
2023 MBIM
Undergraduate Research Symposium

WELCOME

On behalf of the Symposium Planning Committee, it is with great pleasure that we welcome you to the 2023 Microbiology and Immunology Undergraduate Research Symposium (MBIM URS)! After the success of the last five years, we are delighted to share with you the results of our hard work at the sixth annual MBIM URS. Excitingly, this year is the first in-person event we will be hosting since 2020, and we look forward to seeing students showcase their work in a variety of presentation formats. In addition to our traditional 10-minute presentation division, this year's event will also feature the popular 3-minute thesis presentation format from last year as well as a poster session. The organizing committee has been working diligently to make this year's event a possibility and we are excited to be able to share this experience with you all.

This symposium is a product of our hardworking undergraduate student organizing committee, who have put in countless hours to make this event happen in-person. Their contributions cannot be overstated and we would like to thank all of them for their fantastic work in this. We would also like to thank Dr. Evelyn Sun for her tireless leadership and support during the planning of this event, and Dr. David Oliver for contributing his insight and experience in running the event in previous years. Special thanks must also go to our graduate student judges and the staff at the UBC Department of Microbiology & Immunology, for providing their unwavering support in our goal of encouraging scientific outreach. Lastly, we would like to thank all of the symposium participants and attendees for supporting us and seizing this opportunity to advance their scientific experience and education. Congratulations to you all!

We hope you enjoy this symposium and continue supporting undergraduate research in future years. We are looking forward to all of the amazing presentations in April!

Yours sincerely,

Claire Sie & Rachel Cheng

Student Co-Chairs | 2023 MBIM Undergraduate Research Symposium

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ABOUT

The 6th annual MBIM Undergraduate Research Symposium provides undergraduate students with a forum to share their research findings, interact with scientists with related interests, and develop communication skills.

The 2023 MBIM URS will be the first in person meeting since 2022. It will include opening remarks from the URS Co-chairs and a welcome by Dr. Michael Murphy. Additionally, we will feature a keynote address by Dr. Kayla King, evolutionary biologist and a new faculty member in the Department of Microbiology and Immunology at UBC. Furthermore, we will also have lunchtime info sessions with representatives from STEMCELL in attendance!

Our talks 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 both the 10 minute, 3 minute oral presentation, and poster categories!

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

LETTERS

Letter from Dr. Michael Murphy

A message from the Department Head

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 Department Head

Department of Microbiology & Immunology

University of British Columbia

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SPONSORS

We would like to thank our sponsors, STEMCELL technologies and the UBC MBIM department, for their generous contributions to our event.

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KEYNOTE SPEAKER

Dr. Kayla King studies the evolution, ecology, and genomics of host-pathogen interactions. She is new to UBC as Professor in the Departments of Microbiology & Immunology and Zoology at the University of British Columbia. She is also a Professorial Fellow at the University of Oxford. Originally from Nanaimo, Dr. King was awarded her BSc from UBC in Environmental Biology, followed by a MSc from Concordia University in Parasitology. Dr. King went on to earn her PhD from Indiana University in Evolutionary Biology and Genetics where she developed research studying the coevolution of host-parasite interactions.

SYMPOSIUM PROGRAM

April 28, 2022 - UBC Michael Smith Laboratories

Time Period & Events (All times listed in PST)

Time	Presentation Title	Presenters	Location
8:30 - 9:00 AM	**Sign-in & Registration**		MSL 101
9:00 - 9:15 AM	Opening Remarks		MSL 102
9:15 - 10:00 AM	Keynote Talk	Dr. Kayla King	MSL 102
Immunology & Virology Talks			
10:00 - 10:15 AM	Evaluating the effects of calcium flashers on increasing calcium administration rates and improving markers of blood coagulation	Melody Weng	MSL 102
10:15 - 10:30 AM	Towards an *in vitro* model of NLRP3 inflammasome activation via refining measures of IL-1β secretion and inflammasome assembly	Sahar Malekighomi, Iris Zhou, Betty Yao	MSL 102
10:30 - 10:45 AM	Investigation of antiviral activities of SARS-CoV-2 main protease (3CLpro) inhibitors	Ellie Gang	MSL 102
10:45 - 11:00 AM	Break		MSL 101
Concurrent Molecular Microbiology & Environmental Microbiology Talks			

11:00 - 11:15 AM	Optimizing *E. coli* expression, IMAC purification under denaturing conditions, and refolding of recombinant chitinase C of *Pseudomonas aeruginosa*	Soroush Mohebat, Zee Muradi, Pooya Namavari, Negarin Shahtalebi	MSL 102
11:00 - 11:15 AM	Optimization of chitinase C expression from the pM3CRYY plasmid in *Escherichia coli* BL21(DE3) and determination of secretion status	Jinny Choi, Veronika Lewis, Claire Sie, Justin Yap	MSL 101
11:15 - 11:30 AM	Recombinant Chitinase C from *Pseudomonas aeruginosa* is expressed and potentially secreted in *Escherichia coli* BL21 (DE3)	Renée Lim, Ishana Lodhia, Shutong Gang, Azraa Banka	MSL 102
11:15 - 11:30 AM	Optimization of expression in *Escherichia coli* BL21 (DE3), IMAC purification, and preliminary functional characterization of recombinant ChiC from *Pseudomonas aeruginosa*	Haein Kim, Davey Li, Apsara Srinivas, Leonardo Wu	MSL 101
11:30 - 11:45 AM	Development of a chitinolytic activity assay using the DNS method for detection of reducing sugars	Maria Beletsky, Ashleen Khatra, Jenny Shee, Kevin Wang	MSL 102
11:30 - 11:45 AM	Antifungal activity of recombinant ChiC from *E. coli* pM3CRYY BL21(DE3)	Emma Dhaliwal, Karmin Dhindsa, and Vianne Chang	MSL 101
12:00 - 1:00 PM	**Lunch time & Poster session**		MSL 101

1:00 - 1:15 PM	Expression of thermophile C*hloroflexus aurantiacus* photosynthetic reaction centre synthetic genes in the mesophile *Rhodobacter sphaeroides* RCx^R	Meghan Marshall	MSL 102
Microbiome Talks			
1:15 - 1:30 PM	Individuals undergoing westernization with abnormal cardiometabolic statuses are more vulnerable to smoking-induced alterations of the gut microbiome	Ali Reza Nasseri Dehkharghani, Darius Parmar, Mehdi Tabesh, Junqin Wang	MSL 102
1:30 - 1:45 PM	Major factors responsible for diversity in the gut microbiome of Columbian adults	Sana Samadi, Ghazaleh Shoaib, Jen Wang and Martin Joo	MSL 102
1:45 - 2:00 PM	A breast milk exclusive diet promotes dysbiosis in the gut microbiome of six-month-old anemic infants	Jenine Hira, Ekroop Sohal, Apsara Srinivas, Haein Kim	MSL 102
2:00 - 2:15 PM	Iron deficiency anemia is associated with loss of gut microbial diversity in 6 and 12 month old infants	Luiza Lopes Pontual, Mark Josef Huang, Parneet Sekhon, Taylor Bootsma	MSL 102
3x1 Rapid Talks			
2:15 - 2:18 PM	The Road Less Traveled: Exploration of Non-Structural Proteins Using Organoid Models in SARS-CoV-2	Sana Samadi	MSL 102
2:18 - 2:21 PM	Syncytia Formation: A Trademark of Severe SARS-CoV-2 Infection and a Potential Target for Novel Drug Therapies	Loujain Bilal	MSL 102

2:21 - 2:24 PM	Hunting for synthetic lethality: hit validation following CRISPR-based chemogenomic screen	Maya Ruehlen	MSL 102
2:24 - 2:27 PM	Insights into the Structure and Function of SARS-CoV-2 Non-Structural-Protein 6: Implications for Antiviral Drug Development	Parsa Khatami	MSL 102
2:27 - 2:30 PM	The impact of LPS and SCFAs on microglia-neuron interactions	Veronika Lewis	MSL 102
2:30 - 2:50 PM	**MICB 418 Film Fest Showdown**		MSL 102
2:50 - 3:00 PM	**Closing Remarks and Prizes**		MSL 102

ABSTRACTS (10X10)

Presenters will have 10 minutes to present a maximum of 10 slides.
Presenters must include an introduction, methodology, results, and discussion.
This will be followed by a 2-5 minute Q&A period.

