1) The SUbventral-Gland master Regulator (SUGR) of nematode virulence

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Plant-parasitic nematodes are devastating pests that have evolved unique and intimate relationships with their host plants. At the core of these interactions are effectors, nematode secretory products enabling the colonisation of plant tissues. Here we identify and build the first conceptual model for effector regulation in any plant-parasitic nematode. In the beet cyst nematode *Heterodera schachtii,* plant signals within host roots (termed here effectostimulins) activate expression of the *subventral gland regulator1* (*sugr1)*. SUGR1, then, directly binds effector promoters and controls the expression of 297 genes, including 42 effectors expressed at the earliest stages of plant invasion. Concordantly, SUGR1-activated genes include half of all known cell wall degrading enzymes and several known virulence determinants. These effectors, in turn, likely lead to increased cell penetration, releasing yet more effectostimulins, and triggering a feedforward loop for host entry. Importantly, we demonstrate that blocking SUGR1 blocks parasitism and translate these findings to the SUGR1 homologue in *Heterodera glycines*: the number one pathogen of soybean. This suggests that the SUGR1 signalling cascade is a valuable target for crop protection and its discovery could, therefore, form the first step towards much-needed, novel approaches to nematode control targeting effector production. Nematodes also parasitise humans and other animals via the secretion of effectors. This context highlights immense potential impact: disrupting effector production could, in principle, be applied to the fields of human and veterinary medicine, or indeed any pathogen that secrets effectors.

2) Parasitic worms and mosquito immunity

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Mosquitoes serve as both vectors and hosts for filarial nematodes, parasitic worms that cause diseases like lymphatic filariasis. While different parasite species have specific development sites in the mosquito, they all produce infectious third-stage larvae (iL3) that reside in the mosquito's body cavity, surrounded by hemolymph. The mosquito's hemolymph is rich in immune proteins, including a complement-like pathway initiated by pattern recognition proteins and amplified by a cascade of serine proteases. This pathway can ultimately lyse or melanize bacterial, fungal, and protozoan pathogens. Remarkably, iL3 of *Brugia malayi* and *Dirofilaria immitis* can persist in the hemolymph for over 7 days without any loss in fitness, suggesting that the parasites may secrete proteins to evade immune attack. To investigate this hypothesis, proteomic analyses have been conducted on the hemolymph of *Aedes aegypti* mosquitoes carrying iL3 larvae of *B. malayi* or *D. immitis*. We identified 326 *B. malayi* proteins and 207 *D. immitis* proteins. We selected four promising candidates for further characterization as potential mosquito immune modulators: Serine Protease Inhibitors (SRPNs, Bm8446 and Di_g04753), a Galectin (Bm4227), and Venom Allergen-like Protein-1 (VAL-1, Bm4233). Ongoing experiments aim to silence these proteins in vivo using RNAi to understand their role in modulating mosquito immunity. It is anticipated that knocking down these proteins will reduce the survival and emergence success of iL3 from mosquitoes. We are also generating antibodies to localize these proteins in iL3 and identify the mosquito proteins they interact with. Understanding how parasitic worms evade mosquito immunity is crucial for developing new strategies to interrupt disease transmission and control these devastating parasitic infections.

3) Host: parasite immune interplay during *Schistosoma mansoni* infection

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Extracellular proteins produced by parasites are often key mediators of host:pathogen interactions but also the targets of protective host humoral responses. To identify new potential vaccine targets against schistosomiasis, we have compiled a library of ~150 recombinant proteins representing the entire extracellular regions of cell-surface and secreted proteins from the *Schistosoma mansoni* parasite. The protein library was produced in mammalian cells to promote correct folding and biochemical activity of the proteins through the addition of essential post-translational modifications such as disulfide bonds. We have used this resource to identify the targets of immunoreactivity in sera from protected animals in a murine model of schistosomiasis. Using AVEXIS, a high-throughput assay designed to detect low affinity protein:protein interactions, we have also tested our parasite proteins against a collection of 750 human immune surface receptors to identify new host:parasite interactions involved in host immune modulation. Finally, I will discuss the recent expansion of the human receptor library to a genome-scale collection of over 1,500 human membrane-tethered receptors at the University of York, creating a unique research infrastructure designed to systematically detect new extracellular host:pathogen interactions.

