KEYNOTE SPEAKER:
Adolfo García-Sastre, Ph.D.
Professor, Microbiology and Medicine, Icahn School of Medicine at Mount Sinai
Co-Director, Global Health and Emerging Pathogens Institute

DATE: MAY 20th, 2016
McIntyre Medical Building 6th Floor/Amphitheater 522

PRIZES FOR BEST PRESENTATION & POSTER
<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>8h30-9h15</td>
<td>Registration/Breakfast</td>
</tr>
<tr>
<td>9h15-9h30</td>
<td>Welcome Address</td>
</tr>
<tr>
<td>9h30-9h30</td>
<td>Oral Session I</td>
</tr>
<tr>
<td>1. Kaitlin Anstett – The Effect of E157Q in HIV-1 Integrase on R263K-Mediated Dolutegravir Resistance</td>
<td></td>
</tr>
<tr>
<td>2. João Ormonde – REGULATION OF T CELL ACTIVATION AND FUNCTION BY THOUSAND-AND-ONE AMINO ACID KINASE 3</td>
<td></td>
</tr>
<tr>
<td>3. Kristin Van Den Ham - Protein Tyrosine Phosphatase Inhibition Enhances the Generation of Protective Tr1 Cells and Attenuates the Brain Sequestration of Pathogenic T Cells during Experimental Cerebral Malaria</td>
<td></td>
</tr>
<tr>
<td>10h15-10h30</td>
<td>Coffee Break</td>
</tr>
<tr>
<td>10h30-11h30</td>
<td>Keynote Lecture : Dr. Adolfo Garcia-Sastre</td>
</tr>
<tr>
<td>11h30-12h15</td>
<td>Lunch</td>
</tr>
<tr>
<td>12h15-14h15</td>
<td>Poster Session</td>
</tr>
<tr>
<td>14h15-15h00</td>
<td>Oral Session II</td>
</tr>
<tr>
<td>1. Andreea Damian - Prolonged Inflammatory Responses to Staphylococcus aureus in Aryl Hydrocarbon Receptor-Deficient Mice</td>
<td></td>
</tr>
<tr>
<td>2. Nirmin Alsahafi - NEF PROTEINS FROM HIV-1 ELITE CONTROLLERS ARE INEFFICIENT AT PREVENTING ADCC</td>
<td></td>
</tr>
<tr>
<td>3. Travis Ackroyd - Characterizing the role of CFTR in intestinal infection</td>
<td></td>
</tr>
<tr>
<td>15h00-15h15</td>
<td>Coffee Break</td>
</tr>
<tr>
<td>15h15-16h15</td>
<td>Oral Session III</td>
</tr>
<tr>
<td>1. Felix LaRoche-Johnston - Recent horizontal transfer of a bacterial group II intron: Evolutionary relationship and functional adaptation between Ll.LtrB and Ef.PcfG</td>
<td></td>
</tr>
<tr>
<td>2. Saina Beitari - HIV-1 Envelope protein is as potent as glycoprotein G of vesicular stomatitis virus in overcoming the restriction of high levels of SERINC5</td>
<td></td>
</tr>
<tr>
<td>3. Fernando Alvarez - The alarmin IL-33, a novel mechanism for Foxp3+ TREG cells to resist reprogramming in the course of infectious disease</td>
<td></td>
</tr>
<tr>
<td>16h00-16h15</td>
<td>Closing Remarks</td>
</tr>
<tr>
<td>16h30-20h00</td>
<td>Presentation of Awards/Reception</td>
</tr>
</tbody>
</table>

Thomson House – 3650 Rue McTavish
**Table of Contents**

**Oral Presentations:**

The Effect of E157Q in HIV-1 Integrase on R263K-Mediated Dolutegravir Resistance. .................................................................................................................. O01  
*Kaitlin Anstett*

REGULATION OF T CELL ACTIVATION AND FUNCTION BY THOUSAND-AND-ONE AMINO ACID KINASE 3 ........................................................................................................ O02  
*João Ormonde*

Protein Tyrosine Phosphatase Inhibition Enhances the Generation of Protective Tr1 Cells and Attenuates the Brain Sequestration of Pathogenic T Cells during Experimental Cerebral Malaria .................................................................. O03  
*Kristin Van Den Ham*

Prolonged Inflammatory Responses to Staphylococcus aureus in Aryl Hydrocarbon Receptor-Deficient Mice .................................................................................. O04  
*Andreea Damian*

NEF PROTEINS FROM HIV-1 ELITE CONTROLLERS ARE INEFFECTIVE AT PREVENTING ADCC ........................................................................................................ O05  
*Nirmin Alsahafi*

Characterizing the role of CFTR in intestinal infection ........................................ O06  
*Travis Ackroyd*

Recent horizontal transfer of a bacterial group II intron: Evolutionary relationship and functional adaptation between Ll.LtrB and Ef.PcfG ....... O07  
*Felix LaRoche-Johnston*

HIV-1 Envelope protein is as potent as glycoprotein G of vesicular stomatitis virus in overcoming the restriction of high levels of SERINC5.. O08  
*Saina Beitari*

The alarmin IL-33, a novel mechanism for Foxp3+ TREG cells to resist reprogramming in the course of infectious disease................................. O09  
*Fernando Alvarez*
Poster Presentations:

SHP-1-Regulated Hepatocyte Inflammatory Response Is Critical During Septic Shock................................................................. P01
Anupam Adhikari

Leishmanicidal activity and immunomodulatory effects of the Cramoll 1,4 Lectin................................................................. P02
Andrezza Raposo Borges de Melo

Role of Glycosyl Hydrolases in Fungal Biofilms ........................................ P03
Caitlin Zacharias

Reactivation of an HIV-1 latency model by targeting the PKR signalling pathway ................................................................. P04
Roman Labbé

Two-component systems and altered virulence of Citrobacter rodentium. P05
Natalia Giannakopoulou

Using Live Cell SHAPE analysis to Compare the Structure of Dengue Virus (DENV) RNA in Human and Mosquito cells ......................... P06
Michael Rajah

Delineating the role of myeloid cell populations upon early Heligmosomoides polygyrus infection ........................................ P07
Marilena Gentile

Characterizing the T Cell Response to Zika Virus Infection ..................... P08
Ryan Pardy

Investigating the pathways determining intestinal homeostasis versus dysfunction during Citrobacter rodentium infection by genome-wide expression profiling .......................................................... P09
Eugene Kang

Reduced CD8+ T cell antigen sensitivity during the establishment of chronic viral infection is dependent on IL-10 ............................. P10
Logan Smith

RNA Pull-down Strategy to Investigate the Roles of MicroRNA-122-Associated Complexes in Hepatitis C Virus Infection .......................... P11
Annie Bernier

The Contribution of N-Glycolyl Muramyl Dipeptide to Cell-Mediated Immunity Elicited by Complete Freund’s Adjuvant ..................... P12
Jean-Yves Dubé

The role of RNA surveillance proteins on HIV-1 proviral reactivation ...... P13
Shringar Rao

The Anti-HIV-1 Host Protein MxB Promotes Cell Apoptosis....................... P14
Fan Huang
Early HA-immune cell interactions influence the innate immune response to plant-made virus-like particle vaccines for influenza.................................P15
Hilary Hendin

Dissecting the role of the poly(rC)-binding protein 2 in the hepatitis C virus life cycle .................................................................................................P16
Sophie Cousineau

Withdrawal of dolutegravir in early phases of HIV-1 infection in tissue culture does not abrogate antiretroviral activity.................................P17
Nathan Osman

CD109 regulates IL-17 producing gamma delta T cells and psoriatic-like inflammation ..................................................................................P18
Giustino Carnevale

Identification of cellular and immunological mechanisms triggered by Leishmania-derived exosomes in Cutaneous Leishmaniasis .................P19
Alonso Lira Filho

The Role of Mxi1 in the development of CD4+Foxp3+ regulatory T cells ... P20
Nils Pavey
The Effect of E157Q in HIV-1 Integrase on R263K-Mediated Dolutegravir Resistance.

Anstett, K.1,2, Cutillas, V.2, Fusco, R.3, Mesplede, T.2, and Wainberg, M.A.1,2

1 Department of Microbiology and Immunology, Faculty of Medicine, McGill University, Montreal, Quebec, Canada.

2 McGill AIDS Centre, Lady Davis Institute for Medical Research at the Jewish General Hospital, Montreal, Quebec, Canada.

