Parasitic Helminths: New Perspectives in Biology and Infection

Hotel Bratsera, Hydra, Greece

Abstracts for Oral Presentations

6 September 2023

Wednesday 6 September

09:00 – 10:40 Session 7. Human Infection. Chair Padraic Fallon

09:00	**Moses Egesa**	**MRC/UVRI/ LSHTM Uganda**	**Establishing a single sex *Schistosoma mansoni* controlled human infection model for Uganda**
09:40	Emma Houlder	Leiden, NL	Immune responses in controlled human *Schistosoma mansoni* infection, lessons from single and reinfection studies.
10:00	Bridgious Walusimbi	MRC/UVRI/ LSHTM Uganda	Are the effects of helminth infection and urbanisation on one's cardiovascular risk mediated via the gut microbiome?
10:20	Cornellis Hokke	Leiden, NL	In-depth characterisation of *Brugia malayi* glycosylation and unraveling of cross-reactive anti-glycan antibody responses in filarial nematode infections

11:10 – 12:50 Session 8. Immune Activation. Chair Oyebola Oyesola

11:10	**Michalis Barkoulas**	**Imperial College UK**	***C. elegans* as a tractable host to study natural infections by oomycetes**
11:50	Lara Linnemann	BNITM, GER	Characterization of the regulative role of the C-type lectin receptor MINCLE in the initiation of anti-helminth immune responses
12:10	Pedro Papotto	Manchester, UK	Dermal γδ17 T cells orchestrate innate and adaptive immunity in distal organs during nematode infection
12:30	Kyle Cunningham	Glasgow, UK	A family of helminth-derived TGF-β mimics provide key insights to innate and adaptive immune cell activation

16:00 – 17:20 Session 9. Drug Resistance. Chair Murray Selkirk

16:00	Ray Kaplan	St George's, West Indies	Molecular evidence of widespread benzimidazole drug resistance in *Ancylostoma caninum* from domestic dogs throughout the USA and discovery of a novel isotype-1 β-tubulin benzimidazole resistance mutation
16:20	Anne Lespine	INRAE, FR	Role of nematode ABCB transporters and their regulation in anthelmintic resistance
16:40	Stephen Doyle	Sanger, UK	Genomic landscape of drug response reveals mediators of anthelmintic resistance
17:00	Sarah Cobb	Texas, USA	Understanding the basic biology of juvenile schistosomes by studying stem cells

1) Immune responses in controlled human *Schistosoma mansoni* infection, lessons from single and reinfection studies.

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Prior studies have revealed Type-1/Type 2 response in early migrating and maturing *Schistosoma mansoni (Sm)* infection, developing to a Type-2 and regulatory response upon egg production. These findings have been mainly derived from animal (murine) models, as longitudinal assessment how worm-specific immune responses develop humans has not been possible. Here, we have used controlled human infection models to study the development of human immune responses to *Sm*. Specifically, we have infected non-endemic participants with 10-30 *Sm* cercariae in three independent studies of single-sex infection with male (n=17) or female (n=13) *cercariae* as well as male reinfection (n=24). Throughout the studies repeat blood sampling has been performed, with cellular and humoral immune responses assessed via cytometry (mass and spectral) in peripheral blood mononuclear cells (PBMCS). Additionally, multiplex immunoassays, as well as protein and glycan microarrays are being performed with serum. Single infection studies revealed broad similarity in the clinical and immunological profiles of infection with single-sex male or female cercariae. This was characterised by an early inflammatory response at 4 weeks post infection, coinciding with the peak of symptoms. This included expansion of IL-4 expressing $CD4^+$ effector memory T cells, monocyte activation and increases in type-1 inflammatory mediators such as IFNγ and CXCL10 measured in serum. By week 8 post infection inflammatory responses had reduced, while frequencies of $CD4^-CD8^-$ T cells expressing the regulatory cytokine IL-10 as well as serum IL-10 levels increased. Preliminary results from reinfection studies are revealing enhanced worm-specific T helper 2 responses (IL-13) in second and third reinfections. Current work will focus on thorough T cell characterization, as well as using protein and glycan microarrays to understand antibody repertoire. Immunological outputs will be integrated with clinical parameters with the aim of understanding how immune responses contribute to symptom development as well as infection burden. Together, this data reveals detailed insights into the development of human immune responses during schistosome infection.