1. **Optimizing *E. coli* expression, IMAC purification under denaturing conditions, and refolding of recombinant chitinase C of *Pseudomonas aeruginosa***

Soroush Mohebat[1], Zee Muradi[1], Pooya Namavari[1], Negarin Shahtalebi[1]

[1]Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia, Canada
[1]All the authors contributed equally to this study

Chitinase C (ChiC) from *Pseudomonas aeruginosa* can potentially be developed into a bioinsecticide agent, as it can break down chitin, a crucial component of most insect exoskeletons. However, due to the pathogenic potential of *P. aeruginosa*, developing ChiC for use in agricultural industries is not desirable. Therefore, previous studies attempted to genetically engineer *E. coli* BL21, a non-disease-causing *E. coli* that allows for the safe expression of ChiC. For this, they transformed *E. coli* BL21 with pM3CRYY, a pET-28α plasmid containing the *chiC* gene. Although they observed the expression of ChiC in *E. coli*, they did not go further to purify ChiC. Thus, the aim of this study was to obtain purified and functional ChiC. We investigated the yield of ChiC expression under different sets of conditions, followed by purification and refolding of the enzyme. The studied conditions were temperature, Isopropyl β-D-1-thiogalactopyranoside (IPTG) concentration and IPTG induction period. The conditions that led to the highest yield of expressed ChiC were found to be 0.1 mM [IPTG] with 2h induction period at 37°C. At these conditions, the majority of the expressed ChiC was insoluble, meaning that the ChiC was not functional. Therefore, to obtain the functional form of the enzyme we solubilized the expressed ChiC. For this, we purified ChiC from the insoluble fraction of the cell lysates using immobilized metal (Ni2+) affinity chromatography under denaturing conditions. Subsequently, we refolded the enzyme through dialysis, which yielded 11.7g of ChiC per 1L of *E. coli* culture. The limited proteolysis experiment demonstrated that the purified enzyme was refolded into a uniform tertiary structure in dialysis. The successful purification of the refolded ChiC using a chitin-binding column suggested that the enzyme binds to the chitin beads. This suggests that the binding domain of the refolded ChiC is functional. The overall results indicate that functional ChiC can be extracted from insoluble fraction of cell lysates, which paves the way for future biotechnological applications of ChiC in agricultural industries.

2. **Evaluating the effects of calcium flashers on increasing calcium administration rates and improving markers of blood coagulation**

Melody Weng [1, 2]

[1] Trauma Services Department, Vancouver General Hospital
[2] Center for Blood Research, Life Sciences Institute, University of British Columbia

Calcium in human blood catalyzes the late stages of rapid and effective blood clot formation, also known as coagulation. In injured trauma patients with large amounts of blood loss, appropriate coagulation is important to cease further bleeding in a process called hemostasis. To balance out the effects of severe blood loss, these patients receive massive blood transfusions. However, blood components are stored in a citrate-based anticoagulant while in the blood bank, which prevents blood clot formation by binding calcium. Thus, upon transfusion, citrate's calcium-binding capacity leads to hypocalcemia (a lack of calcium) which can negatively affect blood coagulation in the human body, among other adverse effects. Following transfusions in most trauma centers, hypocalcemia is treated once detected, however, a more proactive rather than reactive approach is being tested with a "calcium flasher" system using conveniently visible sticker reminders on packs of blood. This new system implemented at Vancouver General Hospital in November 2019 reminds physicians to administer calcium whenever blood is given, with the aim that patients receive more calcium and improve hemostasis. Our quality improvement project therefore investigates the efficacy of these calcium flasher stickers through improvements in trauma patient outcomes, and may guide us to implement changes to calcium administration protocol to benefit patient care. In this project, we examined trauma patient charts from before and after the introduction of calcium flashers and recorded laboratory values including calcium levels and coagulation testing from routine patient blood tests, patient admitting diagnoses, and patient survival outcomes. After descriptive and statistical analysis of our data, which is currently in the works, we hope to gain insight into the current efficacy of calcium flashers, guiding us towards future interventions if we find hypocalcemia treatment is lacking.

3. **Recombinant chitinase C from *Pseudomonas aeruginosa* is expressed and potentially secreted in *Escherichia coli* BL21 (DE3)**

Renée Lim[1], Ishana Lodhia[1], Shutong Gang[1], Azraa Banka[1]

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

There have been growing efforts to develop natural alternatives to chemical pesticides to mitigate the repercussions of microbiological post-harvest loss on the agricultural industry. Chitin is an attractive target because it is abundant in organisms that are the leading causes of post-harvest loss. Many organisms have evolved chitinases which hydrolyze chitin and may serve in the development of a natural biocontrol agent. In this study, we investigated recombinant chitinase C (ChiC) expressed from the previously established pM3CRYY expression vector. We hypothesized that pM3CRYY(+) *E. coli* BL21 (DE3) expresses and secretes recombinant ChiC. We confirmed the retention of *chiC* in the pM3CRYY expression vector through Sanger Sequencing and evaluated the expression and secretion of ChiC via western blotting. Ultimately, we determined that ChiC is strongly expressed in pM3CRYY(+) *E. coli* and potentially secreted in the extracellular medium. To determine if recombinant ChiC retains its chitinolytic activity, we piloted a functional assay that utilized media clearance on chitin-containing plates as a readout for enzymatic activity. Our findings were inconclusive; however, they serve as an important stepping stone to optimize the chitinolytic assay design. Future studies may implement a positive control to reduce the ambiguity in using media clearance to indicate chitinolytic activity. Furthermore, our study supports the potential of recombinant ChiC expressed in non-pathogenic *E. coli* for the development of a natural biocontrol alternative to synthetic pesticides.

4. **Towards an in vitro model of NLRP3 inflammasome activation via refining measures of IL-1β secretion and inflammasome assembly**

Sahar Malekighomi[1], Iris Zhou[1], Betty Yao[1]

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

The (NOD)-like receptor protein 3 (NLRP3) inflammasome is a multiprotein oligomer that triggers the secretion of pro-inflammatory cytokines IL-1β and IL-18 and inflammatory modes of cell death in response to microbial infection and cellular damage. Aberrant inflammasome function is implicated in various diseases, and understanding the underlying mechanisms is crucial for developing molecular therapies. In this study, we present an updated in vitro cell culture model of the NLRP3 inflammasome using lipopolysaccharide (LPS)-primed, nigericin-activated J774A.1 macrophages. We evaluated changes in cell morphology and mature IL-1β expression using phase-contrast microscopy, Western blotting and ELISA, respectively. Immunocytochemistry was used to detect apoptosis-associated speck-like protein containing a CARD (ASC) speck formation in nigericin-treated macrophages. Although the experimental approaches to mature IL-1β measurement require further development, we observed distinctive, irregular nigericin-induced morphological changes and the formation of ASC specks, which are likely indicators of NLRP3 inflammasome activation. These findings suggest that our cell culture model suitable for future research.