3 Currently at Enanta Pharmaceuticals in Watertown, MA, USA.

Background: Due to its error-prone reverse transcriptase enzyme HIV-1 exists as a quasi-species of different variants. Some of these naturally occurring variants may in turn affect the ability of antiretrovirals (ARVs) to neutralize the virus. This concept was recently demonstrated when a patient failed the integrase inhibitor (INI) raltegravir (RAL), and then subsequently the superior INI dolutegravir (DTG), with only the E157Q polymorphic change reported in integrase (IN). Our laboratory is highly focused on the evolution of DTG resistance, specifically through the canonical resistance mutation R263K. This project aimed to unravel the interplay between positions 157 and 263 in IN, as well as their effects on IN activity and ARV resistance. If synergy between these two mutations is possible it could result in treatment failures for HIV-infected individuals.

Results: R263K decreased biochemical IN strand transfer, DNA binding activities, and viral infectivity by 20%. Neither biochemical function nor infectivity showed a significant decrease from WT when the E157Q mutant was evaluated, and the presence of this substitution in the R263K background partially restored the enzymatic and infectious defects conferred by the latter mutation. Despite the patient report, E157Q-containing viruses were not resistant to RAL, however in combination with R263K high levels of DTG resistance were observed.

Significance: DTG is one of the best current therapies for HIV infection, displaying a high genetic barrier for resistance and few treatment failures to date. However, we show that E157Q is synergistic with the DTG-signature resistance mutation R263K. The presence of this polymorphism at DTG initiation is possible, which could lead to the rapid selection of a fit, R263K-containing virus and subsequent treatment failure. Thus, the outcomes of this research have far reaching implications for the clinical care of HIV-positive individuals.
REGULATION OF T CELL ACTIVATION AND FUNCTION BY THOUSAND-AND-ONE AMINO ACID KINASE 3

JOÃO ORMONDE & JOAQUÍN MADRENAS

Microbiome and Disease Tolerance Centre, Department of Microbiology and Immunology, McGill University, Montréal, QC

Immune responses orchestrated by T cells are of utmost importance for host homeostasis and determine the outcome of infectious diseases, transplants, and tumours. Impaired regulation of these responses may result in the rise of autoimmune diseases. Thus, it is important that T cells maintain their activation and function tightly regulated.

During studies aimed at discovering novel regulators of TCR-dependent T cell activation, our laboratory observed an enrichment of the protein kinase Thousand-And-One Amino Acid 3 (TAO3) in the proteome of the soluble fractions of cell lysates obtained from activated human T cells. TAO3 is constitutively expressed by human peripheral blood T cells and T cell lines (Jurkat cells). To study the role of TAO3 in T cell activation, we generated a TAO3-deficient (TAO3 KO) Jurkat cell line using CRISPR/Cas9 technology. TAO3-deficient T cells exhibited increased basal protein phosphorylation on serine and tyrosine residues. Upon TCR ligation, TAO3 KO cells showed a marked impairment in proximal signaling with reduction of tyrosine phosphorylation of ZAP-70 and decreased activation of downstream targets such as ERK1/2. More importantly, deficiency of TAO3 in Jurkat cells resulted in a significant decrease in IL-2 production in response to signalling through the canonical pathway of TCR ligation. In contrast, activation of these cells through the alternative, Lck-independent TCR signalling pathway or by PMA and ionomycin resulted in slight but significantly enhanced IL-2 secretion.

Our preliminary data suggest that TAO3 acts as a regulator of basal and TCR-dependent T cell responsiveness. We are currently characterizing the mechanism of action of TAO3 in vitro and in vivo.
PROTEIN TYROSINE PHOSPHATASE INHIBITION ENHANCES THE GENERATION OF PROTECTIVE TR1 CELLS AND ATTENUATES THE BRAIN SEQUESTRATION OF PATHOGENIC T CELLS DURING EXPERIMENTAL CEREBRAL MALARIA

Kristin Van Den Ham\textsuperscript{1,2}, Martin Richer\textsuperscript{1} and Martin Olivier\textsuperscript{1,2}

\textsuperscript{1}Microbiology and Immunology, McGill University, Montreal, QC, Canada and \textsuperscript{2}Research Institute of the McGill University Health Centre, Montreal, QC, Canada

Neuropathology induced by \textit{Plasmodium berghei} ANKA infection is dependent on the sequestration of cytotoxic CD8\textsuperscript{+} T cells within the brain microvasculature and augmentation of the inflammatory response. Inflammation is integral to controlling parasitemia, but can result in tissue damage if unregulated. IL-10 has the capacity to suppress inflammation and has also been implicated in limiting tissue parasite burden. Recently, IL-10-producing Foxp3\textsuperscript{+} CD4\textsuperscript{+} T cells were identified in \textit{P. falciparum}-infected children and were determined to have a role in the regulation of pathogenic inflammation. Modulation of protein tyrosine phosphorylation has the capacity to alter the immune response and has previously been demonstrated to mitigate pathology in models of leishmaniasis, asthma and cancer. Here we determined that pharmacological inhibition of protein tyrosine phosphatase (PTP) activity markedly protected mice from developing experimental cerebral malaria (ECM). Protection was concomitant with an increase in IL-10\textsuperscript{+} Foxp3\textsuperscript{+} CD4\textsuperscript{+} T cells, which were shown to be largely comprised of LAG-3\textsuperscript{+}CD49b\textsuperscript{+} type 1 regulatory cells. Moreover, the enhanced IL-10 production was established to be a major component of the protection afforded by PTP inhibition. Additionally, the decreased incidence of ECM was associated with significantly reduced sequestration of CD8\textsuperscript{+} T cells within the brain, and this attenuated accumulation correlated with decreased cell surface expression of CXCR3 on splenic CD8\textsuperscript{+} T cells. Overall, our study suggests that pharmacological modulation of host PTPs could provide a novel mechanism for the development of new immunotherapies to treat parasitic infections.
Prolonged Inflammatory Responses to *Staphylococcus aureus* in Aryl Hydrocarbon Receptor-Deficient Mice. Andreea Damian and Joaquín Madrenas. Microbiome and Disease Tolerance Centre, Department of Microbiology and Immunology, McGill University, Montréal, QC, Canada.

*Staphylococcus aureus* (*S. aureus*) is a Gram-positive bacterium that commonly colonizes healthy human beings. However, *S. aureus* is also a frequent causative agent of infections ranging from skin diseases such as dermatitis to sepsis and toxic shock syndrome. A protective immune response leading to the clearance of *S. aureus* includes initial production of IL-22 and IL-17A, leading to neutrophil and macrophage recruitment and the development of subsequent T\(_{\text{H}17}^1/T\_{\text{H}17}^2\) immune responses. As expected, lack of T\(_{\text{H}17}^1\) differentiation due to STAT3 deficiency leads to susceptibility to *S. aureus*. However, this phenotype is not due to lack of IL-17A as deficiency in this cytokine alone is not associated with increased susceptibility to *S. aureus* infections. This observation suggests that other cytokines in the context of T\(_{\text{H}17}^1\) responses are needed for protection against *S. aureus*. One cytokine that could play this role is IL-22. Several groups have investigated the dependency of AHR for IL-17A and IL-22 production from various cell populations. However, the role of AHR in mediating IL-22 production to *S. aureus* is unknown. We hypothesized that AHR is required for a protective IL-22 response against *S. aureus* infections. Using a skin infection of *S. aureus*, we observed that neutrophils are an important source of IL-22 during the first 2 days of the infection. However, IL-22 production in neutrophils is AHR-independent. Furthermore, using AHR knockout mice, we found that in the absence of AHR, inflammation and IL-22 production following neutrophil recruitment was prolonged. Lastly, T cell numbers were decreased in AHR absence. Our data suggest that AHR plays a critical role in the induction of disease tolerance to *S. aureus* infection although it is not involved in the early neutrophil-dependent response to *S. aureus*. The ability of AHR to promote a disease tolerant state implies that AHR may be a possible target for anti-inflammatory therapies against *S. aureus* infections.
Impairment of Nef function was described in elite controllers (EC), rare HIV-1 infected individuals who suppress plasma viremia to <50 RNA copies/mL without antiretroviral treatment. Understanding viral and host elements that result in the controller phenotype may direct the development of new therapeutic approaches and strategies aimed at attaining a functional cure. Studies have shown a correlation between antibody-dependent cell mediated cytotoxicity (ADCC) and disease progression. Importantly, the elimination of HIV-1 infected cells by ADCC mediated by HIV+ sera requires the presence of envelope glycoproteins (Env) in the CD4-bound conformation at the surface of infected cells. Interestingly, Nef clones isolated from EC are less efficient at downregulating CD4. This raises the possibility that accumulating CD4 could interact with Env at the cell surface and thereby sensitize HIV-1-infected cells to ADCC. Here we evaluated the ability of CD4-induced antibodies and sera from HIV-1-infected individuals to detect and mediate ADCC on primary CD4+ T cells infected with isogenic HIV-1 viruses that expressed nef clones isolated from chronic progressors or elite controllers using a FACS-based ADCC assay. Nef clones from EC were unable to fully downregulate CD4. We observed a significant increase in the exposure of HIV-1 Env epitopes targeted by ADCC-mediating antibodies at the surface of cells expressing Nef isolates from EC that correlated with their enhanced susceptibility to ADCC. Altogether, our results highlight the importance of Nef-mediated CD4 downregulation in preventing the elimination of HIV-1-infected cells by ADCC.
Characterizing the role of CFTR in intestinal infection
1Complex Traits Group, Department of Microbiology and Immunology, McGill University, Montreal, Que., Canada H3G 0B1.
2Cystic Fibrosis Translational Research Center, Department of Physiology, McGill University, Montreal, Que., Canada H3G 1Y6.
3Division of Gastroenterology, Department of Pediatrics, University of British Columbia, Vancouver, BC, Canada V5Z 4H4.
4Department of Pediatrics, Department of Genetics, Case Western Reserve University, Cleveland, OH, USA 44106.

