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UNDERGRADUATE RESEARCH SYMPOSIUM 2024

WELCOME FROM THE CO-CHAIRS

On behalf of the Microbiology & Immunology Undergraduate Research Symposium (MBIM URS) Organising Committee, it is our pleasure to welcome you to the 2024 Symposium. So much ground-breaking research has been completed in the last year through research pursuits in the context of Directed Studies and Honors Projects, Co-op placements, and other laboratory positions. We are excited to welcome UBC undergraduate students to share their research in areas related to Microbiology, Immunology and Biotechnology.

Science communication is a fundamental component of the research process, facilitating crucial engagement with and appreciation of benchtop discoveries. We are honoured to be the Co-Chairs of an event that provides a platform for undergraduates to communicate their discoveries with a broad audience consisting of their peers and members of the greater research community.

We look forward to meeting you, learning about all your hard work and research projects, and sharing new insights on April 29th, 2024.

Sincerely,

Melika Hajimohammadi and Nicholas Viegas
Student Co-Chairs, MBIM URS 2024

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ABOUT THE URS

The 7th annual MBIM Undergraduate Research Symposium provides undergraduate students with an excellent opportunity to share their research findings, develop communication skills, and network with UBC faculty and peers. This year, the symposium will consist of 10-minute and 3-minute oral presentations as well as poster displays.

The event will include opening remarks from the URS Co-chairs and a welcome by department head Dr. Michael Murphy. This year, the URS will also feature a keynote address by Dr. Jim Sun, a tuberculosis researcher and new faculty member in the Department of Microbiology and Immunology. Our generous sponsors will also be boothing and providing insight into their companies throughout our event.

Presentations will feature the work of our undergraduate students done in research laboratories (MICB 448/449/Co-op/WorkLearn), and some of our experiential learning courses (MICB 421/447). There will be prizes for the top presenters in all categories.

We look forward to welcoming you to the 2024 MBIM URS!

https://blogs.ubc.ca/mbimurs/

WELCOME MESSAGE FROM THE DEPARTMENT HEAD

Letter from Dr. Michael Murphy

On behalf of the Department of Microbiology and Immunology welcome to our annual Undergraduate Research Symposium. We welcome students attending UBC and at other institutions to share their research experiences in all aspects of microbiology and immunology. As in past years, the presentations will demonstrate the high-quality science performed by talented students. All students benefit from the practice of communicating their work and appreciating the efforts of their colleagues in science. For some, these early formative research experiences will be early steps to long productive careers in science.

Notably, this symposium is student led and organized. Thank you to the students who contributed to success of this year's symposium and to the staff and faculty for their guidance and support. Student engagement in the symposium is evidence of their desire to learn and to communicate their discoveries with each other and the larger scientific community. I am inspired by the effort to build a community of early career researchers. I look forward to meeting you and learning about your research projects whether through a course-based research experience or work in a research lab.

Michael Murphy, PhD
Professor and Head
Department of Microbiology & Immunology
University of British Columbia

CONTRIBUTORS

Undergraduate Student Organizing Committee

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Xianya Qu

SPONSORS

We'd like to thank our sponsors Genevant, Zymeworks, and the UBC Microbiology & Immunology Department for their generous contributions to our event.

KEYNOTE

URS 2024 Keynote: Dr. Jim Sun

Dr. Jim Sun is a distinguished researcher who has recently joined UBC Microbiology & Immunology with his cellular microbiology lab this year. Dr. Sun's journey in academia began at UBC, obtaining his BSc in 2006 and his PhD in 2012. Following this, he completed a postdoctoral fellowship at the University of Alabama at Birmingham. Dr. Sun has been awarded multiple early career research awards, including those granted from Pfizer Canada, Canadian Society for Immunology, American Society for Microbiology, and Lung Health Foundation.

Between 2017-2023, Dr. Sun established a dynamic tuberculosis research program at the University of Ottawa. Dr. Sun's research has revealed crucial insights into the evasion mechanism of Mycobacterium tuberculosis, leading to the development of novel treatments and the discovery of the tuberculosis necrotizing toxin. Now, Dr. Sun is investigating host immunity to M. tuberculosis and other bacterial pathogens in hopes to develop novel host-directed therapies for tuberculosis and other bacterial infections.

SYMPOSIUM PROGRAM

URS 2024 Draft Schedule

	Presentation Title	Presenter(s)	Location
8:30-9:00AM	Sign-in, Poster pin-ups		LSI Atrium
9:00-9:15AM	Opening Remarks		LSI 2
9:15-10:00AM	Keynote Talk	Dr. Jim Sun	LSI 2
Immunology and Virology			
10:05-10:25AM	FLT3-ligand alone enables the maintenance *in vitro* of human fetal liver hematopoietic stem cells	Keheng Wang	LSI 2
10:05-10:25AM	Endogenous retroviruses reduce neutrophil infiltration during HSV-2 infection	Grace Bodykevich	LSI 1410
10:25-10:45AM	Characterisation of the antiviral response against herpes simplex virus 2 in primary mouse vaginal organoids	Imogen Porter	LSI 2
10:25-10:45AM	Validating *in vivo* mouse models for investigating the role of dopamine in glioblastoma growth	Luiza Lopes Pontual	LSI 1410
10:45-11:05AM	Endogenous retrovirus modulation of macrophage activation	Phoebe McNair-Luxon	LSI 2
10:45-11:05AM	STAT1 restricts viral replication and directs appropriate macrophage activation to prevent liver pathology in a model of intestinal viral infection	Madilyn Portas	LSI 1410
11:05-11:25AM	Using dried blood spots to measure spike-specific SARS-CoV-2 IgG concentration and avidity	Nicholas Viegas	LSI 2
Environmental Microbiology			
11:25-11:45AM	Expression of photosynthetic genes from hot spring thermophiles in the mesophile *Rhodobacter sphaeroides*	Michelle Tong	LSI 2

11:25-11:45AM	Placenta, fetal brain blood flow, and substance use exposures	Jamie Corbett	LSI 1410
11:45-12:05PM	Engineering *Rhodococcus jostii* RHA1 to funnel aromatic compound metabolism through cis,cis-muconate	Lucie Lippuner	LSI 2
11:45-12:05PM	Untitled presentation	Burak Ozkan, Maya Tharp, Aeron Selac, Raj Dhillon	LSI 1410
12:05-1:05PM	Lunch Break and Poster Session		LSI Atrium
Microbiome			
1:05-1:25PM	Dogs are a poor taxonomic model for human inflammatory bowel disease, but are potentially functionally relevant for human ulcerative colitis	Sharisse Chan, Brian Deng, Renz Po, Nicholas Viegas, Max Yang	LSI 2
1:05-1:25PM	The role of the microbiome in the disparity in the prevalence among racial and ethnic groups	Tia Murdoch	LSI 1410
1:25-1:45PM	Surgical intervention correlates with reduced bacterial diversity in Crohn's disease patients without inflammation	Max Bremner, Andrew Feng, James Forward, Rochelle Guan, Sarah Zhang	LSI 2
1:25-1:45PM	The diet of the Hadza tribe is higher in gut microbial diversity, but lower in functional diversity when compared to a westernized diet	Adam Abdirahman Hassan, Farbod Nematifar, Timothy Bernas, Trushaan Bundhoo, Young Ha Jin	LSI 1410
Molecular Microbiology			
1:45-2:05PM	Investigating the role of chaperone protein in *E. coli* membrane stability and autotransporter expression	Fares Burwag, Jamie Corbett, Imogen Porter, Kaila Villarey	LSI 2
1:45-2:05PM	Investigating *in vitro* protein folding of *Acetonema longum*	Gregory Nikas	LSI 1410

	beta-barrel outer membrane proteins BamA and SonA		
2:05-2:25PM	Mapping the *brkA* promoter region on pDO6935 in *Escherichia coli* using pLISA, a novel promoterless GFP reporter plasmid	Emilie Haniak, Annie Tsoromocos, and Hari Arneja	LSI 2
2:05-2:25PM	Engineering the *Bordetella Pertussis* BrkA autotransporter for chitin-binding domain surface expression: Exploring its potential for whole-cell immobilization	Alisa Li, Tiffany Wu, Karen Yeung, Alicia Zhang	LSI 1410
2:25-2:45PM	Engineering an OmpT cleavage site in the BrkA passenger domain to explore the role of the conserved autochaperone region	John Go, David Tan, Cayden Yu, Ali Zaidi	LSI 2
2:25-2:45PM	Evaluating the role of the FkpA periplasmic chaperone on the secretion of the BrkA autotransporter in *Escherichia. coli* strain K-12	Rui Xu, Jason Bie, Ives Chau, Jordan Si	LSI 1410
2:45-3:05PM	*Escherichia coli* secretion of a variant in the *Bordetella pertussis* autotransporter protein BrkA containing a dual polyhistidine-tagged 112 amino acid insert between Asp-57 and Ala-58 of its passenger domain	Nicole Cormack, Hayley Emery, Aiden Simard	LSI 2
2:45-3:05PM	Chaperone DegP is not necessary for the polyhistidine tag-detected secretion of autotransporter BrkA in *Escherichia coli* BW25113	Loujain Bilal, Ayesha Lalani, Màiri MacAulay, and Parvin Malhi	LSI 1410
Everyone move to LSI 2 for rapid fire talks			
3:10- 3:40PM	3x1 Presentations (See order below)		LSI 2
3:40-4:00PM	Final remarks and prizes		LSI 2

3x1 Talks

1	**Investigating the pharmacokinetics of GlycoCaged drugs and establishing a murine colitis Model to assess efficacy** Jamie Corbett
2	**MCT-1 inhibition synergizes with glutamine metabolic inhibition to suppress the growth of SMARCA4/2-deficient ovarian cancer cells** Ran Tao
3	**Pan-□-coronavirus and pan-flavivirus host factor TMEM41B as a potential broad-spectrum host-directed antiviral to suppress virally induced ER membrane Remodelling** Gregory Nikas
4	**The DsbA chaperone protein may be necessary for outer membrane secretion of BrkA** Dima Lim, Luiza Lopes Pontual, Max Yang
5	**A 6xHistidine tag insertion at the N-terminal signal peptide A42 and G43 may interfere with BrkA secretion and expression in *Escherichia coli* DH5α** Shini Chen, Haohua Li, Zhuohui Lin
6	**It's pretty gut-wrenching: evaluating the impact of the human IBD microbiome on microglia morphology and systemic steroid hormone levels** Shreya Gandhi
7	**Development of a flow cytometry method for high throughput analysis of synaptic protein signalling in isolated synaptosomes** Brian Deng
8	**Breastmilk mitigates microbial differences caused by varying modes of infant delivery despite the resistant to change nature of C-section microbiome** Arshia Tavangar
9	**A phase II/III clinical trial investigating the efficacy of ustekinumab as a treatment for recent-onset type 1 diabetes** Monica Leca

