17) The genomes of African *Simulium spp.*: first assembly and broad characterisation

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Blackflies of the genus *Simulium* are the vectors for the filarial parasite *Onchocerca volvulus*, the causative organism of river blindness or onchocerciasis. The taxonomy of blackflies is challenging due to the relative lack of morphologically informative characters in adult flies, particularly amongst the members of the *S. damnosum sensu lato* species complex that includes the most important vector species. Cytotaxonomy based on chromosomal banding patterns suggest a rich underlying genetic diversity but has proven impractical as a routine, "field friendly" method for vector identification. The limited molecular data that are available, primarily using mitochondrial markers, has proven relatively uninformative and is not congruent with cytogenetic taxonomy. Astonishingly, no attempts to sequence the genome of these important vectors has been reported. We report here the first genome assemblies of 3 separate "species" of African *Simulium* blackflies. Two of the species were identified morphologically as *S. damnosum s.l.* but collected from Ghana and Ethiopia respectively, i.e. on different sides of the continent, and the third species was identified morphologically as *S. ethiopiense*, a member of the *S. neavei* species complex from Ethiopia. The assemblies were based on a combination of Illumina short read and Oxford Nanopore data from single flies. All three assemblies are >90% complete by BUSCO, and of approximately 240Mb. The two *S. damnosum* genomes are clearly sufficiently distinct from each other to be considered as separate MOlecular Taxonomic Units but are more closely related to each other than either are to the *S. ethiopiense* genome, which thus constitutes a third MOTU. Further analyses of the gene content and genome arrangement of these genomes will be reported. As an indicator of assembly quality, all three assemblies exceed the quality thresholds set by the Wellcome Trust Darwin Tree of Life project.

18) Molecular Characterization of *Opisthorchis viverrini* TGF-β Homologue and Role in Host-Parasite Interactions

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Opisthorchis viverrini, a human liver fluke, is a causative agent of opisthorchiasis and a significant risk factor for cholangiocarcinoma. Parasites employ various strategies, including secretion of proteins that engage host molecules, to evade the host immune response, making these molecules promising targets for vaccine and drug development. The transforming growth factor-β (TGF-β) signaling pathway plays a pivotal role in numerous cellular processes, including growth, differentiation, apoptosis, motility, invasion, and immune response regulation. While homologues of TGF-β have been identified in helminth parasites, the characterization of *O. viverrini*-derived member of the TGF-β superfamily has been limited.Our study aimed to construct a recombinant TGF-β homologue molecule of *O. viverrini* (OV-TGH) and elucidate its role in host-parasite interactions and immune modulation. The sequence encoding *O. viverrini* TGF-β was cloned into the pSecTag2A expression vector and expressed using the Expi293 mammalian cell expression system. The recombinant OV-TGH molecule's activity was assessed through the MFB-11 assay and its involvement in the TGF-β signaling pathway was investigated. This study contributes to a deeper understanding of the role played by the TGF-β homologue molecule of *O. viverrini* in host survival and immunopathogenesis, shedding light on potential therapeutic targets for opisthorchiasis and associated complications

19) Reduced HIV incidence after elimination of *Wuchereria bancrofti* in Southwest Tanzania

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Prior to the introduction of antihelminthic treatment programs in southwestern Tanzania, our group described a 2.3-fold increase in HIV susceptibility among individuals 14 to 65 years infected with *Wuchereria bancrofti* (WB) compared to WB-negative villagers. Study participants in a prospective cohort study had been tested annually for HIV and circulating filarial antigen, an indicator of WB burden from 2007 to 2011 and the HIV incidence described for this time period. Due to the high prevalence of WB in the region in 2007, ivermectin and albendazole was distributed annually to all villagers as part of government programs from 2009 to 2015. The impact of WB elimination on HIV incidence was investigated by our group during a revisit of the same participants in 2019. Of the 1,299 villagers who were rescreened in 2019, 1,139 had tested HIV-negative at the end of previous surveillance in 2011. This included 848 persons who had never tested positive for WB, 272 participants who were previously – but no longer – WB-positive, and 19 individuals who were still or again WB-positive. During the first surveillance period from 2007 to 2011, 15 HIV seroconversions occurred among WB positive individuals during 871 PYs (1.72 per 100 PY). In the second period from 2011 to 2019, the HIV incidence decreased significantly to 0.73 cases per 100 PY (17 in 2,344 PY, p=0.019) in this group. In all-time-WB-negative individuals we documented 9 HIV seroconversions in 1,298 PY (0.69 per 100 PY) between 2007 to 2011, 39 in 5,724 PY (0.68 per 100 PY), between 2011 and 2019 p=0.963). Interpretation: There was a significant decline in the incidence of HIV in the group of villagers who were previously filarial- positive but were now cured of WB. This reduction in HIV incidence was not observed among those who had never been infected with WB.

