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Poster Presentations
Title: Assessment of the direct contribution of alveolar macrophages to HIV persistence within the lungs during suppressive ART

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Abstract

While antiretroviral therapy (ART) has achieved widespread success reducing morbidity and mortality due to human immunodeficiency viral (HIV) infection, a cure remains elusive due to the obstacle of the latent viral reservoir. Persistence of HIV in particular cell subtypes, such as memory CD4+ T cells, and anatomical sites, such as the gut, lymph nodes, and brain, is well-documented. Before the advent of ART, the lungs were the focus of many studies as people living with HIV (PLWH) often presented with respiratory symptoms and opportunistic lung infections. In the ART era, the lungs have been somewhat overlooked as a potential anatomical reservoir despite the frequency of pulmonary co-morbidities in PLWH and features of the lung conducive to reservoir establishment, such as high cell density. Our group recently showed higher levels of HIV DNA in CD4+ T cells from bronchoalveolar lavage (BAL) fluid compared to peripheral blood; however, in alveolar macrophages (AMs), the major cell type in BAL fluid, HIV DNA levels varied. AM longevity and capacity for self-renewal make them a suitable HIV cellular reservoir candidate, so we propose a comprehensive assessment of their role in viral persistence. We will (1) examine the immunophenotype and origin of AMs based on HIV infection and smoking status; (2) compare the transcriptomic profile of latently and in vitro-infected AMs; (3) prove permissiveness of AMs to HIV infection in vitro; (4) use an adapted viral outgrowth assay (VOA) and viral genome sequencing to ascertain if virus detected in AMs from ART-suppressed HIV+ adults is replication-competent.
Characterization of the host-mediated modification of bacterial effector NleA and its role in virulence

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Enterohemorrhagic and enteropathogenic \textit{Escherichia coli} (EHEC and EPEC) are gastrointestinal pathogens that infect the human gut to cause diarrheal disease. EHEC, EPEC, and the related mouse pathogen \textit{Citrobacter rodentium} belong to a group of related Gram-negative pathogens characterized by their ability to form “attaching and effacing” (A/E) lesions on the surface of host cells during intestinal colonization. Upon adherence, these pathogens inject effector proteins directly into cells of the host intestine. Effector proteins alter host cell biology to help favour survival and replication of the pathogen. One of these injected proteins, the Non-LEE Encoded effector A (NleA), is a major virulence factor necessary for EHEC and EPEC virulence. NleA undergoes a mobility shift upon translocation into the host cell, suggesting a host-mediated modification of the protein. It was determined that NleA is not phosphorylated or ubiquitinated in the host cell. Using a modified CHO cell line with a reversible defect in glycosylation (CHO-LDLD), we have obtained evidence consistent with the modification of NleA by mucin-type O-linked glycosylation. This is also supported by a bioinformatics prediction program that predicts a serine- and threonine-rich region of NleA to be modifiable by O-linked glycosylation. Importantly, whereas the rest of the NleA protein sequence is well conserved between bacterial isolates, we have noted an expansion or contraction of the serine- and threonine-rich region of NleA in some strains. To the best of our knowledge, this is the first example of a bacterial effector protein modified by mucin-type O-linked glycosylation inside host cells. By determining the precise functional role of NleA in the virulence of A/E pathogens, we anticipate uncovering novel insights into host-microbe interactions in the intestine, and opening new avenues for potential drug targets and treatment of the diseases associated with these bacterial infections.
Regulatory mechanisms of eicosanoids in hematopoietic stem cell-mediated trained immunity

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Despite the world-wide application of Bacillus Calmette-Guérin (BCG) vaccination and other anti–Mycobacterium tuberculosis (Mtb) interventions, Mtb remains one of the most successful human pathogens. Approximately 1.6 million people die of tuberculosis annually and eight to ten million new cases of active tuberculosis occur each year. While the BCG vaccination prevents the disseminated form of tuberculosis in childhood, its efficacy in adults is variable. Recently, our group has demonstrated that the access of BCG to the bone marrow initiates a unique program in hematopoietic stem cells (HSCs) to generate memory-like innate immunity (trained immunity) with enhanced protective capacity against Mtb infection. However, our understanding of regulatory mechanisms involved in reprogramming of HSCs and trained immunity is limited. Eicosanoids are host bioactive lipid mediators which play a critical role in the regulation of the immune response to infectious diseases, including Mtb infection. Importantly, they are also key mediators in HSC function. Taken together, these observations lead us to hypothesize that eicosanoid pathways are required for the generation of trained immunity through BCG-induced reprogramming of HSCs. We aim to determine the contributions of key eicosanoid pathways, the prostaglandin pathway (e.g. PGE$_2$) and the lipoxygenase pathway (e.g. LTB$_4$), in HSC reprogramming by BCG. Due to the commercial availability of drugs that target eicosanoid pathways, our study has tremendous potential to develop targeted therapy for tuberculosis.
A Reverse Topology IFITM Protein Enhances Lentiviral Transduction

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Lentiviral transduction of haematopoietic stem cells is an attractive method for the stable, long-term expression of transgenes. However, the intrinsic resistance of these cells to viral infection is a challenge to its broad adoption in clinical settings. Current methods to enhance lentiviral transduction primarily utilize pharmacological agents to antagonize the viral resistance pathways in the target cells. Techniques that enhance the basal infectivity of the incoming lentiviral particles have not yet been explored. We have tested a protein-based method to enhance lentiviral transduction that utilized a reverse-topology IFITM protein called SynDIG1. Incorporation of SynDIG1 onto VSV-G pseudotyped lentiviral particles enhanced lentiviral transduction of SupT1 cells. IFITM3 incorporation inhibited lentiviral transduction to a similar extent. Our study is an initial proof of concept that a reverse topology IFITM protein that is incorporated onto the envelope of lentiviral particles can enhance lentiviral transduction of the target cell.\textsuperscript{a}

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The development of a mesenchymal stromal cell-based cellular vaccine for leishmaniasis

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Vaccination is one of the most effective approaches developed so far for the control of several infectious diseases. Unfortunately, however, vaccines remain unavailable for several epidemic infectious agents including parasites such as the protozoan \textit{Leishmania}. Despite all efforts dedicated for the development of anti-\textit{Leishmania} vaccines, the currently available vaccine candidates remain limited in their effectiveness leaving millions of people and animals threatened by the tropical epidemic disease, leishmaniasis. Novel vaccine strategies are therefore paramount to effectively prevent morbidity caused by this complex infectious agent and/or to improve the outcome of infected subjects.