5. **Expression of Thermophile *Chloroflexus aurantiacus* Photosynthetic Reaction Centre Synthetic Genes in the Mesophile *Rhodobacter sphaeroides* RCx^R**

Meghan Marshall[1]

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

As the global demand for energy continues to rise and transition from fossil fuels becomes increasingly urgent, new technologies are needed to make renewable energy options more competitive. Solar energy has the capability to meet and exceed current energy needs; however, the cost and efficiency of solar cells currently on the market requires improvement. Biophotovoltaics have great potential in retaining the near perfect efficiency of biological energy conversion to generate electricity. To create biophotovoltaics, reaction centres from thermophilic photosynthetic bacteria can be used for enhanced protein stability in direct sunlight and the resultant high temperatures. In previous research, reaction centre genes homologous to *pufL* and *pufM* of the thermophilic bacteria *Chloroflexus aurantiacus* were designed from metagenomic data and inserted into the pMA4 cloning vector. In the current study, restriction enzyme digest of the previously created pMA4::pufLM plasmid was used to transfer the *pufLM* genes into the expression vector pIND4 and synthesize the pIND4::pufLM plasmid. Transformation of S17-1 *E. coli* with pIND4::pufLM, followed by conjugation with *Rhodobacter* (*R.*) *sphaeroides* strain RCx^R generated *R. sphaeroides* RCx^R pIND4::pufLM. The synthetic *pufL* and *pufM* genes were successfully expressed in *R. sphaeroides* RCx^R pIND4::pufLM to produce reaction centre proteins PufL and PufM, which associated with the necessary cofactors. This was confirmed by the presence of all three characteristic absorbance peaks, although the amplitude of some peaks was weak. In future research, additional and alternative thermophile photosynthetic genes may be expressed in *R. sphaeroides* RCx^R. This research serves as a proof of concept for the use of a *Rhodobacter sphaeroides* system for the expression of thermophilic bacteria photosynthetic genes obtained from metagenomic analyses. This system allows for the relatively simple production of novel thermophile reaction centres, which may then be tested for thermostability and efficiency in biophotovoltaics, with the potential to improve current solar energy technology and encourage its widespread adoption.

6. **Optimization of expression in *Escherichia coli* BL21 (DE3), IMAC purification, and preliminary functional characterization of recombinant ChiC from *Pseudomonas aeruginosa***

Haein Kim[1], Davey Li,[1] Apsara Srinivas[1], Leonardo Wu[1]

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

Chitin is a homopolymer that is found in the abdominal lining and exoskeleton of various insects. Given the increasing environmental pollution due to chemical insecticides used in agriculture, there is great interest in the development of bioinsecticides. Proteins that hydrolyze chitin, like chitinases, have potential in this regard. *Pseudomonas aeruginosa* PAO1 Chitinase C (ChiC) is a 55 kDa secreted protein, and the chiC gene has previously been cloned and expressed in *Escherichia coli* BL21 (DE3). However, the optimal isopropyl ß-D-1-thiogalactopyranoside (IPTG) inducer concentration, incubation time, and temperature for soluble recombinant ChiC (rChiC) expression have yet to be elucidated. We used a factorial experiment to optimize the tested conditions for soluble rChiC expression, purified soluble rChiC using immobilized metal affinity chromatography (IMAC) with Ni-NTA resin, and conducted a preliminary functional characterization of rChiC using a chitin-binding assay. We found that the soluble protein is optimally expressed under 0.1 mM IPTG induction, 24 hr incubation, and at 20°C. The present study used histidine in place of the more commonly used imidazole for competitive washing and elution in IMAC, illustrating the potential of histidine as a competitive agent for protein purification. Finally, we observed that the purified rChiC has a functional chitin-binding domain, demonstrated by a chitin-resin binding assay. Overall, our study demonstrates the optimization of expression, purification, and functional characterization of rChiC in *E. coli* BL21 (DE3). This study reveals potential for future research aimed at further optimization of soluble rChiC expression and subsequent functional characterization of the chitinolytic domain of rChiC using in vitro and in vivo systems. Additionally, our findings demonstrate the potential for the large scale production of rChiC as a bioinsecticide to promote sustainable agriculture.

7. **Antifungal activity of recombinant ChiC from *E. coli* pM3CRYY BL21(DE3)**

Emma Dhaliwal[1], Karmin Dhindsa[1], Vianne Chang[1]

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

Chitin, the polymer of β(1,4)-N-acetylglucosamine, is an abundant polysaccharide that plays a structural role in many organisms, including being present in the cell walls of fungal and exoskeletons of insects. As fungi and insects are common pests of plants that pose a threat in agricultural contexts, chitin is a promising biopesticide target. Chitinases produced by various genera of bacteria degrade chitin through hydrolysis, making them a potential biocontrol agent that is a safer alternative to chemical pesticides. One example of a bacterial chitinase with demonstrated chitinolytic activity is ChiC from *Pseudomonas aeruginosa* PAO1. Previously, *chiC* was cloned into the expression vector pET-28a to generate the expression plasmid pM3CRYY. Transforming *Escherichia coli* BL21(DE3) with pM3CRYY produces recombinant ChiC (rChiC), which has recently been shown to retain its chitin-binding activity. However, the chitinolytic activity of rChiC has yet to be demonstrated. Our study aimed to determine if recombinant ChiC from *E. coli* pM3CRYY BL21(DE3) has chitinolytic activity. To approach this question, we conducted a fungal assay to assess whether rChiC-expressing E. coli can inhibit the growth of the fungus *Fusarium oxysporum*. We also purified rChiC using immobilized metal affinity chromatography and performed a DNS assay to assess if purified rChiC can break down colloidal chitin to release reducing sugars. Results from our fungal assay showed that IPTG-induced, rChiC-expressing pM3CRYY *E. coli* had moderate antifungal effects. Our DNS assay, however, showed that purified rChiC could not degrade chitin into reducing sugar monomers. These results suggest that rChiC likely has chitinolytic activity but that either our extraction and purification methods could not produce sufficient amounts of functional protein for detectable in vitro activity or that colloidal chitin is not a suitable substrate for rChiC. These preliminary results demonstrate that rChiC has potential as a biocontrol agent; however, further exploration of methods for producing large amounts of active rChiC and conditions and substrates required for its activity is needed.

8. **Investigation of antiviral activities of SARS-CoV-2 main protease (3CLpro) inhibitors**

Shutong (Ellie) Gang[1], Dr. François Jean[1]

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

The ongoing SARS-CoV-2 pandemic continues to be a significant threat to global health. The most mutated variant is the Omicron variant (B.1.1.529) with high transmissibility and immune evasion ability that has raised global concerns. Herein, we have identified a novel series of molecules that target the 3-chymotrypsin-like protease (3CLpro), the main protease that is pivotal for the replication of SARS-CoV-2. This study aims to evaluate the potency of these molecules against wild type versus P132H Omicron 3CLpro *in vitro* as a groundwork for further development of potential direct-acting antivirals (DAA). Internally quenched fluorogenic substrates assays were used to screen five computer-designed, non-covalent and non-peptidomimetic 3CLpro inhibitors. Subsequent dose-dependent analysis revealed that all inhibitors block 3CLpro activity in the single-digit micromolar range with half maximal inhibitory concentration (IC_{50}) between 1.4 -8.7 µM. Lastly, it confirmed that the P132H mutation in Omicron 3CLpro does not have a discernible effect on small molecule inhibition. In summary, these molecules have the potential to develop into highly specific DAA drugs to inhibit the SARS-CoV-2 replication either alone or in combination with drugs specific for other viral targets.

