Parasitic Helminths: New Perspectives in Biology and Infection

Hotel Bratsera, Hydra, Greece

Abstracts for Poster Session 2

6 September 2023

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Local vs systemic cytokine responses to human hookworm infection in healthy adults

KATE MACLEAN, FRANCESCO VACCA, BRITTANY LAVENDER, MALI CAMBERIS, GRAHAM LE GROS

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It has been long suggested that a reduction of endemic helminth infection in developed nations may contribute to increasing rates of inflammatory conditions such as inflammatory bowel disease, asthma, and allergy. For this reason, proposals have been made to re-establish hookworm infection in individuals to protect against disease. To this end, we conducted a study of the effects of a low-dose human hookworm infection on the host immune system. Of particular interest was the relationship between systemic and local cytokine responses. We conducted a 24 week-long study of healthy volunteers infected with *Necator americanus* and measured the concentration of several cytokines in both plasma and stool at various timepoints across the 24-week infection period. Many of the cytokines investigated showed an increase in concentration in both plasma and stool in the acute phase of the infection, before returning to near pre-infection levels in the chronic phase of infection. This would indicate that despite an initial immune response to *Necator americanus* both locally and systemically, the infection soon becomes tolerated by the immune system to allow for infection chronicity. This therefore shows great promise in moving toward the trial of hookworm infection, a potential novel therapy for treating inflammatory disease.

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Phenotypic screening of compounds identified by molecular docking targeting *Schistosoma mansoni* protein kinases

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Schistosomiasis is a helminthiasis caused by parasites from the S*chistosoma* genus. This disease presents high morbidity rates, and its only treatment is based on praziquantel administration. Yet, due to limitations as low efficacy in parasites' immature stages and worms with reduced sensitivity, studies for new therapeutic approaches and parasite cycle interruption are urgently needed. The discovery and development of a new drug are costly and demand several years of research. Therefore, the initial identification of a potential target, followed by the screening of molecules, provides a crucial advance in the drug discovery process. In this context, functional studies of the PKs SmERK1, SmERK2, SmFES, SmJNK, and Smp38 pointed out that those proteins are involved in *Schistosoma mansoni* maturation, reproduction, and survival*. Also, SmFES might be also linked to intermediate host recognition,* indicating that they could be promising targets for interrupting the parasite cycle. Accordingly, this work searches for new alternative molecules to support schistosomiasis treatment. Hence, three-dimensional structures of the kinase targets were predicted, and molecular docking was employed to identify molecules from the Managed Chemical Compound Collection that could be capable of binding to the ATP binding site of those PKs. Then, 169 molecules were selected to be screened *in vitro* in schistosomula and adult worms. Following this, an *in silico* analysis of ADMET properties was conducted for the compounds. After the *in vitro* screening, 52.1% of the selected molecules induced viability reduction in *S. mansoni* and were considered as active compounds. From those, 10.1% were only active in schistosomula, 30.8% in adult worms, and 11.2% in both stages. In miracidia, among the compounds with predicted affinity to SmFES, three induced reduced attraction to the snail host, which was also demonstrated for SmFES-knockdown miracidia. In conclusion, the prioritization of molecules through a rational model was efficient, since a high number of active molecules were identified and those for SmFES, mimicked the depleted phenotype. Additionally, it was possible to point-out potential molecules to be used in future trials in search of a new schistosomiasis therapeutics or interruption.

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The role of *Toxocara canis* third-stage larvae antigens in immune cell activation and induction of trained immunity