Cystic fibrosis (CF) is a fatal disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) protein. Located in the apical membrane of various epithelial tissues and in immune cells, CFTR plays a critical role in ion transport and fluid secretion. Although often associated with a respiratory phenotype, CF affects multiple organ systems, including the gastrointestinal tract. Mouse models of CF display little lung pathology, but do possess an intestinal phenotype, characterized by obstruction, inflammation, and bacterial overgrowth.

We have shown that ∆F508 CF mice (Cfrtm1Eur) are highly susceptible to intestinal infection with Citrobacter rodentium, with atypical localization and greater colonization than WT mice. There is a greater tissue response to infection, with increased inflammatory cytokines and myeloid cells. Notably, there is also a significantly higher frequency and number of neutrophils in the small intestine of CF mice at steady state. Despite this increase, we have shown that CF neutrophils are defective in their production of reactive oxygen species and have decreased apoptosis compared to WT.

To determine the relative contributions of the CFTR-deficient myeloid and epithelial cell compartments to intestinal infection susceptibility, we generated mice with a myeloid-specific CFTR inactivation and found that they are completely resistant to infection. Furthermore, no differences were observed in myeloid cell frequencies, oxidative burst activity, nor cell death. Currently, we are generating mice with CFTR inactivation in the intestinal epithelium, to test the hypothesis that functional CFTR mediates its protection in an intestinal epithelial cell dependent manner.

C. rodentium infection provides a model to study intestinal disease observed in CF patients. Furthermore, the lack of an appropriate mouse model of CF pulmonary disease renders our research critical to the study of CF infections as a whole.
Recent horizontal transfer of a bacterial group II intron: Evolutionary relationship and functional adaptation between Ll.LtrB and Ef.PcfG

Félix LaRoche-Johnston¹, Caroline Monat¹ and Benoit Cousineau¹

¹Department of Microbiology and Immunology, Microbiome and Disease Tolerance Centre (MDTC), McGill University, Montréal, Québec, Canada H3A 2B4

Abstract

Group II introns are catalytically active RNA elements present in certain eukaryotic organelles, bacteria and archaea. These large ribozymes are capable of self-splicing from the precursor mRNA of interrupted genes and, following their excision, reinsert within target DNA sequences. Current evolutionary theories place group II introns at the origin of the non-LTR retrotransposons, the nuclear spliceosomal introns, and the small nuclear RNAs (snRNAs) present in the spliceosome. Together these genetic elements constitute over a third of our genomes, illustrating the large evolutionary footprint group II introns have left in eukaryotes. Here we characterized the functional and evolutionary relationship between the model group II intron from Lactococcus lactis, Ll.LtrB, and Ef.PcfG a newly discovered group II intron from a clinical strain of Enterococcus faecalis (SF24397). Ef.PcfG was found to be highly homologous to Ll.LtrB and to splice and mobilize in its native host environment as well as in L. lactis. Interestingly, Ef.PcfG was shown to splice at the same level as Ll.LtrB but to be significantly less efficient to invade the Ll.LtrB recognition site. We also demonstrate that specific point mutations between the ORFs of both introns correspond to genetic adaptations which developed in L. lactis as a response to strong selective pressure on mobility efficiency. Taken together, our data thus suggest that Ef.PcfG is ancestral to Ll.LtrB and was introduced, most likely by conjugation, into L. lactis through a single event of horizontal transfer. Repeated instances of additional horizontal transfer and independent intron mobility events in different strains and subspecies of L. lactis then led to the colonization of this bacterium by different Ll.LtrB variants. We thus demonstrate for the first time the functional adaptation of a group II intron following its acquisition by horizontal transfer providing strong experimental support of the theory that group II introns behave mostly as mobile elements in bacteria.
HIV-1 Envelope protein is as potent as glycoprotein G of vesicular stomatitis virus in overcoming the restriction of high levels of SERINC5

Saina Beitari, Qinghua Pan, Chen Liang

Serine incorporator 5 (SERINC5) was recently reported as a potent HIV-1 host restriction factor reducing HIV-1 infectivity; however, both Nef and Env proteins have been shown to counter SERINC5 restriction. Albeit that ectopically expressed SERINC5 by CMV promoter still diminishes the infectivity of the NL4-3 Nef positive HIV-1 by more than 50 fold. The question raised whether there are any HIV-1 strains which are resistant to SERINC5 or not. To find these potential resistant strains, we screened a number of different HIV-1 strains including primary isolates and transmitted founder viruses for their susceptibility to the inhibition by ectopically expressed SERINC5 and we found that two HIV-1 primary isolates named AD8.1 and YU-2 are resistant to SERINC5. After finding the resistant strains, we study the Nef protein from AD8.1 and YU2, we shown that Nef protein from these SERINC5 resistant strains is unable to overcome high levels of SERINC5. Viral envelope protein also been reported to overcome SERINC5 inhibition; therefore, inserting the Env sequence from AD8.1 into NL4-3 generated a chimeric virus that was as resistant to SERINC5 as AD8.1. Further mutagenesis studies revealed that the V3 loop alone from AD8.1 was able to transform the Nef-defective NL4-3 from a SERINC5-sensitive to a SERINC5-resistant virus. Similar levels of resistance to ectopically expressed SERINC5 were also observed when the glycoprotein G of vesicular stomatitis virus was used to pseudotype virus particles. In Conclusion, our studies indicate that HIV-1 Env similar to glycoprotein G of VSV appear to provide an overcoming mechanism to counter the antiviral activity of SERINC5.
The alarmin IL-33, a novel mechanism for Foxp3+ T\textsubscript{REG} cells to resist reprogramming in the course of infectious disease

Fernando Alvarez\textsuperscript{1,2,3,5}, Jorg Fritz\textsuperscript{1,3,4}, C. Piccirillo\textsuperscript{1,2,3,5}

\textsuperscript{1}McGill University, \textsuperscript{2}McGill University Health Center, \textsuperscript{3}Department of Microbiology and Immunology, \textsuperscript{4}Complex Traits Group, \textsuperscript{5}IDIGH

