Parasitic Helminths: New Perspectives in Biology and Infection

ABSTRACTS

Oral Presentations in order of appearance
Sessions 1–12
29 August - 1 September

BRATSERA HOTEL, HYDRA, GREECE

28 August to 2 September 2022
## Parasitic Helminths - New Perspectives in Biology and Infection

28 August - 2 September 2022, Hydra, Greece

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<td>Meta Roestenberg</td>
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<td>Francesco Vacca</td>
<td>Tess Renahan</td>
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<td>Mona Suleiman</td>
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<td>10:00</td>
<td>Tatiana Küster</td>
<td>Marta Campillo Poveda</td>
<td>Matthew Darby</td>
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<td>10:20</td>
<td>Amy Pedersen</td>
<td>Danielle Karo-Atar</td>
<td>Maaike Scheenstra</td>
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<td>Andrew MacDonald</td>
<td>Alex Loukas</td>
<td>Andy Fraser</td>
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<td>William Hornsnell</td>
<td>Roland Ruscher</td>
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<td>Benjamin Dewals</td>
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<td>12:10</td>
<td>Gyaviira Nkurunungi</td>
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<td>Hermelijn Smits</td>
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<td>Teresa Attenborough</td>
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<td>Jianbin Wang</td>
<td>Adrian Streit</td>
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<td>Frederike Sonnet</td>
<td>Gabriel Rinaldi</td>
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Complete immunophenotyping of one year controlled human hookworm infection

FRANCESCO VACCA1, BRITTANY LEWER1, SOPHIA-LOUISE NOBLE2, KATE MACLEAN1, JOHN MAMUM1, BIBEK YUMNAM2, TAMA KAWA1, TOM MULES1, ALISSA CAIT1, SAM OLD1, LAURA FERRER-FONT1, GRAHAM LE GROS1, STEPHEN INNS2, MALI CAMBERIS1.

1 MALAGHAN INSTITUTE OF MEDICAL RESEARCH, WELLINGTON 6012, NEW ZEALAND. 2 DEPARTMENT OF MEDICINE, OTAGO UNIVERSITY, WELLINGTON 6242, NEW ZEALAND.

The prevalence of allergic and autoimmune disorders is often noted to negatively correlate with chronic helminth infection in a population. Helminth survival in the host is predicated on their need to modulate the host immune system to establish a chronic infection. This ability to modulate host immune responses appears in some experimental models to suppress inflammation at distal tissues from the site of infection. This evidence has been used as a rational for trialing helminths such as Necator americanus for the treatment of autoimmune and inflammatory disorders in humans. However, these clinical intervention studies are difficult to develop due to the lack of sufficient knowledge of the immunological interplay between human host and parasite in a controlled infection setting. In this study, healthy volunteers were recruited and infected with N. americanus. Infection was monitored for one year. Immune responses were studied in peripheral blood and serum, and microbiome was analysed from stool samples. All participants were successfully infected as demonstrated by detectable eggs in the feces and adult worms in the intestine. High dimensional analysis of PBMC, inflammatory cell subsets and cytokines was carried out and compared to symptom scores throughout the one-year hosting period. Changes to the microbiome were evaluated through faecal sampling and pill cam and smarty pill analysis of gut physiology and worm behaviour in the gut recorded. The presented data of detailed effects of a controlled hookworm infection in healthy volunteers provides a critical platform for investigating the immunomodulatory properties in patients suffering from chronic inflammatory diseases.

Heartworm disease – Overview, intervention, and industry perspective

TATIANA KÜSTER1, SANDRA NOACK1, JOHN HARRINGTON2, DOUGLAS S. CARITHERS3, RONALD KAMINSKY2, AND PAUL M. SELZER1

1BOEHRINGER INGELHEIM ANIMAL HEALTH, BINGER STR. 173, 55216, INGELHEIM AM RHEIN, GERMANY 2BOEHRINGER INGELHEIM ANIMAL HEALTH, 1730 OLYMPIC DRIVE, 30601, ATHENS, GA, USA 3BOEHRINGER INGELHEIM ANIMAL HEALTH, 3239 SATELLITE BLVD, 30096, DULUTH, GA, USA 4 PARAC CONSULTING, ALTENSTEIN 13, 79685, HAG-EHRSBERG, GERMANY

Dirofilaria immitis (heartworm), the causative agent of dirofilariasis, is a major parasitic threat for dogs and cats around the world. In dogs, the disease is caused by young adult and adult parasites, which are present in the pulmonary arteries approximately 6 months after infection by a mosquito vector. Damage to the pulmonary endothelium and vascular occlusion reduce cardiac output and cause pulmonary hypertension, progressing to right-side heart enlargement and failure. Cats are atypical hosts, presenting more severe symptoms and being often underdiagnosed. Because of its impact on the health and welfare of companion animals, heartworm disease is of huge veterinary and economic importance especially in North America, Europe, Asia, and Australia. Very few products are approved for adulticidal treatments, and the market is dominated by preventive products, all of which contain active components of the same drug class, the macrocyclic lactones (MLs). MLs are potent allosteric agonists of nematode glutamate gated chloride channels, but the treatment concentrations required for phenotypic effects observed in vitro do not reflect the extrapolated tissue concentrations of these compounds in vivo. Studies have shown that MLs halt the cellular process that mediates secretion of excretory/secretory products (ES), from the parasite’s excretory/secretory pore into the host environment. That leads to the parasite’s loss of ability to manipulate the host’s immune response and results in the neutralization of the parasite. ML resistant D. immitis populations have however been reported, creating the need for innovative approaches in searching for drugs affecting filarial targets, such as anti-Wolbachia compounds, which have an indirect but lethal effect on D. immitis. The time and technology now seem also ripe for the development of next-generation vaccines. Ideal novel products should be sustainable and highly effective and should possess a convenient route of administration to encourage owner compliance.
A lab-to-wild rodent model of helminth infection and immunity

Amy B. Pedersen
University of Edinburgh; Edinburgh, UK

Despite great concern about the global health threat of infectious diseases in humans and domestic animals, we still don't have a clear understanding about how ecological heterogeneity determines infection burdens, disease, transmission, or how to successfully control infections in variable populations. Our reliance on highly controlled, laboratory models may underlie some of our failures to adequately manage disease burdens in real-world settings, where individuals compete for food, mates and space; endure seasonal and spatial environmental variability; and are exposed to a vast array of parasites and pathogens. We have established a hybrid wild/laboratory rodent model system in order to investigate the causes and consequences of this ecological heterogeneity for host-parasite interactions. Specifically, this work focuses on *Heligmosoides polygyrus* and its natural host, the wood mice (*Apodemus sylvaticus*), and addresses the following questions: (i) what determines susceptibility and resistance to *H. polygyrus* in the wild? (ii) is process of infection with a host different between *H. polygyrus* and *H. bakeri*, and (iii) how does nutritional availability and coinfection with other gastrointestinal parasites impact *H. polygyrus* infection and immunity. Our results highlight how pairing both the lab and natural setting provides a unique and powerful opportunity to understand the causes and consequences of ecological heterogeneity on infection, immunity and disease control.

Inflammation at the interface: mucosal immune responses during *Schistosoma mansoni* infection

Andrew S. MacDonald
Lydia Becker Institute of Immunology and Inflammation, University of Manchester, Manchester, United Kingdom

During mammalian infection with *Schistosoma mansoni*, parasites migrate through the lung vasculature before maturation in the mesenteric vessels, where they reach patency and begin to produce eggs. Many of these eggs transit from the mesenteric blood vessels, rupturing across the intestinal wall and into the lumen. Remarkably, even though this process causes chronic tissue damage, it does not lead to sepsis in immunocompetent hosts. Thus, schistosomes have evolved potent strategies to promote ‘regulated’ mucosal inflammation to ensure host survival in the face of larval migration and egg transit, and chronic tissue damage. However, pulmonary and intestinal immune responses against schistosomes have so far been poorly defined. We have begun to characterise schistosome-induced mucosal responses, focussing on the immune features that dominate in the lungs and intestines at different stages of infection, and interplay between the parasite and the host microbiota. Our data are beginning to reveal how immunity and tissue repair are regulated in these sites during infection, information that may aid future design of therapies against schistosomiasis, or other mucosal inflammatory diseases.
Maternal type 2 immunity inherently protects offspring from helminth infection

Malika Gabier1, A’ishaah Taliep1, Matthew Darby1, Pia Vornewald4, Jamie Pillaye2, Alisha Chetty3, Benjamin Dewals3, Adam Cunningham2, Menno Oudhoff4, William Horsnell1-2

1: Institute of Infectious Disease and Molecular Medicine and Division of Immunology, University of Cape Town 7925
2: Medical Research Council Centre for Immune Regulation, School of Immunity and Infection & Institute of Microbiology and Infection, University of Birmingham, B15 2TT Birmingham, UK
3: Fundamental and Applied Research in Animals and Health (FARAH), Immunology-Vaccinology, Faculty of Veterinary Medicine (B43b), University of Liège, Belgium.
4: Centre of Molecular Inflammation Research (CEMIR), NTNU, Trondheim, Norway

A clear immune feature of pregnancy/early life is a type 2 immune signature. This has been known for some time but its role in maternal transfer of immunity to offspring is not well understood. We have addressed how this feature of early life is driven by nursing and tested its influence on offspring immunity. To achieve this we have initially nursed wildtype offspring on either wildtype or type 2 deficient mothers (IL-4Ra−/−).

Our findings to date show offspring immunity being influenced by nursing dependent type 2 promoted maternal offsprin9 microchimerism. We have found that offspring nursed on Type 2 immune deficient mothers acquire fewer maternal cells. These offspring subsequently display impaired type 2 immunity and delayed resolution of Nippostrongylus brasiliensis infection.