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Toxocara canis is a zoonotic parasitic nematode that can accidentally infect humans via the fecal-oral route causing an inflammatory reaction in different tissues. Studies using human models to characterize the immune response to *T. canis* are important to understand the immunopathogenesis of human toxocariasis. Additionally, even though frequent exposure to *T. canis* promote granulomatous reactions in mouse models, its potential as an inducer of trained immunity has not been explored. Human peripheral blood leukocytes were culture with *T. canis* somatic antigens (Tc-SA) and excretion/secretion products (Tc-ESP). Increased concentrations of IL-6, IL-8, CCL2 and CCL3 were detected after stimulation with Tc-SA, suggesting an important role of these antigens in the early response to *T. canis*. Furthermore, using PBMCs, Tc-ESP induced an increased production of IL-5 and IL-13 after CD3/CD28 activation. Flow cytometry analysis revealed an increase in CD4+ T cells with effector properties, and a decrease in the Naïve CD4+ T cell population, indicating that Tc-ESP promote T cell effector activation towards a Th2 phenotype. To evaluate the role of *T. canis* as an inducer of trained immunity, human monocytes were stimulated with Tc-ESP as "trainer", after a resting period a second challenge with Tc-ESP, LPS or ovoalbumin was given to the cells. Increased levels of IL-6, IL-8, CCL2 and CCL10 were detected in the supernatant of Tc-ESP trained monocytes compared with untrained cells, suggesting a role of *T. canis* as an inducer of trained immunity. Our results demonstrate the importance of *T. canis* antigens in the induction of a type 2 immune response and as a potential inducer of trained immunity, associated with exacerbated inflammatory responses which are important not only in repeated infections but also in other conditions associated with type 2 inflammation.

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Chromosomal integration of a reporter gene by RNA-guided Cas-enzymes into a predicted genomic safe-harbor site of *Schistosoma mansoni*

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Schistosoma mansoni is a trematode characterized by a complex lifecycle. For investigating the function of a gene of interest (GOI), knock-out models are common. However, in the post-genomic era of schistosome research, no established protocol exists for the stable transformation of this and other platyhelminth parasites. Until now, RNA interference (RNAi) is used as the most suitable method for functional gene characterization. However, RNAi efficiency varies, and it can lead to ectopic effects. CRISPR/Cas-based editing is a newer and powerful tool for gene characterization. To make this technique accessible for trematode research, we established a protocol for editing a bioinformatically predicted genomic safe harbor site (GSH) of *Schistosoma mansoni*. GSHs represent distinct sites in the genome that allow the integration of new genetic material without negatively affecting genome integrity or gene expression. Thus, GSHs should allow constitutive reporter-gene expression. For editing the identified GSH, a 5'C6-PEG10-modified construct encoding an eGFP reporter-gene under a strong native promoter of *S. mansoni* was used as donor repair template. Cas-mediated integration of the transgene was performed by electroporation of eggs. In a comparative approach, we used Cas9 and Cas12a and found differences in the editing efficiencies of both enzymes. Furthermore, we confirmed reporter-gene integration into this GSH using both ribonucleoprotein complexes formed by Cas9 or Cas12a. Finally, detection of eGFP signals in eggs and developing miracidia demonstrated reporter gene activity at the GSH. To summarize, we showed the programmed editing of a predicted schistosomal GSH, transgene knock-in, and expression of a GSH-integrated reporter. The findings provided proof of concept for a new genome-editing approach in *S. mansoni* and that both these Cas nucleases appear to be powerful tools for functional genomics in schistosomes. Our results open a new perspective to close an existing technical gap in trematode research.

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Insights into the molecular IgE-IPSE/alpha-1 interaction responsible for basophil activation

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IPSE/alpha-1 (IL-4 inducing principle of *S. mansoni* eggs) is a dimeric glycoprotein secreted by the eggs of the blood-trematode *S. mansoni*, the causative agent of schistosomiasis, an important neglected tropical disease. Natural IPSE has been shown to bind to IgE, resulting in the release of IL-4 and IL-13 from basophils and mast cells. The classical mechanism of IgE-dependent activation consists of cross-linking IgE by allergen binding to the antigen-recognition variable region of the corresponding immunoglobulin. Homodimeric IPSE appears to activate basophils by binding to IgE without any typical cross-linking. The aim of this study is to investigate the molecular details underlying this unique interaction between IPSE/alpha-1 and IgE. Using site-directed mutagenesis, we created six mutants, based on the knowledge that neither IPSE monomers nor the T92Y/R127L mutant, are able to activate basophils. Proteins were expressed in HEK293-6E suspension cells, followed by affinity chromatography for purification. The ability of all IPSE forms to activate basophils by binding IgE was evaluated using humanized RS-ATL8 rat basophilic leukemia (RBL) reporter cells. Cells were sensitized with either IgE-containing sera or different IgE truncates and luciferase expression was measured after stimulation with IPSE. Ancillary ELISAs using similar truncated IgE forms and IPSE were performed to further determine the binding region. Our results show that all the mutations have an impact on IPSE's capability to interact with IgE, thus lowering the activation of the reporter cells. Only the double mutant T92Y/R127L hampers cell activation completely, leading us to the conclusion that both amino acids must be key residues involved in IgE interaction. Furthermore, we show that IPSE does not bind to all truncated forms of IgE, suggesting that IgE needs all heavy chain domains, with or without light chains, to be successfully bound by IPSE. Ultimately, additional reporter assays including different IgE-forms and cryo-EM of the IgE-IPSE complex are expected to reveal a detailed model of interaction.

