Go to page 5

Eight Annual Quebec Molecular Parasitology Symposium

12th and 13th June, 2008

Complexe des sciences, UQAM

Montreal, Quebec

Program

Thursday, June 12 (Room: SH-3420)

9:00-9:10 Welcome (Dr. Armando Jardim, Director of the CHPI)

9:10-10:00 Keynote speaker: Dr. Richard Bucala (Department of Internal Medicine, Yale University): "Role of Macrophage Migration Inhibitory Factor in malaria pathogenesis".

10:00-10:15 Coffee break (Hall SK01)

Session 1 (SH-3420): Signal Transduction (Chair: Dr. Albert Descoteaux)

10:15-10:35

1. Maria Adelaida Gomez, Irazu Contreras, Laleh Alisaraie, Maxime Halle, Michel L. Tremblay, Albert M. Berghuis, Robert W. McMaster and Martin Olivier: *Leishmania* GP63 is involved in the modulation of macrophage protein tyrosine phosphatases.

10:35-10:55

2. Issa Abu Dayyeh, Benoit Cousineau and Martin Olivier: *Leishmania*-induced IRAK-1 inactivation is mediated by SHP-1 interacting with an evolutionarily conserved KTIM motif.

10:55-11:15

3. Irazú Contreras, Maria Adelaida Gomez, Oliver Nguyen and Martin Olivier: Macrophage AP-1 transcription factor is inactivated by the *Leishmania* surface protein Gp63.

11:15-11:35

4. Adrien F. Vinet, Mitsunori Fukuda and Albert Descoteaux: The exocytosis regulator Synaptotagmin V controls phagocytosis in macrophages.

11:35-11:55

5. Christine Matte, Mélanie Giroux and Albert Descoteaux: Inhibition of the Interferon-γ JAK-STAT1 pathway by *Leishmania donovani* amastigotes.

11:55-12:15

6. Serge Cloutier, Conan Chow, Carole Dumas and Barbara Papadopoulou: Role of eIF2-alpha phosphorylation in the intracellular development of *Leishmania*.

12:15-13:30 Lunch (Hall SH-4200)

Session 2 (SH-3420): Parasite Biology/Biochemistry (Chair: Dr. Martin Olivier)

13:30-13:50

1. Aurélien Dupé, Simon Haile and Barbara Papadopoulou: Stage-regulated mRNA decay in the protozoan parasite *Leishmania*.

13:50-14:10

2. Angana Mukherjee and Marc Ouellette: Gene disruptions indicate an essential function for γ-glutamylcysteine synthetase (γ-GCS) in *Leishmania*.

14:10-14:30

3. Wilfried Moreira, Éric Leblanc and Marc Ouellette: The role of PTR1 and reduced pterins in resistance to oxidative stresses in *Leishmania*.

14:30-14:50

4. Normand Cyr, Kleber P. Madrid, Mark Aurousseau and Armando Jardim: Structural insights into the conformational changes occurring to the *Leishmania donovani* peroxin 14 upon binding to peroxin 5.

14-50-15:10

5. Ana V. Pilar, Rona Strasser and Armando Jardim: Quantitative assessment of the interactions between the PTS2 receptor LmPEX7 and other components of the glycosomal protein import pathway in *Leishmania* sp.

15:10-15:30

6. Janet Yee, Anita Tang, Heather Ritter, Wei-Ling La, Dewald Delport, Melissa Page, Rodney Adam, Miklós Müller and Gang Wu: The core histone genes of *Giardia lamblia*.

15:30-15:50 Coffee Break (Hall SK01)

Students Symposium (15:50-17:50, SH-3420)

Taking the road less traveled: *Alternative career choices for parasitology graduate students*.

Chair: Normand Cyr

15:50-16:20

1. Leslie Payne. Translating health research into action in developing countries: A career with the Canadian International Development Agency.

16:20-16:50

2. Christian Cawthorn. Intellectual property: the new frontier.

16:50-17:20

3. Mathieu Perreault. From science to news.

17:20-17:50

4. Tim Geary. Careers in industry: discovery through development.

17:50-19:00 Poster session and aperitifs (SH-4200)

19:00-21:00 Dinner and poster session (SH-4200)

Friday June 13 (Room: SH-3420)

9:00-9:50 Keynote speaker: Dr. Ricardo Gazzinelli (Centro de Pesquisas René Rachou, Belo Horizonte, Brazil): "Pro-Inflammatory Priming in *Plasmodium falciparum* Malaria Patients".

9:50-10:10 Coffee break (Hall SK01)

Session 3 (SH-3420): Drug Resistance and Targets (Chair: Dr. Paula Ribeiro)

10:10-10:30

1. Claudia Wever and Joseph Dent: Validating acetylcholine-gated chloride channels as novel nematocide targets.

10:30-10:50

2. Aïssatou Diawara, Carli Halpenny, Marilyn Scott and Roger Prichard: Assays to detect benzimidazole-resistance SNPs in soil transmitted helminths.

10:50-11:10

3. Daniel Feingold and Joseph A. Dent: Characterization of three novel Cys loop ligand-gated ion channel subunits in *Drosophila melanogaster*: Potential pesticide targets?

11:10-11:30

4. Vijayaraghava Rao, Roger Prichard and Sean Forrester: Cloning and characterization of a novel ligand-gated chloride channel in *Haemonchus contortus*.

11:30-11:50

5. Fouad El-Shehabi and Paula Ribeiro: Deorphanization of two of novel *Schistosoma mansoni* GPCRs, using a yeast expression system.

11:50-12:10

6. Sonia Edaye, Melissa Richard-Lalonde and Elias Georges: *Plasmodium falciparum* drug resistance: Implication of a putative ABC transporter.

12:10-13:50 Lunch (SH-4200).

Session 4 (SH-3420): Infection and Immunity (Chair: Dr. Florence Dzierszinski)

13:50-14:10

1. Nathalie Trudel, Ravendra Garg, Nadine Messier, Shyam Sundar, Marc Ouellette and Michel J. Tremblay: HIV-1 protease inhibitors interfere with *Leishmania* infection in THP-1 and primary human macrophages.

14:10-14:30

2. Juliette Diou, Mélanie R. Tardif and Michel J. Tremblay: Malaria pigment inhibits the early events of HIV-1 life cycle in monocyte-derived macrophages.

14:30-14:50

3. Maurice Odiere, Kris Koski and Marilyn Scott: Morphological effects, parasitological outcomes and trade-offs of concurrent *Heligmosomoides bakeri* (Nematoda) infection and pregnancy in CD-1 mice.

14:50-15:10

4. Robert Lodge and Michel J. Tremblay: *Leishmania* and HIV-1 coinfections in human macrophages.

15:10-15:30 Coffee break (Hall SK01)

Session 5 (SH-3420): Genomics and Proteomics (Chair: Dr. Reza Salavati)

15:30-15:50

1. Yovany Moreno and Timothy Geary: Proteomic Analysis of the Secretome of *Brugia malayi*.

15:50-16:10

2. Hamed Shateri Najafabadi and Reza Salavati: Homology-independent annotation of *Trypanosoma brucei* genome.

16:10-16:30

3. Houtan Moshiri and Reza Salavati: Fluorescence-based reporter substrate for monitoring RNA editing in Trypanosomatid pathogens.

16:30 Closing.

Posters

Infection and Immunity

1. Stéfany Bazinet, Martin Olivier and Tatiana Scorza: Hemozoin down-regulates IL-12p75 production through a mechanism independent of the P38 MAPK-IL-10 pathway.
2. Benoit Belanger, Mathieu Cambos, Stéfany Bazinet, Lin Leng, Richard Bucala and Tatiana Scorza: Neutralization of macrophage migration inhibitory factor (MIF) results in contrasting effects on pro-inflammatory responses during *Plasmodium c. adami* malaria.
3. Mathieu Cambos, Martin Olivier, Armando Jardim and Tatiana Scorza: The phagocytosis of parasitised red blood cells induces the apoptosis of macrophages during *Plasmodium chabaudi adami* infections.
4. Ravendra Garg, Corinne Barat and Michel J. Tremblay: *Leishmania infantum* amastigotes enhances HIV-1 replication in human dendritic cells and transfer to CD4+ T cells by inducing secretion of cytokines TNF-α and IL-6.
5. Stephanie Goyette, Cynthia Santamaria, Momar Ndao, Gary E. Ward, Terry W. Spithill, Florence S. Dzierszinski and Brian J. Ward: Development of a subunit vaccine against *T. gondii* based on the ectodomain of TgAMA-1.
6. Diane Malaison, Corinne Barat, Marc Bergeron and Michel J. Tremblay: Co-infection of human monocyte-derived microglia-like cells (MDMis) by *Trypanosoma cruzi* and HIV-1.
7. Pranav Kumar, Nathalie Trudel, Robert Lodge and Michel J. Tremblay: Nelfinavir induces DNA fragmentation in *Leishmania donovani*, a hallmark of programmed cell death and apoptosis.

8. Marina T. Shio, Myriam Savaria and Martin Olivier: Hemozoin-induced IL-1β production by human monocytes is dependent on SYK, SRC kinases and inflammasome.
9. Marie-Claire Rioux, Momar Ndao, Brian Ward and Terry Spithill: Identification of markers of response to infection and vaccination to *Fasciola hepatica* in cattle.
10. Anne E. Schwab, Timothy E. Geary, Paul Baillargeon and Gilles Fecteau: Association of BoLA class II alleles with susceptibly to *N. caninum* in Quebec cattle.

Biochemistry/Biology

1. Hiren Banerjee, Michael Nyisztor and Armando Jardim: Interaction of the *Trypanosoma* peroxin 14 with glycosomal membrane.
2. Marie-Josée Bellemare, Colin Nadeau Brosseau, Mara L. Leimanis, Mariane Goldboult, Richard Leonelli, Elias George, Martin Olivier and D. Scott Bohle: Fluorescent phenomenon: Discovering the bright side of malaria pigment.
3. Celia Caballero-Franco, Line Dufresne and Armando Jardim: Biochemical characterization of EspD protein from the enteropathogenic *Escherichia coli*.
4. Christelle Gabriel, Nisha Dogra, Robert W. McMaster and Albert Descoteaux: Impact of *Leishmania donovani* phosphoglycans on the macrophage transcriptome.
5. Joella Joseph and Janet Yee: Functional characterization of a recombinant TATA binding protein (TBP) and a TBP interacting protein in *Giardia lamblia*.
6. Yuan Mao, Hamed Shateri Najafabadi and Reza Salavati: Prediction and characterization of regulatory RNAs in *Trypanosoma brucei*.
7. Nicholas Patocka and Paula Ribeiro: Serotonin signaling in *Schistosoma mansoni*, identifying the players involved.
8. Antonio Ruzzini and Janet Yee: Studies of relative gene expression during the cell cycle of *Giardia lamblia*.
9. Jonathan Solomon, Joe Nabhan and Timothy Geary: The localization and in vitro detection of *Brugia malayi* secreted proteins.
10. Rona Strasser and Armando Jardim: Analysis of the *Leishmania donovani* glycosomal membrane proteome.
11. Amira Taman and Paula Ribeiro: Identification of a novel dopamine receptor in *Schistosoma mansoni*.

Abstracts

Session 1: Signal Transduction

1. Maria Adelaida Gomez[1], Irazu Contreras[1], Laleh Alisaraie[2], Maxime Halle[2], Michel L. Tremblay[2], Albert M. Berghuis[2], Robert W. McMaster[3] and Martin Olivier[1]. [1]Departments of Microbiology and Immunology, [2]Biochemistry, McGill University, Montreal; [3]Department of Medical Genetics, UBC, Vancouver.