We have also identified nursing to direct development of type 2 associated intestinal epithelial cells that can promote resolution of helminth infection and development of type 2 immunity. For example offspring nursed on type 2 deficient mothers displayed reduced numbers of goblet cells and have altered intestinal architecture.

These findings identify a fundamental influence of transfer of Type 2 immunity via nursing on both haematopoietic and non-haematopoietic control of infection.

CD22: a specific surface marker of IL-4-dependent virtual memory CD8+ T cells during helminth infection

YANG BIN1, LAVERGNE ARNAUD2, BAI QIANG2, MARICHAL THOMAS2, NITSCHKE LARS3, DEWALS BENJAMIN G1

1FUNDAMENTAL AND APPLIED RESEARCH IN ANIMALS AND HEALTH (FARAH), IMMUNOLOGY-VACCINOLOGY, FACULTY OF VETERINARY MEDICINE (B43B), UNIVERSITY OF LIÈGE, LIÈGE, BELGIUM, 2LABORATORY OF IMMUNOPHYSIOLOGY, GIGA INSTITUTE, ULÎÈGE, LIÈGE, BELGIUM, 3 DIVISION OF GENETICS, DEPARTMENT OF BIOLOGY, UNIVERSITY OF ERLANGEN, 91058 ERLANGEN, GERMANY

Helminth infection can modulate the immune response to concurrent infections. Recent work from the laboratory has demonstrated that IL-4 during helminth infection could expand CD49dlowCD44high virtual memory CD8 T cells (TVM), which are foreign antigen (Ag)-inexperienced with a memory-like phenotype. Helminth induced expansion of TVM led to a subsequent raised Ag-specific CD8 T cell activation that enhanced control of viral infection. Here, we have further investigated how IL-4 regulates TVM cell expansion via single-cell RNA sequencing of peripheral CD8+ T cells. We have compared the heterogeneity of cellular gene signatures of CD8 T cells after IL-4-complex (IL-4c) treatment or infection with the helminth Heligmosomoides polygyrus. Using mice conditionally deficient for IL-4Ra in peripheral CD8+ T lymphocytes, we could identify a cluster of cells that upregulated signature genes of TVM cells in response to IL-4. Among upregulated genes, we identified Cd22 that encodes the inhibitory receptor sialic acid-binding immunoglobulin-type lectin 2 (siglec-2) and is described as largely restricted to B lymphocytes. CD22 expression could be detected by flow cytometry and was restricted to IL-4-responsive TVM cells in the spleen, mesenteric LN but also nondraining lymphoid tissues. In vivo IL-4c treatment did not induce CD22 expression in thymic T cells, nor in Ag-specific effector/memory T cells during viral infection. Moreover, while IL-4c-induced expansion of TVM cells was maintained over time, CD22 expression was however transient. In addition, in vivo IL-4c treatment upregulated IFNγ expression specifically in CD22+ TVM cells. Bulk RNAseq experiment further revealed that CD22+ TVM cells in response to IL-4c upregulate effector molecules such as Gzma, Gzmm, Ctl2a2a, Ccl4, Xcl1, as well as receptors Ccr2, Ccr5, Klra5, Klrc2, Klrd1, Entpd1 (CD39) or CD160, and proliferation marker Mki67. These data demonstrated that CD22 is a surface marker that further defines IL-4-induced TVM cells and is likely involved in the regulation of TVM responses during helminth infection.
Impact of helminth exposure and the rural-urban tropical environment on gut inflammation, pre-vaccination immunological profile and vaccine-specific responses

GYAVIIRA NKURUNUNGI1,2, MARION H KÖNIG3, LUDOVIKO ZIRIMENYA4, JACENT NASSUUNA1, AGNES NATUKUNDA2, SIMON P JOCHEMS2, MARIA YAZDANBAKHSH4, ALISON M ELLIOTT1,2*

1MRC/UVRI AND LSHTM UGANDA RESEARCH UNIT, ENTEBBE, UGANDA
2LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE, UNITED KINGDOM
3LEIDEN UNIVERSITY MEDICAL CENTER, THE NETHERLANDS
4HYGIENE AND TROPICAL MEDICINE, UNITED KINGDOM
*Equal contribution

Drivers of population differences in vaccine-specific immune responses are incompletely understood. Our trials in rural and urban Uganda seek to address the hypotheses that urban-rural differences in vaccine-specific responses are mediated to an important extent by differential helminth exposure, that parasites act partly via “trans-kingdom” effects including immune modulation resulting from gut microbial translocation; that these exposures impact vaccine response via the pre-vaccination immune profile. We show that circulating markers of gut microbial translocation (MT), such as LPS binding protein and intestinal fatty acid-binding protein, are significantly elevated in the Schistosoma mansoni (Sm)-endemic rural, compared to the low-helminth-exposure urban setting; and that in the rural setting, Sm infection increases, and intensive Sm treatment lowers, levels of plasma and faecal markers of gut MT/inflammation, particularly soluble CD14 and faecal calprotectin and occult blood. Utilising mass cytometry technology, we have also conducted immune system-wide phenotyping of peripheral blood mononuclear cells collected pre- and post-BCG vaccination. Comparing rural and urban, Sm untreated and treated participants, and pre- and post-vaccination timepoints, initial high-dimensional single-cell data analyses reveal distinct cellular distributions within all major immune cell lineages (CD4+, CD8+ and γδ T cells, B cells, myeloid cells and innate lymphoid cells [ILCs, NK]). Higher resolution embedding reveals sub-populations within these lineages whose expression frequencies differ between urban and rural settings, pre- and post-vaccination, and between Sm treated and untreated individuals. For example, pre-vaccination expression frequency of Th2-type CD4+ T cells and KLRG1+ effector memory CD8+ T cells is higher among rural versus urban individuals; NK and NKT cell subpopulations are higher among urban participants and expand post-BCG vaccination in both settings. For both gut inflammation and immunological cellular profiles, upcoming analyses will explore associations with responses to a portfolio of live parenteral (BCG, yellow fever), live oral (typhoid), virus-like particle (HPV) and toxoid (tetanus/diphtheria) vaccines.

Immunoregulation by schistosomes: a tail of secretions, microbiome and tissue responses

Hermelijn H. Smits
Dept of Parasitology, Leiden University Medical Center, The Netherlands.

Schistosomes are master regulators of the host immune response to prolong their own survival. As a side-effect, other inflammatory immune responses are dampened as well, possibly prevention the development of allergic or auto-immune responses. Indeed people living in rural endemic areas experience less allergies or auto-immunities, and animal models have demonstrated a causal link between chronic schistosome infections and reduced development of those inflammatory diseases. We aim to better understand the molecular mechanisms leading to these protective events and the regulatory calibration of the host immune system in order to translate these findings to preventative or curative strategies for chronic inflammatory disorders in the lung. Important aspects include: 1) how immune cell function is influenced during helminth infection, e.g. the development of regulatory cell populations or their exhaustion, in both mouse models and in humans; 2) the characterization of bio-active molecules, including extracellular vesicles, secreted by schistosomes and their effect on the host immune system as well as on structural cells within organs, e.g. the lung; 3) interaction with species or their metabolic products in the gut microbiome and the gut barrier function to elucidate the tripartite interaction between schistosomes, the microbiome and the host.
Meloidogyne hapla: A genetic model for parasitism.

DAVID BIRD1, DAHLIA NIELSEN2 and VALERIE WILLIAMSON2.

1BIOINFORMATICS RESEARCH CENTER, NC STATE UNIVERSITY, RALEIGH NC, USA
2DEPARTMENT OF PLANT PATHOLOGY, UNIVERSITY OF CALIFORNIA, DAVIS CA, USA

Root-knot nematode (RKN, Meloidogyne) is a serious pest of plants important to humans, and a growing research target. However, one tool, forward genetics, is conspicuously absent. This is in part because RKN reproduction across the genus includes sexual and asexual species, with facultative or obligatory mitotic or meiotic modes. We asked “what nematode genes influence expression of what genes in the host.” We have exploited some of the genetic complexities of RKN to build a genetic map of Meloidogyne hapla. In particular, driving reproduction into meiotic parthenogenesis (MP) permitted highly inbred wild isolates to be constructed. A sexual cross enabled recombination (crucial for genetics), followed by a subsequent round of MP to fix those primary recombination events. Each of the ~120 “F2” plant lines approximates a recombinant inbred line. To genotype the worm, we generated whole genome sequences for both parents, and identified SNPs which could differentiate each parent’s contribution to the line. SNPs were confirmed/rejected based on 2X109 RNASeq lanes. Several points are emerging. First, phenotypic variability in the parents is reflected in the diversity of the transcriptomes. Second, the behavior of genes of one host is typically mirrored in another host. Third, the M. hapla genome contains several pleiotropic loci able to modulate multiple plant genes. Although most of these genes currently remain only as map positions, some have been cloned to identity: none of these obviously encode known “effectors.” One M. hapla locus (hem-1), spanning 84kb of a well assembled contig on LG3, regulates expression of more than 150 plant genes. This locus encodes 15 predicted proteins, three of which are expressed in hem-1. These candidates also show extensive polymorphism between parents. These genes may function together, or independently, and conceivably may function in biosynthesis of a communication molecule.

