AMPS 2022

June 10, 2022 | Campus de St-Hyacinthe, Université de Montréal

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Welcome to AMPS 2022

Welcome to the second Annual Montreal Parasitology Symposium (AMPS)!

AMPS is a student initiative which aims to bring together researchers in the field of parasitology, provide trainees and postdoctoral fellows with a platform to present their work and stimulate exchange and collaborations.

AMPS 2022 Organizing Committee

Maude Dagenais, PhD Candidate, Institute of Parasitology, McGill University

Marine Queffeulou, PhD candidate – Université Laval

Audrey Sergerie, PhD candidate – Université Laval

Tanja Stögerer, PhD candidate – INRS-Institut Armand-Frappier

Ana Medina, PhD candidate – INRS-Institut Armand-Frappier

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Audrey Corbeil, PhD candidate – Université de Montréal

Ana Victoria Ibarra Meneses, PhD – Université de Montréal

Professor Christopher Fernandez-Prada, DMV, PhD – Université de Montréal

Professor Maritza Jaramillo, PhD – INRS-Institut Armand-Frappier

For those interested, there will be a pre-conference talk by Nathan Peters, from the University of Calgary.
When: 11:00 on June 9th, 2022
Where: INRS – Centre Armand-Frappier Santé Biotechnologie, 531 Boul. des Prairies, Laval, QC H7V 1B7
Title: Fast, Faster, Fastest: Understanding the importance of timing in protective $CD4^+$ T cell mediated immunity against *Leishmania.*
For more information: maritza.jaramillo@inrs.ca

The main event will be held on June 10th at the picturesque Jardin Daniel A. Séguin, adjacent to the Université de Montréal's Faculty of Veterinary Medicine Campus
3230 Rue Sicotte, Saint-Hyacinthe, QC, J2S 2M2, Canada

Time	Event	Location
8h30 - 9h30	**Registration + coffee**	**Entrance**
9h30 - 9h40	**Welcome and opening remarks** **Maude Dagenais & Dr. Christine Théoret**, Dean of the Faculty of Veterinary Medicine, Université de Montréal	**Rooms A&B**
9h40 - 10h40	**Keynote address: Dr. Jude Uzonna,** University of Manitoba - Changing Dogma?: Immunologic memory to cutaneous leishmaniasis may need constant reminding	**Rooms A&B**
10h40 - 10h55	**Gabriel Reis Ferreira**, Université Laval, QC - Combining multi-level bioinformatics tools to globally assess the diversified critical roles of SIDER2 retroposon elements in *Leishmania* genome ploidy, gene expression and developmental regulation	**Rooms A&B**
10h55 - 11h10	**George Dong,** McGill University, QC - Disruption of *Leishmania major* extracellular vesicle production significantly impedes its infectious capability and disease progression	**Rooms A&B**
11h10 - 11h25	**Ana Medina,** INRS - Centre Armand-Frappier Santé Biotechnologie, QC - Could *Leishmania tarentolae* EVs be a new platform for vaccine?	**Rooms A&B**
11h25 - 11h40	**Sophia Bigot,** Université Laval, QC - Delving in one carbon metabolism in the parasite *Leishmania* through a chemi-genomic screen	**Rooms A&B**

11h40 - 11h55	**Jose Mauricio Ayala Esparza,** McGill University, QC - The role of IRF1 in cutaneous *L. major* infection and driving the inflammatory response in primary macrophages	**Rooms A&B**
11h55 - 13h25	**Lunch**	**Chapiteau Desjardins**
13h30 - 14h15	**Invited speaker: Dr. Karine Thivierge,** Laboratoire de Santé publique du Québec - What's the role of a parasitologist in Quebec's Public Health Laboratory	**Rooms A&B**
14h15 - 14h30	**Kieran Freitag,** Trent University, ON - Characterization of the TATA-binding protein in *Giardia intestinalis*	**Rooms A&B**
14h30 - 14h45	**Melanie Marlow,** Trent University, ON - Analysis of a nuclear localized cytochrome *b*5 in *Giardia intestinalis*	**Rooms A&B**
14h45 - 15h15	**Coffee break**	**Entrance**
15h15 - 15h30	**Sohini Kumar,** McGill University, QC - Droplet digital PCR as a tool to detect resistant isolates of *Dirofilaria immitis*	**Rooms A&B**
15h30 - 15h45	**Susan Westfall,** McGill University, QC - Helminth-induced IFN*g* remodels the intestinal epithelium to compromise host resistance	**Rooms A&B**
15h45 - 16h00	**Nathalia Malacco,** McGill University, QC - *Heligmosomoides polygyrus*-derived metabolites induce tolerogenic dendritic cells and attenuate DSS-induced colitis	**Rooms A&B**
16h00 - 16h15	**Patrick Lypaczewski,** McGill University, QC - Hybridization as a phenotypic adaptation in *Leishmania donovani* is associated with cutaneous leishmaniasis	**Rooms A&B**
16h20 - 17h00	**Roundtable discussion**	**Chapiteau Desjardins**
17h00 - 18h50	**Poster session, happy hour, pizza & cocktail food**	**Chapiteau Desjardins**
18h50	**Closing remarks + best talk & best poster prizes**	**Chapiteau Desjardins**

Oral presentations

10h40 – 10h55

Combining multi-level bioinformatics tools to globally assess the diversified critical roles of SIDER2 retroposon elements in *Leishmania* genome ploidy, gene expression and developmental regulation

Gabriel Reis Ferreira[1], Vanda Gaonac'h-Lovejoy[2], Philippe Leprohon[1], Martin A Smith[2], Barbara Papadopoulou[1]

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[2]CHU Sainte-Justine Research Centre, University of Montreal, Montreal, QC, Canada

Leishmania genome harbors a large number of formerly active retroposons termed SIDERs (Short Interspersed DEgenerated Retroposons). SIDERs are divided into two subfamilies, SIDER1 and SIDER2, playing different functions in the cells. SIDER2 elements were shown to regulate mRNA-turnover. To allow a genome-wide characterization of the SIDER2 subfamily, we combined a multi-level bioinformatics approach with genomic, transcriptomic and proteomic profiling of *L. infantum* promastigotes, axenic amastigotes and macrophage-derived amastigotes. Hidden Markov Model provided a detailed map uncovering 1449 SIDER2 elements regularly dispersed along the chromosomes. 1128 (77.8%) were located within 3'UTRs of which 948 (85%) in sense orientation and 180 (15%) in the antisense orientation. Illumina RNA-seq confirmed 1127 (99, 99%) of the predicted SIDER2-bearing mRNAs and their 3'UTR location. Remarkable, SIDER2-mRNAs were generally less expressed than non-SIDER2 mRNAs ($p < 0.001$) in promastigotes and amastigotes, confirming the major role of SIDER2 elements in mRNA degradation. Interestingly, stage-specific transcriptomic comparison revealed that while organelle envelope related functions were associated with SIDER2 transcripts upregulated solely in axenic amastigotes, an enrichment for transporter activity was observed in intracellular amastigotes. Combination of Illumina DNA-seq to the RNA-seq data supports that the presence of low-expressed SIDER2 transcripts could force chromosome copy number variation as a compensatory mechanism. Label-free quantitative proteomics coupled to RNA-seq showed a good correlation between SIDER2-mRNAs expression and its protein abundance, indicating that SIDER2-transcripts are mostly regulated at the RNA level. These data provided a detailed characterization of SIDER2-elements and demonstrated their central role in regulating gene expression at the post-transcriptional level.

10h55 – 11h10

Disruption of *Leishmania major* extracellular vesicle production significantly impedes its infectious capability and disease progression

George Dong[1], Audrey Corbeil[2], Christopher Fernandez-Prada[2] and Martin Olivier[1]

[1]Research Institute of McGill University Health Centre, McGill University, Montreal, QC, Canada
[2]Université de Montréal Faculté de Médecine Vétérinaire, St-Hyacinthe, QC, Canada

Leishmania is a protozoan parasite that infects mammalian macrophage cells. Our lab has shown that small extracellular vesicles (sEVs) released by *Leishmania* within the sandfly increase infection severity. To assess the impact of inhibiting *Leishmania* sEV production, the gene Vps36, a protein key to sEV production in eukaryotes, was disrupted in *L. major* and sEV production and infectivity were assessed. Vps36 was disrupted using CRISPR/Cas9 and restored using plasmid recombination, generating VPs36null and Vps36addback *L. major* strains. sEVs were collected from cultured *L. major* using differential ultracentrifugation and analyzed using nanoparticle tracking analysis (NTA), Transmission Electron Microscopy (TEM), and LC-MS/MS proteomic analysis. Balb/C mice footpads were injected with WT and Vps36null *L. major* parasite either alone or supplemented with purified WT *L. major* sEVs and lesions were measured. TEM showed Vps36null *L. major* still produced sEVs in culture and after temperature shock, but NTA results suggest they produce significantly less sEVs compared to WT. Vps36addback restored sEV production. Vps36null *L. major* sEVs have a unique protein profile compared to WT *L. major* sEVs whereas there is no significant difference between the whole parasites. Vps36null *L. major* failed to induce a high level of infection in susceptible Balb/C mice even when co-injected with WT *L. major* sEVs compared to severe infection caused by WT *L. major*. Our results suggest Vps36 plays a key role in sEV production by *L. major* and interferes with vesicle packaging, resulting in a severe reduction in infectious capability. This effect is specific to Vps36 disruption.

11h10 – 11h25

Could *Leishmania tarentolae* EVs be a new platform for vaccine?

Ana Medina[1], Hamlet Acevedo[1], Albert Descoteaux[1]

[1]Institut national de la recherche scientifique – Centre Armand-Frappier Santé Biotechnologie, Laval, QC, Canada

Protein subunit vaccines use key antigens to stimulate long-lasting protective immune response. However, exhibits low immunogenicity and requires adjuvant support to potentiate the immune response.

Extracellular vesicles (EVs) are particles formed by a spherical lipid bilayer which are release from all cells. It has been shown that *Leishmania* a protozoan parasite, can actively release EVs which modulate mammalian immune cells. Therefore, we hypothesized that EVs derived from *Leishmania tarentolae*, a non-pathogenic species, have an immunostimulatory effect towards the development of vaccine adjuvants.

We used SARS-CoV-2 Spike (S) protein as a proof of concept. To accomplish this goal, full-length S-glycoprotein was synthetically produced, and codon-optimized for the constitutive expression in *L. tarentolae*. The protein expression was demonstrated by western blot (WB). EV derived from *L. tarentolae*, were isolated by ultracentrifugation and characterized via Nanoparticle tracking analysis, transmission electron microscopy and WB.

We evaluated the inflammatory potential of EV expressing S (EV-S), and we compared between EV which does not express S (EV-WT) and the purified S-protein *in-vitro*, quantifying the expression of IL-6, IL-10, IL-12, TNF-a, IL-1β, IL-1α by RT-qPCR using bone marrow-derived macrophages (BMM) and bone marrow-derived dendritic cells.

We found EVs derived from *L. tarentolae* have an immunostimulatory effect in a dose dependent manner. Further, EV-S had more relative expression than S in BMM. We expect *L. tarentolae* induce specific antibody production against S-protein and produce neutralizing antibodies SARS-CoV2 specific, making EV-derived from *L. tarentolae* a platform, that can be rich by many laboratories around the world.

11h25 – 11h40

Delving in one carbon metabolism in the parasite *Leishmania* through a chemi-genomic screen

Sophia Bigot[1,2], Philippe Leprohon[2], Marc Ouellette[1,2]

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[2] Division of Infectious and Immune Diseases-CHU-Québec, and Centre de Recherche en Infectiologie, Québec, QC, Canada

Studies of *Leishmania* resistant to the drug methotrexate (MTX) has illustrated major differences in one carbon metabolism between the parasite and its host. Some of those differences could be exploited. Genomic screens are now allowing holistic views of metabolic pathways. Here, we applied a screen coupling chemical mutagenesis with sequencing with MTX selection to further our understanding of one carbon metabolism in *Leishmania*. Resistant clones are characterized by next-generation sequencing and recurrent mutations highlighted by a bioinformatics pipeline, in independent mutants are pointing at likely candidates. Candidate mutations are studied by molecular means. Twenty clones of *L. major* with a 2-400-fold decrease in MTX susceptibility in comparison to wild type (WT) cells were sequenced. Recurrent mutations were observed in genes involved in folate metabolism (FT1, DHFR-TS, PTR1, transporters of the FBT family), or genes never associated with it (GULO). The role of point mutations in MTX resistance was validated by gene editing strategies. We showed that cells with the FT1 $^{P555S/+}$, FT1 $^{A430V/-}$ or FT1 $^{G129D/-, A121T/-}$, DHFR-TS $^{E291K/+}$ and DHFR-TS $^{T107I/+}$ versions were 5-fold, 38-fold, 136-fold, 4.4-fold and 2.9-fold more resistant to MTX compared to the WT, respectively. Dominant positive effects were highlighted for three mutations in DHFR-TS and PTR1. Episomal transfection of a WT copy of GULO in one mutant resulted in a 2-fold resensitization to MTX. Our Mut-seq screen has allowed to map all known genes involved in folate metabolism, identified key mutations involved in resistance, and has highlighted novel features.

