Ninth Annual
Quebec Molecular Parasitology Symposium

9ième Symposium Annuel
de Parasitologie Moléculaire du Québec

18th and 19th June, 2009
Leacock Building
McGill University
Department of Microbiology and Immunology
Montréal, Québec

PROGRAM

Thursday, June 18 (Room Leacock 219)

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

9:10-10:00 **Keynote speaker: Dr. Edward Pearce** (University of Pennsylvania, USA)

"Th2 cell responses during chronic schistosomiasis – how does the immune system deal with unresolved infection?"

10:00-10:15 **Coffee break** (Leacock Lobby)

Session 1 (Room Leacock 219): Signal Transduction/Host-Pathogen Interaction
(Chair: Dr. Benoit Cousineau)

10:15-10:40

1. Adrien F. Vinet, Mitsunori Fukuda, Salvatore J. Turco, and Albert Descoteaux: The *Leishmania donovani* lipophosphoglycan excludes the vesicular proton-ATPase from phagosomes by impairing the recruitment of Synaptotagmin V.

10:40-11:05

2. Irazú Contreras, Marina Tiemi Shio, Philippe A. Tessier and Martin Olivier: Macrophage's microbicidal functions induced by MRPs 8/14 are inhibited by *Leishmania* parasites.

11:05-11:30

3. Christelle Gabriel, Denis Girard, Albert Descoteaux: *Leishmania* promastigotes induce and are killed by Neutrophil Extracellular Traps.

11:30-11:55

4. Karen K. Yam, P. Pouliot, F. Hugentobler, A.M. Stern, J-D. Lalande, G. Matlashewski, M. Olivier, B. Cousineau: Generation and evaluation of A2 and LACK Expressing *Lactococcus lactis* Live Vaccines against Leishmaniasis.

LUNCH (11:55-13:30) (Leacock Lobby)

Session 2 (Room Leacock 219): Parasite Biology/Biochemistry
(Chair: Dr. Armando Jardim)

13:30-13:55

1. Normand Cyr, Armando Jardim: Biophysical characterization of the conformational changes taking place on peroxin 14 implicated in the glycosomal translocation mechanism of the parasite *Leishmania donovani*.

13:55-14:20

2. Larbi Dridi, Amin Ahmed Ouameur and Marc Ouellette: SAMT1 the first transporter of S-Adenosyl-methionine in *Leishmania* cells.

14:20-14:45

3. Rona Strasser, A.V. Pilar, J. McLean, N. Cyr, A. Jardim: Association of *Leishmania donovani* glycosomal docking protein Peroxin14 with receptor-cargo complexes modulates its conformation.

14:45-15:10

4. Wen-Wei Zhang, Greg Matlashewski: Expression of *L. donovani* specific genes in *L. major* and their effects on virulence.

15:10-15:35 **Coffee Break** (Leacock Lobby)

Session 3 (Room Leacock 219): Malaria Symposium
(Chair: Tatiana Scorza)

15:35-16:00

1. Petra Rohrbach: Live cell imaging of *Plasmodium falciparum*: Shedding light on biological processes.

16:00-16:25

2. Marina Tiemi Shio, Stephanie C. Eisenbarth, Myriam Savaria, Adrien F. Vinet, Marie-Josée Bellemare, Kenneth W. Harder, Fayyaz S. Sutterwala, D. Scott Bohle, Albert Descoteaux, Richard A. Flavell and Martin Olivier: NLRP3 inflammasome in malaria: role of hemozoin-induced signaling on inflammasome activation.

16:25-16:50

3. Jenny Miu, Maya Saleh, Mary Stevenson: Caspase-12 deficiency results in hyperinflammatory responses to lethal malaria but does not greatly alter the course of infection.

16:50-17:15

4. Mathieu Cambos, Martin Olivier, Armando Jardim and Tatiana Scorza: Hemoglobin uptake following red blood cell phagocytosis during *Plasmodium chabaudi adami DS* infection triggers macrophage apoptosis in a ROS dependent manner.

17:15-17:40

5. Floriana Berretta, Jessica St-Pierre, Ciriaco A. Piccirillo and Mary M. Stevenson: Expansion of $CD4^{+}Foxp3^{+}$ regulatory T cells in the spleen correlates with susceptibility to *Plasmodium chabaudi* AS in mice.

17:40-19:00 **Poster session and aperitifs** (University Centre Building, room 301)

19:00-21:00 **Dinner** (University Centre Building, room 301)

Friday, June 19 (Room Leacock 219)

9:00-9:50 **Keynote speaker: Dr. Dan Zilberstein** (Technion - Israel Institute of Technology)

"Functional genomics of amino acid transporters of trypanosomatid parasites."

9:50-10:10 **Coffee Break** (Leacock Lobby)

Session 4 (Room Leacock 219): Drug Resistance and Targets
(Chair: Paula Ribeiro)

10:10-10:35

1. Claudia M Wever, Patrick Janukavicius, Igor Putrenko Joseph A Dent: Validating Acetylcholine-Gated Chloride Channels as Novel Nematocide Targets.

10:35-11:00

2. Amira Taman and Paula Ribeiro: Investigation of a dopamine receptor in *Schistosoma mansoni:* Functional studies and immunolocalization.

11:00-11:25

3. Daniel Feingold, Joseph A. Dent, Laura Nilson: Characterization of three novel ligand gated ion channel subunits – Potential pesticide targets?

11:25-11:50

4. Dalton, John P.: Malaria neutral aminopeptidases: targets for new anti-malarials?

11:50-12:15

5. Vijayaraghava TS Rao, Salma Z Siddiqui, Roger K Prichard and Sean G Forrester: Dopamine as a neurotransmitter in *Haemonchus contortus.*

LUNCH (12:15-13:50) (Leacock Lobby)

Session 5 (Room Leacock 219): Molecular and Cellular Aspects of Infection and Immunity (Chair: Dr. Petra Rohrbach)

13:50-14:15

1. Ravendra Garg, Corinne Barat, Michel Ouellet, Robert Lodge, Michel J. Tremblay : *Leishmania infantum* Amastigotes Enhance HIV-1 Replication in Cocultures of Human Dendritic Cells and Autologous $CD4^+$ T Cells by Inducing Secretion of IL-6 and TNF-α.

14:15-14:40

2. Pranav Kumar, Robert Lodge, Nathalie Trudel, Michel Ouellet, Marc Ouellette, Michel J. Tremblay: Nelfinavir, a HIV-1 protease inhibitor, induces oxidative stress and caspase-independent apoptosis in *Leishmania* amastigotes.

14:40-15:05

3. Maurice Odiere, Kris Koski, Marilyn Scott: Protein deficiency during pregnancy and lactation impairs resistance to GI nematode and modifies body composition and neonatal growth in CD1 mice.

15:05-15:25 **Coffee Break** (Leacock Lobby)

15:25-15:50

4. Joella Joseph and Janet Yee: Functional Characterization of a Recombinant TATA Binding Protein (TBP) and a TBP Interacting Protein in *Giardia lamblia.*

15:50-16:15

5. Hamed Shateri Najafabadi, Reza Salavati: Functional annotation of the genome of *Trypanosoma brucei* based on regulatory elements of coding sequences and untranslated regions.

16:15 **Closing**

POSTERS

IMMUNE RESPONSE AND HOST-PATHOGEN INTERACTION

1. Abu Dayyeh Issa, Cousineau Benoit, and Olivier Martin: *Leishmania*-Induced IRAK-1 Inactivation is Mediated by SHP-1 Interacting with an Evolutionarily Conserved KTIM motif.

2. Stefany Bazinet, M. Cambos, J. Sanchez Dardon, M. Olivier and T. Scorza: Hemozoin and Hemin modulate IL-12 responses through distinct mechanisms in bone marrow derived macrophages.

3. Pampa Bhaumik, Christian St-Pierre and Sachiko Sato: Role of Galectins in the Innate Immune Response to Leishmaniasis.

4. Stephanie Goyette, Momar Ndao, Brian J. Ward, and Florence S. Dzierszinski: An animal model for reactivated *Toxoplasma gondii* infection.

5. Felix Hugentobler, Karen K. Yam and Benoit Cousineau: Development of a new generation of live vaccines against leishmaniasis using the Gram-positive bacterium *Lactococcus lactis.*

6. Laura-Isobel McCall, Greg Matlashewski: Exploring the interaction between *Leishmania donovani* virulence factor A2 and host macrophages.

7. Fikregabrail Aberra Kassa, Marina Tiemi Shio**,** Marie-Josée Bellemare, Momar Ndao and Martin Olivier: Proteomic analysis of serum proteins from malaria patients and identification of proteins binding to hemozoin.

8. Mukesh Samant, Reema Gupta Shraddha Kumari Pragya Misra, Prashant Khare, Pramod Kumar Kushawaha, Amogh Anant Sahasrabuddhe, and Anuradha Dube: Immunization with the DNA-encoding N-terminal domain of Proteophosphoglycan of *Leishmania donovani* generates Th1-Type immunoprotective response against experimental visceral leishmaniasis.

9. T. Scorza, D. Malu Tshikudi, O. Kevorkova, J. Sanchez Dardon and R. Moreau: Characterization of novel interactions between immune cells and bone metabolism during acute malaria infection.

10. Diane Tshikudi Malu, B. Belanger, J. Sanchez Dardon, A. Satoskar and T. Scorza: Macrophage Migration Inhibitory Factor (MIF) modulates the activation of T cells during early and late infection with *Plasmodium chabaudi adami* parasites.

BIOCHEMICAL AND MOLECULAR PARASITOLOGY

1. Sirin Chaker, Avinash Thadani, Sirinart Ananvoranich: Growth Inhibition of *Toxoplasma gondii* by Securinine and its derivatives.

2. Felipe Dargent, Marilyn E. Scott & Gregor F. Fussmann: Evolution of tolerante and resistance on the *P. reticulata – Gyrodactylus turnbulli*. host parasite system.

3. Vanessa Dufour, Robin N. Beech, Timothy G. Geary: Benzodiazepines and schistosomes: molecular pharmacology of a potential drug target.

4. Pablo Godoy and Roger K. Prichard: Functional analysis of *Haemonchus contortus* P-glycoproteins and interaction with macrocyclic lactones.

5. Michael Holmes, Urszula Liwak, Xiang Wang, Stanislas Tomavo, Sirinart Ananvoranich: Successful knock-down of enolase isoform 2 produced a subtle phenotypic change.

6. Michael Holmes, Sirinart Ananvoranich: Characterization of translational regulation of lactate dehygrogenase 1 in *Toxoplasma gondii*.

7. Smriti Kala, Reza Salavati: Identification of the KREPA4 RNA binding domain and elucidation of its biochemical role in RNA editing.

8. Jose Loaiza, Marilyn Scott, Eldredge Bermingham, Jose Rovira, Jan Conn: Mitochondrial *CO1* gene provides evidence for a Pleistocene expansion of *Anopheles albimanus* in eastern Panama.