A single-cell atlas of the free-living miracidium larva of Schistosoma mansoni

TERESA ATTENBOROUGH*1, KATE RAWLINSON*1, CARMEN LIDIA DIAZ SORIA2, JENNIE GRAHAM1, GEETHA SANKARANARAYAN1, GABRIEL RINALDI1, MATTHEW BERRIMAN1 (*These authors contributed equally)

1WELLCOME SANGER INSTITUTE, UNITED KINGDOM

Infection with parasitic flatworms of the genus Schistosoma affects millions of people annually. The one currently available drug, praziquantel, is crucial for disease treatment, but its efficacy has limitations, and resistance is a concern. Thus, novel treatment and control strategies are desperately needed. A key strategy to advance this goal is to develop a deeper understanding of the fundamental biology of the parasite. Schistosoma mansoni eggs hatch in fresh water, where the free-living miracidia emerge. These miracidia seek out suitable snail hosts, inside which they transform into mother sporocysts, before asexually producing large numbers of daughter sporocysts that generate human-infective cercariae to continue the life-cycle. In this project we seek to build a cell atlas of the miracidium, through single-cell sequencing and analysis. We have established that the miracidium is composed of ~365 cells, and have sequenced enough cells to achieve >10x theoretical cover of each cell. We have identified transcriptional profiles which indicate stem/germinal, muscle, protonephridia, neural, tegumental, and parenchymal cells, and are currently validating these findings via in-situ hybridisations. Within these cell types, we have detected subclusters of cells, which indicate functional heterogeneity, particularly within the neural cells. We have identified miracidia-specific cell types such as the ciliary plates that are key for swimming. Additionally, we have identified sex-specific transcriptional activity that is particularly striking in the stem cell populations of this sexually monomorphic developmental stage. Our focus on single-cell detail in the miracidium provides the foundation for understanding the cell types and transcriptomes that make up Schistosoma mansoni. Furthermore, identifying the cellular composition of this simple and short-lived larval stage will lead to a greater understanding of its infective behaviour that leads to the propagation of the life cycle.
Programmed DNA elimination (PDE) is a notable exception to the paradigm of genome integrity. PDE often occurs during early embryogenesis, where portions of genomic DNA are lost resulting in reduced somatic genomes. First discovered in the horse parasite *Parascaris*, PDE has since been found in single-cell ciliates and a variety of multicellular organisms across animal phyla. Currently, the parasitic nematodes ascarids represent the best known metazoan system to undergo PDE. Compare to the extensive genomic, cytological, and molecular studies, little is known about the mechanisms of PDE in ascarids, due to the difficulty in genetic manipulation and high cost in worm maintenance.

Here, we established a genetic model for PDE in the free-living nematode *Oscheius tipulae*. *O. tipulae* was suggested to eliminate DNA in a genomic study from The Tree of Life project at the Sanger Institute. Using staged embryos, we sequenced and confirmed that *O. tipulae* DNA elimination occurs during early embryogenesis similar to *Ascaris*. Like *Ascaris*, *O. tipulae* eliminates the ends of all chromosomes. However, in contrast to the seemingly sequence-independent break sites for *Ascaris*, we identified a conserved sequence motif for all 12 break sites in *O. tipulae*. Mutation of this motif leads to a “fail-to-eliminate” phenotype only at the modified sites. Our END-seq result reveals that *O. tipulae* breaks can occur at multiple sites within the motif, with the break regions showing extensive resection at both retained and eliminated ends, followed by telomere addition. Interestingly, alternative break sites exist in many wild *O. tipulae* isolates we sequenced, suggesting flexibility in the break sites and the sequence eliminated.

Overall, the compact genome, short life cycle, and amenability to genetic manipulations make *O. tipulae* an excellent comparative model to study the molecular mechanisms of PDE in nematodes, including many important parasites.

The immunomodulatory strategies of hookworms, including type 2 polarization and the induction of a regulatory network in relation to the migration of the hookworm through the host, are well studied in murine models. In humans, however, the dynamics of hookworm-induced immunomodulation remain largely unexplored. Controlled human hookworm infection studies allow for the investigation of synchronized, controlled infections over time and also the assessment of vaccine responses. We performed high-dimensional immunophenotyping of peripheral blood, assessing cellular phenotypes, activation states, and cytokine expression of hookworm-naïve individuals infected with *Necator americanus* (Na) who were followed up for two years. Thereafter, we used chemo-attenuated *Na* to induce immunity and tested efficacy in our developed model. Results of the immune profiling following controlled infection indicate a restructuring of the T cell compartment with a strong increase in specific clusters of regulatory T cells expressing CD38, CTLA4, PD-1, and ICOS. Further, a significant increase of plasmacytoid dendritic cells, basophils, natural killer T cells, and natural killer cells, paralleling the peaks in the production of both type 1 (IFNγ and TNF) and type 2 (IL-4/IL-5/IL-13) cytokines by CD4+ T cells was seen in primary infection. The chemo-attenuated *Na* induced some level of immunity, which correlated with the strong activation of certain immune cell clusters that will be presented. These novel data reveal detailed insights into the development of human type 2 and regulatory networks and the development of protection.
There is more than meets the eye in the multilayered interactions among microorganisms, and deconstructing these intricate relationships in an ecological context has proved strenuous. The necromenic nematode *Pristionchus pacificus* is a genetic and evolutionary model used to elucidate the ubiquitous insect-nematode-bacteria complex. Global *P. pacificus* has a facultative affiliation with myriad scarab beetles and relies on the microbial blooms decomposing the beetle carcasses as a food source, and often must compete with other worms joining the feast. Consequently, as worm population densities increase, resources can become scarce, though *P. pacificus* can respond dynamically by utilizing two polyphenisms: dauer and mouth form. While the developmentally arrested dauer larva enables escape from harsh environmental conditions, *P. pacificus* can also develop a predatory mouth form that expands the worms’ dietary range to include other nematodes in addition to bacteria. We have, for the first time, explored population density and dispersal of *P. pacificus* by performing experiments in the wild on expansive temporal and spatial scales. Beetles were collected, decapitated, and placed in mesh-met al cages that allow for free movement of nematodes while enabling tracking of their dispersion. We found that there are two major dispersal events of dauers from the host carcass that reflect bacterial abundance, but not bacterial type. Intriguingly, all post-dauers worms on the carcass were predators, in contrast to both wild worms that did not develop via dauer and domesticated worms that did develop via dauer. In laboratory studies, we have traced this effect to be partially due to a stage-specific pheromone that induces the predatory mouth form in a density-dependent manner. Yet, this does not fully explain the patterns observed in nature, and ongoing analyses include single worm transcriptomics of wild dauers. We continue to investigate the intricacies of tripartite relationships, particularly the ecological forces influencing plastic phenotypes.

The ability of intestinal helminths to establish long-term infections has been attributed, in part, to their secretion of immunomodulatory proteins acting upon host immune cell populations. However, less is known of their impact on the epithelial layer, which serves as the first point of contact and a critical barrier to infection, and also generates specialised secretory cell types such as tuft and goblet cells required for anti-helminth immunity. We noted that expansion of tuft cells was significantly more marked in mice infected with *Nippostrongylus brasiliensis*, which is expelled in 6-9 days, than in *Heligmosomoides polygyrus*-infected mice which tolerate parasites for many weeks. Moreover, mice harbouring *H. polygyrus* showed abated tuft cell responses both to *N. brasiliensis* infection or administration of the metabolite succinate. Using *in vitro* intestinal organoid (enteroid) cultures, we found that *H. polygyrus* infective (L3) larvae, and adult worm excretory-secretory (HES) products suppressed gene sets expressed by goblet, paneth, and tuft cells, while promoting factors associated with nonsecretory enterocyte cells. Furthermore, organoid morphology was drastically altered, with HES causing a spheroid, proliferative phenotype devoid of crypts and differentiated cells. *In vivo*, HES administration reduced tuft cell induction by succinate, replicating the effects of live infection. Thus, chronic infection with *H. polygyrus* may prevent the development of a crucial chemosensory cell for parasite defence, allowing the parasite to survive, and illustrate how the impact of helminth parasites extends beyond immune control in order to modify the intestinal environment to their advantage.

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**Bacteria and pheromones regulate predation and escape polyphenisms in insect-associated *Pristionchus* nematodes**

**Tess Renahan¹**, Wen-Sui Lo¹, Michael S. Werner², Ralf J. Sommer³ Department for Integrative Evolutionary Biology, Max Planck Institute for Biology Tübingen, Max-Planck Ring 9, Tübingen, 720976, Germany

²Department of Biological Sciences, University of Utah, 257 South 1400 East, Salt Lake City, UT, 84112

³Centre of Molecular Inflammation Research, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, Trondheim, Norway

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**Recasting of intestinal epithelial cell fate by *Heligmosomoides polygyrus* and its secreted products**

**Marta Campillo Poveda¹**, Claire Drurey¹, Hävard Lindholm², Gillian Coakley³, Nicola Harris³, Menno Oudhoff² and Rick Maizels¹

1. Wellcome Centre For Integrative Parasitology, Institute of Infection, Immunology and Inflammation, University of Glasgow, UK

2. Centre of Molecular Inflammation Research, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, Trondheim, Norway

3. Intestinal Immunology Laboratory, Department of Immunology and Pathology, Monash University, Melbourne, Australia
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 in Transit Amplifying cells that is critical for the 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 that also give rise to less secretory cells in the same villi. 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.
Diabetes is recognised as the world’s fastest growing chronic condition globally. Helminth infections have been shown to be associated with a lower prevalence of T2D, mainly due to their ability to induce a type 2 immune response. Therefore, to understand the molecular mechanisms that underlie the development of T2D-induced insulin resistance, we undertook a randomised placebo-controlled clinical trial to assess the impact of experimental Necator americanus infection on patients with metabolic syndrome over a 2-year period. Hookworm infection was well tolerated and induced peripheral blood eosinophilia, consistent with generation of a Type 2 immune response. Critically, while placebo-treated participants displayed stable measures of insulin resistance throughout, hookworm infection was associated with a significantly improved insulin sensitivity index (lowered HOMA-IR). To determine whether hookworms regulate insulin sensitivity by secreting bioactive protein, we treated mice fed on normal or diabetes-promoting diets with excretory/secretory products (ES) from infective larvae and adults of the rodent hookworm Nippostrongylus brasiliensis. Treatment with crude ES products from adult and L3 N. brasiliensis induced a type 2 immune response in adipose tissue and liver, and improved glucose tolerance and attenuated body weight gain in mice fed on a high glycemic index diet. To identify the specific biomolecules involved in this process, we expressed the recombinant N. americanus ES proteome in mammalian cells and in vitro screening for proteins with relevant bioactivities is underway. These data highlight a role for hookworms and their ES products in protecting against metabolic syndrome, and could shed light on new approaches to treating the global type 2 diabetes pandemic.