9. **Major Factors Responsible for Diversity in the Gut Microbiome of Columbian Adults**

Sana Samadi[1], Ghazaleh Shoaib[1], Jen Wang[1] and Martin Joo[1]

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

Over recent years, the gut microbiome has received increased attention due to its role in health and disease. The gut microbiome can negatively influence health, such as its role in the microbiome-gut-brain axis and contribute to developing and progressing diseases, such as Alzheimer's and Parkinson's disease. In contrast, it can also positively impact the host by aiding in immune system regulation. In a previous study, the microbiota of 441 Columbians was characterized using 16S rRNA gene sequencing. Before processing and determining significant predictors, this dataset was separated into groups of phylogenetically and/or functionally related organisms, which were referred to as co-abundant microorganisms (CAGs). This resulted in only 114 out of the 441 individuals in the dataset being analyzed. Considering the strict data filtering and the pre-grouping of species for analysis in the previous study, it is possible that many patterns in abundance differences across species in various metadata categories have yet to be identified. This study aims to address this knowledge gap by taking a more comprehensive approach to analyzing this dataset. We plan to look at alpha and beta diversity differences in all data points across all metadata categories. Following this, model selection will be used to determine the combination of factors that best explain variation in microbiome diversity in this dataset. Finally, this study will combine metrics of obesity, including waist circumference and body fat percentage, to determine whether obesity has any significant effects on beta diversity within the gut microbiome. By providing a comprehensive analysis of this dataset and looking into obesity as a well-established predictor of variation, this study will provide valuable insights into the factors influencing gut microbiome composition and its potential association with obesity.

10. **Development of a chitinolytic activity assay using the DNS method for detection of reducing sugars**

Maria Beletsky[1], Ashleen Khatra[1], Jenny Shee[1], Kevin Wang[1]

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

Chitin is a polymer consisting of N-acetyl-D-glucosamine (GlcNAc) monomers. Chitinolytic organisms such as various *Pseudomonas* spp. are promising in pest biocontrol research due to their ability to break down chitin-containing insect exoskeletons. Prior studies have cloned pM3CRYY, which contains *chiC* from *Pseudomonas aeruginosa* PAO1, into *E. coli* BL21 (DE3). Purified recombinant Chitinase C (rChiC) has been shown to possess a functional chitin binding domain (CBD). However, the chitinolytic activity of rChiC in this model has not been explored. Our study aimed to develop a functional assay to measure chitinolytic activity from *E. coli* BL21 (DE3) pM3CRYY A3 rChiC. We used dinitrosalicylic acid (DNS) reagent to assay for GlcNAc with spectrophotometry, as DNS reagent has traditionally been used to detect reducing sugars at 540 nm. Our substrate was chitosan, a popular chitin derivative in chitin-requiring experiments due to its solubility. We detected measurable chitinolytic activity against chitosan from the *Streptomyces griseus* positive control chitinase and rChiC, which can be inhibited by heat denaturation of the chitinases. The DNS assay also detected a small amount of chitinolytic activity in *E. coli* BL21 (DE3) pM3CRYY culture supernatant, which suggests rChiC may be secreted by *E. coli*. Confirmation that rChiC is functional highlights the potential to develop an eco-friendly insecticide that can be upscaled through an *E. coli* expression system.

11. **Optimization of chitinase C expression from the pM3CRYY plasmid in *Escherichia coli* BL21(DE3) and examination of secretion status**

Jinny Choi[1], Veronika Lewis[1], Claire Sie[1], Justin Yap[1]

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

Chitin is a polysaccharide and essential structural component of fungal cell walls, crustacean shells, and insect exoskeletons. This environmentally abundant polymer can be broken down and recycled into its carbon and nitrogen-based components by chitinolytic organisms, which secrete chitin-degrading enzymes called chitinases. The soil microbe *Pseudomonas aeruginosa* is known to express chitinase C (ChiC), which has been proposed as a safer alternative to conventional pesticides. A previous study generated a ChiC expression plasmid, pM3CRYY, by cloning *chiC* from *P. aeruginosa* PAO1 into a pET28a backbone. Through induction with isopropyl β-D-1-thiogalactopyranoside (IPTG), they demonstrated expression of a 55 kDa protein from pM3CRYY, though the identity of this protein was not confirmed. Additionally, it is unknown if the expressed protein is secreted when expressed in *E. coli* BL21. Thus, we aimed to confirm the identity of the protein via western blot against the 6xHis-tag conjugated to ChiC off pM3CRYY, and to determine the optimal IPTG induction conditions for ChiC expression. We found that *chiC* in pM3CRYY is conjugated to a 6xHis-tag at the 5' region, and confirmed the presence of His-tagged ChiC in IPTG-induced pM3CRYY *Escherichia coli* BL21(DE3) cultures. Optimal conditions for protein expression were determined using SDS-PAGE to be 0.1 mM IPTG for >6 hours at 37ºC. Lysing cells by bead beating yielded higher amounts of protein compared to a boiling lysis method. Interestingly, we also detected 6xHis-tagged ChiC in the soluble fractions of induced cultures. However, it was unclear if the protein was being released into the extracellular space because of active secretion or through the action of cells lysing and releasing their intracellular contents. Therefore, we attempted to determine if ChiC was being secreted under induction conditions by probing for the presence of the putative cytosolic chaperone protein, DnaK. Detection of DnaK along with ChiC in the supernatant would suggest that ChiC was being released not via secretion, but through cell lysis. Although our results suggest cytosolic proteins and ChiC are released into culture supernatants as a result of cell lysis, this experiment should be repeated before concluding the secretion status of ChiC in this expression system.

12. **A breast milk exclusive diet promotes dysbiosis in the gut microbiome of six month old anemic infants.**

Jenine Hira[1], Ekroop Sohal[1], Apsara Srinivas[1], Haein Kim[1]

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

Anemia refers to a physiological condition characterized by a lower-than-average number of red blood cells required to transport oxygen to tissues. Iron deficiency anemia (IDA) has been linked to negative cognitive, developmental, and metabolic effects in infants. As iron is an essential nutrient for many microbial colonizers in the gut, IDA can also be potentially damaging to the gut microbiome. The gut microbiome has been recognized as a key regulator of protective immunity, and thus an area of interest is the relationship between the onset of IDA and the make-up of the microbiota. Diet is known to alter the composition of the gut microbiome, and as few studies have focused on the tri-directional relationship between infant diet, gut microbial composition, and anemia status, we chose to explore this through our study. We analyzed the impacts of a breast milk (BM) exclusive diet, which is low in iron content, versus a complete diet, on the gut microbiome composition of 6-month-old infants that were either anemic or healthy. We found significantly decreased alpha and beta diversity in the microbiomes of anemic infants that were fed a BM diet, whereas these shifts were not seen in healthy infants. Upon investigation of the core microbiome, we discovered unique genera including Actinomyces and Bacteroides only in anemic infants. These results hint at increased volatility of the gut microbiome in response to diet in anemic infants. Further, we found decreased expression of several key commensal genera in BM fed infants compared to those fed a complete diet, along with an upregulation of pathways associated with hemoglobin synthesis and iron scavenging. Overall, our study generates a fundamental understanding of the impact of diet on the gut microbiota in the context of anemic status and provides insight into potential diets that could facilitate a healthy gut microbiome and minimize the risk of IDA.