20) Morbidity in preschool age children in a *Schistosoma mansoni* endemic community in Lake Victoria, Uganda

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Schistosoma mansoni infection is known to cause damage to the liver, however resource and time constraints limit regular ultrasound screenings in endemic areas. Therefore, the number of eggs in faeces is used as a proxy for morbidity and the high infection intensity (400 eggs per gram) threshold to inform and evaluate control programs. However, evidence challenges this link between infection intensity and morbidity, urging a revaluation of these control targets. In a cross-sectional survey in Bugoto, Uganda, involving 287 individuals aged 3-74, *S.* mansoni prevalence and intensity were determined using Kato Katz microscopy and point-of-care antigen tests. Ultrasound and the Niamey protocol assessed periportal fibrosis (PPF), portal vein dilation (PVD), and left parasternal line (PSL) enlargement. Logistic regression models incorporated infection, coinfections, anemia, and symptoms to predict morbidity and infection. PPF prevalence was 9% (B-F) and 4% (C-F), while PVD and PSL prevalence were 34% and 33%. Although 11-14-year-olds had the highest infection intensity, preschool-aged children (PSAC) were more likely to exhibit PVD and PSL morbidities. Current *S. mansoni* infection showed no association with assessed liver morbidity markers. Our study findings add to the growing evidence indicating a lack of association between current *S. mansoni* egg count with morbidity markers, which raises significant implications against the use of eggs per gram as a proxy for morbidity within national programs and policy. The age-related distribution of morbidities observed here, with notable burden of PVD and PSL in PSAC, stresses the critical need to both: a) elucidate the impact and progress of apparent 'subtle morbidities' on host health and its interplay with current and past infection status; and b) accommodate and monitor these youngest age classes in treatment programmes, if we are to ever truly achieve the revised WHO NTD Roadmap schistosomiasis targets of elimination as a public health problem by 2030.

21) High seropositivity for helminths in immunosuppressed patients with peripheral eosinophilia

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Serological tests are commonly used for screening helminthic infections in patients with peripheral eosinophilia. We screened 115 eosinophilic patients with immunosuppressive conditions from OPDs and wards of oncology, paediatrics, pulmonology and rheumatology for IgG antibodies against *Strongyloides*, *Toxocara*, *Trichinella spiralis*, *Echinococcus granulosus*, and *Taenia solium* by ELISA, and *Wuchereria bancrofti* antigen by lateral flow assay. A total of 34/115 (29.6%) patients were seropositive—*Strongyloides* (21, 18.3%), *Toxocara* (n=13, 11.3%), *Trichinella* (n=7, 6.1%), *E. granulosus* (n=6, 5.2%), *W. bancrofti* antigen (n=6, 5.2%), and *T. solium* (n=2, 1.7%). Of the *Strongyloides* seropositives, 8 were only *Strongyloides* positive, 9 also for one other helminth (3 for *Trichinella*, 3 for *Toxocara*, 2 for *E. granulosus* and one for *W. bancrofti*), one for 2 other helminths (*Trichinella* and *Toxocara*), and 3 for 3 other helminths (2 for *Toxocara*, *E. granulosus* and *W. bancrofti*, and one for *Trichinella*, *E. granulosus* and *W. bancrofti*). A total of 16/115 patients were positive for *Strongyloides* DNA by *18S rRNA* real-time PCR in paired stool samples, only 6 of which were seropositive. A subset of 15 *Strongyloides* positive patients (subgroups: 5 ELISA and RT-PCR, 5 only ELISA, 5 only RT-PCR) and 5 healthy controls were assessed for Th1/Th2/Th17 cytokine response. The *Strongyloides* positive patients had significantly higher IL-10 (27.65±6.8pg/ml) and IL-6 (29.39±3.4pg/ml) levels than healthy controls (p-value<0.05), while IL-2, IL-4, TNF, IFN-γ, and IL-17A levels were not significantly different; there was no significant difference between the cytokine levels within the *Strongyloides* positive subgroups. Thus, in this cohort of eosinophilic immunosuppressed patients, a high serological positivity for multiple helminthic infections was observed, *Strongyloides* being the predominant helminth. It is not clear whether the multiple seropositivity is due to co-infections or cross reactions and needs further exploration. The study also highlights the need for more specific serological tests for helminthic infections.