Parasitic helminth infections, while a major cause of neglected tropical disease burden, negatively correlate with the incidence of immune-mediated inflammatory conditions such as inflammatory bowel disease (IBD), which is difficult to treat, and globally on the rise. Helminths have developed sophisticated mechanisms to regulate their host’s immune responses. Controlled experimental human helminth infections have been assessed clinically for treating inflammatory conditions, however such a radical therapeutic modality has challenges. An alternative and safer approach is to harness the immunomodulatory properties within the worm’s excretory-secretory (ES) complement, or its secretome. Yet, an efficient strategy to distill the best drug leads from helminth secretomes is lacking. We therefore utilized a discovery and validation pipeline to screen a library of helmint-secreted proteins. Starting with 91 protein-coding genes obtained from the secretome of a gastrointestinal hookworm, we expressed recombinant proteins and screened them for anti-inflammatory properties in a mouse model of colitis. After statistical filtering and ranking of 20 protective hits, and validation studies that demonstrated ex vivo suppression of inflammatory cytokine secretion by T cells from human ulcerative colitis patient colon biopsies, we identified 3 lead candidates from distinct protein families that offer promise as novel, safe and mechanistically differentiated biologics for treating inflammatory diseases.
ES-62 is a major secreted protein of the rodent filarial nematode, *Acanthocheilonema viteae*. The molecule exists as a large tetramer (MW, ~240kD), which possesses immunomodulatory properties by virtue of multiple phosphorylcholine (PC) moieties attached to N-type glycans. By suppressing inflammatory immune responses, ES-62 can prevent disease development in certain mouse models of allergic and autoimmune conditions, including joint pathology in collagen-induced arthritis (CIA), a model of rheumatoid arthritis (RA). Such protection is associated with functional suppression of “pathogenic” hyper-responsive synovial fibroblasts (SFs), which exhibit an aggressive inflammatory and bone-damaging phenotype induced by their epigenetic rewiring in response to the inflammatory microenvironment of the arthritic joint. Critically, exposure to ES-62 in vivo induces a stably-imprinted CIA-SF phenotype that exhibits functional responses more typical of healthy, Naïve-SFs. Consistent with this, ES-62-na"rewiring" of SFs away from the hyper-responsive phenotype is associated with suppression of each of ERK activation, STAT3 activation and miR-155 upregulation, signals widely associated with SF pathogenesis. Surprisingly however, DNA methylome analysis of Naïve-, CIA- and ES-62-CIA-SF cohorts reveals that rather than simply preventing pathogenic rewiring of SFs, ES-62 induces further changes in DNA methylation under the inflammatory conditions pertaining in the inflamed joint, including targeting genes associated with ciogenesis, to program a novel “resolving” CIA-SF phenotype. Such unique behaviour signposts the potential for developing DNA methylation signatures predictive of pathogenesis and its resolution and hence, candidate mechanisms by which novel therapeutic interventions could prevent SFs from perpetuating joint inflammation and destruction in RA. Pertinent to these translational aspects of ES-62-behavior, novel synthetic small molecule analogues (SMAs) based on ES-62’s active PC-moiety mimic the rewiring of SFs as well as the protection against joint disease in CIA afforded by the parasitic worm product.
Heligmosoides polygyrus both amplifies and suppresses IL-33 responses

FLORENT COLOMB1, ADEFUNKE OGUNKANBI1, DANIELLE J SMYTH1, HENRY J MCSORLEY1
1SCHOOL OF LIFE SCIENCES, UNIVERSITY OF DUNDEE, UNITED KINGDOM

Heligmosoides polygyrus is an intestinal nematode with multiple immunomodulatory activities mediated by its secreted products. We previously identified HpARI, a secreted protein which blocks IL-33, inhibiting type 2 immune responses. HpARI mechanism of action is via dual binding to genomic DNA and directly to IL-33. This allows HpARI to tether IL-33 to genomic DNA within necrotic epithelial cells, and makes it an extremely effective inhibitor of IL-33.

We have since found that H. polygyrus secretes 3 HpARI homologues: HpARI1, HpARI2 and HpARI3. The “original” HpARI protein identified was the second-most abundantly expressed in parasite secretions, therefore was renamed HpARI2. While HpARI2 suppresses IL-33 responses in in vitro type 2 innate lymphoid cell assays, and in in vivo models of allergic asthma, HpARI3 does the opposite, amplifying IL-33-dependent responses in vitro and in vivo. HpARI3 lacks the DNA-binding activity of HpARI2, and has altered binding characteristics for IL-33 – this results in stabilisation of this labile cytokine, extending its half-life and amplifying responses to it. We are currently investigating the hypothesis that this IL-33-amplifying activity allows H. polygyrus to increase IL-33-dependent regulatory T cell responses at later stages of infection.

Selective activation of filarial DAF-12 by host serum to resume iL3 development during infection

Anne Lespine1, Anthony Emile1, Hua Che2, Roger Prichard2 and Rémy Bétous1
1INATHERES, Université de Toulouse, INRAE, ENVT, 31027 Toulouse Cedex 3, France. 2Institute of Parasitology, McGill University, Sainte-Anne-De-Bellevue H9X3V9, QC, Canada.
E-mail: remy.betous@inrae.fr

Filarial nematode parasites such as *Dirofilaria immitis* (*Dim*) and *Brugia Malayi* (*Bma*) enter their definitive host via the bite of an insect vector at the developmentally arrested iL3 stage. This step is critical for establishment of the infection and development to adulthood. Previously, we have shown that the nuclear receptor *Dim*DAF-12 is central for the development of *D. immitis* iL3. Here, we identified and cloned DAF-12 from *B. malayi* and found high sequence identity with *Dim*DAF-12. Interestingly, *Dim* and *Bma* DAF-12 share a remarkably higher sensitivity to their natural ligands such as Δ4-dafachronic acid (Δ4-DA) compared with DAF-12 from the non-filarial nematode species, *Haemonchus contortus* and *Caenorhabditis elegans*. *Dim* and *Bma* DAF-12 were specifically activated by mammalian sera. Remarkably, DAF-12 natural ligand Δ4-DA has been identified in mammalian sera at level above its EC50 for filarial DAF-12. Moreover, we showed that depletion of Δ4-DA from serum by charcoal stripping impairs significantly the development of *D. immitis* iL3 in vitro. Finally, analysis of RNAseq data from *B. malayi* revealed that, at the time of infection, the parasites do not seek to produce their own dafachronic acids. These observations suggest that filarial nematodes have evolved to switch-off their DA synthesis at the infective stage, in order to sense components of the mammalian host environment directly through DAF-12 to resume their development and open the route to novel therapies against filarial infections.
Strikingly, during the sexual reproduction in free-living Strongyloides spp. only female progeny arise. Assuming "normal" male meiosis one would expect that 50% of the sperm formed lack an X chromosome and hence, upon fertilizing oozytes, give rise to male progeny. We had shown earlier by quantitative whole genome sequencing that in Strongyloides spp. nullo-X sperm is absent (in S. papillosus) or underrepresented (in S. ratti) among mature sperm. To study how and when male determining sperm is eliminated we used DIC microscopy, immunohistochemistry, fluorescent in situ hybridization (FISH) and electron microscopy to characterize spermatogenesis in S. ratti, S. papillosus and Parastrongyloides trichosuri. We found the meiotic divisions to occur "normally", as expected for an organism with XO males leading to four spermatocytes, two with and two without an X chromosome. All four products of meiosis are of approximately equal size and we did not observe residual bodies, as they are known from C. elegans.

Further down in the gonad, where the sperm mature, we found most, if not all nuclei to contain an X chromosome and we observed structures that contained protein constituents of sperm, such as actin and major sperm protein (MSP) but no DNA. These structures are reminiscent of C. elegans residual bodies in appearance and may assume their function. We suggest that spermatocytes without an X chromosome undergo some form of programmed cell death and transform into these residual body-like structures. Like in C. elegans, MSP is found in fibrous body-membranous organelles (FB-MOs). However, the FB-MOs in Strongyloides spp. differ from the ones in C. elegans in size, number and appearance. Knocking down MSP by RNAi showed that MSP is essential for viability.

Functional genomics to understand the establishment of sexual dimorphism in schistosomes.