***Leishmania* GP63 is Involved in the Modulation of Macrophage Protein Tyrosine Phosphatases.**

Rapidly following *Leishmania*-macrophage initial interaction, activation of the macrophage protein tyrosine phosphatase (PTP) SHP-1 has been noted as a landmark of the negative regulation exerted by this parasite to successfully establish itself as an intracellular pathogen. Herein we sought to determine whether other host PTPs were involved in this event. PTP profiling of *Leishmania* infected macrophages by in-gel PTP activity assay, showed the appearance of low molecular weight (LMW) PTP bands, further characterized as active PTP cleavage products of SHP-1, PTP1B and TCPTP. PTP cleavage is independent of parasite internalization, but dependent on the expression of *Leishmania* GP63. Interestingly, we found that GP63 can access the host cell and directly cleave target PTPs. *In-silico* modeling experiments show a direct interaction between the host PTP1B and GP63, further confirming the biochemical observations here presented. Collectively, our data show that in addition to SHP-1, PTP1B and TCPTP are modulated upon *Leishmania* infection, contributing to understand the mechanisms that this clever parasite utilizes for negatively regulating signaling pathways and downstream macrophage functions, promoting its successful intracellular survival.

2. Issa Abu Dayyeh, Benoit Cousineau and Martin Olivier. Department of Microbiology and Immunology, Quebec, Canada. ***Leishmania*-induced IRAK-1 inactivation is mediated by SHP-1 interacting with an evolutionarily conserved KTIM motif.**

It is well established that *Leishmania* infection rapidly activates the protein tyrosine phosphatase SHP-1 causing an inhibition of several macrophage functions mostly driven by LPS or IFN stimulation. In addition, work from our laboratory suggested that *Leishmania* can induce the expression of specific chemokines in a TLR4-dependent but MyD88-independent manner. In this study, we were interested to see if the *Leishmania*-induced SHP-1 was able to interfere with MyD88-dependent signaling in infected macrophages by inhibiting the activity of a pivotal kinase in this pathway: IRAK-1. Results showed that *Leishmania* was able to rapidly abrogate IRAK-1 kinase activity. This IRAK-1 inactivation was associated with increased binding of SHP-1 to IRAK-1 and reflected by unresponsiveness to Toll ligand stimulation. Sequence analysis of the IRAK-1 sequence and site-directed mutagenesis revealed that the binding site of SHP-1 to IRAK-1 is an evolutionarily conserved ITIM-like motif located in the kinase domain of IRAK-1 which we proposed to name KTIM. Taken together, this study reports the first demonstration that a pathogen can directly interfere with the MyD88-dependent pathway through shutting down IRAK-1 kinase activity. This allows the parasite to cause selective inflammation that is not detrimental to its survival within the harsh environment of macrophages. Identification of such new evading mechanisms may permit the development of new strategies to control pathogens. Acknowledgment: Lab grant by CIHR, FRSQ for my Ph.D. studentship.

3. Irazú Contreras, Maria Adelaida Gomez, Oliver Nguyen and Martin Olivier. Department of Microbiology and Immunology, McGill University. **Macrophage AP-1transcription factor is inactivated by the *Leishmania* surface protein Gp63.**

Leishmania parasites have evolved sophisticated mechanisms to subvert the macrophage immune response by inactivating host cell signalling, modifying the JAK/STAT pathway and other transcription factors including AP-1, CREB and NF-κB. AP-1 is an important transcription factor regulating pro-inflammatory cytokines, chemokines and nitric oxide production. AP-1 activity within host cells is abolished upon infection with various *Leishmania* species; furthermore 5 of the 7 AP-1 subunits are degraded inside the nucleus after *Leishmania* infection. Of interest c-Jun, the main activator of AP-1, is degraded and cleaved. Furthermore, degradation and cleavage of c-Jun appear to be gp63 (major surface protease of *Leishmania*) -dependent processes. Inhibition of phagocytosis with Cytochalasin D did not recover c-Jun, suggesting that internalization of the parasite might not be necessary to deliver gp63 molecules inside the cell. We corroborated this result by exposing cells to the supernatant of *L. mexicana* (rich in free gp63 molecules) and degradation of c-Jun was still occurring even in the absence of the parasite, suggesting that gp63 could be using endocytosis to enter the cell. In addition, disruption of the lipid raft by Methyl β-Cyclodextrin showed that there was no degradation of c-Jun, although the cleavage fragment was still detected. Furthermore, separating nuclear and cytoplasmic proteins we found that gp63 is present in both cellular compartments. Immunoprecipitation of c-Jun from nuclear extracts showed that gp63 interacts, degrades and cleaves c-Jun inside the nucleus very soon after infection. Together, these results indicate a novel mechanism whereby *Leishmania* uses its surface proteases to subvert the macrophage‘s AP-1 signalling pathway.

4. Adrien F. Vinet[1], Mitsunori Fukuda[2] and Albert Descoteaux[1]. [1]INRS-Institut Armand Frappier and Centre for host-parasite interactions, Laval, QC, Canada, [2] Tohoku University, Sendai, Japan. **The exocytosis regulator Synaptotagmin V controls phagocytosis in macrophages.**

Phagocytosis is initiated through binding of a particle by receptors that trigger actin polymerization at the site of contact. Previous studies showed a focalized exocytosis of membrane from internal vesicles at the phagocytic cup. Components of SNAREs are essential for this process. Synaptotagmins (Syt) are a large family of membrane proteins that contains two Ca^{2+}-C_2 domains which can bind phospholipids as well as SNARE components. Although they were identified and widely studied in neuronal cells for their role in the regulation of neurotransmitters exocytosis, few studies have demonstrated the expression of synaptotagmin isoforms in macrophages. Several signaling molecules, including members of the protein kinase C (PKC) superfamily participate in the regulation of actin polymerisation and phagolysosome biogenesis. Using a proteomic approach, we identified Syt V as a new potential partner to PKC-α in regulating phagocytosis. We showed that Syt V is expressed in macrophages and that a large part is localized on recycling endosomes. Moreover, we observed the recruitment of Syt V to phagosomes containing various particles of wich *Leishmania donovani* indicating that Syt V is recruited independently of the phagocytic receptors involved. We also demonstrated an early recruitment of Syt V in macrophages and an accumulation throughout the maturation process notably for both promastigote and amastigote forms of *Leishmania donovani*. Silencing of Syt V by RNAi revealed a key role for this protein in the regulation of phagocytosis. Collectively, these results showed for the first time the importance of Syt V in the regulation of an important innate function of macrophages and suggest that Syt V acts as a positive modulator of exocytosis with a key role in the regulation of focal exocytosis during phagocytosis.

5. Christine Matte, Mélanie Giroux and Albert Descoteaux. INRS-Institut Armand-Frappier, Université du Québec.

Inhibition of the Interferon-γ JAK-STAT1 pathway by *Leishmania donovani* amastigotes.

Interferon (IFN)-γ is a highly potent macrophage activator that plays a crucial role in the control and resolution of microbial infections, stimulating key intracellular responses such as reactive oxygen and nitrogen species production and Major Histocompatibility Complex Class II (MHC II) molecule expression. IFN-γ-induced signalling events are predominantly centered on the Janus Kinase (JAK)–Signal Transducer and Activator of Transcription 1 (STAT1) pathway, which consequently represents an ideal target for intracellular pathogens. *Leishmania* parasites are renowned for their capacity to prevent the activation of antimicrobial responses in macrophages. Recent evidence from our laboratory demonstrates that the amastigote stage of *L. donovani* prevents IFN-γ-induced Class II Transactivator and MHC II expression. However, the underlying mechanism of this inhibition remains elusive. Here, we infected bone marrow-derived macrophages with *L. donovani* amastigotes prior to IFN-γ stimulation to observe the impact of amastigotes on activation of the JAK-STAT1 pathway. Western Blot analyses showed that *L. donovani* amastigotes inhibit IFN-γ-induced IRF1 expression, an important transcription factor of this pathway, without affecting upstream phosphorylation of JAK2 or STAT1. Western Blot analysis, EMSA and confocal immunofluorescence microscopy indicated that IFN-γ-induced STAT1 nuclear translocation is partially inhibited in *L. donovani* amastigote-infected cells. Experiments aiming to determine the mechanism by which *L. donovani* amastigotes inhibit STAT1 nuclear translocation are currently under way. These studies will provide a more thorough understanding of the mechanisms by which *L. donovani* interferes with IFN-γ-induced macrophage activation and responses. This project is funded by CIHR.

6. Serge Cloutier, Conan Chow, Carole Dumas and Barbara Papadopoulou. Infectious Disease Research Centre, CHUL Research Centre, Faculty of Medicine, Laval University, Quebec, Canada.

Role of eIF2-alpha phosphorylation in the intracellular development of *Leishmania*.

During the infection of the mammalian host, the protozoan parasite *Leishmania* encounters drastic environmental changes, including a high temperature shift and acidic pH. These conditions trigger rapid morphological and biochemical changes that allow the parasite to differentiate, adapt and survive within the phagolysosome of mammalian macrophages. In other eukaryotes, the stress response predominantly involves the phosphorylation of the translation initiation factor eIF2alpha by four distinct kinases leading to the inhibition of protein synthesis and growth suppression. In *Leishmania*, eIF2alpha is constitutively expressed in both life stages of the parasite. However, when the parasites are grown as axenic amastigotes under conditions mimicking macrophage growth (e.g. elevated temperature and acidic pH), eIF2alpha becomes specifically phosphorylated at Threonine 166. EIF2alpha phosphorylation coincides with downregulation of global mRNA translation as shown by polysome profiling and ^{35}S Met-incorporation studies. We have developed a *Leishmania* mutant strain that is deficient in the expression and possibly phosphorylation of the eIF2alpha PERK kinase and showed that this mutant has a defect in eIF2alpha phosphorylation upon stress conditions. Interestingly, this mutant presents a delay in differentiation into its amastigote form inside macrophages, especially at early stages following entry of the parasite into macrophages. These data suggest that there is a link between eIF2alpha phosphorylation and adaptation of the parasite to the harsh environment of the macrophages. Proteomic and transcriptomic analyses are now underway to better characterize this mutant. This work was supported by CIHR funding (MOP-12182) to BP.

Session 2: Parasite Biology/Biochemistry

1. Aurélien Dupé, Simon Haile and Barbara Papadopoulou. Infectious Disease Research Centre, CHUL Research Centre, Faculty of Medicine, Laval University, Quebec, QC. Canada.

Stage-regulated mRNA decay in the protozoan parasite *Leishmania*.

