SOCIETY FOR MUCOSAL IMMUNOLOGY

REVIEW ARTICLE

Effects of helminths on the human immune response and the microbiome

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Helminths have evolved sophisticated immune regulating mechanisms to prevent rejection by their mammalian host. Our understanding of how the human immune system responds to these parasites remains poor compared to mouse models of infection and this limits our ability to develop vaccines as well as harness their unique properties as therapeutic strategies against inflammatory disorders. Here, we review how recent studies on human challenge infections, self-infected individuals, travelers, and endemic populations have improved our understanding of human type 2 immunity and its effects on the microbiome. The heterogeneity of responses between individuals and the limited access to tissue samples beyond the peripheral blood are challenges that limit human studies on helminths, but also provide opportunities to transform our understanding of human immunology. Organoids and single-cell sequencing are exciting new tools for immunological analysis that may aid this pursuit. Learning about the genetic and immunological basis of resistance, tolerance, and pathogenesis to helminth infections may thus uncover mechanisms that can be utilized for therapeutic purposes.

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INTRODUCTION

Helminth infection models in mice have rapidly improved immunologists' understanding of type 2 immune responses, but an understanding of human immune responses to helminths and the ability to reduce the morbidity caused by helminth infections in endemic populations remains poor. Unique features of these parasites include being large multicellular organisms that mature through several larval stages, migrate through different tissues, and have been selected by evolution to produce immune evasion molecules. The type 2 response is particularly important for maintaining a balance between worm expulsion (i.e., resistance) as well as minimizing the virulence of these parasites (i.e., disease tolerance) by repairing the tissue damage caused by the worms.

A large proportion of helminth-infected individuals can be asymptomatic and morbidity typically affects individuals with high worm burdens, who may be more susceptible because of reduced immunity. Pathology also occurs in overly immune reactive individuals from collateral tissue damage, despite low worm burdens. This inter-individual natural variation in immune responses against helminths is poorly understood, but likely a result of interactions between genetic and environmental factors. If live helminths or helminth-produced molecules are to be tested as therapeutics for inflammatory diseases, understanding this heterogeneity in immune responses is likely necessary to maximize clinical benefit. Unfortunately, the most accessible readout of immune function in human populations is the peripheral blood, whereas the tissues in which the helminths reside are more difficult to access for analysis of tissue-resident immune cell phenotype and function.

Technological breakthroughs often precede major advances in the biological understanding of complex systems. Organoid technology to study epithelial cells from human biopsies, as well as the rapid advancement of single-cell sequencing technologies, are two promising tools with the potential to transform our knowledge of human mucosal responses. The ability to generate and maintain epithelial cells close to a native state from pinch biopsies collected from human subjects opens the door toward studying immune cell interactions with different types of epithelial cells. At the same time, single-cell sequencing provides transcriptional profiles by RNA-seq, surface molecules by CITE-seq, and epigenetic states by ATAC-sec, enabling not just phenotyping alone but an understanding of transcriptional regulation at a molecular level. In addition, the ability to freeze and thaw biopsy specimens collected during endoscopies, such that samples can be processed in batches rather than individually, to make organoids and for single-cell sequencing, has also been transformative. These exciting new approaches can now be deployed for clinical studies whereby it is possible to obtain intestinal biopsies, for example, in human challenge infections.

In this review, we discuss bridging the gap between mouse and human immunology of helminth infections, heterogeneity of responses in human populations, roles for the microbiome during helminth infections, and future opportunities in understanding human mucosal responses to helminths through technological advances and human challenge studies. While it is clear that immune responses elicited at tissue sites where pathogens are located are very different from what can be measured in the blood[1], most studies of human helminth infections are based on the analysis of peripheral blood mononuclear cells (PBMCs). There

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are few studies on mucosal immune responses in humans during infection with gastro-intestinal helminth parasites. Consequently, tissue-resident cells such as ILC2, macrophages, and tissue-resident memory T cells are not well studied during human helminth infection. In contrast, mouse studies rarely characterize PBMCs and typically involve the analysis of samples from tissue sites and associated lymphoid tissue. Hence, there is quite a disconnect in our understanding of mouse and human responses to helminth infections.

There are logistical, technical, and ethical issues for accessing tissue samples to study helminth infection in resource low regions where these diseases are endemic. While we can technically obtain a biopsy from tissue sites, these approaches can be applied only when clinical infrastructure is sufficient and ethically justifiable. Experimental challenge helminth infection studies, as well as studies with self-infected individuals, provide the main glimpse of helminth responses in the gut. Perhaps in the future, studies in endemic regions can be coordinated between clinicians and scientists such that tissues harvested following intestinal or elective surgeries, may provide insights into immune responses elicited at tissue sites in helminth-infected individuals. In addition, endoscopies and imaging techniques might be tailored for immunology studies during helminth infection at tissue sites and associated lymphoid tissues[2].

