1) CD160, a crucial regulator for ILC2 function during helminth infection

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Strongyloides ratti, a rodent-specific parasitic nematode, has both tissue-migrating and intestinal life stages. In immunocompetent hosts, infection resolves within 2-4 weeks in the context of an adaptive type II immune response. Termination of infection depends entirely on adaptive immunity and $RAG^{-/-}$ mice lacking T and B cells remain infected for a year. Nevertheless, innate immunity is highly effective in reducing the number of intestinal parasites, from approximately 80 parasites per mouse on day 6 post-infection (p.i.) to only 2-5 parasites on day 10 p.i. and beyond. CD160, which acts as a regulatory receptor expressed on T cells and innate immune cells such as NK cells and ILC1, emerges as an important factor in this context. Comparing *S. ratti* infection kinetics between $RAG^{-/-}$ and $RAG^{-/-}CD160^{-/-}$ mice, a consistent intestinal parasite burden of approximately 50 parasites per mouse was observed from day 6 to day 97 p.i. in CD160-deficient mice. Notably, the absence of CD160 correlated with a lack of intestinal mastocytosis and mucosal mast cell activation that is essential for parasite clearance. We have shown previously that ILC2 promote the initial activation of mast cells during *S. ratti* infection in an IL-9-dependent manner. Here we report for the first time that CD160 is expressed on intestinal ILC2. Expression increased during *S. ratti* infection *in vivo*, coinciding with the expansion of intestinal ILC2. In contrast, $RAG^{-/-}$ $CD160^{-/-}$ mice did not show intestinal ILC2 expansion during infection. Bone marrow-derived CD160-deficient ILC2 produced fewer type 2 cytokines compared to WT ILC2 *in vitro*. Finally, adoptive transfer of intestinal CD160-competent ILC2 reduced the worm burden and induced mucosal mast cell activation in *S. ratti* infected $RAG^{-/-}$ $CD160^{-/-}$ mice. In summary, our findings suggest that CD160-mediated signalling contributes to ILC2 activation and the subsequent innate immune response during intestinal helminth infection.

2) ***H. polygyrus*** **skews intestinal dendritic cell development towards regulatory phenotype to counter the anti-helminth immune response**

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Precise regulation of type 2 immune responses is essential for balancing protective immunity and tissue tolerance during intestinal helminth infections. Dendritic cells (DC) orchestrate this balance by priming effector and regulatory T cells. However, it remains unclear which DC exactly is responsible for each arm of the immune balance during the intestinal type 2 responses. In other tissues, DC2 are critical for regulating type 2 response. Yet, the intestinal DC2 are highly heterogeneous and their role in driving effector or tolerogenic immune responses in the intestine remains ambiguous. Here we have used infection with the helminth *Heligmosomoides polygyrus bakeri (Hpb)* to show that phenotypically distinct populations of intestinal DC2 play different roles in the arms race between pro- and anti-inflammatory immune responses in the same model. While $CD103^-$ DC2 induce anti-parasitic Th2 effector responses, $CD103^+$ DC2 counter this effect by expanding in number, progressively lose their ability to prime effector T cells while also become superior inducers of tissue-protective regulatory T cells. This correlates with upregulation of retinoic acid (RA) synthesis by the tolerogenic DC2. Furthermore, we observed that the expansion of the $CD103^+$ DC2 results from *Hpb* producing molecules that mimic TGFβ, which has been previously shown to be important for their differentiation. Thus, *Hpb* modulates host type 2 immune responses by altering the differentiation and function of local intestinal DCs over the course of a single infection. Our results also underline how context plays a crucial role in the complex biology of DC2 at mucosal surfaces.