2) Are the effects of helminth infection and urbanisation on one's cardiovascular risk mediated via the gut microbiome?

Bridgious Walusimbi[1,2,3]*, Melissa AE Lawson[3], Allison J Bancroft[3], Jacent Nassuuna[1], David P Kateete[2], Emily L Webb[4], Richard K Grencis[3†], Alison M Elliott[1,5†]*

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Preliminary results from our Lake Victoria Island intervention study have shown that *S. mansoni* (*Sm*)-infected individuals had reduced LDL-cholesterol and triglycerides levels. Further, rural individuals showed reduced blood pressures than those in the urban setting. This project was aimed at understanding the mechanisms by which helminth infections and the rural environment alters cardiovascular disease risk. We hypothesized that; by changing the human gut microbiome profiles, helminths and the rural environment may alter one's cardiovascular risk. Firstly, we conducted a meta-analysis using random-effects model for all microbial diversity metrics, to explore the relationship between helminths and human gut microbiome. Secondly, we investigated Sm-and urbanisation-induced changes on the gut microbiome and metabolome, using 16S rRNA sequencing and Liquid chromatography- mass spectrometry of randomly selected faecal samples from 216 individuals whose cardiovascular profiles (LDL-, total-cholesterol, triglycerides and blood pressure levels) and *Sm*-infection status were known. Our systematic review (endorsed for publication in Frontiers in Microbiology), showed a higher alpha diversity among the *Sm*-infected. Further, from our samples, we showed increased alpha-diversity in *Sm*-infected Vs uninfected, and in rural Vs urban participants. Using PCoA, we show significant differences in the gut microbiome beta-diversities of rural vs urban (PERMANOVA, p=0.001) and *Sm*-infected vs uninfected (PERMANOVA, p=0.011). Linear discriminant analysis showed differentially abundant bacteria in rural vs urban, and *Sm*-infected vs uninfected. Correlation analysis showed that differentially abundant bacteria were significantly associated with cardiovascular risk factors for rural vs urban and *Sm*-infected vs uninfected. The metabolome was different for *Sm*-infected Vs *Sm*-uninfected, and rural Vs urban populations. Pathway analysis showed that cholesterol-related metabolic pathways were significantly enriched by the differentially abundant metabolites. This work bolsters our understanding of the possible roles that helminths and urbanisation may play in altering one's gut microbiome and metabolome and how these changes may contribute to the resolution of cardiovascular risk.

3) In-depth characterisation of *Brugia malayi* glycosylation and unraveling of cross-reactive anti-glycan antibody responses in filarial nematode infections

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Millions of people worldwide are infected with filarial nematodes. Elimination programs have resulted in reduction of infections, creating a need for improved diagnostic tools for population surveillance. Glycans from helminths play various crucial roles in host-parasite interactions, and constitute potential antigens for use in diagnostics or as vaccine antigens. Although it has been reported that glycan cross-reactivity between several filarial species occurs, filarial glycosylation is still largely uncharacterised. Thus, we investigated the glycan repertoire and glycan antigenicity of filarial nematodes, using *Brugia malayi* as starting point. Lipid- and protein-linked glycans from the parasite were identified using mass spectrometry(MS) approaches, revealing antigenic motifs containing phosphorylcholine and glucuronic acid. Site-specific glycosylation maps of excretory/secretory glycoproteins were established in view of their immunogenic and diagnostic potential. Isolated glycans were fractionated and printed onto glycan microarrays. Microarray screenings showed strong recognition of various *B. malayi* glycans by serum antibodies during infections. To further address the specificity of IgG binding to these glycans, we screened *B. malayi* glycans with plasma from individuals infected with *Loa loa, Onchocerca volvulus, Mansonella perstans* and *Wuchereria bancrofti*, closely related filarial nematodes. IgG to a subset of *B. malayi* glycans was observed for each of these species. This indicates expression of shared glycans, which for *O. volvulus* was confirmed by MS. IgG to array glycans was most abundant for *O. volvulus* and *M. perstans* infections, with IgG1 and IgG2 as major subclasses. In *B. malayi* infected individuals we observed a marked reduction in IgG2 to parasite glycans post-treatment, suggesting potential diagnostic applications. Altogether, our work provides detailed insights into filarial nematode glycosylation and identifies broadly shared, as well as more specific glycans and glycan motifs that are antigenic in the infected host. This work significantly extends the currently scarce knowledge of filarial nematode glycosylation and the associated host antibody response.