The adaptive immune response has developed complex immunological interactions in order to maintain homeostasis in steady-state conditions and mount inflammatory responses when required. Regulatory T cells (T\textsubscript{REG}) are at the heart of immune tolerance and their transcriptional program requires the fork-head box P3 (FoxP3) transcription factor. Surprisingly, recent infection models revealed that T\textsubscript{REG} cells could lose Foxp3 expression under polarizing conditions. Thus, the concept of plasticity, where a T\textsubscript{REG} converts either terminally or transiently into a pro-inflammatory T cell, has become a hotly debated subject. While searching for processes involved in T\textsubscript{REG} plasticity, we identified IL-33 as an important cytokine involved in the maintenance of stable Foxp3+ T\textsubscript{REG} cells. In our study, the membrane-bound form of the IL-33 receptor (ST2) was found on a subset of thymic-derived T\textsubscript{REG} cells (tT\textsubscript{REG}). ST2+ T\textsubscript{REG} responded to IL-33 by up-regulating Foxp3 and CD25 and by proliferating in vitro. In culture, IL-33 alone was able to induce the expression of its receptor on the surface of tT\textsubscript{REG} cells as a feed back mechanism, a process not seen on CD4+ effector T cells. In the course of a non-lethal influenza A H1N1 infection, ST2+ tT\textsubscript{REG} cells accumulated in the lung after clearance of the virus and resisted expression of a pro-inflammatory phenotype. Remarkably, in a model of chronic disease by the fungi Cryptosporidium neoformans, the ST2+ T\textsubscript{REG} cells that also increased early in the lung were gradually replaced by a subset of Foxp3+ ROR\gamma T+ ST2\textsuperscript{neg} T\textsubscript{REG}. This effect was shown to be IL1 dependent, as IL1R1\textsuperscript{-/-} mice maintained high numbers of ST2+ T\textsubscript{REG} in the lung. Moreover, these mice exhibited increased mortality and an inability to clear the pathogen. These results reveal the ability of T\textsubscript{REG} to resist reprogramming towards a pro-inflammatory phenotype in conditions that require adequate suppression of the adaptive immune system and highlight the importance of T\textsubscript{REG} plasticity in the face of chronic disease.
SHP-1-Regulated Hepatocyte Inflammatory Response Is Critical During Septic Shock

Anupam Adhikari¹, Caroline Martel¹, André Marette² and Martin Olivier¹

¹Departments of Medicine, Microbiology and Immunology (McGill University) and The Research Institute of the McGill University Health Centre, Montréal, QC, Canada.
²Centre de Recherche de l’Hôpital Laval Université Laval, Ste-Foy, QC, Canada.

Liver hepatocytes (Hep) are known to be central players during inflammatory response to systemic infection. Interestingly, protein tyrosine phosphatases (PTP), and in particular the SHP-1 (aka PTPN6), are recognized to be key negative regulator of inflammation, however their role in the control over Hep-mediated inflammatory response is still unravelled. To study the role of SHP-1 in the regulation of Hep-mediated inflammatory response during Septic shock, Cre-Lox mice having Hep-specific Ptpn6 deletion ($Ptpn6^{H-KO}$) were injected with LPS (5-30 µg) and observed over a 72 hrs time period. Interestingly, whereas WT mice died by 24hrs post-inoculation with the highest LPS dose, $Ptpn6^{H-KO}$ mice exhibited increased mortality (6-12 hrs) even with lower doses. This was paralleled with higher amount of metabolic markers (ALT, AST, BUN, Glucose) and pro-inflammatory mediators (LBP, SAA, NO) and cytokines (TNF-$\alpha$, IL-1$\beta$, IL-6) circulating in serum of $Ptpn6^{H-KO}$ mice. At molecular level, we observed that in $Ptpn6^{H-KO}$ mice MAP kinases activation were sustained upon LPS stimulation $in vivo$, this observation being corroborated $in vitro$ using primary Hep from $Ptpn6^{H-KO}$ and WT mice, as well as to show increase secretion of cytokines (TNF-$\alpha$, IL-1$\beta$, and IL-6) and NO by $Ptpn6^{H-KO}$ mice. Peritoneal macrophages from these mice –both having SHP-1- were showing similar pattern of MAP kinases phosphorylation and production of cytokines (TNF-$\alpha$, IL-1$\beta$, IL-6) as expected. Ultimately, using pharmacological approaches to block TNF-$\alpha$ and NO productions, we have been able to protect WT and $Ptpn6^{H-KO}$ mice (60% survival) against deadly LPS-mediated septic shock. Collectively, our study strongly establishes the critical role played by SHP-1 in the regulation of Hep-mediated inflammatory concurring to septic shock physiopathology. Also contributing to the identification of potential therapeutic targets such as signalling molecules critical in the regulation of inflammatory disorders.

*This research is supported by CIHR to MO.
Leishmanicidal activity and immunomodulatory effects of the Cramoll 1,4 Lectin
Andrezza Raposo Borges de Melo and Martin Olivier.
Research Institute of the McGill University Health Centre RI-MUHC, Infectious Diseases and Immunity in Global Health - IDIGH.

Cutaneous leishmaniasis is a neglected vector-borne tropical infection with 1.2 million new cases per year, making it a worldwide concern. Pentavalent antimonials are still the first choice drugs for leishmaniasis treatment that usually presents many adverse effects, toxicity and resistance. Therapeutic response in infectious disease involves host as well as infectious agent and the immune response defines the infection and efficacy of treatment. Therefore, the immunomodulation is considered a promising leishmaniasis therapeutic strategy. Cramoll 1,4 is a lectin extracted from seeds of *Cratylia mollis*, a natural plant from Brazil. Many assays have shown the cytokine expression activity and anti-inflammatory profile of immune cells by this lectin. Our study aimed 1) to investigate leishmanicidal activity of Cramoll against *L. amazonensis* and *L. mexicana*, and 2) to test its immunomodulatory activity on macrophages (B10R) through nitric oxide (NO), TNF-α and IL-12 production. Cramoll exhibited an inhibitory effect on promastigote growth in a dose- and time-dependent manner with IC$_{50}$ values of 11.2 and 10.4 μg/mL against *L. amazonensis* and *L. mexicana* respectively. B10R cells-treated with different concentrations of Cramoll showed higher TNF-α secretion in comparison with untreated cells. TNF-α was secreted early after 12.5 μg/mL of Cramoll treatment but the maximum secretion was significantly higher in O/N treatment (210 pg/mL). However, Cramoll did not induced IL-12 for all concentrations tested except with 12.5 μg/mL O/N (55 pg/mL). In relation to the production of NO between the treatments with different concentrations of Cramoll did not differ from untreated group and was only detected with 6 μg/mL in O/N treatment. LPS treatment was used as positive control. These results showed that Cramoll was toxic to promastigotes in culture and induced NO and TNFα secretion by macrophage. However, further investigation is needed to fully support and understand the action mechanism of Cramoll lectin as leishmanicidal.
Role of Glycosyl Hydrolases in Fungal Biofilms

Aspergillus fumigatus is a ubiquitous saprophytic mold. It propagates asexually by producing conidia, which are released into the environment and dispersed by air currents. Every day the average person inhales hundreds to thousands of these conidia, which are efficiently removed by the innate immune system. However, patients with immune deficiencies such as neutropenia or those recovering from an organ transplant, the conidia may evade immune responses and germinate to form filamentous hyphae that invade and injure lung tissues. This condition, invasive aspergillosis (IA) is associated with mortality as high as 50-80%, even despite the use of systemic antifungals. There is therefore an urgent need for novel therapeutics for IA.

During pulmonary infection Aspergillus hyphae grow within an extracellular matrix which protects the organism from killing by antifungals and host immune cells. A key component biofilm matrix is the exopolysaccharide galactosaminogalactan (GAG). GAG mediates adherence to host tissues and is required for virulence. GAG is produced by the action of the protein products of a 5-gene cluster. Found within this cluster are two genes (sph3 and ega3) predicted to encoding glycoside hydrolases. The function of these hydrolases in the synthesis of GAG is unclear, however, we have discovered that recombinant Ega3 and Sph3 hydrolyse purified and cell wall bound GAG. We therefore hypothesize that these enzymes will inhibit and disrupt A. fumigatus biofilms, and by extension function as therapeutic agents for IA, either alone or in combination with antifungal agents.