MUCOSAL RESPONSES TO INTESTINAL HELMINTH INFECTION IN MICE

Based on gastro-intestinal helminth infection models in mice, the importance of type 2 immune response to expel and resist helminths is well appreciated. This type 2 immune response is characterized by the accumulation of cells like eosinophils, basophils, ILC2, mast cells, alternatively activated macrophages, and CD4+T helper 2 (T_H2) cells, which produce effector type 2 cytokines (e.g., IL-4, IL-13) that changes the epithelial and associated stromal cells for clearance of the parasite. Signaling through IL-4R-alpha and STAT6 in intestinal epithelial cells is important for increasing epithelial cell turnover as part of the "weep and sweep" response, alongside stimulating increased mucus production by goblet cells and changes to the composition of this mucus barrier. This response maintains the mucosal barrier and prevents inflammatory responses triggered by gut bacteria. In addition, type 2 cytokines will increase the contraction of intestinal muscles, which together with the activation and release of mast cell proteases can increase the flow of fluids into the lumen to flush the helminths out of the intestinal tract[3].

Over the last decade, the initiation of type 2 immune responses has become well defined[4]. Tuft cells, a chemosensory cell, were identified as being important in the production of activating cytokines like IL-25[5–7]. Together with the release of IL-33 and TSLP, these alarmins are critical in the activation of type 2 immune response[8–12]. However, the role of these alarmins in human helminth infections is still understudied. Recently, "non-classical" type 2 cells like neutrophils have been shown to play important roles in parasite clearance and inflammation[13–16]. Neutrophils are particularly important during the early phases of helminth infection[17], in the recruitment of other effector cells, as well as in directly killing the parasite, especially at the infective larva stage[15]. An important feature of this innate response is to promote tissue repair[18]. Notably, macrophages become alternatively activated by IL-4Ra/STAT6 signaling to adopt a phenotype that has enhanced anti-inflammatory tissue repair function. Type 2 cytokines will also increase immunoglobulin E production by B cells, which can activate basophils, eosinophils, and mast cells through Fc receptors to amplify type 2 cytokine production. Overall, this tissue repair response in the intestinal tract is important to maintain gut integrity and prevent the leakage of gut bacteria and sepsis.

While these are general features of type 2 responses to intestinal helminths, the different experimental models—*Nippostronyglus brasilensis*, *Heligmosomoides polygyrus bakeri* and *Trichuris muris*, have different lifecycles, reside in different parts of the intestine, hence function as models of different human helminth parasite with different pathogenicity and chronicity pattern[19]. The complex array of immune, epithelial, neuronal, and stromal changes has been previously reviewed[4,19–25]. Notably, there are also important immunometabolic consequences of helminth infections[26,27]. While in general the type 2 immune response is dominant during these infections, it is important to note that all infections also elicit to different degrees type 1 and type 17 cytokines that interact and cross-regulate the type 2 responses during acute and chronic infections.

EFFECTS OF HELMINTH INFECTIONS ON HUMAN PERIPHERAL BLOOD

Contrary to popular assumptions, human peripheral blood responses during natural helminth infection are usually characterized by a mixed population of type 2, regulatory, and type 1 immune cells[28–34]. Increased expression of type 2 cytokines, type 1 cytokines, regulatory cytokines, and markers such as CD161 and CTLA-4 is often observed in helminth-infected individuals compared to dewormed individuals[28,29,32,35–37]. Individuals with stronger type 2 cytokine responses are generally more resistant to re-infection and have lower worm burdens than those with a weaker type 2 response[29,36,38,39], indicating a protective role for type 2 immune responses in parasite clearance and resistance to re-infection. Notably, the proportion of ILC2s is observed to be decreased in the PBMCs of helminth-infected individuals compared to the increase of CD4+ $T_H2$cells[28,40], which is in contrast to helminth-infected mice whereby ILC2s are increased following helminth infection in the tissues and lymphoid organs[41–45]. It is possible that ILC2s migrate from the peripheral blood to tissue sites during infection. In addition, helminth infection in endemic individuals is chronic; hence, the initial innate ILC2 response might have subsided and been replaced by the adaptive T_H2 response, which is a different scenario from acute models of helminth infections in mice. Eosinophils by contrast are innate cells that increase in the blood, hence likely play a different role during helminth infection than ILC2s[46–48]. PBMCs of infected endemic individuals are also associated with increased T regulatory cell (T_{reg}) function, including increased expression of immune checkpoint markers and production of regulatory cytokines[31,32,49], which is especially prominent in children with high worm burden[39]. While these are general features for helminth infections, the different types of worms with different lifecycle and excretory-secretory products also result in a variety of responses that we shall consider below.