Parasites of the genus *Leishmania* are endemic in several tropical and sup-tropical regions around the world and cause leishmaniasis. Their life cycle alternates between developmental forms residing within the insect vector (promastigotes) and the mammalian host (amastigotes). In *Leishmania*, nearly all control of gene expression is post-transcriptional and involves sequences in the 3'-untranslated regions (3'UTRs) of mRNAs. Very little is known as to how these *cis*-elements regulate RNA turnover and translation rates in trypanosomatids and nothing is known about mRNA degradation mechanisms in *Leishmania* in particular. Using the amastin mRNA-an amastigote-specific transcript-as a model we showed that a ~100 nt U-rich element within its 3'UTR significantly accounts for developmental regulation. RNase-H-RNA blot analysis revealed that a major part of the rapid promastigote-specific degradation of the amastin mRNA is not initiated by deadenylation. This is in contrast to the amastin mRNA in amastigotes and to reporter RNAs lacking the URE, which, in common with most eukaryotic mRNAs studied to-date, are deadenylated before being degraded. Moreover, our analysis did not reveal a role for decapping in the stage-specific degradation of the amastin mRNA. Overall, these results suggest that degradation of the amastin mRNA is likely to be bi-phasic, the first phase being stage-specific and dependent on an unusual URE-mediated pathway of mRNA degradation. Using UV crosslinking assays, we detected a ~25 kDa protein that binds specifically to the URE. RNA affinity tagged-RNA and RNA affinity chromatography will be used to identify interacting proteins. The identification of *trans*-acting factors participating in mRNA decay in *Leishmania* would shed light into our understanding of how stage-specific transcripts are regulated in these parasites.

2. Angana Mukherjee and Marc Ouellette. Research Center in Infectious Diseases, Laval University.

Gene disruptions indicate an essential function for γ-glutamylcysteine synthetase (γ-GCS) in *Leishmania*.

γ-glutamylcysteine synthetase (γ-GCS) which catalyzes the ATP-dependent ligation of L-Cys and L-Glu to produce γ-glutamyl cysteine has been demonstrated to be the rate limiting enzyme in the biosynthesis of GSH in mammalian cells and of trypanothione in *Leishmania*. The *GSH1* gene, encoding for γ-GCS appears to be central in GSH and trypanothione homeostasis and we tried to decipher the importance of γ-GCS in *Leishmania* by attempting to generate *GSH1* null mutants by gene disruption using selectable markers in *L. infantum*. *GSH1* is an important gene for parasite survival since our attempts to obtain a single K/O of *GSH1* failed in a strain of *L. infantum*. Attempted removal of even a single wild type allelic copy of *GSH1* in this strain, invariably led to the generation of an extra wild type copy of this locus. However, by first supplementing the WT parasites with a rescue plasmid (harbouring the *L. infantum GSH1*coding sequence in an expression vector) and then going for 2 rounds of inactivation at the chromosomal locus, we succeeded in getting a null mutant at the chromosomal locus, thus proving the essentiality of this gene in *Leishmania*. Parasites with one intact *GSH1* allele at the chromosomal locus lost the plasmid but not the double knock out, when grown in the absence of antibiotic, again indicating the essentiality of the gene. We also tried to obtain a null mutant of *GSH1* in 2 other field isolates, 3049 and 3485 of *L. infantum*. Surprisingly, in these field isolates, inactivation of the first allele at the chromosome was possible, in the absence of a supplementary episome. However, after the second round of replacement, in the absence of the episome, appearance of an extra WT

allele appeared. Phenotypic analyses of the single knock out of *GSH1* in these strains showed that they had less GSH content and more susceptible to oxidative stress when compared to the WT strains.

3. Wilfried Moreira, Éric Leblanc and Marc Ouellette. Infectious Diseases Research Center, CHUL.

The role of PTR1 and reduced pterins in resistance to oxidative stresses in *Leishmania*.

Leishmania is a folate and pterin auxotroph and those compounds are essential for its growth. Pteridine reductase PTR1 is the enzyme of the folate pathway allowing the reduction of pterins and folates and capable of conferring resistance to the antifolate methotrexate in *Leishmania*. During its life cycle, the parasite experiences oxidative stresses when interacting with the macrophage. Reduced pterins are known as scavenger of reactive oxygen and nitrogen species (ROS and RNS). *L. tarentolae PTR1-/-* null mutant were more sensitive to H2O2 and NO-induced oxidative stress. We investigated the role of PTR1 in resistance to oxidative and nitrosative stress by inactivating PTR1 in both *L. infantum* and *L. major* human pathogens. Both *PTR1-/-* null mutants were more sensitive to H2O2 and NO-induced oxidative stress and this growth-inhibition phenotype were reverted by an episomal copy of PTR1 or by reduced pterins supplementation. Those results have been confirmed by measuring the oxidant levels induced within the parasite using DCF-DA probe that shows fluorescence after oxidation and allows relative quantification of ROS induced in the parasite. Our results indicated that *PTR1-/-* null mutants which have been exposed to an oxidative stress released more fluorescence compare to the wild-type, indicating that those mutants accumulated more oxidant molecules. Resistance to oxidative and nitrosative stresses generated by macrophages during the initial step of phagocytosis has also been studied in J774 mouse cell line and human monocyte-derived macrophages (MDM) with the *PTR1-/-* null mutants. Our results in the macrophage-model show that *PTR1-/-* null mutants survived less within the macrophage compare to the wild-type and this decrease in intracellular survival was more pronounced in macrophages that have been activated for oxidants production. This study demonstrates that one physiological role of reduced pterins in *Leishmania* is to deal with oxidatives and nitrosative species.

4. Normand Cyr, Kleber P. Madrid, Mark Aurousseau and Armando Jardim. Institute of Parasitology, McGill University. **Structural insights into the conformational changes occurring to the *Leishmania donovani* peroxin 14 upon binding to peroxin 5.**

Leishmania and other kinetoplastid parasites compartmentalize several essential metabolic pathways, including glycolysis and pyrimidine biosynthesis, in aspecialized organelle called a glycosome. Genetic studies have demonstrated that trafficking of matrix proteins to the glycosome is crucial for the viability of the parasite. Consequently, the protein machinery associated with the biogenesis of the glycosome represents an attractive drug target. Early studies have highlighted essential proteins required for glycosome assembly which include, the *L. donovani* proteins peroxin 5 (LdPEX5), a cytosolic receptor that selectively recognizes proteins that are destined for the glycosome, and peroxin 14 (LdPEX14), a glycosomal membrane protein that allows cargo loaded LdPEX5 to bind to the glycosome surface to facilitate protein import. Quaternary structure analysis revealed that LdPEX14 forms a homo-oligomeric structure that is > 670 kDa. Deletion mutants of LdPEX14 have enabled the implicated a hydrophobic region as well as a coiled-coil motif that are important for the oligomerization of the protein. Moreover, binding of LdPEX5 to LdPEX14 exhibited marked conformational change in LdPEX14 that involved a significant change in the hydrophobic region thought to be important for LdPEX14 oligomerization. We hypothesize that these

structural changes permit LdPEX14 to be involved in the translocation of folded proteins across the glycosomal membrane.

5. Ana Victoria Pilar, Rona Strasser and Armando Jardim. Institute of Parasitology, MacDonald Campus, McGill University. **Quantitative assessment of the interactions between the PTS2 receptor LmPEX7 and other components of the glycosomal protein import pathway in *Leishmania* sp.**

Glycosomes are unique organelles in the Family Trypanosomatidae which includes *Leishmania* and *Trypanosoma*. Glycosomes perform various metabolic functions essential for parasite survival and are considered promising chemotherapeutic targets. Proteins destined for the glycosomal matrix contain signal sequences called PTS1 or PTS2 that are recognized by the receptors PEX5and PEX7. The receptor-cargo complexes interact with the docking protein PEX14 on the glycosomal membrane prior to the translocation of PTS proteins into the lumen. The precise mechanism of glycosomal protein import is unclear. However, this import machinery is crucial for glycosome biogenesis and represents an attractive drug target. This study focuses on the elucidation of the PTS2 import pathway into the glycosomes. The PTS2 receptor PEX7 was previously shown to bind PTS2 proteins, LdPEX5, and LdPEX14 and shuttles between the cytosol and glycosomal lumen. An ELISA-based protein interaction assay was used to characterize these protein-protein interactions. Results showed that the LdPEX5-LmPEX7 complex is modulated by the presence of PTS proteins. The decreased affinity of this receptor complex to LdPEX14 upon PTS binding might be the trigger for the dissociation of the PTS-receptor complexes to translocate into the glycosomal lumen. Tryptic digestions of these complexes showed that LdPEX14 and LdPEX5 undergo conformational changes upon binding LmPEX7 and PTS ligands.

6. Janet Yee[1], Anita Tang[1], Heather Ritter[1], Wei-Ling Lau[1], Dewald Delport[1], Melissa Page[1], Rodney Adam[2], Miklós Müller[3] and Gang Wu[3]. [1]Biochemistry Program, Trent University, [2]Department of Microbiology/Immunology and Medicine, University of Arizona College of Medicine, [3]The Rockefeller University.

The core histone genes of *Giardia lamblia*.

Histones are small, highly conserved proteins that associate tightly with DNA to form chromatin within the nucleus. We detected two copies each of the core histone H2a, H2b and H3 genes, and three copies of the H4 genes at dispersed locations within the genome of *Giardia lamblia*, although we could not recognize a gene encoding a H1 linker histone. The copies of each gene shared extensive DNA sequence identities along their coding and 5' noncoding regions. Transcription start for these genes mapped to sites containing triplet A sequences that are 1-27 nucleotides upstream of the translation start codon for each gene. The promoter for the histone H4 gene was characterized by transfecting *Giardia* with DNA constructs in which the H4 upstream sequence was used to drive the expression of a luciferase reporter gene. A 50 bp region upstream from the start of the histone H4 coding region was the minimal promoter and a highly conserved 15 bp sequence motif called *him* was critical for its activity. The equivalent upstream regions of the three other core histone genes, also containing the *him* sequence, had similar promoter activities as the H4 gene. Proteins from a *Giardia* nuclear extract were shown to bind to a probe containing three tandem repeats of *him* in bandshift assays. Competition experiments suggest that a factor within the protein complex that binds *him* may also be a part of the protein complexes that bind other promoter elements described

previously in *Giardia*. Our findings suggest that the *G. lamblia* core histone genes are constitutively expressed at approximately equivalent levels and that their mRNAs are polyadenylated.

Student Symposium

1. Leslie Payne. **Translating health research into action in developing countries: A career with the Canadian International Development Agency.**

The Canadian International Development Agency (CIDA) administers the majority of Canada's Official Development Assistance through funding to Canadian and international organizations, as well as directly to developing countries. Working as an advisor to CIDA provides the opportunity to apply research skills and experience to determine where Canadian funding will have the greatest impact, to provide technical support to developing countries, to influence the direction of major global partnerships and to evaluate, first-hand, the effectiveness of programs implemented in developing countries. Learn about the ups and downs of a career with the government, what a career with CIDA entails, and tips to get a job with CIDA.

2. Christian Cawthorn. **Intellectual property: The new frontier.**

Discover the various types of intellectual property and their utility. Understand how to use the patent system to bring value to a company. We will explore the patent system and explain it so as to maximize your investment on your innovations. Finally, time permitting, we will explore the profession of a patent agent. Christian Cawthorn is a registered US and Canadian patent agent with more than 12 years of experience in the field. He practices in the areas of biochemistry, biotechnology, molecular biology, proteins, genetics, cloning, pharmaceuticals, nutraceuticals, PCR techniques, tissue culture and enzymatic assays. In addition to providing comprehensive management of client's intellectual property portfolios, Mr. Cawthorn provides support for clients through the entire patent process and beyond, and develops strategies tailored to their specific needs. He focuses on drafting and prosecuting patent applications in the field of biotechnology. He has also participated in due diligence analyses of intellectual property portfolios and prospectus drafting in collaboration with lawyers of our Business Law Group. Before joining the firm, Mr. Cawthorn worked for seven years as a research assistant in the Genetic Unit laboratory of the Shriners Hospital for Children.