Low concentrations of recombinant Ega3 and Sph3 were found to inhibit and rapidly disrupt A. fumigatus biofilms. Further, treatment of A. fumigatus biofilms with either of these hydrolases enhanced the activity of the commonly used antifungals posaconazole, amphotericin B and caspofungin. Kinetic studies using fluorophore-tagged posaconazole suggested that the hydrolase-mediated increase in antifungal activity was likely a consequence of enhanced antifungal penetration of hyphae. Finally, treatment of A. fumigatus with either hydrolase abrogated the ability of this organism to adhere to and damage pulmonary epithelial cells in vitro.
Reactivation of an HIV-1 latency model by targeting the PKR signalling pathway

Latency is one of the major obstacles faced in the development of a curative HIV therapy. Latency is responsible for the persistence of HIV in treated individuals and can be established in different cell types. It has been shown that components of intrinsic immunity play a critical role in the establishment and maintenance of latency.

Protein Kinase R (PKR) is an interferon stimulated gene (ISG). It is a sensor of pathogen associated molecular patterns (PAMP) and is activated by double stranded RNA. Our lab showed that activated PKR inhibits the production of viral proteins from an HIV molecular clone (pNL4-3). We previously demonstrated that PKR is transiently activated during HIV infection but is rapidly deactivated at the peak of infection [1]. We hypothesised that the PKR signalling pathway is involved in the establishment of HIV latency and reactivation of viral reservoirs.

To address the reactivation pattern and the role of the PKR pathway during latency we used an HIV-1 latent model. This model was based on two cell lines; CD4+ T lymphocytes (CEM-T4) and Monocyte Derived Macrophages or MDM (THP-1). The model consists of a modified HIV-1 provirus, integrated within the host cell DNA [2]. Modifications include a GFP reporter fused to the Gag viral protein. GFP expression is monitored to determine levels of reactivation. To induce reactivation, cells were treated with Latency Reversing Agents (LRAs) targeting transcription through the Nuclear Factor κB and Protein Kinase C pathways. Levels of expression and activation of the PKR pathway were assessed by Western Blot.

Our results show that some LRAs modify the expression and activation of PKR in certain cell types. These results suggest that the PKR pathway could play a role in the establishment of latency in specific cellular reservoirs and novel LRAs targeting PKR could be identified.

Two-component systems and altered virulence of *Citrobacter rodentium*

Authors: Natalia Giannakopoulou, Jenny-Lee Thomassin, Lei Zhu, Hervé Le Moual and Samantha Gruenheid

*Citrobacter rodentium* is a murine pathogen used to model the intestinal infection caused by Enteropathogenic and Enterohaemorrhagic *Escherichia coli*, two diarrheal pathogens responsible for morbidity and mortality in developing and developed countries, respectively. During an infection, bacteria must be able to sense and adapt accordingly to the gut environment of the host. In order to adapt to changing environmental cues and modulate expression of specific genes, bacteria can use two-component signal transduction systems (TCS). Here we show that the deletion of several TCSs in *C. rodentium* leads to avirulent (ΔcpxRA), or moderately attenuated (ΔarcA, ΔrscB, ΔzraRS, ΔrstAB, ΔuhpAB) strains. Fecal CFU loads during infection of susceptible mice, as well as immunofluorescence staining of infected intestinal tissue sections and *in vitro* cell adherence assays revealed that the ΔcpxRA and ΔarcA strains are defective in colonization and cell adherence. *In vitro* secretion assays revealed a Type III secretion defect in the ΔarcA mutant. Finally, the ΔrscB mutant strain likely possesses an alteration in surface structures, as real-time qPCR revealed that expression of the wcaA gene, implicated in the generation of colanic acid, is drastically downregulated. The delineation of the mechanisms responsible for the altered virulence of the TCS knock-out strains of *C. rodentium* can open new avenues for potential drug targets against these often lethal pathogens.
Using Live Cell SHAPE analysis to Compare the Structure of Dengue Virus (DENV) RNA in Human and Mosquito cells

Maaran Michael Rajah, Selena M. Sagan

McGill University, Montreal, QC, Canada

Background:
Dengue virus (DENV) is a positive sense RNA virus that belongs to the flavivirus genus within the Flaviviridae family. The DENV genome is a dynamic structure that serves as a template for viral replication, translation, and packaging. In order to carry out all of its diverse functions, the viral RNA must unwind, elongate, and expose different regions of the genome to host and viral proteins. Traditional approaches to interrogating RNA structure have produced important information about the secondary structures in the DENV genome. However, no packaging signals have been identified for DENV and approximately 95% of the genome and the entire negative-strand replicative intermediate remain uncharacterized. It is also unclear how changes in the RNA secondary structure differs between mosquito and human cells. We hypothesize that the DENV genomic RNA forms multiple host-specific dynamic structures that are critical to the viral life cycle.

Purpose:
The purpose of this project is to identify local and long-range RNA-RNA interactions by examining the structure of DENV genomic RNA in live human and mosquito cells.

Method:
We will perform Selective 2’ Hydroxyl Acylation analyzed by Primer Extension (SHAPE) on DENV RNA in live cells using an in vivo SHAPE reagent. Flexible nucleotides are able to react with SHAPE reagents to form 2’-O-adducts that function as stops to reverse transcription initiated using end-labeled primers. The resulting lengths of the 5’-end labeled cDNA fragments can be visualized by gel electrophoresis and used to infer RNA structure.

Results:
We have established the DENV cell culture system in our lab and we have optimized SHAPE experiments in live cells using 5S ribosomal RNA. We are currently working on optimizing oligos for the characterization of the DENV genomic RNA. In vivo click reactive SHAPE (icSHAPE) will be used to couple SHAPE to next generation sequencing.
Delineating the role of myeloid cell populations upon early *Heligmosomoides polygyrus* infection

Helminth infection occurs in over 2 billion people worldwide and has posed a tremendous threat in developing countries for decades. Although a type 2 immune response is critical for protecting the host from helminth infection and repairing parasite-induced tissue damage, the innate immune response to these pathogens are not well characterised. We aim to fill this knowledge gap by assessing the initial immune response during the submucosal larval granuloma stage of the murine helminth *Heligmosomoides polygyrus* until its adult luminal stage. Contrary to our expectations, preliminary immunophenotyping data indicates an increase in Type 1 cytokines such as TNF-alpha and IFN-gamma in the murine duodenum upon early infection. Furthermore, we detect an induction of the neutrophil chemoattractant molecule CXCL1 (KC), as confirmed by qPCR. Our studies have confirmed an accumulation of neutrophils in the duodenum, peaking at day 2 post infection, where they surround the developing larvae within the submucosa. Our preliminary data also indicate a parallel increase in monocytes in the duodenum following infection. We hypothesize that early neutrophil influx to the small intestine plays a critical role in promoting monocyte recruitment that, in turn, minimizes tissue damage induced by invasive helminth infection. Studies are now underway in which neutrophils will be depleted during the course of infection and the impact on adult worm burden and intestinal pathology will be assessed. Characterizing the functional role of myeloid cell populations during the early stages of helminth infection can provide new avenues for therapeutic treatments that minimize tissue damage upon intestinal injury and disease.

*McGill University, Department of Microbiology and Immunology*
Characterizing the T Cell Response to Zika Virus Infection
Ryan D. Pardy¹, Stephanie A. Condotta¹, Maaran M. Rajah¹, Selena M. Sagan¹ and Martin J. Richer¹

Zika virus (ZIKV) is a mosquito-borne virus of the Flaviviridae family that was first isolated in 1947 in the Zika forest of Uganda. Although the first human infection was described as early as 1964, ZIKV received little attention until major outbreaks were reported in Yap Island, Federated States of Micronesia (2007), French Polynesia (2013) and the current ongoing pandemic across South America. In adults, ZIKV typically causes a relatively mild febrile disease, however it has been associated with increases in Guillain-Barré syndrome (GBS), a disorder characterized by ascending muscle weakness and loss of sensation that can lead to respiratory weakness in severe cases. In neonates, ZIKV has been linked to microcephaly from women who were infected with the virus during pregnancy. To date, very little is known about the immune response to ZIKV. Previous attempts to establish a mouse model have focused on observable pathogenesis using immunocompromised, newborn or gene knockout (e.g. interferon α/β receptor deficient) mice. Herein, we describe the establishment of a mouse model for ZIKV infection using wild-type adult immunocompetent C57BL/6 mice. Using this approach, we characterized the kinetics and magnitude of the overall CD4 and CD8 T cell responses using surrogate markers of antigen encounter, which allow for identification of antigen-experienced T cells without prior knowledge of specific viral epitopes. By tracking numbers and percentages of CD49d⁹⁹/CD11a⁺ CD4 T cells and CD11a⁹⁹ CD8 T cells we show that ZIKV infection induces a robust T cell response that peaks at day 7 post-infection. In addition, we have characterized the effector T cell profile induced following infection. Our data is the first survey of the T cell response to ZIKV and will have important translational implications for vaccine design allowing for the identification of key viral epitopes. Further, this data will provide insight into the disease complications associated with ZIKV infection.

