Twelfth Annual Quebec Molecular Parasitology Symposium

12ième Symposium Annuel de Parasitologie Moléculaire du Québec

May 28-29, 2012
INRS – Institut Armand-Frappier
531 boulevard des Prairies, Laval,
H7V 1B7, Québec, Canada

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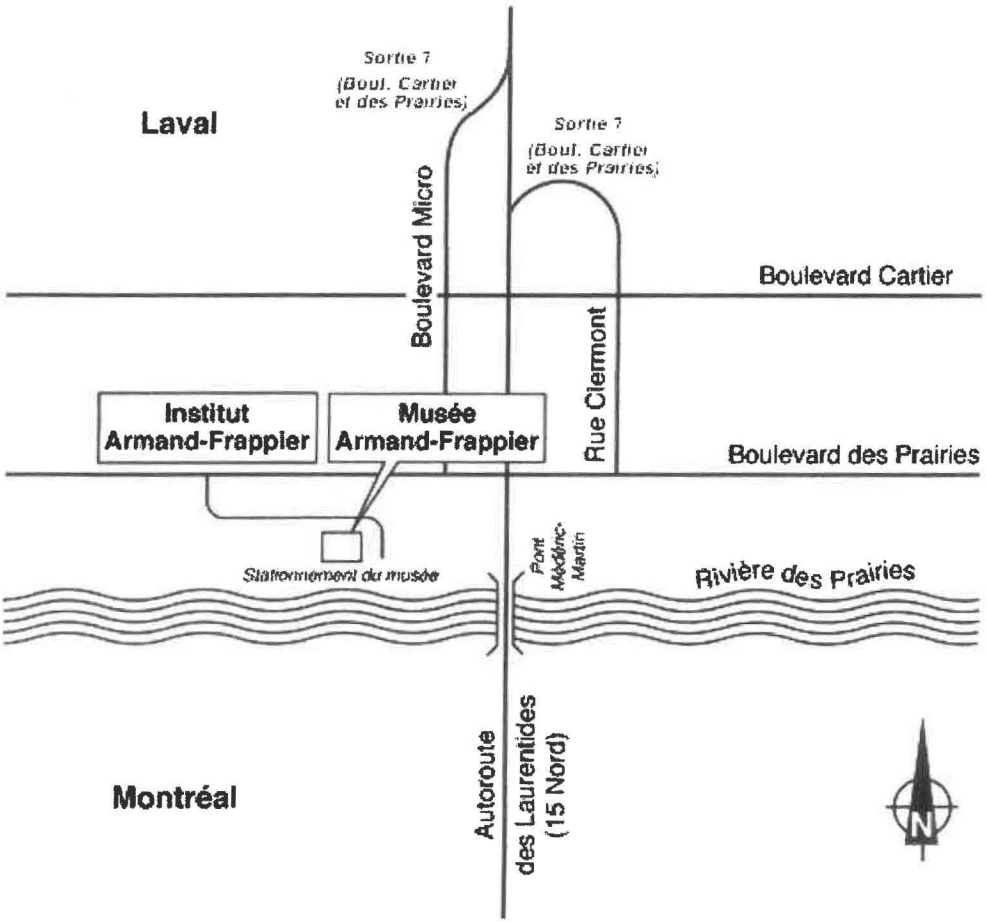

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Metro Cartier in Laval; Bus #20.
Distance from metro Cartier to INRS-IAF is about 1.5 km.

CHPI 2012 Program

Monday, May 28, 2012

9:00 – 9:30	Welcome speech by Drs. Armando Jardim and Albert Descoteaux (Salle Pasteur, Edifice 18)
9:30 – 11:30	**Session 1: Parasite immunology** (Salle Pasteur) Chair: Dr. Tatiana Scorza
9:30 – 9:45	**Felix Hugentobler,** Raphaël B. Di Roberto, Joshua Gillard, and Benoit Cousineau. Oral Immunization Using live *Lactococcus lactis* co-expressing LACK and IL-12 Protects BALB/c mice Against *Leishmania major* Infection.
9:45 – 10:00	**Guillermo Arango Duque**, Mitsunori Fukuda and Albert Descoteaux. Synaptotagmin XI, a negative regulator of cytokine secretion and phagocytosis, is targeted by GP63.
10:00 – 10:15	**Amandine Isnard** and Martin Olivier. Impact of *Leishmania* GP63 on Host Macrophage Nuclear Integrity, Signalling and Functions.
10:15 – 10:30	**Neda Moradin,** Diana Matheoud, Wan Jin Hong, Michel Desjardins, Albert Descoteaux. *Leishmania* inhibits antigen crosspresentation by direct cleavage of the SNARE VAMP8.
10:30 – 11:00	BREAK (Hall)
11:00 – 11:15	**Esther Dalko,** Nilson Quintana, Jaime Sanchez-Dardon, Robert Moreau, Tatiana Scorza. The antagonist effect of free heme on CD4 T cell responses and clearance of *Plasmodium* infection in mice.
11:15 – 11:30	**Rajesh M Valanparambil,** Yovany Moreno, Armando Jardim, Timothy G. Geary, Mary M Stevenson. Identification of the immunomodulatory molecule(s) in the excretory secretory (ES) products of the gastrointestinal nematode *Heligmosomoides polygyrus.*

11:30 – 12:30	**Keynote Speaker** **Dr. David Sacks, NIH-NIAID** Chair: Dr. Simona Stager The Influence of vector transmission on the immune response to *Leishmania major*.
12:30 - 13:30	LUNCH (Hall)
13:30 – 14:30	**Keynote Speaker** **Dr. Tim Gilberger, McMaster University** (Salle Pasteur) Chair: Dave Richard Dissection of the invasion machinery of the malaria parasite.
14:30 - 15:00	BREAK (Hall)
15:00 - 17:00	**Session 2: Biochemistry/Molecular Parasitology** (Salle Pasteur) Chair: Dr. John Dalton
15:00 – 15:15	**Samiah Alam**, Janet Yee, Manon Couture, Shin-ichi J. Takayama, A. Grant Mauk, Steven Rafferty. Expression & characterization of a cytochrome-b_5 from *Giardia lamblia*.
15:15 – 15:30	**Chase Reaume**, Christine Ouellet, and Janet Yee. The application of counterflow centrifugal elutriation and quantitative RT-PCR in the cell cycle analysis of *Giardia lamblia*.
15:30 – 15:45	**Normand Cyr,** Terry K Smith, Isabelle Coppens, and Armando Jardim. The hydrophobic region of the *Leishmania* peroxin 14 is implicated in the formation of a transient pore that mediates protein import into the glycosomal matrix.
15:45 – 16:00	**Rona Strasser,** and Armando Jardim.__*Leishmania donovani* glycosomal proteins form complexes in the cytosol for trafficking to the organelle surface.

16:00 – 16:15	**Erin Dodd**, D. Scott Bohle. The structure of the hemozoin-chloroquine complex: Single crystal structure and solution studies with a gallium heme analog.
16:15 – 16:30	**Rency T. Mathew,** John P. Dalton. The M1 alanyl aminopeptidases of *Plasmodium falciparum* (*Pf*M1AAP) malaria: biochemical and mutational analysis.
16:30 – 16:45	**Pablo Godoy,** Lian, J., Che, H., Beech, R.N. and Prichard, R.K. Interaction of macrocyclic lactones endectocides with *Haemonchus contortus* P-glycoproteins.
16:45 – 17:00	**Mostafa Zamanian**, Prince N Agbedanu, Michael J Kimber, Paula Ribeiro, Tim A Day. Genomic and functional characterization of G protein-coupled Receptors in pathogenic and free-living flatworms.
17:00 - 18:30	**Poster session and drinks** (Atrium PRF)
18:30 -	DINNER (Cafeteria, Edifice 18, basement level)

Tuesday, May 29, 2012

9:00 - 10:00	**Keynote Speaker** (Salle Pasteur, Edifice 18) **Dr. John Parkinson, The Hospital for Sick Children (Sickkids)** Chair: Dr. Albert Descoteaux Charting metabolic diversity in the *Apicomplexa*.
10:00 – 10:30	BREAK (Hall)
10:30 - 13:00	**Session 3: Drug discovery and resistance** (Salle Pasteur) Chair: Tim Geary
10:30 – 10:45	**Sonia Edaye,** Mara L. Leimanis, Sarah Reiling, Juliane Wunderlich, Petra Rohrbach, Elias Georges. A Quinoline-based Drug, MK571, Inhibits P-gh1-mediated Drug Transport and is More Toxic to Choroquine Resistant *Plasmodium falciparum.*
10:45 – 11:00	**Pranav Kumar,** Robert Lodge, Frédéric Raymond, Jean-François Ritt , Marc Ouellette, Michel J. Tremblay. Gene expression modulation and the molecular mechanisms involved in Nelfinavir resistance in *Leishmania donovani* amastigotes.
11:00 – 11:15	**Rubens Monte-Neto,** Raymond F, Légaré D, Corbeil J, Frézard F, Ouellette M. Comparative gene expression analysis and antimony uptake in Sb-sensitive and -resistant New World *Leishmania* species.
11:15 – 11:30	**Claudia M. Wever,** Patrick Janukavicius, Igor Putrenko and Joseph A. Dent. Validating Acetylcholine-Gated Chloride Channels as Novel Nematocide Targets.
11:30 – 11:45	**Patrick Janukavicius,** Ludmel Urdaneta, James Bae, Laura Tiseo, Mi Tan, Roger Prichard, Joseph Dent. *dyf-7* is responsible for the low-levels of ivermectin resistance in the *C. elegans* strains IVR6 and IVR10.

11:45 – 12:00	**Nour Rashwan,** Catherine Bourguinat, Roger Prichard**.** Asymmetrical isothermal amplification method for genotyping mutations, in human soil-transmitted helminths, that have been associated with benzimidazole resistance.
12:00 – 12:15	**Daniel Feingold,** Stephanie Bourque, Patrick Janukavicius, Saima Sidik, Laura Nilson, Joseph Dent. Characterization of three novel Cys-loop ligand-gated ion channel subunits in *Drosophila melanogaster* – Potential pesticide targets?
12:15 – 12:30	**Felipe Dargent,** Marilyn E. Scott, Andrew Hendry & Gregor F. Fussmann. Enemy release does not lead to decreased resistance in hosts .
12:30 – 12:45	**Corine G. Demanga,** Sarah J. Reiling, Juliane Wunderlich, Jenny W. L. Eng, Petra Rohrbach, John P. Dalton**.** A novel method for large-scale culture of *Plasmodium falciparum* in a Wave Bioreactor.
13:00 - 14:00	LUNCH (Hall)
14:00 - 15:00	**Closing remarks + prizes for talks and posters** (Salle Pasteur)

POSTERS

1. **Guadalupe Andreani,** Michel Ouellet and Michel J Tremblay
Leishmania infantum amastigotes trigger human B cells with regulatory functions.

