bovine host response to H5N1 virus infection is key to understanding H5N1 pathogenesis in cattle, finding intervention candidates, and assessing human outbreak risk.

103. *Salmonella* Dublin Strains Evolved to Cause More Pneumonia than Diarrhea in Calves

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The bovine-adapted *Salmonella enterica* subspecies *enterica* serovar Dublin (*Salmonella* Dublin) infects young calves (<6 months) via oral route to cause clinical signs which include diarrhea, pneumonia, and septicemia. It is a major differential diagnosis for acute cases of diarrhea in calves less than 6 months of age. Pneumonia in infected calves is associated with higher mortality rate. Here we provide evidence that certain strains of *Salmonella* Dublin may be naturally selected for causing pneumonia with lack of gastrointestinal illnesses. Epidemiological and postmortem analysis during outbreaks in Western Canada revealed that most of the *Salmonella* Dublin strains primarily caused respiratory infection without severe lesions in the gastrointestinal tract or evidence of diarrhea. Most strains displayed multidrug resistant phenotypes which included resistance to tetracycline, ceftiofur, penicillin and chloramphenicol irrespective of lung or gastrointestinal pathology. We tested the hypothesis that strains causing exclusive lung lesions may have evolved strategies to survive better in mammalian macrophages. It will be interesting to investigate which factors enabled *Salmonella* Dublin to preferentially replicate and survive inside bovine macrophages compared to other linages of *Salmonella* Dublin strains. Currently we have established a method to chromosomally integrate a florescent marker into these multidrug resistant strains to expand work at the cellular level to further understand their interaction with the host.
Keywords: Salmonella Dublin, calves, pneumonia, diarrhea

104. Identifying protein interacting partners of an essential Herpes simplex virus 1 (HSV1) transcription factor through BioID proximity ligation

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Herpes simplex virus (HSV1) is a dsDNA virus that is nuclear-replicating. In the nucleus, the HSV1 genome is chromatinized. Chromatin structures are dynamic, where nucleosomes are constantly assembling and reassembling on DNA. In contrast to cellular chromatin, HSV1 chromatin is more dynamic and unstable.

The HSV1 essential transcription regulator, ICP4, destabilizes chromatin by increasing the net population of histones not bound in chromatin. Whether ICP4-mediated chromatin destabilization regulates HSV1 chromatin instability is not tested. To investigate whether ICP4 regulates HSV1 chromatin, I will first characterize mechanisms whereby ICP4 destabilizes chromatin. Of the 827 proteins identified to interact with ICP4, only three have chromatin regulatory functions. A carboxy-terminal deletion mutant, ICP4 Δ760-1298, also exhibiting chromatin regulatory functions will also be investigated. This mutant only interacts with 528 proteins, but maintains interactions with the three proteins exhibiting chromatin regulatory functions. Methods of identifying these interactions only selected for high-affinity interactions. The lack of transient interactions being identified, alongside a minority of interactors involved in chromatin regulation, indicates the list of ICP4 interactors remains incomplete. Therefore, I hypothesize that ICP4 has more interacting proteins.

I will use BioID proximity ligation to identify ICP4-interacting proteins, including transient or unstable interactions. For this method, a biotin ligase, fused to a protein causes biotinylation of proteins within 10 nm in the presence of biotin. Biotinylated proteins are isolated with affinity chromatography and identified by mass-spectrometry. The biotin ligase, miniTurbo, will be fused to ICP4, ICP4 Δ760-1298, and a control NLS. As ICP4 is cytotoxic a stable inducible cell line will be generated by an inducible expression system and transduced into U2OS cells bylentiviral transduction. Upon induction and incubation with biotin, a list of ICP4 protein interactions will be identified. This list of proteins, including transients, will elucidate previously unknown mechanisms of chromatin destabilization by ICP4.

105. The HIV-1 Vif and APOBEC3F interaction involves the N-terminal domain of APOBEC3F

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APOBEC3, deoxycytidine/cytidine deaminases, are a seven-member family in humans named APOBEC3A through APOBEC3H, excluding APOBEC3E. APOBEC3s are host restriction factors against retroviruses such as HIV-1. They inhibit replication by deaminating cytosine to uracil in single-stranded DNA during reverse transcription in newly infected CD4+ T cells. The HIV-1 Vif protein interacts directly with APOBEC3 enzymes and induces their ubiquitination and proteasomal degradation to enable viral replication. The Vif-APOBEC3F interaction domain was originally mapped in the C-terminal domain of APOBEC3F. However, in 2021, Nchioua *et. al.* demonstrated that an N-terminal domain APOBEC3F mutant (R128T) compared to wild-type APOBEC3F exhibited greater Vif resistance and antiviral activity during infection with the HIV-1 Transmitted/Founder virus CH077. This unexpected result suggested that Vif interacted with the N-terminal domain of APOBEC3F. We hypothesized that the N-terminal domain of APOBEC3F had a role in Vif-mediated degradation. We confirmed this through co-immunoprecipitation with APOBEC3F wild type and mutants. In addition, the greater Vif resistance and antiviral activity of APOBEC3F R128T was observed with multiple Transmitted/Founder viruses. Altogether, our study supports a novel Vif interaction interface in the APOBEC3F N-terminal domain and provides new insights into the molecular mechanisms underlying APOBEC3F antiviral activity.

106. Genetic Strains of *Mycobacterium Tuberculosis* among Indigenous Populations: A molecular epidemiology analysis of First Nation Communities in Northern Saskatchewan.

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Background: Despite national progress towards elimination of new infections, Tuberculosis (TB) remains a significant public health concern in Northern Saskatchewan (NS), Canada disproportionately affecting Indigenous populations. The transmission dynamics and genetic strains of MTB in this population are poorly understood. This study sought to determine the predominant *Mycobacterium* TB (MTB) strain among First Nation Communities in Northern Saskatchewan.

Methods: This cross-sectional study utilized anonymized secondary data extracted from the Roy Romanow Provincial Laboratory (RRPL) database in Saskatchewan. We included all confirmed cases of active Mycobacterium Tuberculosis between 2013 and 2023 who had Mycobacterial Interspersed Repetitive-Unit Variable Number of

Tandem Repeats (MIRU-VNTR) genotyping results. Associations between MTB genetic strains lineages and key variables were tested using multivariate logistic regression and presented as adjusted odds ratios (aOR) with 95% confidence intervals (CI) and statistical significance at *p* value ⩽0.05.

Results: Of the 181 TB cases with genotyping results, mean age was 37.4 ± 17.6, 118 (65.2%) male, 76 (41.9%) from the Far North-East region, and 78 (98.3%) culture positive. Furthermore, 87 (48.1%) were TB vaccinated, majority 179 (98.9%) had initiated treatment with a 78% completion rate and most 166 (91.7%) HIV - negative. The predominant genotype lineage was the Euro-American- L4.4.1.1 found in 70.1% of the cases, others include Euro American- L4.9 (24.3), East-African Indian- L3 (2.8%), Euro American-L4.8 (1.6) and M. Africanum-L9 (0.6%). Individuals residing in the Far North-West were 14 ($p < 0.001$, aOR 14.2; CI: 4.9 – 40.8) times more likely to have the predominant genotype compared to those in the Far North-East.

Conclusions: Although this study determined a molecular homogeneity of MTB in Northern Saskatchewan with the predominant genotype Euro-American L4.4.4.1. Addressing regional differences in strain distribution is relevant for developing culturally appropriate TB programs aimed at eliminating TB among specific Indigenous sub-populations in Northern Saskatchewan.