Gabriel Rinaldi, Teresa Attenborough, Carmen Diaz, Jennie Graham, Magda Lotkowska, Geetha Sankaranarayanan, Kate Rawlinson, Matthew Berriman
Wellcome Sanger Institute, United Kingdom

Schistosomes infect more than 220 million people worldwide, while more than 700 million are at risk of infection in endemic areas. There is only a single effective drug, and the threat of drug resistance is emerging. Cutting-edge genomic and molecular technologies will boost our understanding of schistosome biology, leading to development of novel drugs and vaccines. Unusual among parasitic flatworms, schistosomes have genetically-determined male and female individuals; however, most developmental stages of the parasite do not exhibit sexual dimorphism. The sexual dimorphism becomes only apparent by adulthood. Within the mammalian host males and females undergo separate but concurrent sexual differentiation in both their gonads and somatic tissues. This is a key but poorly understood process in the early intra-mammalian development of schistosomes. By using single cell transcriptomics we aim to accurately identify differentially expressed genes and cell populations between sexes in sexually-monomorphic parasites during infection and early interaction with the mammalian host. Gene perturbation strategies, including RNAi and CRISPR-Cas-based genome editing will be used to functionally characterise candidate genes involved in sexual dimorphism establishment. The establishment of sexual dimorphism was assessed by morphological analysis of individual parasites collected from mice at different time points post infection, followed by sex confirmation by PCR. In addition, single cell RNAseq was performed from single-sex cercariae, schistosomula, and lung-stage parasites using 10X Genomics Chromium platform and sequencing. Seurat was used to identify cell clusters. We showed that the sexual dimorphism is established in vivo from day-13 to day 17 post infection. Single cell transcriptomic analyses from female and male early intra-mammalian developmental stages to define a transcriptomic sexual dimorphism are currently ongoing. Novel genomic and molecular technologies, including single cell transcriptomics, in vitro long-term culture and gene perturbation mediated RNAi and genome editing will shine a light into long-standing questions on schistosome development.
Schistosomiasis is a neglected tropical disease caused by parasitic flatworms of the genus *Schistosoma*. These parasites claim more than 250,000 lives every year and cause chronic, debilitating symptoms in millions more. The morbidity associated with schistosomiasis is driven almost entirely by the female parasite’s massive egg output. The female parasite develops into a sexually mature, egg-laying state only upon pairing with a male parasite. This observation was first made over a century ago, yet there has been little insight into the molecular details of this unique characteristic of schistosome biology. We recently described a male-derived peptide pheromone that controls female schistosome development. In the male schistosome, a zinc-finger transcription factor named GLI1 induces the expression of an enzyme termed non-ribosomal peptide synthetase (SmNRPS). This enzyme makes a small peptide conjugate called β-alanyl tryptamine (BATT); this small molecule is secreted and acts on the female schistosome to induce sexual maturation and is sufficient to induce maturation in the absence of male parasites. Regarding the chemical nature of the BATT molecule, we hypothesize BATT to be a ligand for a G-protein coupled receptor (GPCR) responsible for the induction and maintenance of the female parasite’s mature state. We used bioinformatic methods to identify 163 putative GPCR genes expressed in the female schistosome. Using an RNAi-based screening approach, we have identified several genes involved in BATT-induced female sexual development and egg-laying. Further study of these genes will no doubt yield exciting new insights into the molecular basis of the male-induced sexual development of female schistosomes and aid advancement of pathology-targeted therapeutics.

*Heligmosomoides polygyrus* is a natural rodent parasite which infects the small intestine. Infection leads to an increase in activation and frequency of regulatory T cells (Tregs) which suppress effector mechanisms of immunity. Similarly, Treg responses are prominent in many human parasite infections, which are among the neglected tropical diseases afflicting approximately 1.5 billion people. Critically, little is known of the composition of the Treg response in helminth infection, and how they interact with T effector cell populations. In both humans and mice, host genetics influence susceptibility to infection, as also seen during *H. polygyrus* infection in laboratory mice whereby C57BL/6 mice are susceptible to infection whereas BALB/c mice are partially resistant. This difference in response may be key to understanding why some humans are more susceptible to helminth infection than others and allowing design of new interventions strategies for parasitic diseases. In our study, we are compared the CD3+ T cell populations of *H. polygyrus* infected and naïve BALB/c and C57BL/6 mice using single cell RNA sequencing. This will allow us to build a comprehensive profile of both CD4+ and CD8+ T cells, including Tregs and Th2 cells, at steady-state and in response to infection. From this understanding of the different cell subsets at the transcriptional and protein expression levels, we aim to define key determinants in the T cell population that may confer resistance versus susceptibility to helminth infection.
Platelets are small, anucleate cells which circulate in blood and respond rapidly to tissue damage and vascular inflammation caused by pathogens. They not only maintain tissue integrity and prevent haemorrhage, but also initiate and regulate a variety of immunologic responses. But little is known on the role of platelets in helminth infections, despite the fact that many helminth species are known to cause significant vascular pathology as they migrate through blood in various phases of their life cycle. Based on previous studies showing a tight association between platelets and innate immune responses during bacterial and viral pulmonary infection, we hypothesized that platelets significantly contribute toward acute immunity to helminth infections. We aimed to investigate the role of platelets in regulating pulmonary pathology and acute immune responses following infection with the murine gastro-intestinal nematode *Nippostrongylus brasiliensis* (Nb), commonly used to model human helminthiases. C57/B16 mice were infected with 500 L3 Nb, and the association of platelets with acute innate immune responses in the circulation and lung was confirmed by flow cytometry and immunohistochemistry. Subsequently, mice were depleted of their platelets using anti-CD41 antibodies prior to infection with Nb and the effect of this on pulmonary pathology and innate immune responses was established from flow cytometric and histologic analyses of pulmonary tissues. Lastly, antibodies were used to block specific platelet receptors during Nb infection to gain mechanistic insight into platelet regulation, particularly of neutrophil responses. Infection with Nb caused significant activation of platelets, their localisation into lung tissue and their interaction with innate immune cells. Additionally, platelet-immune cell interaction was associated with changes in the expression of factors known to play a role in driving the early immune response to Nb, including IFN-γ and RELM-α. Furthermore, mice depleted of their platelets prior to infection had significantly enhanced pulmonary pathology and rapidly succumbed to infection. This was associated with changes in neutrophil responses, and depletion of neutrophils together with platelets showed some protection against enhanced pathology caused by platelet depletion. Finally, direct and indirect targeting of the platelet receptors CD62P and CLEC-2 did not result in significantly enhanced pulmonary pathology as was found with anti-CD41 depletion, but these platelet receptors were associated with altered platelet and neutrophil responses. We have shown that platelets associate with protective host responses during acute Nb infection and that their absence correlates with a dysregulated neutrophil response and enhanced helminth–associated pulmonary pathology. This data therefore collectively shows that platelets play important roles in host survival and directing the acute innate immune response to Nb.
Asthma is a chronic inflammatory disease, leading to cough, wheeze, and shortness of breath. The prevalence has increased drastically in Westernized societies and is now increasing in low- and middle-income countries. It has been suggested that, among others, changes in lifestyle and microbial exposure at young age, including parasites, may play a role in the increased prevalence of childhood asthma. Chronic infection with the parasitic helminth, *Schistosoma* (*S.*) *mansoni* protects against allergic airway inflammation (AAI) in mice and is associated with reduced skin prick test positivity to inhaled allergens in humans. Even treatment with *S. mansoni* eggs only, prior to OVA/alum induced AAI, led to significantly reduced AAI.

We now show that peritoneal administration of a single glycoprotein T2-RNase Omega-1, secreted by *S. mansoni* eggs, reduced allergic airway responses upon allergen challenge. This reduction seems independent from the induction of regulatory cell populations in the lungs or draining lymph nodes. To deduce the mechanism of Omega-1-induced protection, we examined the cellular response to Omega-1 in the peritoneal cavity and its effect on the processing and trafficking of subsequent OVA.

Omega-1 is taken up in the peritoneal cavity by dendritic cells (DC), expressing the mannose receptor (CD206), and by peritoneal macrophages, irrespective of their mannose receptor expression, and reduces MHC-II expression on both cell types. In addition, pre-treatment with Omega-1 impairs DC migration to the draining mediastinal lymph nodes, leading to reduced numbers of OVA-positive cDC2 and moDCs in the draining lymph nodes, while halting OVA-positive DC in the peritoneal cavity leading to their accumulation. Omega-1 did not negatively affect antigen presentation by CD206+cDC2; even increased this as shown by increased OVA-DQ levels compared to untreated animals. The activity of Omega-1 is dependent on its RNAse activity, since the RNAse mutant form of Omega-1 was unable to reduce AAI nor affect DC migration. Altogether, the immunomodulatory effects of omega-1 affect the migratory capacity of moDCs to the draining mediastinal lymph nodes in mice, explaining its capacity to prevent allergic airway inflammation in an OVA/Alum model.
Many soil-transmitted helminths survive for long periods in the highly hypoxic environment of the host gut. To do so, they use an unusual form of anaerobic metabolism that relies on the electron carrier rhodoquinone (RQ). RQ is highly related to ubiquinone (UQ) but it is not made or used by the host. RQ synthesis and RQ-dependent metabolism are thus excellent targets for anthelmintics. Over the last 5 years we have been using C. elegans to dissect the pathway for RQ synthesis. We showed that RQ synthesis requires precursors that are generated from tryptophan by the kynurenine pathway and that the key decision to switch from UQ to RQ synthesis is governed by a single alternative splicing event.

More recently we have been using C. elegans to screen for drugs that specifically affect RQ synthesis and RQ-dependent metabolism. We have been doing this in two complementary ways. The first is in vivo screens in C. elegans — we have screened 25k natural products and 40k synthetic compounds and identified several families of compounds that block RQ-dependent metabolism. These include a set of species-selective Complex I inhibitors as well as inhibitors of distinct enzymes in the kynurenine pathway and we will present these data. In parallel to our in vivo screens, we have been using in silico approaches to predict compounds that could act as species selective inhibitors of enzymes that are critical for RQ-dependent metabolism. We identified 4 enzymes that are required for RQ-dependent metabolism and that also have active sites that differ between the vertebrate hosts and the parasite. It should thus be possible to identify compounds that inhibit the parasite enzyme and not the host. We collaborated with Cyclic, a leading AI compounds screening company, to screen >3 billion compounds to identify compounds that tightly bind the parasite active site but not the host and identified clusters of highly related compounds as hits. We will present the results of both the in vivo and in silico screens at the meeting as well as the insights our C. elegans work have given us on the biology of RQ synthesis and use in parasitic helminths.