4) Redundant and nonredundant functions of ILC2 during *Strongyloides ratti* infection in mice

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Strongyloides ratti is a parasitic nematode with tissue-migrating and intestinal life stages. Immunocompetent mice clear the infection within 2-4 weeks by a type II immune response and remain semi-resistant to a second infection. Initial control of parasite load is mediated by innate effectors, but infection clearance depends on adaptive immunity as $RAG^{-/-}$ mice lacking T and B cells remain infected for 1 year. Adaptive type-II immunity is thought to be initiated by group 2 innate lymphoid cells (ILC2), which are activated by alarmin cytokines such as IL-33. Here we analyse the contribution of ILC2 to anti-*S. ratti* immunity, using the $Nmur1^{iCre\text{-}eGFP}Id2^{fl/fl}$ mouse model in which ILC2 are selectively absent in immunocompetent mice. Consistent with published results on *Nippostrongylus brasiliensis* infection, numbers of tissue-migrating larvae and intestinal parasites were increased in *S. ratti*-infected ILC2-deficient mice compared to WT-littermates. ILC2-deficient mice showed reduced eosinophilia and reduced numbers of intestinal goblet and tuft cells. Mice in which the IL-33 receptor ST2 was specifically deleted on ILC2 phenocopied the global ILC2-deficiency. The role of ILC2-derived effector cytokines is under investigation. Interestingly, ILC2-deficient mice cleared *S. ratti* infection with WT kinetics in the context of intact mucosal mast cell activation. Also, induction of $GATA3^{+}$ Th2 cells and production of *S. ratti*-specific type-II cytokines and antibodies were unaffected by ILC2-deficiency. Testing the role of ILC2 in establishing memory responses, initial experiments show a significantly reduced parasite burden in ILC2-deficient mice after second infection compared to first infection. However, protection was incomplete in direct comparison with WT littermates. In conclusion, our results suggest that IL-33-activated ILC2 play a non-redundant role in initiating some aspects of the innate response to helminth parasites, such as eosinophilia and intestinal goblet and tuft cell hyperplasia. However, immunocompetent mice can develop adaptive type-II responses in the absence of ILC2.

5) Spatial transcriptomics reveals focal induction of molecular responses and cellular interactions in the small intestine during *Heligmosomoides polygyrus* infection.

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Many parasitic helminths establish long-term chronic infections despite the host immune response. *Heligmosomoides polygyrus* is a natural intestinal helminth parasite of mice, which can persist for many weeks or months in laboratory strains. Larvae are taken in by the oral route, penetrate the epithelial layer of the small intestine and colonise the submucosa for the first 7-8 days of infection. However, little is understood about immune responses during the early stages of the infection, when the parasite is embedded in the submucosa, beyond the observed formation of granulomas, requiring the recruitment of different immune cell types such as macrophages, eosinophils and neutrophils in an IL-4R-dependent manner. Using spatial transcriptomics, we have studied the localised transcriptional changes within the intestinal epithelium and lamina propria of mice in the early stages of infection with *H. polygyrus*. Molecular characterization of *H. polygyrus* granulomas reveals unique cellular compositions within distinct clusters; while macrophages accumulate close to larval parasites, mast cells are increasingly found at distal sites as infection progresses. Utilizing deconvolution techniques, we uncovered common and infection-specific signatures of cell type colocalization and we identified potential ligand-receptor pairs that may mediate communication between the granuloma tissue and the epithelial crypt cells. Additionally, our study highlights the upregulation of genes such as *Ccl9, Fcer1g* and *Tmsb4x* within granulomas, suggesting roles in type 2 inflammation, and genes (e.g *Reg3b* and *Mxra7*) associated with wound healing and tissue repair. These results not only enhance our understanding of the murine small intestine's transcriptional landscape but also provide a platform for exploring host-pathogen interactions.