22) Structural and Functional Analysis of the TGF-β Mimic TGM-2 and its Receptor-Binding Domains

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The extraordinary prevalence of helminths up to date can largely be attributed to their ability to secrete molecules that manipulate the host immune system, facilitating their survival. A recent investigation into the excretory/secretory products (ESP) of the murine intestinal nematode *Heligmosomoides polygyrus,* unveiled a novel protein with functional resemblance to the mammalian immunosuppressive cytokine, TGF-β. This TGF–β mimic is distinguished by its unique structure comprising five Complement Control Protein (CCP) domains and has been identified as one of ten homologs within the *H. polygyrus* ESP, designated as TGM-1 to TGM-10. Our study aims to dissect the structure and functions of the second homolog of the TGF–β mimic, TGM-2. Through cloning, expression, and purification efforts, we prepared eight distinct protein truncations lacking N- or C-terminal domains for detailed analysis. Our investigations into the mechanism of action of TGM-2 confirm its binding affinity for TGFBRI, TGFBRII, and the co-receptor CD44. By examining the functional roles of various domains within TGM-2, we have observed that domains 4 and 5 exhibit enhanced binding to the CD44 co-receptor compared to the previously reported TGM-1 homologue. Our investigations into the protein interactions through pulldowns and mass spectrometric analysis also indicated a possible interaction with the intracellular protein CDC42. Additionally, full-length TGM-2 demonstrates potent activation of the pSMAD pathway in the MFB-F11 fibroblast cell line at concentrations as low as 1 ng/ml and induces the in vitro conversion of naïve murine CD4+ T cells into Foxp3+ Tregs. Both stimulatory activities diminish significantly in the absence of domains 4 and 5 that interact with CD44, demonstrating the importance of co-receptor binding for the efficacy of the helminth TGF-β mimic.

23) Highly modified and immunoactive N-glycans of canine heartworm and porcine whipworm

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Glycans are key to host-pathogen interactions, whereby recognition by the host and immunomodulation by the pathogen can be mediated by carbohydrate binding proteins, such as lectins of the innate immune system, and their glycoconjugate ligands. Previous studies have shown that excretory-secretory products of nematodes exert immunomodulatory effects in a glycan-dependent manner. To better understand the mechanisms of these interactions, we prepared N-glycans from both *Dirofilaria immitis* and *Trichuris suis* and both analyzed their structures and used them to generate natural glycan microarrays. With these arrays we explored the interactions of glycans with C-type lectins, C-reactive protein and sera from infected animals. In-depth analysis revealed not only fucosylated LacdiNAc motifs with and without phosphorylcholine moieties, but species-specific elements including glucuronylated and chito-oligomer antennae in *D. immitis* and phosphorylcholine-modified mannose and N-acetylhexosamine-substituted fucose residues in *T. suis*, in the context of maximally tetraantennary N-glycan scaffolds. In summary, the glycans of *D. immitis* and *T. suis* are recognized by both the innate and adaptive immune systems, and also exhibit species-specific features distinguishing their glycomes from each other and those of other nematodes.