Posters

1	**Promoter mapping analysis using a GFP reporter plasmid suggests that the lac promoter may drive brkA expression on plasmid pDO6935 in *Escherichia coli*** Nidhin Biju, Sharisse Chan, Dahyeon (Betty) Hong, Renz Po
2	**Suppression of murine inflammatory bowel disease by Tr1 cells via IL-10-mediated inhibition of inflammatory macrophages** Sarah Lim
3	**Mapping potential transcription start sites of the *brkA* gene on pDO6935 in *Escherichia coli* using the ARF-TSS method** Gurpreet Sidhu, Alissa Gama, Sana Alayoubi, Jamila Huseynova
4	**Harnessing the BrkA autotransporter and pDO6935 plasmid for MTT5 display on *E. coli* for zinc biosorption evaluation** Alice Wang, David Lin, Kevin Zhang, Tiffany Wai
5	**Optimization of a Jurkat reporter cell line to measure TGF-β1 secretion by regulatory T cells** Tiffany Wai
6	**Histological analysis of the effect of CRP treatment on the serotonergic profile and injury scar in a porcine model of spinal cord injury** Alexander Pei
7	**Oxalate as a disrupter of the intestinal microbiome and the protective effects of butyrate in kidney stone disease** Naomi Isak
8	**Immune checkpoint inhibitors and tumor characteristics in precision oncology** Asmita Jain
9	**Determining the therapeutic potential of Tr1 cell therapy via fecal lipocalin-2 levels in DSS-induced colitis mouse models** Insha Majeed
10	**Purified Ag43α protein as a potential method for preventing *Escherichia coli* autoaggregation** Samantha K Allum, Kris Chen, Sean Dang, Pranjali Singh
11	**The clinical role of routine bladder biopsies in follow-up after BCG treatment of pure carcinoma in situ of the bladder – a retrospective review** Gabrielle Reznik
12	**Oral microbiome sampling using non-invasive brushing for at-risk oral lesions: S pilot study** Andrew Huang and Catherine Pepin
13	**The association between dietary nutrients and gut microbiome diversity in Parkinson's disease patients** Adam Voss, Gregory Nikas, Kevin Zhang, Louise He, Rob Cloke
14	**Characterization of Skp chaperone knockdown and its effects on *Bordetella pertussis* BrkA secretion in *Escherichia coli* BW25113**

	Sid Ahuja, Jordan Sanders, Haolin Tian, Amber Xia
15	**Peel to Seal: effectiveness of orange peels in growing bacterial cellulose as food packaging** Talia Feng, Mittal Bagul, Maria Lauren
16	**Novel enhancers downstream of MYCN drive increased gene expression in early T-cell progenitor acute lymphoblastic leukemia which sustains a proliferative cell state** Fares Burwag
17	**Low serum saturated fatty acid levels positively associate with microbiota diversity and metabolic pathways in Parkinson's disease patients** Ali Tavakoli Hedayatpour, Parsa Khatami, Jordan Si, David Lin, Josh Zhang

10x10 PRESENTATION ABSTRACTS

Presenters will be given 10 minutes to present a maximum of 10 slides followed by a 2-5 minute question and answer period. 10×10 presentations will be expected to transparently report a study and ought to include an:

- Introduction
- Experimental design/methodology
- Results
- Discussion

Escherichia coli secretion of a variant in the _Bordetella pertussis_ autotransporter protein BrkA containing a dual polyhistidine-tagged 112 amino acid insert between Asp-57 and Ala-58 of its passenger domain

Nicole Cormack[1], Hayley Emery[1], Maya Ruehlen[1], Aiden Simard[1]

[1]Department of Microbiology and Immunology, The University of British Columbia, Vancouver, BC, Canada

Bordetella pertussis is a Gram-negative bacteria which produces Bordetella resistance to killing A (BrkA). BrkA mediates autotransporter functionality and undergoes a processing event to produce a cleaved and uncleaved protein. Despite advancements in the functionality of different regions of this autotransporter, there is a lack of understanding of the mechanistic dynamics driving BrkA translocation. This study aimed to generate a sizable amino acid insert into BrkA passenger domain, analyze the structural layout of the protein, and to determine surface-expression status, regardless of the increase in molecular weight. We hypothesized that inserting a 112 amino acid spacer into the N-terminal domain will not affect BrkA surface expression, but may modulate processing events in an *E. coli* model. We constructed pSERC, containing two 6x histidine tags within a 336 base pair insert on *brkA* passenger region. The amino acid sequence corresponding to the insert revealed passenger residue repetition and potential for a protease cleavage site. Finally, we confirmed that the 112 amino acid insert-containing BrkA passenger retained surface expression in an *E. coli* model and a processing event. Our findings report that BrkA translocation is retained following a substantial amino acid insertion, and further suggests a potential avenue for studying BrkA surface expression and the complex translocation pathway that the passenger follows from cytoplasm to outer-membrane.

Engineering an OmpT cleavage site in the BrkA passenger domain to explore the role of the conserved autochaperone region

John Goh[1], David Tan[1], Cayden Yu[1], Ali Zaidi[1]

[1]Department of Microbiology & Immunology, University of British Columbia

The secretion of autotransporters stands as a distinctive mechanism employed by Gram-negative bacteria for the transportation of proteins to their cell surface. BrkA is an autotransporter protein in *Bordetella pertussis* which plays a pivotal role in conferring serum resistance and host cell adherence. BrkA comprises three key domains: an N-terminal signal peptide domain, a 73 kDa passenger α-domain, and a 30 kDa translocator β-domain. Previous literature has shown that the autochaperone region glutamate^{601}–alanine^{692} is essential for proper protein folding and protection against proteolysis. This region, however, is not necessary for translocation of the protein across the outer membrane. Previous literature has also shown that following translocation out of the cell, cleavage occurs at the asparagine^{731} – alanine^{732} site to dissociate the passenger from the translocator β-domain. However, despite cleavage at the cell surface, the BrkA passenger cannot be detected in the supernatant, suggesting a non-covalent interaction anchoring it to the membrane. In this study, we sought to identify the specific region that may non-covalently interact with the β-barrel region by engineering a secondary outer-membrane protease T (OmpT) cleavage site before glutamate^{601}. Two rounds of site-directed mutagenesis were employed to introduce a polyhistidine tag at the N-terminus of the BrkA passenger and then an OmpT cleavage site at glutamate^{601}. Western blot analysis of whole cell lysates of an OmpT-expressing strain of *E. coli* (UT2300) detected a band corresponding with the BrkA passenger (61 kDa) processed at the OmpT cleavage site, suggesting that it remained cell-bound after cleavage. A 61 kDa band was not observed in Western blots of whole cell lysates in an OmpT deficient strain of *E. coli* (UT5600). A band of 61 kDa using Western blots of filtered culture supernatants was not detected in either strain. It is possible that the concentration of the 61 kDa protein was too low in supernatants for Western blot detection or that the cleaved BrkA passenger was unstable in the absence of the autochaperone region and degraded by extracellular proteases in the media. These findings provide insights into the intricacies of BrkA autotransporter processing and prompt further investigation in this field.

Engineering the Bordetella Pertussis BrkA Autotransporter for Chitin-Binding Domain Surface Expression: Exploring its Potential for Whole-Cell Immobilization

Alisa Li[1], Tiffany Wu[1], Karen Yeung[1], Alicia Zhang[1]

[1]Department of Microbiology and Immunology, University of British Columbia, Vancouver, BC, Canada

The BrkA autotransporter is an important virulence factor in *Bordetella pertussis* that confers serum resistance and mediates cell adherence. Autotransporters are elegant systems because they are self-secreting and only one protein needs to be manipulated. These properties make it a useful secretion pathway that can be exploited to deliver proteins to the surface for applications such as whole-cell immobilization. In this context, the chitin-binding domain derived from *Pseudomonas aeruginosa* PAO1 chitinase C emerges as a promising candidate, given its strong affinity for chitin, an abundant polysaccharide, and its pH-sensitive characteristics that simplifies the process of retrieving cells after a bioreaction. Despite these advantages, its potential in whole-cell immobilization has been relatively unexplored. Furthermore, BrkA has successfully displayed heterologous proteins on bacterial surfaces. As such, we aim to exploit the BrkA autotransporter secretion system to export the chitin-binding domain to the cell surface. By leveraging the binding properties of the chitin-binding domain to chitin, we also aim to explore its potential in whole-cell immobilization. In our study, we successfully engineered a recombinant plasmid (TAAK-A54) by substituting a part of the BrkA passenger domain with the chitin-binding domain. A trypsin accessibility assay confirmed the expression and export of the chitin-binding domain to the cell surface. Lastly, we observed through microscopy that *Escherichia coli* cells expressing the chitin-binding domain can interact with chitin. Additionally, we found that TAAK-A54 samples had decreased turbidity compared to the negative control following incubation with chitin resin, suggesting that cells were also able to be pulled down by chitin. This study enhances the understanding of the repertoire of heterologous proteins that the BrkA autotransporter system can secrete. We demonstrated the feasibility of leveraging the BrkA secretion system for the external presentation of the chitin-binding domain. Our results indicate that the chitin-binding domain is worthy of further study for applications in whole-cell immobilization.

Dogs are a poor taxonomic model for human inflammatory bowel disease, but are potentially functionally relevant for human ulcerative colitis

Sharisse Chan[1], Brian Deng[1], Renz Po[1], Nicholas Viegas[1], Max Yang[1]

[1]Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia, Canada

Inflammatory bowel disease (IBD) is an inflammatory condition affecting the gastrointestinal tract. Human IBD consists of two subtypes, Crohn's Disease (CD) and Ulcerative Colitis (UC), which continue to have large global health and economic implications. Dogs have previously been investigated as a potential research model for human IBD, but not against these specific IBD subtypes. We explored the microbial diversity and abundance, as well as the functional pathways of the gut microbiome in IBD dogs against UC and CD humans, to investigate if IBD dogs may serve as a model for a particular human IBD subtype. We utilized a combined 16S rRNA dataset of IBD dogs and humans to compare diversity metrics, taxonomic abundance, core microbiome, indicator species, differential abundance, and predictive functional analyses. We found a difference in gut microbial composition between dogs and humans, further supported by a lack of common taxonomic groups in both core microbiome and indicator species analyses. In addition, we found a significant reduction in the abundance of taxa in IBD dogs against human disease states during differential abundance analysis. Despite significant species-driven differences, our predictive functional pathway analysis indicates similarity in shared metabolic pathways of IBD dogs and UC humans. The compositional differences between dogs and humans suggest that dogs are not a reliable taxonomic model for inflammatory bowel disease subtypes in humans.

Untitled Presentation

Burak Ozkan, Maya Tharp, Aeron Selac, Raj Dhillon

Our study investigates the role of Trk1p, a potassium import channel, in modulating the activity of Pma1p, a proton ATPase, in *Saccharomyces cerevisiae*. By examining changes in extracellular pH and growth rates at varying D-glucose concentrations, we aimed to evaluate the impact of Trk1p on Pma1p activity and, subsequently, on yeast cell growth. Our experimental design included two main approaches: measuring extracellular pH as a proxy for Pma1p activity and assessing growth rates under different glucose conditions for wild- type and Trk1p-Δ mutant strains. We observed a significant difference in extracellular pH values between the wild-type and mutant strains across varying glucose concentrations, suggesting that Trk1p modulates Pma1p activity. However, this modulation did not translate into significant differences in growth rates between replicates of the same strain, indicating that Pma1p activity changes do not directly affect yeast growth under the conditions tested. These findings propose that other ATPases might compensate for the reduced Pma1p activity in the mutant, highlighting the complexity of ion regulatory mechanisms in yeast. Future research should further explore the intricate network of ion homeostasis and its implications for yeast physiology and pathogenicity, with a particular focus on Pma1p as a potential drug target in pathogenic yeast species like *Candida albicans.*

Endogenous retroviruses reduce neutrophil infiltration during HSV-2 infection

Grace Bodykevich[1]

[1]The University of British Columbia, Department of Microbiology and Immunology

Endogenous retroviruses (ERVs) are genomic sequences that are derived from retroviruses and that have been associated with both detrimental and beneficial effects on the immune system. Previous work from our lab showed that ERVs promote an antiviral state and protect against herpes simplex virus type 2 (HSV-2) disease in mice. However, the mechanism by which ERVs mediate protection against HSV-2 infection remains unknown. In this study, we tested the hypothesis that ERVs protect mice against HSV-2 through modulating the innate immune response within the vaginal mucosa. To test this hypothesis, we intravaginally administered ERVs and measured neutrophils and monocytes in the vaginal lumen of mice before and after HSV-2 infection using flow cytometry. We observed that upon HSV-2 infection, mice treated with ERVs had reduced neutrophil counts in the vaginal lumen, but there was no difference in monocyte recruitment. These data suggest that neutrophils may worsen HSV-2 disease and that the reduction in neutrophil recruitment by ERVs may be a potential mechanism to prevent severe HSV-2 disease.