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Geographic-specific variation in genomic diagnostics targets of soil-transmitted helminths

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Soil-transmitted helminths (STHs) infect billions of humans and livestock worldwide causing severe morbidity in humans and threatening food security and animal health. In humans, STHs are the leading cause of malnutrition, stunted growth, and anaemia. Current diagnostic methods heavily rely on microscopical detection and identification of parasite eggs. Nevertheless, molecular diagnostics (e.g., qPCR) targeting mitochondrial genes and nuclear repetitive elements provide valuable alternatives to microscopy. Thus far, molecular diagnostics have been developed and evaluated using parasite isolates from a limited selection of geographical areas, and it is unknown whether genetic variation among worm populations from other areas might affect the performance of these tools. Recently, we have shown that low-depth shotgun metagenomics sequencing or genome skimming applied directly to DNA extracts from faecal samples from infected individuals can readily confirm helminth infections, whilst also yielding complete mitochondrial genome data for downstream population genetics and phylogenetic analyses. In this study, we analysed ~250 samples originating from 15 countries, and characterised the genetic variation in i) mitochondrial and nuclear ribosomal RNA genes and ii) nuclear repetitive elements. We investigated geography-specific single nucleotide polymorphisms (SNPs) in key binding sites of primers and probes within qPCR and phylogenetic targets. We also explored associations between DNA extract metadata (e.g., extraction kit, egg counts, DNA concentration) and performance of genome skimming to identify factors that may affect DNA recovery from faecal extracts and subsequent sequencing success. Our work highlights the need to better understand genetic variation at diagnostic target sites to limit the risk of false negative results and underlines the importance of standardisation and optimisation of approaches to ensure the development of robust molecular tools for detection of helminth infections.

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Molecular epidemiology and evolution of antigen-coding genes from the multi-host parasite *Schistosoma japonicum*

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The multi-host transmission landscape of *Schistosoma japonicum* is complex and is subject to many ecological and host-related factors, all of which may hamper China's schistosomiasis elimination targets. Understanding the mechanisms and impact of multi-host transmission, particularly at the human-animal interface, could help inform the development of new surveillance and control strategies, including development of an anti-schistosome vaccine. It is important, therefore, to further determine the relative importance of non-human hosts as reservoirs of infection, together with the genetic variability of parasites infecting different host species. Antigens interact directly with the host and are impacted by host-related factors, and therefore, can be used to understand how different host species may influence parasite genetic diversity and antigenic variability, as well as provide clues relating to the evolution of zoonotic schistosomiasis more generally. Antigen diversification in many pathogens has been implicated in alterations in the pathogen's ability to evade detection by the host. Here, we characterised the genetic variability of selected *S. japonicum* antigen coding genes (SjACGs) within and between parasite populations infecting humans and domestic animals, assessed the frequency of shared ACG genotypes between them, and therefore the potential importance of zoonotic transmission in the formation of novel *S. japonicum* antigenic variants. In addition, the impact of the measured variation on antigen protein structure and function, and how this influences antibody recognition and binding within human and animal hosts, was also evaluated to gain insight into potential vaccine efficacy and the immunomodulatory capabilities of these antigens. Three potential vaccine candidate antigens also implicated in host immunomodulatory functions, comprising two tegument-associated (tetraspanin-23 and tegument allergen-like protein-1) and an excreted/secreted antigen (venom allergen-like protein-1), were investigated here. We discuss our findings in terms of their theoretical and applied implications.