3) Altered amino acid metabolism is a central feature of intestinal schistosomiasis with links to type 2 immunity

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The egg shedding stage of *Schistosoma mansoni* infection represents a major challenge to the physiology and immunology of the intestinal tract. Throughout the length of the intestines, shed eggs rupture across the intestinal wall into the lumen, risking exposure to opportunistic microbes and necessitating continuous tissue repair. However, few studies have investigated how the immune response coordinates these vital processes. Here we present a comprehensive characterisation of the intestinal landscape during *S. mansoni* infection in a mouse model. We combine high-parameter flow cytometric profiling of the intestinal immune compartment with transcriptomics, metagenomics and metabolomics. Our analysis demonstrates that infection profoundly reshapes every aspect of the intestinal environment. Our data show how a potent Th2-driven immune response develops in response to increased tissue damage and barrier leakiness. We demonstrate that infection reshapes the microbiota and, using germ-free mice, that this altered composition has the potential to transfer features of Th2 immunity to uninfected individuals. Finally, by combining whole tissue transcriptomics with targeted LC-MS metabolomics we provide evidence that infection drives increased consumption of tryptophan and arginine within the intestinal tissue. Our findings suggest that these key amino acids are metabolised to produce products with the potential to modulate the immune response including supporting the alternative activation phenotype of macrophages. Collectively our data provide a highly detailed picture of the intestinal landscape in response to *S. mansoni* infection and will be invaluable going forward in defining mechanisms of immune regulation in the context of intestinal inflammation more broadly.

4) Changes in lipid metabolism in *C. elegans* resistant to ivermectin

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Resistance to ivermectin (IVM) dangerously compromises the success of anthelmintic therapy in animal and humans. The mechanisms underlying the development of resistance to IVM involve several factors, among which the nuclear hormone receptor NHR-8 has recently emerged as an important driver in *Caenorhabditis elegans* and in the ruminant parasitic nematode *Haemonchus contortus*. Interestingly, NHR-8 plays is central in drug protection and lipid homeostasis in *C. elegans*, and exposure to IVM alters lipid metabolism in mammalian cells and nematodes. We hypothesized that IVM-resistant *C. elegans* would have altered lipid metabolism through ivermectin exposure and activation of nhr-8 regulatory cascade. We have compared wild-type N2B, IVM10 and nhr-8-deficient *C. elegans*, using complementary approaches, based on imaging of triacylglycerol content by oil-red staining or Coherent anti-Stokes Raman scattering (CARS) spectroscopy. RNA sequencing analysis were also performed to identify putative changes in gene expression. Our data revealed that triacylglycerol stores were altered by ivermectin resistance. We identified several abnormally expressed genes involved in IVR-10 resistance and in NHR-8 deficient *C. elegans* that may support the alteration of lipid stores observed in IVR-10 animals. Altered lipid metabolism impacts on fitness and the molecular targets involved in this alteration can be exploited to counteract anthelmintic resistance in parasitic nematodes.

5) Searching for new drugs that block nematode-specific responses to environmental stress

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Many soil-transmitted helminths undergo profound changes to their environment as they transition from free-living stages of their life cycle into their vertebrate host. These include changing from aerobic to highly anaerobic conditions, along with changes in salt and temperature. The ways in which nematodes respond to these stresses is often different to their host — this gives us a potential way to attack the parasite while leaving the host unaffected. Our overall strategy is to establish assays in *C.elegans* for each of these stress responses (e.g. high salt, low oxygen etc) and use the power of *C.elegans* genetics to dissect these responses and to screen for compounds that specifically block them. We initially focused on using *C.elegans* to model the shift in metabolism that occurs as the parasites switch from free-living aerobic metabolism to rhodoquinone-dependent anaerobic metabolism in the host. This is an ideal target since the hosts do not make or use RQ. We used *C.elegans* to identify both the pathway for rhodoquinone (RQ) synthesis, the key molecular switch that drives RQ synthesis, and many of the genes that affect this pathway. We have also carried out drug screens for compounds that either kill *C.elegans* specifically when they rely on RQ for survival or that block RQ synthesis and have presented some of these results in previous meetings. Here I will present a new family of species-selective Complex II inhibitors that can kill nematodes that are resistant to the main class of Complex II inhibitor nematicides like fluopyram. I will also present results from a new and expanded suite of assays where we try to go beyond looking just at nematode-specific anaerobic metabolism but also at the nematode-specific pathways and machineries they use to deal with high salt, high temperature and low pH. Nematodes respond to osmotic stress via a well characterised transcriptional program that drives the synthesis of glycerol to maintain osmolarity. Just as hosts do not use RQ, the host cells also do not use glycerol as an osmolyte — the nematode response is different to the host and thus an attractive target. We have now carried out a drug screen to identify new compounds that kill worms specifically when they experience high salt as an environmental stress. I will present the results of this drug screen which identifies new compounds as well as defining a key role for mitochondria in responding to osmotic shock. I will also describe a key shift during development in the ways in which worms can respond and adapt to these stresses. We believe that targeting this salt response will provide another way to kill nematodes inside their hosts without affecting the host biology.