2. **Hiva Azizi** and Barbara Papadopoulou.
Rapid mRNA decay mediated by SIDER2 retroposons in *Leishmania*

3. **Verena Brand,** Carl Song, Sundar Natarajan, Viviana Pszenny, Denis Reynaud, Michael Leadley, Shaheena Bashir, Michael Grigg, John Parkinson.
A metabolomics approach to characterize the different strains of *Toxoplasma gondii*

4. **Vanessa Dufour,** Claudia Wever, Robin N. Beech, Joseph A. Dent, Paula Ribeiro, Timothy G. Geary.
Characterization of novel glutamate-gated chloride channels in *Schistosoma mansoni*

5. **Nelly El-Sakkary,** Fouad El-Shehabi, Amira Taman, Paula Ribeiro.
Characterization of the Novel Schistosomal Receptor, SmGPR-3.

6. **Astrid Christine Erber,** Catherine Bourguinat, Roger Prichard.
Development of a Field Assay for Monitoring Ivermectin Resistance in Onchocerciasis.

7. **Véronique Gaudreault,** Nilson Quintana, Jaime Sanchez-Dardon, Robert Moreau, Tatiana Scorza.
Heme differentially modulates MHC class II molecule expression in peritoneal B cells and macrophages and may contribute to the selection of B cells with a heme-resistant phenotype in hemolytic disorders.

8. **Hamed Hojjat** and Armando Jardim.
Mapping the Leishmania donovani LdPEX5-LdPEX14 interacting surfaces

9. **Fikregabrail Aberra Kassa,** Marina Tiemi Shio and Martin Olivier
Malaria-related serum proteins modify hemozoin-induced innate immune response

10. **Zhiquan Lu,** Vaibhav Mehta, Hamed Shateri Najafabadi, Reza Salavati.
Characterization of ARE-binding proteins in *Trypanosoma brucei*.

11. **Kevin MacDonald** and Paula Ribeiro.
Acetylcholine-gated Chloride Channel Subunits as Modulators of *S. mansoni* Motor Function.

12. **Christine Matte**, Neda Moradin, Albert Descoteaux.
Alteration of the autophagic response by *Leishmania major* promastigotes.

13. **Laura-Isobel McCall,** Wen-Wei Zhang and Greg Matlashewski.
Immunization with a cutaneous *L. donovani* isolate from Sri Lanka protects against visceral leishmaniasis.

14. **Benjamin Ralph** and Martin Olivier
SHP-1/PTP1B Macrophage Interactome upon *Leishmania mexicana* Infection.

15. **Mohammed Rashid,** Paula Ribeiro.
Characterization of putative cation-selective nicotinic acetylcholine receptors in *Schistosoma mansoni.*

16. **Sarah J. Reiling,** Petra Rohrbach.
Investigating PfMDR1 drug transport in *Plasmodium falciparum.*

17. **Ruiz-Lancheros E,** Jansen G, Thomas D, Geary T.
In situ and in vitro deorphanization *of Caenorhabditis elegans* FMRFamide-like peptides.

18. **Karine Sonzogni-Desautels,** Timothy G. Geary.
Targeting *Cryptosporidium parvum* surface proteins to inhibit host cell infection *in vitro* and to control cryptosporidiosis in a rodent model.

19. **Starr, L.M.,** Halpenny, C., Naylor, J., Koski, K.G., Sinisterra, O., Scott, M.E.
Stunting, Hormones and Cytokines Influence Intestinal Infection of Panamanian Preschool Children.

20. **Pegine B. Walrad,** Paul Capewell, Katelyn Fenn & Keith R. Matthews.
The post-transcriptional *trans*-acting regulator, *Tb*ZFP3, coordinates transmission-stage enriched mRNAs in *Trypanosoma brucei.*

21. **Bahar Zarrabian,** Mifong Tam, Rajesh Valanparambil, Timothy Geary, and Mary M. Stevenson.
Identification of intracellular signaling pathways activated in ESP-treated dendritic cells.

22. **Wen-Wei Zhang,** Laura-Isobel Mccall and Greg Matlashewski,
The cytosolic glyceraldehyde-3-phosphate dehydrogenase in *Leishmania donovani* is involved in glycolysis and required for normal growth and virulence.

ABSTRACTS

Samiah Alam[1], Janet Yee[1,2], Manon Couture[3], Shin-ichi J. Takayama[4], A. Grant Mauk[4], Steven Rafferty[1,2]
[1]Environmental and Life Sciences Graduate Program, [2]Biochemistry Program, Trent University, [3]Département de biochimie, de microbiologie et de bio-informatique, Université Laval, [4]UBC Centre for Blood Research

Expression & characterization of a cytochrome-b_5 from *Giardia lamblia*

Although it is a eukaryote, the parasitic protist *Giardia lamblia* lacks mitochondria and cannot synthesize heme. On this basis, and on the absence of other common heme enzymes such as catalase, it was thought that Giardia lacked heme proteins. This assumption was challenged when it was discovered that the Giardia genome encodes several putative heme proteins, and that recombinant expression of one of them (a flavohemoglobin) yielded an active heme-containing enzyme. Here we describe the cloning and recombinant expression of a Giardia heme protein ($gCYTb_5$) with sequence similarity to the cytochromes b_5. These are small (~15 kDa) heme-binding proteins or modules of larger multidomain complexes. The gene for $gCYTb_5$ was PCR amplified from Giardia genomic DNA, cloned into a pET-14b vector, and expressed in *E. coli* as a 17 kDa protein with heme bound to it. Heme is extractable from $gCYTb_5$ by acid butanone treatment, indicating a noncovalent association between the cofactor and protein. The UV-visible and Resonance-Raman spectra of $gCTYb_5$ in both the oxidized and reduced states resemble those of known *b*-type cytochromes. Sequence alignment and homology modelling of the structure identify a pair of conserved histidine residues as the axial ligands to the heme. The oxidation-reduction potential of the protein is -165 mV (*vs* NHE), which is low compared to other family members. While such cytochromes serve as electron shuttles with well-established roles in electron transfer pathways, the roles of $gCYTb_5$ and means by which Giardia acquires heme, are not yet known.This work is supported by NSERC Discovery Grants (JY, MC, SR), NSERC USRA (SA) and a Queen Elizabeth II Graduate Scholarship in Science and Technology (SA).

Guadalupe Andreani, Michel Ouellet and Michel J Tremblay. Laboratoire d'immuno-rétrovirologie humaine, Centre de Recherche en Infectiologie de l'Université Laval, CHUQ, Pavillon CHUL, 2705 boul. Laurier, Québec, PQ, G1V 4G2

***Leishmania infantum* amastigotes trigger human B cells with regulatory functions.**

Regulatory B cells (Breg) were shown to suppress $CD4^+$ T cells functions via secretion of IL-10. In mice infected by *Leishmania major*, Breg were identified as a major source of IL-10 which was further shown to be required for visceralization of the pathogen. The importance of IL-10 on leishmania visceralization in humans has also been established. In this work, we evaluated the ability of *Leishmania infantum* (Li) amastigotes to activate human tonsillar B cells to secrete IL-10 and study its effect on the immune response. Stimulation of B cells with Li amastigotes induced IL-10 mRNA expression and protein secretion in a dose-dependent manner. This stimulation also led to an increase of the surface expression of CD25, CD69, CD80, CD83 and CD86, indicating B cell activation. Experiments using pharmacological inhibitors showed that signal transduction induced by exposure of Li to tonsillar B cells is mediated at least in part by Syk, p38 and PI3K. This suggests an involvement of the B cell receptor together with one or more Toll-like receptor(s). Supernatants from Li-stimulated B cells strongly inhibited expression of TNF□by primary human $CD4^+$ T cells following CD3 stimulation. In a similar fashion, supernatants from Li-stimulated B cells markedly reduced expression of activation markers CD69 and CD25 and abrogated proliferation of $CD4^+$ T cells following CD3/CD28 costimulation. These results therefore suggest the activation of human Breg in response to Li; however the exact role of IL-10 on the observed modulation of $CD4^+$ T cell functions remains to be elucidated. This work was supported by operating grant #MOP-84555 from the CIHR.

Guillermo ARANGO DUQUE[1], Mitsunori FUKUDA[2] and Albert DESCOTEAUX[1] [1]INRS-Institut Armand-Frappier and CHPI. [2]Department of Developmental Biology and Neurosciences, Tohoku University, Japan.

Synaptotagmin XI, a negative regulator of cytokine secretion and phagocytosis, is targeted by GP63

Leishmania parasites infect phagocytic cells, especially macrophages. An important pathogenesis factor in *Leishmania* is the GP63 metalloprotease. GP63 alters multiple modules of macrophage signalling and cleaves components of the vesicle fusion machinery, ensuing in weakened antimicrobicidal responses. Synaptotagmins (Syts) are membrane proteins that regulate vesicle docking and fusion in processes such as exocytosis and phagocytosis. Syts possess a transmembrane domain, and two conserved tandem Ca^{2+}-binding C2 domains. However, Syts IV and XI possess a conserved serine in their C2A domain that precludes these Syts from binding Ca^{2+}, and from mediating vesicle fusion. Given the importance of vesicular trafficking in macrophages, the objective of this research was to elucidate the role of Syt XI in cytokine secretion and phagocytosis, and to investigate the impact of GP63 on Syt XI function. We demonstrated that Syt is expressed in macrophages, localized in transferrin receptor 1-containing recycling endosomes, and recruited to early phagosomes. Syt XI had a direct effect on the secretion of tumour necrosis factor (TNF) and interleukin 6 (IL-6), and on phagocytosis. Whereas siRNA-mediated knockdown of Syt XI potentiated secretion of these cytokines and particle uptake, overexpression of a Syt XI construct suppressed these processes. On the other hand, Syt XI was not recruited to phagosomes containing *L. major* promastigotes expressing GP63. Upon finding that GP63 caused Syt XI degradation and augmented the release of TNF and IL-6, we showed that secretion of these cytokines depended on Syt XI degradation. Altogether, our data reveal novel roles for Syt XI, and provide a mechanism by which *Leishmania* induces the secretion of proinflammatory cytokines.

Hiva Azizi and Barbara Papadopoulou, Research Centre in Infectious Disease, CHUL Research Centre (CHUQ), Laval University, Quebec, Canada.

Rapid mRNA decay mediated by SIDER2 retroposons in *Leishmania*

Regulation of gene expression in *Leishmania* occurs almost exclusively post-transcriptionally. We have shown previously that widespread truncated versions of formerly active retroposons, SIDER2 (**S**hort **I**nterspersed **DE**generate **R**etroposons), within 3'-untranslated regions (3'UTRs) of *Leishmania* transcripts promote mRNA destabilization without prior deadenylation and that mRNA decay is initiated by a specific endonucleolytic cleavage within the 79-nt conserved SIDER2 signature sequence. Genomic replacement of the *L. infantum* LinJ36.4000 ORF (harboring SIDER2 in its 3'UTR) and of the LinJ36.3990 adjacent gene lacking SIDER2 by the neomycin phosphotransferase marker (*NEO*) showed an increase in *NEO* transcript decay only in the presence of SIDER2. However, no regulation was observed with the *L. major* orthologue (LmjF36.3810), suggesting that the SIDER2-mediated decay process is differentially regulated among different *Leishmania* species. We further investigated whether rapid degradation of SIDER2-containing transcripts requires the translation machinery. The introduction of a hairpin structure upstream of the initiator codon of a luciferase (*LUC*) reporter gene regulated by SIDER2 to block translation increased *LUC* transcript accumulation, suggesting that SIDER2-mediated decay is associated with ongoing translation. To identify the putative endoribonuclease and other protein factors involved in SIDER2-mediated mRNA degradation, we developed a tethering approach using the bacteriophage MS2 coat protein tagged with protein A and protein C domains and its cognate RNA hairpin located within the *LUC* reporter mRNA under the control of SIDER2. Experiments using this approach are under way. Collectively, these results provide new insights into the SIDER2-mediated decay mechanism of unstable mRNAs in *Leishmania*

Verena Brand[1], Carl Song[1], Sundar Natarajan[2], Viviana Pszenny[2], Denis Reynaud[1], Michael Leadley[1], Shaheena Bashir[3], Michael Grigg[2], John Parkinson[1]. [1]Hospital for Sick Children, Toronto, Ontario, Canada, [2]NIH/NIAID, Bethesda, MD, USA, [3]OICR, Toronto, Ontario, Canada

A metabolomics approach to characterize the different strains of *Toxoplasma gondii*

The apicomplexan pathogen *Toxoplasma gondii*, a relative of *Plasmodium* - the causative agent of malaria, is thought to infect one third of the world population. Some therapies exist; however, drug resistance has emerged. Population genetics have identified four clonal subgroups worldwide, designated types I, II, III and X, each with distinct virulence profiles. For example, for type I strains LD50 for mice requires a single parasite, on the other hand for type III, even initial infection with more 10^4 parasites may not be lethal.