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Identification of species-specific glycan antigens of *Schistosoma haematobium*

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Schistosomes are parasitic worms responsible for devastating chronic diseases worldwide. *Schistosoma mansoni* and *S. haematobium* are the major species infecting humans, causing intestinal and urogenital pathologies, respectively. The *S. mansoni* glycome has been studied in detail, revealing complex, immunogenic and life stage-specific glycans crucial in host-parasite interactions. Very little is known, however, regarding the glycosylation and glycan antigenicity for other schistosome species, including *S. haematobium* which is estimated to be responsible for half of the approximately 250 million schistosome infections. Thus, we investigated the glycans expressed on cercariae, worms and eggs of *S. haematobium*. First, protein and lipid-linked glycans were released using enzymatic and chemical techniques and characterized using mass-spectrometry (MS) based approaches. Glycan structures were determined using sequencing techniques including exoglycosidase digestions in combination with MALDI-TOF-MS, and porous graphitized carbon-liquid chromatography-MS for in-depth resolution of complex isomeric structures. Our analysis revealed substantial differences between *S. haematobium* and *S. mansoni* glycosylation. Notably, *S. haematobium* glycosphingolipid (GSL) glycans are built on a trihexosyl core unlike the disaccharide core described in *S. mansoni*, are enriched in terminal acidic residues, but present a lower degree of fucosylation. The protein-linked glycans, on their hand, present core-modifications and terminal motifs identical to *S. mansoni*, although expressed with major quantitative differences. Next, a selection of glycans representative of *S. haematobium* and *S. mansoni* glycomes including a broad coverage of the differential structures was purified and printed on a glycan microarray. Upon array screening, we observed a strong binding to acidic GSL glycans of IgG in sera from *S. haematobium*-infected individuals compared to *S. mansoni*-infected individuals and uninfected controls. These results indicate that the identified species-specific glycans of *S. haematobium* are immunogenic and may play a role in *S. haematobium* specific immunobiology and pathology. Additionally, they constitute a potential diagnostic target specific for *S. haematobium* infections.

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Structural characterization and protective response of host non-homologous epitopes of *Wuchereria bancrofti* Thioredoxin

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Lymphatic filariasis (LF) is a neglected parasite infectious disease causing long-term disability in patients and amounts to a burden of more than 100 million patients worldwide. In this regard, any effort for vaccine development would be a strategy to augment these efforts. But screening of vaccine candidates for LF pose a challenge as many proposed putative targets show homology to human host-proteins possessing allelic variants or immunosuppressive domains. Hence, in this study, we have explored the possibility of screening host-non-homologous regions of filarial Thioredoxin (Wb-TRX), an antioxidant enzyme that plays a pivotal role in survival of the parasite. Further, we also explored the avenues of structural analysis on parasitic antigens to qualitatively improve protective immune response in the human host. Accordingly, recombinant Wb-TRX was purified for X-ray crystallographic structural studies. The three-dimensional structure of Wb-TRX was determined and refined at 1.95 Å resolution. The protective immune responses of synthesized WbTRX host-non-homologous peptides were evaluated in mice/mastomys filarial models. The high-resolution structure provided the insight for conformational epitope analysis. Since whole protein of Wb-TRX has 42% sequence identity with human host, certain putative host-non-homologous regions were selected through epitope analysis. The activity assay performed for Wb-TRX in the presence of anti-sera raised in mice against these regions characteristically reduced activity proposing a novel mechanism of enzyme inhibition depriving antioxidant ambience and challenging parasite survival. Further, parasite challenge studies in Brugia/Mastomys model showed significant protection of 75% for the host-non-homologous TRX peptides compared to the whole antigen (45%). Hence, the study reveals the possibility of selecting host-non-homologous regions as an attractive enhancement strategy in the screening of vaccine candidates for nematode parasitic diseases.