This project focuses on evaluating a new anti-\textit{Leishmania} cellular vaccine using bone marrow-derived mesenchymal stromal cells (\textit{MSCs}) gene-engineered to acquire stable antigen-presenting capabilities (\textit{MSC\textsubscript{x}}). When used in the context of prophylactic vaccination, \textit{MSC\textsubscript{x}} administration led to complete protection in a mouse model of T-cell lymphoma. Based on these observations, we next tested \textit{MSC\textsubscript{x}} in a mouse model of cutaneous leishmaniasis using \textit{Leishmania (L) major} strain. Vaccination using antigen-pulsed \textit{MSC\textsubscript{x}} elicited a good immune response represented by secretion of interferon (\textit{IFN})\textsubscript{γ} and a 50 \% reduction in disease pathogenesis as measured by reduced foot pad inflammation. In addition, data obtained from immunization experiments using \textit{MSC\textsubscript{x}} vs. dendritic cells (\textit{DCs}) indicate that \textit{MSC\textsubscript{x}} present a unique repertoire of peptide for immune cells. We are currently working to further characterize and optimize the immune response triggered by the \textit{MSC\textsubscript{x}} vaccine to achieve better prophylactic protection or use it as a therapeutic vaccine to treat infected subjects.
Unique Properties of Large Cluster Species Contributing to the HIV-1 Epidemic in Quebec

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Background

Viral phylodynamics in the context of HIV epidemics allows us to understand the epidemiological, immunological and evolutionary processes viruses employ to shape phylogenies and drive transmission dynamics. Our phylogenetic studies collected data from the Quebec genotyping program to reconstruct transmission networks and their expansion in real time. We believe that viruses belonging to large cluster species could have unique properties that make them more transmissible or resistant to integrase strand transfer inhibitors (INSTI).

Methods

About 10,000 unique sequences from the provincial genotyping program were analyzed in our lab. Phylogenies were built previously with MEGA 6 using the Neighbour-Joining tree method. Patient isolates were isolated and amplified using CBMCs. We screened some of the viruses for their tropism using a U87 cell line capable of expressing CD4 receptors and either CCR5 or CXCR4 co-receptors. Similarly, MT-2 cells were infected with these viruses to monitor for syncytia formation. Dual-tropic species were selected from this screening for a drug washout experiment. Two C185 WT species and their INSTI resistant mutants (R263K and N155H) cultivated in our lab previously were used for this experiment. MT-2 cells were infected with four different viruses, in the presence of three different INSTI (DTG, CAB, BIC) at 20x their IC90. The drug was removed on the peak day of infection and allowed to grow for 7 more days.

Results

Our phylogeny studies show that between 2002 and 2017, viruses belonging to large cluster (LC) lineages are responsible for sustaining the epidemic. While the number of reported cases has declined about 50% from 2006, there has been a steady increase of patients whose genotype fall within one of our LC clades. Since tropism is one of the most important characteristics of viruses, we tested 45 different HIV-1 samples from patient isolates to determine if they were CCR5 or CXCR4 tropic. This screening revealed that 5/45 isolates showed dual-tropism, from which there were a mix of LC and singleton candidates. One sampled proved to be X4-tropic and the rest were all R5-Tropic. Two isolates from the same cluster C185, both showed similar dual-tropic properties and were selected for further analysis. In our washout experiments, C185.1 and C185.2 peaked 5 days post infection. IC50 values differed according to treatment and virus used. CAB required about 3.00 nM to reach IC50 whereas DTG and BIC had similar values, needing between 0.50 and 1.50 nM. Previous results using laboratory pNL4.3 strains showed that second generation INSTI were efficient following washout in lab strains even in the presence of R263K drug resistant mutation. Data from our clinical isolates show that R263K from C185.1 can rebound after 3 days of drug removal and then continued to do so after a week as observed by the increase of integrated proviral.

Conclusions

LC viruses are responsible for continuing the epidemic in Quebec. Our studies from these kinds of viruses will continue to reveal unique characteristics.
Increasing Lentiviral Vector-Mediated Gene Delivery in The Context of HIV Gene Therapy

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Background: Although combination antiretroviral therapy can prevent the progression of human immunodeficiency virus-1 (HIV) infection to acquired immunodeficiency syndrome, it cannot cure the infection, due to the persistence of latent viral reservoirs. Hematopoietic stem cell (HSC) transplants from resistant donors to HIV positive individuals have led to the only two documented cures of HIV infections. However, compatible resistant donors are rare and allogeneic transplants have high mortality rates. Alternatively, a patient’s HSCs could be modified \textit{ex vivo} to express molecules that inhibit HIV replication and transferred back to the patient where they could serve as a source of HIV-resistant immune cells.

Objective: While HIV-based lentiviral vectors (LVs) are the leading gene delivery tool in HIV gene therapy, clinical trials are hampered by low transduction, in part because anti-HIV molecules can inhibit HIV-based LV production. Our goal is therefore to improve LV transduction of HSCs and to overcome anti-HIV RNA-mediated inhibition of LV production.

Hypothesis: Anti-HIV gene delivery can be increased by using a packaging plasmid:
1. with an optimal HIV-based \textit{gag-pol} sequence.
2. that is not HIV-based.

Results: We have developed a system to compare the production and transduction efficiency of LVs generated using \textit{gag-pol} genes from different HIV strains with the goal of increasing transduction during gene therapy. We have also shown that anti-HIV RNA-mediated inhibition of LV production can be overcome by using a hybrid feline (F)IV-HIV based LV system.

Conclusion: Identifying an optimal \textit{gag-pol} gene for LV production has the potential to enhance gene delivery in many gene therapy applications. Additionally, we have shown that non-HIV based LV systems have potential for use in HIV gene therapy. Our results provide insights that will be helpful when designing and selecting LV delivery systems for gene therapy clinical trials, and specifically for HIV gene therapy.
CRISPR Screen of the Interactions Between HIV-1 and the Cellular Membrane Trafficking Pathway

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**Background** As an obligate intracellular pathogen, the human immunodeficiency virus type 1 (HIV-1) largely relies on host cellular components for replication. The virus has developed an arsenal of methods to productively replicate within cells while evading host defenses, such as hijacking the cellular membrane trafficking pathway. This pathway facilitates the transport and delivery of molecular cargo within intracellular membrane-bound vesicles and is exploited by HIV-1 for its own intracellular trafficking purposes. This exploitation is especially important in the late stage of the replication cycle when nascent viral components travel to the plasma membrane to assemble into immature virions, culminating in the release of fully infectious progeny virions; however, much remains to be understood about the intracellular trafficking of HIV-1.