Our hypothesis is that differences in virulence may, at least in part, be due to differences in metabolic capabilities. Metabolic reconstruction and modeling through flux balance analysis, which integrates microarray data for the three strains – type I, II and III, identified significant differences in metabolic capabilities. To complement these activities extracellular parasites representing these three types were subjected to metabolomic investigation through mass spectrometry (LC-MS). Focusing on eicosanoids, phosphatidylcholines, and sphingolipids, we identified significant differences in the abundance of several key lipids across strains. The abundance of the metabolite dihydroceramide 18:0 was significant higher in extracellular tachyzoites of type I than in the other types. Dihydroceramide is produced from sphinganine by sphingosine-N-acetyltransferase encoded by two different genes. One of these genes was found to possess a polymorphism in the type I strain relative to the other two strains, where the threonine at position 73 in the type II and III strains has been replaced with an alanine. Further investigations are currently ongoing to examine if this polymorphism is responsible for the observed increase in dihydroceramide and potentially virulence in type I strains.

Normand Cyr[1,2], Terry K Smith[3], Isabelle Coppens[4], and Armando Jardim[1,2]. [1]Institute of Parasitology and [2]Centre for Host-Parasite Interactions, McGill University, Montréal, Québec Canada; [3]University of St. Andrews, St. Andrews, Scotland UK; [4]Johns Hopkins University, Baltimore, Maryland USA.

The hydrophobic region of the *Leishmania* peroxin 14 is implicated in the formation of a transient pore that mediates protein import into the glycosomal matrix.

In *Leishmania*, glycolysis and a number of other vital metabolic pathways are segregated in the glycosome, a specialized organelle that is related to the peroxisomes of other eukaryotes. Proteins targeted to the glycosome typically contain either a PTS1 or PTS2 signal sequence that is recognized and is tightly bound by the receptor proteins peroxin 5 (LdPEX5) and peroxin 7 (LdPEX7), respectively. These cargo-receptor complexes are escorted to the glycosome surface where they bind to peroxin 14 (LdPEX14), a pivotal constituent of the docking/translocation machinery. However, little is known about the protein-lipid bilayer interactions involved in the formation of a pore structure that mediates the translocation of PTS1 and PTS2 cargo proteins across the glycosomal membrane. Here we show that the *Leishmania donovani* LdPEX14 forms a macromolecular structure on the glycosome surface resembling a rosette or ring-like structure with a diameter of ~30-40 nm. Studies, using large unilamellar liposomes mimicking the glycosomal membrane, established that an amphipathic region spanning residues 149-179 is critical for membrane binding. Moreover, dye release assay demonstrated that a LdPEX14 fragment encompassing this amphipathic structure is sufficient to pores formation in liposomes mimicking the glycosomal membrane. These results suggest a model in which the binding of cargo loaded LdPEX5 and LdPEX7 to LdPEX14 triggers structural changes that promote insertion of the LdPEX14 amphipathic helix into the glycosomal membrane and formation of a transmembrane pore through which glycosomal matrix proteins are imported.

Esther Dalko, Nilson Quintana, Jaime Sanchez-Dardon, Robert Moreau, Tatiana Scorza, Université du Québec à Montréal

The antagonist effect of free heme on CD4 T cell responses and clearance of *Plasmodium* infection in mice

Patients suffering from hemolytic pathologies as Malaria and Sickle Cell Disease display increased susceptibility to intracellular infections and blunted T helper (T_h) 1 responses. High concentrations of free heme (HE) are characteristic of these hemolytic pathologies but little is known about its effects on adaptive immunity. Herein, we investigated in BALB/c mice the impact of HE administration on factors involved in the establishment and differentiation of T_h2 versus T_h1 responses *in vivo*. Administration of HE increased the number of splenic basophils and the levels of histamine, all indicatives of a pro-T_h2 environment. Impaired IFN-γ responses were measured both in stimulated spleen cells and purified CD4 T cells from HE-conditioned mice, as well as increased production of IL-4. Furthermore, splenic CD4 T cells from mice conditioned with HE *in vivo* displayed altered expression of T-bet, the master transcription factor of T_h1 cells, and hampered *ex vivo* differentiation into T_h1 cells. These results suggest that by hampering the development of T_h1 responses, HE may contribute to increased infections with intracellular pathogens such as *Salmonella* or *Mycobacterium tuberculosis*, and may interfere with the responses to

vaccines designed to stimulate T_h1 immunity. Interestingly, administration of biologically relevant amounts of HE in mice prior to *P. chabaudi adami* DK infection resulted in decreased cumulative and peak parasitemia, suggesting that in the specific case of malaria, HE favors parasite clearance, by a mechanism that is cytokine independent. As red blood cells (RBC) from HE conditioned mice were shown to be more prompt to hemolysis, we suspect that HE modifies RBC membranes and may in this manner interfere with parasite invasion and/or multiplication. This work was partially funded by CIHR and NSERC.

Felipe Dargent[1], Marilyn E. Scott[2], Andrew Hendry[3] & Gregor F. Fussmann[1]. 1.Department of Biology, McGill University, Montréal, Canada QC H3A 1B1; 2.Institute of Parasitology, McGill University, Ste. Anne de Bellevue, Canada QC H9X 3V9; 3.Redpath Museum, McGill University, Montréal, Canada QC H3A 2K6.

Enemy release does not lead to decreased resistance in hosts

Individual hosts with increased ability to defend themselves from parasites are selected when the likelihood of being infected is high and parasites have a negative impact on host fitness. Ecological immunology assumes that defence against parasites is costly and trades-off with other host fitness-enhancing traits. Therefore it has been suggested that when hosts are released from their parasites, individuals that allocate more resources to growth and reproduction, instead of defence, will have higher fitness (evolution of increased competitive ability – EICA). Although theoretical evidence supports these claims, empirical evidence is still limited. In order to test the hypothesis that enemy release leads to decrease parasite resistance we translocated 400 parasite-free guppies (*Poecilia reticulata*) from a site were their monogenean ectoparasite *Gyrodactylus turnbulli* is present and introduced them into two previously guppy-free tributary streams. After eight generations in the wild we sampled 20 females from the three sites and bred them to a second generation under common garden conditions. F2 fish were infected with an isogenic strain of *Gyrodactylus turnbulli* and their parasite loads were assessed every two days for the duration of infection. Our results show that enemy release leads to increased host resistance against parasites. This suggest that defence against parasites could be driven by selection on correlated traits and that EICA is not a necessary outcome of enemy release.

Corine G. Demanga, Sarah J. Reiling, Juliane Wunderlich, Jenny W. L. Eng, Petra Rohrbach, John P. Dalton; Institute of Parasitology, McGill Univ.

A novel method for large-scale culture of *Plasmodium falciparum* in a Wave Bioreactor.

The continuous culture of *Plasmodium falciparum* malaria involves growing parasites in tissue culture flask in the presence of human erythrocytes at low O_2 concentration. However, bulking-up of cultures to obtain workable quantities of parasite material requires establishing multiple flasks, which becomes very time consuming and labour intensive. Over the last decade a series of sterile disposable plastic bioreactors have been developed for cell culture. Controlled wave-induced motion within the bioreactors provides low-shear and favourable hydrodynamic conditions for cell cultivation and very efficient gas transfer. Therefore to obtain workable amounts of parasite material with high consistency, we have adapted the wave bioreactor system to *P. falciparum* culture. The strains 3D7, Dd2 and FCR3 were selected for this study and we have used the wave Bioreactor^{TM} 20/50 EHT (GE). This is composed of a plastic cellbag, which is the cultivation chamber, and a platform that sits on a rocking unit. Several parameters within the bioreactor chamber can be controlled remotely by a touchpad. We first established various basic parameters for maintaining *P.falciparum* culture such as rocking motion, temperature and gas flow. A gas mix of 3% O_2, 5% CO_2 and 92% N was directly fed from a cylinder into the bioreactor via the rocking unit. During the malaria culture process we have analyzed the impact of lactate production and pH of the medium on parasite growth within the bioreactor. Finally, we established a simple protocol for a 1-L culture of healthy *P. falciparum* parasites that retain synchronicity over at least three cycles. This breakthrough method will allow large scale production of malaria parasites for antigen or organelle isolation, high throughput screening of compound libraries and for whole cells blood-stage malaria vaccine development under GMP compliant procedures.

Erin Dodd, D. Scott Bohle, Department of Chemistry, McGill Univ.

The structure of the hemozoin-chloroquine complex: Single crystal structure and solution studies with a gallium heme analog.

The development of new drugs which target the most virulent strain of the malaria parasite, *Plasmodium falciparum*, has been hampered by ambiguity in the prediction of structural features which lead to efficacy in the known 4-aminoquinoline-family antimalarial agents. Despite the spread of malaria strains which are resistant to these therapies, 4-aminoquinoline-based antimalarials remain a potent tool in the treatment of malaria around the world. The crystal structure of the chloroquine – gallium(III) protoporphyrin IX reciprocal dimer complex shows a mechanism of binding that confirms predictions of a quinoline ring that lies flat over the porphyrin and a side chain that interferes with the hydrogen bonding network of the porphyrin acid groups of the dimeric hemozoin analog which is 6-coordinate with a bound solvent molecule. Solution studies by ^{1}H NMR and fluorescence confirm the features of the solid state structure exist in solution in equilibrium with the unbound drug and monomeric metalloporphyrin.

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Characterization of novel glutamate-gated chloride channels in *Schistosoma mansoni*

Cys-loop ligand-gated ion channels (LGIC) are instrumental for nervous system modulation, both in vertebrates and invertebrates. While LGICs are widely described in nematodes and insects, very little is known about these channels in flatworms, including in *Schistosoma mansoni*. We have cloned and characterized 3 glutamate-gated chloride channel (GluCl) subunits from *S. mansoni*; these are the first GluCl subunits from flatworms to be successfully cloned and expressed. Bioinformatics and electrophysiology have revealed that not only are the SmGluCl subunits evolutionarily unique, but their pharmacological and biophysical properties are also distinct from their counterparts in other invertebrates, particularly with regard to their sensitivity to agonists and modulators. In addition, confocal laser microscopy analyses show that these SmGluCl subunits appear to be distributed throughout the central and peripheral nervous system of the worm. Further work is in progress in order to provide a detailed description of SmGluCl distribution in males, females, cercaria and somules. This project has uncovered a completely new aspect of neuronal modulation in flatworms, and brings attention to very appealing new anthelmintic targets which could be used to address the urgent need for new chemotherapeutic options for schistosomiasis.