Affiliations:
¹Department of Microbiology and Immunology, Microbiome and Disease Tolerance Centre, McGill University, Montreal, Canada
Title: Investigating the pathways determining intestinal homeostasis versus dysfunction during *Citrobacter rodentium* infection by genome-wide expression profiling

Authors: Eugene Kang, Samantha Gruenheid

Affiliations: Complex Traits Group and Department of Microbiology and Immunology, McGill University, Montreal, QC, Canada

Inbred mouse strains suffer one of two fates following intestinal *Citrobacter rodentium* infection: self-limiting colitis or fatal diarrheal disease. We previously reported that *Rspo2* is a major determinant of susceptibility to *C. rodentium* infection in mice; *Rspo2* is highly upregulated in susceptible mouse strains, leading to pathological activation of canonical Wnt signaling, loss of intestinal differentiation, and animal death. Conversely, mouse strains that do not induce *Rspo2* upregulation following infection undergo a milder, self-limiting disease with no mortality. However, the mechanism leading to the breakdown of intestinal homeostasis and the development of intestinal dysfunction is poorly understood. In the present study we apply a global approach by performing RNA sequencing (RNAseq) of normal and *C. rodentium*-infected colons of susceptible and resistant mice to determine the *Rspo2*-mediated dysfunction pathway signature and networks that result in the loss of intestinal differentiation and diarrhea.

Susceptible and resistant congenic mice were either left untreated or orally infected with *C. rodentium* and sacrificed at day 9 (peak) of infection. Colons were snap-frozen in liquid nitrogen and total RNA was extracted using TRIzol. Transcriptomes were subsequently determined using RNAseq, and bioinformatics and analytic tools were then applied to systematically extract biological meaning from our gene expression datasets. As a control, we assessed by qPCR and immunoblotting the modulation of some of the differentially regulated genes from previous analyses. We next performed gene set enrichment analysis to compare our datasets to published gene sets and used DAVID bioinformatics resources as well as gene ontology terms to associate our differentially expressed genes to biological processes. We anticipate that these studies will provide new and important insights into the mechanisms regulating intestinal dysfunction and the development of fatal diarrhea.
Reduced CD8⁺ T cell antigen sensitivity during the establishment of chronic viral infection is dependent on IL-10.

Logan K. Smith¹, Giselle Boukhaled², Connie M. Krawczyk¹,², and Martin J. Richer¹.

¹Department of Microbiology and Immunology, Microbiome and Disease Tolerance Centre, McGill University, Montreal, QC.

²Department of Physiology, Rosalind and Morris Goodman Cancer Centre, McGill University, Montreal, QC.

CD8⁺ T cells are specialized cells of the adaptive immune system whose primary function is to recognize and kill infected and cancerous cells. Central to their role as cytotoxic T lymphocytes is their ability to rapidly respond to low levels of antigen, termed antigen sensitivity, despite expressing a T cell receptor with relatively poor affinity for cognate antigen. It has been shown that antigen sensitivity is dynamically tuned by infection-induced inflammatory cytokines. The inflammatory milieu produced by acute viral infections increases CD8⁺ T cell antigen sensitivity, allowing the T cells to respond while the pathogen burden is still low and ultimately aiding in pathogen clearance. However, some viruses are able to establish chronic infections, and are characterized by distinct inflammatory profiles. We hypothesize that viruses that establish chronic infections are able to induce an inflammatory milieu that reduce CD8⁺ T cell antigen sensitivity, thereby allowing the virus to overwhelm the host immune response.

Herein, we demonstrate that viral infection with LCMV strain clone-13 (a virus that establishes a chronic infection in mice) reduces CD8⁺ T cell antigen sensitivity and that interleukin-10 (IL-10) plays a central role in this regulation. CD8⁺ T cell antigen sensitivity in LCMV clone-13 infected wild-type hosts were significantly lower compared to LCMV clone-13 infected IL-10 deficient hosts. Importantly, surface expression of PD-1 (a molecule associated with immune impairment during chronic infection) was not impacted in IL-10 deficient hosts, suggesting the effects of IL-10 on antigen sensitivity are independent of inhibitory receptor expression. Mechanistically, we demonstrate that IL-10 reduces CD8⁺ T cell antigen sensitivity by impairing T cell receptor signalling. These data help to elucidate how pathogens manipulate CD8⁺ T cell biology, and further our understanding of the first steps in the establishment of chronic infection.
RNA Pull-down Strategy to Investigate the Roles of MicroRNA-122-Associated Complexes in Hepatitis C Virus Infection

Annie Bernier, Selena M. Sagan
McGill University, Department of Microbiology and Immunology

Approximately 200 million individuals worldwide are infected by hepatitis C virus (HCV). MicroRNA-122 (miR-122) is a highly abundant liver-specific microRNA shown to interact at two "tandem" microRNA-binding sites in the 5’ end of the HCV genome. This unusual interaction promotes HCV RNA accumulation in both HCV-infected cells and the livers of infected patients. Mutation, truncation, or exchange of the 3’ terminal ribonucleotides of miR-122 for deoxynucleotides reduces HCV RNA accumulation. However, these nucleotides are not required for canonical miRNA activities.

We hypothesize that miR-122 forms a distinct complex with host and/or viral proteins that together mediate HCV RNA accumulation. Hence, we aim at identifying and characterizing host and viral factors associated with non-canonical miR-122 complexes in HCV-infected cells to understand miRNA-mediated viral RNA accumulation and identify novel antiviral targets.

Alkyne-tagged miR-122 molecules are transfected into HCV RNA-harboring Huh-7 cells. Following miR-122 biotinylation by a click reaction, miR-122 ribonucleoprotein complexes from naïve and HCV-infected cells are isolated by streptavidin affinity purification. MiR-122-associated proteins are then analyzed by SDS-PAGE and liquid chromatography tandem mass spectrometry. We demonstrate that alkyne-tagged miR-122 molecules are functional in mediating HCV RNA accumulation in Huh-7 cells. We show that the click reaction permits efficient labeling and affinity purification of miR-122 molecules in cell lysates. Western blot of affinity purified miR-122 complexes show enrichment in the RNA-induced silencing complex (RISC) protein Argonaute 2.

We expect that the results will provide insight into a novel microRNA ‘capping complex’ as well as a non-canonical ‘microRNA enhancing complex’. We anticipate that we will identify novel host-virus interactions important for viral replication that will provide new targets for therapeutic intervention.
Title
The Contribution of N-Glycolyl Muramyl Dipeptide to Cell-Mediated Immunity Elicited by Complete Freund’s Adjuvant

Authors
Jean-Yves R.J. Dubé¹², Damien J.C. Montamat-Sicotte²³, Marcel A. Behr¹²³⁴

Authors’ Affiliations
1, Department of Microbiology and Immunology, McGill University
2, Research Institute of the McGill University Health Centre
3, McGill International TB Centre
4, Department of Medicine, McGill University Health Centre

ABSTRACT
The capacity for the mycobacterial cell to act as an adjuvant has long been recognized, notably in the case of complete Freund’s adjuvant (CFA, killed Mycobacterium tuberculosis bacilli in mineral oil). However, much work remains to define the molecular entities unique to mycobacteria that are essential for the strong adjuvancy of CFA, particularly in their capacity for cell-mediated immunity (CMI) first demonstrated by Chase and Landsteiner in the 1940s. Work by Lederer and colleagues in the 1970s suggested that the peptidoglycan fragment N-acetyl muramyl dipeptide (MDP) was the minimal component for CFA adjuvancy. However, other studies have highlighted the rare ability of mycobacteria to catalyze N-glycolylation of their peptidoglycan muramic acid. N-glycolyl MDP was recently shown by our group to superiorly elicit pro-inflammatory cytokine production in macrophages, but how this occurs is still unknown. We hypothesize that N-glycolyl MDP more strongly elicits CMI than N-acetyl MDP, thereby contributing to the adjuvancy of CFA. Our in vitro data suggest N-glycolyl MDP may contribute strongly to CFA adjuvancy through dendritic-cell functions. Conversely, neutrophils did not discriminate between N-acetyl and N-glycolyl forms, highlighting a complexity in MDP recognition that is cell-type specific, and suggesting differential MDP recognition may involve more than just quantitatively different ligation to the supposed host receptor NOD2. We are also examining the adjuvancy of MDPs with a murine model of CMI, where we are ‘completing’ Freund’s incomplete adjuvant (mineral oil without mycobacteria) with the addition of MDPs and other mycobacterial pathogen-associated molecular patterns. In future experiments, we intend to explore mechanistic aspects of MDP recognition, at the level of cellular architecture important during natural mycobacterial infection (i.e. the phagosome), and at the molecular level regarding MDP structure and host molecules involved.
The role of RNA surveillance proteins on HIV-1 proviral reactivation.