**Methods** To investigate the interactions between HIV-1 and the membrane trafficking pathway, we employed a sophisticated CRISPR-Cas9 arrayed genetic screen causing loss-of-function in 140 genes of the membrane trafficking family. The screen was performed in the HIV-1 reporter cell line, TZM-bl, which we modified to stably express the Cas9 endonuclease. Our screen focused on host factors that significantly alter HIV-1 infectivity, measured by Tat-dependent LTR-driven luciferase activity, and virion production, quantified by p24 capsid protein present in the cell-free supernatant.

**Results** We have uncovered several candidate genes that proved to be viable knockouts and displayed significant reduction in HIV-1 infection when compared to the non-targeting control (selected threshold: more than 2-fold decrease in viral infectivity and virus production). Protein interaction studies indicate that many of these candidate genes are enriched in clathrin-mediated endocytosis. To further our inquiry into these candidate genes, we will generate stable CRISPR-knockout cell lines in a T-cell model, SUP-T1, and use protein expression studies and microscopic visualization techniques to investigate the underlying molecular mechanisms.

**Conclusion** Deciphering the relationships between HIV-1 and the membrane trafficking pathway using a revolutionary CRISPR-based genetic screening approach will not only improve our current understanding of viral pathogenesis, but may also translate into novel antiviral targets towards an HIV-1 cure.
Characterization of the implication of the Idd2 locus in type 1 diabetes development

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Type 1 diabetes (T1D) is an autoimmune disease characterized by the destruction of the β cells of the pancreas. Genetic susceptibility to T1D is conferred by complex traits. More than 20 genetic loci, termed Idd for “insulin dependent diabetes”, are linked to T1D in the NOD mice, a model which spontaneously develops T1D. The second genetic locus, namely Idd2, was identified in the 1980s, but its contribution to T1D susceptibility was never validated. We generated a NOD.B10-Chr9 congenic mouse, where the Chr9 locus encompasses part of the Idd2 locus, enabling us to assess the specific contribution of Idd2 in T1D development. Using this congenic strain, we validate that Idd2 confers resistance to insulitis and, consequently, to T1D in the NOD mouse. By generating bone marrow chimeras and performing spleen cell transfers, we find that the resistance is conferred in an immune cell-intrinsic manner. Moreover, we demonstrate that Idd2 contributes to the formation of germinal centers as well as the expression of the major histocompatibility complex (MHC) on B cells. Together, these data not only validate Idd2 as a genetic locus contributing to T1D susceptibility, it strongly supports recent evidence of the contribution of the humoral response in T1D susceptibility.
Effects of Amino Acid Restriction on Macrophage Function

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Acute lung injury (ALI) is a deadly clinical condition characterized by the breakdown of the lung alveolar-capillary membrane and respiratory failure that can occur after a direct (e.g., pneumonia) or indirect (e.g., sepsis) inflammatory insult. Macrophages play an important role in ALI, and their activity is regulated by changes in metabolism and gene transcription required for stress responses and cytokine production. These are tightly regulated by the protein kinase ‘mammalian target of rapamycin’ (mTOR), which nucleates two highly conserved macromolecular complexes – mTOR complex-1 (mTORC1) and mTORC2. Clinical studies demonstrate an association between protein availability and clinical outcomes in the critically ill, and mTORC1 is a cellular sensor of essential amino acid (EAA) availability. We therefore proposed that the availability of EAAs, particularly leucine and/or arginine, is critical for metabolic, stress, and inflammatory responses in macrophages that are related to ALI. Bone marrow-derived macrophages (BMDMs) were exposed to control media or that lacking leucine, arginine, and lysine (EAA restriction), in the presence or absence of saline or E. coli lipopolysaccharide and interferon-gamma (LPS/IFN-γ) for 6 h. The induction of interleukin-6 (IL-6) mRNA and protein by LPS/IFN-γ was inhibited by EAA restriction; IL-6 induction was restored by the addition of leucine alone. Moreover, IL-6 induction was inhibited by exposure to media lacking leucine alone. EAA restriction enhanced the induction of ER stress response genes (i.e., CHOP, GADD34) by LPS/IFN-γ. Our results point to mTOR and its leucine sensing mechanism as an important regulator of macrophage stress and inflammatory responses and form the basis for future studies identifying novel clinical biomarkers or therapeutic targets in ALI.
The circadian regulation of *Plasmodium* spp. infection

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Malaria is a serious vector-borne disease characterized by periodic episodes of high fever and strong immune responses. Circadian clocks are known to regulate inflammatory responses, but the implications of this for *Plasmodium* parasite infection (which causes malaria) are unknown. We hypothesized that there is a rhythm of the response to *Plasmodium* infection and that the outcome of the infection is impacted by circadian disruption. First, we are analyzing whether there are daily variations on the *in vivo* infection and pathological signs by using a model of cerebral malaria. Mice were infected at 2 different time points (ZT1 and ZT13) with infected red blood cells. Starting on day 3 after infection, parasitemia in the blood was measured daily, and progression of the disease was followed using clinical scores and survival. We observed a trend for higher parasitemia and earlier lethality in animals infected at ZT13 compared to ZT1. We also tested the impact of environmental disruption in disease development by applying a protocol of chronic jet lag (4 consecutive weekly shifts of 6h-advance of the light-dark cycle). Mice were then infected and the same parameters were evaluated. We observed a trend in circadian disrupted animals to develop earlier lethality and clinical signs, higher parasitemia and significantly higher parasite load in the blood at day 6 post-infection. This project will represent the first detailed study of malaria in the context of circadian rhythms and it may lead to better therapeutics and preventive approaches for this disease.
The Role of Secreted Acid Phosphatase in *Leishmania donovani* Infection

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The protozoan parasite, *Leishmania donovani*, is the causative agent of the neglected tropical disease known as visceral Leishmaniasis. This disease can be lethal when untreated however, even with access to the correct drugs many patients are non-compliant due to the possibly painful and lengthy treatment process. Moreover, there is an emergence of drug resistant parasites making the discovery of new therapeutics key. To date, there are no vaccines for leishmaniasis. Studying *Leishmania* virulence factors is crucial in understanding how the parasite causes disease. *Leishmania’s* most abundantly secreted and highly conserved protein is an acid phosphatase (SAcP). Our goal is to determine the function of SAcP through experimentally deleting the genes encoding SAcP with the aims of using the mutant to (i) infect macrophages in vitro, (ii) infect mice in vivo and (iii) use confocal microscopy to determine the localization of the protein in infected macrophages. We will use the molecular biology tool CRISPR-Cas9 to delete all 16 copies of the SAcP gene family. We hypothesize that *L. donovani* SAcP plays an important role in cell infection and/or parasite survival. Determining the function of SAcP will help us to better understand virulence and disease pathology. On a large scale, this can lead to improved treatment options and perhaps preventative measures for visceral Leishmaniasis.
Contemporary Zika virus isolates induce more dsRNA replicative intermediate in human astrocytoma cells