24) Modelling host: parasitic nematode interactions with ovine 'mini-gut' organoids.

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Teladorsagia circumcincta is one of the most predominant gastrointestinal (GI) nematodes of sheep in temperate regions. Reported resistance to anthelmintics is increasing and therefore research into new control strategies (e.g. vaccination) is vital. One area of interest for identification of potential vaccine candidates are extracellular vesicles (EVs). Extracellular vesicles are lipid membrane-enclosed packages which contain effector proteins and immune modulators and play important roles in establishing helminth infections. However, there are challenges in studying these interactions between the host and GI nematodes due to the lack of accessibility of the infection site and the need to rely on infection models which have ethical implications. Recently, ovine gastrointestinal organoids have been developed which allow host-parasitic interactions to be studied in a physiologically-relevant and host-specific *in vitro* cell culture system. The overall aim of the project is to use ovine abomasum organoids to identify and characterise active components of *T. circumcincta* EVs at different infective life stages. The separation and characterisation of EVs from adult and larval stage 4 excretory/secretory products has been achieved. Protein characterisation of these EVs has revealed a consistency with proteins found in other nematodes (e.g. M13 metallopeptidases, actin, tetraspanins) which further supports the presence of EVs. To progress understanding of these proteins on the host, the uptake of EVs by organoids must be confirmed. Further investigations are underway to look at the interactions and potential implications of these EVs at the host epithelial cell interface using species- and tissue-specific ovine abomasal organoids.

25) Breaking the silence of dirofilariasis: immune insights amidst wartime challenges

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Dirofilariasis, caused by *D. repens* and *D. immitis*, is a significant concern in both veterinary and human medicine. Climate change and human activities contribute to the spread of these zoonoses to new regions. This study, conducted during the Russian invasion on Ukraine, examines the prevalence of stray dogs and cats, highlighting the crisis's impact on OneHealth and the potential for zoonotic transmission. Using Real-Time PCR and species-specific primers we tested over 450 stray animals, detecting *D. repens*, *D. immitis*, *A. reconditum* and various co-infections yielding an overall prevalence 27,8%. Previous data collected up to 2012 reported nearly 1500 cases of human dirofilariasis in this region. This underscores the parasites' zoonotic potential, especially in uncontrolled conditions like wartime. Despite *D. repens* being responsible for 70% of infections worldwide, cases of *D. immitis* infections in humans have been established. Given these circumstances, the absence of a vaccine seems to be a growing concern. Unfortunately, very little is known about the immune responses induced during *Dirofilaria* infections, especially in the case of *D. repens*. Helminths are master manipulators of their host's immune system, honing this ability through extensive host-parasite co-evolution. Central to this interaction are antigen-presenting cells, specifically macrophages and dendritic cells (DCs), which encounter helminth-derived molecules that can significantly alter their maturation and polarization states. In our study, we utilized an in vitro model comprising primary monocyte-derived human dendritic cells (moDCs) and macrophages to investigate the effects of somatic antigens from *Dirofilaria repens* (DrSA) and *Dirofilaria immitis* (DiSA) on cytokine secretion by LPS-stimulated moDCs and M1 macrophages. Our findings indicate that exposure to DrSA and DiSA dampens the inflammatory response in human moDCs. Additionally, we assessed the functional changes in these cells following exposure to the helminth antigens.

26) Worms to the rescue: probing *Schistosoma mansoni* eggs for tolerogenic products