9. Mirna Nascimento, Sara Gosline, David Thomas, Michael Hallett and Greg Matlashewski: Absence of conventional Unfolded Protein Response in Parasites; A Potential Therapeutic Target for Leishmaniasis.

10. Amber Olson, Kin Chan, Tom Edge, Chi-Yip Ho, and Janet Yee: Gene expression profiling of *Giardia lamblia* isolates.

11. Nicholas Patocka, Paula Ribeiro: Serotonin in Action; Dissecting Serotonin Signaling in *Schistosoma mansoni.*

12. Rushini Perera, Robin Beech**:** Gene duplication among the ligand-gated ion-channels in the genome of *Haemonchus contortus*.

13. Andrea Rauba, Mike Osei-Atweneboana, Roger Prichard: Macrocyclic lactone selection on ABC-transporter genes in *Onchocerca volvulus* from Ghana.

14. Marie-Claire Rioux, Tim Geary and Terry Spithill: Comparative proteomic analysis of *Fasciola hepatica* and *Fasciola gigantica* newly excysted juvenile and adult excretory-secretory products.

15. Isabelle Rouiller, Kelly Sears, Hojatollah Vali: The McGill Facility for Electron Microscopy Research (FEMR).

ABSTRACTS

Abu Dayyeh, Issa, Cousineau, Benoit, and Olivier, Martin. 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.

Stefany Bazinet[1], M. Cambos[1], J. Sanchez Dardon[1], M. Olivier[2] and T. Scorza[1]. [1]Departement of Biological Sciences, UQAM; [2]Departement of Microbiology and Immunology, McGill University.

Hemozoin and Hemin modulate IL-12 responses through distinct mechanisms in bone marrow derived macrophages.

Hemozoin (HZ), issued from the conversion of heme in protoporphyrin IX crystals and protecting *Plasmodium* parasites against free heme toxicity, modulates the inflammatory function in macrophages by oxidative stress dependent and independent mechanisms. We compared the oxidant properties of HZ and hemin (HE) as well as their capacity to modulate IL-12 (p40 and p70) and IL-10 responses in bone marrow derived macrophages (BMDM). Our data suggests that in respect to HE, HZ is weak inducer of reactive oxygen species (ROS) and interestingly, drastically decreases ROS, and increases total glutathione and intracellular GSH/GSSG ratio once accumulated for 16-18 hours. Albeit sharing with HE moderate inhibitory effects on IL-12p40 production, HZ inhibits in a more pronounced manner the IL-12p70 response to LPS and IFN-gamma/LPS, hampering also IL-10 secretion. Treatment with N-acetyl-cysteine (NAC) or neutralization of IL-10 significantly corrects the inhibition induced by HE, without modifying the detrimental effects of HZ. P38 MAPK inhibition blocks IL-10 responses and significantly restores IL-12p70 responses to IFN-gamma/LPS in HE-conditioned BMDM, failing to modify the effect of HZ. In conclusion, our data suggests that in contrast to HE, HZ inhibits IL-12p70 through a mechanism independent of oxidative stress or IL-10 induction through the p38 MAPK pathway. This work has been supported by a Discovery grant (NSERC).

Floriana Berretta[1,2], Jessica St-Pierre[2], Ciriaco A. Piccirillo[1,2] and Mary M. Stevenson[1]. [1]Departments of Medicine and [2]Microbiology & Immunology, McGill University

Expansion of CD4$^+$Foxp3$^+$ regulatory T cells in the spleen correlates with susceptibility to *Plasmodium chabaudi* AS in mice.

Foxp3$^+$ regulatory T cells (Tregs) are important in the maintenance of peripheral T cell tolerance as well as in regulating immunity to pathogens including *Plasmodium*, the causative agent of malaria. Studies indicate that Tregs may proliferate in response to various inflammatory conditions; expansion of these cells may be required for their suppressive activity *in vivo*. Whether Tregs expand in response to malaria infection remains unknown. Recently, we observed significantly higher blood parasitemia and reduced survival in transgenic mice over-expressing *Foxp3* (Foxp3Tg) compared to wild-type (WT) C57BL/6 (B6) mice following *P. chabaudi* AS (PcAS) infection, a finding confirmed by adoptive transfer of CD4$^+$Foxp3$^+$ T cells from naïve Foxp3Tg mice to recipient WT mice. Here, we investigated the contribution of Tregs to lethal PcAS infection in A/J mice. FACS analysis revealed higher frequencies of proliferating splenic Tregs in susceptible A/J compared to resistant B6 mice in association with increasing blood parasitemia during acute infection. *In vivo* proliferation of effector CD4$^+$ T cells in the spleen of infected A/J was observed to be lower compared to B6 mice and was inversely correlated with the number of splenic Tregs at days 5 and 7 post infection (p.i.). We also analyzed NK cell proliferation and IFNγ secretion during early PcAS infection. The increased number of splenic Tregs in susceptible A/J mice was coincident with a lower frequency of proliferating NK cells and of CD3$^-$DX5$^+$IFNγ$^+$ cells at day 5 p.i. compared to resistant B6 mice. There was also a decrease in B cell proliferation *in vivo* in the spleen of A/J mice but not B6 mice during infection. Together, these findings indicate that Tregs impair the development of protective Th1 responses to malaria by suppressing innate and adaptive immunity. (Supported by FQRNT Projet de recherche en équipe (MMS, CAP) and CIHR (CAP).

Pampa Bhaumik, Christian St-Pierre and Sachiko Sato. Glycobiology Laboratory, Research Center for Infectious Diseases, CHUL-CHUQ, Laval University, Quebec City, Canada

Role of Galectins in the Innate Immune Response to Leishmaniasis.

Galectins are a class of novel carbohydrate (beta-galactoside) binding animal lectins that have been shown to be involved in key events of the immune and inflammatory response. The role of galectins in the context of leishmaniasis, however, remains undefined. We have previously shown that lack of galectin-3 (chimera-type galectin) affected the leukocyte recruitment in infected lungs during infection by *Streptococcus pneumoniae*. Using galectin-3 Knock-Out mice, we have been able to detect a similar defect in leukocyte especially in neutrophil recruitment in the case of cutaneous infection by *Leishmania major*. Interestingly, the lack of availability of these innate immune cells seem to alter the course of *L. major*-induced pathogenesis as a result of a modulation in the development of immunity towards the parasite. This is in addition to our work that showed the involvement of galectin-3 in the species-specific recognition of leishmania. Our current work involves a way of characterizing the importance of host galectins in regulating the early events of *L. major* infection and in determining the nature of response that lead to the outcome of disease. This work is supported by the CIHR and FQRNT.

[1]Mathieu Cambos, [2]Martin Olivier, [2]Armando Jardim and [1]Tatiana Scorza. [1] Département Sciences Biologiques de l'UQAM,[2] Parasitology Institute McGill University.

Hemoglobin uptake following red blood cell phagocytosis during *Plasmodium chabaudi adami DS* infection triggers macrophage apoptosis in a ROS dependent manner.

Macrophages (MP) play a crucial role in eliminating parasitized red blood cells (RBC) and initiating specific immune response against *Plasmodium* parasites during blood stage malaria. While fighting the infection, MP phagocytose numerous parasitized as well as damaged RBC and accumulate the malaria pigment Hemozoin (HZ). This phagocytosis could be responsible for MP apoptosis measured during *Plasmodium* infections.

Herein, we evaluated the ability of RBC recovered from naïve (NRBC) or *P.c adami DS* infected Balb/c mice (DSRBC) as well as synthetic HZ to induce apoptosis in Bone Marrow Derived Macrophages (BMDM) and in J774 monocyte/macrophage cells. Our results indicate that BMDM and J774 ingest important amounts of DSRBC and HZ but few NRBC. Whereas incubation with NRBC or uptake of HZ did not affect MP viability, phagocytosis of DSRBC induces significant MP apoptosis in a dose dependent manner. MP apoptosis seemed consequent to oxidative stress as uptake of DSRBC induces reactive oxygen species (ROS) and treatment with antioxidants (NAC and GSH) partially corrected it. Oxidation of NRBC with $CuSO_4$ and L-ascorbate significantly enhanced their phagocytotsis and induced MP apoptosis, suggesting that cell death can be induced by RBC specific components. Moreover, treatment of J774 with increasing concentrations of Hemoglobin (Hb) induced MP apoptosis, whereas uptake of DSRBC Ghosts (Hb free) did not affect phagocyte viability. In conclusion, ingestion of Hb following DSRBC phagocytosis is responsible for MP apoptosis during *Plasmodium* infection through a ROS dependent mechanism.

Sirin Chaker, Avinash Thadani, Sirinart Ananvoranich. Department of Chemistry and Biochemistry, University of Windsor.

Growth Inhibition of *Toxoplasma gondii* by Securinine and its derivatives.

Toxoplasma gondii is an apicomplexan parasite with worldwide distribution. The outcomes of *Toxoplasma* infections in humans range from severe in neonates and immunocompromised individuals to asymptomatic in immune-competent hosts. Current medications for the prevention and treatment of *Toxoplasma* infection are the combinations of pyrimethamine, sulfadiazine and/or clindamycin. These medications have potential side effects, including bone marrow suppression, liver toxicity and diarrhea. More effective and less harmful chemotherapy is thus needed. Securinega alkaloids, produced by the *Securinega* and *Phyllanthus* species of the *Euphorbiaceae* plant, have been shown to have potent biological activities, including antitumor, antimalarial, and antibacterial effects. Recently, novel derivatives of securinega alkaloids have been synthesized. Here we investigate the usefulness of these novel compounds as anti-*Toxoplasma* agents by testing them for *in vitro* efficacy against different steps of the lytic cycle of *Toxoplasma* tachyzoites. Using the tachyzoites constitutively expressing β-galactosidase and human fibroblast host cells, the compounds will be evaluated for parasite growth inhibition and cytotoxicity, inhibition of replication and inhibition of parasite invasion of host cells.

Irazú Contreras[1], Marina Tiemi Shio[1], Philippe A. Tessier[2] and Martin Olivier[1]. [1]Department of Microbiology and Immunology, McGill University, Montreal QC, Canada; [2]Centre de Recherche en Infectiologie, Universite Laval, Quebec, QC, Canada.

Macrophage's microbicidal functions induced by MRPs 8/14 are inhibited by *Leishmania* parasites.