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Zika virus (ZIKV) is a mosquito-borne flavivirus whose recent emergence and rapid geographic expansion poses a significant challenge for global public health. Although historical ZIKV infections caused only mild febrile illness, recent ZIKV outbreaks are associated with severe neurological complications, including Guillain-Barré syndrome and fetal microcephaly. We hypothesized that contemporary ZIKV isolates have evolved ways to increase viral fitness in human cells. Here we demonstrate that two contemporary (2015) ZIKV isolates from Puerto Rico and Brazil display increased replicative fitness in human astrocytoma cells. Over a single infectious cycle, the Brazilian isolate replicates to higher titers and induces more severe cytopathic effects in astrocytoma cells than the historical African reference strain or an early Asian lineage isolate. In addition, despite similar numbers of infected cells across isolates, both contemporary ZIKV isolates induce significantly more double-stranded RNA (dsRNA) replicative intermediate per infected cell. However, over multiple rounds of infection, contemporary ZIKV isolates appear to be impaired in cell spread, infecting a lower proportion of cells at a low MOI despite replicating to similar or higher titers. Overall, our data suggests that contemporary ZIKV isolates may have evolved mechanisms to increase viral replicative fitness in human cells. Current work is focused on understanding how evolutionary-acquired genetic differences between ZIKV isolates affect viral replication, dsRNA accumulation, and evasion of host antiviral responses. By assessing the role(s) of individual amino acid polymorphisms in viral fitness, we anticipate that these studies will provide insight into the roles of individual ZIKV proteins in the viral life cycle in human cells. Additionally, we anticipate that we will identify viral factors that have contributed to the rapid spread and increased pathogenesis seen in recent ZIKV outbreaks.
Preliminary data on the effect of HIV/CMV co-infection on CD8+ T lymphocyte replicative senescence
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Background: HIV/CMV co-infection has been associated with an increase in cardiovascular disease. CMV infection induces proliferation of CD8+ T cells, a low CD4:CD8 T cell ratio and downregulates expression of CD28 on T cells. This phenotype is known as the immune risk profile (IRP), is found most frequently in the elderly, and is associated with an increased risk of aging-related mortality and morbidity. CMV is known to impact immune aging. One postulated mechanism is by increasing T cell replicative senescence, however, only limited data supporting this view are available. We how therefore aimed at exploring HIV/CMV co-infection impacts CD8+ T cells, and how this factors into immune aging in a small pilot study.

Methods: Twelve individuals, 3 from each of four groups were evaluated: HIV-/CMV-, HIV-/CMV+, HIV+/CMV-, and HIV+/CMV+. The following were studied: 1) Immune senescent phenotypic profile utilizing flow cytometry (CD57, PD-1, CXCR3R1). 2) A functional assay for immune senescence evaluating population doublings of activated CD8+ T cells. 3) A pro-angiogenic cytokine profile measured via ELISAs (IL-6, IL-10, GM-CSF, and TNF-alpha).

Results: CMV appeared to be associated with a lower CD4:CD8 T cell ratio in the context of HIV. There were no remarkable differences in the immune phenotypes between the different groups. However, CMV infection appeared to decrease population doublings independently of HIV infection.

Conclusion: The study was exploratory and not designed to show significance. Due to a limited availability of CMV seronegative individuals, the sample size was small. The preliminary data hint that those with CMV, whether infected with HIV or not, exhibit changes in the functionality of CD8+ T cells. A larger study to further elucidate these changes is underway.

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Investigating the Role of PTP1B and TC-PTP in Natural Killer Cell Cytotoxicity

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Natural killer (NK) cells are important effectors in cell-based onco-immunotherapy with a major advantage of MHC-unrestricted cytotoxicity and low risks to develop cytokine release syndrome (CRS). In the past decades, genetically modified NK cells bearing chimeric antigen receptors (CAR-NK) have been under investigations with cell-base sources from established human cell line NK-92, bone marrow derived primary cells, umbilical cord blood, peripheral blood and induced pluripotent stem cells (iPSCs). Although CAR-NK cells have shown potent anti-tumor activities against certain malignant hematopoietic cells and refractory solid tumors at a comparable level to conventional CAR-T cells, improvements of CAR-NK in vivo survival, proliferation, and cytotoxicity require further investigations. Protein tyrosine phosphatases (PTPs) are important signaling regulators in maintaining cell homeostasis. PTP1B and TC-PTP are closely related non-receptor PTPs which negatively regulate cytokine signaling in T cells, B cells, Macrophages and Dendritic cells via dephosphorylating JAK/STAT signaling pathways. However, the role of PTP1B and TC-PTP in NK cytotoxic signaling remains obscure. Previous results in our lab have shown that deficiency of PTP1B and TC-PTP sensitizes immune cells to extracellular cytokines and growth factor stimulations. We hypothesize that in NK cells, deficiency of PTP1B and TC-PTP will enhance anti-tumor activities by sensitizing activating receptors to ligands binding. To date, our preliminary data has shown that chemical inhibition of PTP1B and TC-PTP improved NK cell direct cytotoxicity against human chronic myelogenous leukemia cells K-562 in vitro. Our ongoing investigations on NK cytotoxicity include measurement of degranulation, cytokine production and specific lysis of multiple malignant cells in human NK cell lines NK-92 and NKL as well as in murine derived, lymphokine activated killer cells (LAK) models.
The microorganisms in our intestines, called the gut microbiota, are essential for health. Mainly consisting of bacteria, the composition of the gut microbiota is altered in disease, though little is known about how these changes arise. One factor which could influence changes in microbial composition during disease is bacterial replication. However, there are no established techniques for identifying replicating bacterial members in complex communities such as the gut.

In this study, we aimed to address this gap by optimizing an experimental pipeline to identify replicating gut bacteria using 5-ethynyl-2′-deoxyuridine (EdU) click chemistry paired with flow cytometry. EdU incorporates into replicating DNA and fluoresces when linked to a fluorescently labeled azide. Fluorescent (EdU+) cells can be quantified using flow cytometry. As a proof of concept, we conducted EdU labeling of three known gut bacterial isolates: *Escherichia coli*, *Enterococcus faecalis*, and *Bacteroides fragilis*.