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Food allergy is considered the “second wave” of the allergy epidemic after asthma and allergic rhinitis. Absence of early childhood pathogen stimulation embodied by the Hygiene Hypothesis is one explanation, and the eradication of parasitic helminths could be at play. Infections with *Schistosoma* spp. have a negative correlation with allergic diseases. Schistosomes achieve host immunomodulation through the release of excretory/secretory products such as extracellular vesicles (EVs), which are modulated by internal and membrane-bound cargos (protein, microRNA, metabolites, lipids). Research on *Schistosoma* spp. egg-derived EVs is minimal, with studies focused the modulatory mechanisms of on *S. japonicum* egg EVs in the liver and as Schistosomiasis vaccine candidates. These studies didn’t follow the MISEV2018 guidelines, making it difficult to confirm any nanoparticles identified are EVs. Additionally, the “native” structure of *S. mansoni* egg EVs has not been characterised. We isolated EVs from cultured eggs of *S. mansoni* using a novel scalable liquid chromatography method. Purified EVs were characterised using cryogenic transmission electron microscopy, nanoparticle tracking analysis, and western blotting. Lipid-proteomic, metabolomic, and microRNA cargo were analysed using mass spectrometry and small-RNA sequencing. We report the discovery of a population of novel globular lipoprotein-like nanoparticles (10-30 nm) that are distinct from other helminth-derived EVs in their structure, size, and cargo. These nanoparticles exist in low abundance, do not possess typical double membrane structures, or have a typical circular shape, and appear to aggregate significantly. This study provides novel insights into the extracellular nanoparticles produced by *S. mansoni* eggs and suggest they may not produce typical exosome-like nanoparticles like that of its other life stages. By elucidating the molecular mechanisms by which *S. mansoni* egg EVs may promote tolerance in the gut microenvironment, this work provides an exciting avenue for the identification of novel therapeutic moieties that promote tolerance and treat food allergy.

27) Helminth derived recombinant protein alleviates inflammation in a model of early-life inflammatory bowel disease

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Inflammatory bowel diseases (IBD), including ulcerative colitis and Crohn's disease, are chronic inflammatory disorders that have no cure and impact millions of individuals worldwide. Notably, pediatric IBD contributes to 25% of the overall IBD cases and tends to manifest as a more aggressive disease than in adult-onset IBD. In unison with the increased prevalence of inflammatory diseases like pediatric IBD, exposure to gastrointestinal helminths has vastly decreased in the western world. These complex multicellular parasites have co-evolved with humans for millennia, and drive immunoregulatory pathways in the host by secreting various bioactive molecules. To explore their anti-inflammatory potential further, we have expressed individual recombinant proteins derived from hookworms, aiming to identify proteins with promising regulatory properties during chronic inflammatory conditions. Out of a set of recombinant proteins with potent anti-inflammatory capacity in adult onset IBD that we recently published, one protein of particular interest effectively reduced disease severity and alleviated inflammation induced by experimental colitis in mice before sexual maturity. This is important, because the developing intestinal immune system in early life differs significantly from that of adults, and pediatric IBD is not only even more devastating but also more difficult to treat than adult onset IBD. We now seek to discern the mechanism of action of this protein by assessing cellular binding sites, intestinal barrier integrity and effects on intestinal immune cells. Ultimately, we aim to identify a novel and safe therapeutic to alleviate chronic inflammatory responses during pediatric IBD.

28) Effective helminth vaccination via abrogation of IL-33 pathway blockade

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The murine intestinal nematode *Heligmosmoides polygyrus bakeri* (Hpb) can powerfully modulate the host immune response. Hpb modulates IL-33 responses via the release of two families of molecules: the HpARI family, which affects IL-33 and comprises of HpARI1, HpARI2 and HpARI3. The HpBARI family affects the IL-33 receptor (or ST2) and has HpBARI and HpBARI_Hom2 as its members. This immunomodulation is most evident in the first week of infection, when the infective larvae enter from the lumen of the gut into the gut wall and begin moulting. This immunomodulation can be tracked by the abrogation of detection of the IL-33 receptor ST2, mediated by the HpBARI family. ST2 is undetectable on the surface of immune cells throughout the host during the first week of infection but recovers to normal levels once the parasite leaves the intestinal wall. We used a vaccination approach to block the effects of individual members of the HpARI and HpBARI families, to determine their effects in vivo. Recombinantly expressed HpARI or HpBARI family members were administered in an alum adjuvant prior to infection with 200 infective Hpb larvae: parasite ejection was monitored by fecal egg counts and day 28 adult worm counts. Surprisingly, whilst there is sequence similarity between the molecules in each family, only HpARI2 and HpBARI offered protection against subsequent parasite infection, whilst the remainders offered no protection. Excellent protection against infection was afforded by a combination HpARI2+HpBARI+HpBARI_Hom2 vaccination, with ST2 suppression being abrogated, and dramatically increased Th2 and ILC2 responses against the parasite. Vaccinated serum could block the effects of relevant HpARI and HpBARI family members tested in vitro, abrogating the ST2 suppressive effect of total parasite secretions (HES). Therefore, this study offers a proof-of-principal that vaccination with immunomodulatory recombinant proteins can affect potent protection against infection, while providing a tool to abrogate the activity of specific immunomodulatory proteins.