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A possible role for helminth-derived prostaglandin in regulating intestinal permeability and colitis-associated colorectal cancer development

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Approximately 10-15% of patients with inflammatory bowel disease go on to develop colorectal cancer; otherwise termed colitis-associated colorectal cancer (CAC). Recently identified risk factors for CAC include a diet rich in linoleic acid (ω-6) and infection with the small intestinal nematode *Heligmosomoides polygyrus bakeri* (Hpb). During infection with Hpb, inhibition of the cyclooxygenase (COX) metabolites, derived from linoleic acid, significantly reduced tumour formation in a murine model of CAC. Significantly, activation of COX-derived prostaglandin E_2 receptors prior to initiation of disease exacerbated tumour formation in a similar manner to Hpb infection. How Hpb infection is able to manipulate PUFA metabolism, and whether this causes exacerbation of CAC remains unknown. Analysis of the Hpb genome using WormBase ParaSite has revealed a protein with 42% amino acid identity to human prostaglandin E_2 synthase 2 (PGES2), which produces the pro-inflammatory metabolite prostaglandin E_2 (PGE_2). This protein has a known PGES2 orthologue in *C. elegans* and several highly similar paralogues in related nematode families, including Haemonchidae and Strongylida. PCR analysis demonstrated that the PGES2 homologue is preferentially expressed within the luminal adult life-stage of Hpb. Similar to the established role of PGE_2 in promoting intestinal permeability via prostaglandin receptor (EP) signalling, we completely ablated HES-mediated increases in permeability of a colorectal cancer cell line using antagonists of EP2 and EP4 receptor signalling. We therefore hypothesize that a PGES2 homologue is encoded in the Hpb genome enabling secretion of a PGE_2 mimic, which leads to subsequent breakdown of the intestinal barrier and exacerbation of CAC.

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Effects of 24-nor-ursodeoxycholic and ursodeoxycholic acid on mitochondrial dynamics in the liver of Schistosoma mansoni infected mice

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Background and aims: Hepatic fibrosis and granuloma formation around tissue entrapped eggs characterize the pathology of *Schistosoma mansoni* (*S.m.*) infection. *S.m.* infection affects mitochondrial biogenesis, mitochondrial dynamics (fusion/fission), and regulates innate and adaptive immune responses. We have already shown that 24-*nor*-ursodeoxycholic acid (*nor*UDCA) has anti-inflammatory and anti-fibrotic effects in *S.m.* induced liver injury. The mechanism behind this is not yet fully understood. We therefore aimed to investigate whether *nor*UDCA exerts its beneficial effects on liver fibrosis in murine schistosomiasis by compensating mitochondrial dysfunction. Methods: NMRI mice were infected with 50 *S.m.* cercariae and after 12 weeks received either *nor*UDCA- or ursodeoxycholic acid (UDCA)-enriched diet (0.5% wt/wt) for 4 weeks to evaluate liver pathology, as well as analyze mitochondrial dynamic genes expression level and respiration in isolated hepatocyte mitochondria using high-resolution respirometry. Results: *Nor*UDCA improved mitochondrial dynamics by reduction of mitochondrial fragmentation and enhancement of mitochondrial inner and outer membrane fusion. Moreover, *nor*UDCA but not UDCA treatment of infected animals significantly improved OXPHOS capacity and ratio of respiration in the uncoupled state, and additionally increases the electron transport system capacity and cytochrome C oxidase function. Conclusion: Our results demonstrate protective effects of *nor*UDCA on hepatocyte mitochondria function which in turn contributes another piece to the puzzle of the broad effects of *nor*UDCA on *S.m.* associated liver pathology.

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Heterogeneity of immune response during schistosomiasis in inbred mouse strains