4) Characterization of the regulative role of the c-type lectin receptor MINCLE in the initiation of anti-helminth immune responses

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Strongyloides ratti is a parasitic nematode that actively penetrates the skin of its rodent host and migrates via skin, lung and head tissue before embedding into the intestine. Initiation of protective immune responses against helminths involve their recognition by pattern recognition receptors (PRR), like C-type lectin receptors (CLR). Screening a CLR-hFc library, we found yet unidentified *S. ratti*-derived ligand(s) that bind to Macrophage inhibitory C type lectin receptor (MINCLE) and signal into a MINCLE reporter cell line. MINCLE recognizes endogenous damage-associated ligands and conserved bacterial, fungal or protist parasite-derived ligands. It is expressed by eosinophils, neutrophils and macrophages which contribute to the eradication of tissue-migrating *S. ratti* L3. Comparing the course of *S. ratti* infection in wildtype and MINCLE-deficient mice we did not observe changes in the numbers of tissue-migrating L3. Surprisingly, numbers of intestinal parasite burden were reduced in MINCLE-deficient mice, suggesting that absence of this PRR did not impair but improved the intestinal host defense. This was not explained by differences in mucosal mast cell activation, quantity and quality of *S. ratti*-specific Th2 cell responses, establishment of protective memory or composition of intestinal microbiota. However, frequency of eosinophils recruited to lungs and intestine of *S. ratti*-infected mice were increased while neutrophil frequencies were reciprocally decreased in MINCLE-deficient mice. To further analyse the role of these cell population in intestinal immunity, we depleted eosinophils or neutrophils starting after the tissue migration. This led to a significantly increased worm burden in both genotypes, implicating for the first time an important role for granulocytes during the intestinal phase of a *S. ratti* infection. To further analyse the role of MINCLE we are currently comparing the function of MINCLE-competent and MINCLE-deficient granulocytes and macrophages *in vitro*. First results suggest that *S. ratti*-derived MINCLE-ligands interfere with simultaneous stimulation by described MINCLE agonists.

5) Dermal γδ17 T cells orchestrate innate and adaptive immunity in distal organs during nematode infection

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During the course of an infection, different types of immune responses are sequentially employed by the host in order to achieve pathogen clearance without causing excessive collateral tissue damage. Whilst the molecular mechanisms and effector cell types underlying protective immunity have been extensively studied, how the immune system coordinates an immune response across tissues is still poorly understood. Experimental nematode infections provide a great tool to investigate this process, as infective larvae migrate across different organs. We have established a natural *Nippostrongylus brasiliensis* infection model that allow us to fully recapitulate all the steps of the infection and investigate the cellular and molecular aspects of inter-tissue communication during helminth infections. High-dimension profiling of the initial immune response in the skin shows an expected recruitment of inflammatory cells. However, different immune cell subsets, such as dermal IL-17-producing γδ (γδ17) T cells, appear to leave the tissue soon after parasite invasion. Employment of tissue-restricted gene reporters suggested that dermal γδ17 T cells migrate from the skin to the lungs, soon after *N. brasiliensis* arrival to the pulmonary tissue. Interestingly, bypass of the skin phase of infection, or conditional ablation of IL-17 on skin-resident lymphocytes resulted in marked transcriptional and functional changes in the early neutrophilic/type-2 immune responses to migrating larvae in the lungs. Furthermore, ablation of skin-derived IL-17 prior to the skin migration phase resulted in a subsequent decrease in lung T_H2 immunity and impaired T cell priming in skin draining lymph nodes. Altogether, our data indicate that dermal γδ17 T cells are responsible not only for kick-starting the innate immune responses in the lungs but also contribute to adaptive immunity formation during *N. brasiliensis* infection. Thus, dermal γδ17 T cells may have previously undiscovered roles in mediating immune-communication between tissues during helminth infections.