Acknowledgements: Maria Tokuyama and everyone within the Tokuyama Lab. UBC Flow Facility, UBC Modified Barrier Facility, and funding from the UBC Microbiology and Immunology Department as well as CIHR.

Investigating the mechanisms of Acetonema longum β-barrel outer membrane protein biogenesis

Gregory Nikas[1], Polina Beskrovnaya[1], & Elitza Tocheva[1]

[1]Department of Microbiology & Immunology, University of British Columbia, Vancouver, Canada

Bacterial endospore formation, a pivotal survival strategy in members of the phylum Firmicutes, involves extensive membrane and peptidoglycan (PG) remodelling. The monoderm Bacillus subtilis, encapsulated by a single lipid membrane, has been extensively studied as a model organism for endospore formation. However, recent discovery of sporulating diderms, such as Acetonema longum, raised new questions regarding the processes behind the cell envelope remodelling. Characterization of A. longum with high-resolution imaging and omics approaches highlighted the unprecedented de novo outer membrane (OM) biogenesis that takes place during germination in diderm spore-formers. In these organisms, an inner membrane (IM)-like lipid bilayer is transformed into a canonical OM containing lipopolysaccharides (LPS) and β-barrel outer membrane proteins (bbOMPs). Folding and insertion of bbOMPs is mediated by the β-barrel Assembly Machinery (BAM) complex. The absence of the BAM complex in dormant A. longum spores further indicated that the insertion of its main pore protein BamA into the outer spore membrane (OsM) also occurs during germination and via a mechanism which cannot be accounted for by the known pathways. Here, we propose that the uncharacterized bbOMP SonA mediates the de novo insertion of A. longum BamA into the OsM during germination. To assess our hypothesis, we expressed and purified recombinant BamA and SonA and conducted in vitro assays to analyze their folding and incorporation into phospholipid liposomes. Our preliminary results suggest that these proteins may not be able to self-incorporate into lipid bilayers under physiological conditions, suggesting that other, possibly novel proteins may assist in de novo bbOMP insertion in germinating spores. Altogether, this study sheds light on the role of bbOMPs proteins in OM assembly and expands our knowledge of OM remodelling, potentially informing the divergence of monoderm and diderm Firmicutes.

Mapping the *brkA* promoter region on pDO6935 in *Escherichia coli* using pLISA, a novel promoterless GFP reporter plasmid

Emilie Haniak[1], Annie Tsoromocos[1], and Hari Arneja[1]

[1]Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia, Canada

The lactose, or *lac*, operon regulatory system is known for its ability to control gene expression via transcriptional regulation in *Escherichia coli*. Previous studies have demonstrated the expression of *Bordetella* resistance to killing A (B*rkA)* from pDO6935 in *E. coli*, but have not identified the promoter responsible for its regulation. pDO6935 harbours a *lac* regulatory region, including the *lac* promoter, operator, and catabolite activator protein binding site (CAP BS), between its origin of replication (*ori*) and the 5' end of *brkA* which may be implicated in *brkA* expression. To characterize the *brkA* promoter for *E. coli* on pDO6935, we created pLISA, a promoterless green fluorescent protein (GFP) vector to be used in a promoter trap experiment. In our work, various regions upstream of the *brkA* locus on pDO6935, either inclusive or exclusive of the *lac* regulatory region, were cloned into pLISA. GFP expression was observed as an indicator of promoter activity. We showed that GFP expression was induced when amplicons with the full *lac* regulatory region were present. This observation suggests that the *lac* regulatory region is both necessary and sufficient to drive *brkA* expression from pDO6935.

Characterisation of the antiviral response against herpes simplex virus 2 in primary mouse vaginal organoids

Imogen Porter[1], Ananya Saraph[1], Maria Tokuyama[1]

[1]Department of Microbiology and Immunology, University of British Columbia, Vancouver, BC, Canada

Herpes simplex virus 2 (HSV-2) is the agent responsible for genital herpes, a sexually transmitted disease of significant public health concern. Although women are affected disproportionately more than men, and infection during pregnancy introduces the risk of severe neonatal transmission, we lack a well-established female reproductive model with which to study HSV-2 infection. This study introduces a vaginal organoid model with which to study vaginal epithelial biology, grown from primary murine vaginal cells. We demonstrate the capacity of these organoids to HSV-2 infection, quantified through RT-qPCR of viral genes at 24 and 48 hours post infection (hpi) compared to an uninfected control. Additionally, the host innate immune response to infection is characterised through the quantification of interferon-stimulated gene (ISG) expression. Our results show high viral gene expression at both time points, suggesting extensive infection, with no significant change in ISG expression compared to uninfected organoids. These data suggest that the systemic infection of organoids by 24 hpi may repress an interferon-stimulated response, or that no such response is induced in our model. Further studies utilising this new model at earlier timepoints may prove valuable in confirming this effect. The organoid model therefore proves promising as a new avenue for HSV-2 and other viral infection studies of the vaginal epithelium, currently in progress at the Tokuyama lab.

Investigating the role of chaperone protein in *E. coli* membrane stability and autotransporter expression

Fares Burwag[1], Jamie Corbett[1], Imogen Porter[1], Kaila Villarey[1]

[1]Department of Microbiology and Immunology, University of British Columbia, Vancouver, BC, Canada

Autotransporters are virulence factors that are expressed on the surface of gram-negative bacteria through the type V secretion systems. One such example is BrkA of *Bordetella pertussis*, a type V autotransporter responsible for adhesion, serum resistance, and cellular invasion. The assistance of chaperone proteins is required to translocate autotransporters across the bacterial membranes and enable proper folding into functional structures on the cell surface. However, the exact chaperones required for BrkA surface expression are currently unclear. In this study, we transformed a panel of *E. coli* chaperone knockout strains with a brkA expression vector to investigate the importance of these chaperones in stable autotransporter surface expression. Through trypsin accessibility assays we found that only BamB was necessary for the expression of cleaved BrkA on the outer membrane. Additionally, formaldehyde crosslinking suggested direct interactions between BrkA and BamB, as well as potentially FkpA. Our data supports prior investigations into the role of chaperone proteins in BrkA expression while marking the first attempt to perform a wide panel screen for inter-chaperone comparisons. Insights derived from this study are also relevant to immunotherapeutic and vaccine development, where BrkA is an attractive target due to its surface presentation and its role as a virulence factor.

Placenta, fetal brain blood flow, and substance use exposures

Jamie Corbett[1,2,3]

[1]Families in Recovery (FIR) Unit, BC Women's Hospital and Health Centre, Vancouver, BC, Canada
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In only twenty-two years, 645,000 overdose deaths were connected to the use of opioids in North America. Opioid use disorder (OUD) refers to a chronic condition wherein an individual is more susceptible to continuous use or relapse into the use of opioids. The current standard of care for individuals with OUD is opioid agonist therapy (OAT), which is a life-saving, evidence-based, pharmacologic intervention treatment plan. OAT in pregnancy is an understudied area of research that requires greater information to provide people and physicians with the information to make informed medical decisions. In response, the Placenta, Fetal Brain Blood Flow, and Substance Use Exposures (PEBBLES) pilot study has been initiated through a preliminary literature review, study design, and the first study visit. Through a scoping literature review, we have highlighted the dynamic changes in OAT dosage across the years and a gap in the literature that describes the effect that OAT alone plays on the placenta. The ongoing pilot study will conduct two ultrasounds before and after OAT dosage while the participant is between 30 to 36 weeks gestation. The findings from this study will provide greater insight into the influence, or lack thereof, of OAT on the placenta and fetal brain blood flow and pave the way for future work to help strengthen our understanding of OAT in pregnancy.

FLT3-Ligand Alone in Vitro Maintains Human Fetal Liver Hematopoietic Stem Cells

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Hematopoietic stem cells (HSCs) are a unique cell population that is able to self-renew and give rise to all types of blood cells. Current findings indicate that they are responsible for lifelong blood production. HSCs can be first detected within the first month of development in humans, and are largely created prior to birth, after which they expand their numbers in response to physiological demands for normal or enhanced blood cell output requirements. Cells with long-term (>6 month) HSC regenerative properties present in the developing fetal liver (FL) are a source of HSCs that possess a much higher *in vivo* regenerative capability than those from older donor sources, such as adult bone marrow or even cord blood (CB). Among which, it has been found that human fetal liver derived $GPI80^{+}CD90^{+}CD38^{-}CD45RA^{-}CD34^{+}CD45^{+}$ cell subset is enriched for cells with high *in vivo* regenerative capability. However, in the past this capability was found to be lost *ex vivo*. Hence, it is of great importance to identify *in vitro* culture conditions that are able to maintain FL HSCs with long term regenerative capability. The long term culture-initiating cell (LTC-IC) assay using stromal cell-containing cultures is believed to mimic the *in vivo* stem cell niche, and may predict long-term hematopoietic cell output capabilities obtained *in vivo*. In this study, single-cell LTC-IC assay of hFL $GPI80^{+}$ cells before (freshly thawed) and after various culture conditions was performed to assess the long term hematopoietic output. It was found that 5-day Flt3L culture alone in vitro produces primitive hematopoietic outputs for the longest time and highest outputs. This finding suggests the potential capability of the Flt3L culture condition to maintain human FL HSC populations *in vitro*, setting the stage for future development of strategies to exploit human FL cells therapeutically and may also be critical to designing conditions that will best support the *ex vivo* maintenance of HSCs at and after birth.

Engineering *Rhodococcus jostii* RHA1 to funnel aromatic compound metabolism through *cis*,*cis*-muconate

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Lignin is a biopolymer that occurs in the woody material of plants to increase their structure and rigidity. In pulp and paper mills, lignin is burned to drive the pulping process as its heterogeneous structure renders transformation difficult. Recently, new strategies to valorize lignin have emerged, in which lignin is depolymerized into lignin-derived aromatic compounds (LDACs), such as vanillate, which can be metabolized by the β-ketoadipate pathway present in microbes such as *Rhodococcus jostii* RHA1. The β-ketoadipate pathway is composed of the catechol and protocatechuate branches which funnel into the TCA cycle. A metabolite of the catechol branch is *cis,cis*-muconate (muconate), a valuable chemical for bioplastic synthesis. The aim of this study is to modify the β-ketoadipate pathway in RHA1 such that the protocatechuate branch is blocked and metabolites are funneled into the catechol branch to maximize muconate production. To accomplish this, genes from *Enterobacter cloacae* encoding a protocatechuate decarboxylase AroY and its accessory enzymes, EcdBD, were assembled into the integrative plasmid pRIME. The plasmid was then transformed into Δ*pcaHG* RHA1, a mutant that is unable to grow on aromatic substrates due to a blockage in the protocatechuate branch. Introduction of *aroY* and *ecdBD* into the Δ*pcaHG* mutant rescued the ability to grow on vanillate, a substrate metabolized by the protocatechuate branch, indicating that the flow of carbon had been directed to the catechol branch of the β-ketoadipate pathway. Previous literature has shown that EcdD provides a prenylated flavin mononucleotide co-factor to AroY allowing for enhanced decarboxylase activity. However, its necessity in RHA1 remains to be investigated. Overall, this work shows the first steps in developing an RHA1-based microbial cell factory for the valorization of lignin into muconate.