Shringar Rao\textsuperscript{1,2}, Raquel Amorim\textsuperscript{1,3} and Andrew J. Mouland\textsuperscript{1,2,3}

\textsuperscript{1}HIV-1 RNA Trafficking Laboratory, Lady Davis Institute at the JGH; \textsuperscript{2}Departments of Microbiology and Immunology and \textsuperscript{3}Medicine, Division of Experimental Medicine, McGill University, Montreal, Quebec.

ABSTRACT:

Latent HIV-1 infected cells that constitute the viral reservoir, the main hurdle in curing HIV-1 infections, contain viral RNA that do not lead to the production of viral particles. This may be due to a post-transcriptional block to gene expression, either a result of defective splicing, mistrafficking of the viral RNA, inhibition of its nucleocytoplasmic export or hindered translation. In this study, we investigate the role of RNA surveillance proteins, especially UPF1, on viral RNA metabolism and the subsequent effects on virus production and reactivation of latent HIV-1 infected cells. We observed that in latent T cell derived models an overexpression of UPF1 did not increase the levels of viral RNA transcription but induced a reactivation in 23.8% of cells. This phenomenon was observed only when residual viral RNA was present in the latent cells, indicating that UPF1 was causing a reactivation at a post-transcriptional level. The knockdown of UPF1 also led to decreased viral RNA levels, indicating that UPF1 contributes to viral RNA stability. These results identify a role for the RNA surveillance proteins in HIV-1 proviral reactivation and highlight the significance of post transcriptional events in the maintenance of HIV-1 latency.
The Anti-HIV-1 Host Protein MxB Promotes Cell Apoptosis

Fan Huang\textsuperscript{1,2}; Chen Liang\textsuperscript{1,2}

\textsuperscript{1}Department of Microbiology and Immunology, McGill University
\textsuperscript{2}McGill University AIDS Centre, Lady Davis Institute, Jewish General Hospital

Abstract:

MxB is an interferon-inducible myxovirus resistance (Mx) protein that has recently been reported to restrict HIV-1 infection. Before the discovery of its anti-HIV-1 activity, MxB was believed to be non-antiviral but to serve normal cellular functions. However, the role of MxB in regulating cell apoptosis has not been reported.

In our study, overexpression of MxB resulted in a dramatically increased apoptotic cell number in U87-CD4\textsuperscript{+}/CXCR4\textsuperscript{+} cells. Annexin V staining revealed that MxB induces apoptosis in a dose-dependent manner. An additive effect on apoptosis was observed when these MxB-expressing U87 cells were exposed to treatment of sodium arsenate. Moreover, knockout of endogenous MxB expression reduced oxidative stress-induced apoptosis in U87MG cells upon IFN\textalpha induction. In addition, SupT1 cells expressing MxB was also shown to be more sensitive to stress-induced apoptosis as MxB accelerated the apoptosis inducement under the condition of sodium arsenate treatment. Deleting the N-terminal region, disrupting the GTPase activity, and blocking the higher-ordered oligomerization of MxB resulted in a reduced apoptosis promotion activity. Completely blocking the dimerization of MxB, however, significantly enhanced its activity in apoptosis promotion. These data help identify the functional regions of MxB that are involved in its apoptotic promotion activity. Taken together, results of our study suggest that MxB promotes cell apoptosis and enhances the sensitivity of different cell types to stress-induced apoptosis.

Future studies would aim to illustrate the mechanism of MxB-induced cell apoptosis. We will also investigate the possible link between the pro-apoptosis activity and the antiviral function of MxB.
Title: Early HA-immune cell interactions influence the innate immune response to plant-made virus-like particle vaccines for influenza

Authors: H. Hendin\textsuperscript{1,2}, A. Lara\textsuperscript{3}, S. Pillet\textsuperscript{2,5}, S. Chierzi\textsuperscript{2,4}, T. Talarico\textsuperscript{6}, N. Charland\textsuperscript{5}, M.A. D’Aoust\textsuperscript{5}, N. Landry\textsuperscript{5}, B.J. Ward\textsuperscript{2,5}

Affiliations: \textsuperscript{1}Department of Microbiology and Immunology, McGill University, Montreal, QC, Canada; \textsuperscript{2}Research Institute of the McGill University Health Centre, Montreal, QC, Canada; \textsuperscript{3}Department of Infectious Diseases, University of São Paulo School of Medicine, São Paulo, Brazil; \textsuperscript{4}Department of Neuroscience, McGill University, Montreal, QC, Canada; \textsuperscript{5}Medicago Inc., Quebec, QC, Canada; \textsuperscript{6}Medicago USA, Durham, NC, USA

Background: There is great interest in the role of innate immunity in shaping adaptive responses, particularly in the context of vaccination. Medicago’s plant-made virus-like particle (VLP) vaccines for influenza can be highly immunogenic without an adjuvant, however the underlying mechanism remains poorly defined. We evaluated early innate responses elicited by two VLPs bearing different HA proteins.

Methods: Peripheral blood mononuclear cells (PBMC) isolated from healthy adults were stimulated with 1-5 \textmu g/mL VLPs targeting H1N1 (A/Cali/2009) or H5N1 (A/Indonesia/2005). VLP-immune cell interactions were characterized by confocal microscopy 30min after stimulation with DiD-labeled VLP. Expression of CD69 and pro-inflammatory cytokines were used to assess innate activation 6h after stimulation and proliferative responses were evaluated by Ki67 expression at 48h.

Results: H1 and H5 VLPs elicited a robust innate immune response including the induction of IL-6, IL-8, IL-1\beta, and TNF\alpha in CD14+ monocytes. The most striking difference between responses was aggregation of PBMC within minutes of adding the H1 VLP. The H1 VLP preferentially associated with CD19+ B cells resulting in CD69 expression and proliferation of this cell subset. PBMC aggregation was less apparent after stimulation with the H5 VLP, which almost exclusively associated with monocytes. The robust B cell response elicited by the H1 VLP was abrogated in the absence of early VLP-B cell interactions.

Conclusions: Plant-made VLP vaccines bearing influenza H1 or H5 rapidly elicit immune activation and cytokine production in human PBMC. Aggregation of PBMCs was marked following exposure to the H1 VLP vaccine. Differences in the magnitude and kinetics of these responses suggest that features of the HA proteins such as receptor specificity influence both innate and adaptive responses. Further investigations will assess how HA binding, affinity and glycosylation are involved in plant-made VLP vaccine immunogenicity.
Title: Dissecting the role of the poly(rC)-binding protein 2 in the hepatitis C virus life cycle

Authors and Affiliations: Sophie Cousineau and Selena M. Sagan. Department of Microbiology and Immunology, McGill University.

Background: The hepatitis C virus (HCV) uses a number of cellular elements - including proteins and microRNAs - to promote its own replication and to protect itself from cellular molecular defenses against viruses. However, the exact molecular mechanisms behind many of these effects are still unknown. One particular cellular RNA-binding protein, the poly(rC)-binding protein 2 (PCBP2), is known to mediate the stability and expression of cellular transcripts, and is also known to be co-opted by picornaviruses to promote their replication. Six PCBP2 binding sites have been identified on the HCV genome, including in areas of the 5' and 3' untranslated regions with annotated roles in HCV translation and RNA replication. However, the exact mechanism by which PCBP2 plays affects HCV replication still remains to be elucidated.

Purpose: We aim to define the role of PCBP2 in the HCV life cycle, and to identify the PCBP2 KH domains necessary for this interaction.