11h40 – 11h55

The role of IRF1 in cutaneous *L. major* infection and driving the inflammatory response in primary macrophages

Jose Mauricio Ayala Esparza[1,2], Mathieu Mancini[1,2], George Dong[2,3,6], Rayoun Ramendra[2,3], Tianyuan Lu[5,6], Hamed Najafabadi[1], Martin Olivier[2,3,6], David Langlais[1,2,3]

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Cutaneous leishmaniasis refers to a group of heterogenous parasitic infections affecting primarily low-income countries, causing disfiguring lesions with high morbidities due to ineffective treatments. In early stages of infection, these parasites infect phagocytic cells, primarily macrophages, which are also major effector cells in its elimination. IRF1 is a transcription factor whose role in macrophage activation is essential and non-redundant with other IRFs. Unsurprisingly, *Leishmania* parasites have developed mechanisms to inhibit the expression of IRF1. Therefore, a better understanding of how IRF1 drives its inflammatory transcriptional programs and how this impacts *Leishmania* infections could lead to novel treatment avenues. Here, we performed *in vivo* footpad infections with *L. major* on both WT and *Irf1*$^{-/-}$ mice, revealing a dependency on IRF1 for controlling footpad lesion size and parasitemia. We also provide the first ChIP-seq characterization of early and late IRF1 binding dynamics in primary macrophages, where upon 15 minutes of IFN-γ stimulation, IRF1 binds to hundreds of new sites associated to cytokine, chemokine and immune cell activation, with recruitment increasing and peaking at 3–6 hours. Finally, using ATAC-seq, we detect an increase in chromatin accessibility at these sites at 3 hours post-IFN-γ stimulation, which steadily increases until 48h in an IRF1-dependent manner, suggesting long-term epigenetic reprogramming. In summary, this work highlights the relevance for IRF1 in controlling cutaneous *L. major* infection and expands our knowledge on the mechanisms by which IRF1 drives inflammatory responses in macrophages.

14h15 - 14h30

Characterization of the TATA-binding protein in *Giardia intestinalis*

Kieran Freitag[1], Jessica Chorolovski[1], Evan Schuett[1], Ally Yang[2], Timothy Hughes[2], and Janet Yee[1]

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Giardia intestinalis is a protozoan parasite that causes giardiasis, a diarrheal disease in mammals. The Giardia genome is compact, has few introns, and encodes streamlined pathways for essential cellular processes such as DNA replication and transcription. In many eukaryotes, the TATA-binding protein (TBP) is a crucial transcription factor that binds to an AT rich consensus sequence called the TATA box in gene promoters and is responsible for directing assembly of the transcription pre-initiation complex. Giardia possesses a highly divergent TBP (gTBP) that has substitutions in three out of four critical phenylalanine residues that contribute to the "kink" induced in the DNA sequence upon TBP binding to the TATA-box in other eukaryotes. Furthermore, Giardia lacks TATA boxes in its gene promotors. Prior DNA binding tests that used recombinant GST-tagged gTBP in electrophoretic mobility shift assays (EMSA) showed that gTBP does not bind to TATA boxes or other AT rich sequences. Further exploration was performed using a high-throughput method to screen all possible 8-nucleotide DNA sequences to test for gTBP DNA-binding preference. Intriguingly, an AT-deficient motif consisting of (A/C)NNGGGGNN(A/C) was identified to be the highest ranked recognition sequence. A search of the *Giardia* genome identified 424 *Giardia* genes with this motif within 75 bp of their upstream sequences, one of which is the histone H2B gene. Competitive EMSA confirmed that gTBP binds to the consensus sequence identified in the H2B promoter, and that the core GGGG segment is the most important element for binding.

14h30 - 14h45

Analysis of a nuclear localized cytochrome b_5 in Giardia intestinalis

Melanie Marlow[1], G. William Batoff[1], Guillem Dayer[1], Steven Rafferty[2], and Janet Yee[1]

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[2]Chemistry Department, Trent University, Peterborough, ON, Canada

Giardia intestinalis encodes several heme proteins, including four cytochrome b_5 isotypes (gCYTB5-I – IV) of unknown function. Each of the cytochrome b_5 isotypes is in a different cellular location, with isotype I in the nucleolus, isotype II associated with endocytic vesicles, isotype III in the nucleoplasm, and isotype IV found in the posterior flagella axonemes. Co-immunoprecipitation (coIP) experiments with gCYTB5-III, the isotype found in the nucleoplasm, identified other nuclear proteins such as RNA polymerase, DNA topoisomerase, histones, and histone modifying enzymes. Intriguingly, these coIP experiments also identified more than 40% of the known proteome of the mitosome, an organelle that is the site of the assembly of iron-sulfur clusters (ISCs), which are essential cofactors for many redox proteins. One of the mitosomal proteins found in the immunoprecipitate is the flavoenzyme GiOR-1, which has been shown to mediate electron transfer from NADPH to recombinant gCYTB5-III *in vitro*. Moreover, exposure of Giardia to a low concentration of formaldehyde (a DNA crosslinker that causes DNA damage) showed a transient increase in gCYTB5-III. This observation, together with the coIP results and other evidence of gCYTB5-III's tight association with chromatin, suggest that gCYTB5-III may have a role in a DNA damage response in Giardia. Since many enzymes involved in repairing DNA damage contain ISCs, gCYTB5-III may be a link in sensing DNA damage in the nucleus to the up-regulation of ISC assembly that originates in the mitosomes.

15h15 - 15h30

The host cell secretory pathway mediates the export of *Leishmania* virulence factors
Droplet digital PCR as a tool to detect resistant isolates of *Dirofilaria immitis*

Sohini Kumar[1], Roger K. Prichard[1], Thavy Long[1]

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Dirofilaria immitis is a filarial nematode that infects companion animals and can be fatal if not diagnosed early. Heartworm infection in dogs has become an emerging concern in many parts of the world due to the recent emergence of resistance to ML preventives. Previous studies have reported that the development of drug resistance has a genetic origin. Indeed, whole-genome sequencing techniques showed that the genetic profiles of suspected ML resistant *D. immitis* isolates were distinct compared to susceptible *D. immitis* isolates. Recently, eight single nucleotide polymorphism (SNP) markers have been shown to correlate with resistance phenotype and therefore, those could differentiate resistant and susceptible populations of *D. immitis* using Miseq sequencing. In our study, we evaluated the potential of the droplet digital PCR (ddPCR) technology to detect alternate allele frequencies across four SNP marker sites that have previously been correlated with drug resistance. For this assay, specific hydrolysis probes were designed to quantify alternative nucleotide (resistant) subpopulations from genomic DNA templates of eight different susceptible or resistant isolates. We have optimized the assay with four SNPs of interest and have confirmed the presence of these four SNPs at different frequencies in these eight isolates. Our results suggest that ddPCR could be employed to accurately distinguish between resistant and susceptible populations of *D. immitis*. Further, our results also support the use of ddPCR based probe competition assay for rapid detection of isolates with ML susceptible, resistant, and mixed genotype, in a laboratory setting.

15h30 - 15h45

Helminth-induced IFNg remodels the intestinal epithelium to compromise host resistance

Susan Westfall[1], Marilena Gentile[2], Danielle Atar-Karo[1], Garrie Peng[1], Ryan Pardy[1], Martin Richer[3], Alex Gregorieff[1], Irah King[1]

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[2]University of Pennsylvania
[3]Indiana University

Repair of the intestinal epithelial barrier is a dynamic process involving the intestinal stem cell (ISC) niche and local immune responses. The intestinal helminth *Heligomosomoides polygyrus bakeri* (*Hpb*) disrupts the epithelial monolayer resulting in a robust IFNg-mediated type 1 immune response during the tissue-invasive phase of infection. While we have previously shown that IFNg production promotes disease tolerance to *Hpb* infection, it remains unclear how a type 1 immune response is orchestrated and its relevance to host resistance. Here we hypothesize that an early type 1 immune response to *Hpb* promotes disease tolerance at the expense of compromising host resistance to infection. Using a combination of cell transfer, bone marrow chimera and gnotobiotic approaches, we identify a tissue-resident CD8 T cell population that secretes IFNg following breach of the epithelial wall by *Hpb* larvae. Notably, activation of intestinal CD8+ T cells occurs in an antigen-independent manner but requires exposure to the commensal microbiota. These events coincide with a loss of proliferating homeostatic Lgr5+ ISCs responsible for epithelial repair. Deletion of IFNg receptor signaling results in accelerated regeneration of the Lgr5+ ISC compartment, elevated numbers of mucus-producing goblet cells and reduced parasite burden. Together, our results indicate that *Hpb* exploits a gut microbiota-dependent type 1 immune response to alter the epithelial "weep and sweep" response and facilitate chronic infection.

15h45 - 16h00

Heligmosomoides polygyrus-derived metabolites induce tolerogenic dendritic cells and attenuate DSS-induced colitis

Nathalia Malacco[1], Elizabeth Siciliani[1], Ana Madrigal[1], Tamara Sternlieb[1], Igor Cestari[1], Armando Jardim[1], Mary Stevenson[1], Fernando Lopes[1]

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The low incidence of helminth parasite infections in Western societies is inversely correlated with the high incidence of inflammatory bowel diseases (IBD), considering the ability of helminth parasites to induce tolerance in the host. We aimed to evaluate the tolerogenic response induced by helminth-derived metabolites (HDM) in DCs, and its ability to alleviate DSS-induced colitis. *Heligmosomoides polygyrus* worms were cultured for 24h and HDM was isolated by chromatography. DCs were incubated with HDM for 20h, and then stimulated with LPS or adoptively transferred to DSS-induced colitogenic mice. Cytokine secretion was measured by ELISA and protein expression was analyzed by flow cytometry. Transcriptomics and metabolomics were also assessed. HDM-treated DC presented decreased LPS-induced TNF and increased IL-10 release. Moreover, there was a decreased expression of MHC-II, CD86, and CD40 in DCs treated *in vitro* and *in vivo* with HDM. Mice adoptively transferred with HDM-treated DCs presented lower disease activity scores, less colon shortening, decreased weight loss, and healthier histopathology compared to control mice. These results indicate that HDM induced tolerogenic DC profile, and adoptive transfer of HDM-treated DCs ameliorates colitis. RNAseq results showed that HDM upregulated 183 and downregulated 76 genes in DCs, including upregulation of genes classically upregulated in dexamethasone-induced tolerogenic DCs. Metabolomics revealed downregulated metabolites related to terpenoid backbone metabolism in HDM-treated DCs. These differentially expressed genes and enriched metabolites may indicate a novel mechanism by which helminths induce a tolerogenic profile in DCs. In conclusion, HDM induces tolerogenic DCs and alleviates DSS-induced colitis.

16h00 - 16h15

Hybridization as a phenotypic adaptation in *Leishmania donovani* is associated with cutaneous leishmaniasis

Patrick Lypaczewski[1], Lovlesh Thakur[2], Aklank Jain[2], Sandhya Kumari[3], Kayla Paulini[1], Manju Jain[4], Greg Matlashewski[1]

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[3]Indira Gandhi Medical College, Shimla, Himachal Pradesh, India
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Leishmaniasis is a neglected tropical disease endemic in over 90 countries. The disease has two main pathologies; cutaneous leishmaniasis (CL) that generally self-heals, and visceral leishmaniasis (VL) can be fatal if untreated. The majority of VL cases, concentrated on the Indian subcontinent (ISC) and East Africa, are caused by *Leishmania donovani*. However, recent foci of CL on the ISC have been attributed as an atypical phenotype of *L. donovani* infection including outbreaks in Sri Lanka and in Himachal Pradesh, India.

Whole genome sequencing of novel isolates, and datamining of existing publicly available sequenced isolates was performed, followed by phylogenetic analysis to understand the unique phenotype of these CL causing *L. donovani* parasites. Hybridization signals were detected by heterozygous SNP analysis and where possible, determination of the parental genotypes was performed.

Here we demonstrate that multiple independent hybridization events, both intra- and inter-species, involving *L. donovani* appears to have resulted in progeny with a cutaneous disease phenotype. Indeed, we identified in Sri Lanka the progeny of hybridization between *L. donovani* of Ethiopian origins and *L. major,* the same *L. donovani* and *L. tropica*. Further, we identified in India the progeny of hybridization between two ISC1 “Yeti” strains with Nepalese Highlands origins.