The myeloid-related proteins (MRPs) belong to the S100 proteins. Among MRPs, the MRP-8 and MRP-14 exhibit antimicrobicidal and pro-inflammatory properties. We and others have previously reported that MRPs can induce microbicidal phagocyte functions, such as iNOS expression and its subsequent nitric oxide (NO) production. MRPs trigger activation and phosphorylation of several MAP kinases as well as rapid NF-κB nuclear translocation (NT) concurring to induce pro-inflammatory cytokines. These signaling pathways are involved in the control of parasitic infections such as Leishmaniasis. On the other hand, *Leishmania* survives within phagocytes, strongly blocks NO production and induces a rapid dephosphorylation of MAPKs. However, the role of MRPs in the innate immune response upon *Leishmania* infection and survival within the macrophages is still unrevealed. Here we showed that priming of macrophages with different doses of MRPs prior to infection with *Leishmania major* was favoring NO production comparable with the levels found in macrophages stimulated *per se* with MRPs. However, *L. major*-infected macrophages prior to MRPs stimulation showed a significant decrease of NO production, as well as a reduced iNOS protein expression. In parallel, killing assays showed that priming of macrophages with MRPs reduced the parasitic load after 24 hrs by 25% in a dose dependent manner. We also investigated NF-κB, STAT1α and AP-1 NT in the same context. EMSAs showed that primed microphages could induce the translocation of NF-κB and AP-1 at comparable levels to these cells only stimulated with MRPs. However, infected cells prior to MRP stimulation showed reduced NT of these transcription factors. Our preliminary results showed that in the mouse air-pouch model there is secretion of MRPs 8/14 by the neutrophils recruited to the air-pouch after *Leishmania* infection and LPS stimulation possibly helping to control the progression of the infection. Collectively, our results allow to postulate that whereas rapid secretion of MRPs by neutrophils at site of infection may concur to protect uninfected macrophages from *Leishmania* infection during the innate immune response, it is clear that this pathogen has developed strategies to rapidly abrogate the host immune machinery induced by MRPs and therefore favoring its installment and propagation within its mammalian host.

Normand Cyr, Armando Jardim. Institute of Parasitology, McGill University.

Biophysical characterization of the conformational changes taking place on peroxin 14 implicated in the glycosomal translocation mechanism of the parasite *Leishmania donovani*.

The protozoan parasite *Leishmania*, and other kinetoplastids, compartmentalize glycolysis, and other vital metabolic pathways, in the glycosome, an organelle that is evolutionarily related to peroxisomes of higher eukaryotes. The translocation of enzymes across the glycosomal membrane is dependent on a class of peroxisomal biogenesis proteins known as peroxins which includes peroxin 14 (PEX14), a peripheral membrane protein anchored to the cytosolic face glycosome where it functions as a docking platform. Previous studies have demonstrated that proper targeting of proteins to the glycosome is essential for parasite and represents an interesting therapeutic target. However, little is known about the structural aspects involved in the translocation of proteins across the glycosomal membrane. Previous quaternary structure analysis revealed that *L. donovani* PEX14 forms a large homo-oligomeric structure that is > 670 kDa in solution. Furthermore, attachment of LdPEX5 to LdPEX14 caused marked conformational changes, including the solvation of a predicted hydrophobic region, which is hypothesized to penetrate into the glycosomal membrane. Indeed, spectrofluorometric analysis of a PEX14-phospholipid bilayer complex revealed that this ~30 amino acid stretch migrates to a more non-polar environment. This opens the door to a better understanding of the structural implications in the glycosomal translocation machinery.

Dalton, John P. Institute of Parasitology, McGill University.

Malaria neutral aminopeptidases: targets for new anti-malarials?

Malaria is a significant cause of morbidity and mortality worldwide with an estimated 1-2 million people dying from this disease every year. Drug resistance is widespread, and with a safe and effective vaccine still many years away, new chemotherapeutic agents are required to ensure that cheap and effective treatment remains widely available. We have recently validated two neutral aminopeptidases of the most lethal human malaria parasite, *Plasmodium falciparum (Pf)*, as targets for the development of novel antimalarial drugs. These enzymes (*Pf*M1AAP and *Pf*M17LAP), are located within the cytoplasm and we suggest that they act in the final stages of haemoglobin catabolism, generating amino acids that are essential for parasite growth and development. They have overlapping but distinctive functions and therefore may have additional role to play.

Lead inhibitors of both enzymes are lethal to *P. falciparum* in culture, and kill the murine malaria *P. chabaudi in vivo*. Lead optimization studies are now underway and are being guided by detailed biochemical, structural and functional studies together with structure-activity relationships identified by high throughput inhibition screening. Here I will discuss the rationale for choosing the *Pf*M1AAP and *Pf*M17LAP as targets for anti-malarial development and discuss our recent advances towards this goal.

Felipe Dargent, Marilyn E. Scott & Gregor F. Fussmann. Department of Biology, McGill University, Montréal, Canada QC H3A 1B1; Institute of Parasitology, McGill University, Ste. Anne de Bellevue, Canada QC H9X 3V9.

Evolution of tolerante and resistance on the *P. reticulata – Gyrodactylus turnbulli*. host parasite system.

The two main components of host antipathogen defense are tolerance and resistance, which act by reducing the fitness decreasing effects of an infection. Resistance reduces the fitness of parasites while tolerance involves a reduction of the effects of disease on the host, thus their expression is expected to tradeoff if both are costly to express. Although their evolution and expression has been explored in plants, research on animals is scarce. My research focuses on understanding the rate and nature of the evolution of these components, as well as parasitism's significance as a major evolutionary driver interacting with predation pressure in the Trinidadian guppy (*Poecilia reticulata*) system and their natural co-occurring Monogenean ectoparasite *Gyrodactylus turbulli*. Six generations of *P. reticulata* from four populations with differences in parasite burden and predation pressure are subject to experimental infections and challenge infections to assess the evolution of tolerance and resistance through health and fitness indicators of the host in response to parasite population dynamics.

Larbi Dridi, Amin Ahmed Ouameur and Marc Ouellette. Centre de Recherche en Infectiologie, Université Laval.

SAMT1 the first transporter of S-Adenosyl-methionine in *Leishmania* cells.

S-Adenosyl-methionine (SAM, also known as AdoMet) is an important biological methyl donor. It is also the precursor of aminopropyl groups utilized in polyamine biosynthesis and also involved in the glutathione and trypanothione synthesis in trypanosomatids by the *trans*-sulfuration pathway of cysteine. The methionine adenosyltransferase is an important enzyme for the metabolic synthesis of SAM in *Leishmania*, but the parasite is also able to acquire SAM by active transport from the outside. Sinefungin (SF), a nucleoside antibiotic produced by *Streptomyces griseolus* is structurally related to AdoMet and very effective against strains of *Leishmania*. To identify the SAM transporter, we have generated in vitro SF resistant *Leishmania major LV39* strain. We have observed in this mutant a loss of SAM accumulation related to SF resistance. Resistance to SF was correlated with gene rearrangement of some members of the folate/biopterin transporter. One of these genes *SAMT*, was found by gene transfection, gene inactivation and transport studies to be the main SAM transporter. Intriguingly, not all *Leishmania* strains are SF sensitive and this does not appear to be related to SNPs in *SAMT* but to gene expression level. This study, has allowed the characterization of the first SAM importer in parasites.

Vanessa Dufour, Robin N. Beech, Timothy G. Geary. Institute of Parasitology, McGill Univ.

Benzodiazepines and schistosomes: molecular pharmacology of a potential drug target.

Praziquantel (PZ) is currently the only drug commercially available for the treatment of schistosomiasis. However, suboptimal efficacy of the drug against schistosomes has been reported in Egypt and Senegal. Alternatives treatments must be developed as soon as possible to be deployed when resistance to PZ becomes widespread. Activity in a chemical series already shown to be tolerated in humans likely offers the fastest developmental route for a new drug Anterior studies have shown that 3-methylclonazepam (3MC), a benzodiazepine (BZ), exhibit potent schistosomicidal activity against S. mansoni. 3MC seems to compromise neuromotor activity in S. mansoni, possibly by altering GABAA receptor (GABAAR) activity of the worm, reminiscent of BZ activity in humans. Synthesis and distribution of GABA have been demonstrated in schistosomes, although no $GABA_AR$ have been characterized to date in this parasite. In addition, BZD modulation in schistosomes appears to be distinct from both nematodes and mammals. We aim to determine BZD molecular target of and mode, of action in schistosomes. The analysis of the *S.* mansoni genome has revealed 4 genes encoding putative $GABA_AR$ subunits. We are currently assessing the complete sequence of these genes and cloning the full length cDNA. Ultimately, we will characterize the pharmacological properties, the modulation by BZD and the functional roles in of these BZD receptors in *S. mansoni*. This work was supported by FQRNT.

Daniel Feingold, Joseph A. Dent, Laura Nilson. Department of Biology, McGill Univ.

Characterization of three novel ligand gated ion channel subunits – Potential pesticide targets?

Cys-loop ligand gated ion channels (LGICs) are pentameric neurotransmitter receptors that are ubiquitous in both vertebrate and invertebrate nervous systems. Their diversity as well as their central role in mediating rapid synaptic transmission has made these channels attractive targets for pesticides. Despite the relative success of such pesticides, issues regarding drug specificity and resistance continue to pose serious problems in regions that rely on pesticides for crop protection and prevention against disease. We are characterizing three novel Cys- loop LGIC subunits; CG7589, CG6927 and CG11340 in *Drosophila melanogaster*. These genes are of particular interest because they exhibit little homology among invertebrates and do not possess any orthologs in vertebrate systems (Dent, 2006). Consequently, pesticides that target channels formed by these genes are predicted to be safe and have low risk for cross-resistance with existing compounds. In order to determine if these LGIC subunits would be suitable drug targets, we generated putative knockouts of all three genes. Preliminary evidence suggests that mutations in CG7589 are semi-lethal. Gene expression profiles have been examined via *in situ* hybridization. CG7589 and CG11340 are expressed in the midgut and tubules, while CG6927 is expressed in the ovaries. Electrophysiological experiments conducted in *Xenopus* oocytes reveal that CG11340 forms a homomeric channel that is sensitive to pH. Similarly, CG7589 and CG6927 form a heteromeric channel that is pH sensitive. CG7589 also forms a homomeric channels that appears to be inhibited by Tyramine. Based on the relative divergence of these genes from other Cys-loop LGIC subunits, as well as the potential semi-lethal phenotype associated with deletions in CG7589, these putative LGIC subunits may provide a promising target for the development of a novel class of highly selective and efficient pesticides.

Christelle Gabriel, Denis Girard, Albert Descoteaux. INRS-Institut Armand-Frappier.

***Leishmania* promastigotes induce and are killed by Neutrophil Extracellular Traps.**

The role of neutrophils during early stage of infection by *Leishmania* is not clear. Due to their rapid recruitment to the site of inoculation, neutrophils are the first immune cells to interact with the parasite. Recent evidence suggest that neutrophils can phagocyte promastigotes which interestingly survive into cells during few days. Initially, we investigated how promastigotes resist human neutrophil oxidative killing. Unexpectedly, we found that in contact to *Leishmania* promastigotes, neutrophils released structures which seemed to be Neutrophil Extracellular Traps (NETs). Production of NETs is a recent discovered microbicidal mechanism borrowed by neutrophils and is characterized by the release of DNA and some granular proteins from stimulated-neutrophils. This study was conducted to determine whether *Leishmania* promastigotes induce the production of NETs by human neutrophils and the impact of those structures on the parasite. Using confocal microscopy and DNA quantification we observed that independently strains and species, *Leishmania* quickly induced in a ratio dependant-manner the formation of NETs. Using defective promastigotes on lipophosphoglycan, we also observed that this important virulence factor was not involved in this process. Then, quantification of the promastigotes survival following contact with neutrophils showed an efficient killing of *Leishmania*. Thus, our results strongly describe NETs as a novel host response against *Leishmania*.