3. Mathieu Perreault. **From science to news.**

Mathieu Perreault studied chemical engineering at Polytechnique (Université de Montréal), then started a masters in Arabic philosophy. He made an internship at La Presse in 1995. He was then able to have that opportunity in part because of articles he wrote that were related to his engineering studies. He remembers fondly his first article, about his honour’s project at Polytechnique. Then Mathieu became specialized in science topics, although he has also delved in most of the newspaper's inserts. He thinks his scientific formation enabled him to approach any subject with a scientific eye. During his presentation, he will highlight the capacities a science student must exploit if he wants a career in journalism, and the pitfalls he must avoid.

4. Tim Geary. **Careers in industry: discovery through development**

The generation of new chemical and biological interventions for the amelioration of disease and enhanced agricultural productivity is a major economic enterprise. This industrial sector includes pharmaceuticals for human and animal health, vaccines for the same markets, and products that enhance the productivity or utility of plants and microbes. It includes both traditional, chemically-based approaches as well as biotechnology. The industry offers a wide variety of career opportunities for science graduates in fields as diverse as identification of drug targets, screening, development, registration, intellectual property and marketing. Although industrial jobs in parasitology per se are not overly abundant, the scientific training developed in graduate school parasitology programs can be readily adapted for a career in the industry. Tim Geary spent 20 years in the pharmaceutical industry in an antiparasitic discovery group before joining the Institute of Parasitology in 2005.

Session 3: Drug Resistance and Targets.

1. Wever Claudia and Joseph Dent. Department of Biology, McGill University.

Validating Acetylcholine-Gated Chloride Channels as Novel Nematocide Targets.

We have identified a novel class of acetylcholine-gated chloride (ACC) channels in *Caenorhabditis elegans (C. elegans*). These channels are specific to nematodes and are not targets of previously known pesticides. A drug that targets these channels is therefore predicted to be effective and safe, and consequently, we think the ACCs are promising drug target candidates. One part of the project is to determine the pattern of expression of all 8 ACC subunits. This will be done via microinjection of promoter-GFP gene fusion constructs. A second part of the project is to determine the outcome of over-exciting the ACC channels. Since these are anion (chloride) channels, permanent over-excitation of the ACC channels would cause the cells to remain in a permanently unexcitable state. To test what occurs when ACCs are overexcited, we will exploit an already well-known system; Ivermectin is known to overexcite glutamate gated chloride channels (GluCl). In order to simulate over-excitation of ACCs, we will express GluCls in tissues that normally express ACCs. We will do this by microinjecting GluCl coding DNA with ACC promoters into worms that lack all four endogenous GluCl subunits. We will then expose these worms to Ivermectin and observe any effects on the worm. A third part of the project is to perform high-throughput drug screens to determine what chemicals function as agonists of these channels. We will report the expression pattern for 6 of the 8 subunits. Some subunits show promising expression patterns; two subunits, Y71D11A.5 and F47A4.1, are expressed in approximately 20 neurons in C. elegans. Due to the expression of ACCs in a significant fraction of the nervous system, a drug that targets these channels by over-activating them promises to have highly deleterious effects on nematode physiology. Therefore, we conclude that ACCs merit further investigation as antiparasitic drug targets. This work was supported by NSERC and Chemtura Co.

2. Aïssatou Diawara, Carli Halpenny, Marilyn Scott and Roger Prichard. Institute of Parasitology, McGill University.

Assays to detect benzimidazole-resistance SNPs in soil transmitted helminths.

Human soil-transmitted helminths (STH), cause considerable morbidity. Efforts are being made to “Deworm the World” through large-scale use of benzimidazole anthelmintic drugs for school-aged children in developing countries. The benzimidazole drugs, albendazole and mebendazole are used as a single annual dose in areas where the burden is high. Unfortunately, there is concern that increased use of anthelmintics in children could select for

resistant populations of these nematode parasites. We have developed pyrosequencer assays for codon 200 in β-tubulin of *Trichuris trichiura*, *Ascaris lumbricoides*, and the hookworm *Necator americanus* to screen for the (TTC → TAC) single nucleotide polymorphism (SNP). Assays for this resistance-associated SNP could be useful for monitoring for anthelmintic resistance in control programs. These assays have been tested on adult worms from a benzimidazle-naïve population in Kenya. Following this, these assays have been applied on individual worms, pooled eggs and pooled larvae from people in East Africa, the Caribbean and Central America where mass anthelmintic programs have been implemented. The 200Tyr SNP was detected in *T. trichiura* and *N. americanus*. However, no codon 200 polymorphism has yet been detected in *A. lumbricoides* samples.

3. Daniel Feingold and Joseph A. Dent. Department of Biology, McGill University. **Characterization of three novel Cys loop ligand-gated ion channel subunits in *Drosophila melanogaster*: Potential pesticide targets?**

Cys-loop ligand gated ion channels are pentameric neurotransmitter receptors that are ubiquitous in both vertebrate and invertebrate nervous systems. Their large diversity as well as their central role in mediating rapid synaptic transmission has made these channels attractive molecular targets for a number of pesticides. Despite the widespread use of such pesticides, there remains a pressing need for the development of effective pesticides that pose minimal risk to non-target species and the environment. The goal of my research project is to identify a molecular target for a novel class of pesticides that will be safe for non-target animals and have low environmental impact. We are characterizing three novel Cys- loop LGIC subunits - CG7589 and CG6927 and CG11340 – in the *Drosophila melanogaster*. These genes are of particular interest because they exhibit little homology among other invertebrate or vertebrate species (Dent 2006). In order to determine whether CG7589, CG6927 and CG11340 would be suitable pesticide targets, it is important to understand their functional role in vivo. The first component of my research has involved generating flies deficient for my genes of interest. We have successfully generated deletions in CG7589 and CG6927 via P-element excision mutagenesis. Preliminary results indicate that mutations in CG7589 are semi-lethal. Any mutant phenotype that negatively impacts fly viability and/or fertility would validate these channel subunits as putative pesticide targets. Gene expression assays using in situ hybridization suggest that both CG7589 and CG11340 are expressed in the gut while CG11340 exhibits expression in the imaginal disks as well. Based on the expression data and potential mutant phenotypes, these putative ion channel subunits may provide a promising molecular target for the development of a novel class of highly selective and efficient pesticides.

4. Vijayaraghava Rao[1], Roger Prichard[1] and Sean Forrester*[1,2]. [1]Institute of Parasitology, McGill University, [2]Faculty of Science, University of Ontario Institute of Technology.

Cloning and characterization of a novel ligand-gated chloride channel in *Haemonchus contortus*.

Haemonchus contortus, a parasitic nematode of great economic importance, is a major challenge for the livestock industries. The parasite is controlled by nematocidal drugs, many of which target ligand-gated chloride channels (LGCCs). However, drug resistance has become a major deterrent in controlling this parasite. Therefore, the discovery of new drug targets is desirable to maintain the level of parasite control required in modern agriculture. We have isolated a novel LGCC gene, which has been named *HcGGR3*. Putative protein sequence analysis indicates that this channel is anion selective and possesses all the signature motifs of a chloride channel subunit. Analysis of the cDNA sequence shows putative micro-RNA recognition sites which could be important in relation to

developmental expression of this subunit. qPCR of *HcGGR3* showed that it is differentially expressed among the various life-stages and the rank order of expression was eggs > adult female > larvae > adult male. In addition, *HcGGR3* is down regulated in macrocyclic lactone resistant laboratory strains of *Haemonchus contortus*. Immunolocalisation of this subunit in adult worms has revealed some differences in the expression patterns between males and females. In females, the localization is distinctly punctuate around the cervical papillae, suggesting a possible role in mechanosensation. In males, expression was observed around the cervical papillae and some other unidentified cells. Functional analysis of this subunit will lead to a further understanding the role of LGCC's in the biology of parasitic nematodes. This project has received funding support from the following: NSERC, Fort Dodge Animal Health and FQRNT

5. Fouad El-Shehabi and Ribeiro Paula. Institute of Parasitology, McGill University.

Deorphanization of two of novel *Schistosoma mansoni* GPCRs, using a yeast expression system.

A bioinformatics analysis of the *S. mansoni* genome database has revealed many new G protein-coupled receptors (GPCRs) that share some sequence similarity with the biogenic amine receptor family but have no identifiable mammalian orthologues. These GPCRs have been designated as "*orphan*" receptors and may be schistosome-specific. We have cloned three of these receptors, one of which was previously shown to be activated by the biogenic amine, histamine (HA). Here we describe the functional analysis of the other two GPCRs (Smp_043290 and Smp_043340). To test for receptor activity, we have used a recently described yeast system, which is uniquely designed for functional expression of GPCRs (Wang et al, 2006, Methods in Molecular Biology, 332:115-127) and can be easily adapted to highthroughput ligand screens. A first assessment of this system revealed that it was suitable for expression of *S. mansoni* GPCRs, even those receptors that could not be expressed in other heterologous systems, such as mammalian cells. Smp_043290 and Smp_043340 were both expressed in yeast and then tested with all known biogenic amines at various concentrations. Smp_043290 was activated by dopamine (DA) and, to a lesser extent, the structurally related catecholamines, adrenaline (A) and noradrenaline (NA), but not by any of the other biogenic amines tested. Further analyses with various classical agonists/antagonists showed the receptor was selective for dopaminergic ligands and their IC50 values were determined. The second GPCR, Smp_043340, was found to be preferentially stimulated by histamine (HA) and 3-methyl histamine, a common agonist of histamine receptors. Several histamine antagonists were tested and the results confirmed the specificity of this response. These results describe a powerful new system for de-orphanization of schistosome GPCRs and for large-scale screens of receptor blockers.

6. Sonia Edaye, Melissa Richard-Lalonde and Elias George. Institute of Parasitology, McGill University.

***Plasmodium falciparum* drug resistance: Implication of a putative ABC transporter.**

The ATP-binding cassette (ABC) transporters are a family of proteins which are known to play a major role in multi drug resistance (MDR) in mammalian cancer cells. In the past few decades, the emergence of antimalarial drug resistance in *Plasmodium falciparum* has led to the identification of different factors involved in the parasite resistance. PfMDR1 and PfMDR2 (*Plasmodium falciparum* multidrug resistance 1 and 2) were initially identified and characterized in *Plasmodium*. Similar to its homolog in mammalian cells, PfMDR1 was shown to mediate resistance to mefliquine and other related quinolines in *P. falciparum*. However, the mechanism by which PfMDR1

mediates drug resistance in *P. falciparum* remains unclear. The sequencing of *Plasmodium falciparum* genome and subsequent comparison allowed us to identify among other putative ABC transporters, a transporter which has some similarity with human ABCG2. This transporter (named by us as "PfABCG2") is a half transporter (1 ATP-binding domain+6 transmembrane domains) and is expressed in the gametocyte stage of the parasite. Given the role of ABCG2 in drug resistance in mammalian cells, it was of interest to determine its role in *P. falciparum* drug resistance. In this report, we determined the expression level of PfABCG2 gene by Real-time PCR in *P. falciparum* chloroquine (CQ) sensitive strain (3D7) and CQ resistant strains (K1, 7G8K, FCR3). Our results demonstrate that PfABCG2 expression level is down regulated (1.5-3 fold) in the resistant strains compared to the sensitive strain (3D7). Indeed, there was a much better correlation between CQ resistance and PfABCG2 expression than that of PfMDR1 expression in the same drug sensitive and resistant stains. These initial findings suggest that PfABCG2 could play a role in *P. falciparum* multidrug resistance. Work is ongoing to further character the PfABCG2 protein in *P.falciparum*.