Plant-parasitic nematodes (PPNs) are a clear threat to global food security and their management is critical in optimizing crop yields. While effective in combatting PPN infestation, many commercial nematicides are facing increasing restrictions and bans due to poor phylum-selectivity. Towards developing safe and effective agricultural nematicides, we aim to identify and characterize novel small molecules with nematode-selective toxicity. To this end, we performed a screen in the model nematode Caenorhabditis elegans for molecular scaffolds that are metabolically bioactivated into a toxic product by cytochrome P450s (CYPs) within the worm. These molecules act as pro-nematicides and offer potential for nematode-selective activity due to the phylogenetic diversity of CYP enzymes. We identified numerous bioactivated molecular scaffolds featuring a disubstituted oxadiazole core structure. These hit scaffolds include the commercial nematicide Tioxazafen and a novel compound we have named Cyproside. Cyproside is broadly active in vitro across all nematode species tested and does not disrupt viability in the non-target systems examined to date. We tracked the metabolism of Cyproside using HPLC and found it to be robustly metabolized by C. elegans and diverse PPN species including Meloidogyne hapla, Ditylenchus dipsaci, and Pratylenchus penetrans. Cyproside is not metabolized in the non-target systems examined to date. Using chemical CYP inhibitors, we have found that Cyproside kills PPNs in a CYP-dependent manner, suggesting that CYP-catalyzed bioactivation is required for activity in PPNs. We conclude that the nematode-selective activity of Cyproside is likely achieved via nematode-specific metabolic conversion into a lethal product. Currently, we are further characterizing the Cyproside metabolites produced in C. elegans and PPN species using LC-MS/MS to better understand the metabolic bioactivation and potential mechanism-of-action of this novel selective nematicide.
Muscular movement, controlled by pentameric ligand-gated ion-channels (pLGICs) is a defining feature of animals and a major target for many anthelmintic drugs. New receptor classes, especially those unique to parasites, present interesting novel drug targets. Details of the mechanisms by which duplicate genes evolve to form new receptors and how subunit composition is regulated are not currently understood. We have identified a unique, novel receptor in C. elegans that arose by gene duplication of a likely homomeric ancestor to form a heteromeric receptor that responds to a different neurotransmitter. One subunit remains alpha-type while the other has lost the alpha-type YXCC motif. This provides an ideal system in which to investigate how the switch to a different neurotransmitter occurred as well as the mechanisms by which subunit organization within a receptor is regulated. Phylogenetic reconstruction identified the arctic nematode, Plectus sambesi with subunits of this new receptor that have only a few amino acid differences from the ancestral state. Characterizing this alongside C. elegans by electrophysiology of reconstituted receptors ex-vivo found that only one subunit from P. sambesi can form a homomeric receptor despite both having the alpha-type YXCC motif. Currents when both subunits are present are much more robust. C. elegans can only form a heteromeric channel. The P. sambesi receptor responds fully to the new ligand. Taken together this suggests that the switch to a new ligand occurred in the homomeric ancestor before gene duplication and that the switch from alpha- to non-alpha type was in response to selective pressure and is not dependent on the YXCC motif.

Mechanisms and molecules associated with the evasion of the immune response by parasitic helminths can be exploited for the treatment of type 2 immune response disorders. Key mediators in type 2 inflammatory diseases, particularly in therapy-resistant airway disease are bioactive metabolites of arachidonic acid (AA). Here, we identified an immune-modulatory glutamate dehydrogenase (GDH) enzyme in the larval extract of the helminth Heligmosomoides polygyrus (Hpb). We particularly assessed whether Hpb GDH regulates type 2 immune responses (i.e. allergy or anti-helmint immunity) by modulating immune cell metabolism. Effects of Hpb GDH on the metabolism of monocyte derived macrophages (MDM), were quantified by mediator profiling by LC-MS/MS (eicosanoids, TCA metabolites) and seahorse analysis. Furthermore, Hpb GDH treated MDM were subjected to RNA sequencing to assess effects on gene expression profile. For characterization of immune regulatory effects in vivo, mice were treated with Hpb GDH during house dust mite (HDM)-induced allergic airway inflammation or during infection with different parasites.In macrophages, Hpb GDH induced the production of prostanoids and 2-hydroxyglutarate (2-HG), which contributed to the suppression of pro-inflammatory cysteiny1 leukotrienes. Moreover, Hpb GDH treated MDM showed an induction of regulatory and type 2 suppressive genes, which partially depended on histone acetylation via p300 HAT. Treatment of mice with Hpb GDH attenuated allergic airway inflammation in mice, while the treatment during helminth infection results in a significant increase in worm burdens. Our findings thus suggest that helminthic GDH regulates type 2 immune responses by modulating the eicosanoid and TCA metabolite output as well as gene expression in macrophages. Thus, anti-inflammatory modulation of the macrophage metabolism by Hpb GDH may be translated into new immunomodulatory strategies for the treatment of inflammatory diseases.
Current chemotherapy against alveolar echinococcosis (AE) relies on albendazole (ABZ), which targets $\alpha$-tubulin of the causative agent, the metacestode of *Echinococcus multilocularis*. ABZ chemotherapy, however, is parasitostatic only, has to be given for prolonged time periods (often life-long), and is associated with adverse side effects. We show that ABZ primarily acts on differentiated *Echinococcus* cells ( tegument, muscle, nerve cells), whereas the parasite’s stem cell department, the only proliferative cell type, is largely resistant to ABZ treatment. This provides a cell biological explanation why ABZ chemotherapy of echinococcosis acts parasitostatic only. We also show that parasite stem cells specifically express a mammalian-type ($Y_{200}$) $\alpha$-tubulin, $\text{Tub}_2$, towards which ABZ has only little affinity. When heterologously expressed in *C. elegans*, $\text{Tub}_2$ complements a mutation in the neuron specific Tubb4 and shows much less sensitivity towards ABZ than $\text{Tub}_2$ with an F200Y mutation, indicating that Tyr200 is the critical residue in $\text{Tub}_2$, conferring ABZ resistance. We further show that triclabendazole (TCBZ), which is widely used for the control of fascioliasis, ideally complements ABZ through effective elimination of stem cells in *Echinococcus* metacestode vesicles and cell cultures at concentrations which do not affect cultured mammalian cells. When incubated in the presence of ABZ and TCBZ, metacestode vesicles showed a striking phenotype with complete detachment of parasite tissue from the laminated layer. Furthermore, cell preparations of such vesicles were incapable of proliferation and metacestode formation. These data have important implications on future chemotherapeutic strategies against echinococcosis and introduce combined chemotherapy with ABZ and TCBZ as a highly promising approach towards curative treatment of the disease.

### Shotgun metagenome sequencing of microbiome-humanised vs. wildtype rodents reveals likely opposing roles of intestinal bacteria in the pathophysiology of schistosomiasis mansoni

Alba Cortés1,2, John Martin3, Bruce A. Rosa3, Klara A. Stark1, Simon Clare4, Trevor Lawley5, Makedonka Mitreva3, Matthew Berriman2,4^& & Cinzia Cantacessi1,2***

1Department of Veterinary Medicine, University of Cambridge, Madingley Road CB3 0ES, United Kingdom
2Departament de Farmàcia, Tecnologia Farmacèutica i Parasitologia, Facultat de Farmàcia, Universitat de València, 46100, Burjassot, València, Spain
3Division of Infectious Diseases, Department of Medicine, Washington University School of Medicine, St. Louis, MO, United States
4Department of Medicine, University of Cambridge, Addenbrookes Hospital CB2 0QQ, United Kingdom
5Wellcome Trust Sanger Institute, Wellcome Genome Campus, Hinxton, United Kingdom

*Correspondence: matt.berriman@glasgow.ac.uk (MB), gr10@sanger.ac.uk (GR), cc779@cam.ac.uk (CC)

**Current address: Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, United Kingdom

^Equal contributions

The pathophysiology of schistosomiasis is mainly related to the inflammatory granulomatous response triggered by parasite eggs trapped in host tissues. Over the last few years, evidence has emerged that the host gut microbiota might be (at least partially) involved in the immunological cascade that culminates with the formation of schistosome egg-induced intestinal granulomas. In this study, we investigated the impact of *Schistosoma mansoni* (*Sm*) infection on the gut microbial composition and predicted function of microbiome-humanised mice and compared the findings with those obtained from schistosome-infected wild type mice. *S. mansoni* infection induced profound gut microbiome alterations in both rodent hosts. In spite of substantial differences in microbiome composition at baseline, selected pathways were consistently affected by parasite infection, which points towards a likely connection between *S. mansoni* infection and the host gut microbiome. Such pathways included enhanced production of tryptophan metabolites and butyrate, and subsequent activation of aryl hydrocarbon receptor (AhR) signaling and additional butyrate-regulated pathways, that might be involved in prevention of excessive injuries caused by migrating parasite eggs. Together, data from this and previous studies suggest that the host gut microbiome may play a dual role in the pathophysiology of schistosomiasis, where intestinal bacteria may contribute to egg-associated intestinal pathology while, in turn, protecting the intestinal epithelium from uncontrolled tissue damage.
Filarial nematodes have a major impact worldwide on human and animal health. We are interested in developing new strategies to reduce the burden of filarial disease by disrupting the parasite’s lifecycle in its intermediate host and vector. Towards this aim, we study the molecular interactions between *Dirofilaria immitis*, the agent of canine heartworm disease, and *Aedes aegypti* mosquitoes. The “Blackeye” strain of *A. aegypti*, is susceptible to *D. immitis* infection and supports the development of infectious third-stage larvae (iL3) capable of infecting another dog. Briefly, microfilariae ingested during blood feeding migrate to the Malpighian tubules and eventually develop to L3. L3 larvae exit the Malpighian tubules and migrate through the body cavity (hemocoel) to the proboscis. Despite being awash with immune components abundant in mosquito hemolymph, L3 can persist in the labial sheath of the proboscis for days until the mosquito takes another blood meal, at which time iL3 emerge onto the skin. We have recently shown that the mosquito immune response, if experimentally elevated, can block parasite development. Given that other parasitic nematodes have been shown to secrete proteins and small RNA molecules in excretory/secretory (ES) products to modulate vertebrate immunity, we hypothesize that *D. immitis* similarly uses ES products to manipulate mosquitoes. We have used label-free quantitative mass spectrometry of hemolymph isolated from mosquitoes with iL3 and detected several *D. immitis* proteins. Ongoing analysis suggests that iL3 may modulate the *A. aegypti* immune response since we also observe changes in hemolymph proteins in the same samples compared to controls that were fed with uninfected blood. We have begun to functionally characterize *D. immitis* proteins found in the hemolymph using an RNA-interference protocol developed by the Kimber laboratory to target filarial genes in mosquitoes. We hypothesize that, if any of the *D. immitis* proteins in the hemolymph are important immune modulators, we will detect an increase in *A. aegypti* immune gene expression following silencing and a decrease in iL3 persistence or emergence.