Validating *in vivo* mouse models for investigating the role of dopamine in glioblastoma growth

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Glioblastoma (GBM) is the most common and lethal glioma, proving resistant to all therapeutic strategies attempted to date. Previous research from our lab has found that inhibiting dopamine (DA) receptor D4 leads to apoptosis of GBM stem cells (GSCs) *in vitro* and attenuates growth *in vivo*. Our group is using two mouse models to investigate GBM in the context of either enhanced DAergic activity or DA depletion *in vivo*. We are utilizing a technique called optogenetics to stimulate release of DA from DAergic neurons in DAT::Cre mice. Conversely, to study GBM growth in the context of DA deficiency, we are using a model of Parkinson's disease to ablate DAergic neurons on one side of the mouse brain. This project's main objectives were to validate the stimulation of DAergic neurons in the optogenetics system and characterize the tumours that grew in the DA ablation model. To validate the optogenetics system, we used immunofluorescent (IF) staining to visualize the expression of the light-activated channel (ChR2) and quantify the level of activity after optogenetic stimulation using c-Fos. In the DA depletion model, we engrafted GSCs into either the normal DA or DA depleted side of the brain. We then collected the resulting endpoint tumours for bulk RNA sequencing and IF staining to characterize their growth and proliferation status. Using c-Fos quantification, we found a comparative increase in the optogenetically stimulated vs. the control mice. We also found a small but not significant trend towards a prolonged survival with the tumours grown on the DA depleted side of the brain. We are now analyzing the corresponding RNA sequencing to identify other characteristic differences between the tumours of both groups. Validating these mouse models is a key step toward understanding the effects of DA on GBM *in vivo*, which may eventually contribute to repurposing current therapeutics that modulate DA for patients with GBM.

STAT1 restricts viral replication and directs appropriate macrophage activation to prevent liver pathology in a model of intestinal viral infection

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Murine norovirus strain CR6 (CR6) establishes a persistent asymptomatic infection in the mouse intestine. Similar to commensal bacteria, CR6 has been shown to benefit the host, but unlike commensal bacteria, little is known about how the host regulates this viral infection. STAT1 is a key antiviral signalling factor, and STAT1-deficient mice fail to restrict CR6 to the intestine. Without STAT1, CR6 disseminates beyond the intestinal mucosa, resulting in severe disease. Our recent data establishes that this disease is not a function of adaptive immune-mediated pathology but rather failure to restrict viral replication in infected cells. The lethality of CR6 in STAT1-deficient mice has been linked with severe liver pathology and the infiltration of highly infected innate immune cells into the liver. We hypothesize that STAT1 expression in macrophages directs their appropriate activation, preventing fatal hepatic pathology. In the absence of STAT1, macrophages fail to activate appropriate antiviral pathways, leading to intracellular CR6 replication and inflammatory pathology. In this study, STAT1-deficient bone marrow-derived macrophages (BMDMs) were infected with CR6 in vitro. Analyses revealed decreased expression of antigen presentation-related markers and increased cell death, supporting the hypothesis that STAT1 is essential for macrophages' effective activation and function during viral infection. Gene set enrichment analysis suggested that the STAT1-deficient macrophages may be of a unique activation phenotype, displaying upregulated expression of both M1 and M2 macrophage-associated genes. M1 and M2 transcriptional profiles are classically considered to be opposing, both in their means of activation and resulting activity, further elucidating the integral role of STAT1 in typical immune cell function regulation. Subsequent coculturing of infected STAT1-deficient BMDMs with hepatocytes resulted in significantly decreased hepatocyte proliferation compared to STAT1-expressing controls. These data suggest that CR6-infected STAT1-deficient macrophages may limit the ability of hepatocytes to survive immune infiltration and inflammation, potentially through dysregulated activation. Collectively, our data further delineate the significance of STAT1 in restricting viral replication and cellular processes for appropriate immune cell activity. In the case of a viral commensal, such as CR6, STAT1 prevents immune dysregulation and associated fatal immunopathology through proper activation and antiviral protection of immune cells.

Chaperone DegP is not necessary for the polyhistidine tag-detected secretion of autotransporter BrkA in *Escherichia coli* BW25113

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Autotransporters are virulence-associated proteins capable of independent secretion, present on the outer membranes of gram-negative bacteria. *Bordetella pertussis* is the causative agent of whooping cough and expresses the 103 kDa type Va autotransporter Bordetella resistance to killing (BrkA), which contributes to the bacterium's resistance against the bactericidal activity of the complement pathway. The exact folding mechanisms, including the role of periplasmic chaperones involved in the translocation of BrkA, are not well understood. DegP is a highly conserved periplasmic chaperone that has been implicated in the folding and secretion of other autotransporters but has yet to be characterized in the expression of BrkA. We therefore hypothesized that DegP would be necessary for BrkA secretion. To investigate this, a 6x histidine tag was inserted in the unstructured region of the BrkA passenger domain for detection by immunoblot analysis. We compared the expression between wildtype BW25113 and mutant JW0157 (Δ*degP*) cells and observed BrkA surface expression in both conditions, indicating that DegP is not necessary for BrkA secretion. This study provides insight into the secretion process of the virulence factor BrkA and bacterial membrane transport systems as a whole which holds the potential for the development of new clinical interventions and biotechnological applications.

Expression of photosynthetic genes from hot spring thermophiles in the mesophile *Rhodobacter sphaeroides*

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As global electric energy consumption rises and the environmental toll of fossil fuels becomes increasingly evident, the need to transition to renewable energy intensifies. Solar power emerges as a prominent renewable energy source, yet its efficiency remains a challenge. Biophotovoltaics, leveraging the ability of photosynthetic microorganisms to convert light into energy, presents a promising alternative that greatly surpasses the efficiency of traditional photovoltaic systems. However, biophotovoltaic systems suffer from overheating due to sunlight exposure, compromising performance and protein integrity. Hot spring thermophiles, living in temperatures ranging from 40°C to 85°C, represent a reservoir for potentially valuable thermotolerant proteins. Notably, their photosynthetic proteins can resist thermal degradation, exhibiting potential for biophotovoltaic applications. Cultivating and conducting genetic experiments on hot spring thermophiles are challenging in a laboratory setting, and most genetic data stems from bulk DNA extraction and metagenomic analyses. To address this, the well-characterized model organism *Rhodobacter sphaeroides* was leveraged to express genes from hot spring thermophiles *Chloroflexus islandicus, Chloroflexus aurantiacus* and *Chloroflexota bacterium L.E.CH.39_1 (L.E.CH.39_1)*. This strategy can be employed to use genetic approaches to address biochemical questions regarding the photosynthetic proteins of hot spring thermophiles. Synthetic gene fragments encoding the reaction center (RC), the light harvesting complex (LHC) and other photosynthetic proteins were synthesized by the Joint Genome Institute (JGI) and conjugated into *R. sphaeroides*. All synthetic genes were successfully expressed in *R. sphaeroides*, with genes from *C. aurantiacus* and *L.E.CH.39_1* producing possibly functional reaction center and light-harvesting proteins that associated with chlorophylls. This was confirmed via the restoration of characteristic absorbance peaks of photosynthetic complexes. This research project underscores the use of *R. sphaeroides* for the expression of photosynthetic genes from hot spring thermophiles, sourced from metagenomic data. The utilization of this system enables the production and purification of novel thermotolerant photosynthetic proteins, offering potential enhancements to biophotovoltaic systems and advancements in current solar energy technology.

Using dried blood spots to measure spike-specific SARS-CoV-2 IgG concentration and avidity

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19), has caused millions of deaths globally. Spike protein-specific immunoglobulin G (IgG) antibody protects against COVID-19 via multiple mechanisms. Dried blood spots (DBS) provide an exciting and accessible alternative to venous blood sampling. They are a reliable source of samples that may be used to surveil hard-to-reach communities and increase participation in clinical studies, and ultimately, can be incorporated into assays that measure the concentration and avidity of protein-specific antibodies. This project evaluated COVID-19 vaccine-induced antibody response using dried blood spot (DBS) samples (n=101) in community dwelling adults aged 50 years and above one-month after their first booster dose. Detection of index virus SARS-CoV-2 spike-specific IgG concentration was performed using an enzyme-linked immunosorbent assay (ELISA). Antibody concentration was measured in binding antibody units per milliliter (BAU/mL) and reported as the geometric mean concentration (GMC). Antibody avidity, the collective binding strength of antibody-antigen complexes, which has been recommended to be considered alongside antibody concentration for the determination of a correlate of protection for COVID-19 vaccines, was determined via a modified ELISA protocol. Statistical analyses demonstrated significant differences in index virus SARS-CoV-2 spike-specific IgG (S-IgG) concentration and avidity outputs across many factors, including age, vaccine series received, and body mass index (BMI). This project is of significance as a proof of concept for the use of DBS in immune-surveillance research.

Endogenous retrovirus modulation of macrophage activation

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Endogenous retroviruses (ERVs) are viral elements within the genomes of modern-day organisms that originate from ancient exogenous retroviral infections. These infections became fixed in the genome through stable inheritance, accounting for 8% of the modern human genome (four times that of protein coding regions). ERVs are known to affect the immune system by inducing proinflammatory cytokines and activating innate sensors. However, the mechanism by which ERVs modulate innate immune responses remains unclear. Based on evidence of ERVs as proinflammatory agents, we hypothesize that they predispose innate cells to greater activation upon stimulation. To test this, we established bone marrow-derived macrophage (BMDM) cultures using four genotypes of mice with varying ERV levels. We then stimulated each genotype with lipopolysaccharide (LPS), a model toll-like receptor (TLR) 4 ligand. To determine macrophage activation levels, we stained BMDMs for activation markers and used flow cytometry for analysis. We found that ERV levels did not impact baseline activation state, but that upon LPS stimulation, BMDMs with higher ERV levels were more activated**.** These results demonstrate that ERVs enhance macrophage activation upon stimulation. Future research may lead to therapeutic strategies targeting ERVs in disease pathogenesis or autoimmunity related to macrophage activation.

Evaluating the role of the FkpA periplasmic chaperone on the secretion of the BrkA autotransporter in *Escherichia. coli* strain K-12

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BrkA autotransporter, a virulence factor produced by *Bordetella pertussis*, is a causative agent of whooping cough that can induce serum resistance and promote cell adhesion. BrkA is translocated across the inner membrane (IM) and outer membrane (OM) using its N-terminal signaling sequence and C-terminal β-barrel, respectively. However, the detailed process of BrkA biogenesis within the periplasm is still not fully understood. Recent studies have demonstrated the importance of periplasmic chaperones in the folding and translocation of Type 5 Secretion System (T5SS) autotransporters. FkpA is one of the periplasmic chaperones that was reported to interact with the passenger domain of certain T5SS autotransporters by stabilizing their nonnative conformation in the periplasm. We aimed to investigate the necessity of FkpA on BrkA biogenesis. We transformed *Escherichia coli* (*E. coli*) strains—wildtype (BW25113) and *fkpA* knockout (JW3309)—with pPALM plasmids that contain a polyhistidine tag. We then compared BrkA expression between examined strains using western blot analysis via polyhistidine-tag specific antigen binding. We observed a significant decrease in the BrkA signal from the *fkpA* knockout strain compared to the wild-type strain. Our data suggest that FkpA is crucial for BrkA secretion, likely facilitating the folding of the BrkA β-domain and maintaining it in a translocation-competent state. Future research could explore whether BrkA secretion depends on the interaction of FkpA with other periplasmic chaperones, thus facilitating BrkA secretion.

Does the microbiome mediate differences in the prevalence of asthma among racial and ethnic groups?

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Asthma prevalence in industrialized nations is increasing, however, some groups are more greatly impacted than others. Racial and ethnic minorities consistently have higher rates of asthma with greater morbidity. This disparity does not arise from genetic features, but rather the differential access to social determinants of health that exists among racial and ethnic groups. The development and severity of asthma are well established to be influenced by the microbiome and differences in the microbiome exist among ethnic and racial groups. Thus, the microbiome may act as a driver behind the disparity of asthma among racial and ethnic groups. This paper explores the interplay between race and ethnicity, the microbiome, and asthma prevalence by investigating the questions of (1) how does dysbiosis of the microbiome increase asthma risk? (2) how is the microbiome affected by ethnicity? (3) are ethnic variations in microbiome composition a potential driver of the disproportionate rates of asthma in different racial/ethnic groups? The questions are answered through a multi-disciplinary narrative literature review. Differential exposure to biological, behavioural, and environmental actors, born from the systemic unequal distribution of social determinants of health, drives differences in microbiome composition among racial and ethnic groups. These differences in the microbiome may be contributing to increased asthma prevalence and morbidity in racial and ethnic minorities. A shorter duration of breastfeeding, Cesarian section delivery and antibiotic treatment influence the development and the composition of the microbiome and more greatly affect racial and ethnic minorities. These factors are also associated with an increased risk of developing asthma later in life. Additionally, later life factors are unequally distributed among ethnic and racial groups affect the severity and control of asthma, potentially mediated by changes to the microbiome. These findings provide a potential mechanism to explain the disparity of asthma prevalence and severity among racial and ethnic groups. Furthermore, this project may provide insight into how to utilize the social determinants of health framework to understand differences in microbiome composition among social groups.