6) A family of helminth-derived TGF-β mimics provide key insights to innate and adaptive immune cell activation.

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Helminth parasites have evolved sophisticated methods for manipulating the host immune response to benefit their long-term survival and circumvent therapeutic interventions. A pivotal mechanism for dampening protective immunity is through the secretion of immunomodulatory proteins. Studies on the secreted products of *Heligmosomoides polygyrus* have identified a novel mimic of TGF-β (TGM-1), organised as a 5-domain modular protein. *In vitro,* TGM-1 induces the differentiation of murine and human Foxp3$^+$ T regulatory (Treg) cells via signalling through the canonical TGF-β receptor/SMAD pathway in both murine and human T cells, despite sharing no structural homology to any member of the TGF-β family. Treg induction requires domains 1-3, while domains 4 and 5 increase the potency of the mimic. Nine additional proteins with significant sequence similarity to TGM-1 are also found in the secretomes of adult (TGMs 2-6) and larval (TGMs 7-10) life stages. These TGM family members display contrasting abilities to induce or inhibit Treg cell induction *in vitro,* vary in TGF-β signalling in different cell types, and induce markedly disparate surface expression of key activation markers, including CD39, CD103 and PD-L1. Recently, through co-precipitation, followed by mass spectrometry, a novel co-receptor for TGM-1 has been identified as CD44, a cell surface marker found on many cell types, including effector T cells and macrophages, which is involved in the sensing of hyaluronan upon cellular damage. T cells from CD44 knockout mice have a significantly impaired ability to differentiate into Treg cells in response to TGM-1, but have no such impairment in response to TGF-β. As myeloid cells express particularly high levels of CD44, we also investigated *in vitro* stimulation of macrophages, finding that TGM-1 induces an anti-inflammatory state, suppressing secretion of pro-inflammatory cytokines in response to LPS co-stimulation. Therefore, *H. polygyrus* has evolved to secrete TGM-1 to act preferentially on cells which specifically co-express TGF-β receptors and CD44. With the identification of additional co-receptors for other members of the TGM family, we postulate that these products are tailored to interact with different host cell populations to maximize the parasite's ability to prolong infection.

7) Molecular evidence of widespread benzimidazole drug resistance in *Ancylostoma caninum* from domestic dogs throughout the USA and discovery of a novel isotype-1 β-tubulin benzimidazole resistance mutation

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The canine hookworm, *Ancylostoma caninum,* is an important zoonotic gastrointestinal nematode of dogs worldwide, and is a close relative of human hookworms. We recently confirmed the presence of multiple-drug resistant (MDR) *A*. *caninum* in dogs in the USA, and demonstrated that MDR *A. caninum* were extremely common in racing greyhound dogs. We therefore were interested in determining the prevalence, distribution, and molecular epidemiology of drug-resistant *A. caninum* in the overall USA pet dog population. In greyhounds, benzimidazole resistance in *A*. *caninum* was associated with a high frequency of the canonical F167Y(TTC>TAC) isotype-1 β-tubulin mutation. The isotype-1 β-tubulin gene was sequenced from 685 hookworm-positive fecal samples collected from dogs across the USA using a deep amplicon sequencing assay. In this work, we show that benzimidazole resistance is remarkably widespread in *A*. *caninum* in domestic dogs across the USA, with the F167Y(TTC>TAC) mutation demonstrating a prevalence of 49.7% (overall mean frequency 54.0%). We also identified and showed the functional significance of a novel benzimidazole isotype-1 β-tubulin resistance mutation, Q134H(CAA>CAT) not previously reported from any eukaryotic pathogen in the field. Several benzimidazole resistant *A*. *caninum* isolates from greyhounds with a low frequency of the F167Y(TTC>TAC) mutation had a high frequency of a Q134H(CAA>CAT) mutation. Structural modeling predicted that the Q134 residue is directly involved in benzimidazole drug binding, and that the 134H substitution would significantly reduce binding affinity. Furthermore, introduction of the Q134H substitution into the *C. elegans* β-tubulin gene *ben-1*, by CRISPR-Cas9 editing, conferred similar levels of resistance as a *ben-1* null allele. The Q134H(CAA>CAT) mutation was less common, with a prevalence of 31.1% (overall mean frequency 16.4%). Canonical codon 198 and 200 benzimidazole resistance mutations were absent. This work has important implications for companion animal parasite control and the potential emergence of drug resistance in human hookworms.