29) Ecological survey and molecular profiling of *Caenorhabditis* Nematodes in Bangladesh

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The *Caenorhabditis elegans* serves as a key laboratory model system, contributing significantly towards advancements in molecular biology, cell biology, drug discovery, neurobiology and environmental sciences. Despite it being a prominent research model focus globally, Bangladesh has yet to explore this field comprehensively. This study aimed to investigate *C. elegans* and cogeneric nematodes through molecular detection, understanding their ecology in ephemeral resources and their genetic diversity. Small pieces of tomato and carambola were buried for three days, 5 inches below the surface in proximity to cooler habitats in Thakurgoan and Mymensingh districts. Rotting baits were shipped to the *C. elegans* Model Science and Technology Lab, BAU and transferred to NGM petri plates seeded with OP50 *E. coli*, then live nematodes were moved to new plates. After two days, growing nematodes were isolated and allowed to proliferate under controlled temperature. Ecological data and morphological descriptions were documented. The identification process combined microscopic examination and PCR targeting 18S rDNA (SSU) of Rhabditids and between 5.8S and 28S rDNA (ITS2) of *Caenorhabditis* for precision. After morphometric identification, eight isolates were domesticated and allowed to self-replicate, then subjected to cryopreservation for future investigations. Worm's DNA succeeded to amplify SSU and ITS2 primers which indicate they belongs to *Caenorhabditis* genera. This pioneering investigation address availability and unresolved queries surrounding natural populations of *Caenorhabditis* species across several locations in Bangladesh. Research of this kind can uncover signatures of local adaptation in *Caenorhabditis* populations and provide important insights into spatiotemporal genetics.

30) *Ex-vivo* and *in-vitro* investigation of host-pathogen interaction in human strongyloidiasis

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In humans, *Strongyloides stercoralis* infection can persist lifelong due to the peculiar auto-infective cycle. The limited knowledge of the mechanisms underpinning this chronic infection is a key issue in disease management and control. To gain novel insights into disease pathogenesis, we are studying host-pathogen interaction in strongyloidiasis *ex-vivo* and *in-vitro.* For the *ex-vivo* experiments, we investigated the systemic modulation in protein expression and serum-derived extracellular vesicles (EVs) induced by chronic strongyloidiasis using immunoassays and proteomics. In a cohort of Italians with long-lasting strongyloidiasis, we did not observe the classical shift towards a type 2 immune response, but rather decreased chemokines, suggesting that immune cell recruitment might be dampened in these patients. The untargeted proteomics investigation performed on both serum and serum-derived EVs revealed few proteins as significantly modulated in patients at baseline compared to post-treatment (n=14) or uninfected controls (n=22). Such results suggest that, at the systemic level, important mechanisms of adaptation might have established for the host to tolerate the chronic presence of *S. stercoralis*, which will be studied more in depth by extending our analyses to subjects with recently acquired infections. *In vitro* investigations were also carried out to explore the local interaction between the parasite and its host. Human intestinal epithelial cells (Caco-2) were exposed to clinically isolated infective larvae (iL3) or iL3-derived EVs. Preliminary analyses revealed that both larvae and EVs induce the release of CXCL10 from Caco-2 cells in a time-dependent manner, while iL3 also induce a significant release of IL-8, similarly to the profile reported in patients from endemic areas. The gene expression modulation induced by iL3 and iL3-EVs on human cells will be examined more in depth using 'omics' approaches to expand our understanding of host-pathogen interaction in human strongyloidiasis.