The diet of the Hadza tribe is higher in gut microbial diversity, but lower in functional diversity when compared to a westernized diet.

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The Western diet has recently been a point of scrutiny in the scientific community due to its association with various metabolic diseases such as obesity, type 2 diabetes, cardiovascular disease, and certain cancers. However, the unique hunter-gatherer diet of the Hadza tribe is protective against such diseases primarily due to its high fibre content, which shapes their gut microbial composition through short-chain fatty acid (SCFA) production. While previous studies have compared the gut microbial compositions of the Hadza to those of Western countries, very few have looked into the differences in microbiome functional diversity between the 2 different diet types, and have not focused on the specific metabolic pathways that are upregulated. In our study, we confirm the literature's findings that the Hadza gut microbial composition tends to be higher in both raw taxa and across diversity metrics (alpha and beta) when compared to a westernized gut microbiome. Additionally, we found multiple beneficial taxa relating to SCFA production to be present in higher amounts in the Hadza gut microbiome, and also found more disease-associated species and less SCFA-producers in the westernized one. Our study also indicated that the Hadza have lower overall functional diversity, while the westernized dataset contained more upregulated metabolic pathways related to sugar metabolism. We show that higher overall gut microbial diversity does not correlate with having increased functional diversity. These findings indicate that the high-fibre diet of the Hadza plays a role in shaping their unique microbiome, ultimately contributing to resistance against the various metabolic diseases that greatly affect the West.

3x1 PRESENTATION ABSTRACTS

Presenters will be given 3 minutes to present 1 slide with no question period. 3×1 presentations may include full research projects, proposals, or working thesis.

Breastmilk mitigates microbial differences caused by varying modes of infant delivery despite the resistant to change nature of C-section microbiome

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Early life infancy is a crucial period for shaping the microbiota as many factors during this period are responsible for driving the assembly of the infant microbiome. Two of the most studied early-life habits and practices that have drastic impacts on the infant microbiome are the mode of infant delivery and the feeding mode of the infant. Over the years, cesarean section delivery rates have been on a rise globally. Although microbial differences resulting from the mode of delivery become less striking later on in life, the microbiomes of C-section infants have notable differences in composition and diversity compared to those born vaginally. Of note, C-section deliveries have been consistently correlated with an increased chance of developing autoimmune disorders. Beta diversity analysis established feed as having a stronger influence on the microbiota compared to mode of delivery. Accordingly, breastmilk, to an extent, was able to mitigate differences caused by varying modes of delivery where C-section infants supplemented with breastmilk had a more similar profile to those born vaginally compared to C-section infants that were fed formula, as illustrated by core microbiome analysis. Differential abundance analysis further established the microbiome of C-section infants as being more resistant to changes caused by external factors compared to their vaginally delivered counterparts. This study highlights the importance of diet as a major factor influencing the infant microbiome and establishes breastmilk as the preferred feed over formula for C-sectionally delivered infants.

Pan-□-Coronavirus and Pan-Flavivirus host factor TMEM41B as a potential broad-spectrum host-directed antiviral to suppress virally induced ER membrane remodelling

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Autophagy is an evolutionarily conserved cellular process whereby damaged cellular contents are sequestered and targeted for lysosomal degradation. Macroautophagy is a major autophagy pathway which involves the formation of an autophagosome derived from intracellular membranes. Lipid droplets (LDs) are cellular organelles responsible for intracellular lipid storage whereby a phospholipid monolayer shields hydrophobic lipids in the neutral core. Both macroautophagy and LD biosynthesis begin with intracellular membrane remodeling primarily initiated from budding at the endoplasmic reticulum (ER). Despite the importance of macroautophagy and LDs on cellular homeostasis, □-Coronaviruses and Flaviviruses, hijack and exploit these pathways as part of their life cycles. One common characteristic of these viruses is the manipulation of intracellular membranes to form Replication Organelles. Although, the mechanism and the extent by which these viruses modify and dysregulate lipid membranes is not entirely understood, it is evident that manipulation of macroautophagy and LD biosynthesis through ER lipid remodeling is essential for viral replication. □-Coronaviruses, such as SARS-CoV-2, remodel the ER membranes to form double-membrane vesicles while Flaviviruses form replication complexes by invaginating ER membranes. Viral induced membrane remodeling functions to create a protective microenvironment for immune evasion and replication. Interestingly, recent studies using CRISPR genome screening discovered a pan-proviral host factor, TMEM41B, that is essential for viral replication of □-Coronavirus and Flaviviruses. TMEM41B is a transmembrane protein phospholipid scramblase ubiquitously expressed in ER membranes and plays an important role in early autophagosome, lipoprotein, and LD formation. However, the direct mechanisms of TMEM41B in remodeling ER membranes during viral infection and the viral proteins responsible for exploiting and hijacking TMEM41B is still in question. Additionally, the potential and feasibility of whether targeting TMEM41B to act as a broad-spectrum host-directed antiviral requires further research. Exploring these questions is crucial especially with rising concern over the future of SARS-CoV-2 and the imminent global onslaught of mosquito-borne Flaviviruses due to global warming.

Investigating the pharmacokinetics of GlycoCaged drugs and establishing a murine colitis Model to assess efficacy

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Inflammatory bowel disease (IBD) is an inflammatory disease of the gastrointestinal tract that is increasing in prevalence, as such the use of oral small-molecule drugs has also increased. These drugs are limited in their efficacy due to off-target effects and the high dose required to reach the lower gastrointestinal tract (GI) which is the most common site of disease. As an alternative, our laboratory, in collaboration with Dr. Brumer's team, has designed a strategy of "GlycoCaging" small molecule drugs to a sugar fragment derived from a common dietary fibre to protect drugs from premature absorption. The GlycoCaged drug is targeted to the lower GI where commensal gut microbes, particularly Bacteroides spp., express endo-xyloglucanases that cleave the sugar component releasing the active drug. Here, we applied this strategy by GlycoCaging the corticosteroid dexamethasone with a sugar fragment derived from xyloglucan (XXXG-Dex). We showed a limited concentration of dexamethasone (Dex) in circulation after XXXG-Dex treatment compared to the uncaged Dex in the SHIP deficient (SHIP-/-) mouse model of Crohn's disease-like ileitis by liquid chromatography-tandem mass spectrometry. We also established the T cell transfer model of colitis in the Sly laboratory for future studies to demonstrate the efficacy of XXXG-Dex. Our findings provide the foundation for future investigation into the implementation of GlycoCage drug development for IBD therapeutics.

A 6xHistidine tag insertion at the N-terminal signal peptide A^{42} and G^{43} may interfere with BrkA secretion and expression in *Escherichia coli* DH5α

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BrkA is a type Vα autotransporter on the gram-negative human pathogen *Bordetella pertussis.* BrkA mediates serum resistance and cellular adherence; however, the secretion mechanism of BrkA has yet to be fully elucidated. We aimed to investigate the secretion of BrkA into the periplasm and extracellular space. We inserted a 6xHis-tag at the N-terminal signal peptide A^{42} and G^{43} on *brkA*-containing pDO6935. We then conducted an immunoblot assay using our test plasmid but did not observe BrkA secretion in *Escherichia coli* (*E. coli*) DH5α. Follow-up DNA sequencing of the newly constructed 6xHis-tagged BrkA coding plasmid showed that a frameshift mutation had been introduced in the *brkA* sequence, which resulted in a truncated and possibly unstable protein product. Since we were unable to isolate clones containing the desired 6xHis tag insertion in BrkA, we speculate that the 6xHis tag insertion near the signal sequence cleavage site may have resulted in the production of a toxic BrkA variant, which may have stalled in the Sec translocon resulting in cell death. Our research, despite the unexpected outcomes, offers critical insights that can guide future attempts to clone His-tagged proteins, particularly when modifying regions near signal sequences that are essential for protein translocation

The DsbA chaperone protein may be necessary for outer membrane secretion of BrkA

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Gram-negative bacteria utilize specialized secretion systems to transport vital proteins across their outer membrane and into the extracellular environment. Among these systems, autotransporter proteins, like BrkA in *Bordetella pertussis*, autonomously localize to the bacterial surface, enhancing virulence and inhibiting bactericidal immune responses from hosts. While chaperone proteins have been shown to be involved in autotransporter biogenesis, the specific interactions between these factors and BrkA as it translocates through the periplasm present a gap in knowledge. DsbA is a chaperone protein found in the periplasm of gram-negative bacteria that may play a role in the functional pathway of BrkA expression. We aimed to explore whether DsbA affects BrkA surface expression in Escherichia coli models. We hypothesized that DsbA assists with BrkA secretion and, thus, a *dsbA* knockout should impede secretion. We employed engineered histidine tags, trypsin digestion time series and western blot analysis to investigate the BrkA secretion pathway. Our results suggest that DsbA may be necessary to facilitate BrkA secretion. We speculate that DsbA may interact with BrkA's hydrophobic secretion facilitator which promotes its translocation. This finding highlights the potentially significant role of DsbA and other periplasmic factors during BrkA translocation and provides novel insight into how periplasmic chaperones may facilitate autotransporter protein translocation. In developing a comprehensive understanding of periplasmic factor interactions in BrkA secretion, we can better understand BrkA, and contribute to the development of strategies against other pathogenic bacteria to create more effective and accessible therapeutics.

MCT-1 inhibition synergizes with glutamine metabolic inhibition to suppress the growth of SMARCA4/2-deficient ovarian cancer cells

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Small-cell carcinoma of the ovary, hypercalcemic type (SCCOHT) is a highly aggressive ovarian cancer type that primarily affects young women. Nearly all SCCOHT are characterized by the concurrent loss of both SMARCA4 and SMARCA2 (SMARCA4/2), two mutually exclusive ATPase subunits of the SWI/SNF chromatin remodelling complex that regulate cellular transcriptional activities. We have previously discovered significant metabolic shifts in SMARCA4/2-deficient SCCOHT cells, particularly decreased import of glucose and subsequent suppressed glycolysis; adaptive to this, SMARCA4/2-deficient ovarian cancer cells utilize glutamine to feed the citric acid cycle (TCA) cycle and mitochondria oxidative phosphorylation for biogenesis and energy production. Furthermore, we discovered that inhibition of glutamine uptake and metabolism by alanine and CB-839, respectively, are putative potent therapeutic approaches for SCCOHT treatment. Notably, while de novo pyruvate production is compromised due to repressed glycolysis, cancer cells can uptake pyruvate and lactate through monocarboxylate transporters (MCT), which can be converted into pyruvate, from extracellular microenvironment and import them into mitochondria to feed into the TCA cycle, thereby limiting the potency of targeting glutamine uptake or metabolism alone. Thus, MCT1 inhibition should synergize with approaches blocking glutamine uptake or metabolism to suppress SMARCA4/2-deficient ovarian cancer cell growth. Pyruvate rescue assays are done on SCCOHT cells treated with alanine and CB-839 to determine how pyruvate would interfere with drug candidates that affect glutamine uptake and metabolism. In addition, *in vitro* drug combination assays, between MCT1 inhibitor, AZD3965, and alanine or CB-839, were conducted in a panel of SMARCA4/2-deficient and proficient cell lines to observe if drug synergism occurs. It was found that pyruvate significantly rescues the inhibitory effect of both alanine and CB-839 in SCCOHT cells, showing how the presence of extracellular pyruvate impedes anti-cancer drug potency. Drug combination results revealed significant synergisms between both AZD3965 & alanine and AZD3965 & CB-839 in SMARCA4/2-deficient cell lines. Moreover, the drug combination shows no synergism in SMARCA4/2-proficient non-tumour cells, suggesting these drug combinations are tumor specific. Preliminary *in vitro* data suggests MCT-1 inhibitor, AZD3965, can be coupled to glutamine metabolism inhibitors as a novel, potent drug treatment for SCCOHT. As SMARCA4/2 deficiency is observed in other cancer types, this drug combination has potential broader implications.