Session 4: Infection and Immunity

1. Nathalie Trudel[1], Ravendra Garg[1], Nadine Messier[1], Shyam Sundar[2], Marc Ouellette[1] and Michel J. Tremblay[1]. [1]Centre de Recherche en Infectiologie, Centre Hospitalier de l'Université Laval, and Département de Biologie Médicale, Université Laval, Québec, Canada; [2]Institute of Medical Sciences, Banaras Hindu University, Varanasi 221 005, India.

HIV-1 protease inhibitors interfere with *Leishmania* infection in THP-1 and primary human macrophages.

Visceral leishmaniasis is now recognized as an opportunistic disease in individuals infected with human immunodeficiency virus type-1 (HIV-1). Whereas the usefulness of HIV-1 protease inhibitors (PIs) in antiretroviral regimen is well documented, little is known about their potential impact in the setting of *Leishmania*-HIV-1 co-infections. We now report that, although selected PIs do not inhibit the growth of *Leishmania infantum* promastigotes alone in culture, these drugs significantly inhibit the intracellular survival of parasites in PMA-differentiated THP-1 macrophages and human primary monocyte-derived macrophages (MDMs). Furthermore, a field isolate of *Leishmania donovani* resistant to sodium stibogluconate (SbV), one of the most commonly used drugs to treat leishmaniasis, is equally susceptible to the tested PIs when compared to a sensitive strain, thus suggesting that resistance to SbV does not provide cross-resistance to PIs. The efficacy of PIs to reduce the intracellular growth of *Leishmania* is also observed in MDMs co-infected with HIV-1. This work was made possible through a Strategic new initiative team grant to M.J.T. and M.O. from the FQRNT Centre for Host-Parasite Interactions

2. Juliette Diou, Mélanie R. Tardif and Michel J. Tremblay. Infectious Disease Research Center, Laval University.

Malaria pigment inhibits the early events of HIV-1 life cycle in monocyte-derived macrophages.

Malaria/HIV coinfection results in a raise of viral load and an acceleration of disease progression. The aims of this study were first investigating whether hemozoin (Hz) exposure influences HIV-1 infection of primary human monocyte-derived macrophages (MDM) and then understanding the mechanisms by which viral infection is modulated. To this end, human peripheral blood mononuclear cells were isolated from healthy donors by Ficoll-Hypaque. Monocytes were purified and induced to differentiate in MDM. Synthetic Hz was chemically produced using polymerization of heme while HIV-1 particles were produced by transient calcium phosphate transfection of

293T cells. Hz-fed and unfed MDMs were exposed to HIV-1 particles and the infections were measured by luciferase activity or by p24 ELISA. Results have demonstrated that Hz-loaded macrophages have a reduced ability to replicate HIV-1. Surprisingly, Hz-loaded monocytic cell line THP1 has an opposing effect on HIV-1 infection, which is increased in these cells. Knowing this, we focused on primary cells only and demonstrate here that diminution of HIV-1 infection, in response to Hz engulfment, is not caused by the induction of primary human macrophage apoptosis, alteration of the cellular membrane or contamination by LPS. Data from a time-course experiment suggest that Hz is affecting the early steps in HIV-1 replication. However, results from entry assays have clearly indicated that virus internalization is not affected in Hz-loaded macrophages. In contrast, integration of the viral genome is severely decreased. Altogether our findings demonstrate that treatment of MDM with Hz leads to a diminution of HIV-1 infection. The cause of that phenomenon could be explained by the production of some specific molecules (e.g. cytokines, chemokines) and/or by inhibition of reverse transcription through a yet to be defined mechanism. This work was supported by CIHR Doctoral Award.

3. Maurice Odiere[1], Kris Koski[2] and Marylyn Scott[1]. [1]Institute of Parasitology and [2]School of Dietetics and Human Nutrition, McGill Univ. **Morphological effects, parasitological outcomes and trade-offs of concurrent *Heligmosomoides bakeri* (Nematoda) infection and pregnancy in CD-1 mice.**

There is a growing interest in the link between individual immune system performance and fitness-related traits such as reproductive effort. This study provides an insight into how animals cope with simultaneous energy demands in the form of infection and reproduction, and the costs and trade-offs of these interactions. A 2 x 3 factorial design involving pregnancy (pregnant and non-pregnant) and 3 levels of infection dose (0 L_3=sham, 50 L_3=low and 100 L_3=high) was used. Mice were infected at day 6, 11 and 16 of pregnancy. *H. bakeri* egg output was estimated from complete 24 h stool collections at day 18 of pregnancy. At necropsy (day 19 of pregnancy), a c-section was performed; fetuses were removed and killed by exsanguination. Maternal organ masses and reproductive outcomes (fetal count, fetal weight, crown-rump length, placental weight and tissue resorptions) were determined immediately thereafter. Small intestines were excised, the gender and number of adult worms and 4th-stage larvae (L_4) were determined. Pregnancy increased L_4, adult worms and fecal egg count (FEC) indicating immunosuppression, confirmed by decreases in Th2 cytokines (IL-4, IL-5 and IL-13) using Luminex. Both infection dose and pregnancy increased organ masses (heart, lungs, spleen, liver, kidneys, pancreas, small intestines). There was a dose-dependent decrease in fetal crown-rump length, but no effect on fetal weight. The periparturient immunosuppression observed ensured that costly immune responses were suppressed and the released resources from this were then adaptively reallocated to the prioritized reproductive function (trade-off: reproduction prioritized over immunity). The reduced crown-rump length mimics stunting that is seen in children with chronic helminth infections, making this model ideal to study such effects.

4. Robert Lodge and Michel J. Tremblay. Centre de Recherche en Infectiologie, Centre de Recherche du CHUL, and Département de biologie médicale, Faculté de Médecine, Université Laval, Québec, Canada.

***Leishmania* and HIV-1 coinfections in human macrophages.**

Visceral leishmaniasis, albeit in itself a major parasitic disease, is today recognized as an important infection afflicting HIV-1-seropositive individuals. Indeed, *Leishmania* infection enhances HIV-1 replication and accelerates

the onset of AIDS. Particularly relevant is the fact that both pathogens infect cells of the monocyte/macrophage lineage. Our group has previously reported that HIV-1 production is increased in macrophages infected with *L. infantum*. Conversely, we have also shown that HIV-1 infection enhances *L. infantum* amastigote intake and replication in macrophages. However, these studies focused on the overall impact of HIV-1 on the parasite's survival in macrophages, since as little as 5-10% of the cells were actually co-infected. We therefore set out to determine the mechanisms by which HIV-1 enhances *Leishmania* replication in co-infected macrophage populations. Human monocyte-derived macrophages (MDMs) are infected with a CCR5-tropic HIV-1 virus also encoding HSA (NL4-3BALHSA), a murine cell surface constituent. This enables us to easily identify virus-producing MDMs. Cells are then exposed to *L. infantum* parasites. The amounts of parasites internalized in HIV-1-infected, bystander (i.e. HIV uninfected cells in HIV-infected cultures) and control (uninfected) macrophages are then compared using confocal microscopy. We show that both phagocytosis and multiplication of *L. infantum* are greatly enhanced in the bystander cells as compared to HIV-1-infected macrophages. Furthermore, we observe the same effect in macrophages exposed to virus-inactivated supernatants of HIV-1-infected MDMs. These observations suggest that HIV-1-infected macrophages secrete some unidentified viral or cellular factor(s) that enhance *Leishmania* replication. Possible candidate factors will be presented. This work is funded by a CIHR operating grant to MJT (MOP-84555).

Session 5: Genomics and Proteomics

1. Yovany Moreno and Geary Timothy. Institute of Parasitology, McGill University.

Proteomic Analysis of the Secretome of *Brugia malayi*.

To succeed in infection, parasites must develop ways to reach the host, penetrate its tissues and avoid its defense system by deploying mechanisms to evade or suppress host defense responses. Although it is generally accepted that parasitic nematodes release a variety of products that are essential for establishing and maintaining the host-parasite interaction, relatively little is known about the mechanisms involved in these processes. In order to better understand the roles performed by secreted products from parasitic nematodes and the contribution of the different stages and genders to the progression of the infection, we characterized the secretome of *Brugia malayi*, one of the etiological agents of limphatic filariasis, a disease that infects 128 million people and puts at risk a population currently estimated to be 1307. By using a combination of 1D-SDS gel electrophoresis with LC/MS-MS, we identified a total of 228 proteins secreted by the microfilariae, female and male worms of *B. malayi*. In addition to the identification of new proteins secreted by this parasite, a comparative analysis of the secretome at each stage and gender reveals common points but also clear differences in the roles and scenarios at which these proteins may be involved.

This work was supported by an operating grant of NSERC and CRC.

2. Hamed Shateri Najafabadi and Reza Salavati. Institute of Parasitology, McGill University

Homology-independent annotation of *Trypanosoma brucei* genome.

Trypanosoma brucei causes sleeping sickness in human and Nagana in livestock. The biology of this organism is clouded by the lack of homology between a major portion of its genome and any gene from any other organism outside the trypanosomatids clade. Identification of physical and/or functional interactions of proteins can lead to new insights regarding the functions of currently uncharacterized proteins, and help precise annotation of the genomes of trypanosomatids. However, most of the current computational methods either require cross-species

homology or experimental data which are not available for the majority of trypanosomatid genes. We have applied several computational methods for identification of functional linkages between proteins in *T. brucei*, two of which are novel methods that do not require cross-species homology. One of them uses the similarity of codon usage as a measure for prediction of functional linkages, while the other one seeks for function-specific regulatory motifs at the 5' and 3' UTRs. Using these methods, we have shown that uncharacterized trypanosomatid-specific genes can be assigned to known functions with high reliability. Furthermore, we have predicted several interesting regulatory *cis*-elements in both 5' and 3' UTRs of *T. brucei* genes, providing new insights on the regulatory mechanisms of protein expression in this organism.

3. Houtan Moshiri and Reza Salavati. Institute of Parasitology, McGill University.

Fluorescence-based reporter substrate for monitoring RNA editing in Trypanosomatid pathogens.

Mitochondrial gene expression in trypanosomatid pathogens requires extensive post transcriptional modification called RNA editing. This unique molecular mechanism, catalyzed by a multiprotein complex (the editosome), generates translatable transcripts for essential components of parasite respiratory complex. How editosome proteins are assembled and perform RNA editing is not fully understood. Also, previous studies have shown that editosome proteins are essential for parasite survival, which makes editosome as a suitable target for drug discovery. Currently, researchers use Radiolabeled based assays to monitor RNA editing process. However, these assays are not suitable for high throughput screening of editosome inhibitors, have low detection limits, and cannot monitor RNA editing in real time. Therefore, to develop a sensitive high throughput RNA editing assay, we have designed a sensitive hammerhead ribozyme based fluorescence assay. Ribozyme structure was remodelled by adding or removing uridylates in its conserved catalytic core to make an inactivate ribozyme. In the presence of the editosome, inactive ribozyme is edited to an active ribozyme. Consequently, hammerhead ribozyme activity can be measured by cleaving its fluorescently labelled substrate. We showed that higher sensitivity is achieved using fluorescent based assay than conventional radio-labelled assay. Moreover, we can use this assay for rapid identification and characterization of the editosome inhibitors against RNA editing activities in trypanosomatids.