We then applied our approach to whole fecal bacterial communities from healthy C57BL/6 mice, and show that with a 3-hour incubation in bovine heart infusion (BHI) broth with 50 uM EdU, up to 47% of bacteria are EdU+ cells. Furthermore, restriction fragment length polymorphism on the V4 region of the bacterial 16S rDNA shows that our incubation time with EdU does not considerably alter the original bacterial population. Next, we separated EdU+ and EdU- cells using fluorescence-activated cell sorting (FACS) and identified the number of cells required to accurately reflect gut bacterial diversity. This will enable the valid characterization of these populations using 16S rDNA sequencing. These findings confirm the validity of EdU labeling for identifying replicating gut bacteria.

This technique will be instrumental for elucidating gut microbial dynamics, especially for deepening our understanding of bacterial activity during disease.
IL-1 and IL-33 differentially regulate the functional specialization of mucosal Foxp3+ regulatory T cells

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CD4+ regulatory T (T\textsubscript{REG}) cells are critical mediators of peripheral immune tolerance and homeostasis and express the forkhead box p3 (Foxp3) transcription factor. T\textsubscript{REG} cells are abundant at mucosal surfaces, where they respond to local danger signals in order to adapt their transcriptional program. The effect of these signals on T\textsubscript{REG} cells function remain largely unknown.

To understand the processes involved, we compared the mRNA signature of Foxp3\textsuperscript{+} and exFoxp3\textsuperscript{+} T cells isolated from a model developed to study the reprogramming of T\textsubscript{REG} cells into inflammatory T cells. We uncovered that the IL-33 receptor (IL-33R, ST2) was prominently expressed by T cells that maintained Foxp3 expression, while the IL-1 receptor (IL1R1) was expressed on cells that lost Foxp3. Interestingly, both ST2\textsuperscript{+} and IL1R1\textsuperscript{+} T\textsubscript{REG} cells populations compete for expansion under inflammatory conditions: the absence of IL1R1 expression (IL1R1\textsuperscript{−/−}) leads to the accumulation of ST2\textsuperscript{+} T\textsubscript{REG} cells at mucosal surfaces, while IL-33 injections facilitate the accumulation of ST2\textsuperscript{+} T\textsubscript{REG} cells and reduce the onset of exFoxp3 T cells. Using lung infection models, we demonstrate that ST2-expressing T\textsubscript{REG} cells resist production of inflammatory cytokines, whereas IL1R1-expressing T\textsubscript{REG} cells express RORyT and proinflammatory IL-17A. While IL-1 signalling impairs T\textsubscript{REG} cell suppressive function, IL-33 is required for the successful prevention of T-cell dependent colitis.

Thus, ST2\textsuperscript{+} and IL1R1\textsuperscript{+} T\textsubscript{REG} represent two distinct populations of reprogrammed T\textsubscript{REG} cells. These observations demonstrate that IL-1 and IL-33 produced during immune challenge exert distinct roles on the functional adaptation of Foxp3\textsuperscript{+} T\textsubscript{REG} cells at mucosal surfaces during infections.
In DOCK8 deficiency migration-induced immune cell shattering induces a type-2 CD4 T effector differentiation bias.

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Immune cells are the only cells in the body able to migrate between and within tissues. While it is increasingly clear that immune cell movement is critical to immune system function, it remains less well understood how migratory processes impact CD4 T cell effector differentiation. Here we studied a specific migration defect caused by the deficiency of the guanine exchange factor DOCK8, where loss-of-function in humans leads to immunodeficiency as well as allergies, eczema and dermatitis. We show that DOCK8−/− mice have a profound type-2 CD4 T cell effector (Th2) bias upon pulmonary infection with Cryptococcus neoformans, mirroring what is observed in humans. Unexpectedly, this type-2 skew did not require loss of DOCK8 in dendritic cells or T cells and recruitment of CD4 T cells to the lung was unimpaired, despite the role of DOCK8 in T cell migration. However, activated CD4 T cells produced significantly more GM-CSF in DOCK8−/− mice than in wild type mice, and blocking GM-CSF eliminated the type-2 bias. We demonstrated that DOCK8−/− mice also produced markedly more IL-1 in the lung. Inhibition of IL-1 signalling in CD4+ T cells diminished GM-CSF production and abolished the Th2 bias. Interestingly, in DOCK8−/− mice there was a significant increase in cell death at the site of infection. We determined that DOCK8-deficient myeloid cell populations develop stretched, dysregulated morphologies and frequently fragment during migration. This migration-induced cell shattering led to IL-1β production both directly by migrating cells and indirectly through release of DAMPs. Thus, our work reveals an important role for cell death in driving type-2 signals within tissue sites of infection, which may have implications understanding the etiology of type-2 responses in other settings such as allergies.
Characterizing the Inflammatory Response Following Interaction between Ebola Virus and Host Cells