6) A minimal kynurenine pathway was preserved for rhodoquinone but not for *de novo* NAD^+ biosynthesis in parasitic worms: the essential role of NAD^+ rescue pathways

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ATP and NAD^+/NADH are the energy and redox currencies of life. Helminths obtain ATP using an alternative electron transport chain in which NADH serves as electron donor, rhodoquinone (RQ) as electron transporter and fumarate as electron acceptor. This allows worms to harvest energy and maintain their redox balance under hypoxia, in the gastrointestinal tract of their hosts. In *C. elegans*, the kynurenine pathway generates the precursors for RQ and de novo NAD^+ biosynthesis. We use comparative genomics, metabolic labeling, HPLC-MS targeted metabolomics, and enzyme inhibitors to define pathways that lead to rhodoquinone and NAD^+ biosynthesis in helminths. Of the kynurenine pathway genes, only the kynureninase (KYNU) and tryptophan/indoleamine dioxygenases are encoded by all helminths. The absence of kynurenine formamidase and kynurenine monooxygenase genes did not preclude RQ biosynthesis in species lacking these genes, such as the cestode *Mesocestoides corti*. Furthermore, *M. corti* metabolic labeling with 13C-Tryptophane revealed that RQ derives from this amino acid. In addition, we found that most helminths lack the enzyme 3-hydroxyanthranilate 3,4-dioxygenase (HAAO), downstream of KYNU in the kynurenine pathway. A *C. elegans haao-1* mutant strain and *M. corti*, which lacks this enzyme, were unable to synthesize de novo NAD^+ from labeled 13CTrp. Our results indicate that the absence of the *haao* gene precludes de novo NAD^+ biosynthesis and that most helminths rely exclusively on NAD^+ recycling pathways. Cestodes can neither recycle nicotinic acid nor nicotinamide riboside, and depend solely on nicotinamide salvage pathway for NAD^+ synthesis. Importantly, the inhibition of the NAD^+ recycling enzyme nicotinamide phosphoribosyl transferase (NMPRT) with FK866 led cultured *M. corti* to death. Our results demonstrate that a minimal kynurenine pathway was evolutionary maintained for rhodoquinone and not for de novo NAD^+ biosynthesis in helminths, and shed light on the essentiality of NAD^+ rescue pathways in helminths.

7) Chronic small intestinal helminth infection perturbs bile acid homeostasis and disrupts bile acid signaling in the murine small intestine

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Intestinal helminths have evolved an abundance of mechanisms to enable chronic infections in their mammalian hosts. To manipulate host immune responses, helminths can exert functional changes in the intestinal metabolome, either directly or through impacts on the bacterial microbiota. We have shown that mice infected with the small intestinal roundworm *Heligmosomoides polygyrus* have reduced concentrations of specific taurine-conjugated primary bile acids (including T-CDCA) in their small intestinal luminal contents compared to uninfected mice. We show that *H. polygyrus* has the capacity to directly de-conjugate T-CDCA in ex vivo cultures, suggesting an evolutionary benefit to the worm to be able to modify bile acids directly. Bile acids can engage with a variety of surface and nuclear receptors on host intestinal cells, and we report that there is reduced signaling through the nuclear bile acid receptor FXR in the small intestine of mice during a small intestinal helminth infection. Together, our data reveal disruptions to bile acid homeostasis and signaling in the small intestine during helminth infection. As bile acids are known to impact many aspects of mucosal physiology and immunity, examining the functional consequences of bile acid disruptions during helminth infection will be an important avenue for future research. Our ongoing work is exploring the impact of bile acid-supplemented diets on immunity to murine helminth infections.