13. **Individuals with abnormal cardiometabolic statuses are more vulnerable to smoking-induced alterations of the gut microbiome in a Colombian population undergoing Westernization**

Ali Reza Nasseri Dehkharghani[1], Darius Parmar[1], Mehdi Tabesh[1], Junqin Wang[1]

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The “Western diet” is of increasing scientific interest due to its rising global prevalence and potential implications for human gut health. The Western diet is associated with a decline in microbiome diversity and pro-inflammatory gut responses, which can lead to metabolic dysregulation and abnormal cardiometabolic status. The development of cardiovascular disease contributes to pro-inflammatory conditions in the body, which are likely to worsen metabolic health. Smoking has been implicated in increased morbidity and mortality from cardiovascular diseases and metabolic risk factors. Illustrating this link, smoking has been associated with increased insulin resistance, atherosclerosis, dyslipidemia, and diabetes. Using the dataset generated from 441 samples from Colombians undergoing Westernization by Jacobo de la Cuesta-Zuluaga and his research group, we aimed to determine the effects of smoking on the gut microbiome diversity and composition of individuals with abnormal cardiometabolic status. To gather data, stool samples were collected and 16s rRNA sequencing was performed using the V4 gene region. For those with an abnormal cardiometabolic status, smoking was associated with reduced richness, particularly in rare species, and a distinct reduction in eight core microbiome members that included commensal genera such as *bifidobacteria*, *christensenellaceae* and *blautia*. Smoking was associated with reduced relative abundances of bacterial ASVs in both healthy and abnormal cardiometabolic statuses, but the number of ASVs which decreased was more pronounced in those with abnormal cardiometabolic status. The relative reduction in ASVs was primarily observed in *firmicutes* and *proteobacteria* phylum. Further, abnormal cardiometabolic status was associated with harmful bacterial species such as *desulfovibrio piger* and *bacteroides ovatus*, which are prevalent in inflammatory bowel disease patients. These findings showcase that individuals with abnormal cardiometabolic status are susceptible to smoking-induced changes in their gut microbiome. This highlights that Westernizing individuals with abnormal cardiometabolic statuses should be cautious of smoking due to the potential negative impacts in their gut microbiome.

14. **Iron deficiency anemia is associated with loss of gut microbial diversity in 6 and 12 month old infants**

Luiza Lopes Pontual[1], Mark Josef Huang[1], Parneet Sekhon[1], Taylor Bootsma[1]

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Iron-deficiency anemia (IDA) is the most common form of anemia, arising from an inadequate quantity of iron in the body. Iron-deficiency anemia is especially concerning for young children, as it is linked to neurological dysfunction, immunological deficiencies, and increased mortality. Previous studies have demonstrated a link between gut microbiota alterations and infant anemia. As early life is associated with changes in the human microbiome, we aimed to investigate the effects of IDA on the gut microbiome of 6- and 12-month-old infants. We used a previously generated dataset of 16S rRNA gene sequences to compare diversity metrics, core microbiome analyses, and indicator taxa between IDA and healthy infants of both age groups. We found that both 6- and 12-month-old infants with IDA had significantly lower microbiome diversities compared to healthy infants. Additionally, the indicator taxa differed between 6- and 12-month-old infants with IDA and healthy controls, suggesting that IDA may have differential, age-associated impacts on infants. Loss of gut microbial diversity in infants with iron-deficiency anemia offers a potential explanation for the cognitive deficits associated with the condition. However, the nature of this relationship remains to be further clarified.

ABSTRACTS (Posters)

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

1. **Increased pathological severity of Familial Dysautonomia enriches the murine gut microbial composition through rare pathogenic species**

Ashleen Khatra[1], Jenny Shee[1], Aidan Wang[1], Kevin Wang[1]

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Familial dysautonomia (FD) is the most prevalent type of hereditary sensory and autonomic neuropathies, which are rare genetic neurological disorders that affect both the peripheral and central nervous systems. Previous studies have found significant alterations in the gut microbiome and metabolome in FD patients compared to healthy individuals. However, it is unclear how the gut microbial composition differs with varying levels of pathological FD severity. Here, we aim to investigate whether there are differences in gut microbial compositions among murine models with mild, moderate, and severe FD pathology. These pathologies correspond to stool samples from C57BL/6 mice with *Tuba1a-Cre+*; *Elp1loxp/loxp* mutation constituting FD, which were binned from scores of 0-4, 5-7, and 8-10 respectively. Through comparing within pathology, we first showed a significant increase in species richness as severity increases. Moreover, beta diversity analysis shows that there are increasing compositional differences as severity increases. A differential abundance analysis then revealed a significant downregulation of core commensal microbes in mice with severe FD compared to healthy controls. Additionally, indicator species analysis showed the presence of unique pathogenic species, such as *Clostridium* spp., indicating a relationship between specific bacterial species and FD pathologies. Overall, our findings suggest that the increase in gut microbial richness observed in murine models with severe FD is due to the downregulation of commensal microbes and the introduction of unique pathogenic species. These results suggest that the gut-brain and gut-metabolism axes may be promising targets for developing therapies for FD.

2. Ex vivo programming of murine type 1 regulatory T cells

Andrew Sze[1], Chad Poloni, Msc[1], Sarah Lim[1], Xiaojiao Wang[1], Ted Steiner, MD[1]
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Regulatory T cells (Tregs) suppress immune responses to maintain self-tolerance and homeostasis. Type 1 regulatory T cells (Tr1) are a subset of Tregs that secrete high amounts of the anti-inflammatory cytokine IL-10. Tr1 cells show therapeutic potential in cancer, autoimmune diseases, and chronic inflammation. However, Tr1 cells constitute a very small proportion of immune cells in humans and mice. To generate enough cells for therapeutic purposes, we must be able to differentiate naive CD4+ T cells into Tr1 cells ex vivo. In addition, naturally occuring Tr1 cells are a heterogenous population, requiring cytokines and polarizing factors to expand and program these cells. Literature has demonstrated the importance of IL-27 in inducing Tr1 cells, but the role of IL-7, a hematopoietic growth factor, is less understood. We hypothesized that stimulation of murine CD4+ cells in the presence of IL-7 & IL-27 would induce a subset of Tr1 cells. To optimize Tr1 expansion, we isolated CD4+ T cells from FOXP3:GFP and IL-10:GPF reporter mice via immunomagnetic cell separation, stimulated them with anti-CD3/CD28 for 2 days, and sorted for cells that are $CD44^{high}$ $FOXP3:GFP^{(neg)}$ or $CD25^{(neg)}$ $IL\text{-}10^{high}$. We continued to culture these under different cytokine conditions and analyzed supernatants for anti & pro-inflammatory cytokines using ELISAs and cytometric bead arrays after 1, 2, or 8 days. Following stimulation, $CD44^{high}$ $FOXP3\text{-}GFP^{(neg)}$ and $CD25^{(neg)}$ $IL\text{-}10^{high}$ sorted cells produced significantly increased levels of IL-10 in the presence of IL-7. However, IL-7 also appeared to increase levels of pro- inflammatory Th2 cytokine IL-4 in the $CD44^{high}$ $FOXP3\text{-}GFP^{(neg)}$ population. The presence of IL-4 and other pro-inflammatory cytokines suggests contamination from Th2 CD4+ T cells, which is associated with allergic type reactions. Taken together, these results suggest IL-7 can be used in expanding $CD25^{(neg)}$ $IL\text{-}10^{high}$, but not $CD44^{high}$ $FOXP3\text{-}GFP^{(neg)}$ cells. We recommend using $CD25^{(neg)}$ $IL\text{-}10^{high}$ sorted cells in downstream experiments evaluating Tr1 therapy for inflammatory mediated diseases.

3. Investigating microbial contributions to hormonal comorbidities in pediatric inflammatory bowel disease

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Inflammatory bowel disease (IBD) is an increasing public health concern in Canada. In addition to colitis-related symptoms, patients with IBD have been reported to experience higher rates of hormonal comorbidities, such as decreased serum hormone levels, hormone-associated mood disorders, and delayed puberty. Recently, the role of the gut microbiota in regulating circulating hormone levels in the host has been investigated as a potential driver behind diseases which exhibit differences in presentation between sexes. However, the function of the IBD microbiome in driving the extra-colonic pathologies associated with the disease has not yet been elucidated. In this study, we utilize both inflammation-inducing chemical reagents and human microbiome-induced (hIBD) mouse models of IBD to decipher several potential mechanisms behind this phenomenon. We demonstrate that chronic administration of the chemical colitogen dextran sodium sulphate (DSS) to mice decreases seminal vesicles weight and shifts the composition of the gut microbiota to downregulate microbes with known anti-inflammatory functions. Interestingly, mice colonized with microbiota derived from an IBD patient experience significant pubertal delay and decreased serum estradiol concentrations. Functional metagenomic predictions revealed upregulation of β-glucuronidases and β-glucosidases – enzymes which can reactivate hormones in the gastrointestinal tract – in DSS-treated mice. Finally, genes associated with the mycolate biosynthesis pathway were found to be significantly upregulated in the hIBD microbiota, which may suggest potential involvement of *Mycobacterium* spp. in the pathology of the model. Overall, this study provides a crucial first step into the microbial mechanisms which may underlie the hormonal symptoms that accompany IBD pathogenesis.