This work was supported by the Canadian Institutes of Health Research and the Canada Research Chair Program.

Ravendra Garg, Corinne Barat, Michel Ouellet, Robert Lodge, Michel J. Tremblay. Centre de Recherche en Infectiologie, Centre Hospitalier de l'Université Laval, and Faculté de Médecine, Université Laval, Québec (Québec), Canada

***Leishmania infantum* Amastigotes Enhance HIV-1 Replication in Cocultures of Human Dendritic Cells and Autologous $CD4^+$ T Cells by Inducing Secretion of IL-6 and TNF-α**

Visceral leishmaniasis has emerged as an important opportunistic disease among patients infected with HIV-1. Both HIV-1 and the protozoan parasite *Leishmania* can productively infect cells of the macrophage-dendritic cell lineage. Here we demonstrate that *Leishmania infantum* amastigotes increase HIV-1 production when human primary dendritic cells (DCs) are cocultured together with autologous $CD4^+$ T cells. Interestingly, the promastigote form of the parasite does not modulate virus replication. Moreover, we report that amastigotes promote virus replication in both cell types. Our results indicate that this process is due to secretion of parasite-induced soluble factors by DCs. Luminex micro-beads array system analyses indicate that *Leishmania infantum* amastigotes induce a higher secretion of several cytokines (i.e. IL-1α, IL-2, IL-6, IL-10 and TNF-α) and chemokines (MIP-1α, MIP-1β and RANTES) in these cells. Studies conducted with pentoxifylline and neutralizing antibodies revealed that the *Leishmania*-dependent augmentation in HIV-1 replication is due to a higher secretion of IL-6 and TNF-α. Altogether, these findings suggest that the presence of *Leishmania* within DC/T-cell conjugates leads to an enhancement of virus production and demonstrate that HIV-1 and *Leishmania* can establish complex interactions in such a cellular microenvironment.

Stephanie Goyette[1,2], Momar Ndao[2], Brian J. Ward[2], and Florence S. Dzierszinski[1] . [1]Institute of Parasitology, McGill University, [2]MUHC, McGill University.

An animal model for reactivated *Toxoplasma gondii* infection.

T. gondii is a ubiquitous parasite and a global zoonotic pathogen. Upon infection, lytic forms called tachyzoites multiply and spread to reach target organs, where they differentiate into dormant encysted bradyzoites. Bradyzoites sustain the chronic phase of infection and persist for the lifetime of the host. Although *T. gondii* infection typically causes mild disease in healthy adults, toxoplasmosis can be serious in the congenitally-infected fetus or immunocompromised host. Much of the morbidity and mortality associated with this organism occurs as a result of reactivation, but the mechanisms that lead to cyst rupture and recrudescent infection are poorly understood. We have established a murine reactivation model in order to investigate how *T. gondii* stimulates and interferes with the immune response in its vertebrate hosts and better understand the mechanisms that trigger reactivation. Briefly, to induce reactivation, chronically infected mice are treated with the dexamethasone until they show signs of disease. It has been previously established that T-cells are present at the site of infection and that these control latent *T. gondii* stages. However, their phenotype, antigen specificity, and the mechanisms by which they interact with cysts are unknown. In order to track these cells, we have generated transgenic *T. gondii* lines that express the model antigen ovalbumin (OVA). PruΔsag1P30OVA and PruΔbag1P30OVA secrete OVA under the control of tachyzoite and bradyzoite-specific promoters, respectively. The experimental reactivation model described above will be utilized in combination with these transgenic parasites in order to study T-cell kinetics during recrudescent *T. gondii* infection. Future directions include characterization of OVA-specific T-cells by staining with various immunological markers (*e.g.* activation, memory, *etc.*). We will also conduct proteomic studies using serum and CSF from experimentally reactivated mice to isolate parasite and host factors that interact and ultimately influence the outcome of infection.

Godoy, P. and Prichard, R.K. Institute of Parasitology, McGill University. Functional analysis of ***Haemonchus contortus*** **P-glycoproteins and interaction with macrocyclic lactones.**

There is consistent evidence of macrocyclic lactone (ML) drug resistance in the parasitic nematode, *Haemonchus contortus.* One of the mechanisms involved, is the over expression of P-glycoproteins (Pgps). These transmembrane proteins may efflux MLs, affecting the bioavailability and pharmacokinetic of these drugs. Additionally, drug transport assays in LLC-PK1 cells transfected with the murine gene *mdr1-a,* which over express Pgp, measuring the intracellular accumulation of the substrate Rhodamine 123, at increasing concentrations of MLs, found that Ivermectin (IVM) was a very potent inhibitor of Rhodamine efflux, based on the concentration for half maximal effect (IC_{50}). In *H. contortus* six Pgps have been described (from A to F). *Hc*Pgp-A, E and C have shown over expression in field and experimentally selected drug resistant strains. We have focused on *Hc*Pgp-A, cloning the full length sequence and making a construct for stable transfection in LLC-PK1 cells. The goal of this project is to transfect and over express this *Hc*Pgp-A in LLC-PK1 cells and see the interaction with increased concentrations of macrocyclic lactones. Preliminary results in drug transport assays controls in LLC-PK1 transfected with the murine gene *mdr1-a* show a consistent 10 folds higher inhibition of Rhodamine 123 transport by IVM in comparison with Moxidectin (MOX).

Michael Holmes, Urszula Liwak, Xiang Wang, Stanislas Tomavo, Sirinart Ananvoranich. Department of Chemistry and Biochemistry, University of Windsor.

Successful knock-down of enolase isoform 2 produced a subtle phenotypic change.

In *Toxoplasma gondii,* an intracellular parasite of the phylum Apicomplexa, two isoforms of enolase (*ENO1* and *ENO2*) are expressed in stage-specific manner. *ENO2* is expressed only in rapidly growing tachyzoites, while *ENO1* is in slowly growing bradyzoites. Interestingly the localization of *ENO1* and *ENO2* in the nuclear compartment has suggested possible roles of the proteins in gene regulation and/or cell cycle. To understand the physiological role of *ENO2* in *T. gondii*, the expression of *ENO2* was silenced using a homologous gene silencing procedure. Both transient and stable gene silencing of *ENO2* were successful at the levels of transcripts and proteins. While there was no change in the growth rate of both tachyzoites and bradyzoites, a subtle change in the parasite phenotype was observed in the localization of the enolase gene product in the bradyzoite stage. Moreover the effectiveness and specificity of homologous gene silencing will be discussed.

Michael Holmes, Sirinart Ananvoranich. Department of Chemistry and Biochemistry, University of Windsor.

Characterization of translational regulation of lactate dehygrogenase 1 in *Toxoplasma gondii*.

Toxoplasma gondii is an obligate intracellular protozoan parasite that infects any warm-blooded animals including over one third of humans worldwide. In healthy individuals, the immune system keeps the parasite in an asymptomatic state of non-proliferation called the bradyzoite stage. However, in people with compromised immune systems, this otherwise latent infection is able to alternate to the tachyzoite form and circulate freely in the blood stream, allowing the parasite to infect new cells. This is an important cause of brain lesions in diagnosed AIDS patients as well as neonates.

T. gondii expresses two isoforms of lactate dehydrogenase (LDH1 and LDH2). LDH1 is only present in the tachyzoite stage whereas LDH2 is expressed exclusively in bradyzoites. Interestingly, the mRNA of LDH1 is present in both stages. This suggests either a translational repression or posttranslational degradation system for LDH1. Although, both of these methods of stage-specific repression have been previously described in other protozoan parasites, no such mechanisms have been previously described in *T. gondii*. In order to determine which system is responsible for LDH1 regulation, we have generated stable lines with constructs in which the LDH1 open reading frame has been replaced with a reporter gene (GFP) all while maintaining the 5'- and 3'-untranslated regions (UTRs). Thus far, preliminary results suggest that translation of the reporter construct appears to be reduced or does not occur under bradyzoite conditions, therefore suggesting that the LDH1 UTRs are involved in a translational repression system. The results on the presence of *cis* elements and its role will be discussed.

Felix Hugentobler, Karen K. Yam and Benoit Cousineau. Department of Microbiology and Immunology, McGill University

Development of a new generation of live vaccines against leishmaniasis using the Gram-positive bacterium *Lactococcus lactis*

Lactococcus lactis is a non-pathogenic and non-colonizing Gram-positive lactic acid bacterium (LAB). Strains of *L. lactis* have been used for decades in the dairy industry and this bacterium was also given Generally Recognized As Safe (GRAS) status by the American Food and Drug Administration (FDA). Furthermore, we recently showed that *L. lactis* exhibits innate inflammatory effects, indicating a capacity for adjuvanticity. These characteristics support the use of *L. lactis* as an antigen carrying live vaccine. Our main interest is to engineer *L. lactis* live vaccines against leishmaniasis in the model systems of *Leishmania* in BALB/c mice. Leishmaniasis, caused by the human parasite *Leishmania*, affects over 12 million individuals worldwide. Nevertheless, no vaccines are currently available against this parasitic disease. It is well established that immunity in mice against various forms of leishmaniasis depends on the activation of T-helper (Th) type 1 mediated immunity, which is induced by the essential cytokine interleukin 12 (IL-12). Therefore, we will engineer *L. lactis* strains co-expressing one of two known protective *Leishmania* antigens, LACK or A2, alone or in combination with IL-12. We will evaluate these strains for their capacity to induce protection against different infection models of *Leishmania* in mice. Ultimately, the antigens, along with IL-12, will be relocated to the bacterial chromosome. This will prevent the use of plasmids conferring antibiotic resistances and will therefore generate even safer live vaccines. These *Leishmania* live vaccines will be easy and cheap to produce, safe, "food grade" and biologically containable. Furthermore, these *L. lactis* strains could be used as a platform to develop live vaccines against different human pathogens.

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.*

The TATA-binding Protein (TBP) is required for transcription initiation by all three RNA polymerases in eukaryotes. TBP binds to the TATA sequence found in the majority of gene promoters by insertion of four highly conserved phenylalanine residues between the base pairs of the sequence, which distorts the DNA and signals the recruitment of the conserved general transcription factors TFIIA and TFIIB. *Giardia lamblia,* an enteric protozoan parasite, encodes a highly divergent TBP (gTBP) which contains substitutions for three of the four conserved phenylalanine residues. However, *Giardia* lacks TATA sequences in its gene promoters and does not encode a homolog of TFIIA. In our previous work on the characterization of proteins that bind to a *Giardia* histone-gene promoter element, we identified TIP49, a putative DNA helicase that shows high sequence identity to a member of a family of TBP-interacting proteins. In order to gain insight into the roles of these proteins in *Giardia* transcription, we have expressed these two proteins and have attempted to characterize their functions through gel mobility shift assays, gene expression studies and, for TIP49, helicase assays. The results of this work, as well as implications and future directions, will be discussed.