6) Deciphering the immunomodulatory code: FABP isoforms and their role in *Fasciola hepatica's* lifecycle and host immune evasion

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In the nuanced interplay between host immune defenses and parasitic mechanisms of evasion, *Fasciola hepatica*, the liver fluke, stands as a sophisticated adversary. It employs a diverse repertoire of immunomodulatory molecules to elude immune detection, among which Fatty Acid Binding Proteins (FABPs) play a pivotal role. These proteins, significant in the parasite's excretory/secretory mechanisms, are known for their anti-inflammatory effects. Yet, the detailed landscape of FABP isoforms within *F. hepatica* and their specific functions have remained largely uncharted. Our research delves into this unexplored terrain, aiming to elucidate the expression dynamics and immunomodulatory roles of FABP isoforms across the parasite's lifecycle. Employing a comprehensive methodological approach, we examined the effects of recombinant *F. hepatica* FABPs, produced in *Pichia pastoris*, on monocyte-derived human dendritic cells (moDCs). This included dendritic cell-allogenic T cell co-cultures and in-depth DC phenotyping through transcriptomic, proteomic, and FACS analyses, complemented by quantitative Real-Time PCR (qRT-PCR) to quantify the expression of FABP isoforms. A novel facet of our investigation focused on the binding affinities of different Long-Chain Fatty Acids (LCFAs) to FABP isoforms, revealing distinct ligand specificity patterns. Our findings indicate significant variations in LCFA binding affinity and isoform expression at different stages of the parasite's life cycle. Notably, our data suggest that the FABP1 isoform uniquely engages Thrombospondin-1 (TSP-1) in its immunomodulatory influence on antigen-presenting cells, highlighting a specific pathway through which this isoform may modulate host immune responses. This intricate examination of FABP isoforms not only enriches our understanding of *F. hepatica's* immune evasion strategies but also opens new avenues for targeted therapeutic interventions against fascioliasis. By uncovering the specific roles and interactions of FABP isoforms within the parasite's lifecycle, our study invites further exploration into the molecular intricacies of host-parasite interactions, setting the stage for groundbreaking advancements in the field of parasitology.

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7) Glycoengineering of *Schistosoma mansoni* using mannosidase inhibitors

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Schistosomiasis is a neglected tropical disease (NTD) caused by blood flukes of the genus *Schistosoma*. Worldwide, at least 250 million people are infected with *Schistosoma* parasites and an estimated 779 million people are at risk of infection. Understanding of the intricate parasite-host interaction is crucial for targeting this disease. The parasite is known to modulate the host's immune response by expressing a variety of antigens and releasing specific molecules, including glycoconjugates. Glycans from helminths play crucial roles in host-parasite interactions, and constitute potential antigens for use in diagnostics or as vaccine antigens. Cellular and molecular processes such as protein folding and intercellular communication are dependent on glycosylation, suggesting that glycans may play pivotal roles in schistosome development also. Our research aims to develop glycoengineered living adult *Schistosoma mansoni* worms and schistosomula and subsequently employ these to study how different glycans might contribute to the parasite development and the host immune responses against parasite-derived molecules. Glycoengineered *S. mansoni* worms and schistosomula are generated in *ex vivo* and *in vitro* cultures using kifunensine and swainsonine, chemical compounds that inhibit specific α-mannosidases involved in the N-glycosylation pathway. We show that these compounds gradually alter the N-glycosylation profile from complex type glycans to hybrid and oligomannosidic N-glycan forms without apparent changes in worm motility or morphology. Extracts of the glycoengineered worms are used to stimulate monocytes in *in vitro* model systems and study the differences in induced immune responses compared to untreated worms. We conclude that glycoengineered schistosomes are a promising tool to further elucidate the role of glycans during parasite development and in induction of host immune responses.

9) A *cki* homolog of unusual origin is a tumor suppressor in *Schistosoma mansoni*