Based on our observations first in Sri Lanka, and more recently in India, we suggest that hybridization constitutes a rapid evolutionary adaptation mechanism to a yet unidentified environmental pressure for *L. donovani* to associate with CL.

Poster session

17h00 - 18h50 – Chapiteau Desjardins

Poster #1

Exploring the role of the gut microbiota in tissue repair during intestinal helminth infection

Garrie Peng[1,2], Gabriel Russell[1,2], Susan Westfall[1,2], Cynthia Faubert[3], Siegfried Hapfelmeier[4], Irah King[1,2,3]

[1]Meakins-Christie Laboratories, Department of Medicine, McGill University Health Centre, Montreal, QC, Canada
[2]Department of Microbiology and Immunology, McGill University, Montreal, QC, Canada
[3]McGill Interdisciplinary Initiative in Infection and Immunity, Montreal, QC, Canada
[4]Institute for Infectious Diseases, University of Bern, Switzerland

Intestinal helminth infection is a neglected tropical disease affecting over 2 billion people worldwide. While this chronic disease is rarely fatal, it can cause morbidities such as anemia, and intestinal obstruction. As the gut microbiome can regulate tissue homeostasis and maintenance, the interactions between intestinal parasites and the microbiome need to be elucidated. Our lab has developed a model of axenic helminth infection that can fully isolate effects of the microbiome and helminth infection. Our previous study showed that the presence of the gut microbiota was able to reduce excess type 2 immune response that would otherwise be detrimental to resolution of intestinal inflammation. We and others have previously shown that an early type 1 immune response contributes to tissue repair and reduction of intestinal bleeding. Thus, we hypothesize that the gut microbiota regulates type 2 protective immunity to helminth infection through promotion of the type 1 immune response. Our preliminary results showed an enhanced expression of type-2-associated genes, Arg1, Chil3 and Reltnb, during chronic axenic helminth infection. In contrast, early germ-free helminth infection induced a decreased expression of type-1-associated genes, Cxcl9 and Ly6a, and a reduced production of lipocalin 2, an inflammatory molecule produced by neutrophils. To examine isolated effects of the type 1 immune response during helminth-induced tissue inflammation, we employed interferon gamma (IFNy)-receptor-knockout mice, where we observed that IFNy signalling may promote granuloma resolution. In conclusion, our preliminary results show that the microbial-induced type 1 immune response may inhibit over-exuberant type 2 immunity and subsequent pathological fibrosis.

Poster #2

Loss-of-function screens for drug mode of action and resistance mechanisms in Leishmania

Marine Queffeulou[1,2], Ana Maria Mejia Jaramillo[1], Christopher Fernandez Prada [1], Philippe Leprohon[1], Marc Ouellette[1,2]

[1]CRCHUQ-Université Laval, Québec, QC, Canada
[2]Département de Microbiologie-Infectiologie-Immunologie, Université Laval

The few drugs available against Leishmania are poorly understood in terms of mode of action (MOA) and their use can be complicated by the emergence of drug resistance. Here, we describe two loss-of-function genomic screens that we developed for deciphering MOA and resistance mechanisms for licensed and experimental drugs. One deals with CRISPR-Cas9 where we constructed a plasmid library of six guide RNAs (gRNAs)/gene against the Leishmania infantum genome. The expression of this library along with the Cas9 nuclease allowed L. infantum to grow in the presence of the antileishmanial drug Amphotericin B (AMB). The gRNAs enriched in this AMB-resistant population were identified by next-generation sequencing. This revealed a steady enrichment of different gRNAs targeting genes coding for sterol 24-c- methyltransferases, whose inactivation is known to confer AMB resistance, hence validating our approach. A new candidate gene, coding for a hypothetical protein, was also highlighted. We carried out additional screens with miltefosine (MIL) and the experimental drug GSK143295. The MIL screen highlighted the MIL transporter (MT1) but also a RING-variant domain protein. The GSK143295 screen highlighted several candidates including the lorien protein. The second loss-of-function screen relies on RNAi in *L. braziliensis*. The design involves DNA constructs harboring 500 bp inserts flanked by inward-facing ribosomal promotors for the expression of double stranded RNA. Preliminary attempts with constructs targeting MT1 led to MIL resistance. We are currently furthering this strategy with additional genes and drugs. Our loss-of-function screens will help refine our understanding of drug MOA and may reveal new drug targets.

Poster #3

Creatine *de novo* biosynthesis signature of Dexamethasone-induced tolerogenic dendritic cells

Seyed Alipour[1], Nathalia Malacco[1], Fernando Lopes[1]

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Introduction

Tolerogenic dendritic cells (tolDCs) are anti-inflammatory antigen-presenting cells that regulate immunotolerance towards various antigens. TolDCs maintain the homeostasis of immune responses, the dysregulation of which causes inflammatory disorders like Inflammatory Bowel Diseases (IBD). Elucidating the metabolic pathways necessary for tolDC polarization can reveal potential targets for developing new IBD treatments. It has been reported that creatine promotes anti-inflammatory macrophage polarization but it has not been studied in DCs. This study aims to examine the occurrence and necessity of creatine de novo biosynthesis during tolDC-polarization.

Methods

TNF cytokine secretion levels of dexamethasone (DEX) treated DCs and their ability to induce Foxp3+ Treg cells upon LPS exposure were used confirm the tolDCs polarizing ability of DEX. Creatine assays were used to quantify DC intracellular creatine concentrations and examine changes upon DEX and/or LPS treatments. Ornithine was used as an inhibitor of an intermediate enzyme in the creatine biosynthetic pathway to examine the necessity of creatine de novo biosynthesis during tolDC polarization.

Results

DEX-treated DCs secreted lower LPS-induced levels of TNF, had a higher ability to induce Treg polarization, and a diminished ability to prime TH1 inflammatory cells. However, preliminary assay did not detect creatine biosynthesis by DCs treated with DEX nor LPS.

Discussion

DEX is an effective tolDCs-polarizing treatment. Due to sensitivity of the assay, it is inconclusive if creatine de novo biosynthesis is required for tolDCs to self-perpetuate their phenotype. Future metabolomics approaches will determine the role of creatine biosynthesis in DEX induced tolDC-polarization – or may reveal other metabolites implicated.

Poster #4

Building the protein-protein interaction map of the *trypanosoma cruzi*

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Kinetoplastid parasites are the causative agents of deadly diseases in humans and cattle. While their genome was published in 2005, the function of more than one-third of their genome is not known. Protein-protein interaction (PPI) networks accelerate functional annotation by transferring functional information from annotated genes to unannotated ones based on the "guilt-by-association" principle. The physical interaction (or co-complex membership) of hundreds of proteins can be investigated by resolving the protein complexes in native conditions and identifying the proteins that migrate together. First, the protein complexes extracted in native conditions are resolved by size exclusion chromatography (SEC), glycerol gradient (GG), or native PAGE. Next, the proteins in each fraction (co-migrating proteins) are identified and quantified using mass spectrometry. Machine learning algorithms can be trained to infer the protein complex map from the co-fractionation data. In this study, the cytosolic proteins of *Trypanosoma cruzi* Sylvio X-10 were fractionated into 18 fractions using GG and 45 fractions by SEC. Label-free MS quantitation identified more than 1000 proteins. The PPI map was built using the PCprophet software based on a random forest algorithm. The preliminary analysis identified more than 50 protein complexes, including many proteins with unknown functions. Building the PPI maps with the co-fractionation-MS is a successful approach for the functional annotation of the genome of the trypanosomes. The experiments for the co-fractionation of the mitochondrial, nuclear, and membrane-bound proteins are ongoing, which will expand the PPI map of the trypanosomatids and the functional annotation of their genomes.

Poster #5

Three-dimensional analysis of telomeric expression side chromatin in trypanosomes

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Trypanosoma brucei expresses a homogeneous coat of variant surface glycoproteins (VSG), which they periodically switch to evade the host antibody response by antigenic variation. *T. brucei* has about 2,500 VSG genes, but only one VSG gene is transcribed at a time from a telomeric expression site (ES). Antigenic variation occurs by transcriptional switching between telomeric ESs or VSG gene recombination. The proteins repressor activator protein 1 (RAP1) and phosphatidylinositol phosphate 5-phosphatase (PIP5Pase) associate with telomeric ESs in repetitive regions flanking the VSG gene, namely 75 bp repeats and telomeric repeats. Knockdown of RAP1 or PIP5Pase results in transcription of all silent VSG genes. Moreover, PIP5Pase activity controls RAP1 association with telomeric ESs and hence its repressive function via a phosphoinositide-regulatory system. We propose a model in which telomeric ES three-dimensional organization plays a role in repressing the transcription of VSG genes. We use chromatin immunoprecipitation with RAP1-HA or PIP5Pase-V5 to enrich telomeric ES sequences and perform chromatin interaction analysis with paired-end tag sequencing (ChIA-PET) followed by nanopore sequencing and computational analysis to identify ES DNA interacting sites. We propose to compare interaction analysis in bloodstream forms expressing only one VSG gene, in mutant cells that express all VSG genes, and in procyclic cells that do not express VSG genes. Our data will provide insights into the telomeric ES chromatin folding and its role in VSG gene regulation.

Poster #6

Heme metabolism at the *Leishmania donovani*-host-cell interaction

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The *Leishmania*-macrophage interaction is a bidirectional one, of which the outcome of is important for determining the parasite fate. *Leishmania* spp. can modulate the host cell at epigenetic, transcriptional, and metabolic levels. Relatively little attention has been paid to the manipulation of host nutrient and energy sources by *Leishmania* despite the competition of both organisms for identical resources. Mitochondria are host organelles that rearrange in response to changes in nutrient availability, playing a crucial role regulating energy production, cell cycle, and immune signaling. We recently showed that *L. donovani* induces mitochondria biogenesis during infection. Therefore, we hypothesize that *Leishmania*-induced mitochondriogenesis could be an outcome of the auxotroph parasite's heme requirements. To address this hypothesis, we infected bone-marrow-derived macrophages with *L. donovani* metacyclic promastigotes and lesion-derived amastigotes and assessed the expression kinetics of host genes controlling the heme synthesis pathway by using RT-qPCR. Our results indicate that *L. donovani* induces an up-regulation of two key enzymes of the heme biosynthetic pathway, namely the aminolevulinic acid synthase and ferrochelatase. GM-MS analysis revealed an increase in aminolevulinic acid synthesis, the first intermediate of the heme biosynthetic pathway. Pharmacological inhibition the conversion of aminolevulinic acid into porphobilinogen impaired the intracellular growth of *L. donovani*, illustrating the importance of the host cell heme biosynthetic pathway for the intracellular replication of *L. donovani*. This study provides novel insight into how *Leishmania* takes advantage of the host mitochondrial biology for sustenance.

Poster #7

Identifying telomeric expression site protein interactions at peptide resolution using cross-linking and mass spectrometry in trypanosomes

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Trypanosoma brucei evade the host antibody response by antigenic variation, periodically switching the expressed variant surface glycoprotein (VSG). *T. brucei* have over 2,500 VSG genes and pseudogenes; however, they only express one VSG gene at a time from one of the 20 telomeric expression sites (ESs). We previously identified a telomeric expression site protein complex (TESC) that functions in VSG silencing via a phosphoinositide-regulatory system. The TESC includes the enzyme phosphatidylinositol 5-phosphatase (PIP5Pase), repressor activated protein 1 (RAP1), DNA/RNA-binding proteins, nuclear lamina proteins, and protein kinases and phosphatases. To understand the composition and organization of the TESC, we used a combination of chemical crosslinking, affinity purification, and mass spectrometry. Our preliminary data identified 22 out of 44 previously identified TESC proteins, with about 40% of crosslinked peptides obtained at a 1% false discovery rate. An additional 183 proteins were identified crosslinked to TESC proteins revealing an extensive network of telomeric ES protein interactions. The preliminary results indicate direct interactions of the TESC at peptide resolution, including protein domains involved. Additional work will explore changes in the TESC interactions associated with VSG silencing and activation. Our findings yield insights into the composition and topology of the TESC in trypanosomes.

Poster #8

Role of phosphoinositides in endocytosis and hemoglobin transport in the malaria parasite, *Plasmodium falciparum*

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A critical step in the life cycle of the malaria parasite *Plasmodium falciparum* is the invasion of an erythrocyte. Once inside its host cell, the parasite survives thanks to the endocytosis of hemoglobin, which provides essential nutrients. It is therefore a therapeutic target of choice. However, little is known about the processes involved. Our hypothesis is that phosphoinositides play a role in the endocytosis and the transport of hemoglobin to the food vacuole, where it is digested.