Smriti Kala, Reza Salavati. Institute of Parasitology, McGill University.

Identification of the KREPA4 RNA binding domain and elucidation of its biochemical role in RNA editing

Mitochondrial mRNAs in trypanosomes undergo RNA editing by insertion and deletion of uridylates (Us) to produce mature functional mRNAs. The editing process is carried out by a multi-protein complex, the editosome that has not yet been fully characterized. KREPA4 (Kinetoplastid RNA Editing Protein A4) is an essential protein component of the editosome. It is potentially associated with both insertion and deletion editing subcomplexes and may play a role in the stability and perhaps assembly of the editosome. Structural prediction and compositional analysis of KREPA4 has identified a conserved oligonucleotide binding fold (OB fold) at the C-terminal, and two low compositional complexity regions (LCRs) at the N-terminal of KREPA4. Concurrent with these predictions, recombinant KREPA4 has been shown to bind to synthetic guide RNA *in vitro*, specifically the gRNA 3'-(U)-tail. Here we report the RNA binding domain of KREPA4 by analyzing the RNA binding activities of the full length and truncated versions of this protein. Our results suggest that the RNA binding activity of KREPA4 is mainly localized to the OB fold at the C-terminal of the protein, with additional contribution from the LCRs which might further stabilize the interaction. We also show that KREPA4 has RNA annealing activity as it is able to stimulate gRNA/pre-mRNA hybrid formation.

Fikregabrail Aberra Kassa[1], Marina Tiemi Shio[1], Marie-Josée Bellemare[1], Momar Ndao[2] and Martin Olivier[1]. [1]Department of Microbiology and Immunology and Center for the Study of Host Resistance, McGill University, Montreal, Quebec, Canada; [2]National Reference Centre for Parasitology, Research Institute of the McGill University Health Centre, Montreal, Quebec, Canada.

Proteomic analysis of serum proteins from malaria patients and identification of proteins binding to hemozoin

After being released from infected red blood cells, the malarial hemozoin comes in contact with different serum proteins. However, the identity of the serum proteins interacting with hemozoin remains unrevealed. Furthermore, the proteomic profile of the serum proteins from malaria patients remains to be uncovered. In the present study, sera from malaria patients and healthy individuals were bound to protein chips (CM10 and IMAC) and were used to detect serum proteomic patterns using SELDI-TOF. A total of 171 protein peaks were identified. Further analysis revealed that some of the proteins were up-regulated in malaria patients, while others were down-regulated. Apart from the global study of the serum protein profiles, we investigated the proteins which are able to interact with malarial pigment hemozoin. A total of 39 proteins were identified. The major groups of proteins identified were immunoglobulins, complement proteins, lipid binding and transport proteins, acute phase proteins and hemoglobin subunits. The common molecular function of the proteins is binding, as the hemozoin crystal has hydrophilic, lipophilic as well as amphiphilic surfaces. Other functions include enzyme regulatory activity, transporter activity, catalytic activity, antioxidant activity and molecular transducer activity. Understanding the serum proteomic profile of malaria patients and identification of biomarkers enables us to understand the role of serum proteins in the malaria pathology. This work was supported by an operating grant of CIHR to MO and the RI-MUHC studentship to FAK.

Pranav Kumar, Robert Lodge, Nathalie Trudel, Michel Ouellet, Marc Ouellette, Michel J. Tremblay/ Centre de Recherche en Infectiologie, Centre Hospitalier de l'Université Laval, Québec, Canada

Nelfinavir, a HIV-1 protease inhibitor, induces oxidative stress and caspase-independent apoptosis in *Leishmania* amastigotes.

Visceral leishmaniasis has now emerged as an important opportunistic disease in patients infected with human immunodeficiency virus (HIV-1). Recently, we and others have found that several HIV-1 protease inhibitors (PIs) inhibit *Leishmania* survival in human macrophages. The molecular mechanism(s) that contributes to antileishmanial activity of PIs is still unknown. Here, we show that nelfinavir, a potent HIV-1 protease inhibitor, generates oxidative stress in the parasite leading to altered physiological parameters such as an increase in the sub-G1 DNA content, nuclear DNA fragmentation and loss of mitochondrial potential which are characteristics of apoptosis. Pretreatment of axenic amastigotes with the caspase inhibitor zVAD-fmk did not inhibit the increase in sub-G1 DNA content of PI-treated parasites suggesting that nelfinavir does not kill *Leishmania* amastigotes in a caspase-dependent manner. Furthermore, we observed that the mitochondrial resident protein endonuclease G is involved in this caspase-independent apoptosis. We also show that parasites overexpressing the gene coding for GSH1 (the rate limiting enzyme of glutathione biosynthesis) were more resistant to nelfinavir when compared to untransfected controls. These data suggest that nelfinavir induces an oxidative stress, culminating into a caspase-independent apoptosis mediated by a novel effector molecule endonuclease G. Understanding the nelfinavir-mediated death signalling pathway may eventually help in the design of new therapeutic strategies against the *Leishmania* parasite and for HIV-1/*Leishmania* co-infected individuals. This work is supported by a Strategic new initiative team grant to M. J.Tremblay and Marc Ouellette from the FQRNT Centre for Host-Parasite Interactions.

Jose Loaiza[1] Marilyn Scott[1] Eldredge Bermingham[2] Jose Rovira[3] Jan Conn[4]. [1] The Institute of Parasitology, McGill University, [2] Smithsonian Tropical Research Institute, [4] Instituto Conmemorativo Gorgas de Estudios de la Salud, [5] Wadsworth Center, New York State Department of Health.

Mitochondrial *CO1* gene provides evidence for a Pleistocene expansion of *Anopheles albimanus* in eastern Panama

Anopheles albimanus is an important malaria vector throughout the northern Neotropics, and physical barriers to gene flow are expected to restrict its dispersal. We hypothesize that *An. albimanus* is not at demographic equilibrium likely due to population growth and historical geographic fragmentation at a regional scale. To analyze the structure of genetic variation and demographic history of *Anopheles albimanus*, we used partial sequences of the mitochondrial DNA *CO1* gene and 265 mosquitoes from 16 localities in Costa Rica, Panama and northern Colombia. Three groups of haplotypes were depicted in the statistical parsimnony network, each separated by 7 mutational steps, although not related to geography. An analysis of molecular variance (AMOVA), which provided evidence for differences in the haplotype frequencies at this geographic scale, further supported the haplotype partition. Three sympatric and genetically divergent groups of *An. albimanus* may be the result of several dispersal events across Costa Rica and Panama. Furthermore, a population expansion in *An. albimanus* appears to have occured around 22 939 years ago (95% CI 10 183 – 47 869) in eastern Panama. Our findings do not support physical barriers to gene flow, but instead, Pleistocene geographic separation in eastern Panama and northern Colombia is the likely cause of population structure in *An. albimanus*.

Laura-Isobel McCall, Greg Matlashewski. Dept. of Microbiology and Immunology, McGill Univ.

Exploring the interaction between *Leishmania donovani* virulence factor A2 and host macrophages.

L. donovani causes visceral leishmaniasis in humans, which is invariably fatal if untreated. There are 500,000 new cases of visceral leishmaniasis yearly, and *Leishmania*-HIV co-infections are also a growing concern. The amastigote-specific protein A2 has been shown to be an essential *L. donovani* virulence factor and to play a role in the visceralization of the parasite. However, the details of its function have so far remained unknown and are the focus of this study. Promastigotes were transferred to amastigote conditions and the culture supernatant was analyzed by Western blot for A2; A2 was secreted by *L. donovani*. Bone marrow-derived macrophages and B10R cells were infected with *L. donovani* for 12 or 24 hours with either promastigotes or amastigotes and immunofluorescence experiments were performed to detect A2. A2 was located in discrete foci that colocalize with the phagolysosome. Moreover, not all *L. donovani* parasites were expressing A2 during infection, with the highest expression 12 hours post-infection. Macrophages were infected for 12 hours with *L. donovani* amastigotes and A2 was immunoprecipitated using a monoclonal anti-A2 antibody. Co-immunoprecipitated proteins were detected by silver staining. Mass spectrometry of excised bands showed that A2 interacts with cytoskeletal proteins, including myosin IX. We also investigated by Western blot whether A2 interacts with parasite myosin or host myosin. These results provide an insight into the function of A2 and into *Leishmania* host-pathogen interactions. Study of *L. donovani* virulence factors might provide valuable new drug targets to treat visceral leishmaniasis. This work was supported by NSERC and CIHR.

Jenny Miu, Maya Saleh, Mary Stevenson. Department of Medicine, Research Institute of the McGill University Health Centre, Mcgill University.

Caspase-12 deficiency results in hyperinflammatory responses to lethal malaria but does not greatly alter the course of infection.

Malaria is a severe disease resulting in high morbidity and mortality, and particularly affects people of developing countries. The host response to the parasite can determine the severity of disease and the final outcome. In particular, a fine balance between pro- and anti-inflammatory immunological responses is thought to be critical in malaria. Caspase-12 deficient ($C12^{-/-}$) mice are hyperinflammatory due to the loss of inhibition on caspase-1, an inflammatory caspase. Although $C12^{-/-}$ mice inoculated with lethal *P. yoelii* XL (PylXL) succumb to the disease at a similar time-point as WT mice, they show various signs of heightened immunopathological responses. $C12^{-/-}$ mice exhibited higher numbers of parasitized RBCs, enhanced liver pathology, and differing numbers of TUNEL-positive cells in the spleen. PylXL-infected $C12^{-/-}$ mice also had higher levels of pro- and anti-inflammatory cytokines in the serum; this was reflected in the splenic production of certain cytokines. Overall, WT and $C12^{-/-}$ mice inoculated with PylXL revealed elevated levels of a broad range of pro-inflammatory and pro-apoptotic genes in the liver but downregulation of these genes in the spleen. These patterns may simply reflect the lethality of the parasite, and moreover, these differences appear too subtle to confer any survival advantages to the $C12^{-/-}$ mice. Finally, the lower levels of EPO protein detected in the serum and organ homogenates of $C12^{-/-}$ mice, despite the higher endogenous levels of the EPO transcript, suggests possible aberrations in the EPO production pathway. Overall, deficiency in C12, while exacerbating several immunopathological responses to a lethal malaria parasite, did not appear to affect pathways critical to the survival of the host and thus did not greatly alter the progression and outcome of lethal murine malaria. Supported by grants from CIHR and FRSQ.