4. Prior infection and vaccine dose effect on ACE2 inhibition 18 months post-initial vaccination with BNT162b2 and mRNA-1273 in Canadian paramedics

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The COVID-19 pandemic, caused by the SARS-CoV-2 virus has rapidly evolved since late 2019, where highly transmissible circulating Omicron variants have emerged. While most Canadian paramedics are COVID-19 vaccinated, optimal ongoing vaccination is unclear. We investigated humoral immunogenicity 18-months post-initial vaccination, stratified by past SARS-CoV-2 infection and number of vaccine doses. We used demographic, vaccination, SARS-CoV-2 infection data, and serum samples, from paramedics enrolled in the "COVID-19 Occupational Risks, Seroprevalence, and Immunity among Paramedics in Canada" study between January 2021 and November 2022. We included those who provided a blood sample 18 months after initial mRNA vaccination (BNT162b2 or mRNA-1273), which were the most widely used vaccines. Outcome measures were percent inhibition against the predominant circulating variant Omicron BA.4/BA.5 and wild-type (WT) antigens using an ACE2 assay, a surrogate for viral neutralization. We compared outcomes based on the number of vaccine doses (2 vs. 3) and previous SARS-CoV-2 infection status. From 657 paramedics, median age was 40 years (IQR, 33-50) and overall median percent inhibition to BA.4/5 was 71.61% (IQR 39.44-92.82). Those with past SARS-CoV-2 infection had a higher median percent inhibition to BA.4/BA.5, when compared to uninfected individuals, overall and when stratified by 2 or 3 vaccine doses. Among COVID negative participants, when comparing 2 vs. 3 WT vaccine doses, we did not detect a difference in BA.4/BA.5 percent inhibition, but there was a difference in WT percent inhibition. Among those with previous SARS-CoV-2 infections, when comparing 2 vs. 3 WT vaccine doses, there was no difference in either group. These findings demonstrate that additional doses of the original WT vaccines does not improve the immune response to BA.4/BA.5 regardless of previous infection history.

5. Understanding the biological pathways of CCL11 and its pathological effects on the central nervous system following COVID-19 infection

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"Brain fog", an informal term that encapsulates various neurological symptoms including impaired attention, concentration, speed of information processing, memory, and executive functions, is a persistent symptom common among 1/4 of COVID-19 survivors. While severe COVID-19 can result in other symptoms such as multi-organ disease, even mild COVID-19 can result in neuroinflammatory responses. COVID-19 infection has shown to also increase the risks of later developing other neurodegenerative disorders such as Alzheimer's disease. Considering the continued prevalence of COVID-19 around the globe and emerging variants such as Omicron, these neurological symptoms present a major public health concern. Recent studies have detected elevated levels of pro-inflammatory chemokine CCL11 only in those experiencing cognitive impairments after COVID-19 infection, establishing a strong link between CCL11 and the central nervous system (CNS). CCL11 plays a major role in eosinophilic inflammation and has shown to limit neurogenesis and contribute to other cognitive and psychiatric illnesses, such as multiple sclerosis, Alzheimer's, major depression, bipolar disorder, and schizophrenia. However, the biological mechanisms of CCL11 over-expression as a result of COVID-19 infection remain poorly understood. This poster will investigate the biological pathways of CCL11 from its upregulation following COVID-19 infection to its entry through the blood-brain barrier, outlining the CCL11 producers and targets within the plasma and CNS. Additionally, it will discuss the subsequent pathological effects on the CNS, including eosinophil degranulation, activation of microglia, inhibition of oligodendrocyte precursors, and production of reactive oxygen species. These inquiries play a key role in understanding CCL11 functions within the brain and evaluating the potential of CCL11 as a new therapeutic target. If this therapeutic strategy is successful, it will improve health outcomes not only for post-COVID "brain fog", but also for other neurological disorders related to inflammatory dysregulation.

6. Cloning and expression of *Pseudomonas Aeruginosa* ChiC chitin-binding domain of in pET-28a(+)

Renee Boldut[1], Lanyin Mao[1], Sana Samadi[1] and Rebecca Saunders[1]

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The function of chitinase in bacteria is to hydrolyze chitin into usable forms of carbon and nitrogen. PAO1 Chitinase C (ChiC) is a *Pseudomonas aeruginosa*-derived chitin-hydrolyzing enzyme with three functional domains: a catalytic domain, a fibronectin type III domain, and a chitin-binding domain (CBD). The CBD of PAO1 ChiC is a novel type of CBD that shows promise for use in purification assays. Historically, CBDs have been employed as tags in purification processes. Still, there is a need for CBD tags with new properties, such as increased stability at high temperatures or increased binding affinity, which the PAO1 ChiC CBD could provide. Therefore, this study aimed to rationally design a gene block of the PAO1 ChiC CBD and insert it into an expression vector to confirm CBD functionality separate from its native protein and its potential as a tag. This gene block was also designed for cloning into a BioBrick vector, a standardized vector for interchangeable parts. Insertion into a BioBrick would allow a standardised protocol for CBD tagging various proteins through different combinations of BioBrick parts. To explore the functional relevance of the sole CBD cysteine, a second gene block was designed with the substitution of alanine in place of the cysteine. This study describes the experimental design for cloning a CBD-encoded gene block into a pET-28a(+), evaluating its functional activity using a chitin-binding assay and then cloning the gene block upon functional activity verification into a BioBrick. We designed these gene constructs and experimentally showed two sets of primers that could amplify the gene construct, which can be used in future study designs outlined in this paper. If the gene construct containing the functional CBD of ChiC is successfully inserted into a vector in the future, there are implications for researchers to use this CBD in the assembly of unique protein sequences. This could have applications in producing chitin-based materials for use in industries such as biomedicine and agriculture.

7. Non-Breast milk diet increases gut microbial diversity and inflammation, in six month old infants

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*All the authors contributed equally to this study

Alteration to the infant microbiome is linked with development of conditions like inflammatory bowel disease (IBD) and asthma. Microbiome is also shown to be associated with inflammation. Further, exposure to inflammation in early childhood can impair the full developmental potential of children, especially brain functioning and cognitive development. Additionally, anemia is prevalent in infants globally, and it is found to impact both inflammation state and microbiome. While past research has illustrated the effect of anemia and diet on inflammation state and microbiome composition; their effects in infants have not been extensively studied. Thus, we aimed to investigate how anemia and diet, specifically breast milk (BM) versus non-breast milk (non-BM) diets, impacts the interplay between gut microbiome and inflammation in infants. We reported that in six-month-old infants, anemia does not significantly alter the gut microbial alpha diversity. Moreover, the inflammation level, measured by C-reactive protein (CRP), was not significantly changed by anemia in six month old infants. However, we found that a non-breast milk diet is associated with significantly higher alpha diversity and CRP levels, compared to breast milk diet. The core microbiome analysis showed a higher number of unique species in the gut microbiome of infants taking non-BM diets, and those who had elevated CRP levels. Furthermore, indicator species analysis revealed three common indicator genera between gut microbiota of infants taking non-BM and the microbiota of those with elevated CRP levels. Interestingly, two of these genera, Erysipelatoclostridium and Tyzzerella, are associated with Crohn's disease, which is a type of IBD. This suggests that non-BM in six-month-old infants results in an increase of microbial species associated with pro-inflammatory effects, which likely leads to the observed elevated inflammation. Our study illustrates the importance of diet for maintaining a healthy gut microbiome and inflammation level in infants, and highlights the need for formulating non-BM diets that promote healthy gut microbiome and inflammation status in infants.