31) Utilizing equine enteroid-derived monolayers for studying parasitic intestinal nematode infection

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Organoid cultures derived from stem cells have become increasingly popular as experimental models for studying infections caused by various gastrointestinal pathogens in different host species. However, the size of infectious nematode larvae and the closed structure of 3-dimensional organoids often pose challenges when studying the natural route of infection. In order to address this issue, the present study utilized enteroids, organoids derived from the equine small intestine, to establish monolayer cultures on the apical surface of the epithelium, allowing for easier administration of infectious agents. To evaluate the functionality of these monolayers, they were stimulated with IL-4 and IL-13, and/or exposed to infectious stage larvae of equine nematodes such as *Parascaris univalens*, cyathostominae, and *Strongylus vulgaris*. The effects of these stimuli were assessed through qPCR analysis, histochemistry, immunofluorescence, live-cell imaging, and scanning electron microscopy. These analyses revealed that the monolayers were heterogeneous, consisting of both immature and differentiated cells including tuft cells and mucus-producing goblet cells. Stimulation with IL-4/IL-13 led to an increase in the differentiation of tuft and goblet cells, as evidenced by the expression of DCLK1 and MUC2. Co-culture with *P. univalens* further enhanced the expression of MUC2 in these cytokine-primed monolayers. Additionally, live-cell imaging showed morphological changes in the epithelial cells upon exposure to larvae, even in the absence of cytokine stimulation. Overall, this study presents the design, characterization, and usability of an experimental model representing the equine nematode-infected small intestinal epithelium. The presence of tuft cells and goblet cells, whose mucus production is influenced by Th2 cytokines and/or the presence of larvae, provides an opportunity for mechanistic studies on the physical interactions between nematodes and the equine intestinal mucosa.

32) Isoform-specific targeting of insulin receptor *Ss*-DAF-2 in *Strongyloides stercoralis* with theophylline-dependent hammerhead ribozyme

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The current main strategy for identifying function at the gene-level in *Strongyloides stercoralis* has been to knock out or overexpress the gene of interest. However, this becomes more challenging at the isoform-level. Previous studies have shown that the insulin receptor *Ss-daf-2* from *S. stercoralis* is expressed in two spliced isoforms, *Ss-daf-2a* and *Ss-daf-2b*, with only one exon difference between them. Due to limitations in gene editing methods, the function of the insulin receptor *Ss*-DAF-2 has not yet been revealed. Here, we used a theophylline-dependent hammerhead ribozyme for gene expression regulation in *S. stercoralis*. Ribozymes are catalytic RNA molecules capable of catalyzing chemical reactions. To knock down a target gene, both binding arms of trans-cleaving hammerhead ribozymes need to be complementary to the target mRNA simultaneously. Importantly, this allows targeting of alternative splicing events. The data from average fluorescence intensity and real-time PCR showed that ribozyme's ability to regulate exogenous GFP reporter gene expression in various somatic cells of *S. stercoralis* increases, enhanced by increasing the theophylline concentration. Subsequently, targeting of the endogenous gene *Ss-unc-22* in *S. stercoralis* revealed that the addition of theophylline resulted in defective movement, confirming the applicability of the ribozyme in regulating the expression of endogenous genes in *S. stercoralis*. Respective knockdown of *Ss-daf-2a* and *Ss-daf-2b* showed a significant increase in body length and width of *Ss-daf-2a* knockdown strain, while the *Ss-daf-2b* knockdown strain showed a slight decrease in body width. Additionally, the study found that human insulin induced recovery of the dauer-like iL3 in *S. stercoralis* by activating orthologs of the insulin receptor *Ss*-DAF-2. This study reveals that the use of theophylline-dependent hammerhead ribozyme effectively manipulated endogenous genes in *S. stercoralis*. It provides a sound foundation for investigating key gene functions during the development of parasitic nematodes.