Methods: We are using the HCV cell culture system to assess how viral protein synthesis, viral RNA accumulation, and the production of infectious viral particles is affected by knockdown of endogenous PCBP2 or the overexpression of a FLAG-tagged PCBP2 construct. To identify which PCBP2 domains are necessary - and which ones are dispensable - for this interaction, we will overexpress PCBP2 KH domain-deleted constructs.

Results: We show that siRNA-mediated PCBP2 knockdown inhibits HCV RNA accumulation and protein expression. We will show preliminary data of the effect of PCBP2-FLAG overexpression on viral RNA accumulation and protein expression.

Conclusions: PCBP2 is playing a role in the HCV life cycle, but it is currently unclear if this protein affects viral translation, replication, or both. Future experiments will aim to tease apart which step of the viral life cycle is affected by PCBP2, and our PCBP2 domain-deleted constructs will be informative as to which KH domains participate in PCBP2's effect on HCV.
Withdrawal of dolutegravir in early phases of HIV-1 infection in tissue culture does not abrogate antiretroviral activity

N. Osman¹,², T. Mesplede¹, M. Oliveira¹, S. Hassounah¹,³, K. Anstett¹,², M.A. Wainberg¹,²,³

¹ McGill AIDS Centre, Lady Davis Institute, Jewish General Hospital, Montreal, Canada, ² McGill University, Microbiology and Immunology, Faculty of Medicine, Montreal, Canada, ³ McGill University, Experimental Medicine, Faculty of Medicine, Montreal, Canada

Background: Dolutegravir (DTG) has shown greater efficacy than Raltegravir (RAL) in suppressing HIV-1 replication in treatment-experienced individuals. Biochemical experiments studying the dissociative half-lives of these Integrase Strand-Transfer Inhibitors (INSTIs) showed significant differences between them, and that common mutations involved in resistance against INSTIs were associated with lower dissociative half-lives. Accordingly, we are investigating whether drug removal from INSTI-treated HIV-1 infected cells results in different times to viral rebound depending on the INSTI, and whether the R263K mutation affects the time to viral rebound.

Methods: MT-2 cells treated with DTG, RAL or EVG were infected with HIV-1(WT) or HIV-1 IN(R263K), and drugs were removed after different times of treatment. Viral replication was monitored by measuring reverse transcriptase (RT) activity in culture fluids. Viral integration and 2-LTR circles production were measured by qPCR.

Results: We observed a slower increase in RT activity after removal of DTG compared to the two other INSTIs, resulting in an up to 5 days shift to reach the same level of RT activity. The incubation time before the drug was removed also had an impact on RT activity. Using HIV-1IN(R263K) did not have any impact on this long-acting DTG effect.

Conclusions: These results suggest that there is a more prolonged anti-HIV effect following washout of DTG than the two other INSTIs. This can be explained by a later resumption of viral DNA integration. The R263K mutation did not impact the effects when using DTG. These findings underline another benefit of using DTG as part of a first-line regimen and may support strategies that will aid in HIV elimination from latency.
CD109 regulates IL-17 producing γδ T cells and psoriatic-like skin inflammation

Giustino Carnevale¹, Émile Fortier¹, Marilena Gentile¹, Ghislaine Fontes¹, Irah King¹

¹Department of Microbiology & Immunology, McGill University, Montréal, QC

Interleukin-17 production by γδ T cells (T17 cells) plays an important role in cutaneous immunosurveillance and host defense, but can also lead to immune-driven inflammatory skin diseases such as psoriasis. Uncovering mechanisms that regulate γδ T17 cells is key to developing targeted therapies that alleviate the pathology associated with psoriasis and promote skin health. The GPI-anchored surface protein CD109 promotes wound healing and regulates skin inflammation via TGF-β and STAT-3-dependent signalling. However, the role of CD109 in cutaneous immunity has not been investigated. We hypothesized that CD109 acts as a negative regulator of skin γδ T17 cells and psoriasiform-like inflammation. Examination of mice genetically deficient in CD109 (CD109−/−) revealed spontaneous epidermal hyperplasia, transcriptional activation of the IL-23/IL-17 immune axis and enhanced T cell infiltration into both epidermal and dermal compartments compared to wild-type mice. In addition, CD109−/− mice harboured an increased number of γδ T17 cells in the dermis and cutaneous lymph nodes under steady state conditions. Upon induction of psoriatic-like inflammation following topical imiquimod treatment, CD109−/− mice exhibited exacerbated epidermal thickening and increased accumulation of γδ T17 cells compared to wildtype controls. Importantly, bone marrow chimera studies determined that expression of CD109 by a radioresistant cell type was critical for controlling γδ T17 cell responses and skin inflammation. Collectively, these results identify a previously unknown cell-extrinsic pathway regulating IL-17 producing γδ T cells in the skin. Studies are now underway to determine the cellular and molecular mechanisms by which CD109 controls skin immunity and tissue homeostasis.
Identification of cellular and immunological mechanisms triggered by Leishmania-derived exosomes in Cutaneous Leishmaniasis

Alonso Lira Filho, Vanessa Atayde, Salman Qureshi· Maziar Divangahi, Martin Olivier.

1 - Research Institute of the McGill University Health Centre – RI-MUHC.

*Leishmania* spp. is a protozoan parasite transmitted by the bite of infected sand flies leading to a wide-range of diseases called leishmaniasis considered by the World Health Organization a Neglected Tropical Disease. In some cases, it can produce a self-healing wound to a potentially lethal internal organ infection. In the last few years, we have reported that *Leishmania* exosomes (*Leish Exo*) were found to be involved in cell-cell communication between the parasite and its host macrophages. Being secreted during *Leishmania* growth in culture or in response to temperature shock, *Leish Exo* were found to influence macrophage microbicidal (NO) and inflammation related functions by manipulating host cell signalling. Recently, we published a seminal work demonstrating that *Leish Exo* were released in the midgut lumen of its sand fly vector and to be co-inoculated with *Leishmania* during blood meal. *Leish Exo* were found to stimulate a Th17 inflammatory response conducting to exacerbated cutaneous leishmaniasis skin pathology.

At the view of these ground-breaking findings, the aims of this study is to identify the cellular and immunological mechanisms underlying this capacity of *Leish Exo* to trigger the IL-17a inflammatory cytokines and related signalling. First, we have been interested to identify immune sensors that could be involved. Using TLRendo3/7/9 and TLR3 ko mice, we have been able to test whether single/double stranded RNA or DNA being enriched in *Leish Exo* could be the triggering agents. Mice being injected with *L. major* +/- *Leish Exo* in their right-hind footpads have been followed for at least 15 weeks and the footpads swelling measured on a weekly basis. Unfortunately, no phenotype was observed, both WT and ko mice showing increased swelling in response to *Leish Exo* additional signal. At the actual time we are pursuing our screening and further observations will be presented at the 2016 MIMM Research Day.
The Role of Mxi1 in the development of CD4+Foxp3+ regulatory T cells

Gene expression analyses conventionally examine differential mRNA abundance among cell subsets. The poly-ribosome-associated microarray measures the relative abundance of mRNAs being actively translated into protein, giving a more true indication of complete gene expression. We used this technique to study preferentially translated mRNAs in TCR-activated T regulatory (Treg) compared to T effector (Teff) cells. Mxi1, an anti-proliferative transcription factor involved in tumor protection, was found to be translated at higher levels in activated Treg compared to Teff cells. With this, I hypothesize that Mxi1 plays a vital role in the development and function of Treg cells. Overexpression of the Mxi1-SRβ isoform suppressed the proliferation of Teff cells following *in-vitro* TCR-stimulation. Moreover, Teff cells overexpressing Mxi1-SRβ produced less IFN-γ, TNF-α and IL-2 both *in-vitro* following adoptive transfer. These cells also had enhanced Foxp3-induction and commitment to the Treg lineage both *in-vitro* and *in-vivo*. Finally, Mxi1-SRβ reduced the pathogenic potential of diabetogenic Teff cells by reducing their proliferative capacity and promoting Foxp3 induction. These results indicate that Mxi1 is likely an important contributor to Foxp3+ Treg lineage commitment and Treg cell development. Regulating Mxi1 expression in autoreactive Teff cells could be used as a therapeutic to treat inflammatory autoimmune diseases.