Small RNAs (sRNA) are short non-coding RNAs important for the regulation of gene expression via post-transcriptional gene silencing. The majority of sRNA research within nematodes has been carried out in the Clade V free-living model organism *Caenorhabditis elegans* which possess all three classes of sRNAs. Recent studies have shown that sRNA pathways are highly diverged in nematodes and *C. elegans* does not closely represent the sRNAs used by more distantly related nematodes, including parasitic species. For example, the PIWI pathway and PIWI-interacting RNAs (piRNAs) are involved in regulating and silencing transposable elements (TE) in most animals but have been lost in nematodes outside of the *C. elegans* group (Clade V). Little is therefore known about how nematodes outside of this clade regulate TEs in the absence of the PIWI pathway. Here, we investigated the role of sRNAs in the Clade IV parasitic nematode *Strongyloides ratti* by comparing two genetically identical adult stages (the parasitic female and free-living female). We identified putative small-interfering RNAs that are differentially expressed between the two adult stages. A parasite-associated class of 21-22 nucleotide long sRNAs with a uridine 5’-monophosphate (21-22Us) were predicted to regulate and target TE activity. The 21-22Us show striking resemblance to the 21U PIWI-interacting RNAs found in *C. elegans*, including an AT rich upstream sequence, overlapping loci and physical clustering in the genome. This is the first report of a piRNA-like sRNA class of 21-22Us that have been identified specifically in the PF of *Strongyloides* and in nematodes outside of Clade V.
Exploring miRNAs in *Wolbachia*-Host symbiosis

**DENIS VORONIN, BRENT EDWARDS, ELODIE GHEDIN**

SYSTEMS GENOMICS SECTION, LABORATORY OF PARASITIC DISEASES, NIAID, NIH, BETHESDA, USA

Human lymphatic filariasis and onchocerciasis are two of the world’s most debilitating and neglected tropical diseases. The filarial parasites, causative agents of these diseases, have evolved a mutualistic association with the endosymbiotic bacteria, *Wolbachia*, essential for worm development, reproduction, and survival. The mechanism for such dependency is being studied to explore the unique potential of using *Wolbachia* as a novel chemotherapeutic target against human filarial infections. Recent studies in arthropods revealed that to survive in their insect hosts, *Wolbachia* manipulate host microRNAs (miRNAs) to control host gene expression. We propose *Wolbachia* of filarial worms play a similar role to maintain a mutualistic relationship with their worm host. We compared the expression of Bm-miRNAs in worms treated with doxycycline (anti-*Wolbachia* agent) vs control. We identified 13 upregulated and 20 downregulated miRNAs in worms treated with doxycycline, as compared to controls, four of which are unique to *Brugia* with no homologues in other filaria. This observation suggests that these parasites could encode miRNAs specific to *Wolbachia*-harboring filarial worms and regulate functions not present in filarial worms that lack an endosymbiont (such as *L. loa*). We used miRNA-mediated interference to silence specific miRNAs highly expressed in adult female worms to study their role in *Wolbachia* and worm biology. The treatment with miRNA inhibitors significantly reduced *Wolbachia* numbers and induced apoptosis in treated worms, as compared to control worms treated with scrambled miRNA inhibitor. Using a miRNA target prediction algorithm (miRanda), we identified potential target genes and characterized their expression in *B. malayi* treated with specific miRNA inhibitors or doxycycline and controls. We will discuss regulatory mechanisms involved in the *Wolbachia*-filaria mutualistic relationship.

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

Youssef Hamway¹, Marcel Blommers², Kaspar Zimmermann², Cécile Häberli³, Shashank Kulkarni⁵, Susanna Skalicky⁶, Matthias Hackl⁶, Marjo Götte², Jennifer Keiser³, Clarissa Prazeres da Costa¹, Thomas Spangenberg⁷

¹Kamal Azzaoui Institute for Medical Microbiology, Immunology and Hygiene, Technical University of Munich, Munich, Germany; ²Saverna Therapeutics AG, Gewerbestrasse 24, Allschwil, Switzerland; ³Department of Medical Parasitology and Infection Biology, Swiss Tropical and Public Health Institute Socinstr. 57, CH-4051 Basel; ⁴University of Basel, Switzerland E; ⁵MD Serono Research & Development Institute, Inc. (a Business of Merck KGaA, Darmstadt, Germany), Billerica, Massachusetts 01821, United States; ⁶TAmiRNA GmbH, Leberstraße 20, 1110 Wien, Österreich; ⁷Global Health Institute of Merck, Ares Trading S.A., a subsidiary of Merck KGaA, Darmstadt, Switzerland

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 ¹⁹F-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.
Heligmosoides polygyrus bakeri (H. bakeri) is a gastrointestinal nematode that secretes diverse molecules including proteins, lipids, RNAs and extracellular vesicles (EVs) to modulate mouse (host) cells. The intestinal epithelium is both a key initiator and effector of anti-helminth immunity and previous work has demonstrated that H. bakeri excreted/secreted products (HES) directly modify epithelial cells. We aimed to characterize the interactions that adult H. bakeri makes with host intestinal epithelial cells, accounting for differences between apical versus basolateral polarity. The development of intestinal organoid cultures, which recapitulate the intestinal epithelium, has allowed better investigation of this barrier site in vitro. However, accessibility to the apical side of the epithelial barrier is challenging using traditional 3D organoid cultures. The apical and basolateral sides of the intestinal barrier have specialised biological roles, the apical side is the secretion site of mucus and antimicrobial peptides, creating a physical barrier that limits access to epithelial cells. The basolateral side of epithelial cells is packed with receptors, important for signalling with underlying stromal, immune and nerve cells. In order to mimic in vivo interactions between the luminal H. bakeri and the intestinal epithelium we developed methods for growing organoids in an open culture system that allows for localisation of H. bakeri, or its secreted products, to either the basolateral or apical side of the epithelium. Using this model, we found differences in gene expression changes when H. bakeri adult worms were apical or basolateral to the organoids, with basolateral worms inducing stronger transcriptional responses in the host epithelial cells. We observed downregulation of key genes related to host response to helminth infection (defensins and mucins), hormones (somatostatin & ghrelin) and tissue homeostasis (stemness).

Infection with the food-borne liver fluke Opisthorchis viverrini is the principal risk factor for cholangiocarcinoma (CCA) in the Mekong Basin countries of Thailand, Lao PDR, Vietnam, Myanmar and Cambodia. Using a novel model of CCA, involving infection of hamsters with gene-edited liver flukes and concurrent exposure to a dietary nitrosamine, we explored the role of the fluke granulin-like growth factor Ov-GRN-1 in malignancy. We produced programmed gene knockout flukes (ΔOv-grn-1) by delivery of a CRISPR/Cas9/gRNA system by electroporation. Genome sequencing confirmed Cas9-catalyzed mutations in the targeted gene, which was accompanied by rapid depletion of transcript and the cognate protein. Whereas Ov-grn-1 gene-edited parasites colonized the biliary tract and developed into adult flukes, less hepatobiliary tract disease manifested during chronic infection with ΔOv-grn-1 worms in comparison to hamsters infected with control parasites. Specifically, immunohistochemical analysis of thin sections of livers revealed markedly less periductal fibrosis surrounding the flukes and less liver fibrosis globally during infection with ΔOv-grn-1 genotype worms, minimal biliary epithelial cell proliferation, and markedly fewer mutations of TP53 in biliary epithelial cells. Moreover, fewer hamsters developed high-grade CCA when infected with the ΔOv-grn-1 flukes compared to controls. The clinically-relevant, pathophysiologial phenotype of the hepatobiliary tract confirmed a role for this secreted growth factor in malignancy and morbidity during chronic opisthorchiasis.
Saving diabetic feet from chronic wounds: developing a wound healing peptide inspired by the *Opisthorchis viverrini* fluke granulin growth factor.

MJ SMOUT¹, NL DALY¹, G ZHAO¹, P BRINDLEY², A LOUKAS¹.

¹ AUSTRALIAN INSTITUTE FOR TROPICAL HEALTH AND MEDICINE AND CENTRE FOR BIODISCOVERY & MOLECULAR DEVELOPMENT OF THERAPEUTICS, JAMES COOK UNIVERSITY, CAIRNS, QLD, AUSTRALIA; ² GEORGE WASHINGTON UNIVERSITY, WASHINGTON DC, USA

Parasitic worms are large, invasive pathogens. To combat the pathogenesis they induce, worms, such as *Opisthorchis viverrini*, evolved strategies to promote wound repair in infected hosts. While long term infection is harmful, we see potential to harness evolution by using the fluke products as a starting point to develop wound healing medicines of the new millennium. One fluke protein with therapeutic potential is the granulin growth factor that we have shown to promote wound healing in mice and cell culture. Inspired by this healing stimulation, we have developed a novel peptide based on granulin. This peptide shows potent wound healing activity and may be a useful weapon against chronic non-healing wounds, a growing problem for diabetics, smokers, and the elderly. These wounds cost the world $50 billion annually to treat and result in devastating consequences with ~10% patients requiring amputations (~1 million annually). To develop this peptide we have “improved on nature” by removing prolines that induce “structural kinks” and nearly doubled both the healing stimulation and production yields. To support commercial viability we have produced the peptide in the lab and with commercial suppliers, and shown by NMR spectroscopy and HPLC analyses that the products are identical. Both lab and commercially produced peptides significantly stimulate human fibroblast proliferation over a surprisingly wide 0.1-1000 nM range. Keratinocyte scratch assay healing is seen over a similar 0.01-100 nM range for both normal and high glucose “diabetic like” conditions. Human ex-vivo skin healing shows significant 35% healing stimulation with 200 nM peptide. Ongoing pig trial results will be discussed. We plan to develop this unique peptide product as a treatment to help heal the millions of patients worldwide in the war against diabetes and other chronic non-healing wounds.