Posters

Infection and Immunity

1. Stéfany Bazinet[1], Martin Olivier[2] and Tatiana Scorza[1]. [1]Dept. of Biological Sciences, University of Quebec in Montreal, QC; [2]Dept. of Microbiology and Immunology, McGill University, Montreal, QC, Canada. **Hemozoin down-regulates IL-12p75 production through a mechanism independent of the P38 MAPK-IL-10 pathway.**

P38 MAPK are activated by most environmental stresses and inflammatory stimuli including oxidative stress. Recent studies indicate that activation of the p38 MAPK by LPS may have dual effects on IL-12 and IL-10 production by macrophages (Mϕ), and that inhibition of the P38 MAPK may enhance IL-12 responses in conditions associated with robust IL-10 production. Hemozoin (HZ) has been shown to strongly stimulate IL-10 secretion in human monocytes which in turn inhibits IL-12p40 gene expression. A comparable effect is exerted by oxidants such as hemin, through a decrease in intracellular reduced glutathione (GSH) and the concomitant enhancement of IL-10 production. The effect of HZ on IL12p40 and IL-12p75 production was studied in bone marrow derived-Mϕ (BMMϕs). In contrast to hemin, HZ was shown to be a poor ROS-inducer and a less efficient stimulator of IL-10, although both compounds

significantly inhibited IL-12p75 in response to IFN-γ/LPS stimulation. P38 MAPK inhibition resulted in a comparable up-regulation of IL-12p75 and down-regulation of IL-10 in control and HZ-conditioned BMMϕs stimulated with LPS. The inhibitory effect of HZ on IL-12p75 production was refractory to treatment with GSH or N-acetyl cysteine (NAC). Surprisingly, HZ increased intracellular levels of GSH but altered the responses of BMMϕs to exogenous GSH or NAC. Our results suggest that HZ down-regulates IL-12p75 production in murine BMMϕs through a mechanism that seems independent of the p38 MAP kinase-IL-10 pathway but that may rely on an uncharacterized effect on GSH regulation. This work was funded by NSERC.

2. Benoit Belanger[1], Mathieu Cambos[1], Stéfany Bazinet[1], Lin Leng[2], Richard Bucala[2] and Tatiana Scorza[1].
[1]Department of Biological Sciences, University of Quebec in Montreal, QC, Canada and [2]Department of Internal Medicine, Yale University School of Medicine, New Haven, CT, USA. **Neutralization of MIF upregulates TNF-α and changes the time course of IFN-γ responses during *Plasmodium c. adami* infection.**

Malaria is an inflammatory disease characterized by a potent production of TNF-α, MIF and IFN-γ, cytokines that inhibit erythropoiesis in the bone marrow. In this context, the macrophage migration inhibitory factor (MIF) is readily secreted during *Plasmodium* infection, and synergises with IFN-γ and TNF-α to inhibit erythroid differentiation and haemoglobin production. In our laboratory, in vivo neutralisation of MIF during *P. c. adami* infection led to a significant and reproducible decrease in peak parasitemia and to the up-regulation of TNF-α production at early and peak infection. Treatment with anti-MIF Mab antibody had contrasting effects on IFN-γ and IL-10 during the time course of infection. At the time of low parasitemia, a significant decrease in IFN-γ production, accompanied by an important drop in IL-10 was measured in the spleen of MIF-neutralized infected mice. In contrast, at peak parasitemia, IFN-γ and IL-10 responses were significantly increased by the treatment. Besides the inhibitory effects on peak parasitemia, MIF neutralization led to a significant drop in the percentage of circulating reticulocytes at early infection, and this effect was accompanied by a mild decrease in Ery A (TERhighCD71highFSChigh) precursors in the spleen but not in the bone marrow and decreased anaemia. Our results suggest that neutralization of MIF reduces the inflammatory response at early infection, and contributes to the control of parasitemia and anaemia. Research funded by NSERC and NIH.

3. [1]Mathieu Cambos, [2]Martin Olivier, [2]Armando Jardim and [1]Tatiana Scorza. [1]Université du Quebec à Montréal, [2]McGill University. **The Phagocytosis of parasisitised red blood cells induces the apoptosis of macrophages during *Plasmodium chabaudi adami* infections.**

The phagocytosis of complement-opsonized targets is a primary function at inflammation sites, and macrophages, in contrast to neutrophils, can repeat the phagocytic cycle and oxidative burst without entering apoptosis. Interestingly, *Plasmodium* infections are characterized by a significant increase in the percentages of apoptotic macrophages (Mθ) which are cells that avidly ingest infected red blood cells (iRBC) and accumulate hemozoin (HZ) the malaria pigment in their cytoplasm. The effect of naïve and parasitized red blood cells (PRBC) as well as of synthetic B-hematin on the apoptotic state of murine bone marrow derived Mθ (BMMθ) and J774 cells were studied. Our results indicate that phagocytosis of PRBC but not synthetic HZ or naïve RBC triggered the apoptosis of J774 cells and BMMθ. The effect was shown to be dose dependent and became evident at ratios as low as 25 PRBC per Mθ. In

addition to their pro-apoptotic capacity, the phagocytosis of PRBC induced the secretion of soluble immunosuppressive factors inhibiting the proliferation of CD4+ and CD8+ T cell in response to stimulation with Concanavalin A. We are currently investigating the nature of the signaling events triggering macrophage apoptosis as well as the possible relationship between programmed cell death and the release of immunosuppressive factors

4. Ravendra Garg, Corinne Barat and Michel J. Tremblay. Centre de Recherche en Infectiologie, Centre Hospitalier de l'Université Laval, and Département de Biologie Médicale, Université Laval, Québec. ***Leishmania infantum*** **amastigotes enhances HIV-1 replication in human dendritic cells and transfer to CD4+ T cells by inducing secretion of cytokines TNF-α and IL-6.**

Visceral leishmaniasis has emerged as an important potential opportunistic disease among patients infected with HIV-1. Both HIV-1 and *Leishmania* can productively infect cells of the macrophage-dendritic cell lineage. Here we demonstrate that *Leishmania* amastigotes promote HIV-1 transfer and replication in human monocyte-derived dendritic cells. Interestingly, this process is not affected by the promastigote form of the parasite, thus suggesting that the major surface constituent LPG is not involved in this phenomenon. We provide evidence that amastigotes exert an effect on both early and late phase transfer events. Additional studies indicate that this effect is mediated through secretion of parasite-induced soluble factors by dendritic cells. Bio-Plex studies show that the parasite stimulates higher secretion of cytokines (IL-1β, IL-2, IL-6, IL-10, TNF-α) and chemokines (MIP1α, MIP1β, RANTES) from dendritic cells. Studies conducted with pentoxifylline and neutralizing Abs revealed that the *Leishmania*-mediated increase in HIV-1 replication was mediated through higher production of TNF-α and IL-6. Altogether, these findings show that the presence of *Leishmania* within the same cellular microenvironment leads to an enhancement of HIV-1 transmission and demonstrate that HIV-1 and *Leishmania* can establish complex interactions in their common natural host dendritic cells. This work was funded through grants to M.J.T. from the Canadian Institutes of Health Research (CIHR) (MOP-84555). R.G. is the recipient of a fellowship award from the CIHR HIV/AIDS Research Program.

5. Stephanie Goyette[1], Cynthia Santamaria[1], Momar Ndao[2], Gary E. Ward[3], Terry W. Spithill[4], Florence S. Dzierszinski[1] and Brian J. Ward[2]. [1]Institute of Parasitology, McGill University, [2]MUHC, McGill University, [3]Department of Microbiology and Molecular Genetics, University of Vermont, [4]School of Animal and Veterinary Sciences, Charles Sturt University. **Development of a subunit vaccine against *T. gondii* based on the ectodomain of TgAMA-1.**

T. gondii is a ubiquitous protozoan parasite and a global zoonotic pathogen. Although infection typically causes mild disease in healthy adults, it can be life threatening in the fetus and immunocompromised host. It is also an important veterinary pathogen that causes abortion in sheep and goats. Drugs used to treat acute toxoplasmosis commonly cause adverse effects. In addition, there is no effective treatment against tissue cysts, which sustain the chronic phase of infection and persist for the lifetime of the host. While a vaccine based on a live-attenuated strain has been developed, its use remains restricted to veterinary settings, and is hindered by its cost and short shelf-life. We developed a subunit vaccine based on the ectodomain of *T. gondii* apical membrane antigen-1 (TgAMA1). The *Plasmodium* ortholog is a component of many 'cocktail' anti-malaria vaccine efforts. Early attempts at expressing recombinant whole AMA-1 failed due to the complex folding of these proteins. We established a method that allowed expression of large quantities of conformationally relevant recombinant TgAMA1 in *S. cerevisiae*, and

formulated an intra-nasal Protollin-TgAMA1 vaccine. Initial safety and immunogenicity studies conducted in a murine model demonstrated strong humoral and cell-mediated responses, indicating that TgAMA1 could be a good candidate for inclusion in a cocktail vaccine. Although this formulation was not protective against experimental challenge with a highly virulent laboratory strain of *T. gondii* (RH), we are currently developing a model that mimics natural conditions of infection. We are also assessing the effect of vaccine responses on live parasites *in vitro* (e.g. invasion/growth inhibition assays).

6. Pranav Kumar, Nathalie Trudel, Robert Lodge and Michel J. Tremblay. Centre de Recherche en Infectiologie, Centre Hospitalier de l'Université Laval, Québec, Canada. **Nelfinavir induces DNA fragmentation in *Leishmania donovani*, a hallmark of programmed cell death and apoptosis.**

Besides being a major tropical disease, leishmaniasis is now also emerging as an important opportunistic disease found in patients infected with human immunodeficiency virus type-1 (HIV-1). Nelfinavir is a well characterized anti-HIV drug that targets the viral protease. Recently, our group (see abstract from Trudel *et al.*) has found that several HIV-1 protease inhibitors (PIs) inhibit *Leishmania* survival in macrophages. We have now set out to understand the mechanisms by which this HIV-1 PI acts as an antileishmanial agent. We report that *Leishmania donovani* (Ld9518) axenic amastigotes are sensitive to Nelfinavir in culture. Results suggest that Nelfinavir induces programmed cell death, as we observed increasing DNA fragmentation in Nelfinavir-treated *Leishmania* amastigotes in a time-dependent manner. The minimal concentrations of Nelfinavir tested that induced DNA fragmentation were in the range of 25-30 µM. In another set of experiments, in order to get more insight on the mechanism of drug action in *Leishmania*, we are currently trying to obtain *in vitro* Nelfinavir resistance by direct drug pressure on cultured *L. donovani* parasites. Understanding the Nelfinavir-mediated death signalling pathway may eventually help in the design of new therapeutic strategies against the *Leishmania* parasite. This work is supported by a Strategic new initiative team grant to M.J.T. and M.O. from the FQRNT Centre for Host-Parasite Interactions.

7. Diane Malaison, Corinne Barat, Marc Bergeron and Michel J. Tremblay. CRI, CRCHUL, Université Laval, Québec, Canada.

Co-infection of Human Monocyte-Derived Microglia-Like Cells (MDMis) by *Trypanosoma cruzi* and HIV-1.