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Schistosomes are parasitic flatworms responsible for the neglected tropical disease schistosomiasis, afflicting over 200 million of the world's poorest people. The parasites reside within their definitive host's bloodstream where they are protected by their skin-like tegument. The tegument is made and maintained by a population of somatic stem cells called "neoblasts". Several factors have been identified that regulate the production of tegument via neoblasts, including a schistosome homolog of the tumor suppressor TP53. Schistosomes have two TP53 homologs: 1) p53-1, a true TP53 ortholog that is required for maintenance of tegument-producing neoblasts, and 2) p53-2, a p53-1 paralog found only in parasitic flatworms that is involved in the schistosome's DNA damage response pathway. Nothing is known about the mechanism by how these TP53 homologs function. One common mechanism by which several metazoan TP53 homologs function is by inducing the expression of cyclin dependent kinase inhibitors (CKIs), a family of proteins that regulates cell cycle. We identified a *cki*-like gene in the schistosome genome that, like *p53-1*, is expressed in schistosome neoblasts and tegument progenitor cells. RNAi of *cki* resulted in an increase in neoblast number as well as a concomitant loss of tegument progenitor cells. When knocked down together with *p53-1*, we observed robust tumor formation in the parasite's head. Surprisingly, while *cki* homologs were identified in nearly all parasitic flatworms, they were conspicuously absent from all free-living flatworm "cousins", suggesting an unusual origin of *cki*. Together, this suggests that 1) *cki* may be a transcriptional target of *p53-1* that helps to regulate neoblast-mediated tegument production, 2) *cki* is a *bona fide* tumor suppressor and 3) *cki* does not appear to have evolved via normal vertical inheritance.

10) A snail miniprotein regulates the Venus Kinase Receptor activity in the host-parasite relationship *Biomphalaria glabrata-Schistosoma mansoni*

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Venus Kinase Receptors (VKRs) are an atypical family of receptor tyrosine kinases present in most invertebrates, except nematodes, and originally discovered in the parasite *Schistosoma mansoni*. These single-span membrane receptors are composed of an intracellular tyrosine kinase domain similar to that of the insulin receptor and an extracellular domain harboring a Venus Flytrap (VFT) module capable of binding small ligands such as ions, amino acids or peptides. The two VKRs identified in *S. mansoni*, SmVKR1 and SmVKR2, have been extensively studied at the adult stage. Their important role in the development of parasitic gonads and gametogenesis has been demonstrated as well as in other species such as mosquitoes or grasshoppers. Additionally, VKRs are also expressed in larval stages of *S. mansoni* and particularly in sporocysts developing in *Biomphalaria glabrata*, the intermediate snail host, but with an as yet unknown function. Interestingly*, B. glabrata* also expresses its own VKR (BgVKR), which is mainly located in the gonads and can be activated by arginine. Unexpectedly, we discovered that a small protein of 80 amino acids produced by the mollusk is capable of regulating the activity of BgVKR but also of the parasite's two VKRs. Using state-of-the-art protein chemical synthesis, we successfully produced this miniprotein in its folded conformation, stabilized by four disulfide bonds. We resolved its X-ray crystal structure displaying a compact scaffold that appears unique with no homolog in the pdb database. The chemical synthesis of a biotinylated version of this miniprotein made it possible to demonstrate its interaction with the VFT domains of the VKR of mollusks and parasites. We will highlight the discovery of the unexpected activity of this miniprotein which already has a very long and controversial history in the field. We are currently exploring how this miniprotein regulates infection and sporocyst development in *B. glabrata*.

11) Characterizing the host sensory pathway that governs parasitic nematode infections

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The options to treat nematode parasitism rely on classes of drugs (e.g., ivermectin) that have become increasingly susceptible to resistance and, unfortunately, only target feeding stages and adult parasites, leaving a reservoir of infectious stages unaffected. Previously, we had shown that upon contact with the host, parasites sense their new environment and activate a host-specific developmental program that is dependent on a parasite nuclear hormone receptor, called DAF-12. Here, we developed the concept of treating parasitism by targeting either the DAF-12 receptor or the rate-limiting enzyme, DAF-9, that synthesizes the DAF-12 hormonal ligand. Focusing on the often-lethal human parasite *Strongyloides stercoralis*, we demonstrated the therapeutic potential of using a DAF-12 ligand to kill parasites at the infectious stage and thereby effectively treat both latent autoinfections and lethal hyperinfections in a relevant preclinical model. Remarkably, this approach was more effective than ivermectin, which alone has only a ~60% survival rate for the hyperinfection because it fails to kill the autoinfectious stage that causes the disease. Likewise, we also show that inhibiting the DAF-9 biosynthetic enzyme is also an efficacious anthelmintic approach. Finally, we have now characterized the mechanism by which parasites sense their host environment to activate the DAF-9/DAF-12 pathway, revealing another potential novel therapeutic target.