Sonia EDAYE, Mara L. Leimanis, Sarah Reiling, Juliane Wunderlich, Petra Rohrbach, Elias Georges. Institute of Parasitology, McGill University.

A Quinoline-based Drug, MK571, Inhibits P-gh1-mediated Drug Transport and is More Toxic to Choroquine Resistant *Plasmodium falciparum*

The high incidence of malaria and drug-resistance poses a major problem in malaria control. Two membrane transport proteins, Pgh-1 (P-glycoprotein homologue 1) and PfCRT (*P. falciparum* chloroquine resistance transporter), are strongly associated with resistance of *P. falciparum* to several antimalarial drugs. Genetic studies have confirmed a link between mutations in *pfcrt* gene and resistance to chloroquine. Although Pgh-1 is not essential for chloroquine resistance, it has been shown to modulate the resistance to other quinoline drugs. Both Pgh-1 and PfCRT proteins are localized to the membrane of the digestive vacuole and appear to mediate drug transport across the DV membrane, but likely in opposite directions. In this study it was of interest to determine if the quinoline analogue, MK571, is a substrate to Pgh-1 and/or PfCRT. Our findings demonstrate that MK571 is more toxic to the chloroquine-resistant than the -sensitive parasite strains. Moreover, MK571 targets an organelle that appears to be the digestive vacuole, as increasing concentrations of the drug inhibit the formation of beta-hematin and increase the steady-state pH of the parasite's digestive vacuole. Furthermore, verapamil, which has been shown to specifically reverse PfCRT resistance to chloroquine, had no effect on the sensitivity of chloroquine susceptible or –resistant parasite strains to MK571. However, MK571 blocked the transport of Pgh-1 substrate (e.g., Fluo-4AM) into the digestive vacuole. Taken together, the findings in this study

suggest that MK571 binds to Pgh-1 and modulates the import of drugs or solutes into the parasite's digestive vacuole. Studies are ongoing to further characterize MK571 interactions with Pgh-1.

Nelly El-Sakkary, Fouad El-Shehabi, Amira Taman, Paula Ribeiro, Institute of Parasitology, McGill Univ.,
Characterization of the Novel Schistosomal Receptor, SmGPR-3.
The main causative agent of schistosomiasis, a disease which infects over 200 million people worldwide, is the parasite Schistosoma mansoni. Treatment of the disease is primarily with praziquantel (PZQ). There is an increasing fear that with the widespread use of the drug, and the lack of an available alternative, PZQ will lose its effectiveness. We are currently researching the schistosome nervous system to gain insight into this area. The nervous system coordinates many vital functions in the worm and is considered to be an excellent target for anti-schistosomal drugs. Recently, we have discovered a new group of schistosomal biogenic amine (BA) G-Protein Coupled Receptors (GPCRs), the smGPRs, which likely have a neuronal function. SmGPR-1, -2 and -3 have been cloned, and their pharmacological profiles determined by our lab. These receptors differ from the BA GPCRs of the human host in both sequence and function. SmGPR-3 was immunolocalized in the adult worm and shown to be expressed abundantly in the central and peripheral nervous system, including peripheral neurons which innervate the worm musculature, indicating a neuromuscular role. To further characterize smGPR-3, a predicted binding site (D3.32) was mutated and functional expression studies were performed, to assess receptor activity. The wild-type and mutant plasmid constructs were individually expressed in yeast cells which, upon successful interaction with ligand and receptor activation, express a reporter gene, allowing for the cells to be selectively grown in media. Cell growth is then quantitatively assayed, using the Alamar Blue fluorescence assay, as a measure of receptor activation. The mutants showed varied levels of responsiveness to ligand, indicating the site's importance. RNAi knock-down of smGPR-1, -2, and -3 in schistosomulae, followed by video analysis was performed. Targeting of smGPR-3 results in a decrease in worm motility by approximately half, as compared to the control, indicating a role in motility for the receptor.

Astrid Christine Erber, Catherine Bourguinat, Roger Prichard, Institute of Parasitology, McGill University
Development of a Field Assay for Monitoring Ivermectin Resistance in Onchocerciasis
Onchocerciasis, or River Blindness, is a parasitic disease in humans caused by the nematode *Onchocerca volvulus* and is transmitted by the black fly (*Simulium spp.*). Up to 37 millions of people are infected, 99% of which live in Africa. Symptoms are intense itching, dermatitis, depigmentation and atrophy of the skin. Inflammatory reactions can lead to irreversible blindness. Other complications are lymphadenitis, and possibly epilepsy, hyposexual dwarfism and excess mortality. Regular distribution of the macrocyclic lactone ivermectin, donated by Merck and Co. since 1988, was included in all major programs to fight onchocerciasis. The reduction of onchocerciasis-associated blindness and excess mortality in Sub-Saharan Africa already led to major socio-economic improvements. However, despite up to more than two decades of treatment in some areas, infections persist. Recent evidence of sub-optimal responses and genetic selection have raised concerns that a form of ivermectin resistance is arising. The project's specific aim is to develop a tool to allow for resistance monitoring by analysis of specific SNP markers. Adapted to adverse conditions, it will be suitable for immediate use in the field. After initial optimization under laboratory conditions, a small-scale pilot study will be performed in the field. Among the candidates for resistance markers are ABC transporter and β-tubulin genes, and possibly GABA- and glutamate-gated chloride channel genes. The research project is part of an existing research collaboration with institutes in Canada, Europe, Australia and disease endemic countries in Africa. Its broader aim is to address the causes of the sub-optimal responses to ivermectin treatment by genetic analysis, and validation of the parasite populations and DNA markers for ivermectin resistance.

Daniel Feingold, Stephanie Bourque, Patrick Janukavicius, Saima Sidik, Laura Nilson, Joseph Dent. McGill University.

Characterization of three novel Cys-loop ligand-gated ion channel subunits in *Drosophila melanogaster* – Potential pesticide targets?

Cys-loop ligand gated ion channels (LGICs) are pentameric neurotransmitter receptors that are ubiquitous in both vertebrate and invertebrate nervous systems. Their large diversity as well as their central role in mediating rapid synaptic transmission has made these channels attractive molecular targets for various pesticides. Despite the widespread use 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* to determine their potential as pesticide targets. These genes are of particular interest because they are specific to arthropods 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 off-target effects. Electrophysiological tests indicate that CG11340 can form a functional homomeric channel while CG7589 and CG6927 can form a heteromeric channel. We generated loss of function alleles for all three genes and mutations in CG7589 and CG11340 exhibit lethal phenotypes. CG7589 and CG11340 are expressed in the midgut and Malpighian tubules - tissues involved in osmoregulation - and CG6927 appears to be expressed in the salivary glands. Consistent with the CG11340 expression data, preliminary findings indicate that CG11340 mutants are sensitive to osmotic stress. Based on the divergence of these genes from other Cys-loop LGIC subunits as well as the lethal phenotypes associated with the corresponding mutants, these channel subunits may provide a promising target for a novel class of highly selective and efficient pesticides.

Véronique Gaudreault, Nilson Quintana, Jaime Sanchez-Dardon, Robert Moreau, Tatiana Scorza. Université du Québec à Montréal.

Heme differentially modulates MHC class II molecule expression in peritoneal B cells and macrophages and may contribute to the selection of B cells with a heme-resistant phenotype in hemolytic disorders.

The hemolytic events in malaria and sickle cell disease release heme (HE), a potent oxidant and pro-inflammatory molecule. As these pathologies are characterized by oxidative stress, inflammation and altered immunity, it is pivotal to understand the impact of free HE on the interactions bridging innate with adaptive immunity. Herein, we assessed the short-term effects of HE on MHC class II expression in peritoneal B cells and macrophages using serum free conditions. Treatment with HE (7.5-15μM) led to rapid drops in the side scatter profiles of macrophages, which was accompanied by a significant increase in MHC class II expression. In contrast, HE decreased the constitutive expression of MHC class II molecules in B cells, causing also drops in CD19 expression. The effect of HE on macrophages and B cells was blocked by hemopexin and albumin at equimolar concentrations, suggesting that these scavengers efficiently abrogate its reactivity with antigen presenting cells. Intra-peritoneal administration of HE led to a drastic depletion of peritoneal macrophages and to significant drops in the percentages of peritoneal B cells, but interestingly, the remaining B cells became refractory to the inhibitory effects of HE on MHC class II expression or CD19 down-regulation. These results suggest that hemopexin and albumin efficiently protect against HE reactivity with B cells and macrophages, but that *in vivo*, high concentrations of HE may bypass this protection. The effects of intravenous administration of HE on splenic and peritoneal macrophages and B cells remains to be assessed, as well as its impact on the induction of B cells with a HE-resistance phenotype. This work was partially funded by CIHR and NSERC.

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Interaction of macrocyclic lactones endectocides with *Haemonchus contortus* P-glycoproteins.

Haemonchus contortus is an important parasitic nematode of small ruminants that has developed anthelmintic resistance, which represents a serious problem worldwide. Macrocyclic lactones (ML) anthelmintics such as Ivermectin (IVM) and Moxidectin (MOX) have been extensively used against ecto and endoparasites in livestock. There is accumulative evidence that *H. contortus* has developed ML resistance due to in part, to the involvement of constitutive over expression of ABC transporters such as P-glycoproteins (P-gp), which may be influencing the concentration of MLs that reaches the target inside

the worm. The goal of this work was to clone and express *H. contortus* P-glyoproteins in mammalian cells and establish the interaction of these transporters with IVM or MOX. After the characterization and stable expression of these *H. contortus* P-gp's, the inhibition of the transport function by the different MLs was assessed, measuring the intracellular accumulation of fluorophores. HcP-gp's showed consistent less fluorescence in comparison with untransfected parental cells, meaning that the nematode P-gp's transfected in mammalian cells, are expressed and actively transport. Following this, the effect of the MLs on the fluorophores transport was assessed. We found that IVM and MOX produced markedly different responses on the nematode transporters, with IVM being a much more effective inhibitor of the nematode P-gp's transport than was MOX. The results indicate that nematode P-gp's may play an important role in regulating the concentration of avermectins, such as IVM, that reaches the receptors in the parasite, and thus the development of resistance, whereas for MOX, these transporters seems to be less involved in resistance. Research supported by CONICYT-Chile, NSERC, FQRNT, Centre Host-Parasite

Hamed Hojjat and Armando Jardim, Institute of Parasitology, McGill University.