Infection by *Trypanosoma cruzi (T.Cruzi)*, the Chagas sickness causal agent, is endemic in many countries of South America. Moreover, the frequency of *T. cruzi* /HIV-1 co-infections is on the rise. In general, in chronically *T. cruzi*-infected patients, parasite reactivation causes cardiac or digestive pathologies. However, in patients co-infected with HIV-1 and *T. cruzi*, reactivation manifests itself often in the central nervous system (CNS), causing meningo-encephalitis, a symptom associated with AIDS. The goal of this study was to analyze the interactions between both pathogens in HIV-1/*T. cruzi* co-infections of MDMis, microglia being the main immune cells in the CNS. We therefore established an *in vitro* model for studying the co-infection of *T. cruzi* and HIV-1 in MDMis. Primary human MDMis were initially infected by different HIV-1 strains, in combination with *T. cruzi* (Ecua0 strain), and parasite entry and growth was evaluated by microscopy, differential coloration and/or immunocytofluorescence. The parasite-mediated effect on HIV-1 infection was measured using an ELISA against the p24 capsid protein of HIV-1. We observed that *T. cruzi* infection seems to inhibit HIV-1 replication in MDMis. On the opposite, HIV-1 infection leads to an increase in *T. cruzi* replication. Moreover, preliminary results seem to show an increased *T. cruzi* entry in

presence of HIV-1. Therefore, HIV-1 infection may induce cell activation in such a way that stimulates *T. cruzi* proliferation, or may hinder cell defense mechanisms against parasite invasion. These observations suggest that HIV-1 could probably have a role in *T. cruzi* reactivation in the CNS of co-infected patients. Subsequent studies will be aimed at a better understanding of the fine mechanisms of this co-infection, e.g. looking for the influence of the various cytokines secreted by infected cells as well as parasite soluble factors.

8. Marina T. Shio, Myriam Savaria and Martin Olivier. Department of Microbiology and Immunology, Centre for the Study of Host Resistance, the Research Institute of the McGill University Health Centre, Montréal, QC, Canada.

Hemozoin-induced IL-1β production by human monocytes is dependent on SYK, SRC kinases and inflammasome.

Hemozoin (Hz) is a metabolic waste resulting from heme detoxification by *Plasmodium* sps and released during Malaria process. Once in circulation, Hz is engulfed by phagocytes and induces pro-inflammatory mediators. However, molecular mechanisms underlying phagocyte activation by Hz are still unclear. PMA-differentiated THP-1 human monocytes were stimulated with Hz. Supernatant and cell extracts were analyzed by western to detect IL-1β, Caspase-1 and several kinases. Here we showed that Hz rapidly induced Syk kinase phosphorylation that is inhibited by Src family kinase inhibitors (PP1 and PP2). To evaluate the role of Syk in the inflammatory response we used Syk inhibitor (piceatannol) and observed that Syk up-regulate the Hz-induced IL-1β. Both Syk phosphorylation and IL-1β induced by Hz were dependent on phagocytosis that is inhibited by cytochalasin D. Furthermore, IL-1β maturation was dependent on Caspase-1 activation, as the Caspase-1 inhibitor (Y-Vad-Cho) abrogated Hz-induced IL-1β production. Interestingly, Caspase-1/IL-1β cleavages are markers of inflammasome activation. Therefore to confirm inflammasome involvement, we used the HSP-90 inhibitor geldanamycin and K+ efflux (by high concentration of extra cellular potassium) both known to regulate IL-1β resulting from inflammasome activation. As expected, Hz-induced IL-1β was inhibited in cells treated with geldanamycin or high concentration of potassium. Collectively, our findings provide strong evidence that Hz can activate macrophage inflammasome concurring to IL-1β secretion known to be a key inflammatory mediators concurring to augment Malaria morbidity. Acknowledgment: Canadian Institute of Health Research, CNPq.

9. Marie-Claire Rioux[1], M. Ndao[2], B. Ward[2], and Terry Spithill[2,3]. [1]Institute of Parasitology, McGill University, [2]National Reference Centre for Parasitology, McGill University, and [2,3]Charles Sturt University.

Identification of markers of response to infection and vaccination to *Fasciola hepatica* in cattle.

Fasciolosis is an economically important disease of sheep and cattle, responsible for 3.2B USD in losses annually. In order to gain a greater understanding of the complex host-parasite interactions during the first 14 weeks of disease, serum samples from cattle trickle-infected with *Fasciola hepatica* were profiled using Surface-Enhanced-Laser-Desorption-Ionisation Time-of-Flight Mass Spectrometry (SELDI-TOF MS). In the preliminary study of pooled sera from weeks 0-14 post-infection (p.i.), 28 markers decreased after infection, of which 17 were peptides (1.5-10 kDa) and 11 were proteins (10-200 kDa). Twenty-three markers increased after infection, of which 6 were peptides and 17 were proteins. Markers at 1852 Da, 3242 Da, 8364 Da and 100 kDa were validated statistically in two independent analyses. The 8364 Da marker has a kinetic profile that corresponds with the phases of the life cycle in the mammalian host: its intensity decreases at weeks 3, 6, and 10 p.i., the acute liver stage, and increases at week 14 p.i.,

the chronic bile duct stage. The validated markers will be purified and identified and confirmed using immunodetection to gain a greater understanding of their role in the pathogenesis of Fasciolosis.

Acknowledgments: Cattle samples were kindly provided by Dave Nicholas from Pfizer (U.K.)

10. Anne E. Schwab[1], Timothy E. Geary[2], Paul Baillargeon[3] and Gilles Fecteau[1]. [1]Département des sciences cliniques, Faculté de Médecine Vétérinaire, Université de Montreal, [2]Institute of Parasitology, McGill University, [3]Pfizer Animal Heatlh. **Association BoLA class II alleles with susceptibly to *N. caninum* in Quebec cattle.** *Neospora caninum* is the causative agent of neosporosis. Infection with this coccidian protozoan parasite leads to high levels of abortion in cattle. The disease may be transmitted vertically from a cow to her foetus. In this case, some animals abort their foetus, while others produce clinically healthy, but congenitally infected calves. The reasons for these different outcomes remain poorly understood. Resistance against *Toxoplasma gondii*, a closely related apicomplexan parasite, has been shown to be associated with the MHC class 1 and class II genes. In cattle, MHC (BoLA) class I and class II genes have been associated with resistance against mastitis, ticks, Bovine Leukemia Virus and bovine dermatophilosis. Such host resistance genes may also be involved in protection against *N. caninum* infection or parasite induced abortion. It was the objective of this study to determine whether BoLA class II DRB3 alleles were associated with susceptibility to infection with *N. caninum* in Quebec cattle. We obtained serum samples from 18 cattle: 9 uninfected and 9 infected with the parasite. We extracted and restored damaged serum DNA in order to amplify DRB3 exon 2 for sequence based typing. We identified 12 different alleles in the 18 cattle genotyped. Nine of these matched previously identified alleles, whereas 3 alleles differed from known alleles at 3 positions each. Only one of the animals genotyped was a homozygote. There were no significant differences in allele frequency between infected and uninfected animals. We would like to thank the Fonds de Recherche sur la Nature et les Technologies Quebec (FQRNT) for financial support of this research.

Biochemistry and Biology

1. Hiren Banerjee, Michael Nyisztor and Armando Jardim. Institute of Parasitology, McGill University.

Interaction of the *Trypanosoma* Peroxin 14 with glycosomal membrane.

Trypanosomatid group of human parasites including *Leishmania* and *Trypanosoma* compartmentalize biosynthetic and other nutritional pathways in a unique organelle referred to as glycosome. It is a microbody organelle containing a single phospholipid layer and is evolutionarily related to peroxisomes of yeast and mammals. Glycosome perform important functions including glycolysis, purine salvage, pyrimidine and ether lipid biosynthesis and β-oxidation of fatty acids. Proteins are imported into glycosomes using either a carboxy-terminal tripeptide or an amino terminal signal sequence that is recognized by the receptor proteins Peroxin 5 or Peroxin 7 respectively. These receptor proteins must dock at the glycosome membrane to deliver their cargo into the glycosomal lumen. PEX-14 is a critical glycosomal protein that helps in receptor recognition and trafficking of the cargo protein into glycomsomal matrix. In *Leishmania*, PEX-14 is a peripheral membrane protein that associates with the cytosolic face of the glycosomal membrane while in *Trypanosoma* it has a physicochemical behavior consistent with an integral membrane protein. The present study aims to characterize the interaction of *Leishmania* PEX-14 with the glycosomal membrane using mutagenesis mapping of the of N'-terminal domain of *Leishmania* PEX-14.

2. Marie-Josée Bellemare *, Colin-Nadeau Brosseau‡, Mara L. Leimanis†, Mariane Godboult§, Richard Leonelli‡, Elias Georges†, Martin Olivier§, D. Scott Bohle*. *Department of Chemistry, McGill University, Montréal, Québec, Canada. ‡Département de Physique, Université de Montréal, Montréal, Québec, Canada. †Department of Parasitology, McGill University, Ste Anne de Bellevue, Québec, Canada. §Research Institute of McGill University Health Centre, Centre for the Study of Host Resistance, Departments of Medicine, Microbiology and Immunology, McGill University, Montréal, Québec, Canada.

Fluorescence Phenomenon: Discovering the Bright Side of Malaria Pigment.

The heme released by the malaria parasite upon catabolism of hemoglobin, is oxidized and aggregated into insoluble, prismatic, black crystals named hemozoin. As heme possess a paramagnetic Fe(III) centre, it is most often encountered as a quencher. To our surprise, hemozoin crystals comprised of heme dimers, as well as its synthetic analog, hematin anhydride, were discovered to be fluorescent. Auto-fluorescence of hemozoin was observed *in vitro* as well as *in vivo*. Hypothesis toward complete explanation of this luminescence phenomenon will be presented. This work was supported by NSERC, CIHR and Wellcome Borough Fund.

3. Celia Caballero-Franco, Line Dufresne and Armando Jardim. Institute of Parasitology, McGill University.

Biochemical characterization of EspD protein from the enteropathogenic *Escherichia coli*.

Enteropathogenic *Escherichia coli* (EPEC) is an intestinal pathogen that causes diarrhea and gastroenteritis in humans. EPEC delivers bacterial virulence factors into the human intestinal epithelia through a syringe-like complex, knows as the type III secretory system (TTSS). One important component of this complex, EspD, is a 40 kDa soluble protein that is reported to associate with the plasma membrane of infected cells and to facilitate translocation of bacterial effector proteins directly into the cytosol of intestinal epithelial cells. To develop reagent to investigate the molecular mechanisms mediating the insertion of the soluble form of EspD into the host plasma membrane a recombinant form of EspD containing an N-terminal hexahistidine and C-terminal FLAG epitope (His6-EspD-FLAG) was overexpressed in EPEC strain E2348/69 and in non-pathogenic E. coli strain ER2566 His_6-EspD-FLAG was purified using metal affinity chromatography Ni^{2+}-NTA affinity column. Functional analysis demonstrated that recombinant His_6-EspD-FLAG associates with membrane fractions of HeLa cells in a manner similar to native EspD protein secreted by EPEC.

4. [a]Christelle Gabriel, [b]Nisha Dogra, [b]Robert W. McMaster and [a]Albert Descoteaux. [a]INRS-Institut Armand-Frappier, Laval; [b]University of British Columbia, Vancouver.

Impact of *Leishmania donovani* phosphoglycans on the macrophage transcriptome.