8) Role of nematode ABCB transporters and their regulation in anthelmintic resistance

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The multidrug resistance (MDR) transporter ABCB1 is involved in the pharmacokinetic and toxicokinetic of many xenobiotics, including ivermectin, an anthelmintic drug commonly used to treat parasitic diseases. Several *abcb* gene homologs are present in parasitic nematodes infecting humans and animals and some of them have been associated with resistance to ivermectin, but their individual role remains to be elucidated. Being overexpressed in response to chemotherapy, notably to ivermectin-based treatment in nematodes, they lead to loss of drug efficacy by decreasing drug concentration at the target site. Using *Caenorhabditis elegans* as a nematode model, we decipherer structural and functional details of the transporters in parasites of interest. We developed complementary approaches to identify the role of individual transporter in drug transport and lipid accumulation, using imaging approaches. Overall, we identified a transcription factor regulating the expression of ABC transporters and lipid homeostasis genes as new original target to counter drug resistance. Increasing knowledge of the function of ABC transporters and their regulation is important for delaying the spread of anthelmintic resistance in helminths and to improve anthelmintic-based therapy.

9) Genomic landscape of drug response reveals mediators of anthelmintic resistance

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The evolution of anthelmintic resistance, particularly by livestock-infective helminths, has been rapid and widespread and represents a credible threat to the control of human infective helminths. Despite significant and sustained efforts, the genetic basis for resistance is poorly understood but remains key to tracking its spread and improving the efficacy and sustainability of parasite control. We have used an *in vivo* genetic cross between drug-susceptible and multi-drug-resistant strains of *Haemonchus contortus* in a natural host-parasite system together with drug selection and whole genome sequencing to simultaneously map resistance loci for the three major classes of anthelmintics. This approach has identified new alleles for resistance to benzimidazoles and levamisole and implicates the transcription factor *cky-1* in ivermectin resistance. This gene is within a locus under selection in ivermectin-resistant populations of *H. contortus* worldwide; expression analyses and functional validation using knockdown experiments in *Caenorhabditis elegans* support that *cky-1* is associated with ivermectin survival. Our work demonstrates the feasibility of high-resolution forward genetics in a parasitic nematode and prioritises variants for developing molecular diagnostics to combat drug resistance in the field.

10) Understanding the basic biology of juvenile schistosomes by studying stem cells

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Schistosomiasis afflicts over 240 million people, killing hundreds of thousands every year and causing socioeconomic damage rivaling malaria. Schistosomes, the causative agent, are parasitic flatworms with a complex two-host life cycle. Treatment relies upon a single drug, praziquantel (PZQ), that has significant drawbacks including a lack of efficacy against juvenile parasites. Indeed, the insensitivity of juvenile worms to PZQ is thought to be a major contributor to the limited efficacy of PZQ in the field. It is unclear why juvenile parasites are refractory to PZQ. The drug causes tissue damage in both juvenile and adult animals and this damage can be at least partially repaired *in vitro*. One potential explanation for the juvenile's hardiness comes from the observation that juvenile schistosomes have much higher numbers of somatic stem cells (termed "neoblasts") compared to adult parasites. In adult schistosomes, these neoblasts are thought to be required for the parasite's survival in the host by homeostatically maintaining tissues such as the skin and the gut, but their role in the juvenile is less clear. We hypothesize that the increased number of neoblasts in the juveniles allows them to repair PZQ-mediated damage better than adult parasites, leading to the lack of efficacy against juvenile animals. In order to study the role of juvenile neoblasts in praziquantel resistance, we must first improve our understanding of the basic biology of the juvenile parasite. To this end, we are employing techniques such as single-cell RNAseq, *in situ* hybridization, and EdU-based fate mapping experiments to understand the molecular identity and function of neoblasts in this important stage of the schistosome lifecycle.