Mapping the Leishmania donovani LdPEX5-LdPEX14 interacting surfaces

Interaction between LdPEX5 and LdPEX14 is a critical step in the translocation of PTS1 proteins into the glycosomes, and essential for the viability of the *Leishmania*. Employing a bacterial two hybrid (BTH) platform a systematic deletion mutagenesis analysis of LdPEX14 was performed to localized the minimum region of this protein require for LdPEX5 interaction. These studies revealed that a fragment encompassing residues 35 to 80 is necessary for retaining a stable protein-protein interaction. To further fine mapping the amino acid that are critical for this association a random mutagenesis library of the open reading frame encoding this fragment was generated and analyzed using a quantitative BTH assay, isothermal titration calorimetry (ITC), and ELISA experiments demonstrated that residues F40, V46, and F57 are critical for stabilizing the LdPEX5-LdPEX14 interaction. Surprisingly, circular dichroism, size-exclusion chromatography, and native gel electrophoresis, biophysical techniques used to examine the secondary and quaternary structure of proteins showed that none of the mutations at these positions had a significant impact on the LdPEX14 conformation, suggesting that the LdPEX5-LdPEX14 interaction may be preferentially stabilized by side chain, rather than backbone contacts.

Felix Hugentobler, Raphaël B. Di Roberto, Joshua Gillard, and Benoit Cousineau, Microbiology and Immunology, McGill University

Oral Immunization Using live *Lactococcus lactis* co-expressing LACK and IL-12 Protects BALB/c mice Against *Leishmania major* Infection

Leishmaniasis is a parasitic disease affecting over 12 million individuals worldwide. Current treatments are laborious, expensive, cause severe side effects, and emerging drug resistance has been reported. While vaccination is the most cost-effective means to control infectious diseases there is no human vaccine currently available against *Leishmania* infections. *Lactococcus lactis* is a non-pathogenic, non-colonizing Gram-positive lactic acid bacterium commonly used in the dairy industry. In this study, we report the generation of *L. lactis* strains that co-express the protective *Leishmania* antigen, LACK, either in the cytoplasm, secreted or anchored to the bacterial cell wall along with mouse IL-12. We show that oral immunization using live *L. lactis*, secreting both LACK and IL-12 was the only regimen protecting BALB/c mice against subsequent *Leishmania major* challenge. Protected animals displayed a delay in footpad swelling, which correlated with a significant reduction of parasite burden compared to control groups. Immunization with the *L. lactis* strain secreting both LACK and IL-12 induced an antigen-specific mucosal and a LACK-specific systemic T_H1 immune response. Further, protection in immunized animals correlated with a strong *Leishmania*-specific T_H1 immune response post-challenge, detectable in splenocytes and lymph node cells draining the site of infection. This work highlights the importance of temporal and physical proximity of the delivered antigen and adjuvant for optimal immune priming by oral immunization. The *L. lactis* vaccine strains generated in this study provide the basis for the development of an inexpensive and safe oral live vaccine against the human parasite *Leishmania*.

Amandine Isnard and Martin Olivier. MIMM & RIMUHC, McGill University.

Impact of *Leishmania* GP63 on Host Macrophage Nuclear Integrity, Signalling and Functions

During the early stages of infection, the *Leishmania* protease GP63 induces macrophage (MΦ) protein tyrosine phosphatases (PTPs), affecting the host cell signalling pathways. We recently discovered that GP63 reaches the MΦ nucleus affecting specific transcription factors (TFs) such as AP-1. In the present study, we want to decipher the mechanisms whereby GP63 enters into MΦ nucleus and its nuclear impact. Using an optimized nuclear extraction protocol in the LM1 MΦ cell line, we first confirmed that infection by *L. major* wild-type alters the nuclear translocation of TF (NFkB, AP-1), while after infection with *L. major GP63-/-*, TF act as in non-infected cells. We also confirmed that GP63 is responsible for the activation by cleavage of the nuclear PTPs SHP-1 and TCPTP. Interestingly, a *L. major* mutant for a Nuclear Localisation Signal (NLS)-like sequence identified in GP63 shows an intermediate pattern in both cases, suggesting that the classical nuclear transport via a NLS sequence is involved in the entry of GP63 in the nucleus, but not only. Indeed, we found that GP63 degrades two nucleoporins forming the NPC while entering the nucleus. This NPC degradation is time-dependent with *Leishmania* infection and correlates with the increase of nuclear GP63. Different *Leishmania* species cleave NPC, but not *L. tarentolae* in which GP63 is inactive, nor the *L. major GP63-/-*. In conclusion, the entrance of GP63 into the nucleus appears to involve an NLS-like sequence and the degradation of nucleoporins. Collectively, this study will extend our understanding on how the *Leishmania* virulence factor GP63 affects host cell integrity and functions.

Research funded by a grant from CIHR.

Patrick Janukavicius, Ludmel Urdaneta, James Bae, Laura Tiseo, Mi Tan, Roger Prichard, Joseph Dent, McGill University

***dyf-7* is responsible for the low-levels of ivermectin resistance in the *C. elegans* strains IVR6 and IVR10**

Ivermectin is a widely used nematocidal drug. Researchers Catherine James and Mary Davey selected for the ivermectin resistant strains IVR6 and IVR10 by growing them in the presence of ivermectin. They propose that the ivermectin resistance is due to an increase in the expression of the ABC transporters, which are believed to efflux the drug. Our lab has investigated the mechanisms of ivermectin resistance in the IVR6 and IVR10 strains. We uncovered that both strains are Dyf (Dye-Filling Defective), have the same level of ivermectin resistance, and have a frame shift mutation in the *dyf-7* gene. To show that *dyf-7* is responsible for ivermectin resistance we performed a mapping experiment which agreed with the location of *dyf-7* as the source of resistance. Next, we showed that ivermectin selects for the Dyf phenotype. The Dyf phenotype in IVR6 and IVR10 is not fully penetrant. The strains are normally ~80% Dyf but on 10 ng/ml ivermectin the adults are 100% Dyf. This shows that the Dyf phenotype is required for ivermectin resistance. We tested four other strains with mutations in Dyf genes, including a strain with a different mutant allele of *dyf-7*, and all were ivermectin resistant. Preliminary observations indicate that *dyf-7* mutations confer levamisole resistance, suggesting an alternate mechanism for multidrug resistance.

Fikregabrail Aberra Kassa, Marina Tiemi Shio and Martin Olivier. Department of Microbiology and Immunology; Center for the Study of Host Resistance and The Research Institute of McGill University Health Center, McGill University, Montréal, Québec.

Malaria-related serum proteins modify hemozoin-induced innate immune response

Recently, using novel proteomic and biochemical approaches, we have identified the host serum proteins that interact with the malarial hemozoin (HZ). We have shown that hemozoin specifically interacts with apolipoprotein E (ApoE), serum amyloid A (SAA), and LPS binding protein (LBP). In our current work, we were interested to determine the impact of these identified proteins on the macrophage's innate immune response triggered by HZ. HZ induces IL-1β production via the NLRP3 inflammasome. Using PMA-differentiated human monocytic cells (THP-1 cells), we looked at the production of IL-1β. This latter, along with TNF-a, is considered to be the major contributor for malaria pathology. Our results show that SAA bound to HZ induces the production of IL-1β in a dose dependant manner, whereas increasing concentration of ApoE abrogates this concentration-dependent production of IL-1β. Furthermore, our results show that HZ-SAA and hemozoin-ApoE complexes phosphorylate the MAPKs specifically ERK1/2 and JNK. This is in agreement with the observation that SAA interacting with CD36 activates downstream

signaling pathways via LYN resulting in the activation of JNK and ERK1/2. HZ bound serum proteins also modulate ROS production and phagocytosis in PMA-differentiated THP-1 cells. Interestingly, LBP enhances the recognition and phagocytosis of HZ by THP-1 cells. Overall, our results show that serum inflammatory proteins adhering on HZ modify its capacity to stimulate phagocytosis, IL-1β and ROS productions by THP-1 cells upon stimulation and therefore strongly suggest that such cooperation could greatly influence the development of malaria-related pathologies. This work is supported by an operating grant from CIHR.

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Gene expression modulation and the molecular mechanisms involved in Nelfinavir resistance in *Leishmania donovani* amastigotes.

Drug resistance is a major public health challenge in Leishmaniasis chemotherapy. Recently, we have delineated the mechanism of cell death induced by a HIV-1 protease inhibitor, Nelfinavir, in the *Leishmania* parasite. In order to investigate the underlying molecular mechanism involved in Nelfinavir resistance, we have developed *in vitro* Nelfinavir-resistant amastigotes by direct drug pressure in culture. In the present study, we performed RNA expression profiling analyses of closely related *Leishmania* species. *Leishmania infantum* was used as a screening tool to compare Nelfinavir-resistant and -sensitive *Leishmania donovani* in order to identify candidate genes involved in Nelfinavir resistance. Several genes were found to be differentially expressed between the two strains. We also carried out comparative gene hybridization (CGH) analyses of Nelfinavir-resistant and -sensitive *Leishmania donovani* using whole-genome 60-mer oligonucleotide microarrays. RNA expression profiles and the CGH analyses of Nelfinavir resistant *vs* sensitive *Leishmania donovani* amastigotes suggest that *Leishmania* increase or decrease mRNA levels using two mechanisms: first, via gene deletion or gene duplication by homologous recombination; second, by gene copy number increase or decrease through the formation of supernumerary chromosomes or loss of chromosomes. Interestingly, supernumerary chromosomes 6 and 11 in the resistant parasites are accountable for the up regulation of the ABCG and ABCA classes of ABC transporters, which are involved in vesicular trafficking. Transporter assays using radiolabeled Nelfinavir suggest that the drug accumulates in greater amounts in the resistant parasites and in a time dependent manner. These results suggest that Nelfinavir is rapidly and dramatically sequestered in intracellular vesicles. This study provides the first insights into the mechanisms of drug resistance to Nelfinavir in the *Leishmania donovani* amastigotes.

Zhiquan Lu, Vaibhav Mehta, Hamed Shateri Najafabadi, Reza Salavati, Institute of Parasitology, McGill University.

Characterization of ARE-binding proteins in *Trypanosoma brucei*.

In trypanosomes, gene expression is regulated mainly through post-transcriptional mechanisms. RNA-binding proteins are known to play a key role in post-transcriptional gene regulation by modulating mRNA half-life through interactions with *cis*-regulatory elements (i.e. 3' UTRs) of mRNAs. Our computational studies identified a highly conserved AU-rich element (ARE) in the 3' UTRs of certain *T. brucei* mRNAs. Transcripts containing the ARE in their 3' UTR are protected from degradation, suggesting that the motif promotes the stabilization of mRNAs. The expression of ARE-containing transcripts positively correlated with three RNA-binding proteins identified using PSI-BLAST as potential remote homologs of ELAV **(embryonic lethal, abnormal vision)** proteins. Proteins of the ELAV-like family bind to AREs and stabilize mRNA by protecting deadenylated transcripts against degradative enzymes. We confirmed the binding capability of *T. brucei* ELAV-like proteins to AREs, *in vitro*, using recombinant proteins. To further investigate the role of these proteins we created transgenic *T. brucei* cells lines which express tagged proteins. We are currently using these cell lines to purify the tagged ELAV-like proteins to identify both the RNA substrates and the proteins they associate with. Moreover, to determine whether the ELAV-like proteins are life-stage specific, the differential expression and RNAi knockdown of these proteins is being studied in both the bloodstream and the procyclic forms of *T. brucei*. Ongoing studies on the effect of RNAi knockdown and over-expression will help elucidate their role in gene regulation and if they are essential for cell survival.