To characterize the role of PfENTH, a protein containing a putative phosphoinositide binding domain, in hemoglobin endocytosis and trafficking, a strain expressing the protein linked to a mislocalizer was generated. This construction allows the conditional mislocalization of the protein from its action site by *Knock Sideways* and will permit to demonstrate its role. The localization of PfENTH will be determined by a GFP tag and immunofluorescence via colocalization assays with various markers of parasite structures. Finally, protein interaction partners will be studied.

The PfENTH protein appears to localize close to a structure called cytostome. The latter has a role in the endocytosis of hemoglobin. A PIP-Strip assay revealed that the ENTH domain binds PIPs and in particular PI (4,5) P2, recognized for its role in endocytosis in eukaryotes. Finally, the mislocalization of the protein leads to a decrease in parasitemia showing that PfENTH is essential for the parasite survival.

Our results could allow us to demonstrate the role of phosphoinositides in the process of hemoglobin endocytosis and therefore in the survival of the parasite.

Poster #9

Evidence that prior exposure to chemical infection cues reduce parasite transmission in Gyrodactylus-Guppy model

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The reliance on chemical communication is established for evading predation in aquatic systems, yet, only a few studies have found evidence that chemical cues released from animals infected with parasites alter conspecific behaviour. Furthermore, the link between putative chemical cues and susceptibility to infection has not been studied. We aimed to determine if exposure to *Gyrodactylus turnbulli*-infected guppies (*Poecilia reticulata*) at various times post-infection resulted in altered behaviour of uninfected conspecifics, and if prior exposure to this putative infection cue reduced transmission. Guppies exposed to cues from infected guppies spent less time in the center of the tank than the periphery, and males remained still when first exposed to this cue. Continuous exposure of guppy shoals to infection cues resulted in shorter inter-fish distance in later stages of infection but did not impact their overall activity. These subtle responses in individual and shoal behaviour were unexpected based on previous research but do indicate that guppies responded to this putative infection cue. We showed for the first time that exposure of uninfected shoals over 16 days to the cue provided partial protection when the shoal was experimentally infected. Shoals did become infected, but the first wave of infection increased in intensity more slowly and to a lower peak compared with shoals exposed to the control cue. These results indicate that guppies may respond to both short-term and ongoing infection cues by altering individual and shoaling behaviours, and that infection cues may reduce the intensity of epidemics.

Poster #10

Dysregulation of the Leukemia Inhibitory Factor (LIF) pathway in macrophages infected with the parasite *Toxoplasma gondii*

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Toxoplasma gondii is an intracellular parasite responsible for toxoplasmosis. It is estimated that 30% to 40% of the world population is infected by *T. gondii*. Even though the infection is asymptomatic in immunocompetent individuals, immunodeficient patients have an increased risk of developing severe pathologies. Innate immune cells, including macrophages, are essential to control the infection. However, *T. gondii* can dysregulate host gene expression to avoid the activation of efficient immune responses.

We previously reported that in addition to affecting transcription, *T. gondii* down-regulates translation of specific mRNAs in infected macrophages. Among these transcripts, we identified *Lifr* that encodes the Leukemia inhibitory factor receptor alpha subunit (LIFRα).

Accumulating evidence indicates that the LIF-LIFR pathway plays an important role in the control of inflammation and the progression of viral/bacterial infections. However, the modulation of LIF-LIFR-dependent signalling has not been investigated during parasite infections. Our *in vitro* data indicate that *T. gondii* blocks the LIF-LIFR pathway in macrophages by decreasing the expression of LIFRα. Consistent with this, the phosphorylation and nuclear translocation of STAT3 in response to LIF was reduced in *T. gondii*-infected macrophages.

Ongoing experiments in our laboratory using omics approaches (i.e. RNA-seq and ChIP-seq) and STAT3 KO macrophages aim to identify changes in the expression of LIF-LIFR-STAT3-regulated genes that favour *T. gondii* infection.

Our long-term goal is to determine whether inhibition of LIF-dependent transcriptional programs and biological functions contribute to the dysregulation of inflammatory responses and immune-pathogenesis during toxoplasmosis using *in vitro* and *in vivo* models of infection.

Poster #11

Retromer function in apical complex biogenesis of the major malaria parasite *Plasmodium falciparum*

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Malaria was responsible for 627,000 deaths and 241 million infections in 2020 alone. Drug resistance, and the poor efficicacy of the only available vaccine are strong arguments supporting the need for the identification of new therapeutic targets. The malaria parasite Plasmodium falciparum (Pf) invades erythrocytes and multiplies inside them. To do so, it secretes invasion proteins located inside organelles, like micronemes, that are part of an apical complex. Proteic trafficking from the Golgi apparatus to these organelles is dependent on the PfSortilin protein. In the yeast, this protein is recycled between the target organelles and the Golgi Apparatus by a protein complex called retromer. This complex is composed of Vacuolar Sorting Proteins (Vps) including Vps29. We hypothesized that PfVps29 plays a key role in apical complex biogenesis by recycling PfSortilin to the Golgi apparatus. To verify the hypothesis, we have generated a strain coding for the fusion protein PfVps29-2xFKBP-GFP. We verified by PCR, Western Blot, and fluorescence microscopy the integration of the PfVps29-GFP coding gene at the right locus and its expression throughout its life cycle. We showed that PfVps29-GFP localizes at endosome-like structures and partially at micronemes. Finally, a strain where PfVps29 could be functionally mislocalized has been generated. This technique will allow us to assess its essentiality. According to our hypothesis, because the apical complex is essential for Plasmodium, we are expecting a decrease in parasitemia after PfVps29-GFP mislocalization. Our work will possibly lead to the characterization of new antimalarial drugs targets.

Poster #12

Targeting phosphoinositides and their effectors to disarm the malaria parasite *Plasmodium falciparum*

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Malaria is one of the most widespread infectious diseases in the world. The only vaccine currently available has limited efficiency and resistance to antimalarials is becoming more prevalent. Identification of new therapeutic targets is therefore essential. *Plasmodium falciparum* (*Pf*) is responsible for the most virulent form of this disease. The latter has an apicoplast that synthesizes the precursors of isoprenoids necessary for the survival of the parasite. Consequently, the inhibition of the biogenesis of this organelle represents an interesting target for the development of antimalarials. Our hypothesis is that membrane lipids, called phosphoinositides (PIPs), are responsible for the cellular identity of organelles and that they could be involved in the differential trafficking of proteins to them. My goal is to characterize the role of *Pf*PH1, a putative PIP binding protein containing a Pleckstrin homology (PH) domain, in the biology of the parasite. Using a protein-lipid superposition protocol and mammalian cells, we showed that *Pf*PH1 interacts with PIPs and more specifically with phosphatidylinositol-3-phosphate (PI3P). Immunofluorescence assays on *Pf* suggest that *Pf*PH1 colocalizes transiently with the cis-Golgi and with the apicoplast. A parasite line allowing the conditional expression of *Pf*PH1 has been generated and conditional inactivation of the protein causes the death of the parasite, indicating that it is essential. PI3P being enriched in the membranes of the apicoplast, the results obtained so far suggest that *Pf*PH1 might be involved in the trafficking of proteins from the Golgi apparatus to the apicoplast. Interruption of this traffic is a promissing therapeutic target.

Poster #13

Predicting putative target genes of micro-RNAs isolated from *Schistosoma mansoni* extracellular vesicles

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Schistosoma mansoni causes schistosomiasis in hundreds of millions of people worldwide. The parasitic flatworm has been documented to live for decades in its host, raising the question of their longevity. The goal of my project is to better understand how these parasites live so long by potentially evading the host immune response through micro-RNA (miRNA) mediated immune response modulation. miRNAs are a class of small non-coding RNA primarily involved in gene regulation at the post-transcriptional level by binding to the 3'-UTR of target genes. In a previous study, miRNAs were isolated, quantified, and sequenced from S. mansoni extracellular vesicles that are typically released into human hosts. Because several miRNAs can target one gene and several genes can be regulated by a single miRNA, a wide variety of computational algorithms have been developed to predict miRNA target genes from a given sequence. The S. mansoni miRNAs were tested by multiple miRNA target gene prediction algorithms including TargetScan, miRDB, and PicTar to develop an aggregate table of predicted target genes for each miRNA sequence. Because the predicted target genes were involved in a variety of biological processes, genes were filtered using the Panther Classification System to include ones only involved in the immune response pathway. miRNA such as Sma-miR-125b and Sma-let-7-3p predicted to target genes such as IL16 and GATA3 respectively provides insight into helminths potentially modulating and avoiding the host immune response. This also provides potential therapeutic targets for treatment of schistosomiasis.

Poster #14

Quinolines induce the formation of cytosolic Hz-compartments emerging from the digestive vacuole of *Plasmodium falciparum*

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Decreased susceptibility of *Plasmodium falciparum* to current antimalarial drugs is a critical impediment to malaria management. How the parasite develops mechanisms to respond and adapt to chemotherapeutic agents is still a matter of speculation. Here, we investigated the effect of quinoline antimalarials on cellular response and morphological characterization of CQ-sensitive (CQS; 3D7 strain) and CQ-resistant (CQR; Dd2 strain) parasites. This study mainly addressed the formation of cytosolic Hz-containing compartments; a phenotypic response previously described as a result of CQ exposure. We demonstrated that quinine (QN), mefloquine (MQ) and amodiaquine (AQ) also induce this phenotype in CQS parasites. CQR parasites required higher concentration of drug to exhibit this response. Further, we investigated the role of PfCRT, a transmembrane protein that spans the digestive vacuole (DV) membrane, in the formation of these cytosolic Hz-containing compartments. CQR parasites bearing mutant PfCRT is thought to reduce intravacuolar drug accumulation at a given external CQ concentration ($[CQ]_{ex}$) when compared to CQS parasites, enhancing cell survival. Our study shows that *pfcrt*-modified CQS parasite clones harboring mutant PfCRT reduced the production of cytosolic Hz-compartments. Finally, time-lapse live cell imaging enabled us to determine the DV as the origin of compartments. Our results suggest that rearrangement of the membrane and compartmentalization in the DV may be the result of dynamics the parasite employs to maintain membrane integrity and delay cell death. However, as the dual role of autophagic mechanisms suggests, earlier initiation of these events may render CQS parasites more susceptible to cell death-inducing compounds, compared to CQR parasites.

Poster #15

Kinetics of Zn (II) PPIX demetallation under *P. falciparum* (malaria parasite) hemozoin forming vacuole acidic conditions

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When *P. falciparum* (Malaria parasite) invades red blood cells (RBC), it feeds on haemoglobin (Hb) as a source of nutrients and amino acids. The proteolysis of Hb is carried out inside the digestive food vacuole, an acidic organelle found within the parasite. During this process, the metalated tetrapyrrole system of heme (Iron (III) in a porphyrin IX (PPIX)) present in Hb is released and converted into dimer crystals (hemozoin, Hz) as protection against toxic oxidizing free heme. In Iron-deficient patients (anemia) the lower uptake of Fe in Hb leaves more room for increased Zn levels in RBC. The presence of Zn (II) PPIX instead of Fe (III) PPIX is often noted in patient case with extreme iron deficiency or under lead poisoning. Yet, the one advantage in malaria-induced anemia is that the binding mechanism for Zn (II) PPIX inhibits Hz crystallization, acting in a similar way to the antimalarial quinolines. However, the effect of Zn (II) PPIX and its interactions are yet to be fully understood. The objective of this research is to explore in more detail the interaction of Zn (II) PPIX under the parasite's digestive vacuole acidic conditions. In this work, Zn demetallation equilibrium is explored using UV-vis spectrometry to give insight into how the parasite acidic vacuole can affects the Zn complex. Overall, we want to understand how stable Zn (II) PPIX is under the parasite's biological conditions and how long can it stay active and toxic.