During its life cycle, the biphasic organism *Leishmania donovani* infects macrophages. Depending on the parasite species and developmental stage as well as on the activation stage of its host cell, *L. donovani* can prevent or activate cellular signalling pathway and the resulting cellular responses. The promastigote of *L. donovani* is coated by a glycocalyx composed of glycoconjugates containing the repeating unit $Gal\beta_{14}Man\text{-}PO_4$. Previous studies have shown that lipophosphoglycan (LPG), the major surface glycoconjugate of *Leishmania donovani* promastigotes, plays a critical role for the macrophage infection. Indeed, LPG strongly modulate expression of specific host cell genes and proteins. Nevertheless, little is known about the global effect of *L. donovani* glycoconjugates on macrophage gene expression. In this study, we therefore used microarrays to compare genetic expression profiles of macrophages

infected with wild-type promastigotes or mutants (lpg2-KO) defective for the synthesis of glycoconjugates containing repeating unit of Galβ_{14}Man-PO_4. Infected macrophages were either naive or previously stimulated with IFN-γ. After RNA extraction at different times post-infection and transformation into cDNA, samples were analysed by Affymetrix GeneChip Expression Arrays. The analysis shows that the majority of regulated genes by the glyconcojugates are implicated in critical functions such as signal transduction, transcription and metabolism. To validate the results of the arrays, quantitative PCR for some genes are currently under way.

5. Joella Joseph and Janet Yee. Biochemistry Program, Trent University. **Functional Characterization of a Recombinant TATA Binding Protein (TBP) and a TBP Interacting Protein in *Giardia lamblia*.**

In all eukaryotes studied to date, transcription of all three classes of RNAs is dependent upon the binding of the central transcription factor, the TATA-binding protein (TBP), to the promoter region. TBPs contain four highly conserved phenylalanine residues that interact with the minor groove formed by the TATA box sequence, and causes a sharp "bend" in the DNA. This bend serves as a landmark that recruits two other transcription factors, TFIIA and TFIIB, to this region. The interaction of these two transcription factors with TBP stabilizes its binding to the TATA-box. The *Giardia* TBP is highly divergent from those in other eukaryotes, and most interestingly, it has substitutions in three out of four key phenylalanine residues. Moreover, the promoter regions of most *Giardia* genes lack TATA sequences, and a gene encoding a homolog of TFIIA could not be located in this parasite's genome. In our previous work on the characterization of proteins that bind to a histone-gene-specific promoter element, we identified a *Giardia* protein that shows high sequence identity to a family of TBP-interacting proteins, called TIP49. Our goal is to express and characterize recombinant proteins from the *Giardia* TBP and TIP49 genes, and to determine their roles in the transcription of *Giardia* genes.

6. Yuan Mao, Hamed Shateri Najafabadi and Reza Salavati. Institute of Parasitology, McGill University.

Prediction and characterization of regulatory RNAs in *Trypanosoma brucei*.

Trypanosoma brucei is a protozoan parasite that is transmitted to humans by the infected tsetse flies and causes serious illness in the people of many African countries. Its genetic machinery enables the organism to survive and shift between two different host environments of the insect midgut and the human bloodstream. *T. brucei* has a few known regulatory transcription factors and its messenger RNAs are mass transcribed as polycistronic RNA precursors. Therefore, most of its genes are regulated at the post-transcriptional level, relying on RNA turnover and translational control. Knowing that microRNAs (miRNAs) can induce down-regulation of mRNAs in a sequence-specific fashion, we will search the conserved sequences of *T. brucei* for the presence of the regions that are likely non-coding RNA genes, using QRNA. Then, using the newly developed MC-Fold, we will find candidates that have similar structural characteristics to known miRNAs. In addition, we hypothesize that we can identify miRNA sequences that are complementary to the regulatory motifs that we predicted in the untranslated regions of *T. brucei* genes. Finally, we will validate the potential miRNAs and regulatory motifs using reporter genes or RNase H activating anti-sense DNA oligonucleotides. Our preliminary studies show that, using the mentioned algorithms, we are able to achieve high specificity as well as sensitivity for computational prediction of miRNA genes in *T. brucei*.

7. Nicholas Patocka and Paula Ribeiro. Institute of Parasitology, McGill University.

Serotonin signaling in *Schistosoma mansoni*, identifying the players involved.

Serotonin (5:hydroxytryptamine: 5HT has been shown to be an important modulator of neuromuscular function and metabolism in flatworms, including the bloodfluke *Schistosoma mansoni*. Exogenous application of 5HT to intact schistosomes causes contraction of the body wall musculature and a robust increase in motor activity. It is unknown at present if the exogenous 5HT is acting on surface (tegumental) receptors via the worm's sensory nervous system, or if it is transported by a surface carrier to act on internal receptors. Previous work showed the presence of a 5HT - specific transporter (SERT) in S. mansoni. The parasite SERT was shown to mediate the uptake of exogenous 5-HT in live parasites (Patocka and Ribeiro, 2007), suggesting it may be located on the surface. In addition to this transporter, we have recently identified two 5HT-like receptor sequences in schistosomes. One of these receptors was cloned and shown to respond to 5HT through the activation of cAMP when expressed in mammalian cells. Localization of the receptor in vivo shows it to be situated on the tegument, namely the tubercles of the adult worm, as well as weakly on day 0 schistosomula. In order to determine if either the SERT or 5HT-like receptor are mediating behavioural responses to exogenous 5HT, we developed an assay to test for mobility in cultured schistosomula. Using live imaging, we were able to quantify movement of schistosomula in the presence and absence of exogenous 5HT. Parasites are now being treated with known SERT or 5HT receptor blockers to test whether any of these drugs can inhibit the response to 5HT. The results of these pharmacological studies will be discussed.

8. Antonio Ruzzini and Janet Yee. Biochemistry Program, Trent University.

Studies of relative gene expression during the cell cycle of *Giardia lamblia*.

The persistence and severity of giardiasis depends on the extent of proliferation within the host's digestive tract. Furthermore, transmission of this disease depends on cellular differentiation of trophozoites within the small intestine. Cellular division and differentiation proceed via the cell cycle, with differentiation occurring at a specific point in the cycle. The cell cycle is under tight and timely regulation by specific proteins, several of which are regulated at the level of transcription. Data will be presented on our test of several drugs on the cell cycle of *Giardia*. We evaluated the ability of each drug to block the growth of *Giardia* trophozoite cultures, and the ability of these cultures to resume the cell cycle upon drug removal. The drugs that were identified to be the most effective in these "block-and-release" experiments were subsequently used to synchronize *Giardia* cultures for time-course experiments. At regular time intervals after the removal of the drug, aliquots of synchronized cultures were analyzed by flow cytometry to determine the cell cycle stage. RNA was extracted from another aliquot of cells removed at each time point, and this RNA was used in qRT-PCR assays. We determined the relative expression of genes expected to be regulated by the cell cycle, and genes expected to be constitutively expressed. The results and significance of these experiments will be discussed.

9. Jonathan Solomon, Joe Nabhan and Timothy Geary. Institute of Parasitology, McGill University.

The localization and in vitro detection of *Brugia malayi* secreted proteins.

Lymphatic filariasis (LF) is caused by the parasitic nematodes *Wucheria bancrofti*, *Brugia malayi* and *B. timori*. Over 120 million people around the world in 83 different countries are affected. LF is a debilitating disease that causes elephantiasis, lymphedema and genital pathology. Products secreted or excreted (S-E) by parasitic nematodes may contribute to the processes of infection and tissue migration of the parasite, modulating the host immune response and host physiology. These S-E products allow the parasite to persist and survive in conditions that would otherwise bar or destroy them. Four known secreted proteins of *B. malayi* have been cloned, expressed in *E. coli* and purified. These proteins were chosen because they have been reported to be secreted and play a role in immune evasion. These proteins include: macrophage inhibitory factor 1 (MIF-1), macrophage inhibitory factor 2 (MIF2), a tumor protein homologue (TPH-1) and a cysteine protease inhibitor (CPI-2). Antibodies were raised to all of these secreted proteins. The secreted protein CPI-2 has been localized to a specific region of microfilariae of *B. malayi* and the basic anatomy of this parasitic stage has been observed by means of confocal microscopy. The protein was found to be localized to the secretory pore, which appears to be regulated by musculature. Biotinylated Sandwich ELISAS have been developed to detect these secreted proteins in cultures of various stages and sexes of *B. malayi*. The standard curve for detection of purified TPH-1 ranges from 1000 to 3.2 ng/ml, and the protein has been detected in female worm ES with this assay. Other standard curves are currently being optimized and tested. Further studies will be conducted with the assays already established to determine what role, if any, the anthelmintic drug ivermectin plays in the secretion of these proteins and if the structure that retains them is regulated by this drug.

10. Rona Strasser and Jardim, Armando. Institute of Parasitology, McGill Univ.

Analysis of the *Leishmania donovani* glycosomal membrane proteome.

Leishmaniasis is a disease that affects approximately 12 million people in tropical and sub-tropical regions. The etiological agents of disease are the protozoan parasite *Leishmania*. These organisms contain a unique subcellular organelle called a glycosome. The glycosome compartmentalises several key metabolic and biosynthetic pathways, including glycolysis, that are crucial for parasite viability. The faithful trafficking of enzymes in these pathways to the glycosome is crucial for parasite survival. In *Leishmania*, proteins destined for the glycosome are translated on cytosolic ribosomes and rapidly bound by the receptor proteins peroxin 5 or 7 (LdPEX5 or LdPEX7), which escort the nascent polypeptides to the membrane surface of the glycosome. These cargo protein-receptor complexes then bind to peroxin 14 (LdPEX14), a glycosome membrane associated protein that mediates protein import into the organelle, by a mechanism that has not yet been described. Whether additional glycosomal membrane proteins are involved in the translocation of folded proteins across the lipid bilayer is unclear. To address this question a process has been developed to produce highly purified glycosomal membranes that will be used to examine the glycosomal membrane proteome. These membrane preparations will also provide the starting material to define other proteins that selectively interact with LdPEX14 and LdPEX5.

11. Amira Taman and Paula Ribeiro. Institute of Parasitology, McGill University.

Identification of a novel dopamine receptor in *Schistosoma mansoni*.

Schistosoma mansoni is a parasitic flatworm that infects 200 million people worldwide and causes about 130,000 deaths per year. Schistosomiasis is among the top five disease priorities of the WHO. At present there is only one drug treatment for schistosomiasis, (praziquantel), and this is increasingly threatened by the prospect of drug resistance. Thus there is an immediate need to identify new drug targets and to develop new therapies. The worm nervous system, especially neurotransmitter receptors, is good targets for schistosomicidal drugs. Previous studies have shown that the neurotransmitter dopamine plays a key role in the control of neuromuscular function in flatworms and thus could prove to be a useful target for drugs aimed at paralyzing the worm. Dopamine receptors have been identified in nematodes and free-living flatworms (Planaria) but not schistosomes, although tyrosine hydroxylase, the rate limiting enzyme in dopamine biosynthesis has been cloned from adult *S. mansoni*. Here we report the cloning of the first dopamine receptor from *S. mansoni*. The receptor has the classical heptahelical topology of a class A (rhodopsin-like) G protein-coupled receptor (GPCR), including many of the sequence motifs associated with GPCR activity, but its overall homology with mammalian dopamine receptors is low. Of note is an exceptionally long 3rd intracellular loop that has several low-complexity motifs, not seen in mammalian receptors. Expression of the full length cDNA in mammalian HEK-293 cells revealed that the receptor is responsive to dopamine in a dose-dependent manner, whereas other structurally related amines had no effect; the same results were obtained by expressing the cDNA encoding the receptor protein in the yeast strain *Saccharomyces cerevisiae*. A preliminary pharmacological study suggests the *S.mansoni* dopamine receptor has a unique drug profile and does not recognize several classical dopaminergic antagonists. Full pharmacological characterization and localization of the receptor in the intact worm are still in progress.