Mirna Nascimento[1], Sara Gosline[2], David Thomas[3], Michael Hallett[2] and Greg Matlashewski[1]. 1. Microbiology & Immunology, 2. Centre for Bioinformatics, 3. Biochemistry Depts., McGill University. **Absence of conventional Unfolded Protein Response in Parasites; A Potential Therapeutic Target for Leishmaniasis**

Traditional approaches toward the development of anti-infective agents involve targeting a unique property of the parasite (e.g. virulence) through the development of drugs that inhibit this function. In the present study we have undertaken a conceptually converse strategy that exploits the absence of a critical biochemical pathway in parasites. Bioinformatics analysis revealed that parasitic protozoa including *Plasmodium*, *Trypanosomes* and *Leishmania* all lack the canonical genes associated with biochemical pathways that respond to the accumulation of unfolded proteins, the unfolded protein response (UPR). The UPR, present in all higher eukaryotic cells, induces the transcription of genes encoding protein-folding chaperones, protein-transporters, translation inhibitors, and cell cycle inhibitors ensuring that unfolded proteins are not toxic to cells and are folded properly prior to re-initiation of translation and cell proliferation. To determine if there was any transcriptional up-regulation of ER chaperones despite an apparent lack of UPR signaling genes in *Leishmania*, we treated promastigotes and intracellular amastigotes with tunicamycin and DTT that induce protein unfolding, and confirmed that this did not result in increased levels of the BiP chaperone protein which is an indicator of the UPR in eukaryotes. Moreover, *L. donovani* exhibited reduced viability in the presence of DTT concentrations where host macrophage viability was not affected. These results establish the lack of a classical UPR in *Leishmania* as a potential therapeutic target. The testing of other UPR-inducing chemicals and the further exploration of other cytoprotective biochemical pathways that are absent in lower eukaryotic pathogens could reveal novel therapies for parasitic diseases.

Maurice Odiere[1], Kris Koski[2], Marilyn Scott[1]. [1]Institute of Parasitology and [2]School of Dietetics and Human Nutrition, McGill Univ.

Protein deficiency during pregnancy and lactation impairs resistance to GI nematode and modifies body composition and neonatal growth in CD1 mice.

Periods of increased energy demand or reduced nutrient intake during reproductive phase induce changes in maternal condition, immune function and behavior that may be costly post-partum. This study illustrates how animals cope with simultaneous energy demands in the form of protein deficiency and nematode infection during reproduction. A 2 x 2 factorial design involving diet (protein sufficient, PS and protein deficient, PD) and infection (0 L_3=sham and 100 L_3=infected) was used. Mice were infected with *Heligmosomoides bakeri* at day 14 of pregnancy and days 2, 9, and 16 post-partum (PP). *H. bakeri* egg output (EPG) was estimated from 3 h stool collections at days 5 and 18 PP. Maternal body and bone composition was determined using dual-energy X-ray absorptiometry (DXA). Resting metabolic rate (RMR) was determined at day 17 of pregnancy and day 15 PP. Mice (dams) were killed at day 20 PP and organ masses and number of adult worms and 4th-stage larvae (L_4) were determined. Reproductive outcomes (litter size, pup mass and crown-rump length) were determined at birth and days 2, 7, 14 and 21 PP. PD increased EPG and percentage of adult worms but decreased percentage of L_4, body mass, lean mass, bone area, bone mineral content and fat mass, RMR, body temperature, organ masses (heart, lungs, spleen, liver, kidneys, pancreas, thymus, small intestines), whereas infection increased bone mineral density, small intestine length and mass of small intestine and lungs. Litter size, pup mass and crown-rump lengths were lower in PD mice across time. The reduced resistance in PD infected mice served to adaptively reallocate resources from immunity towards the prioritized reproductive function. Energy saving in PD mice was achieved through reduction in body temperature, litter size and reduction in lean mass.

Amber Olson[1], Kin Chan[2], Tom Edge[3], Chi-Yip Ho[2], and Janet Yee[1]. [1]Biochemistry Program, Trent University, [2]Microarray Laboratory, Samuel Lunenfeld Research Institute, University of Toronto, [3]Environment Canada, National Water Research Institute.

Gene expression profiling of *Giardia lamblia* isolates

In Canada, the majority of the waterborne disease outbreaks linked to protozoa is caused by *Giardia lamblia*, an enteric parasite that infects a wide range of vertebrate hosts. The potential of zoonotic transmission of *Giardia* reveals the importance of genotyping and tracking the sources of contaminations. However, profiling studies of *Giardia* by the use of genomic polymorphisms do not provide any information on the regulation or dynamics of gene expression among the isolates.

We extracted RNA from laboratory cultures established originally from two human isolates of *Giardia* (WB and GS) and one isolate from beaver. Fluorescent-labeled cDNA were generated and used in hybridization experiments with microarray slides containing 9115 genes and ORFs within the *Giardia* genome. Among the differentially expressed genes that were identified between the *Giardia* WB and beaver isolates, several encode the Variant Surface Protein (VSP), which is the major protein on the surface of *Giardia* trophozoites. The second group of differentially expressed genes encodes ribosomal proteins, metabolic enzymes and cell signaling molecules. The third group of genes encodes hypothetical proteins that do not have high sequence similarity to known proteins in other organisms. We will discuss the correlation of these results to the differences in the growth and host adaptations between these *Giardia* isolates.

Nicholas Patocka, Paula Ribeiro. Institute of Parasitology, McGill Univ.

Serotonin in Action; Dissecting Serotonin Signaling in *Schistosoma mansoni.*

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. Lack of serum in culturing also leads to death of the parasites, suggesting a crucial role of serotonin in their development. It is unknown at present whether the effect of exogenous serotonin is mediated by activation of surface (tegumental) receptors leading to downstream signaling via the worm's sensory nervous system, or if 5HT 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. This response was specific to serotonin with minimal response to any of the other biogenic amines. Localization of the receptor *in vivo* shows it to be situated on the tegument, namely the tubercles of the adult worm, as well as to the nervous system of developing 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.

Rushini Perera, Robin Beech. Institute of Parasitology, McGill Univ.

Gene duplication among the ligand-gated ion-channels in the genome of *Haemonchus contortus.*

Control of the nervous system and musculature of eukaryotes involves the transduction of a chemical signal in the synapse into an electrical signal in the post-synaptic cell. Ligand-gated ion-channels play a central role in this process and represent an ancient signaling mechanism that predates the origin of eukaryotes. These channels are also important drug targets for anesthetics, anti-depressants and anthelmintics. The function of a particular channel depends critically on the subunits that make up its pentameric structure and with more than 100 different subunits in the model nematode Caenorhabditis elegans this provides the potential for finely discriminating control of the neuromusculature. The parasitic nematode H. contortus is the focus of an ongoing genome project that currently contains sequence of about 85% of the genome in which more than 70 different LGIC subunit genes have been identified to date. Many genes have a single homolog in C. elegans. Others appear to be missing in one or other organism and represent deletion in one lineage or the other. Interestingly the genes targeted by the anthelmintic levamisole in C. elegans appear to have been duplicated independently in H. contortus after divergence of the two organisms.We are currently characterizing tandemly duplicated copies of two lev-1 and four unc-29 genes from H. contortus. cDNA clones have been generated by targeted PCR amplification and microinjection into Xenopus oocytes will be used to examine the electrophysiology of the channels formed by different subunit composition and their sensitivity to the natural ligand, acetylcholine and the specific antagonists, nicotine, biphenium and the anthelmintic levamisole which differentiate the three known acetylcholine receptor subtypes. Understanding the mechanisms which produce new subunit types and the evolutionary changes that shape neuromusculature control will provide a clearer picture of the eukaryotic nervous system and an important anthelmintic target in this parasitic nematode.

Vijayaraghava TS Rao[1,2], Salma Z Siddiqui[2], Roger K Prichard[1], and Sean G Forrester[1,2]. [1]Institute of Parasitology, McGill University, 21111 Lakeshore Road, Ste. Anne de Bellevue, Quebec, Canada H9X 3V9. [2] University of Ontario Institute of Technology, Faculty of Science, 2000 Simcoe St North, Oshawa, Ontario, Canada, L1H 7K4

Dopamine as a neurotransmitter in *Haemonchus contortus*

Dopamine is a biogenic amine known commonly to function as a neurotransmitter as well as a neuromodulator in the vertebrate brain. Dopamine mainly functions by binding to G protein coupled receptors (GPCRs) in vertebrates. Likewise in *C. elegans*, dopamine also exerts its effect through GPCRs and is an important component of the mechanosensory function. In parasitic nematodes like *H. contortus,* however, there is very little data on the function of this neurotransmitter. In the present study, we have discovered a novel role for dopamine in the parasitic nematode *H. contortus*. Through the process of identifying novel ligand-gated chloride channels, we have identified a subunit that when expressed in *X. laevis* oocytes forms a chloride channel that predominately responded to dopamine. This implies that dopamine could have a direct role in the control of membrane chloride conductances in nematodes. Immunolocalization of this subunit in adult worms revealed the presence of this subunit in neuronal support cells surrounding mechanosensory structures called deirids. In addition, localization of antibodies specific to dopamine indicated that this molecule is present mainly in the neuronal commissures connecting the lateral and the sub-lateral nerve cords in adult female worms. The localization was restricted to the mid-body region. Female worms (in RPMI medium) exposed to exogenously supplied dopamine showed complete paralysis of the midbody region. This study highlights the unique role of dopamine in this parasite, a role which is divergent and hence one of interest for the elucidation of novel drug targets. This work received funding support from NSERC, FQRNT, Fort Dodge Animal Health and UOIT.

Andrea Rauba, Mike Osei-Atweneboana, Roger Prichard. Institute of Parasitology, McGill Univ.

Macrocyclic lactone selection on ABC-transporter genes in *Onchocerca volvulus* from Ghana.

Onchocerciasis is a disease of the skin and eyes that is caused by the filarial nematode *Onchocerca volvulus*. The disease is the second leading cause of infectious blindness and significantly reduces life expectancy, carrying a disease burden of one million DALY's. Infected persons live primarily in Africa where extremely successful control programs based on the annual mass administration of ivermectin have been in place for approximately two decades. Recent reports of persistent microfilaridermas and a quicker return to reproduction in the adult female worm bring about concerns of resistance to the only drug available for mass treatment. Resistance to the macrocyclic lactones in *O. volvulus* is believed to be multigenic. Genes of interest include α and β-tubulin, ligand-gated ion channels and ABC-transporters. Using RFLP and SSCP techniques, the ABC-transporter P-glycoprotein-1 (Pgp-1) and the half-size p-glycoprotein-like-protein (Plp-1) have been shown to be under selection upon repeated exposure to ivermectin. Using PCR amplification and sequencing, this study examines the frequencies of single nucleotide polymorphisms (SNPs) in these genes which have previously shown significant evidence of selection. Samples examined are adult worms obtained during the study conducted by Osei-Atweneboana *et al.* (2007), which put forth the first epidemiological evidence of resistance in these parasites. This work was supported by CIHR, NSERC, CHPI, McGill University and the Nova Scotia Agricultural College (Dalhousie Univ.).

Rioux, Marie-Claire[1], Tim Geary[1] and Terry Spithill[1,2] . [1]Institute of Parasitology, McGill University, [2]Charles Sturt University.

Comparative proteomic analysis of *Fasciola hepatica* and *Fasciola gigantica* newly excysted juvenile and adult excretory-secretory products.