Poster #16

Impact of DDX3 RNA helicase in the mitochondrial energetic metabolism in *Leishmania*

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DDX3 is a highly conserved member of ATP-dependent DEAD-box RNA helicases with multiple functions in RNA metabolism and cellular signaling. We have previously shown that its genetic inactivation in *Leishmania* results in the accumulation of reactive oxygen species, leading to mitochondrial dysfunction. We showed that the pyruvate decarboxylase (PDC) contributes to the energic balance between glycolysis and TCA cycle, along with the Pumilio protein 1 (PUF1) involved in translation regulation were downregulated in DDX3 KO. We hypothesize that DDX3 regulates translation of mRNAs coding for central proteins in mitochondrial metabolism. To better understand DDX3's role in mitochondrial metabolism, we will determine whether DDX3 regulates PDC and PUF1 translation. To evaluate translation efficiency of PDC and PUF1 mRNAs in the presence/absence of DDX3, we will conduct polysome profiles followed by Northern blot hybridization. To confirm downregulation in the absence of DDX3, PUF1 and PDC will be HA-tagged at their genomic locus in WT and DDX3 KO. Immunoprecipitation and MS/MS studies will identify PDC and PUF1 protein interactions in the presence/absence of DDX3. To assess the role of PUF1 and PDC in mitochondrial metabolism, we will realize genetic inactivation. To investigate whether PUF1 impacts translation of mitochondrial transcripts, we will perform RNA-seq and proteomic analyses in WT and PUF1 KO strains. Finally, to better understand the function of PDC in *Leishmania* metabolism, we will carry out NMR assays in WT and PDC KO. In summary, this study will allow a better understanding of the novel function of DDX3 in *Leishmania* mitochondrial metabolism.

Poster #17

Extracellular vesicles (EVs) at the origin of a new gene exchange mechanism in protozoa

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Although antimony resistance in Leishmania (the protozoan responsible for leishmaniasis, a neglected zoonotic disease) is multifactorial, gene amplification is a common resistance mechanism. Extrachromosomal amplifications (circular amplicons), containing the mrpA resistance gene, are known and characterized in the antimony-resistant strain (Ldi-Sb2000.1). Also, in Leishmania, extracellular vesicles (EVs) are key players in parasite biology by increasing their survival and aggravating lesions. In addition, EVs can carry Leishmania RNA virus 1 and transmit it in the extracellular medium.

The hypothesis of my Ph.D. project is that EVs have a key role in the spread of antimicrobial resistance and can serve as biomarkers for the rapid diagnosis of resistant strains.

We have isolated and studied the EVs of resistant parasites. The protein profile of the EVs was determined by LC-MS/MS. Next-generation sequencing offered valuable insights into the DNA of EVs. Cultures of the Transwell type and purified parasite-EVs were carried out and the recipient strains were studied.

We performed the first comparative characterization of Leishmania EVs in the context of drug resistance and showed that resistance induces changes in the morphology, size, distribution and protein content of EVs. We demonstrate for the first time the presence of resistance genes in EVs and show the existence of a new mechanism of resistance gene transfer, between protozoa, by EVs.

These findings are of major importance in understanding resistance; show the key role of EVs in the spread of resistance; and will allow the development of new diagnostic tests and new therapeutic approaches.

Poster #18

Toxoplasma gondii represses FOXO3a-dependent transcriptional programs to prevent autophagy mediated killing

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The obligate intracellular parasite *Toxoplasma gondii* modulates host transcription factor activity to establish a safe replicative niche. Activation of AKT signalling by *T. gondii* prevents autophagic targeting of the parasitophorous vacuole (PV); however, the molecular underpinnings are not fully understood. The transcription factor Forkhead box O3a (FOXO3a) controls numerous cellular processes including autophagy. Interestingly, AKT-mediated phosphorylation of FOXO3a inhibits its transcriptional activity. To test the hypothesis that *T. gondii* downregulates FOXO3a-dependent induction of autophagy through AKT activity, we combined pharmacological and forwards-genetics approaches to prevent AKT-sensitive phosphorylation of FOXO3a in human foreskin fibroblasts (HFFs). We report that *T. gondii* promotes AKT-dependent phosphorylation and nuclear exclusion FOXO3a, thereby repressing its transcriptional activity in HFFs. Mechanistically, AKT-sensitive phosphorylation of FOXO3a in *T. gondii*-infected HFFs required the activity of PI3K but was independent of EGFR, PKCα, and mTOR. Moreover, exposure of HFFs to soluble *T. gondii* antigens indicated the necessity of live infection. Expression of autophagy-related transcriptional targets of FOXO3a was reduced during *T. gondii* infection in an AKT-dependent fashion. Consistent with this, *T. gondii* failed to inhibit the recruitment of the PV to the lysosome upon pharmacological blockade of AKT or overexpression of the AKT-insensitive form of FOXO3a. In all, we provide evidence that *T. gondii* suppresses FOXO3a-regulated transcriptional programs to prevent autophagy-mediated killing within the host cell. Ongoing and future work in our laboratory will contribute to better understand how *T. gondii* manipulates both the nuclear and cytoplasmic activities of FOXO family members to subvert host cell functions and promote infection.

Poster #19

Using yeast surface display to identify phosphoinositide-binding proteins in trypanosomes

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Phosphoinositide (PI) signalling plays a central role in the control of immune evasion and life stage development in trypanosomes. The PI system entails ~30 proteins involved in the synthesis, transport, and modification of soluble inositol phosphates (IPs) or membrane-bound PIs via phosphorylation or dephosphorylation. PI kinases and phosphatases and their substrates and products have been identified in the plasma membrane, cytosol, and organelles including in the nucleus. Dissecting the regulatory function of the PI signalling system requires identifying proteins that interact with IPs or PIs. However, a limited number of proteins have known domains that bind phosphoinositide metabolites, including pleckstrin homology or FYVE domains. We propose using yeast surface display (YSD) to identify proteins that bind to IPs and PIs. We generated a *Trypanosoma brucei* genome-wide library for YSD. The library represents ~7-fold of the parasite genome and has DNA fragments ranging from 0.5 to 3Kb. Nanopore sequencing showed that the library includes every parasite gene and computational analysis predicted the expression of ~180,000 distinct polypeptides, including complete proteins or fragments. Western blot also confirmed the expression of library proteins in yeast. We are screening the YSD library to identify proteins that bind to IPs or PIs, e.g., P(4,5)P2 and Ins(1,4,5)P3. Our approach involves magnetic activated cell sorting followed by nanopore sequencing of library sequences from selected yeast. This work will shed light on PI signalling and regulation in trypanosomes.

Poster #20

Targeting PfVps15 to better understand hemoglobin endocytosis in the malaria parasite *Plasmodium falciparum*

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The parasite responsible for the most virulent form of malaria is *Plasmodium falciparum* (*Pf*). *Pf* belongs to the phylum Apicomplexa, a phylum composed of obligate intracellular parasites characterized by the presence of an apical complex. During the erythrocytic cycle, *Pf* invades and develops within red blood cells (RBC). In the RBC, the parasite proceeds to the endocytosis and digestion of hemoglobin within its digestive vacuole (DV). Several studies suggest that the sole PfPI3K likely plays a role in the transport of hemoglobin to the DV and in apicoplast homeostasis. The DV and the apicoplast both possess membranes enriched in PI3P. Inactivation of PfPI3K has not yet been possible, therefore its precise function remains elusive. In yeast the only PI3K, Vps34, is known to form a complex with Vps15 and other effectors required for endosomal trafficking. Our hypothesis is that PfVps15, through its interaction with PfPI3K, is likely essential for parasite survival. My goal is to characterize the role of PfVps15 during the erythrocytic cycle of *P. falciparum* by determining if its presence is essential for parasite growth and survival. We were able to generate a strain allowing the conditional inactivation PfVps15. Fluorescence microscopy observation of the tagged strain confirmed expression of PfVps15 most prominently during trophozoite and schizont stages. Conditional inactivation of PfVps15 demonstrated that removal of this protein results in a significant decrease in parasitemia in comparison to controls. Further exploration of the phenotype generated following inactivation of PfVps15 will be done to determine its potential role in parasite biology. Also, identification of PfVps15 interaction partners will be done.

Poster #21

Complete genome assembly of antimony resistant *Leishmania infantum*: understanding the formation of a circular amplicon carrying the *mrpA* gene

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Leishmaniasis, a disease caused by a parasite *Leishmania* spp, affects ~350 million people around the world. Among different forms of Leishmaniasis, *L. infantum* causes visceral leishmaniasis (VL), which can be lethal if remains untreated. The first line of treatment for VL has been antimonial (Sb) drugs for over 60 years but unfortunately, the parasite became resistant to it. This study focuses on understanding the resistance mechanism of *L. infantum* against Sb drugs. First, I performed a complete genome assembly of a wild type and an Sb resistant mutant strain of *L. infantum* using a hybrid method combining short-read Illumina sequencing and long-read Oxford Nanopore sequencing. Next, I explored various resistance mechanisms (copy number variation, SNPs, etc.) by performing comparative genomics analyses between the newly assembled genomes. Since one of the signature resistant mechanisms of *L. infantum* involves the extrachromosomal circular amplification of the *mrpA* locus in chromosome LinJ23, the next goal was to understand the amplicon formation by homologous recombination between the direct repeats flanking a locus of four genes including the *mrp*A. Using the Nanopore long reads, I identified the repeats taking part in the recombination and the sequence of the junction after the amplicon formation, which remained unknown thus far. The results of this study will be useful to understand the resistance mechanism of *L. infantum* against Sb drugs which will help develop new therapeutic strategies. Furthermore, the complete genome assemblies will be a valuable resource for studying the resistance mechanism in *L. infantum* in the future.

Poster #22

Reprogramming host and parasite eIF4A-dependent mRNA translation to control *Leishmania* infection

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Protozoan parasites of the genus *Leishmania* are the causative agents of leishmaniasis, a spectrum of tropical neglected diseases. The lack of efficient vaccines and the failure to control emerging parasite resistance reflect the need to identify novel targets for therapeutic intervention. Our laboratory demonstrated that one third of protein-coding mRNAs in macrophages are differentially translated upon infection by *Leishmania donovani*. In silico analysis indicated activated translation dependent on the mRNA helicase eIF4A upon infection. Notably, genetic or pharmacological inhibition of eIF4A reduced *L. donovani* survival within macrophages. Selective inhibitors of eIF4A, named rocaglates, have immunomodulatory and antimicrobial properties associated with their ability to fine-tune macrophage functions. Interestingly, some rocaglates target non-mammalian eIF4A activity. Hence, we postulate that pharmacological inhibition of host and parasite eIF4A-dependent translational programs contributes to control *Leishmania* infection. To test this hypothesis, we will assess the leishmanicidal activity of a panel of well-characterized rocaglates that target either mammalian or non-mammalian eIF4A. We are currently developing fluorescence- and luminescence-based semi-high throughput screening assays to test the microbicidal activity of these compounds on extracellular promastigotes and intramacrophage amastigotes of different *Leishmania* spp. Selected compounds will be then tested *in vivo* using experimental models of cutaneous and visceral leishmaniasis. In parallel, we will identify eIF4A-dependent translational programs and functions that are altered in promastigotes and infected macrophages treated or not with selected rocaglates, using polysome-profiling and RNA-seq. Our long-term goal is to provide insight on the mechanistic basis and therapeutic potential of modulating host and parasite eIF4A-dependent translational programs to reduce morbidity and mortality associated with visceral and cutaneous leishmaniasis.

Poster #23

SEC14 proteins, a new therapeutic target against malaria?

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Plasmodium falciparum is the parasite responsible for the majority of malaria cases worldwide and more and more strains resistant to current treatments are being reported; it is therefore of utmost importance to search for new therapeutic targets. Sec14p is an essential lipid transporter protein, first discovered in yeast, involved in many cellular mechanisms. Proteins homologous to Sec14p exist in all eukaryotes and notably in *P. falciparum*. In the latter, there are 6 SEC14 proteins. Our hypothesis is that these proteins are essential for the survival of the parasite. To demonstrate this, we have characterized two of them (PfSEC14-1 and PfSEC14-5) by establishing genetically modified parasite lines. These lines allow to inactivate a target gene and also to determine the localization of the protein thanks to the addition of a fluorescent tag in the c-terminal of the gene of interest. Inactivation of the PfSEC14-1 gene leads to a 75% growth defect during the intraerythrocytic cycle. Abolishing the expression of the PfSEC14-5 gene has no effect on the growth of the parasite. The PfSEC14-1 protein is present in the cytoplasm and localizes partially to the endoplasmic reticulum and mitochondrial membrane. PfSEC14-5 is found in the cytoplasm and at the plasma membrane of the parasite. These results show that PfSEC14 proteins appear to be essential for parasite survival and could therefore serve as a new target for antimalarial drugs.

Poster #24

Finding new drugs to combat Human African trypanosomiasis

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Human African Trypanosomiasis, sleeping sickness, is a neglected tropical disease caused by a parasite infection. This disease can cause severe problems and ultimately death if left untreated. Drug discovery for sleeping sickness has been slow, and consequently, ancient drugs like suramin and melarsoprol are still being used. The emergence of resistance against current treatments and their toxicity and inefficiencies warns us to find a novel trypanocidal before it strikes again.

Thus, we employed both phenotypic and target-based screening approaches to screening a focused library of substituted naphthalenes and quinolinediones, agents tested to be non-toxic on mammalian cells.