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Schistosomiasis is a chronic helminth disease that can progress to severe fibrosis, hepatosplenomegaly and eventually death in some individuals but not others. This heterogeneity of immune responses and susceptibility to infection is associated with genetic factors that are poorly understood. First, we evaluated mortality and morbidity of different inbred strains of mice after *S. mansoni* infection. C57BL/6 mice are more resistant to infection than all other inbred strains of mice. Next, we compared C57BL/6 with the more susceptible Balb/c strain for immunopathology. Compared to C57BL/6 mice, spleen weight, size and cell numbers were greater in Balb/c mice at 7 wpi with *S. mansoni*. In the blood, we observed lymphopenia and neutrophilia in the Balb/c mice. We speculated that infection of Balb/c mice could be associated with increased extramedullary hematopoiesis in the spleen. Compared to C57BL/6 mice, HSC, CMP and GMP progenitors, and their proliferation were significantly increased in the spleen from Balb/c mice, confirming the positive correlation between spleen weight and extramedullary hematopoiesis. Also, we observed increased neutrophils hematopoiesis which is correlated with increased GM-CSF production by $CD4^+$ in the spleen of Balb/c mice, as well the α-GM-CSF treatment reduces the spleen weight and neutrophils hematopoiesis in Balb/c mice. Next, we evaluated the immunopathology in the first (F1) generation of mice. Compared to Balb/c mice, spleen weight, neutrophils hematopoiesis and $CD4^+$ $GM\text{-}CSF^+$ cells were reduced in F1 mice at 7 wpi with *S. mansoni*, suggesting that C57BL/6 background has a dominant characteristic during murine schistosomiasis. We hypothesize that these immunological phenotypes are associated with schistosomiasis mortality and gene regulatory elements (GREs) that differ between C57BL/6 and Balb/c mice regulates the immune response and the development of splenomegaly during *S. mansoni* infection. Future work is directed at identifying specific GREs that regulate these phenotypes and disease outcomes.

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Identification of small molecules interacting with a microRNA present in extracellular vesicles of Schistosomes to study the host–parasite interaction

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We aimed to identify small molecules that can be used as tools to study the role of some schistosome microRNAs in the interaction with their host immune system. To that end, we selected miR-10 a known parasite-specific microRNAs linked to the pathways involved in EV host-parasite and immune system. The latter microRNA was then evaluated for the ability to form a bulge region favourable for small molecules to bind by means of computational tools. Then, a fragment-based library was screened with ^{19}F-NMR to identify binders. The binding fragments were connected using a machine learning platform and larger molecules were then procured. Ultimately selected molecules were tested on miR-10 in NMR as well as in relevant *in vitro* assays to study the host–parasite interaction.

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Investigating the genetic diversity of the *Schistosoma mansoni* Transient Receptor Potential Melastatin ($SmTRPM_{PZQ}$) channel in response to praziquantel treatment in natural Ugandan *S. mansoni* populations

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Schistosomiasis is a neglected tropical disease (NTD) caused by parasitic trematodes from the genus *Schistosoma*, with the highest burden in sub-Saharan Africa. The disease is treated with praziquantel (PZQ) which is typically delivered annually through large scale mass drug administration programs in endemic countries. These programs have been successful in reducing the prevalence and intensity of infection but their impact on the evolution of drug resistance is unknown. As control efforts to eliminate schistosomiasis intensify to reach the WHO NTD roadmap targets, there is a need to detect and track PZQ resistant *Schistosoma* isolates. The transient receptor potential melastatin channel ($SmTRPM_{PZQ}$) is now recognised to be involved in the mode of action of PZQ with mutations in this target resulting in drug resistance. However, this was conducted in a single Brazilian *S. mansoni* laboratory-selected resistant strain; it is unclear if this mechanism will be conserved in African *S. mansoni* populations, in which little is known about the extent of genetic variation of $SmTRPM_{PZQ}$ in natural *Schistosoma* populations. This project aims to analyse natural Ugandan *S. mansoni* populations (pre- and post-PZQ treatment) to identify polymorphisms associated with drug resistance. We sequenced the whole genomes of single *S. mansoni* miracidia from a clinical trial in Lake Albert, Uganda. We developed a high throughput amplicon deep sequencing method to screen genomic regions encoding the PZQ binding site to identify PZQ resistance polymorphisms. Overall, our preliminary baseline (pre-treatment) data suggests that there is limited genetic diversity within the PZQ binding site in $SmTRPM_{PZQ}$. Our data will provide valuable insights into the genetic variation $SmTRPM_{PZQ}$ in field collected schistosome populations and suggest how this may affect treatment efficacy. Monitoring for changes in the frequency of PZQ resistance mutations will reveal how natural *S. mansoni* populations are evolving in response to PZQ treatment.