Fasciola hepatica and *Fasciola gigantica* are the causative agents of fasciolosis, an economically important disease. While there are many similarities between the two species, *F. hepatica* is much more successful in establishing and sustaining infection in its definitive hosts. This difference in pathogenicity is thought to be mediated by differences in expression of factors that either protect *F. hepatica* against the host response or suppress the host immune response. In order to assess the differences in protein expression between the two species, we profiled the excretory-secretory (ES) products from the newly excysted juvenile (NEJ) and adult stages of *F. hepatica* and *F. gigantica* parasites using 1D SDS-PAGE and LC-MS/MS. A total of 157 unique proteins were identified in the dataset. Overall there are greater similarities between the two species than between life stage; 42% of the proteins are shared between *Fasciola spp.* while only 18% of the proteins are shared between NEJ and adult life stages. Proteases had clear stage-specificity with NEJ- and adult-specific clades of Cathepsin B and L identified. Antioxidant defense (AOD) enzymes demonstrated both stage-specific and species-specific expression profiles, with increased expression of Glutathione S-transferase in adult parasites and higher levels of AOD enzymes in *F. gigantica* NEJ and adult ES. These profiles confirm significant differences in protein expression between *Fasciola spp*. and represent promising avenues to explore the differences in pathogenicity of these *parasites*.

We thank Line Roy, Nathalie Hamel, Daniel Boismenu and Sylvie LaBoissière for their help with data analysis and the technical support of McGill University Genome Quebec Innovation Centre. We also thank Dr. Weiyu Zhang for *F. gigantica* metacercariae and adult ES and Dr. Janelle Wright for *F. hepatica* adult ES.

Petra Rohrbach. Institute of Parasitology, McGill University

Live cell imaging of *Plasmodium falciparum*: Shedding light on biological processes

The confocal laser-scanning microscopy (CLSM) has enormous potential in many biological fields. We have used this technique to study various aspects of malaria infection in the erythrocytic stages of *Plasmodium falciparum*, including:

(1) investigating the baseline steady-state pH of the cytoplasm and digestive vacuole of a chloroquine-sensitive (HB3) and a chloroquine-resistant (Dd2) parasite using a pH-sensitive green fluorescent protein, pHluorin. This technique allows for *in vivo* pH measurements in intact *P. falciparum*-infected erythrocytes under physiological conditions.
(2) examining multi-drug resistance in various parasitic strains using a surrogate assay for PfMDR-1 function based on the subcellular distribution of Fluo-4 acetoxymethylester and its free fluorochrome.
(3) monitoring protein trafficking in real time using a conditional protein export system in *P. falciparum*, based on the previously described conditional aggregation domain (CAD domain) that self-aggregates in the endoplasmic reticulum in a manner that is reversible by the addition of a small molecule.

This presentation is thought to give a small overview of several live cell imaging techniques that have been used to better understand malaria and that can be applied to almost any organism.

Isabelle Rouiller, Kelly Sears, Hojatollah Vali. FEMR, McGill University.

The McGill Facility for Electron Microscopy Research (FEMR) is the largest and most comprehensive facility for electron microscopy in Quebec. Its mission is to promote and advance the science and practice of all microscopic imaging, analysis and micro-diffraction techniques useful for elucidating the ultra-structure and function of diverse materials. The FEMR supports and fosters the collaborative and multidisciplinary research activities of over hundred investigators from more than twenty departments in four faculties at McGill in the areas of biological, life, materials, and physical sciences.

The main equipments available are confocal microscope, scanning electron microscopes, transmission electron microscopes (TEM; used for example for morphological studies using resin embedding and heavy metal staining and for sub-cellular localization of proteins using immunogold labeling techniques), cryo-TEMs (used in combination with single particle image analysis techniques for structural studies of purified macromolecular complexes and in combination with tomographic reconstruction for the studies of cellular processes). The two cryo-TEMs, a Tecnai F20 and a Titan Krios, are a recent addition to the FEMR and are top of the range cryo-microscopes.

Mukesh Samant, Reema Gupta Shraddha Kumari Pragya Misra, Prashant Khare, Pramod Kumar Kushawaha, Amogh Anant Sahasrabuddhe, and Anuradha Dube. Central Drug Research Institute, Lucknow, India

Immunization with the DNA-encoding N-terminal domain of Proteophosphoglycan of *Leishmania donovani* generates Th1-Type immunoprotective response against experimental visceral leishmaniasis.

Leishmania produce several types of mucin-like glycoproteins called proteophosphoglycans (PPGs) which exist as secretory as well as surface-bound forms in both promastigotes and amastigotes. The structure and function of PPGs have been reported to be species and stage specific as in the case of *Leishmania major* and *Leishmania mexicana*; there has been no such information available for *Leishmania donovani*. We have recently demonstrated that PPG is differentially expressed in sodium stibogluconatesensitive and -resistant clinical isolates of *L. donovani*. To further elucidate the structure and function of the *ppg* gene of *L.donovani*, a partial sequence of its N-terminal domain of 1.6 kb containing the majority of antigenic determinants, was successfully cloned and expressed in prokaryotic as well as mammalian cells. We further evaluated the DNA-encoding N-terminal domain of the *ppg* gene as a vaccine in golden hamsters (*Mesocricetus auratus*) against the *L. donovani* challenge. The prophylactic efficacy to the tune of ~80% was observed in vaccinated hamsters and all of them could survive beyond 6 mo after challenge. The efficacy was supported by a surge in inducible NO synthase, IFN-γ, TNF-α, and IL-12 mRNA levels along with extreme down-regulation of TGF-β IL-4, and IL-10. A rise in the level of *Leishmania*-specific IgG2 was also observed which was indicative of enhanced cellular immune response. The results suggest the N-terminal domain of *L. donovani ppg* as a potential DNA vaccine against visceral leishmaniasis**.**

T. Scorza, D. Malu Tshikudi, O. Kevorkova, J. Sanchez Dardon and R. Moreau. Department of Biological Sciences, UQAM, Montreal, Canada.

Characterization of novel interactions between immune cells and bone metabolism during acute malaria infection.

Enhanced bone resorption is frequently associated with chronic inflammation, which suggests interactions between the immune system and bone metabolism. In order to further understand these interactions, using the *Plasmodium chabaudi adami* (DK) model in Balb/c mice, we evaluated the effect of acute inflammation on the function of bone-forming cells (osteoblasts) and bone-resorbing cells (osteoclasts). *P.c. adami* infection is characterized by systemic inflammation, parasitemia profiles reaching maximal values at 8-9 days, splenomegaly and anaemia. The impact of daily treatment with the hypocalcemic hormone calcitonin on the progression of infection and erythropoiesis was assessed in parallel. Our results indicate profound inhibition of bone resorption and bone formation during *P.c. adami* infection. Administration of calcitonin exacerbated parasitemia and decreased splenic white blood cell numbers, also modifying hematopoietic cell (HSC) numbers and erythropoiesis in the spleen. In conclusion malaria infections drastically affect bone metabolism and calcitonin modulates the progression of splenomegaly and splenic erythropoiesis during infection.

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

Functional annotation of the genome of *Trypanosoma brucei* based on regulatory elements of coding sequences and untranslated regions

The genomes of trypanosomatids contain many trypanosomatid-specific genes whose functions cannot be determined using homology-dependent annotation methods. This is while a very limited set of homology-independent computational methods are available for predicting gene function, all of which require additional data beyond genome sequences. Here, we present two novel homology-independent methods to directly infer biological functions of genes solely based on their sequences. The first method is based on the premise that coexpressed genes have similar synonymous codon usages. Using rigorous statistical analysis, we have shown that codon usage may regulate the expression pattern of proteins in a wide range of organisms, and have provided experimental data supporting this hypothesis. Knowing the high correlation between expression pattern and gene function, we have developed a method for prediction of function based on codon usage. The second method uses a set of function-specific regulatory motifs in 5' and 3' untranslated regions in order to predict gene function. We have shown that the combination of these two methods provides a powerful homology-independent annotation tool. Applying these methods to the genome sequence of *Trypanosoma brucei*, we have been able to predict the functions of many hypothetical proteins. When applicable, these predictions are usually corroborated by predicted interactome of *T. brucei* and/or other sequence features of genes. This work was supported by an operating grant from CIHR.

Strasser,R., Pilar,A.V., McLean,J., Cyr,N., Jardim,A. Institute of Parasitology, McGill.

Association of *Leishmania donovani* glycosomal docking protein Peroxin14 with receptor-cargo complexes modulates its conformation.

The glycosomes of *Leishmania donovani* are unique microbody organelles that compartmentalize a variety of metabolic pathways essential for parasite survival. Trafficking and import of newly synthesized proteins to the glycosome is dependent on the receptor proteins peroxin 5 (LdPEX5) and peroxin 7 (LdPEX7), and the docking protein peroxin 14 (LdPEX14), a peripheral membrane protein anchored to the glycosomal membrane. Biochemical analysis revealed that in the cytosol LdPEX5-LPEX7 form a diverse array of heteromeric complexes loaded with PTS1 and PTS2 cargo proteins. The PTS1-PEX5-PEX7-PTS2 complexes are conjectured to dock to LdPEX14, an event required for translocation of the PTS1 and PTS2 cargo proteins across the glycosomal membrane. However, little is known about this import mechanism, and the full set of proteins that mediate the biogenesis of the glycosome. Partial tryptic digests reveal that docking of the receptor-cargo complexes at the glycosome surface rendered LdPEX14, but not LdPEX5 or LPEX7, extremely resistant to trypsin digestion. Density centrifugation experiments showed that binding of LdPEX5-LPEX7 oligomers loaded with PTS1 and PTS2 ligands to the docking platform on the glycosome altered the size of the LdPEX14 containing complexes. Collectively these data suggest that the binding of LdPEX5 and LPEX7 triggered a structural change in LdPEX14 that may result in glycosomal membrane insertion or association with glycosomal integral membrane proteins including, but not limited to, LdPEX12, which protected LdPEX14 from proteolytic degradation.

Amira Taman and Paula Ribeiro. Institute of Parasitology- McGill University

Investigation of a dopamine receptor in *Schistosoma mansoni:* Functional studies and immunolocalization

A dopamine receptor (SmD2) was cloned from adult *Schistosoma mansoni*. The receptor has the classical heptahelical topology of class A (rhodopsin-like) G Protein-Coupled Receptors (GPCR) and shares sequence homology with D2-like receptors from other species. The full length SmD2 cDNA was expressed in the yeast *Saccharomyces cerevisiae* and mammalian HEK293 cells. Functional assays in both expression systems revealed that SmD2 was responsive to dopamine in a dose-dependent manner, whereas other structurally related amines had no effect. Activation of SmD2 in mammalian cells caused an elevation in intracellular cAMP but not calcium, suggesting that the receptor coupled to Gs and the stimulation of adenylate cyclase. Pharmacological studies showed that the *S. mansoni* dopamine receptor was inhibited by apomorphine, a classical dopamine agonist, as well as known dopaminergic antagonists, including chlorpromazine, spiperone and haloperidol. SmD2 immunoreactivity was detected in membrane protein fractions of *S. mansoni* cercaria, *in vitro* transformed schistosomula and adult parasites, using a specific peptide antibody. When tested by confocal immunofluorescence, SmD2 was detected in the subtegumental somatic musculature and acetabulum of all larval stages tested. In the adults, SmD2 was enriched in the somatic muscles and, to a lesser extent, the muscular lining of the caecum. The results suggest that SmD2 is an important component of the neuromuscular system in schistosomes.