Alamar blue assay, an established method for assessing the viability of the parasites, was utilized in the phenotypic screen with compounds at 15 µM in a 96 well-plate format. The primary phenotypic screen yielded 9 hits. The hits were cherry-picked from the mother plates and serially diluted. EC50 of the selected hits was defined against the Trypanosoma brucei, ranging from 80 nM to 3 µM.

The target-based screening was against RNA editing, an essential target only present in the parasite. Some agents could inhibit Trypanosoma brucei RNA editing at the low micromolar range. In addition, we found the compounds interfered with RNA protein interaction as suggested by the mode of action analysis.

Hits from these two screens can serve as new starting points for discovery of drugs that kill the parasite causing human African trypanosomiasis.

Poster #25

The intercellular communication between B cells exposed to *Leishmania donovani*

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Polyclonal B cell activation and resulting hypergammaglobulinemia are a detrimental consequence of visceral leishmaniasis, caused by the protozoan parasite *Leishmania donovani*; however, the mechanisms underlying this excessive production of non-protective antibodies are still poorly understood.

While studying the interaction of B cells with *L. donovani* in search of the mechanism underlying polyclonal B cell activation, we observed B cells to form long tubular protrusions when exposed to the parasite. We have characterized these protrusions to be primarily actin-based with lower content of tubulin, which is in line with literature descriptions of tunneling nanotubules (TNTs). These TNTs represent a novel way of intercellular communication via the passage of material along these formed connections, which has been implicated in the spread of some pathogens. Interestingly, we have observed parasites to be situated on these intercellular protrusions between splenic B cells exposed to *L. donovani*, suggesting that amastigotes may be gliding from one cell to another using TNTs. To elucidate a possible role of these connections in the propagation of parasite cell activation, we studied whether activation could be passed on through soluble messengers such as cytokines and found this to be insufficient to pass on the activation state between B cells. On the other hand, preliminary experiments allowing for cell-to-cell contact suggest that parasites and the activation state can be passed from cell to cell.

Taken together, we provide novel insights about TNT formation in primary splenic B cells and the possibility that this mechanism may contribute to polyclonal B cell activation.

Poster #26

The role of cytochrome b_5 isotype I in the nucleolus of *Giardia intestinalis*

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Giardia intestinalis is a protist responsible for one of the most common causes of infectious diarrhea worldwide. It has five heme proteins, four of which are isotypes or paralogues of cytochrome b_5. As these four cytochrome b_5 isotypes are found in different cellular compartments in Giardia they likely have different functions. Giardia cytochrome b_5 isotype I (gCYTB5-I) is localized to the nucleolus. Previous work in our lab suggests that gCYTB5-I has a role in nucleolar stress response, as its protein levels increases and it preferentially translocates out of nucleolus when Giardia trophozoite cultures are exposed to low levels of nitrosative stressors. We also used gCYTB5-I along with CBF5 pseudouridine synthase as markers of the nucleolus to show that the nucleolus structure dismantles during encystation, which is the developmental process where the trophozoite form of Giardia transforms into its infectious cyst form. Furthermore, we observed that the formation of the nucleolus occurs at late S and early G2 phase of the cell cycle, corresponding to those stages where we also observed the highest level of *de novo* rRNA synthesis. Our results indicate that the nucleolus is a dynamic organelle in Giardia that responds to cellular stress, as well as changes in the demands for ribosome synthesis and protein production associated with Giardia encystation and the cell cycle.

Poster #27

Insights into the roles of plant growth-regulating cytokinins in the protozoan parasite *Giardia intestinalis*

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Giardia intestinalis is a protozoan parasite responsible for the diarrheal disease in mammals called giardiasis, or 'beaver fever', but the mechanisms of disease pathogenesis are unclear. While proteins secreted by *Giardia* can affect the physiology and response of host cells, the impact of any potential hormone-like molecules secreted by *Giardia* have not been studied. Cytokinins (CKs) are adenine-derived molecules that were first identified as potent regulators of plant growth and development. More recently, CKs have been detected across multiple kingdoms of life, including protists; however, the roles of CKs in organisms beyond plants have yet to be fully defined. Although CKs were found within *Giardia* cultures, the detection of any CKs that might be secreted by *Giardia* is confounded by high background CK contents of the culture growth medium. Consequently, synthetic forms of CKs were added to the culture medium to examine *Giardia*'s ability to metabolize these exogenous CKs. These experiments showed an ability by *Giardia* to convert CK precursors (ribosides) into the active CK free base forms. We also supplemented *Giardia* cells in a minimal maintenance medium with CKs to determine if they could stimulate *Giardia* growth. To look for impacts beyond macro-changes (*i.e.* growth rates, visible phenotypes) samples of these cultures will be subjected to metabolomic profiling using HPLC-HRMS/MS to examine the fate of the added CKs and their effect on other metabolites.

Poster #28

Exosome isolation from serum samples for neurocysticercosis biomarker research: Realtime challenges

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Introduction
Exosomal-protein biomarkers would potentially be used to diagnose Neurocysticercosis (NCC) in human, but it is challenging to isolate exosome from blood serum. Here, we are addressing some challenges we faced during isolation of exosome from 20 blood serum collected from NCC patient and apparently healthy people (control) in Burkina Faso between 2012-13.

Methods
For this study, the exosomes were isolated from serum samples using total exosome isolation kit and concentrations were determined using nano-particle tracking analysis. Then, the total protein concentrations of isolated exosomes were quantified using microBCA assay.

Results
During this study, we faced some challenges to isolate exosome from serum sample using conventional protocol, such as – foam formation during centrifugation, extra-large pellet formation, difficulty in albumin precipitation etc. But, after several modification of conventional protocol at different stages we were able to isolate exosome with average diameter of 152.4 nm (range: 130.1-179.6) and concentration of 2.9e+11 particle/mL (range: 6.0e+09-1.7e+12). Besides, we found the mean of the total exosomal-protein concentration was 45292 µg/mL (range: 27424-126966.7) and protein-particle ratio was 7.2e+07 (range: 4.7e+05-4.6e+10) for analysed samples.

Discussion
Apparently, the size and concentration of exosome seems good, but the purity was not satisfactory. It may be due to the high concentration of serum albumin, repeated thawing of samples, collection procedure and the storage time of the samples, etc. Although further studies are needed to evaluate the roles of assumed causes behind the aforementioned challenges, still, this study will help future researchers to understand and address the challenges they may encounter.

Poster #29

A genome-wide yeast surface display screen to identify vaccine targets for Chagas disease

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Chagas disease affects about 8 million people and is caused by infection with the protozoan parasite *Trypanosoma cruzi*. The infection results in an acute disease with flu-like symptoms that often develop into a chronic cardiac disease. There are no vaccines for Chagas disease, and the available drugs, benznidazole or nifurtimox, are highly toxic and their efficacy is limited to the acute disease. To identify potential vaccine targets for Chagas disease, we generated a genome-wide library of *T. cruzi* for yeast surface display (YSD) to screen for parasite proteins that react with Chagas disease patient's antibodies. The genome-wide library includes fragments ranging from 0.5 to 3 kb fragments and represents 24-fold of the parasite genome. Nanopore sequencing confirmed that the library covered all chromosomes, and each parasite gene was represented by multiple clones. We generated a computational tool to predict the library-expressed proteins using nanopore sequences and found that the library potentially produces 83,000 unique polypeptides, including protein fragments that might help identify immunogenic epitopes. Using Western and flow cytometry, we confirmed the surface expression of library proteins in over 95% of the yeast. Screening of the *T. cruzi* YSD library with a pool of Chagas disease patient serum showed that 14% of the yeast library reacted with patients' antibodies. Magnetic-assisted cell sorting and nanopore sequencing are in progress to identify the antigens targeted by the patient's antibodies. The identified antigens will be validated as potential vaccine target candidates in animal models.

Poster #30

Assembling genome-wide libraries for yeast surface display screen of drug targets in protozoan pathogens

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Genome-wide genetic screens are powerful approaches to identifying drug or vaccine targets. However, the limited genetic tractability of many pathogens has hindered the use of genome-wide approaches. To overcome this limitation, we exploited yeast genetics as a tool for drug or vaccine target discovery. We have developed an efficient approach to constructing genome-wide libraries for yeast surface display (YSD). In this system, each yeast cell expresses on its surface ~10^5 copies of a single parasite protein, and thus a complete parasite proteome can be represented in a yeast population for ligand screening assays. We show the robustness of our method by generating libraries for *Trypanosoma brucei*, *Trypanosoma cruzi*, and *Giardia lamblia*. Each library has a diversity of ~10^5 to 10^6 clones, representing ~6 to 24-fold of the parasite genomes. Nanopore sequencing confirmed the libraries' genome coverage and showed that each parasite gene was represented by multiple clones. Computational analysis of the nanopore sequencing predicted over 80,000 surface proteins for each library, and Western and flow cytometry confirmed the expression of library proteins by the yeast. We developed a YSD fitness screening assay for drug target discovery. Using the YSD assay, we identified bonafide interactors of metronidazole, a drug used to treat giardiasis, and new candidate proteins to help identify metronidazole targets and mechanisms of action. The libraries are valuable biological resources for discovering drug or vaccine targets, ligand receptors, protein-protein interactions, and investigating pathogen-host interactions. The library assembly approach can be seamlessly applied to other organisms or expression systems.

Poster #31

Elucidating the role of cholesterol in the male-female interplay of *Schistosoma mansoni*

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Schistosomiasis is a parasitic disease caused by schistosomes which affect over 240 million people worldwide. Schistosomes have separate sexes and are unique in that adult worms always live pair together resulting in egg production, which is responsible for the pathology of the disease. The treatment relies on the mass drug administration of a single drug, Praziquantel, which raises concerns about drug resistance. Therefore, it is urgent to identify novel molecules that can be targeted in alternative therapies. Understanding the reproductive biology of schistosomes will contribute to identify such molecules. The male/female pairing is essential to the sexual maturation of the female, but the signals involved in this pairing remains unknown. Schistosomes are unable to synthesize cholesterol; however male and female worms have been shown to exchange exogenous cholesterol suggesting that they may exploit the host cholesterol for their reproduction. Our research focuses on the role of cholesterol on the reproduction and survival of the parasite. We are characterizing schistosome proteins that interact with cholesterol *in vivo* using RNA interference gene expression on adult schistosomes and immature schistosomula. We are also identifying potential signalling pathways that may be regulated by cholesterol by analysing the effect of cholesterol on gene expression in schistosomes using RNA sequencing. This project will contribute to a better understanding of the biology of schistosomes as well as any other parasitic worms that may exploit cholesterol and ultimately, to provide insight into finding new targets and developing novel anthelmintics to combat schistosomiasis.

Poster #32

CXCL16-dependent regulation of macrophage anti-inflammatory responses during *Toxoplasma gondii* infection

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The protozoan parasite *Toxoplasma gondii* is the causative agent of toxoplasmosis, a potentially deleterious infection in immunocompromised individuals and pregnant women. Subversion of the host chemokine system is a common parasite survival strategy. CXCL16 is a multifaceted chemokine produced by macrophages and other immune cells in response to infection. Here, we show that type I (RH) and II (ME49) *T. gondii* strains induce the expression of the membrane-bound and the secreted forms of CXCL16 in mouse bone marrow-derived macrophages (BMDMs). Kinetics analyses of mRNA and protein expression in combination with pharmacological assays hint at mTOR-dependent post-transcriptional regulation of CXCL16 during *T. gondii* infection. Exposure of BMDMs to soluble *T. gondii* antigens and to excreted proteins indicates that CXCL16 induction does not require live infection and host cell invasion. Conditioned media from *T. gondii*-infected BMDMs failed to promote migration of cells expressing CXCR6, the cognate receptor of CXCL16. We then investigated the anti-apoptotic and cell polarizing properties of CXCL16 during *T. gondii* infection using wild-type and CXCL16 knockout BMDMs. The ability of *T. gondii* to block actinomycin D-induced apoptosis in macrophages was independent of CXCL16. In contrast, CXCL16 was involved in the induction of the anti-inflammatory gene *Ym1* by *T. gondii* RH. Interestingly, *T. gondii* RH infection enhanced the accumulation of *Ym1* in response to the M2-polarizing cytokines IL-4 and IL-13 in a CXCL16-dependent manner. Ongoing and future investigation will determine whether CXCL16 contributes to the immunopathogenesis of toxoplasmosis, including the subversion of macrophage polarization, and to elucidate the underlying molecular mechanisms.