Kevin MacDonald and Paula Ribeiro, Institute of Parasitology, McGill University.

Acetylcholine-gated Chloride Channel Subunits as Modulators of *S. mansoni* Motor Function

Single drug treatment of *S. mansoni* infections with praziquantel poses two challenges- the possible emergence of drug resistance and the ineffectiveness of praziquantel against larval schistosomulae. Therefore, discovery of new drug targets, particularly those effective against schistosomulae, is a high priority. Previous studies on neuromuscular modulators in schistosomes have demonstrated that functional nicotinic acetylcholine receptors (nAChRs) are present and play a role in motor function. Our preliminary studies suggest that several of these schistosome nAChR subunits may form anion-selective channels, which are promising drug targets due to their low homology to other nAChRs. The goal of the present study is to clone, characterize and immunolocalize the putative anion-selective nAChR subunits of *S. mansoni*. We first performed a bioinformatics analysis to identify putative anion-selective nAChR subunits. The resulting targets were screened by RNAi to determine if knockdown produced a behavioural phenotype in schistosomulae. In the initial screen, all 5 nAChR subunits tested yielded a significant increase ($P<0.01$) in baseline contraction frequency and overall length in the siRNA-treated samples compared to the negative control. The screen was then repeated on the two subunits producing the strongest phenotypes, Smp_142690 and Smp_176310, with similar results; knockdown of both Smp_142690 and Smp_176310 has been confirmed by qPCR. Peptide-derived antibodies were then generated against Smp_142690 and Smp_176310 and used for confocal microscopy. Data suggest that Smp_176310 localizes to the peripheral nervous system of the parasite, most notably the sub muscular peripheral nerve plexus and the oral sucker. Smp_142690 also localized to the peripheral nervous system, as well as to the tegument of the parasite.

Rency T. Mathew, John P. Dalton. Institute of Parasitology, McGill University.

The M1 alanyl aminopeptidases of *Plasmodium falciparum* (*Pf*M1AAP) malaria: biochemical and mutational analysis.

The *P. falciparum* alanyl aminopeptidase (*Pf*M1AAP) plays a role in the terminal stages of hemoglobin digestion and is a potential anti-malarial drug target. The molecular structure of *Pf*M1AAP complexed with two aminopeptidase inhibitors, bestatin and compound 4, has been determined and revealed that 16 active site residues are involved in the interaction of the enzyme with these inhibitors. When the sequence of *Pf*M1AAP is aligned with homologs of other malaria parasites we found that all these 16 residues are highly conserved. However, when we align the sequence of *Pf*M1AAP with homologs from other apicomplexan parasites (*T. gondii*, *C. parvum*, *C. hominis*, *T. parva*, *B. bovis*) several different alterations appear at specific positions. In this study, we substituted the residues of *Pf*M1AAP to corresponding residues of these other apicomplexan parasite homologs. We found that substitutions corresponding to residues observed in *Cryptosporidium parvum*, *Cryptosporidium hominis* and *Babesia* enhanced the catalytic efficiency (K_{cat}/K_m) of the enzyme while changes that corresponded to *Toxoplasma gondii* homologs and *Theileria* had detrimental effect on the catalytic efficiency of the enzyme. Based on these results we identified positions that were crucial for the activity of the enzyme, and for inhibitor binding. In order to confirm these observations, we generated additional mutants of *Pf*M1AAP with changes at these significant positions. Interestingly, we found that subtle changes in the active site residues either increased or lowered the catalytic efficiency of the enzyme. The inhibitor constant, K_i of the enzyme against 4 inhibitors bestatin, compound 4, tosedostat and H-Leu-chloromethylketone was obtained. Collectively, we have identified critical residue in substrate and inhibitor binding that could be important to understanding the potential drug resistance to aminopeptidase inhibitor.

Christine Matte, Neda Moradin, Albert Descoteaux, INRS-Institut Armand-Frappier and Centre for Host-Parasite Interactions

Alteration of the autophagic response by *Leishmania major* promastigotes

The protozoan parasite *Leishmania* causes a spectrum of diseases in humans, ranging from self-healing skin ulcers to life-threatening visceral infection. These parasites primarily infect macrophages and are renowned for their ability to sabotage host-cell signal transduction pathways. The Akt/mammalian Target Of Rapamycin (mTOR) axis plays a pivotal role in the regulation of multiple cellular processes, including protein synthesis, cytokine secretion, apoptosis, and autophagy. It is therefore a major target of infectious

pathogens. In this study, we aimed to investigate the impact of *L. major* promastigotes on the Ak/mTOR axis and downstream autophagy-related events. Infection of bone marrow-derived macrophages with *L. major* promastigotes caused rapid, time-dependent degradation of key components of the Akt/mTOR signaling axis, including Akt, mTOR and the Tuberous Sclerosis Complex-2 (TSC-2). Disruption of this pathway by *L. major* was dependent on the GPI-anchored zinc-dependent metalloprotease GP63, an important virulence factor of this parasite. Interestingly, recruitment of the autophagic marker LC3 to the parasitophorous vacuole of *L. major* promastigotes was inhibited by GP63, possibly due to GP63-mediated cleavage of vesicle-associated membrane protein 8 (VAMP8). Indeed, absence of VAMP8 resulted in inhibition of LC3 recruitment to phagosomes containing GP63-deficient parasites. This study highlights a novel pathogenic mechanism used by *L. major* to interfere with the autophagic response and will provide a better understanding of *Leishmania* pathogenesis. *Supported by CIHR*

Laura-Isobel McCall, Wen-Wei Zhang and Greg Matlashewski, Dept. of Microbiology & Immunology, McGill University.

Immunization with a cutaneous *L. donovani* isolate from Sri Lanka protects against visceral leishmaniasis

Leishmaniasis is a tropical disease caused by *Leishmania* protozoa and associated with a range of disease manifestations, from self-resolving cutaneous lesions to the much more severe visceral form of the disease. Disease phenotype is determined in part by the infecting species, with *L. donovani* normally causing visceral leishmaniasis. However, in Sri Lanka, *L. donovani* has been associated with many cases of cutaneous leishmaniasis and in contrast visceral disease is rare. We investigate here whether prior infection with a cutaenous strain of the parasite protects against visceral disease. Two clinical isolates were obtained from Sri Lanka, one from a cutaneous lesion (SL-CL) and one from a visceral leishmaniasis patient (SL-VL). BALB/c mice were immunized subcutaneously with 10^3-10^6 SL-CL promastigotes. Seven weeks following immunization, mice were challenged intravenously with SL-VL. Liver protection was observed for mice immunized with SL-CL doses of 10^4 and higher. In contrast, no significant difference in spleen parasite burden was observed between the groups, but a trend towards decreased spleen parasitemia was observed in mice immunized with 10^4 SL-CL. With regards to the immune response following challenge, no difference in IFNg production was observed between the different groups. However, IL4 and IL10 levels were higher in mice immunized with protective doses of SL-CL. Overall, these results provide a possible explanation for the low levels of visceral disease in Sri Lanka.

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Comparative gene expression analysis and antimony uptake in Sb-sensitive and -resistant New World *Leishmania* species.

DNA microarray is a suitable approach to study simultaneous events leading to drug-resistance, a major problem in leishmaniasis chemotherapy. Here we investigated the RNA expression profiling by DNA microarrays and the antimony (Sb^{III}) uptake in promastigote forms of *L.* (*L.*) *amazonensis*, *L.* (*V.*) *braziliensis* and *L.* (*V.*) *guyanensis*. A comparative analysis of shared modulated genes, between at least 2 species, revealed 156 upregulated and 68 down-regulated genes. Only 6 genes were commonly overexpressed in the three species studied. Among the upregulated hits we highlighted the presence of genes encoding proteins previously linked to drug resistance as the multi-drug resistance associated protein A (MRPA), trypanothione synthase/reductase, tryparedoxin peroxidase and S-adenosylhomocysteine hydrolase, related to redox homeostasis and drug inactivation. However, gene encoding proteins not yet related to drug resistance were also found to be upregulated. Work is now in progress in trying to functionally link these genes to the resistance phenotype. The Sb^{III} intake was performed using an atomic absorption technique and revealed a drastically reduction of Sb^{III} accumulation in the Sb^{III}-resistant mutants when compared to their parental sensitive wild-type strains, suggesting that either a reduced uptake or increased efflux was present on these cells. When using a metabolic inhibitor or low temperature, the Sb^{III} uptake was reduced in species from the *Viannia* subgenus but not in *L.* (*Leishmania*) *amazonensis* suggesting differences among species. These findings are helpful to better

understand the drug resistance mechanisms in *Leishmania* parasites. This work was supported by CIHR (Canada), CNPq and FAPEMIG (Brazil)

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***Leishmania* inhibits antigen crosspresentation by direct cleavage of the SNARE VAMP8**

Phagosomes play a key role in immunity by killing microbes and processing their antigens for T cell activation. Some intracellular pathogens such as *Leishmania* inhibit these steps and prevent phagosome maturation through different mechanisms. In the present study, we investigated the impact of infection on SNAREs (soluble *N*-ethylmaleimide-sensitive-factor attachment protein receptor) proteins involved in the trafficking to and from the phagosome. We discovered that upon infection, VAMP8, VAMP3, SNAP23, and syntaxin-4 were cleaved by GPI-anchored zinc metalloprotease GP63. Using a *L. major* gp63-KO mutant, we showed that this parasite protease is responsible for the cleavage of VAMP8 and other SNAREs in infected macrophages. We also found that *Leishmania* promastigotes inhibit antigen crosspresentation in a GP63-dependent manner. Using cells from VAMP8-deficient mice, we confirmed that this SNARE protein is required for antigen crosspresentation. Also, the phagosomal exclusion of Sec22b, one of the regulators in phagosome maturation and antigen cross presentation, was observed upon infection with *L. major* WT and gp63-KO add-back. Thus, we uncovered the existence of a novel mechanism used by *Leishmania* promastigotes to evade recognition by the immune system, whereby the parasites impair crosspresentation by degrading key regulators of vesicular trafficking.

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SHP-1/PTP1B Macrophage Interactome upon *Leishmania mexicana* Infection

Leishmania is the causative agent of leishmaniasis, a disease that affects 12 million people worldwide. It is an intracellular parasite that grows inside various cell types, mainly the macrophage. Once inside the host, it can alter intracellular signaling and decrease cellular activation by exploiting the host's protein tyrosine phosphatases (PTP). Previous work from our lab has shown that *Leishmania* species cleave PTPs resulting in their activation, thus leading to alteration of macrophage functions and signaling.[1,2] Therefore, the goal of this study was to identify PTP substrates with which they interact during a *Leishmania* infection. We have examined the presence of the major cytosolic PTPs, PTP-1B and SHP-1, in the plasma membrane and cytoplasm of the macrophage before and after infection with *Leishmania* using various cell fractionation techniques. We have also identified the cytosolic substrates of these PTPs by mass spectrometry. Our results show that the PTPs not only interact with the classic signaling proteins but also with multiple housekeeping proteins such as certain types of myosin and ribosomal proteins. This may indicate further how *Leishmania* is able to halt the cell functions such as phagocytosis or endosome trafficking that is facilitated by myosin. These findings further suggest that after activation due to cleavage by *Leishmania*, PTP-1B and SHP-1 primarily interact with cytoplasmic proteins and that the PTPs may regulate macrophage function via processes other than those already known. Future directions include a thorough investigation of the roles of interacting proteins during an infection.