Marina Tiemi Shio[1], Stephanie C. Eisenbarth[2], Myriam Savaria[1], Adrien F. Vinet[3], Marie-Josée Bellemare[1], Kenneth W. Harder[4] Fayyaz S. Sutterwala[5], D. Scott Bohle[6], A***lbert Descoteaux***[3], Richard A. Flavell[2] and Martin Olivier[1]. [1]Dept of Medicine, Microbiology and Immunology, McGill University Health Centre, Canada; [2] Dept of Immunobiology and Laboratory Medicine Yale University, USA; [3]INRS-Institut Armand-Frappier, Canada; [4] Dept of Microbiology and Immunology, University of British Columbia, Canada, [5] Dept of Medicine, University of Iowa, USA; [6] Dept of Chemistry, McGill University, Canada.

NLRP3 inflammasome in malaria: role of hemozoin-induced signaling on inflammasome activation.

The intraerythrocytic *Plasmodium* parasite - the causative agent of Malaria - produces an inorganic crystal called hemozoin (Hz) during heme detoxification process, which is released into the circulation during erythrocyte lysis. Hz is rapidly ingested by phagocytes and induces the production of several pro-inflammatory mediators such as interleukin-1β (IL-1β). However, the mechanism regulating Hz recognition and IL-1β maturation has not been identified. Here, we show that Hz induces IL-1β production dependent on NOD-like receptor containing pyrin domain 3 (NLRP3) inflammasome, ASC and caspase-1, but not NLRC4 (NLR containing CARD domain). Furthermore, we observe that the absence of NLRP3 or IL-1β augmented mice survival to malaria caused by *P. chabaudi* AS. We further demonstrate, using pharmacological and genetic intervention, that the tyrosine kinases Syk and Lyn play a critical role in this inflammasome activation. These findings not only identify one way by which the immune system is alerted to malaria infection but also are the first to suggest a role for tyrosine kinase signaling pathways in regulation of the NLPR3 inflammasome and its role in the control the malaria progression. This work was supported by an operating grant of CIHR to MO. CNPq/Brazil and Research Institute of the McGill University Health Centre fellowship to MTS.

D. Tshikudi Malu[1], B. Belanger[2], J. Sanchez Dardon[1], A. Satoskar[3] and T. Scorza[1]. [1]Department of Biological Sciences, University of Quebec in Montreal; [2]University of Quebec, INRS-Institut Armand-Frappier; [3]Department of Microbiology, Ohio State University.

Macrophage Migration Inhibitory Factor (MIF) modulates the activation of T cells during early and late infection with *Plasmodium chabaudi adami* parasites.

Malaria is characterized by potent release of pro-inflammatory cytokines which are involved in pathology and anaemia. Macrophage Migration Inhibitory Factor (MIF) is a pleiotropic cytokine readily released during malaria infection that besides its pro-inflammatory activity has been shown to modulate T cell activation by mitogens and antigens. As MIF neutralization during *P.c. adami* (DK) infection significantly reduced peak parasitemia and inhibited early IFN-gamma production by splenic T cells and had an opposite stimulatory effect at peak infection, we hypothesized that MIF modulated the early and late T cell response through distinct mechanisms. Our recent data mice confirms an important reduction in peak parasitemia, accompanied by delayed resolution of infection in MIF deficient (MIF KO), which we suggest may correlate with an improved T helper 1 response and delayed B cell activation. IFN-gamma production and TLR2 TLR4 expression by splenic T cells were significantly decreased at day 4 post-infection in MIF KO mice, suggesting early interactions between MIF and T cells.

Adrien F. Vinet[1], Mitsunori Fukuda[2], Salvatore J. Turco[3], and Albert Descoteaux[1]. [1]INRS-Institut Armand-Frappier and Centre for host-parasite interactions, Laval, QC, H7V 1B7 Canada; [2]Department of Developmental Biology and Neurosciences, Tohoku University, Sendai, Miyagi 980-8578, Japan; [3]Department of Biochemistry, University of Kentucky, KY, 40536, USA

The *Leishmania donovani* lipophosphoglycan excludes the vesicular proton-ATPase from phagosomes by impairing the recruitment of Synaptotagmin V

Upon their internalization by macrophages, *Leishmania donovani* promastigotes inhibit phagolysosome biogenesis. This inhibition is mediated by the virulence glycolipid lipophosphoglycan (LPG), a polymer of the Galβ1,4Manα1-PO_4 units attached to the promastigote surface via an unusual glycosylphosphatidylinositol anchor. We recently showed that the exocytosis regulator Synaptotagmin (Syt) V controls early steps of phagocytosis, and remains associated to the phagosome during the maturation process. Here, we show that Syt V contributes to phagolysosome biogenesis by regulating the acquisition of cathepsin D and the vesicular proton-ATPase. Insertion of LPG into ganglioside GM1-containing microdomains excluded Syt V from phagosome membranes, enabling *L. donovani* promatigotes to inhibit the recruitment of the vesicular proton-ATPase to phagosomes, preventing their acidification.
Collectively, these results reveal a novel function for Syt V in phagolysosome biogenesis and provide novel insight into the mechanism of vesicular proton-ATPase recruitment to maturing phagosomes. We also provide novel finding into the mechanism of *Leishmania* pathogenesis, whereby targeting of Syt V is part of the strategy used by *L. donovani* promastigotes to prevent phagosome acidification.

Claudia M Wever, Patrick Janukavicius, Igor Putrenko Joseph A Dent. Department of Biology, McGill University.

Validating Acetylcholine-Gated Chloride Channels as Novel Nematocide Targets.

Nematodes cause serious diseases in both humans and animals. Although there are successful anti-parasitic drugs, resistance is beginning to reduce their effectiveness. We have been characterizing a novel class of acetylcholine-gated chloride (ACC) channels in Caenorhabditis elegans (C. elegans) as potential drug targets. These channels are specific to nematodes and are not targets of previously known anthelmintics. The class of ACCs comprises 8 subunit genes; a beneficial characteristic since a gene family that represents multiple targets of a single drug may slow the onset of resistance. 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. To test the validity of these ACC subunits as targets for nematocides, we determinined the expression pattern of 7 out of 8 ACC subunit genes using promoter::GFP fusion constructs, and have been characterizing the electrophysiological properties of ACC channels formed from different subunits. Most of the subunits are expressed in roughly 20 neurons in C. elegans. The expression pattern of ACC-1 is especially promising; it is expressed in MC, a neuron critical for pharyngeal pumping. ACC-1, ACC-3, F47A4.1 and Y71D11A.5 are subunits that are expressed in many neurons, including some in the ventral nerve cord . We have also shown that K10D6.1 forms a functional homomeric channel that responds to ACh and that ACC3 and F47A4.1 interact to form a heteromeric ACh-sensitive channel. 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 anti-parasitic drug targets.
This work is supported by NSERC and Chemtura Co.

Karen K. Yam, P. Pouliot, F. Hugentobler, A.M. Stern, J-D. Lalande, G. Matlashewski, M. Olivier, B. Cousineau. Dept. of Microbiology and Immunology, McGill University

Generation and evaluation of A2 and LACK Expressing *Lactococcus lactis* Live Vaccines against Leishmaniasis

Lactococcus lactis is a non-colonizing, non-pathogenic, Gram-positive lactic acid bacterium commonly used in the food industry. We recently showed that *L. lactis* exhibits innate inflammatory effects, which indicate a capacity for adjuvanticity. These reasons rationalize the use of *L. lactis* to generate live vaccines. The parasite *Leishmania* is the causative agent of leishmaniasis, a disease which affects over 12 million individuals worldwide. The objectives of this study are to engineer strains of *L. lactis* to express protective *Leishmania* antigens, and to evaluate these strains as live vaccines against leishmaniasis in mice. We selected two known protective antigens against *Leishmania*: LACK and A2. The LACK gene was cloned directly from *Leishmania major* genomic DNA. Alternatively, we engineered a synthetic gene encoding for ten copies of the major antigenic repeat of A2. Both antigens were engineered for expression at differential subcellular localizations of *L. lactis*: in the cytoplasm, secreted into the media, or anchored to the cell wall. Proper expression and localization of *Leishmania* antigens in *L. lactis* was confirmed by Western blot and whole cell ELISA analyses. Next, the strains of *L. lactis* were subcutaneously injected into BALB/c mice to assess induction of antigen-specific immune responses and to evaluate protection against *Leishmania* infection. LACK- and A2-specific humoral immune responses were successfully induced in mice immunized with antigen-expressing strains of *L. lactis*. Furthermore, following administration of lactococcal vaccines, mice displayed a reduction in footpad swelling and parasitemia, which indicated a protection against *Leishmania* infection. We demonstrate that *L. lactis*-based vaccines are an effective approach in the generation of live vaccines against leishmaniasis.

Wen-Wei Zhang, Greg Matlashewski. Dept. of Microbiology and Immunology, McGill Univ.

Expression of *L.donovani* specific genes in *L.major* and their effects on virulence

We have previously observed that cross species transfection of the *L. donovani* specific gene A2 into *L. major* could render *L. major* more virulent in visceral organs but less virulent at cutaneous site, suggesting species-specific genes could play an important role in virulence and pathology of *Leishmania* infection(1,2). Comparison of recently completed *L. infantum* and *L. braziliensis* genomes with *L. major* genome has identified total 25 *L. infantum* specific genes (including A2) which are absent or present as pseudogenes in *L. major* and *L. braziliensis* (3). To investigate whether these species specific genes are involved in tissue tropism of *Leishmania* infection, we successfully cloned 18 ortholog genes of the total 25 *L .infantum* specific genes from *L. donovani* (the genetically closely related visceral *Leishmania* species to *L. infantum*) and introduced them into *L. major* to see whether expression of these *L. donovani* specific genes in *L. major* would alter its virulence in visceral infection in BALB/c mice. Interestingly, two of these *L. donovani* specific genes, LinJ15.0890 encodes a putative nucleotide-sugar transporter and LinJ28.0330 a hypothetical protein, were found to be able to significantly increase liver and spleen *L. major* parasite burdens in BALB/c mice. The nucleotide-sugar transporter is expectedly localized to the *Leishmania* Golgi apparatus while the hypothetical protein is present in cytoplasm. Gene targeting study revealed that while both LinJ28.0330 and LinJ15.0890 are nonessential for *L.donovani*, knock out of LinJ28.0330 gene significantly impair *L.donovani* growth in culture and render *L.donovani* completely avirulent in mice.