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*Staphylococcus aureus* induces *Trichuris muris* egg hatching through rapid and asymmetric dissolution of the polar plug

AMICHA ROBERTSON¹,², MERCIEN VENZON¹,², XUHUI ZHENG², CHRIS PETZOLD², JOSPEH SALL³, MICHAEL CAMMER³, VICTOR TORRES², FENG-XIA LIANG³, KEN CADWELL¹,²

¹ KIMMEL CENTER FOR BIOLOGY AND MEDICINE AT THE SKIRBALL INSTITUTE, NEW YORK UNIVERSITY GROSSMAN SCHOOL OF MEDICINE, NEW YORK, NY, USA
² DEPARTMENT OF MICROBIOLOGY, NEW YORK UNIVERSITY GROSSMAN SCHOOL OF MEDICINE, NEW YORK, NY, USA
³ THE MICROSCOPY LABORATORY, NEW YORK UNIVERSITY GROSSMAN SCHOOL OF MEDICINE, NEW YORK, NY, USA

The life cycle of the murine intestinal parasite, *Trichuris muris*, begins with an egg hatching step in the caecum. Gram-negative members of the gut microbiota trigger this hatching process by attaching to the egg via fimbriae. However, previous studies and our data show that the non-fimbriated Gram-positive bacterial species, *Staphylococcus aureus*, can also trigger egg hatching *in vitro* through an unknown mechanism. Given the increasing evidence implicating *S. aureus* as an important variable within the human gut microbiota community, especially during childhood development, we used an *in vitro* hatching assay to investigate how *S. aureus* affects this critical stage of the helminth life cycle. We show that physical contact is necessary for hatching to take place. Scanning electron microscopy and confocal microscopy experiments show that this contact predominantly occurs at the polar regions of the egg. Furthermore, Serial Block Face – Scanning Electron Microscopy shows that under conditions that promote hatching there is a difference in electron density between the two polar plugs of the egg. We show that eggs exposed to higher concentrations of bacteria hatch at a faster rate and that under conditions with faster rates of hatching, there is a higher degree of association between bacteria and eggs. Furthermore, we also show that live bacterial cells need to be present in the mixture for hatching to occur and that active protein synthesis in *S. aureus* is important for hatching. These findings provide insight into a transkingdom interaction that might influence the development of intestinal helminth infections.
Tuft cells (TCs) are of major interest in mucosal immunology due to their proposed function in sensing changes in the gut lumen environment and initiating the Type-2 T-helper immune response to gastro-intestinal nematodes (GIN) in mouse models. Murine TCs have been characterised by immunohistochemistry (IHC) and single cell RNA-sequencing (scRNA-seq), providing insight into their function. Moreover, some of the genes involved in murine small intestinal (SI) TC effector function have been shown to be conserved in human TCs. However, less is known about TCs in other species and in other regions of the GIT. As anthelmintic resistance is becoming an increasing challenge for controlling livestock parasites, it is important to improve our understanding of host immune mechanisms to aid development of new control methods. We used antibodies to murine TC markers to define ovine TC dynamics in the abomasum (gastric stomach) following infection with important ovine GIN, Teladorsagia circumcincta and Haemonchus contortus. scRNA-seq was used to characterise gene expression of ovine abomasal cells following T. circumcincta infection. This demonstrated that murine genes involved in TC function are conserved in ovine abomasal TCs, e.g. genes involved in taste receptor signalling and eicosanoid biosynthesis. However, the surface receptor repertoire differed from murine SI TCs. Distinct sub-populations of ovine TCs were also identified, likely reflecting different stages of maturation. scRNA-seq was validated using RNAscope in situ hybridisation, which confirmed co-expression of TC genes identified by transcriptomics. For the first time, TCs have been identified in the ovine abomasum and shown to increase in number over the course of GIN infection, with a distinctive gene expression profile marking their differentiation. Identification of cell surface markers will aid understanding of the mechanisms by which TC respond to GIN challenge. Current studies are extending to ovine SI TCs following infection with the intestinal parasite Trichostrongylus colubriformis.
Helminth infection studies using mice have underpinned a revolution in how we understand macrophage biology during type 2 immune responses. Using the filarial nematode *Litomosoides sigmodontis*, which resides in the pleural space, we showed that alternatively activated serous cavity macrophages can proliferate in the tissues without requiring input from the bone marrow. That finding coincided with several seminal studies which demonstrated the important difference between macrophages of recent bone marrow origin and those which have acquired a tissue resident phenotype. However, these advances were largely conducted in only one strain of mice, the reference C57BL/6 strain. Further, with regard to *L. sigmodontis*, infection displays strain-specific outcome with C57BL/6 mice being relatively resistant to infection, killing worms by sequestration in granulomas at an earlier stage than in BALB/c mice where worms reach sexual maturity and produce microfilariae. Critically, these two strains also display remarkable differences in macrophage populations prior to parasite death. Here, we provide a comprehensive analysis of immune cells in the pleural cavity using both C57BL/6 and BALB/c mice. Unlike C57BL/6 mice, naïve tissue-resident pleural macrophages of BALB/c mice failed to fully implement the tissue residency program. Following infection with *L. sigmodontis* these pre-existing differences were accentuated with tissue-resident pleural macrophage expansion occurring in C57BL/6 but not BALB/c mice. While infection drove monocyte recruitment in both strains, only in C57BL/6 mice were monocytes able to efficiently integrate into the tissue-resident pool. Monocyte to macrophage conversion required both T cells and IL-4Rα signalling. Host genetics are therefore a key influence on tissue resident macrophage biology, and during nematode infection Th2 cells control the differentiation pathway of tissue resident macrophages.

**Vaccination of human participants with attenuated *Necator americanus* hookworm larvae and human challenge in Australia: a dose-finding study and randomised, placebo-controlled, phase 1 trial**


Clinical Tropical Medicine and Immunology and Infection Laboratory, QIMR Berghofer Medical Research Institute, Herston, QLD, Australia. Infectious Diseases Unit, Royal Brisbane and Women’s Hospital, Herston, QLD, Australia. Centre for Molecular Therapeutics, Australian Institute of Tropical Health and Medicine, James Cook University, Cairns, QLD, Australia.

Control of human hookworm infection would be greatly aided by the development of an effective human vaccine. A radiation attenuated hookworm larval vaccine has previously been successfully developed and commercialised for use in dogs. We replicated this animal model, developing an ultra-violet light attenuated *Necator americanus* vaccine for use in humans. In this work we reviewed the historical origins of experimental human hookworm infection, developed *N. americanus* larval culture methods, characterised the larvae and developed methods for UV attenuation, for use in a phase 1b clinical trial. We demonstrated that larval viability (assessed by thermally induced motility assay at day 14 post-harvest) was inversely associated with UV light exposure. We then performed a two-stage clinical trial. In stage 1, participants received derrmally applied larvae attenuated with either 700µJ or 1000µJ of UV light. We demonstrated that fewer larvae penetrated the skin when attenuated with 1000µJ than with 700µJ of UV light. In stage 2 we performed a phase 1b randomised, controlled, challenge study, comparing safety and tolerability, immune responses, and efficacy of vaccination with 2 doses of dermally applied larvae attenuated with 700µJ UV light to placebo (tabasco sauce). We demonstrated that attenuated larvae are safe and well tolerated. Vaccination was associated with the development of an antigen specific IgG response. Polymorphonuclear blood cells of vaccinated participants produced significantly greater levels of IFNγ, TNFα, IL-2, IL-4, and IL-5 than the PBMC of unvaccinated participants following stimulation with larval antigens. Following challenge with normal larvae, all participants developed patent infection. Significantly fewer larvae per g of faeces were recovered in the vaccine group than in the placebo group. This is the first published soil transmitted helminth human challenge model and the first trial exploring an attenuated hookworm vaccine in humans.
Nurture over Nature: past experience determines future responses to vaccination


[1] Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, UK,
[2] The Moredun Research Institute, Pentlands Science Park, UK,
[3] Roslin Technologies Limited, Roslin Innovation Centre, University of Edinburgh, UK
[4] Institute of Evolutionary Biology, University of Edinburgh, UK

Individuals vary in their responses to immunisation, but the underlying causes of such variation and what to do about it are poorly understood. As a consequence, how effective a immunisation campaigns will be in the target population is extremely hard to predict – especially for vaccines that induce partial immunity, as is typical for anti-helminth vaccines. Such unpredictability is even more concerning when it results in the failure of vaccine candidates when moving from controlled laboratory animal models to the real world during clinical trials. Therefore, predicting how individuals in their normal habitat are likely to respond to immunisation given their demographic, physiological, and immunological history has the potential to foster a step change in vaccine design and disease control. I will present work on our progress in moving from the current standard of descriptive post-hoc analysis of vaccine responses towards predicting (a) how an individual is likely to respond to immunisation and, (b) the likely impacts of interventions on parallel causes of variation in vaccine immunogenicity. To do so, we combine machine learning, pathway analysis, and structural causal modelling to identify the main correlates and causes of vaccination outcomes within complex transcriptomic and/or demographic data in sheep and in wood mice immunised against the parasitic nematode Teladorsagia circumcincta and diptheria toxoid, respectively. We identify which immune pathways predict anti-helminth immunity in sheep and how they vary according to age and vaccination, and show that a small set of demographic metrics can be sufficient to predict how individuals are likely to respond to vaccination in the wild.