Mohammed Rashid, Paula Ribeiro , Institute of Parasitology, McGill University.

Characterization of putative cation-selective nicotinic acetylcholine receptors in *Schistosoma mansoni*.

Praziquantel, the mainstay of treatment against the parasitic disease, is ineffective against larval stages of the parasite. Moreover, evidence of reduced efficacy of the drug has already been documented in endemic areas. As a result, the search for novel chemotherapeutic drugs is of utmost importance. Helminth ion channels have been the subject of much research in recent years due to their potential for drug targeting. However, this work has focused almost exclusively on nematodes. Here, we propose to take advantage of recent advances in Schistosome genomics to conduct the first broad analysis of these important proteins in the parasitic flatworm *Schistosoma mansoni*. Of the 9 putative nicotinic acetylcholine receptors (nAChRs), which contribute to formation of acetylcholine gated ion channels in the parasite, smAR1α, smAR1β and smp_180570 are predicted to form cation-selective channel(s).

Cation-selective nicotinic acetylcholine receptors have been classically associated with excitation of muscle fibres in both vertebrates and invertebrates. A chemotherapeutic agent that effectively blocks parasite-specific channels would be expected to interfere with locomotion of the parasite and clear the infection. In this study we describe a first functional analysis of cation-selective nAChRs in S. mansoni. The three predicted channel subunits have been cloned and are being analyzed in vitro by heterologous expression in *Xenopus* oocytes and cultured mammalian cells. Other studies are being attempted to elucidate the biological role of these channels, such as using RNA interference (RNAi) and immunolocalization methods in adult and larval (schistosomula) stages of the parasite.

Nour Rashwan, Catherine Bourguinat, Roger Prichard. Institute of Parasitology, McGill University.

Asymmetrical isothermal amplification method for genotyping mutations, in human soil-transmitted helminths, that have been associated with benzimidazole resistance

Soil-transmitted helminths (STHs), *Ascaris lumbricoides* and *Necator americanus,* are gastrointestinal nematodes causing human morbidity in tropical areas of the world. Benzimidazole (BZ) drugs have been used extensively for large-scale treatment of STHs. A growing concern is that extensive use of anthelmintics to control human parasites is likely to exert selection on parasite populations. The egg reduction rate has been used to monitor drug efficacy and to detect the development of resistance in the field. This assay is very insensitive for the detection of low levels of drug resistance. Previous molecular assays for putative resistance mutations have been based mainly on sequencing. However, sequencing is a time consuming and complicated procedure, not suitable for routine clinical use or for resource constrained situations. Therefore, development of simple, rapid and cost-effective molecular tools for detecting BZ resistance, that could be adaptable to field conditions, would be very helpful for sustainable control of STHs. We developed a novel genotyping assay based on the Smart Amplification Process (SmartAmp2) to detect mutations of the β-tubulin isotype 1 gene associated with BZ resistance under isothermal conditions. For experimental development, real-time PCR monitoring of the amplification was achieved within 40-60 min with suppression of the mismatch amplification. Wild-type and mutant plasmids were employed to develop and optimize the assay. The assays were applied to analyze fecal samples of eggs and larvae using full-match and mismatched primer sets. A SmartAmp2 assay was developed for genotyping the mutations in the β-tubulin gene and the reliability of the method was validated using the conventional PCR method. Work is being conducted to use end point detection system to enable this technique to detect mutations associated with BZ resistance in the field.

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The application of counterflow centrifugal elutriation and quantitative RT-PCR in the cell cycle analysis of *Giardia lamblia*

The protozoa *Giardia lamblia* is one of the leading causes of waterborne parasitic infection worldwide. Proliferation of the trophozoite form of Giardia in the intestine of a host contributes to giardiasis, a condition characterized by severe diarrhea and abdominal pain. As such, the cell cycle of trophozoites is an important area of research. Gene expression analysis of the cell cycle requires the manipulation of trophozoite cultures such that all cells are progressing through the cell cycle in synchrony, allowing fractions representing different cycle stages to be collected. Previous attempts at Giardia synchronization have involved the use of the drugs nocodazole combined with aphidicolin and aphidicolin alone in "arrest-and-release" experiments. These methods were effective in obtaining semi-synchronous Giardia cultures, but also produced cellular perturbations, most notably DNA damage, that could affect subsequent gene expression analysis. We are developing counterflow centrifugal elutriation (CCE) as a method to obtain samples of Giardia cultures that are enriched in cells from different stages of the cell cycle. As cells progress through the cell cycle from G1, S to G2, they undergo an increase in overall size due to the replication of genetic material and other cellular components. CCE uses these differences in cell size and density to effectively separate asynchronous cell cultures into the individual phases of the cell cycle. Our results indicate that this technique is capable of separating Giardia trophozoites into fractions of increasing cell size that are enriched in the stages of G1, S, and G2/M. Immunofluorescence assay of the presence of phosphorylated histone H2AX, a marker of double-stranded DNA damage, in the nuclei of

elutriated cells showed that negative effects produced by CCE are minimal compared to aphidicolin treatment. Additionally, quantitative PCR (qPCR) analysis of CCE fractions demonstrated that mRNA levels of numerous genes change during the cell cycle, including the core Giardia histone and cyclin genes, which were found to peak in fractions containing a majority of S- and G2/M-phase cells, respectively. Further results from our CCE experiments and quantitative PCR analysis of the cell cycle fractions obtained from this procedure will be presented and discussed.

This work is supported by a NSERC Discovery Grant (JY) and a NSERC Alexander Graham Bell Canada Graduate Scholarship (CR).

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In situ and in vitro deorphanization *of Caenorhabditis elegans* FMRFamide-like peptides

Neuropeptides, including FMRFamide-like peptides (FLPs), play critical roles in the nervous system of free-living and parasitic nematodes. FLPs are involved in a variety of behaviours essential for parasite viability and maintenance in a host. This makes FLP receptors useful targets for anthelmintic discovery. To deorphanize (match) potent *C. elegans* FLPs with their cognate G-protein-coupled receptors (GPCRs), we designed a novel *in situ* bioassay for screening for receptor ligands in *C. elegans.* We also optimized a system for the heterologous expression of FLP-GPCRs in *Saccharomyces cerevisiae* for receptor activation bioassays. For *in situ* matching, we identified characteristic phenotypes in bisected wild-type (WT) worms upon exposure to individual FLPs. Those phenotypes were used as a read-out in a low-throughput screen of candidate FLP-GPCRs in *C. elegans* strains with loss-of-function mutations in individual GPCR genes. Each tested FLP was matched with one or two GPCRs, as their respective knockout strain did not retain the FLP-phenotype observed in WT worms. The ligand–receptor associations were confirmed by expressing GPCRs in modified strains of *S. cerevisiae* for receptor activation bioassays. To improve and confirm the expression and cellular localization of recombinant GPCRs in yeast, the N-termini of receptors were modified with a peptide from the yeast GPCR Ste2 and the C-termini were fusion with the yeast MEKK ste11p. Activation of the yeast High-Osmolarity Glycerol (HOG) pathway was used as a reporter to monitor plasma membrane localization of GPCRs. Data from *in vivo* matches and heterologous systems suggest our strategy is suitable to identify nematode peptide-receptor associations. Our results accomplish a key step in the discovery of new anthelmintics that are non-peptide ligands for invertebrate neuropeptide GPCRs. This work was supported by NSERC, Canada Research Chairs, CIHR, the Bill & Melinda Gates foundation and a Tomlinson Fellowship from McGill.

Sarah J. Reiling, Petra Rohrbach, Institute of Parasitology, McGill University

Investigating PfMDR1 drug transport in *Plasmodium falciparum*.

The human malaria parasite *Plasmodium falciparum*, the causative agent of the most severe form of malaria, is responsible for an estimated 300 million clinical cases and 1.2 million deaths annually. The replication of the parasites in red blood cells causes the clinical symptoms of the disease and is where most anti-malarial drugs are targeted. Drug resistant strains of the parasite are widespread and the immediate prospect of a useful vaccine is uncertain, accentuating the need for a better understanding of drug resistance. Investigations into the mechanism of anti-malarial drug resistance suggest two malaria transporter proteins: the *P. falciparum* chloroquine resistance transporter (PfCRT), and the *P. falciparum* P-glycoprotein homolog 1 (Pgh-1, or PfMDR1). While there is consensus with PfCRT mutations and chloroquine resistance, little is known about the influence of PfMDR1 mutations and gene amplifications on drug resistance. By using an established functional fluorescence assay for PfMDR1 transport, we show that both amino acid mutations and *pfmdr1* gene amplification influence the transport properties of PfMDR1. More specifically, the *pfmdr1* mutation N86Y appears to be the main mediator of enhanced fluorescence influx into the digestive vacuole. Gene duplication further increases PfMDR1-mediated transport into this organelle.

Karine Sonzogni-Desautels, Timothy G. Geary, Institute of Parasitology, McGill University.

Targeting *Cryptosporidium parvum* surface proteins to inhibit host cell infection *in vitro* and to control cryptosporidiosis in a rodent model.

Bovine cryptosporidiosis is a real concern in the dairy and beef industries. *C. parvum* is a major cause of neonatal ruminant diarrhea, which can lead to a 10% mortality rate among newborn calves. There is no preventive or therapeutic protocol that can efficiently kill *C. parvum* oocysts or limit the infection once established. Because calves are infected during the first week of age, active immunization at birth is useless. *C. parvum* is a zoonotic protozoan which has a complex life cycle in host intestinal cells. The host cell invasion process requires specific surface proteins. Among them, p23, CP2, p30, gp15, gp45 and gp900 are the most important. Experiments with antibodies targeted against only one of these proteins failed to control infection. Our hypotheses are that a vaccine made of six different *C. parvum* surface proteins will better protect against cryptosporidiosis and that immunizing cows before calving will passively immunize calves against *C. parvum* infection. Portions of p23, CP2, p30, gp15, gp45 and gp900 *C. parvum* genes were cloned in pET-28a (+) for purification with a HisPur cobalt column. So far, p23 and CP2 have been prepared for antibody preparation and the protocol for p30 expression is being optimized. The sensitivity and specificity of these antibodies will be defined using Western blots. Their ability to inhibit *C. parvum* infection will be evaluated in cultures of Madin-Darby Bovine Kidney cells. Finally, I will test their ability to prevent and/or treat cryptosporidiosis in interferon gamma receptor knockout mice. Promising results may lead to the elaboration of a vaccine which will decrease cryptosporidiosis-related economic losses in dairy and beef industries while improving newborn calves' health status. This work is supported by NSERC and I am receiving a FQRNT fellowship.

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Stunting, Hormones and Cytokines Influence Intestinal Infection of Panamanian Preschool Children.