It’s pretty gut-wrenching: evaluating the impact of the human IBD microbiome on microglia morphology and systemic steroid hormone levels

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Inflammatory bowel disease (IBD) is identified by chronic gastrointestinal inflammation attributed to impaired production of microbiota-derived metabolites and . IBD patients show an increased incidence of psychiatric comorbidities associated with modifications in brain structure and function. Microglia, the central nervous system's innate immune cells, shape neural circuit activity; microglial development and activity are regulated through local and peripheral signals shaped by the microbiome, alluding to a possible pathophysiological mechanism of IBD. In this study, we gavaged germ-free, adult male and female mice with microbiomes derived from a human IBD patient or a healthy control. Microglia morphology was qualitatively assessed by immunohistochemistry and circulating steroid hormone concentrations were determined with mass spectrometry to identify potential mediators of altered microglial morphology. Our findings indicate that microglia transition from a ramified to an ameboid morphology in adult mice gavaged with an IBD-microbiome, and that these mice gain weight despite consistent steroid hormone profiles with their healthy control counterparts. These data suggest that microglia respond to inflammatory signals or altered microbial signalling derived from the gut, establishing a foundation for future research aiming to assess the risk of and treat neuropsychiatric disorders in IBD patients.

Development of a flow cytometry method for high throughput analysis of synaptic protein signalling in isolated synaptosomes

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Synapses are points of contact between neurons that transmit organized signals and are crucial for normal brain function. Synaptic dysfunction is a major determinant in several neurodevelopmental and neurodegenerative disorders, often affecting neuron function and memory. In many disorders, communication between neuronal synapses through action potentials that lead to downstream protein signalling is disrupted. Synaptosomes are an accessible model for studying synaptic signalling molecules, as they consist of detached but intact synaptic terminals that are functionally active. This *ex vivo* model retains its catalogue of *in vivo* proteins and can be activated using potassium chloride (KCl) to simulate an action potential. Although activity-dependent protein signalling has been previously explored in neurological diseases, current tools to evaluate synaptic protein expression are limited. Here, we developed a flow cytometry protocol to evaluate protein expression in thousands of isolated mouse synaptosomes. Synaptosomes were isolated from murine neurons, activated with 30 mM KCl, and then stained with antibodies for flow cytometry analysis. Our results demonstrate antibody validation for presynaptic and postsynaptic markers to verify intact synaptosomes and offer a high-throughput method to evaluate rapid changes in synaptic proteins. Future genetic manipulations in neurons in the context of disease using this assay will help elucidate the role of various epigenetic factors in regulating neurological disorders.

UST1D2: A phase II/III clinical trial investigating the efficacy of ustekinumab as a treatment for recent-onset type 1 diabetes

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Type 1 diabetes (T1D) is an autoimmune disorder which stems from T cell-driven destruction of insulin-producing beta cells in the pancreas. Insulin is essential for blood glucose regulation and untreated T1D can have severe life-threatening implications. Common treatment for T1D is insulin therapy, which addresses symptoms by providing exogenous insulin. However, standard treatment does not address the destruction of beta cells and therefore does not prevent disease progression. Other treatment for T1D includes broad immunosuppression to control the beta cell-targeting T cells. However, broad immunosuppression can have dangerous side effects by dampening the immune response to harmful pathogens and malignancies. Ustekinumab is a monoclonal antibody currently used to treat other autoimmune diseases including psoriasis and Crohn's disease. Ustekinumab targets two inflammatory cytokines (IL-12 and IL-23) which are essential to the development of a small subset of T cells called Th17.1 cells. Th17.1 cells have been implicated in beta-cell loss in T1D, so we hypothesize that ustekinumab can inhibit the development of Th17.1 cells and prevent further beta-cell destruction. UST1D2 is a phase II/III, double-blind and controlled clinical trial studying the efficacy of ustekinumab as a treatment for recent-onset T1D. Eligible patients are young adults aged 18-25 who have been diagnosed within 100 days of screening. Patients receive a loading dose of 6 mg/kg ustekinumab or placebo at week 0 followed by 90 mg maintenance doses at weeks 8, 16, 24, 32, 40 and 48 (7 doses total). The primary endpoint for this trial is preservation of endogenous insulin production as C-peptide levels in the blood (a by-product of endogenous insulin production). This trial is currently ongoing and data is actively being collected. The approval of medications like ustekinumab is crucial for treatment of T1D and can provide opportunities for patients to protect their remaining beta cells, and prevent the development of T1D symptoms. Utekinumab's excellent safety profile and specific immunosuppression mechanism has the potential to drastically improve the lives of T1D patients by reducing the need for exogenous insulin treatment and reducing their symptoms.

POSTER ABSTRACTS

Presenters will be given 5 minutes to present their poster followed by a 2 minute question period. May include full research projects, proposals, or a working thesis.

Histological Analysis of the Effect of CRP Treatment on the Serotonergic Profile and Injury Scar in a Porcine Model of Spinal Cord Injury.

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Chondroitin sulfate proteoglycans (CSPGs) are major components of the injury scar that are upregulated after spinal cord injury (SCI) and play an inhibitory role in axonal sprouting and plasticity. This inspired the development of a novel peptide, **CSPG Reducing Peptide** (**CRP**), that degrades CSPGs. Following promising rodent SCI studies, in this study, the efficacy of CRP was evaluated using a porcine SCI model, which is an essential step before proceeding to clinical trials. The efficacy of CRP treatment in the porcine SCI model was analyzed from a histological perspective by evaluating changes in serotonergic profiles and injury scar composition. This study used n=20 female Yucatan pigs, with n=11 treated with CRP and n=7 serving as control animals without treatment. Two weeks after a T10 SCI, CRP-treated animals received an intrathecal infusion of FITC-CRP for 12 weeks. At 14 weeks post-SCI, spinal cord samples were dissected, snap-frozen and cryo-sectioned in 20µm thick coronal sections. Spinal cords from n=2 non-SCI non-CRP controls with similar body weights were also collected. NeuN, ChAT, and 5-HT antibodies were used to visualize alpha motor neurons and the serotonergic immunoreactive profile. Furthermore, an automated image analysis method was used to segment and analyze the multi-channel fluorescent microscopy images. CS56 and GFAP antibodies were used to visualize CSPGs and astrocytes in the injury scar. 5-HT immunostaining was observed within the ventral horn as punctuate deposits (varicosities) around alpha motor neurons and along presumptive axonal processes. Additionally, diffuse 5-HT staining was observed around the soma of alpha motor neurons. However, 5-HT intensity around the soma of the alpha motor neurons was increased after CRP treatment, while the amount of 5-HT varicosities was decreased. CSPG and Astrocyte expression along the scar border and within the perilesional area was unchanged following CRP treatment but was highly upregulated after SCI compared to no-SCI controls. These preliminary findings indicate a minimal CRP treatment effect from a histological perspective but show that the serotonergic profile and injury scar following SCI are similar to that of humans. This validates the use of the pig model as a valuable translational model.

Low serum saturated fatty acid levels positively associate with microbiota diversity and metabolic pathways in Parkinson's disease patients

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Parkinson’s disease is the fastest-growing neurodegenerative disease worldwide. Gut microbiome dysbiosis can precede the onset of Parkinson’s disease symptoms by 20 years. The ketogenic diet has shown beneficial impacts as an intervention in the treatment and modulation of the microbiome in Parkinson’s disease. While the ketogenic diet improves Parkinson’s disease symptoms, it increases the serum levels of saturated, monounsaturated, and polyunsaturated fatty acids. The current literature indicates conflicting results with the increase in intake and serum levels of fatty acids and the gut microbiome in Parkinson’s disease. We analyzed the data of 197 Parkinson’s disease patients and 103 healthy controls to unveil associations between serum levels of saturated, mono-unsaturated, and polyunsaturated fatty acids and the microbiome. Our results indicate that saturated fatty acids are positively significantly associated with the Shannon diversity of gut microbiome in Parkinson’s disease subjects. Mono-unsaturated and poly-unsaturated fatty acids were not significantly associated with the microbiome diversity. Additionally, we identified low saturated fatty acids associated with the *Akkermansia, Bifidobacterium*, *Faecalibacterium*, and *Haemophilus* genera, with implications in Parkinson’s disease progression and gut dysbiosis. Our analysis also shows low saturated fatty acid positively associates with metabolic pathways such as menaquinol and L-methionine, both having been highlighted as beneficial for Parkinson’s disease. Together, our study indicates low levels of serum saturated fatty acids associate with genus changes and pathways implicated in Parkinson’s disease. These results can be used to improve and uncover new interventions aimed at treating and modulating the gut microbiome of Parkinson’s disease patients.

Harnessing the BrkA autotransporter and pDO6935 plasmid for MTT5 display on *E. coli* for zinc biosorption evaluation

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The Type Va autotransporter secretion system, represented by BrkA in *Bordetella pertussis*, presents an attractive avenue for bioremediation strategies targeting heavy metal contamination. Heavy metals like cadmium (Cd) and zinc (Zn) pose significant environmental and health hazards, warranting effective remediation approaches. Previous studies have demonstrated successful surface expression of MTT5, a metal-binding protein, in *Escherichia coli*, leading to enhanced adsorption of Cd. However, challenges arise from potential membrane disruption. Leveraging the Type Va autotransporter system could circumvent these limitations, ensuring membrane integrity and bacterial viability. Our study aims to exploit the BrkA autotransporter to effectively surface-display MTT5 on *E. coli* UT5600 cells, facilitating efficient zinc ion adsorption. We hypothesize that this strategy will enhance zinc biosorption capabilities in *E. coli* cells expressing MTT5. We constructed a vector using Gibson assembly with MTT5 and 6X His-tag inserted to the pDO6935 backbone, verified the expression of the MTT5 protein using Western Blot and assessed MTT5 adsorption ability using *Arabidopsis thaliana* as a model seed organism. Overall, our research aims to contribute to the development of effective bioremediation strategies for heavy metal contamination using bacterial surface display systems.

Oral microbiome sampling using non-invasive brushing for at-risk oral lesions – A pilot study

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Oral cancer (OC) is amongst the most prevalent cancers worldwide with incidence rates increasing rapidly in many countries. Moreover, there has been minimal improvement in prognosis over the last 50 years which has heightened the need to develop new strategies for early detection and diagnosis. There is increasing evidence suggesting an important connection between oral microbiome dysbiosis and the development of OC and oral premalignant lesions (OPL). Thus, there is potential to use the oral microbiome as a predictor for oral lesion progression and or to identify biomarkers to infer oral health. To this end, we explored the feasibility of brush samples to detect the oral microbiome and assess differences between lesional and non-lesional sites. We characterized the oral microbiome of 26 patients with varying degrees of OPLs by taking brush samples of the mucosal lesion site, along with a non-lesional or contralateral mucosal surface as a control for each patient in anticipation for downstream paired analyses. DNA was extracted using the QIAmp DNA Microbiome Kit and quantified at UBC's Biofactorial High-Throughput Biology Facility. Primers for the V4-V5 hypervariable region of the 16s rRNA were used during Next-generation sequencing on the Illumina MiSeq platform. The resulting amplicon sequence variants were used in taxonomic analysis with R statistical software. When comparing lesional and non-lesional brush samples, microbiome diversity can give an insightful overview of the data. Beta diversity visualized on a multidimensional scaling plot representing the oral microbiome composition of lesional and non-lesional brush samples showed slight segregation between the two groups. Further, when considering degree of pathology, we saw a slight downwards trend in alpha diversity of samples from low-grade OPLs compared to those from high-grade OPLs to OC. Taxonomic analysis revealed a relative increase in Firmicutes and a decrease in Proteobacteria in lesional samples compared to non-lesional samples at the phylum level. Some notable species that have an increased abundance in lesional samples include *Fusobacterium nucleatum*, *Streptococcus anginosus,* and *Parvimonas micra*, which have been associated with cancer progression.Overall, non-invasive brushing samples show promise for characterizing oral microbiomes in oral cancer and premalignancy settings.