8) Untargeted metabolomics links alterations of host tyrosine metabolism with susceptibility to *Schistosoma mansoni* infection

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Hepatosplenic schistosomiasis caused by *Schistosoma mansoni* (Sm) remains a significant public health concern. Understanding the metabolic changes induced by Sm infection is crucial for uncovering host signatures of the infection and disease pathogenesis. In this study, we employed LC-MS untargeted metabolomic profiling on plasma samples obtained from school-aged children in areas of low and moderate endemicity for Sm in Cameroon, strongholds of parasite persistent transmission. Diagnosis of Sm infection was conducted using the Kato Katz (KK) method and complemented by circulating anodic antigen assay. Liver morbidity was assessed by ultrasonography. Children were stratified based on infection and liver fibrosis status. Three successive batches of individuals were screened by untargeted metabolomics probing of isolated plasma to uncover differentially abundant metabolites and associated pathways during Sm infection and/or associated liver fibrosis. Every hit was searched for robust occurrence in the list of differential metabolites through a first 'discovery' metabolomics run, a 'validation' run, and then a 'stability in front of polyparasitism' run (individuals coinfected with malaria or hepatitis). Comparative analyses were performed using various statistical methods, including fold change analysis, t-tests, sPLSDA, VIP score, heatmap visualization, and ROC curve analysis. Our profiling identified significant alterations in multiple metabolic pathways associated with Sm infection and liver fibrosis susceptibility. Strikingly, the alteration of Tyrosine metabolism emerged as a robustly perturbed pathway across the discovery and the validation runs during Sm infection, suggesting its potential as a candidate biomarker for Sm infection. Moreover, in the polyparasitism run, the persistence of alterations in tyrosine metabolism in Sm-infected hosts highlighted the resilience of this pathway as a biomarker of Sm infection in the field. This study highlights the consistent perturbation of tyrosine metabolism in the plasma of Sm-infected hosts and its potential as a harnessable pathognomonic characteristic of Schistosomiasis to inform adjunct diagnostic, monitoring or therapeutic tools.

9) Identification of *Brugia malayi* miRNAs involved in causing human lymphatic filariasis pathology

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Lymphatic filariasis (common name, elephantiasis) is one of the most debilitating yet neglected infectious diseases in the world today. More than 120 million of "the poorest of the poor" are victims of these filarial scourges. The parasitic nematodes that cause these diseases— *Brugia malayi*, *Wuchereria bancrofti* —produce severe pathologies: hydrocoele, lymphoedema, and elephantiasis. Recent studies showed that parasitic worms secrete their miRNAs, which can be detected in the biofluids of infected humans and animals. Our hypothesis is that parasites living in lymphatic systems secrete miRNAs that disturb lymphatic endothelial cells. First, we used published data sources and bioinformatic analyses to select parasite miRNAs that are secreted from *B. malayi* and that could potentially regulate processes in human cells. We prioritized the human targets that encode proteins involved in cell-to-cell connection and the extracellular matrix: fibronectin, integrins, as well as proteins of tight junctions (claudins) and adherens junctions (VE-cadherin). We identified 2 *Brugia* miRNAs—bma-mir-86 and bma-mir-5864—that have potential target sites in these genes. To validate the interactions predicted and determine their effects on target genes, we treated LECs with miRNA-mimics, which mimic selected parasite miRNAs, and analyzed the expression of the human proteins to determine if there was suppression. Results showed that parasite miRNAs significantly suppress expression of human proteins responsible for cell-to-cell interactions and adhesion. The parasite-mediated disruption of cell-to-cell connections compromises the integrity of the cellular endothelial barrier of the lymphatic system, contributing to pathology. Our study helps to understand the mechanisms of the host-parasite interaction and suggest an alternative treatment strategy to coup severe pathology of disease in human.