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Utilisation of 'Omics' to unmask the interactions of adult male and female *Schistosoma mansoni* with their host

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Introduction: *Schistosoma* spp. are gonochoristic trematodes that cause one of the most devastating worm parasitoses in the world. To date, there is no effective vaccine to protect against the infection, and drug treatment with praziquantel has limited success in endemic areas. In the search for new therapeutic approaches to combat this disease, adult female schistosomes have so far been neglected because they have little direct contact with the environment and thus with the host during their mating with male worms.

Objectives: Using "Omic" technologies, we aim to study the interactions between adult male and female *Schistosoma mansoni* and their host to find targets for new therapeutic strategies.

Materials & methods: First, we studied the structure of the tegument of male and female unpaired and paired schistosomes after contact with human serum as the host interface. Comparative analyses were performed by electron microscopy and immunohistochemistry. In addition, the tegument proteome of male and female unpaired and paired schistosomes was examined using a novel and highly sensitive workflow by LC-MS/MS analysis. In addition, the effect of circulating antigens from male and female schistosomes on the host immune response was investigated. For this purpose, the transcriptome and immune cell populations in the spleens of unisexually infected mice were analyzed.

Results: After incubation in human serum, adult male and female schistosomes exhibited marked surface enlargement, and females showed shedding of their outer surface. Using proteomic analyses, we identified 1519 tegument proteins for male and female unpaired and paired schistosomes. We identified more proteins in male than in female worms, regardless of whether they were derived from mating or from unisexual infection. For transcriptomic analyses, 22,207 transcripts were examined in the spleens of unisexually infected mice. Principal component analysis showed clear clustering of experimental groups. Our studies suggest that male and female *S. mansoni* elicit an egg-independent, non-polarized Th1/Th2 immune response in the host, with males having a significantly greater immunoregulatory influence on gene regulation in the spleen and thus on the host.

Conclusion: Our initial preliminary findings need to be investigated to discover and understand new metabolic pathways and immunomodulatory mechanisms of adult worms to contribute to the development of new therapeutic strategies against schistosomiasis.

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Macrophages release extracellular traps against *Strongyloides stercoralis* larvae via nuclear envelope budding

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Macrophage extracellular traps（METs） are DNA-based fibrous structures decorated with cytoplasmic proteins. Various researches have indicated that METs play a vital role in immune elimination of pathogens. *Strongyloides stercoralis* is a parasitic nematode infecting humans and dogs by its skin-penetrating infective 3rd stage larvae (iL3s). Macrophages are essential for killing iL3s, and this process is dependent on METs. However, the mechanism of iL3s inducing MET formation is still unknown. In the present study, we found that iL3s of *S. stercoralis* induced METs both in mice peritoneal macrophages and Raw264.7 macrophage cell line. These METs showed typical morphological characteristics with DNA frameworks decorated with histone 3 (H3) and myeloperoxidase (MPO). Interestingly, the process of MET formation was not dependent on NADPH oxidase, MPO, elastase or Ca^{2+}, which was distinctive from neutrophil extracellular trap formation. Within 30 minutes of iL3s stimulation, we observed the nuclear envelope budding under transmission electron microscope and detected a rapid DNA release from Raw264.7 macrophages by dsDNA quantitative assay. Since inhibition of transcription by Actinomycin D failed to attenuate MET formation, protein phosphorylation/dephosphorylation, a fast post-translational modification, was speculated to be involved in this rapid process. We quantitatively profiled phospho-proteomes of non-stimulated and iL3s stimulated Raw264.7 macrophages, which revealed that 65.81% differentially phosphorylated proteins were present in nucleus of Raw264.7 macrophages. KOG functional classification and GO functional enrichment suggested that chromatin structure and dynamics, cytoskeleton, and MAPK signaling transduction may be involved in MET formation. We demonstrated by pharmacological inhibition that F-actin depolymerization and histone acetylation are required for iL3s induced MET formation. This study reveals the characteristics and mechanisms of MET formation induced by *S. stercoralis* iL3s, expanding our understanding of innate immunity against parasitic nematode infections.