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Ebola virus (EBOV) is an RNA virus of the \textit{Filoviridae} family that is responsible for outbreaks of hemorrhagic fevers in primates with a lethality rate as high as 90\%. It has been shown that EBOV primarily targets host macrophages, and this interaction leads to activation and disease pathogenesis, although our knowledge of the pro-inflammatory response is limited to epithelial cell studies. We were interested to further elucidate the cellular and molecular events underlying the EBOV-induced cytokine storm. To study EBOV without BSL4 containment, we generated EBOV virus-like particles (VLPs) expressing the viral glycoprotein (GP). \textit{In vitro} and \textit{in vivo} experiments were performed using these particles to stimulate inflammatory cells in culture and in mice. Results from stimulation of murine B10R macrophages and human THP-1 cells with VLP-GP showed an induced inflammatory response, characterized by increased production of pro-inflammatory cytokines and chemokines compared to control VLPs (no GP expression). Cells obtained from mouse intraperitoneal (IP) lavages were analyzed by flow cytometry six hours post-IP injection with PBS, VLP-GP, or control VLP. An increase in the presence of neutrophils was observed in a GP-dependent manner, accompanied by a simultaneous decrease in the recruitment and presence of CD4\(^+\) and CD8\(^+\) T cells and B cells. In parallel, serum from two rhesus macaques infected with EBOV was obtained to study the inflammatory events occurring in a progressive infection. Following size exclusion chromatography isolation, NanoSight analysis indicated an increase in the total number of exosomes in circulation at the peak of \textit{in vivo} infection. Analysis of inflammatory serum biomarkers by multiplex array also corroborated our \textit{in vitro} work. This highlights the importance of viral factors, such as GP, and host-derived extracellular vesicles in the inflammatory cascade and pathogenesis of EBOV, which can collectively be further exploited for antiviral development.
DOCK8 is a guanine nucleotide exchange factor expressed specifically in immune cells. Clinically, DOCK8 loss-of-function mutations manifest as an immunodeficiency characterized by severe viral skin infections, allergies, hyper IgE syndrome, and lymphopenia. Loss of DOCK8 results in dysregulated morphology of lymphoid and myeloid cells during migration that ultimately leads to cell death, particularly in dense tissues. Both in vivo and ex vivo in collagen matrices, T cells become extremely stretched with nuclei that are similarly deformed and elongated. In contrast, BMDCs lose the typical dendritic cell morphology; they become catastrophically stretched in a multipolar manner, and their nuclei often fragment into several lobes. In both cell types, the dysregulation in cellular migration phenotype is associated with altered microtubule distribution. Cellular migration induces considerable mechanical stress on the nucleus, and can result in DNA damage. Unexpectedly, despite the dysregulated nuclear morphology in DOCK8-deficient cells, the levels of γH2AX are decreased, indicating that the cells are not initiating DNA damage repair to the same extent as wild type cells. Moreover, the decrease in DNA damage repair is more prominent in myeloid cells than in T cells. Migration is a critical function for all cells of the immune system, and our results suggest that DOCK8 is an important factor in maintaining nuclear integrity during their movement through tight tissue spaces.
ROLE OF DIACYLGLYCEROL ACYLTRANSFERASE 2 (DGAT2) IN GROUP 2 INNATE LYMPHOID CELL (ILC2) FUNCTION

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Background: Group 2 innate lymphoid cells (ILC2) are a resident cell population at body surfaces. These cells are important mediators of type 2 immunity, which is critical for wound healing and responding to extracellular pathogens. However, when dysregulated, it can lead to immunopathologies such as allergy and asthma. Upon activation by the alarmins interleukin (IL)-25, IL-33, and thymic stromal lymphopoietin (TSLP), ILC2 proliferate and secrete large amounts of type 2 signature cytokines, including IL-4, IL-5 and IL-13, to orchestrate innate and adaptive type 2 immune responses. Nevertheless, many mechanisms governing their regulation and metabolism remain unknown.

Rationale and hypothesis: Using a yet-unpublished RNA-sequencing approach, we observed that diacylglycerol O-acyltransferase 2 (Dgat2) mRNA is highly induced in murine ILC2s upon activation. Dgat2 synthesizes triacylglycerol (TAG), which localizes in lipid droplets (LDs) for storage. We thus hypothesized that Dgat2-mediated TAG synthesis plays a key role in ILC2 activation and function.

Experimental approach: Since Dgat2⁻/⁻ mice die shortly after birth, we used commercially available Dgat2 inhibitors to treat ILC2 ex vivo. Upon Dgat2 inhibition in ILC2, we measured proliferation, apoptosis, cytokine production, and reactive oxygen species (ROS) production. Additionally, we investigated LD formation and lipid uptake upon ILC2 activation.

Results and conclusion: We show that Dgat2 inhibition ex vivo significantly alters ILC2 function. Furthermore, we show an increase in free fatty acid (FFA) uptake and LD content upon ILC2 activation. We propose that Dgat2 is upregulated upon ILC2 activation to prevent toxic effects of rapid FFA uptake, and that TAG breakdown is the preferred metabolic pathway for energy generation in ILC2.
Respiratory syncytial virus (RSV) infection leads to millions of hospitalizations and many thousands of deaths per year. Early childhood RSV infection is also associated with development of wheezing & asthma. Well-established sex-differences exist in both asthma epidemiology and morbidity. Asthma prevalence is greater in boys than girls but switches around the time of puberty to become greater in women than men. Currently, neither antivirals nor vaccines are available for RSV; thus, novel preventative and therapeutic strategies are urgently needed. A potential preventative strategy is a cell-penetrating peptide we developed called STAT6-IP, which inhibits Type 2 inflammation in allergy and RSV infection models. To understand the potential impact of STAT6-IP in the induction of asthma associated with respiratory virus infection, we have developed a clinically relevant murine model to examine the impact of early life RSV infection on house dust mite- (HDM-) induced Type 2 inflammatory responses in the lung. Our data demonstrate that prior exposure to RSV markedly enhances HDM-induced lung eosinophil (Eos) numbers and activation as well as production of Type 2 cytokines by ex vivo-stimulated lung draining lymph node (DLN) cultures. RSV infection enhances responses in mice exposed acutely to HDM or in response to a series of HDM challenges to induce Th2 adaptive immunity. The degree of Eos activation is more prominent in females. Delivery of STAT6-IP selectively at the time of RSV infection reduces both lung and DLN inflammatory responses induced by HDM. These data suggest that STAT6-IP has the potential to provide protection against the development of allergic airways disease associated with early life RSV infection.
Ago2:miR-122 interactions with the HCV genome alter the secondary structure of the viral 5' terminus and promote functional IRES formation

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Hepatitis C virus (HCV) is a positive-sense RNA virus that interacts with a liver specific microRNA, miR-122. miR-122 binds to two sites on the 5' untranslated region (UTR) of the viral genome and promotes HCV RNA accumulation, although the precise mechanism(s) of miR-122 in the HCV life cycle remain unclear. Using biophysical analyses and Selective 2' Hydroxyl Acylation analyzed by Primer Extension (SHAPE), we show that hAgo2:miR-122 binding to the wild-type HCV 5' UTR suppresses an alternative structure (termed stem-loop (SL) IIalt) and promotes formation of the functional internal ribosomal entry site (IRES, SLII-SLIV). In contrast, the G28A mutation, which was initially isolated from patients who underwent antisense miR-122 inhibitor therapy and was subsequently shown to have reduced reliance on miR-122, was shown to favour formation of the IRES even in the absence of miR-122. Furthermore, despite the close proximity between the miR-122 sites, two hAgo2:miR-122 complexes are able to bind to the HCV 5' terminus simultaneously and SHAPE analyses revealed further alterations to the structure of the 5' UTR to accommodate these complexes. Computational modeling of the hAgo2:miR-122 complexes with the IRES and 40S subunit of the ribosome suggests that hAgo2 is likely to form additional interactions with the HCV IRES, which is supported by SHAPE analyses, and these interactions may further stabilize the viral IRES and promote HCV translation. Our data provides a new model for hAgo2:miR-122 interactions with the HCV genome. Specifically, we predict that the HCV 5' UTR initially takes on an alternative conformation in the absence of miR-122 (SLIIalt). Binding of hAgo2:miR-122 to Site 2 acts in an RNA chaperone-like manner to convert the 5' terminus to SLII, allowing formation of the viral IRES (SLII-IV). The SLII conformation then allows recruitment of hAgo2:miR-122 to Site 1, which protects the 5' triphosphate moiety from pyrophosphatase activity and viral RNA decay. To accommodate hAgo2:miR-122 at Site 1, the auxiliary interactions with the Site 2-bound hAgo2:miR-122 are destabilized, but this complex is further stabilized by interactions with the HCV IRES. This model is supported by our biophysical data and provides novel insight into the mechanisms of miR-122-mediated viral RNA accumulation.
Dendritic cells (DCs), cells of the innate immune system, are found in a steady state poised to respond to activating stimuli. Once stimulated they rapidly undergo dynamic changes in gene expression to adopt an activated phenotype capable of stimulating immune responses. Our lab and others have shown that negative regulators of gene expression, such as the epigenetic factor Polycomb Group Factor 6 (PCGF6), maintain DCs in the steady state until their expression is reduced upon activation. A group of factors that can rapidly downregulate the expression of multiple targets are MicroRNAs (miRNA), which are emerging as important regulators of immune function due to their ability to regulate programs of gene expression.