Immune Checkpoint Inhibitors and Tumor Characteristics in Precision Oncology

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Cancer is a complex disease that is driven by several mechanisms resulting in genomic alterations and augmented tissue microenvironment. With new advancements in genome sequencing, we can now perform whole genome and transcriptome analysis (WGTA) to understand the underlying factors that drive tumorigenesis that help us target the driver events and deliver precision medicine tailored to individual patients. With growing insights into the genomic drivers of cancer, there is burgeoning interest in immune checkpoint inhibitors (ICIs) for the treatment of various tumour types. This class of drugs works by blocking inhibitory immune pathways such as PD-1/PD-L1 or CTLA-4, which are exploited by cancer cells to evade immune detection and destruction. More importantly, robust biomarkers of response to ICI remain elusive despite its now widespread use in the clinic. In this project, we leveraged data collected from patients enrolled in the Personalized Onco-Genomics (POG) program at BC Cancer to evaluate the impact of the tumour microenvironment and mutation burden in response to immune checkpoint inhibitors. Anonymized data from over 1000 patients in the POG program was reviewed to identify patients who had received ICIs as part of their cancer treatment. Treatment information including time on therapy and response to therapy were compiled. RNA-Seq data was interrogated using the CIBERSORT algorithm to examine the tumour immune environment. Whole genome sequence data was analysed to compute the tumour mutation burden. We hypothesized that patients with higher TMB and CD8+ T cell scores are more likely to experience clinical benefit from immune checkpoint inhibitor treatment. Over 200 ICI-treated POG patients were identified. Tumour types were predominantly breast, eye, head and neck, lung, pancreatic, and skin cancers. The best responses to ICIs were generally in eye and skin cancers. Trends were observed in CD8+ T cell scores and TMB in patients with clinical benefit to ICIs. WGTA will be further leveraged to investigate alterations that may contribute to response/resistance to immune checkpoint inhibitors, which may also identify novel biomarkers to guide treatment decisions in the clinic.

Novel enhancers downstream of *MYCN* drive increased gene expression in early T-cell progenitor acute lymphoblastic leukemia which sustains a proliferative cell state.

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Acute lymphoblastic leukemia (ALL) is an aggressive neoplasm characterized by the dysregulated proliferation of immature lymphocytes in blood. Early T-cell progenitor acute lymphoblastic leukemia (ETP-ALL) is a subset of ALL where leukemic arrest occurs at a very early stage of T-cell differentiation. This subset of T-ALL is marked by particularly poor clinical outcomes including low event-free survival and high disease refractoriness. Recent work has suggested that *MYCN*, a transcription factor and established oncogene, is overexpressed in particularly aggressive cases of ALL, including ETP- ALL. Functionally, *MYCN* is involved in sustaining a transcriptional program that supports a self-renewing and proliferative cell state. Surprisingly, the mechanisms regulating MYCN are virtually unexplored in ETP-ALL. Using an engineered human cord blood derived ETP-ALL model, our lab identified two putative novel enhancers downstream of *MYCN* based on H3K27 acetylation and H3K4 monomethylation marks. These enhancers were unique to ETP-ALL and thus we hypothesized that they drive the overexpression of *MYCN* in ETP- ALL. To test our hypothesis, we employed a paired gRNA CRISPR-Cas9 strategy to delete each of the two enhancers in addition to a third distal enhancer previously characterized in literature. Following confirmation of the deletion with high efficiency, we quantified MYCN expression using RT-qPCR and identified a significant reduction in *MYCN* expression associated with loss of one of the enhancers. Given its role in driving cellular proliferation, we also expected to observe a reduction in cellular growth associated with a reduction in *MYCN* expression. This was measured using high throughput amplicon sequencing over multiple time points to determine changes in the proportion of enhancer-deficient populations. Based on this, we identified a significant loss of fitness associated with loss of the distal enhancer and one of the two novel putative enhancer sequences. Our study better characterizes the molecular mechanisms driving *MYCN* overexpression in ETP-ALL and potentially provides foundations for future research targeting the epigenome to treat aggressive ALL cases.

The clinical role of routine bladder biopsies in follow-up after Bacillus Calmette-Guérin treatment of pure carcinoma in situ of the bladder

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Carcinoma in situ (CIS) of the bladder is characterized by flat mucosal lesions that are high-grade and non-invasive. It is effectively treated with intravesical Bacillus Calmette-Guérin (BCG), a form of immunotherapy. Patients are monitored with cystoscopy and urine cytology, but recurrent or persistent lesions may be invisible or indistinguishable from inflammation. Missed CIS can progress rapidly to muscle-invasive cancer which is potentially lethal. Therefore, it is important to identify recurrent CIS during follow-up. Guidelines recommend bladder biopsies only if cystoscopy shows suspicious findings or if urine cytology is positive for malignant cells. However, the diagnostic value of cystoscopy and urine cytology can be compromised by the bladder inflammation induced by BCG. The present study investigated whether routine biopsy after adequate BCG therapy increases early identification of disease persistence and prevents disease progression in patients with pure CIS. A retrospective chart review was conducted of all patients with isolated CIS treated with BCG between January 1, 2011 and January 31, 2024. The outcomes of patients treated with the intent to perform routine bladder biopsies at 6 months, regardless of cystoscopy and urine cytology results, were compared to the outcomes of those who underwent biopsy only if the cystoscopy or urine cytology was abnormal (“for cause”). 50 patients were included, with 26 patients treated with routine biopsy and 24 patients treated with for-cause biopsies. Recurrent or persistent disease within 6 months was identified in 19% and 17% of routine and for-cause biopsy patients, respectively ($p = 1.0$). Early CIS persistence was detected in 19% of routine biopsy patients compared to 4% of for-cause biopsy patients ($p = 0.19$). Disease progression was observed in 8% of routine biopsy patients compared to 21% of for-cause patients ($p = 0.24$). The results suggest that routine and for-cause biopsy strategies identify similar numbers of early disease recurrence overall. However, there was a trend towards a higher rate of early detection of CIS persistence, specifically, and a lower rate of disease progression for patients treated with routine biopsies. Given the limited sample size, further research is recommended.

Nutrients may play a complex role in influencing microbiota composition and function in Parkinson’s disease patients

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Parkinson's disease is a neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons, leading to motor and non-motor symptoms. Previous studies have shown reduced gut microbial diversity and imbalances in specific populations in Parkinson's disease patients including increases in inflammatory microbes and decreases in commensals. However, the impact of dietary nutrients on gut microbiota composition in Parkinson's disease patients remains unclear. By analyzing published microbiota data containing nutrient intakes of Parkinson's disease patients and non-Parkinson's disease controls, we aimed to identify nutrients that are associated with gut microbiota composition in Parkinson's disease patients. We identified key nutrients associated with altered beta diversity in Parkinson's disease patients compared to the control. We then separated Parkinson's disease and controls based on consumption of each of these nutrients and performed taxonomic analyses by comparing core microbiomes, indicator taxa, differential abundance, and functional pathways analysis comparing the upper and lower nutrient consumption groups for Parkinson's disease and controls. From these analyses, we identified unique and differentially abundant microbial taxa and functional pathways to Parkinson's disease compared to control. Ultimately, our survey into nutrients driving changes in microbial diversity in Parkinson's disease patients may help guide future research into nutrient intake catered to the prevention and management of Parkinson's disease.

Mapping potential transcription start sites of the *brkA* gene on pDO6935 in *Escherichia coli* using the ARF-TSS method

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Autotransporters are proteins expressed on the outer membrane of gram-negative bacteria. BrkA is an autotransporter in the whooping cough-causing bacterium *Bordetella pertussis*. It acts as a virulence factor by inhibiting our immune system from killing the bacterium. Previous studies aimed at characterizing the autotransporter BrkA have used *Escherichia coli* (*E. coli*) cells expressing the plasmid pDO6935 which contains a *brkA* gene; however, the promoter driving *brkA* gene expression in these cells remains unknown. Our study aims to determine the transcription start site (TSS) of the *brkA* gene in pDO6935 in *E. coli* (DH5α). Identification of the TSS will provide a better characterization of the promoter region. To map the TSS, we implemented the adaptor- and radioactivity-free identification of the transcription start site method (ARF-TSS), which involves reverse transcribing *brkA* mRNA into cDNA using a 5'-phosphorylated primer, circularizing the cDNA, amplifying the cDNA with PCR, and inserting the amplified fragments into a vector, before sending them for sequencing to determine the TSS. We more precisely mapped the region of the plasmid in which the transcription start site driving the expression of the *brkA* gene in pDO6935 is located: a region more than 270 bp upstream of the *brkA* translation start site. Finding the transcription start site aids in locating the promoter and contributes to our understanding of gene expression and regulation. This knowledge can help improve future studies aimed at characterizing BrkA autotransporter using pDO6935-expressing *E. coli* cells.

Determining the therapeutic potential of Tr1 cell therapy via fecal lipocalin-2 levels in DSS-Induced colitis mouse models

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Inflammatory Bowel Disease (IBD) is a chronic disease involving recurrent gastrointestinal inflammation, leading to abdominal pain, diarrhea, and fibrosis. A prospective targeted approach to address gut inflammation without compromising overall immune function utilizes Type 1 regulatory T cells (Tr1 cells). Tr1 cells are known for their ability to produce large amounts of interleukin-10, an anti-inflammatory cytokine, and aid in reducing inflammatory related damage. Tr1 cell therapy has previously shown efficacy in mitigating inflammation in two mouse models of IBD. However, the impact of this therapy on Fecal Lipocalin 2 (LCN-2), a validated biomarker for colonic epithelial tissue inflammation in IBD, remains unexplored. We hypothesize that Tr1 cell therapy will reduce inflammation, leading to a corresponding decrease in LCN-2 levels. C57BL/6 mice were adoptively transferred with 1x10^6 Tr1 cells before induction of Dextran sulfate (DSS) colitis, with control mice receiving PBS in parallel. Following 7-days of DSS treatment, mice were allowed to recover, and stool samples were collected on days 7 and 14. LCN-2 levels were quantified using a Mouse Lipocalin-2/NGAL ELISA. Anticipated results include the manifestation of colitis in mice, with inflammation peaking at day 7, leading to elevated LCN-2 levels. Tr1 cell-treated mice are expected to exhibit reduced inflammation and lower LCN-2 levels compared to control mice when exposed to the DSS solution. These findings offer crucial insights into how Tr1 cell therapy effectively treats IBD by modulating specific biomarkers like Fecal Lipocalin 2 (LCN-2) and reducing colonic epithelial tissue inflammation.