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The impact of co-infection on host resistance and tolerance differs in mice of different genotypes

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To combat infection, hosts develop two defence strategies: resistance and tolerance. Both strategies are known to be at least partly genetically controlled. To date, the relationship between resistance and tolerance has only been studied in the context of a single infection. The aim of the study was to characterise the phenotypic variation of host resistance and tolerance to *Heligmosomoides polygyrus* in genetically diverse mice during co-infection (*H. polygyrus* and Theiler's murine encephalomyelitis virus, TMEV). Three strains of mice were used: SJL mice (resistant to *H. polygyrus* but susceptible to TMEV), BALB/c mice (susceptible to both pathogens*)*, and C57BL/6 (susceptible to *H. polygyrus* but resistant to TMEV). Mice of each strain were infected with a single pathogen challenge (*H. polygyrus*) or double pathogen challenge (*H. polygyrus* and TMEV) or were sham infected (*n*=15). Both pathogens were administered at the subclinical level (200 L_3 *H. polygyrus* in 0.2ml water and an avirulent TMEV at 10^6 pfu in 0.2 ml DMEM). Mice were euthanised at 14 dpi and 42 dpi to represent nematode establishment period and nematode clearance period in the resistant genotype. When compared to parasitised only C57BL/6 mice, co-infected (CI) C57BL/6 mice had lower FEC (P<0.001), EIC (P<0.001), and worm counts (P<0.05), whereas CI BALB/c mice showed elevated FEC (P<0.001), EIC (P<0.05) and worm counts (P<0.05). CI did not have any impact on SJL mice. CI C57BL/6 had lower parasite burden compared to *H.polygyrus* only infected counterparts at later period. On the contrary CI penalised parasite clearance in BALB/c, with CI mice having high parasite burden compared to *H. polygyrus* only challenged mice. An analysis of covariance (ANCOVA) was used to assess genetic and treatment variation in tolerance. The tolerance was measured using two different fitness traits: performance (carcass weight) and indirect immune response (spleen/carcass ratio); whereas total worms (direct) and total EIC (indirect) were used as pathogen load measurement. Compared to parasitised only SJL mice, CI SJL mice were more tolerant (P=0.05). CI C57BL/6 mice tended to be more tolerant whereas CI BALB/c mice tended to be less tolerant than their parasitised only counterparts. We have shown significant variation in the impact of co-infection on host resistance and tolerance to *H.polygyrus* among three inbred mouse strains. The underlying mechanisms are elucidated.

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Plant-based production of protective vaccine antigen against the bovine abomasal parasite *Ostertagia ostertagi.*

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Gastro-intestinal nematode infections pose a persistent threat to livestock health, welfare, and farm productivity. The conventional approach to control these infections involves the periodic use of anthelmintic drugs, but the increasing prevalence of anthelmintic resistance renders this approach unsustainable in the long term. With limited new drug compounds in the pipeline, immunological control of nematode infections through vaccination has been explored over the years and is considered promising with regards to sustainability and cost-effectiveness. However, progress in this area has been disappointing, especially with recombinant produced antigens. The reason for failure is often attributed to the inability of the expression systems to reconstitute the native antigens in terms of protein folding and post-translational modifications, such as N-glycosylation. Research on an experimental vaccine against the bovine abomasal parasite *Ostertagia ostertagi* recently indicated that the N-linked glycans on the native antigen were important in antibody recognition, in particular the presence of a core α1,3-fucose residue. By adapting the post-translational machinery of *Nicotiana benthamiana*, it was possible to produce recombinant glycoforms of the *O. ostertagi* ASP carrying the same hybrid-type glycans as the native version, including core fucose. ELISA assays performed with serum from calves vaccinated with the native ASP showed a higher affinity for the *N. benthamiana* versions of the antigen compared to a non-protective recombinant version produced in *Pichia pastoris*. Subsequent vaccination and challenge studies in cattle with the newly expressed recombinants resulted in a significant reduction (39%) of faecal egg output. These findings are highly promising for the field of anti-nematode vaccine development, as the transition from native to recombinant subunit vaccines for various parasitic nematodes has been difficult.