MicroRNA-9 (miR-9) has been shown to be upregulated in human DCs as well as monocytes and macrophages following stimulation with pro-inflammatory stimuli. Analysis of putative targets revealed that miR-9 targets PCGF6 along with other negative regulators.

We propose that miR-9 promotes DC activation through targeting these negative regulators. We found that DCs overexpressing miR-9 showed an increased activation phenotype whereas DCs sequestering miR-9 showed blunted activation. Co-culture of miR-9 sequestering DCs with T cells led to decreased T-cell activation, whereas miR-9 overexpressing DCs promoted cell activation. Mice immunized with miR-9 overexpressing DCs following injection with B16-OVA melanoma cells displayed decreased tumour volume compared to controls. This work demonstrates that miR-9 promotes the activation and function of DCs, adding to the growing evidence that miRNAs are involved in the regulation of immune responses.
Understanding how pathoadaptation of *Pseudomonas aeruginosa* in Cystic Fibrosis alters host-microbe interactions in the airway epithelium

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Chronic *Pseudomonas aeruginosa* (PA) infections occur in 60-80% of all adult Cystic Fibrosis (CF) patients and are associated with an accelerated lung function decline, excessive, non-resolving inflammation and increased mortality. While PA is traditionally considered an extracellular pathogen, we have recently demonstrated the presence of intracellular PA within airway epithelial cells (AECs) of CF lung explants, indicating the possible presence of an intracellular reservoir *in vivo*. In this project, we hypothesize that genetic adaptations commonly observed in PA isolates from chronic infections promote increased lung inflammation and an intracellular PA lifestyle.

Our first aim is to analyze the impact of LasR (quorum sensing transcriptional activator) loss of function on ICAM-1, an important pro-inflammatory mediator. Preliminary results show that expression of ICAM-1 on AEC is upregulated in response to *lasR* mutant filtrates compared to wildtype filtrates and that this upregulation enhances neutrophil binding to AECs *in vitro*.

Our second aim is to examine whether LasR and Type 3 secretion system (T3SS) loss of function, two common pathoadaptations, modulate internalization of PA into AECs as well as its intracellular persistence *in vitro*. So far, we have observed that *lasR* mutation promotes internalization into primary CF AECs. We have further found that loss of T3SS injectisome function enhances bacterial persistence in AECs for up to 120h.

In conclusion, our results suggest that loss of LasR might increase lung inflammation and thus contribute to CF lung function decline. Furthermore, loss of LasR and T3SS function, two phenotypic adaptations commonly observed in PA strains from chronic CF infections, promote an intracellular PA lifestyle, potentially allowing bacteria to persist. Consequently, our research might provide further insight into how common pathoadaptations contribute to disease progression and PA persistence.
Detection of *Fusobacterium nucleatum* subspecies in saliva and colorectal cancer tissue, using matrix-assisted laser desorption ionization/time of flight tandem mass spectrometry (MALDI-TOF/TOF MS)

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Abstract

Colorectal cancer (CRC) is known to be one of the most aggressive types of cancer. While the gut is a diverse ecological inhabitant of thousands of bacterial species, rising evidence links *Fusobacterium nucleatum* with the disease. Whether this bridging bacterium is a causative factor for the disease or a species that merely happens to flourish in the CRC environment, is not fully determined. Recently, the development of MALDI-TOF/TOF MS technology has been introduced as a rapid, efficient and accurate method of detecting bacterial pathogens at microbiology clinical diagnostic laboratories. After MALDI-TOF MS has been used to detect the *Fusobacterium nucleatum* (*F. nucleatum*) four genetically different subspecies, *nucleatum*, *polymorphum*, *animalis*, and *vincintii* in pure cultures, in this study, we are trying to explore the capability of MALDI-TOF/TOF MS to detect them in CRC patient’s colon tissue and saliva and explore the possible similarities among these specimen. Detecting *F. nucleatum* subspecies in CRC-free individuals’ colon tissues and saliva will be also investigated, as saliva has been suggested as a possible route of *F. nucleatum* strains to reach and colonize the CRC tissue. The possible identification of a specific *F. nucleatum* pathogen may allow future targeting of this pathogen as a method of detection of, treatment or prevention of CRC.
Leishmaniasis is caused by the parasite *Leishmania* known to affect millions worldwide. In recent years, we have established the role played by *Leishmania* zinc-metalloprotease gp63 (GP63) in the modulation of host macrophage signaling and functions. In an immunological context, it favors survival of the parasite within its host. Whereas *Leishmania major* knock out for GP63 caused limited infection in mice, it is still unclear how GP63 may influence the innate inflammatory response and parasite survival in an in vivo context. Therefore, we were interested in analyzing the early innate inflammatory events occurring upon *Leishmania* inoculation and to establish whether *Leishmania* GP63 influences this initial inflammatory response.