8. Towards the optimization of a NLRP3 inflammasome model system in J774A.1 murine macrophages and THP-1 human monocytes

Ali Reza Nasseri Dehkharghani[1], Darius Parmar[1], Mehdi Tabesh[1], Junqin Wang[1]

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The NLRP3 inflammasome is a cytosolic, multimeric protein complex, which detects and responds to tissue homeostasis disruptions such as pathogen detection, tissue damage, and environmental stressors. Dysregulated NLRP3 inflammasome activity has been linked to various autoinflammatory and autoimmune diseases including diabetes and Alzheimer's disease, rendering the inflammasome an attractive potential therapeutic target. However, NLRP3 activation mechanisms are complex and can be induced by a multitude of stimulation conditions, leading to challenges with developing a reliable model system. In this study, we aimed to optimize appropriate conditions to detect NLRP3 inflammasome activation in both J774A.1 murine macrophages and THP-1 human monocytes. In J774A.1 cells, we detected upregulated expression of pro-IL-1β following LPS-treatment, indicative of NLRP3 inflammasome priming. Following nigericin stimulation, we observed diminished pro-IL-1β levels and no mature IL-1β. This could be an indication of pro-IL-1β becoming cleaved and released in the cell media due to cell death. We were able to differentiate THP-1 monocytic cells into macrophage-like phenotypes using 500nM PMA treatment for 24 hours. Following nigericin treatment, ASC levels decreased in LPS-undifferentiated, and increased in PMA-differentiated THP-1 cells relative to the respective untreated conditions. We observed nigericin-induced, duration-dependent increases in cell death for both THP-1 phenotypes using a cell viability assay. In this study, we aimed to establish an in-vitro NLRP3 inflammasome cell model. We confirmed prior findings regarding J774A.1 NLRP3 inflammasome signaling and initiated the optimization of NLRP3 inflammasome activation in THP-1 cells. Further enhancements are required to conclusively identify NLRP3 activation and optimize a reliable cell line model system.

9. Microbial Diversity and Population Density is Positively Correlated in New York State Freshwater Wetlands

Tanya Balaji[1], **Azraa Banka**[1], **Daven Kuder**[1], **Raveen Sidhu**[1]
[1] Department of Microbiology and Immunology, The University of British Columbia

Increasing anthropogenic activities, particularly those associated with urbanization, have increased ecological strain on near-urban freshwater wetlands. Despite wetlands being ecologically significant environments with key functions like habitat preservation, water quality enhancement, nutrient cycling properties, and its regulatory roles in maintaining greenhouse gas budgets, research characterizing wetland soils with regards to population density is not well established. Furthermore, microbes in wetland soils are significant contributors to overall wetland functionality, however, the microbial community structure and functional diversity remains unknown. To address this gap, this paper aims to investigate the impact of proximity to population centers on the microbial community and environmental conditions of wetlands. To explore the relationships between human population density and freshwater wetland microbial environments, alpha and beta diversity metrics between urban and rural sites in the state of New York were assessed. Moreover, differential abundance of microbes between sites were quantified, and a correlation analysis between pH, carbon content, nitrogen content and soil saturation was conducted. We show that richness and evenness are significantly higher in the urban sites compared to the rural sites. In addition, differential analysis revealed an enrichment of taxa such as Spirochaetales and Methanosarcinales at urban and rural sites, respectively. Lastly a positive correlation was observed with soil saturation and pH across genera *Aidingimonas*, *Flavobacterium*, and *Sphingomonas* in Owego, a rural site. These findings demonstrate that diversity in urban sites increase compared to rural sites which permits the selection of various taxa. Ultimately, this paper highlights the intricate nature of microbial soil systems, emphasizing the need for further research efforts that seek to characterize wetland soils.

10. Towards the development of an in-vitro model of HD-ALL leukemogenesis

Samuel Salitra [1]

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B-cell Precursor Acute Lymphoblastic Leukemia (B-ALL) is the most common pediatric malignancy, accounting for roughly 25% of childhood cancer diagnoses in Canada. Almost one-third of cases present with the hyperdiploid sub-type (HD-ALL), making it the largest contributor to the ALL incidence peak in 3–5-year-olds. HD-ALL begins in-utero, where B cell precursors acquire chromosomal abnormalities, arresting their development and giving rise to a small population of clinically silent Leukemia-initiating-cells (LIC). Subsequent oncogenic events transform the LIC into a malignant state, initiating a program of uncontrolled proliferation in the bone marrow, blood, and extra-medullary sites.

Our lab has established that LIC can be sustained for many weeks by co-culture on bone marrow stromal cells (BMSC). These extended cultures may be sufficient to observe transformation of LIC into HD-ALL cells for the first time in-vitro. However, a standardized analysis of this transformation has not been conducted and the associated mechanisms remain unclear. To address this, we performed a longitudinal analysis of LIC in long-term co-culture with BMSC. LIC isolated from the bone marrow and spleen of Eμ-Ret mice, which are predisposed to HD-ALL, were seeded on established BMSC cultures. Cell number, viability, and immuno-phenotypic markers were recorded weekly for 25 to 70 days. After this time, cells were tested for markers of HD-ALL transformation via flow cytometry.

LIC remained stable in number and viability throughout the first month of co-culture, which was followed by a period of expansion after day 35. Prior to day 35, 0/11 co-cultures had doubled in cell number. However, by day 50, 5/9 co-cultures had more than doubled their cell count on day 35. Changes in count corresponded to a shift away from the baseline LIC immunophenotype.

Cultured LIC are currently being evaluated for full transformation via growth factor dependency assays and adoptive transfer into BALB/c mice. Subsequently, a comparative analysis of LIC in-vitro versus ex-vivo will be conducted using bulk RNA-seq. A robust understanding of these processes will aid the development of an in-vitro model of leukemogenesis. This will improve our understanding of HD-ALL biology and facilitate the discovery of points at which the disease can be arrested.

11. Synergistic effects of antibiofilm peptides and conventional antibiotics on *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* biofilms

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Biofilm is developed when a cluster of bacterial cells adheres to a surface and forms a surrounding extracellular matrix layer. As this layer provides protection to bacteria from antibiotics, biofilm-associated infections is a substantial health problem due to the resistance of biofilm-producing cells to current antibiotic treatments. Amidst the need for new treatments for these infections, studies have shown that a synthetic antibiofilm peptide, called DJK-5, can not only prevent biofilm formation but also degrade established biofilms. Beyond the direct activity of the peptide, synergistic effects with conventional antibiotics have also been reported in the literature against planktonic bacterial cells. However, it is unclear whether these relationships between antibiofilm peptides and antibiotics exist against cells growing within a biofilm. Hence, using eradication and inhibition assays, we examined the synergistic relationships between DJK-5-derived antibiofilm peptides and conventional antibiotics on biofilms formed by the Gram-negative *Pseudomonas aeruginosa* (PAO1) and the Gram-positive Methicillin-resistant *Staphylococcus aureus* (USA 300 LAC). Indeed, we found that peptides, when combined with aminoglycosides or topical antimicrobials, exerted better antibiofilm effects against the two strains while decreasing the working concentration of each compound alone. Specifically, peptides and antibiotics show a 4-8 fold decrease and 4-16 fold decrease in working concentration when combined together. Future directions of this experiment involves investigation of more peptides/antibiotics combinations and validation of synergy in more sophisticated infection models.

ABSTRACTS (3X1)

Presenters will be given 3 minutes to present 1 slide.

There will be no Q&A period after.

May include full research projects, proposals, or a working thesis.