Despite control programs, intestinal parasitic infections remain a public health concern in rural Panama. We aimed to relate the presence of these infections in preschool children 3 months following albendazole treatment to anthropometric measures and hormone and cytokine concentrations prior to treatment. This longitudinal study evaluated intestinal nematode and *Giardia* infections pre- and post- treatment with a single dose of albendazole in 74 Panamanian schoolchildren ages 12-55 months for whom anthropometry and serum samples were obtained pre-treatment. Cytokine and hormone concentrations were measured using multiplex Luminex assays. Pre-treatment, 34% of the children were infected with at least one nematode (*Ascaris lumbricoides, Trichuris trichuria*, hookworm and/or *Strongyloides stercoralis*), with *Ascaris* being the most common (28% prevalence). *Giardia* infections were present in 15% of the children. Three months after albendazole treatment, the prevalence of nematode infection was 7%. *Giardia* prevalence was 20% and 9 of the 13 *Giardia*-infected children had been uninfected pre-treatment. Mean height-for-age Z-scores (HAZ) were significantly lower for children who were infected with any of these parasites after treatment (-2.96 ± 0.2446) compared with those with no infection post-treatment (-2.19 ± 0.1403) ($P<0.05$). In a multiple logistic regression model, lower IL-5 pre-treatment emerged as a risk factor for being infected after treatment and in multiple linear regression, IL-6 was a predictor of low HAZ. Our data suggest that the presence of intestinal infection three months following albendazole treatment is more likely in more severely stunted children, possibly due to over-active or inappropriate immune responses.

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***Leishmania donovani* glycosomal proteins form complexes in the cytosol for trafficking to the organelle surface.**

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 into the glycosome is dependent on the cytosolic receptor proteins peroxin 5 (LdPEX5) and peroxin 7 (LdPEX7), and the docking protein peroxin 14 (LdPEX14), a peripheral membrane protein anchored to the surface of the glycosomal membrane. This process is essential for viable parasites, as

disruption of this pathway is fatal. To understand the molecular events required for the sorting and trafficking of nascent polypeptides for import into the glycosome, we employed a variety of techniques to examine the structure of native LdPEX5, LdPEX7, and LdPEX14. Biochemical analysis revealed that in the cytosol of *L. donovani* LdPEX5 and LdPEX7 form structurally diverse heteromeric complexes loaded with various PTS1 and PTS2 cargo proteins. These complexes have been recapitulated *in vitro* using recombinant receptor proteins and model PTS proteins, where the various members of the recpetor-cargo complexes migrate together. The glycosomally targeted cargo proteins are thus trafficked to the surface of the glycosome, into which they are then imported via LdPEX14, the glycosomal docking protein, by an unknown mechanism that potentially involves recruitment of other glycosomal membrane proteins. Investigation of the effects of receptor-cargo complex binding to LdPEX14 revealed that docking of receptor-cargo complexes causes conformational changes in LdPEX14, and LdPEX5 and LdPEX7, including causing them to transform from peripheral membrane and cytosolic proteins, respectively, into integral membrane proteins. This work supported by grants from FQRNT and CIHR.

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Identification of the immunomodulatory molecule(s) in the excretory secretory (ES) products of the gastrointestinal nematode *Heligmosomoides polygyrus.*

Heligmosomoides polygyrus (Hp) is a natural gastrointestinal nematode of mice distinguished by its strong immunosuppressive nature. Hp excretory secretory products (HpES) suppresses the immune system by modulating dendritic cells (DC) and inducing a robust anti-inflammatory network involving regulatory T cells and alternatively activated macrophages. Induction of these mechanisms are considered responsible for the potent ability of this parasite to suppress immune responses to by-stander antigen including infections with unrelated pathogens, inflammatory diseases and allergy. To identify and characterize the molecule(s) in HpES that have immunomodulatory properties, we performed HPLC (size exclusion chromatography) to separate HpES proteins based on molecular weight .The immunosuppressive activity of the separated proteins was assessed by measuring IL-12p70 secretion by bone marrow-derived dendritic cells (BMDC) pre-treated with ES fractions prior to stimulation with TLR9 ligand, CpG-ODN. We identified 12 fractions that suppressed IL-12p70 induction by CpG-ODN. These fractions were pooled and subjected to ion-exchange chromatography using an anionic exchange column. Testing of these fractions on BMDC stimulated with CpG ODN showed that 3 of the fractions suppressed IL-12p70 secretion. Analysis of these fractions by LC-MS/MS identified a total of 9 candidate proteins. One of the proteins identified had an ankyrin repeat which is known to inhibit the NFκB pathway. Our goal is to clone and purify all the candidate proteins identified, to confirm the immunosuppressive nature of these proteins and to test their ability to suppress immune responses *in vivo* to an unrelated antigen.

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The post-transcriptional *trans*-acting regulator, *Tb*ZFP3, coordinates transmission-stage enriched mRNAs in *Trypanosoma brucei*.

Post-transcriptional gene regulation is essential to eukaryotic development. This is particularly emphasized in trypanosome parasites where genes are co-transcribed in polycistronic arrays but not necessarily co-regulated. The small CCCH protein, *Tb*ZFP3, has been identified as a *trans*-acting post-transcriptional regulator of Procyclin surface antigen expression in *T. brucei*. To investigate the wider role of *Tb*ZFP3 in parasite transmission, a global analysis of associating transcripts was carried out. Examination of selected transcripts revealed their increased abundance through mRNA stabilization upon *Tb*ZFP3 ectopic overexpression, dependent upon the integrity of the CCCH zinc finger domain. Reporter assays demonstrated that this regulation was mediated through 3'-*UTR* sequences for two target transcripts. Global developmental expression profiling of the cohort of *Tb*ZFP3-selected transcripts revealed their significant enrichment in transmissible stumpy forms of the parasite. Immunofluorescent assays demonstrate that *Tb*ZFP3 colocalises with a P body marker in starvation granules, and with a subset of *procyclin* transcripts in stumpy stage parasites. This analysis of the specific mRNAs selected by the *Tb*ZFP3mRNP provides evidence for a developmental regulon with the potential to stabilize and

coordinate genes important in parasite transmission. This work was supported by a Wellcome Trust Programme Grant.

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

Validating Acetylcholine-Gated Chloride Channels as Novel Nematocide Targets.

We have identified a novel class of ligand gated ion channels, the acetylcholine-gated chloride channels (ACCs), in *Caenorhabditis elegans* (*C. elegans*) that we believe would be a good targets for the design of new anthelmintic drugs. This ACC family is specific to nematodes (Dent, 2006) and is not a target of previously known nematocides. There are eight ACC-like subunit genes; a beneficial characteristic since a gene family that represents multiple targets of a single drug may slow the onset of resistance. To test the validity of these ACC subunits as targets for nematocides, we determined the expression pattern of the 8 ACC subunit genes using promoter::GFP fusion constructs and have been characterizing the electrophysiological properties of ACC channels formed by the different subunits. Most of the subunits are expressed in a non-overlapping subset of approximately 20 neurons in *C. elegans*. We have shown that four of the eight ACC-like subunits form functional homomeric channels that respond to acetylcholine (ACh) in oocytes and we have shown that they are chloride channels. We also have evidence that ACC-1 and F47A4.1 subunits can form a heteromeric ACh-senitive channel in oocytes. 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. We have shown that chronically inhibiting ACC-1, ACC-2, ACC-3 and F47A4.1- expressing tissues is lethal to the worm and have begun experiments to validate the remaining ACC subunits as good drug targets. Because of this, and the fact that the ACCs are nematode-specific, we conclude that ACCs merit further investigation as anti-parasitic drug targets and we hypothesize that a drug that targets these channels would be a safe and effective nematocide.

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Genomic and functional characterization of G protein-coupled Receptors in pathogenic and free-living flatworms

G protein-coupled receptors (GPCRs) represent the largest known superfamily of membrane proteins extending throughout the Metazoa, with prominent roles in signal transduction and as targets for chemotherapeutic intervention. There exists ample motivation to characterize this receptor complement in the Platyhelminthes (flatworms), given the health burden caused by pathogenic flatworms, as well as the role of free-living flatworms as model organisms for developmental biology. We describe the application of a comprehensive *in silico* protocol to identify GPCRs in the genomes of the human parasite Schistosoma *mansoni* and the model planarian *Schmidtea mediterranea*. This protocol led to the discovery of 117 *S. mansoni* and 460 *S. mediterranea* receptors, which were classified using phylogenetic, homology-based, and machine-learning approaches. We highlight phylum and parasite-specific innovations, focusing on the presence of novel GPCR clades. Lastly, we briefly describe an RNAi-based approach to identifying ligands for orphan receptors.

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Identification of intracellular signaling pathways activated in ESP-treated dendritic cells.

The murine nematode *Heligmosomoides polygyrus* serves as a model for studying human gastrointestinal helminth infections, which are highly endemic in areas of poor sanitation worldwide. *H. polygyrus* is recognized for its potent modulatory effects on host immune responses to unrelated antigens. Excretory-secretory products (ESP) released from adult *H. polygyrus* worms have been shown to be responsible for the immunosuppressive effects. Our previous studies showed that *H. polygyrus*-derived ESP modulates immune responses to unrelated antigens by inhibiting dendritic cell (DC) maturation and cytokine production in response to potent TLR ligands, but the intracellular signaling pathway(s) by which ESP

modulates DC function are unknown. To identify the signaling pathways involved, bone marrow-derived dendritic cells (BMDC) were stimulated in vitro with medium, CpG-ODN, ESP, or ESP prior to CpG-ODN. Following overnight culture, BMDC lysates were prepared and analyzed by Western blotting for differential signaling cascades, namely, the MAPK and spleen tyrosine kinase (Syk) pathways. Neither p38 MAPK, Erk 1/2, nor pSAPK/JNK were differentially regulated as a result of DC stimulation with ESP. However, we observed increased PLC-γ1 expression, indicating activation of the Syk pathway known to be involved in response to C-type lectins. Collectively, our data suggest that DC may recognize ESP in a TLR-independent manner but that *H. polygyrus*-derived ESP may induce signaling in DC via the Syk pathway. In conclusion, these observations provide novel information that may be useful in identifying *H.* polygyrus-derived ESP proteins involved in modulating DC function.

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The cytosolic glyceraldehyde-3-phosphate dehydrogenase in *Leishmania donovani* is involved in glycolysis and required for normal growth and virulence

While the first seven steps of glycolytic pathway from glucose to 3-phosphoglycerate are mainly localized in the glycosomes in protozoan *Leishmania*, the cytosolic counterpart of glyceraldehyde-3-phosphate dehydrogenase (cGAPDH) is found to be present in *L. donovani* complex species and *L. mexicana* but a pseudogene in *L. major* and absent in *L. braziliensis*. To investigate whether the cGAPDH is still functional, in this study, we generated a *L. donovani* cGAPDH null mutant by gene deletion targeting. Surprisingly, although cGAPDH is not essential and *L. donovani* cGAPDH null mutant was able to grow at the same rate as the wild type parasite in glucose deficient medium, *L. donovani* cGAPDH null mutant proliferated much more slowly in glucose containing medium and displayed greatly reduced virulence in infecting mice. This demonstrates the partial parallel glycolytic pathway from the sixth to seventh steps coexisting in the *L. donovani* cytosol is functional and required for *L. donovani* normal growth in both extracellular promastigotes and intracellular amastigotes and virulence. Interestingly, however, unlike in *L. donovani* and for not yet known reason, restoration of the cytosolic GAPDH activity had adverse effect on *L. major* normal growth in glucose containing medium, which may help explain why the cGAPDH has evolved into a pseudogene in *L. major*.