Oxalate as a disrupter of the intestinal microbiome and the protective effects of butyrate in kidney stone disease

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Kidney stone disease (KSD) is characterized by the recurrent formation of kidney stones and can cause intense pain or tissue damage. The most common type of kidney stones are made of calcium and oxalate, which crystallize into solid stones (CaC_2O_4) in the kidney and urinary tract. Previous research found that probiotic supplementation of O. *formigenes*, an oxalate degrading bacterium depleted in KSD patients, did not alleviate hyperoxaluria (PMID: 28718073). In support of additional studies where KSD patients had fewer butyrate producers, our lab recently found that tributyrin supplementation reduces CaOx crystal deposits in the kidney (PMID: 32381601). Supplementation with inulin, a prebiotic fermented into butyrate by the microbiome, didn't produce the same effect. Consequently, this project aims to explore additional roles that oxalate has on butyrate production by the intestinal microbiome in the context of KSD. In our experiment, we developed a mouse model for KSD by supplementing dietary sodium oxalate. Mice were fed this diet for 14 days in combination with either a control fibre (cellulose) or one of two butyrate supplements: inulin (prebiotic) or tributyrin (postbiotic). Stool, cecal contents, urine, and tissue (kidney, intestine) samples were collected at 2 time points. Short chain fatty acids were quantified by gas chromatography and the intestinal microbiome was profiled using 16S rRNA sequencing in both stool and cecal contents samples. It was found that oxalate significantly disrupts the diversity and composition of the intestinal microbiome. This included increasing the relative abundance of opportunistic pathogens. Moreover, there was significantly less butyrate in the stool and cecal contents of mice supplemented with inulin and oxalate compared to inulin alone even though butyrate producers were abundant in both diet groups. As butyrate is known to mediate inflammation, we hypothesize that oxalate induced damage to the brush border and microbiome of the intestine leads to increased butyrate usage by the colonocytes. Hence, we are currently measuring the degree of inflammation in the intestinal tissue of the mice in our model and evaluating the expression of gene pathways in this process. Overall, butyrate supplementation shows potential therapeutic promise in mitigating KSD symptoms.

Promoter mapping analysis using a GFP reporter plasmid suggests that the *lac*

promoter may drive *brkA* expression on plasmid pDO6935 in *Escherichia coli*

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Autotransporters (AT) are a class of bacterial proteins that play a crucial role in bacterial virulence and pathogenesis. Understanding these proteins is essential to advance research in infectious diseases and identify possible targets for therapeutic interventions. *Bordetella pertussis* autotransporter, BrkA belongs to the AT-1 subfamily of autotransporter proteins in gram-negative bacteria. The mechanism by which *brkA* is expressed in pDO6935, including its promoter, is yet to be fully elucidated. In order to map this region, we used a promoter trapping method wherein different sections of the plasmid were cloned into a reporter plasmid. An analysis of pDO6935 gene sequence revealed the presence of a *lac* operon upstream of *brkA*. The *lac* operon is known to exhibit basal expression in the absence of lactose or the presence of a repressor. We studied whether the leaky expression caused by the *lac* operon may result in the expression of *brkA* in an *Escherichia coli* system. In our study, we utilized pSPPH21, a promoterless reporter plasmid that was designed to contain green fluorescent protein (GFP). Using this vector, we inserted regions of putative promoter sequences upstream *brkA* in pDO6935. Fluorescence imaging and quantification on a plate reader suggested that the *lac* operon may be driving *brkA* expression in pDO6935. We further investigated this argument upon treatment with glucose, a known catabolite repressor of the *lac* operon. Glucose treatment resulted in repressed levels of GFP, providing additional evidence to our findings. Characterizing the promoter sequence will increase the capabilities of pDO6935 to better fit future studies.

The preliminary findings of purified Ag43α protein as a potential method for preventing *Escherichia coli* autoaggregation

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The pervasive nature of *Escherichia coli* (*E. coli*) autoaggregation and biofilm formation poses significant challenges in clinical and environmental contexts, contributing to the bacterium's resistance against immune responses and antibiotic therapies. Autotransporters, particularly Antigen 43 (Ag43), a prominent outer membrane protein in Gram-negative bacteria like *E. coli*, facilitate bacterial aggregation through their distinct structural domains, which include a secreted passenger domain (Ag43α) and a β-barrel domain anchoring the secreted protein to the outer membrane. Prior studies have established the role of the Ag43α subunit in promoting self-recognition and aggregation, hence we hypothesised that the application of exogenously purified Ag43α could interrupt these interactions, thereby inhibiting autoaggregation. Our methodological approach involved expressing the Ag43α subunit in BL21(DE3) *E. coli* cells using the pEEKABOO plasmid from Leong et al., followed by the purification of the expressed protein. We subsequently examined the effects of introducing purified Ag43α at varying concentrations into cultures of DH5α *E. coli* strains that naturally express Ag43. The objective was to verify the expression and proper folding of Ag43α and to understand its interaction with *E. coli* cells by evaluating its influence on their autoaggregation. The results demonstrated the expression of Ag43α in *E. coli*, with the purified protein adopting a stable tertiary structure post-translation. Preliminary results from a single-trial aggregation assay revealed that introducing 200 μg/mL of purified Ag43α significantly attenuated autoaggregation in acidic environments underscoring the protein’s potential in modulating *E. coli*'s autoaggregation capabilities. The significance of this research extends broadly, introducing a novel approach to combating E. coli infections and contamination driven by Ag43 aggregation, paving the way for the creation of innovative antibacterial strategies. These findings highlight the potential of manipulating Ag43 bacterial aggregation to mitigate infection but also open avenues for the exploration of microbial behaviour, offering a promising direction for future antibacterial research and therapy development.

Suppression of murine inflammatory bowel disease by Tr1 cells via IL-10-mediated inhibition of inflammatory macrophages

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Inflammatory bowel disease (IBD) can be characterized by chronic inflammation of the gastrointestinal tract. Existing therapies for IBD are non-specific and fail to reverse fibrosis, leading to greater interest in regulatory T cell therapies as they target the source of inflammation directly. Specifically, type 1 regulatory (Tr1) cells have been identified as a therapeutic candidate due to their high production of anti-inflammatory cytokines such as IL-10. Moreover, macrophage-mediated production of interleukin-1beta (IL-1β), a key proinflammatory cytokine in IBD development, has been shown to be inhibited through Tr1 cells. This study aims to explore the Tr1-mediated suppression of inflammatory macrophages and elucidate the underlying mechanism. Bone marrow-derived macrophages from an IBD mouse model will be grown and stimulated. Suppressive activity will be assessed by both culturing macrophages in varying ratios of Tr1 supernatant and co-culturing macrophages with Tr1 cells, with changes in IL-1β production captured using an ELISA. Characterization of these macrophages using flow cytometry will allow us to determine effects on cell viability following growth in Tr1 supernatant. We expect to see a dose-dependent decrease in IL-1β levels by inflammatory macrophages when exposed to different dilutions of Tr1-conditioned media or to Tr1 cells. Negation of this Tr1-mediated suppressive effect when anti-IL-10 antibodies are present should be observed, indicating IL-10 dependency. This research could prove foundational in the development of a novel Tr1-mediated therapy for IBD. Further, it can shed light on key immunoregulatory mechanisms in inflammation, leading to more effective and comprehensive therapeutics for other autoimmune conditions.

Characterization of Skp chaperone knockdown and its effects on *Bordetella pertussis* BrkA secretion in *Escherichia coli* BW25113

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Bordetella pertussis (*B. pertussis*) is a well-studied Gram-negative bacterium and the causative agent of whooping cough, a highly contagious respiratory disease found in humans. A key aspect of *B. pertussis* pathogenicity is the secretion of Bordetella Resistance to Killing (BrkA) virulence factor that inhibits the bactericidal activity of complement. As a Type Va autotransporter, the *brkA* sequence contains a β-domain, forming a β barrel structure embedded into the bacteria's outer membrane (OM). While crossing the inner membrane (IM) and the periplasmic space, the protein must remain in its unfolded form; this process is potentially modulated by bacterial periplasmic chaperones such as Seventeen-Kilodalton Protein (Skp). Previous studies utilized Δ*skp* mutant cells and found that Skp is required to solubilize outer membrane proteins (OMPs) in periplasms. However, research regarding the relationship and protein-protein interactions between Skp and BrkA is limited. This study aims to elucidate the role of the Skp chaperone in the secretion of the BrkA autotransporter while characterizing *skp* knockdown *Escherichia coli* (*E. coli*) cells. We employed an anti-sense RNA (asRNA) vector, under the control of a lacO operator, to knockdown *skp* expression. The asRNA sequence was designed to be complementary to a 40 base-pair (bp) region containing the Shine-Dalgarno (SD) ribosome binding site as well as the first four codons. Previous studies also found Δ*skp* mutants to be more susceptible to hydrophobic antibiotics; a zone of inhibition assay was used to determine if Skp protein levels were reduced in transformants. Knockdown effects were characterized using microscopy and growth curves, while BrkA secretion was quantified using enzyme-linked immunoassays (ELISA). We successfully created a *skp* asRNA vector and are currently compiling data on growth and inhibition zones, with BrkA secretion assays underway. Preliminary results suggest that Skp knockdown correlates minimally with altered growth patterns. This study provides a deeper understanding of *B. pertussis* pathogenesis and may guide the development of novel therapeutic strategies against the pathogen. Additionally, the implications of these findings could guide future research of bacterial chaperone systems and their role in infectious diseases.

Peel to seal: effectiveness of orange peels in growing bacterial cellulose as food packaging

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Bacterial Cellulose (BC) is a biodegradable alternative to single-use plastics that can be grown from food waste. With single-use plastics being found on 64% of products in Canadian grocery stores], and nearly 20% of Canadian food being wasted due to preventable food losses, BC provides a solution to a major sustainability issue. Having been proven to slow food spoilage by up to six days at room temperature, bacterial cellulose can replace plastic packaging like bags or cling wrap. Using food scraps to grow BC cuts down on food waste further by utilizing an otherwise wasted organic material. Orange peels and other acidic fruit scraps can be used as growth mediums by creating the ideal pH range of 4-7 needed for BC synthesis. *Komagataeibacter medellinensis* was the chosen bacterial strain in this experiment as it naturally occurs on acidic fruits. After being cultivated, the BC was strengthened through mercerization, a chemical treatment process, increasing the tensile strength. The outcomes of this study indicate that BC produced from orange peels are comparable to BC grown in conventional media, such as Hestrin-Schram. Using food scraps in growth mediums is an economical and sustainable way of manufacturing bacterial cellulose. Furthermore, pectins derived from the acid hydrolysis of fruit scraps can be commercially distributed to generate economic returns. From a community perspective, the use of other food scraps to grow packaging offers opportunities for households to reduce food waste.

Optimization of a Jurkat reporter cell line to measure TGF-β1 secretion by regulatory T cells

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CD4+ regulatory T cells (Tregs), identified by expression of FOXP3, CD25, and CTLA-4, have demonstrated effectiveness in suppressing undesirable immune responses. Transforming Growth Factor beta 1 (TGF-β1) signalling is pivotal for Treg development, and its autocrine or paracrine actions further contribute to immune homeostasis. TGF-β is produced as an inactive protein, requiring further proteolysis of latency-associated peptide (LAP) to release it. However, it is currently challenging to quantify active TGF-β1. Thus, this study aims to develop a precise method for quantifying TGF-β1 produced by CD4+ regulatory T cells (Tregs) using a T-cell receptor (TCR) knock-out, triple parameter reporter (TPR) Jurkat cell line as a reporter system. In response to stimulus, TPR Jurkats express eCFP, eGFP, and mCherry via inherent genetic reporter constructs responsive to NFκB, NFAT, and AP-1 transcription factors. TPR Jurkats were transduced via lentivirus and cells expressing different levels of a Chimeric Antigen Receptor (CAR) specific for active TGF-β1 were sorted. Utilizing flow cytometry, we confirmed the cells' ability to respond to recombinant active TGF-β1 by detecting the expression of eCFP, eGFP, and mCherry. The TGF-β1 reporter Jurkats could also successfully detect active TGF-β1 produced by Tregs which had been stimulated through TCR. A higher CAR level on Jurkat cells was found to have a more pronounced response to TGF-β1 and a lower detection limit. These TGF-β1-detecting TPR Jurkats thus represent a new tool to quantify biologically active TGF-β1 produced by Tregs and will provide insights on the role of this cytokine in Treg suppression and therapeutic applications.