Poster #33

A theoretical approach to understanding electronic transfers of hemozoin formation in the malaria parasite

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Introduction

The objective of this project is to use a computational approach to study hemozoin formation. The focus of the study is on the unusual semi-conductor properties of helozoin and the impact that has on heme reduction in the digestive vacuole. A key problem is understanding the process by which iron gets oxidised during hemozoin formation.

Methods

Calculations are run using the gaussian software package.

Results & Discussion

Work has been done in identifying the best way to represent hemozoin with a quantum mechanics model that isn't too demanding. Periodic boundary conditions on a crystal the size of hemozoin are very demanding, and so crystal nucleation must be studied at a smaller scale. A number of DFT parametres have been tested in order to identify the best functional and basis sets to calculate IR and UV-vis spectra in a number of free-base and metalated porphyrins. IR spectra are replicated with a good accuracy, especially for the characteristic C-O stretches, with a variety of basis sets. The tried 6-31g(d,p) basis set is used to choose a functional that most accurately represents exited states, which is evaluated by simulated UV-vis spectra.

The simpler B97D functional gives surprisingly more accurate Soret bands than the more advanced hybrid wB97XD basis set. Q-bands however vary compared to experimental values, and comparison to the Goutermann 4 orbital models, through the use of natural transition orbitals, should help elucidate them.

Poster #34

Mouse models of passive immunization to study vaccine candidates against bovine cryptosporidiosis

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Bovine cryptosporidiosis can be frequent, severe, and even life-threatening for newborn dairy calves. There is an urgent need to develop a vaccine able to immunize mother cows to passively protect newborn dairy calves via colostrum. The goal of this research is to study the passive transfer of immunity, mimicking the cow/calf reality, in mouse models of *C. parvum* infection. First, to determine the role of humoral or cellular immunity in preventing cryptosporidiosis, serum or splenocytes of vaccinated Bl/6 mice tolerant to *C. parvum* infection were injected intravenously to interferon gamma receptor knock-out (IFNγR-KO) mice which were then infected orally with *C. parvum* oocysts. At the peak of infection, the effect of passive immunization was assessed by evaluating intestinal parasite burdens of IFNγR-KO mice by flow cytometry and qPCR analysis. Second, to reflect the cow/calf reality, pregnant IFNγR-KO mice were vaccinated twice during pregnancy. The protective effect of immune colostrum was established by determining intestinal parasite burdens of newborn IFNγR-KO pups challenged with *C. parvum* oocysts. Third, IFNγR-KO mice received polyclonal antibodies orally and were challenged with *C. parvum* oocysts. Results obtained using these three mouse models of passive immunization identified promising vaccine candidates to protect against *C. parvum* infection. Our preliminary results pave the way for further studies to test the potential of these vaccine candidates to immunize pregnant cows to passively protect via colostrum newborn dairy calves from this severe diarrheal disease.

Poster #35

Identifying the pseudogenes misidentified as functional genes in *T. brucei* genome

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After coming up with some pseudogenes misidentified as Protein-Coding Genes (PCG), we aimed to characterize the pseudogenes by assuming that the parent functional gene of each pseudogene is available in the genome. We focused on two types of pseudogenes, those Lacking a Part of the functional ORF (LPO) and those with a FrameShift (FS).

Methods

Genomic sequences containing flanking regions plus the ORF of each PCG in the genome of *T. brucei* were searched against the organism proteome by blastx. Fragments passing the following filters were reported as pseudogenes:

• Alignment of the fragment to a protein included the flanking regions of the gene in the query fragment (hypothetical LPOs) or a part of the coding part of the fragment aligned with a protein in a frame other than the predicted coding frame (hypothetical FSs).

• The aligned area on the target sequence contained was a Highly Confident Protein (HCP) coding sequence. We assumed sequences with an InterPro domain or significant homology to trusted protein databases such as Swissport or PDB are HCPs.

• Flanking region of the query (for LPOs) or query in another frame than the predicted one contained HCP (for FSs).

Results

More than 100 pseudogenes were identified. More than half were previously known as frequent Pseudogenes (VSG and RHS).

Discussion

The function of about a third of the *T.brucei* genome is unknown. This study shows by identifying pseudogenes, the number of unannotated genes will be reduced. Besides, it shows sequence models such as InterPro can aid in identifying pseudogenes.

Poster #36

An adenovirus-vectored schistosomiasis vaccine reduces parasite burden and pathology

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Introduction
Schistosomiasis is a neglected tropical disease which affects over 236.6 million people worldwide. Affected individuals manifest chronic pathology due to egg granuloma formation, destroying the liver over time. The only FDA approved drug, praziquantel, does not protect individuals from reinfection, highlighting the need for a prophylactic vaccine. *Schistosoma mansoni* Cathepsin B (SmCB) is a parasitic gut peptidase necessary for helminth development and confers protection as a vaccine target for intestinal schistosomiasis.

Methods
An SmCB expressing human adenovirus serotype 5 (AdSmCB) was constructed and delivered intramuscularly to female C57BL/6 mice in a heterologous prime-and-boost vaccine with recombinant protein. Vaccine induced immunity was described and subsequent protection was assessed by analysing parasite burden and liver pathology after infection.

Results
Substantially higher humoral and cell-mediated immune responses, consisting of IgG2c, Th1 effectors, and polyfunctional CD4+ T cells, were induced by the heterologous administration of AdSmCB when compared to the other regimens. Though immune responses favoured Th1 immunity, Th2 responses provided by SmCB protein boosts were maintained. This mixed immune response resulted in significant parasite burden reduction and schistosomiasis associated liver pathology (egg granuloma size, and liver fibrosis) was also prevented.

Discussion
Our study provides missing preclinical data supporting the use of adenoviral vectoring in vaccines for *S. mansoni* infection. Our vaccination method significantly reduces parasite burden and its associated liver pathology - both of which are critical considerations for this helminth vaccine – using a platform which can be easily scaled up for this global disease.

Poster #37

Allosteric regulation of repressor-activator protein 1 by PI(3,4,5)P3 is essential for silencing telomeric expression sites in trypanosomes

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Trypanosoma brucei evades the host antibody clearance by switching its variant surface glycoprotein (VSG) coat. *T. brucei* has hundreds of VSG genes; however, only one VSG gene is transcribed at a time from one of the 20 telomeric expression sites (ESs). Antigenic variation occurs by transcriptional switching between ESs or by VSG gene recombination. We show that VSG monogenic expression and switching entail a phosphoinositide-regulatory system. This system includes a nuclear phosphatidylinositol phosphate 5-phosphatase (PIP5Pase) enzyme that binds to repressor-activator protein 1 (RAP1) and controls VSG transcription. We show here that catalytic mutation (D360A/N362A) of PIP5Pase results in transcription of all silent telomeric VSG genes. ChIP-seq analysis with RAP1 showed that it interacts with ES sequences flanking VSG genes, namely 75 bp and telomeric repeats. Using gel shift and kinetic analysis, we found that RAP1 C-terminal Myb or Myb-like domains bind directly to 75 bp or telomeric repeats. In contrast, the rRAP1 N-terminal domain binds specifically to the PIP5Pase substrate, PI(3,4,5)P3. Notably, PI(3,4,5)P3 disrupts rRAP1 association with telomeric or 75 bp repeats. To investigate how PI(3,4,5)P3 regulates RAP1 association with ES sequences *in vivo*, we performed ChIP-seq with RAP1-HA in *T. brucei* cells that exclusively express the catalytic mutant PIP5Pase. The inactivation of PIP5Pase abolished RAP1 association with telomeric and 75 bp repeats and resulted in transcription of all VSG genes. We propose that PI(3,4,5)P3 is an allosteric regulator of RAP1 and controls its association with telomeric ESs sequences flanking VSG genes, which is essential for VSG silencing.

Poster #38

Analysis of the presence of M1/M2 and exhausted macrophages in the spleen of dogs naturally infected with *Leishmania infantum*

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Visceral leishmaniasis (VL) is an important public health problem and the spleen has been described as one of the most affected organs. The dog is considered an urban reservoir animal and may present progressive infection and high parasite load in several tissues. This study aims to analyze M1, M2 and exhausted macrophages in the spleen of dogs naturally infected with *Leishmania infantum*, correlating the data with the level of splenic white pulp disorganization (SWPD), parasite load and symptomatology. 34 animals with VL were included in this study. The animals were clinically evaluated for clinical score classification. Histopathological analysis was performed to evaluate SWPD and qPCR to quantify the parasite load. SWPD was observed in most of the animals. $iNOS^+$, $Arginase1^+$, $STAT3P^+$, $Mannose^+$, and $TGF\text{-}\beta^+$ cells, were detected demonstrating the participation of both M1 and M2 in the response to infection. M1/M2 ratio decreased in those animals that presented more clinical signs and high parasite load. We also detected significant PD-L1 expression in macrophages in the spleen with SWPD and low parasite load, a profile of cell exhaustion. Therefore, the maintenance of M2 cells amidst the intense inflammatory infiltrate in the parasitized spleen, associated with the reduction of M1 cells, could provide safe sites of replication for the parasite, leading to the persistence of the parasite and the permanent stimulation of the inflammatory response. The persistence of the antigenic and inflammatory stimulus would lead to chronic tissue damage, which in the spleen is characterized by SWPD, and worsening of the disease.

Poster #39

Differential regulation of host and pathogen gene expression by the c-Rel transcription factor determines the outcome of cardiac and neurotropic infections

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Infectious pathogens are distinguished by their tissue tropism, or preferential replication in a given organ. For example, lethal neuroinflammation in the brain can arise during herpes simplex virus (HSV) encephalitis, or during cerebral malaria caused by *Plasmodium* parasite infection. In contrast, coxsackieviruses (CVB) can establish productive infection in the heart, and can result in lethal cardiomyopathy. These diseases are strongly influenced by host genetics, where infection outcome depends on the protective or pathological role of tissue-resident and infiltrating cells. In a mouse mutagenesis screen for HSV susceptibility, we identified a truncating mutation in the *Rel* gene (Rel^{C307X}), encoding for the NF-kB transcription factor subunit c-Rel, as a cause of lethal HSV encephalitis (HSE). On the other hand, mutant Rel^{C307X} mice do not develop experimental cerebral malaria during *Plasmodium berghei* ANKA infection, and also resist CVB cardiac infection. To address the divergent role of the Rel^{C307X} mutation in the regulation of these infections, we performed dual host-pathogen RNA sequencing on HSV-infected brainstems and CVB-infected hearts. Two days prior to HSE symptom onset, the Rel^{C307X} hindbrain was characterized by elevated viral RNA transcription and excess interferon-dependent and inflammatory gene expression, triggering lethal lymphocyte and monocyte infiltration. Yet, the Rel^{C307X} heart exhibited improved control of early CVB transcription, and a later reduction in inflammatory gene expression and in damaging infiltration. Ultimately, our findings identify c-Rel as a key regulator of pathogen replication and inflammation during infection, and highlight that a balanced and tissue-appropriate inflammatory response is critical to resolving infection and limiting pathology.

Poster #40

The RNA ligation mechanism of post-transcriptional editing in *Trypanosoma brucei*

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Introduction

Uridine insertion/deletion RNA editing is a unique biochemical feature of the early-diverged kinetoplastid parasites of *Trypanosoma* and *Leishmania spp*. The RNA editing ligase 1 (L1) and interacting partner A2 are essential enzymes in the process of ligating nicked sites post-editing. L1 ligation involves the catalysis of ATP to L1-AMP intermediate for the formation of a phosphodiesterase bond between the 3'OH and 5' PO4 of the RNA strands. L1 and A2 have diverged in their structure and function from human DNA ligases and mRNA capping enzymes. Our work aims to characterize the RNA ligation mechanism of kinetoplastid L1 and A2.

Methods

L1and A2 mutants were expressed using *in vitro* coupled transcription translation. They were assayed for ligation activity in the presence and absence of A2 using both radioactive and fluorescent labelling. Furthermore, calmodulin affinity immunoprecipitation was used to pull down L1 with tagged A2 to study how these two proteins interact.

Results

The C-terminal domain (CTD) of L1 proves to be a key component to the ligation mechanism in both function and structure. CTD-truncated mutants are inactive in ligation and for the formation of the L1-AMP intermediate. In the presence of A2, there is an increase in the L1-AMP intermediate and the overall L1 ligation activity is enhanced. CTD Truncations have narrowed down amino acids 384-410 of L1 to be the interacting site of A2 and further group mutations of this helix significantly reduced its pulldown to A2.

Conclusion

L1 harbours a diverged CTD to other ligases that is essential for the ATP catalysis. Its interacting partner A2 interacts with this through electrostatic interactions and in doing so enhances L1's ligation activity.