Experimentally, 4 groups of mice were injected intraperitoneally with PBS, *L. major* WT, *L. major* gp63KO or *L. major* gp63Add-back. Six-hours post-inoculation, intraperitoneal lavages were performed and collected. The number of recruited inflammatory cells was counted and cytospin preparations were used to identify cell type recruited. In addition, the collected cells were plated and studied to determine the percentage of infected cells over time. Supernatant was retrieved and cytokine/chemokine contents were measured. Data collected to date suggest that all *L. major* cause inflammatory cells recruitment, however cytokine/chemokine results do not explain why gp63 KO parasite cause a reduced in vivo infection. It is also observed that gp63 may be involved in the internalization of promastigotes during early infection, where less gp63 KO amastigotes are found within host cells. Collectively this study will provide a clear analysis of innate inflammatory events occurring during *L. major* infection and the role of gp63 in this immunological event, as well as permit the development of new ways to protect against leishmaniasis. This research is supported by a CIHR Project grant to MO.
DipM, a potential replication and polar-organizing regulator in Caulobacter crescentus

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Screening for novel replication control genes in C. crescentus has unexpectedly identified a temperature sensitive allele of the dipM gene. In the published literature, DipM has been identified as an important endopeptidase that aids in peptidoglycan cell remodelling. We are interested in determining the role of this cell division-related gene in chromosome replication.

DipM-mutant cells and dipM knockout cells (ΔdipM) are sensitive to nalidixic acid (Nal). This finding was unexpected because WT cells are innately resistant to Nal. An independent study proposes that Nal sensitivity stems from a loss of polarity and mislocalization of an efflux pump. This observation is pertinent because polarity is disrupted in ΔdipM cells.

A potential defect in replication prompted us to investigate the replisome complex and parABS chromosome partitioning system in ΔdipM cells. Within the replisome, the processivity clamp DnaN locks pol III to DNA and the chromosome partitioning component ParB translocates the replicated chromosome to the opposite pole. By following DnaN-YFP and ParB-GFP reporters individually in WT and ΔdipM cells, the results show that ΔdipM cells do not exhibit significant over-replication per volume of cytoplasm.

Interestingly, three distinct foci are visualized in the majority of ΔdipM-ParB-GFP cells, which are located at the two poles and mid-cell. The ParB-chromosome complex is anchored to the cell pole through direct binding of ParB to the pole-organizing protein PopZ. Markedly, a study shows that ΔdipM cells have PopZ abnormally localized to the mid-cell. Therefore, ParB-GFP is likely anchored to the mid-cell by PopZ.

Moreover, we observed that the combination of ΔdipM with ParB-GFP or DnaN-YFP leads to more pronounced defects in morphology and growth compared to ΔdipM alone. This observation provides another link for interactions between cell cycle regulators and DipM.

Lastly, preliminary work suggests that intact dipM is required to maintain autonomously replicating plasmids, since dipM cannot be deleted when simultaneously selecting for plasmid maintenance. The high frequency of recovering WT cells demonstrates the functional importance of DipM in plasmid replication.

Based on these and other lines of evidence, we hypothesize that DipM is involved in chromosome replication by influencing polar organization. The Caulobacter origin of replication is polarly localized but the significance is not known. DipM may offer new insights on the coordination between chromosome replication and division.
INVESTIGATING THE ROLE OF PD-1 EXPRESSION IN MEMORY CD8 T CELL GENERATION FOLLOWING ACUTE INFECTION. Tracey D. Beauchamp¹,², Esther Tarrab³, Alain Lamarre³, and Martin J. Richer¹,².¹ Department of Microbiology & Immunology, McGill University, QC. ² Rosalind & Morris Goodman Cancer Research Centre, McGill University, Montreal, QC. ³ Immunovirology Laboratory, INRS-Institut Armand-Frappier, Laval, QC.

The generation of long-lived memory CD8 T cells is critical for host protection against reinfection. The development of memory CD8 T cells is not a stochastic event, but rather commitment to memory fate occurs early during the activation of CD8 T cells. However, the pathways defining CD8 T cell differentiation remain incompletely understood. Programmed cell death protein 1 (PD-1) is a co-inhibitory receptor that plays a role in mediating CD8 T cell exhaustion during chronic infections and cancer. Conversely, the inhibitory activity of PD-1 is crucial to maintain self-tolerance during acute infection. While stimulation of the T cell receptor (TCR) regulates the expression of PD-1, the full extent of the relationship between PD-1 and CD8 T cell activation remains unknown. PD-1 has been shown to inhibit signaling through both the TCR and the co-stimulatory molecule CD28, both of which are required to activate CD8 T cells. It is unclear whether the affinity of a particular CD8 T cell clone for its antigen, as well as the magnitude of co-stimulatory and inflammatory signals received during infection play a role in the fate decision of naïve CD8 T cells. Herein, we demonstrate that PD-1 is differentially expressed on memory precursor cells (MPC) compared to terminal effector cell (TEC) subsets and is preferentially expressed on CD8 T cell clones with higher sensitivity for their cognate antigen. Despite these differences, our results suggest that PD-1 signaling does not play a functional role in memory progression of CD8 T cells. Although its role may not be functional, differing expression of PD-1 on MPC compared to TEC subpopulations indicates intrinsic differences in signal strength being received by these subsets. This suggests that increased TCR signal strength may play a role in clonal selection of CD8 T cells which will go on to seed the long-lived memory pool.
Gram-negative intestinal infection induces the formation and infiltration of anti-mitochondrial T cells in the CNS of \textit{Pink1}^{-/-} mice

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Current models for familial forms of Parkinson’s disease (PD) caused by PINK1 mutations suggest that dysfunctional mitochondria are responsible for neuronal death. This is based on the finding that PINK1 plays a key role in mitophagy; however, this has proven difficult to validate in mice. This model also fails to consider additional factors involved in PD onset including the role of the gut-brain axis and emerging evidence implicating the immune system in neuronal degeneration. Recently, it has been shown that LPS can stimulate anti-mitochondrial T cells in the absence of PINK1, suggesting it plays a role in the protection against mitochondrial autoimmunity. We hypothesized that a Gram-negative intestinal infection will stimulate anti-mitochondrial T cells in \textit{Pink1}^{-/-} mice that can enter the CNS and damage dopaminergic neurons. Infection of \textit{Pink1}^{-/-} mice with \textit{Citrobacter rodentium}, a model Gram-negative murine pathogen, resulted in an increase in autoreactive T cells compared to WT littermates. Although infection resulted in infiltration of T cells in the CNS regardless of genotype, autoreactive T cells were uniquely found in \textit{Pink1}^{-/-} mice. Remarkably, \textit{Pink1}^{-/-} mice serially infected 4 times developed movement disorders. Administration of L-DOPA significantly decreased these movement deficits. Maintaining mice 1-year post-infection showed a reversal of phenotypes, suggesting this effect is transient.