1. **The road less traveled: exploration of non-structural proteins using organoid models in SARS-CoV-2**

Sana Samadi[1]

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Until recently, the study of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pathogenesis has primarily focused on surface structural proteins, with non-structural proteins (NSPs) generally overlooked. However, recent findings highlighting the important role of NSP6 in the replication organelle, shed light on these lesser-explored proteins. This paper proposed using organoids to explore NSP functions in SARS CoV-2. The study addresses two main questions: 1) how can organoids be further applied to allow SARS-CoV-2 research advancement with NSPs, and 2) what NSPs of SARS-CoV-2 need further exploration? A systems biology approach is suggested, involving the overexpression of NSPs in cells forming the organoid and analyses using transcription screening, proteomics, and microRNA profiling. Exploring all 16 NSPs using the proposed approach will provide a more robust understanding of SARS-CoV-2 mechanisms and could lead to the development of therapeutic agents. This research also has implications for "long COVID" research, in which various organoids can be used to model changes in different affected organs. Furthermore, it has implications for developing antiviral nanoparticles targeting viral or host chaperone proteins. By implementing the proposed approaches, we can move closer to understanding SARS CoV-2 mechanisms.

2. Syncytia formation: a trademark of severe SARS-CoV-2 infection and a potential target for novel drug therapies

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of the recent coronavirus disease (COVID-19) pandemic. COVID-19 is characterized by symptoms and complications primarily affecting the upper respiratory tract. Infected multinucleated syncytial pneumocytes have been observed in severe COVID-19 cases, alongside lung damage and lymphocytopenia. The formation of syncytia allows the virus to evade the immune system, disseminate without cell exocytosis and cause cytopathicity and cell death. The mechanism of cell-cell fusion in SARS-CoV-2 infected cells is well documented as it relies on the same machinery as cell entry. However, the biological composition of these multinucleated cells is not well characterized. At present, there are no approved antivirals that directly target syncytia formation in SARS-CoV-2 or other syncytial viruses. This makes syncytia inhibition an overlooked yet promising area for new drug therapy development. Naturally, these shortcomings lead to the following questions that this literature review aims to answer by using previous research conducted on multinucleated cell models in various organisms; 1) what is the cellular and molecular composition of multinucleated syncytia, and 2) how can syncytia be targeted to produce broad spectrum multidrug antivirals and reduce viral pathogenesis. Studies that modeled syncytia morphology in other organisms observed that numerous syncytial nuclei underwent morphological changes involving a lock-and-key arrangement and membrane invagination. Furthermore, a transcriptome analysis of syncytia in soybean root cells reported an upregulation in the expression of genes associated with a high metabolic activity suggesting syncytial capability to alter gene regulation. Separate findings identified small molecules acting as either furin inhibitors or TMEM16 inhibitors to be promising candidates for blocking syncytia formation in spike-expressing cells. Cell-cell fusion is not unique to SARS-CoV-2 as many other enveloped viruses are known to induce syncytia in infected cells. Therefore, the impact of this research extends beyond treating SARS-CoV-2 lung pathogenesis and can pave the way for the development of broad-spectrum antivirals that target syncytia formation by other enveloped viruses. A better understanding of the cellular composition and biological functions of syncytia is necessary to reduce the burden that syncytial viruses have on public health, including the ongoing COVID-19 pandemic.

3. Hunting for synthetic lethality: Hit validation following CRISPR-based chemogenomic screen

Maya Ruehlen[1,2], Prasamit Baruah[1], Matias Casas-Selves[1]

[1]Repare Therapeutics, [2] University of British Columbia, Vancouver, British Columbia

Protein kinases are key enzymes for the regulation and progression of the cell cycle. It has been shown that in certain genetic contexts (e.g.: loss of gene A), cancer cells are especially vulnerable to the inhibition of a specific cell cycle kinase (kinase B). In order to identify novel genetic lesions that would render cancer cells selectively sensitive to kinase B inhibition, we carried out a genome-wide, loss of function chemogenic screen with a proprietary kinase B inhibitor. From this screen, several putative synthetic lethal hits were identified. We chose to validate two genes (gene X and Y), which provided a strong biological rationale. For validation, we utilized siRNA-mediated knockdown and overexpression of genes X and Y in the presence and absence of a proprietary kinase B inhibitor and analyzed the biological effect by clonogenic survival assays. Our results show that there is clear cell growth inhibition in the presence of the kinase B inhibitor in wild type, knockdown and overexpression populations; thus, suggesting no synthetic lethality relationship between these genes and kinase B inhibition. While these specific hits were determined to be false positives, there remains a number of genetic lesions identified by this particular screen that offer potential. It is plausible that the validation of further hits could expand the clinical pool of cancer patients able to receive treatment from this drug, which would have a significant impact within the oncological field.

4. Insights into the Structure and Function of SARS-CoV-2 Non-Structural-Protein 6: Implications for Antiviral Drug Development

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It has been over three years since the World Health Organization (WHO) declared COVID19 a pandemic. Since that time, numerous SARS-CoV-2 variants of concern (VOC) have emerged. These VOC harbor large numbers of immune evasive mutations within their Spike (S) glycoprotein. Despite this, a substantial amount of effort has been allocated to studying and targeting the spike, while the 16 non-structural proteins known to be encoded by the SARS-CoV- 2 genome have received limited attention. A recent article utilizing chimeric recombinant constructs of SARS-CoV-2 showed Non-structural protein 6 (NSP6) to be a major determinant of pathogenicity. NSP6 is a transmembrane protein with a pleiotropic function; mediating ER zippering, double membrane vesicle (DMV) organization, lipid droplet (LD) tethering, and immune modulation. Though the significance of NSP6 in biogenesis and pathogenicity is evident, there remains a current knowledge gap in its function and evolution while the crystal structure has not been resolved yet. My presentation will review, 1) The various domains, motifs, and mutations of NSP6 that have a pivotal role in function and pathogenicity, and 2) Explore potential antiviral targets within the NSP6 structure. Understanding the molecular structure and function of NSP6 constitutes a new frontier for investigation and can lead to development of antivirals that can mitigate the clinical burden caused by VOC.

5. The impact of LPS and SCFAs on microglia-neuron interactions

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Bacterial infection in the gut causes local inflammation through molecules such as lipopolysaccharide (LPS). These molecules can then travel into circulation where they either directly or indirectly exert influence on other areas of the body such as the brain, contributing to the pathogenesis of various neurodegenerative and neurodevelopmental diseases including Parkinson's disease and autism spectrum disorders. Short-chain fatty acids (SCFAs), by-products of healthy gut microbe metabolism, are known to play anti-inflammatory roles in the gut and cross the blood brain barrier (BBB). While there is strong evidence of a link between the gut microbiota and processes in the brain, the exact mechanisms by which gut microbes modulate brain activity and cellular processes such as neurogenesis and microglial activation remain unclear. In this study, we investigate the role that SCFAs such as butyrate have on neurogenesis and microglia activation. We collected samples from *in vitro* single and co-culture models to compare cytokine levels and proliferation using various assays. We found no evidence that butyrate and LPS influence neuronal proliferation or maturation in contrast to what we anticipated, but did find changes in M1 phase microglia markers interleukin 6 (IL-6) and tumour necrosis factor (TNF). Butyrate in combination with LPS was seen to rescue IL-6 levels compared to when cells were treated with LPS only. Additionally, we found preliminarily evidence that only butyrate exhibits the propensity to partially rescue IL-6 levels unlike acetate and propionate, two other abundant SCFAs. Lastly, we also found that butyrate increases the levels of the anti-inflammatory M2 phase microglia marker IL-10 in the presence of LPS. Overall, these results support that gut-derived molecules influence cellular processes in the brain and provide a foundation for understanding these mechanisms more in depth. A deeper understanding could translate into the future potential to prevent or mitigate neurodevelopmental or neurological diseases associated with the gut microbiota.


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