Poster #41

Modulation of macrophage mitochondrial biogenesis and metabolism by *Leishmania donovani* requires the surface coat glycolipid lipophosphoglycan and type I IFN

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To colonize macrophages, *Leishmania* metacyclic promastigotes employ a panoply of virulence factors, including lipophosphoglycan (LPG), which impairs different host cell processes and rewires host cell metabolism creating a metabolically-adapted microenvironment required for pathogen replication. Whereas previous studies revealed that *Leishmania* alters signaling axes that regulate mitochondrial function, scarce attention has been paid to the characterization of host cell mitochondrial metabolism during *Leishmania* infection and to the effectors involved therein. Here we investigated the mechanisms governing the modulation of macrophage mitochondrial properties by the vacuolar pathogen *Leishmania*. We obtained *in vitro* and *in vivo* evidence that induction of mitochondrial biogenesis by *L. donovani* requires the virulence glycolipid lipophosphoglycan, which mediates the expression of key transcriptional regulators and structural genes associated with the electron transport chain. *Leishmania*-induced mitochondriogenesis also requires a lipophosphoglycan-independent pathway involving type I IFN receptor signalling. Stimulation of oxidative phosphorylation by *L. donovani* is also dependent on lipophosphoglycan and is supported by glycolysis and the electron transport chain, but in contrast to mitochondrial biogenesis does not require type I IFN signalling. The observation that pharmacological induction of mitochondrial biogenesis enables an avirulent lipophosphoglycan-defective *L. donovani* mutant to survive and replicate in macrophages supports the notion that mitochondrial biogenesis contributes to the creation of a metabolically-adapted environment propitious to the replication of the parasite. This study provides novel insight into the complex mechanism by which *Leishmania* metacyclic promastigotes alter host cell mitochondrial biogenesis and metabolism during the colonization process.

Poster #42

Helminth-induced reprogramming of the stem cell niche inhibits type 2 immunity

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Intestinal helminths remain one of the most pervasive parasites of the animal kingdom by stimulating host defense pathways that prioritize tissue adaptation over parasite expulsion. Although helminths form intimate interactions with the intestinal epithelium, little is known about the ability of helminths to directly shape the fate of this barrier tissue. Here we show that infection of mice with *Heligmosomoides polygyrus bakeri* (*Hpb*) induces a fetal-like state in the intestinal stem cell niche coincident with adult parasite adherence to intestinal villi. This reprogramming event is characterized by a regenerative Hippo pathway transcriptional signature and the emergence of Clusterin-expressing 'revival' stem cells (revSC) previously shown to drive intestinal repair following acute injury. Organoid-based studies using parasite-derived excretory/secretory products reveal that *Hpb*-mediated revSC generation occurs independent of host-derived immune signals and inhibits type 2 cytokine-driven differentiation of secretory epithelial lineages that promote worm expulsion. A more in-depth analysis revealed that helminth-secreted products induce an oxidative stress response that is critical for this fetal-reversion of the intestinal stem cell niche. Furthermore, lineage-tracing studies confirm the presence of revSC-derived progeny along the villi of *Hpb*-colonized animals. By contrast, type 2 cytokines inhibit revSC development and the fetal gene program both *in vitro* and *in vivo*, while deletion of type 2 cytokine signaling *in vivo* lead to an enhanced fetal host response, increased host susceptibility to infection and improved worm fitness. Collectively, our study reveals how a helminth parasite co-opts a tissue development program to counter type 2 immune-mediated expulsion and maintain chronic infection.

Poster #43

Development of rapid *in vitro* colorimetric enzymatic activity assay to differentiate macrocyclic lactone susceptible and resistant *Dirofilaria immitis* isolates

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Dirofilaria immitis causes dirofilariosis, a potentially fatal pulmonary condition in canids. Dirofilariosis can be prevented by treatment with prophylactic macrocyclic lactones (ML). ML-resistant *D. immitis* isolates, genetically distinct from the wildtype population, have been confirmed via molecular markers. A simple *in vitro* biological test is lacking to differentiate *D. immitis* infections resulting from inadequate adherence to recommended prophylaxis regimens from those caused by resistant isolates. The goal of the current study was to develop a minimally invasive rapid diagnostic *in vitro* biological assay to differentiate ML-susceptible from ML-resistant *D. immitis* isolates. The *in vitro* diagnostic assay assesses the effects of ivermectin (IVM) exposure on the secretion of enzymes by the *D. immitis* mf at a concentration that distinguishes the ML-susceptible from ML-resistant isolates. The metabolic enzyme, triosephosphate isomerase (TPI), was chosen due to high abundance in the *D. immitis* secretome. The *in vitro* TPI enzymatic assay was optimized and tested in eight laboratory-maintained isolates. We observed mixed results, the susceptible Missouri and Berkeley had statistically significant decreases in TPI activity. The three resistant isolates, JYD-34, Metairie, and WildCat showed no change in TPI activity. The susceptible, or putative susceptible Georgia II, Georgia III, and Big Head had a phenotypic response consistent with ML-resistance based on the *in vitro* assay. However, increasing genotypic evidence has presented a mixed genotype for the three isolates. The measurement of changes in enzymatic activity consequently does not appear to be a reliable detection method for ML-resistance but may be useful for identifying fully susceptible isolates.

Poster #44

Severe genetic iron overload hampers development of cutaneous leishmaniasis in mouse footpads

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Leishmania sp. are a group of intra-macrophage protozoan parasites which rely on the essential micro-nutrient iron for survival. Proper access and regulation of iron is vital for parasite growth and virulence. Macrophages, *Leishmania*'s host cell, may divert iron flux by reducing expression of iron importers or by increasing expression of the cell surface iron exporter ferroportin. How conditions of severe iron dysregulation affect parasite growth remains unclear.

To study the role of iron in leishmaniasis, we employed mice deficient in hemojuvelin (Hjv-/-). Lack of this Bmp co-receptor limits the production of hepcidin, the iron peptide hormone which induces the degradation of ferroportin. Thus, impaired production of hepcidin leads to severe iron overload due to unrestricted dietary iron uptake with paradoxical iron deficiency within macrophages which fail to store iron. Hjv-/- mice were injected with *Leishmania major* either in hind footpads and followed for up to 10 weeks or intraperitoneally before collection of peritoneal lavages after 6 hours.

Hjv-/- mice displayed delayed growth of *L. major* in hind footpads when compared to wild type controls with a significant difference in parasite burden observed at 4 weeks of infection. Following acute intraperitoneal exposure to *L. major*, Hjv-/- peritoneal macrophages had elevated transcription of cytokines and chemokines. Thus, iron overload and hemojuvelin deficiency appear to play important roles in early development of infection. Understanding the role of iron in parasite metabolism may help us develop novel treatments and understand the effects of dietary iron supplementation for iron deficiency anemia in parasite endemic regions.

Poster #45

The relationship between chemosensory neuron morphology and ivermectin resistance in *Caenorhabditis elegans*

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Nematode parasitic infections present a massive disease burden to livestock and human populations in developing countries. Ivermectin is one of the most successful nematocidal antiparasitic drugs however, its success is threatened by the development of drug resistance among parasites. This project aims to understand the genetic and mechanistic basis of ivermectin resistance, to halt the spread of resistance and restore the efficacy of ivermectin. Having identified a genetic marker of resistance affecting the morphology of chemosensory neurons, we are examining two hypotheses explaining why defective sensory neuron morphology confers drug resistance: 1) defective sensory neurons activate an inappropriate stress response that counteracts drug effects, or 2) affected sensory endings are important for drug entry. To test hypothesis one, we are mutating various stress pathways to test whether they are required for resistance. If this hypothesis is correct, we expect mutations affecting stress response pathways to alter ivermectin sensitivity. To determine whether drug entry is affected, we are using autoradiography to examine the ability of wild type and resistant worms to take up radiolabelled ivermectin. If this second hypothesis is correct, we predict that sensory morphology mutants will take up less ivermectin than wild type. Given the importance of livestock as a crucial source of income and food for individuals living in poverty, it is imperative that we uncover the mechanisms underlying antiparasitic drug resistance. Our findings will inform the development of new antiparasitic drugs to get ahead of the problems presented by ivermectin resistance.

Poster #46

Determination of confounders with DAGs: the example of *Taenia solium*

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Introduction
Taenia solium cysticercosis/taeniasis is a parasitic disease transmitted between humans and pigs. Numerous factors in its complex life cycle are modifiable and therefore promote elimination efforts. However, this complexity renders accurate measurement of the true effect of risk factors difficult.

Methods
A systematic review of epidemiologic studies of *Taenia solium* was conducted. One hundred and fifty-one studies containing a total of 183 potentially causal association were included. Two directed acyclic graphs (DAGs) were constructed using two levels of causality established by experts: one DAG containing associations with strong evidence and one with weak evidence. These DAGs were used to identify confounders of the association between knowledge of *Taenia solium* and seropositivity in 3579 people in 60 villages in Burkina Faso. This association was measuring using logistic regression models including confounders identified by the strong and weak DAGs.

Results
No confounders were identified using the strong DAG. Three confounders (age, gender, and current pig ownership) were identified by the weak DAG. Having heard of taeniasis was positively associated with cysticercosis (OR 2.7, 95%CI 1.7;4.3) when no confounders were included in the analysis. This association remained significant in the model that included the three confounders (OR 1.7, 95%CI 1.1;2.8).

Conclusions
Knowledge of *Taenia solium* is associated with higher prevalence of cysticercosis in our models based on both strong and weak criteria for causality. However, the strength of association is greater in the strong-causal model. The impact of choosing other measures of *Taenia solium* knowledge will be discussed in our presentation.

Poster #47

The impact of metronidazole on the nitric oxide dioxygenase activity of the parasitic protist *Giardia intestinalis*

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Giardia intestinalis is an anaerobic parasitic protist that causes the sickness known as giardiasis. Although Giardia is unable to synthesize heme, it still possesses several heme proteins, one of which is a flavohemoglobin (gFlHb). The proposed function of gFlHb is to act as a nitric oxide dioxygenase (NOD) that converts toxic nitric oxide produced by the host's immune system to harmless nitrate. Infections by Giardia are often treated with metronidazole, a prodrug which is activated by its partial reduction, producing damaging free radicals. Recently, an association between metronidazole resistance and genetic diversity in gFlHb has been characterized. The purpose of this research is to determine whether metronidazole has a direct effect on the NOD activity of gFlHb. To investigate this possibility, I expressed and purified recombinant gFlHb and measured its NOD activity with a free radical analyzer equipped with a NO-sensitive electrode. The reaction is initiated by addition of the NO donor, PROLI-NONOate, and the half-life of NO is obtained from a plot of current versus time. Three sets of experiments were conducted: control with no protein or inhibitor ($t_{½}$ =214 ± 15 s), enzyme-catalyzed with 1 nM gFlHb ($t_{½}$ =7.7 ± 0.8 s), and enzyme-catalyzed with 1 nM gFlHb and 100 µM metronidazole ($t_{½}$ = 6.4 ± 1.3 s). Under the aerobic conditions of these experiments, metronidazole does not inhibit the NOD activity of gFlHb. Anaerobic conditions will be investigated in the future as the active drug may be inactivated in the presence of oxygen.

Poster #48

The role of LAG-3-expressing CD4 T cells in visceral leishmaniasis

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Maintenance of CD4 T-cells during chronic infections is vital for effective host response. Expression of inhibitory receptors by CD4 T-cells following immune activation regulates their proliferation. One such inhibitory receptor Lymphocyte activation gene 3 (LAG-3), has often been described as marker associated with regulatory T cells and in the context of T cell exhaustion. Using a mouse model of visceral leishmaniasis (VL), we identified a new subset of LAG-3+ splenic CD4 T-cells that express CXCR5, the chemokine receptor associated with migration to B cell follicles. Interestingly, LAG-3+ CXCR5+ T-cells also express other markers similar to T-follicular helper cells (TFH) such as TCF-1, ICOS and PD-1. However, these cells have intermediate PD-1 expression as opposed to TFH that are PD-1^{high}. Using bulk-RNA sequencing, we compared the transcriptomic profile of the LAG-3+ CXCR5+ CD4 T-cells versus TFH and found that this cell population has ~5000 differentially expressed genes compared to TFH, including several cytokine, inhibitory receptors, effector molecules, and chemokines. We have identified that these cells express gene signatures associated with self-renewal capacity, suggesting hematopoietic stem cell-like properties. When transferred into a RAG-/- recipient mice followed by challenge with *Leishmania donovani*, these cells expanded more than naïve CD4 T-cells in frequency and number and were able to differentiate into IL-10+, IFN-γ+ and IL-10+ IFN-γ+ CD4 T-cells. Taken together, we have shown that this new CD4 T-cell subset may play a key role in maintaining CD4 T-cell responses during persistent infections, owing to their proliferative and differentiation capacity.

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