Helminth infections can be broadly separated into soil-transmitted intestinal helminths (including hookworm, the whipworm *Trichuris trichiura*, and roundworms such as *Strongyloides stercoralis* and *Ascaris spp*) from the tissue dwelling helminths that have intermediate hosts (e.g., insect vectors and snails) such as filarial parasites (e.g., *Brugia malayi* and *Onchocerca volvulus*) and flukes (e.g., *Schistosoma spp*). For hookworm infection, PBMCs of infected individuals are characterized by increased circulating Foxp3+ T_{reg} cells that express markers such as CTLA-4 and GITR, as well as cytokines such as IL-10, transforming growth factor β (TGF-β), and IL-17[31,32,35,49]. Cellular and cytokine response are often mixed with higher type 2 cytokines like IL-13 and IL-5, inflammatory mediators like TNF-α and Interferon-γ as well as regulatory cytokines like IL-10[34,36,49]. *S. stercoralis* infection is also associated with higher circulating levels of type 2 cytokines like IL-4, IL-5, IL-9, and IL-13 and lower levels of type 1 cytokines like IFN-γ and TNF-α, which is reversed following deworming in infected individuals compared to uninfected individuals[50]. Infection with

Ascaris lumbricoides is also skewed toward a type 2 response with increased production of type 2 cytokines in infected individuals[51,52]. In contrast, *T. trichiura* infection is characterized by a mixed immune response with skewing toward a regulatory and type 1 immune response[52]. More recently, we found that circulating levels of TGF-β were the strongest immune predictor of infection with *T. trichiura*[53].

For tissue dwelling helminths such as filarial nematodes, an increased proportion of CD4+ $CD25^{hi}$ T_{reg} cells in infected individuals is fairly typical[33,54] and increased proportion of IL-13 producing ILC2 cells has been observed[37]. In vitro assays indicated that these T_{reg} cells can suppress cytokine production and lymphocyte proliferative capacity, which may be important for protection[33,55,56]. For example, *O. volvulus* infection is characterized by T cell hypo-responsiveness to parasite antigen and an increase in regulatory response with the production of regulatory cytokines like IL-10 and TGF-β[57]. An increased proportion and expansion of adaptive T_{reg} cells is also observed during infection with *Schistosomes*[54,58,59]. For these parasites, the T_{reg} phenotype and proportion is correlated with the level of infection and age of the individual[58,59]. In vitro assays indicate that T_{reg} cells can suppress parasite-specific responses including lymphocyte proliferative capacity and cytokine production[60]. During schistosomiasis, cytokine responses are also mixed and dependent on the stage and chronicity of the infection, with T_H2 responses predominating during the chronic fibrotic stage of infection[30] and persisting even after the infection is cleared[61].

Hence, PBMC responses during infection with roundworms like *Ascaris spp* and *Strongyloides spp* are more skewed toward a type 2 response[50,51], while infection with tissue dwelling helminths is more mixed with predominantly a T_{reg} response, type 2 and type 1 immune response[34,54]. Cytokine profiles are also dependent on the age of the host and other factors including the chronicity of infection and co-infection with other pathogens[29,34]. Indeed, many factors contribute toward the heterogeneity of immune responses during helminth infections, including age of the individual during helminth infection[29,39,58,59,62], host genetic factors[63–68], presence of concurrent infection with another pathogen or parasite[34,52,53,62], stage of the infection (whether acute or chronic phase)[29,30,52,61], infectious dose and intensity[29,39,69,70], presence of underlying inflammatory and/or autoimmune condition[71,72], type of helminth parasite[46,48] and/or the micro and macro-environment of the individual[73]. The relative contribution and interaction between these variables remain difficult to clearly establish. One approach that we have started to take to study the relative contributions of genetic and environmental variables toward heterogeneity in immune responses is to utilize "re-wilded" mice[74,75], but that is a subject of a different review[76].

MUCOSAL IMMUNE RESPONSE DURING *TRICHURIS TRICHIURA* INFECTION

An individual self-infected with *T. trichiura* to treat his own symptoms of ulcerative colitis provided an opportunity to characterize intestinal immune responses during infection[71]. Consistent with previous reports[77–81], biopsies from tissues with *T. trichiura* were characterized by infiltration of eosinophils and lymphocytes into the lamina propria and submucosa of the tissues by histopathologic analysis. The infiltration of lymphocytes might be associated with an increase in plasma cells in the tissue sites during *T. trichiura* infection, as overall T cell numbers can be unaltered in the intraepithelial tissue during *T. trichiura* infection[78,82]. However, the nature of the T cells in the tissues is altered during self-infection. Biopsies from a healthy subject self-infected with *T. trichiura*, showed an increase in type 2, regulatory, Th22, and even Th17 response in the cecum and colon[72]. Remission of symptoms in the individual with ulcerative colitis following a new infection with *T. trichiura* was also associated with Th2 cytokine production (IL-4) and Th22 T cell response (IL-22). Notably, a prominent Th17 T cell response associated with neutrophilic infiltration during symptomatic flares of ulcerative colitis was reduced by a new *T. trichiura* infection cycle. Type 2 cytokines and IL-22 may be important for goblet cell hyperplasia, increased mucous production the repair of epithelial cells and intestinal tissues[71]. Goblet cell hyperplasia has been reported in the cecum of those with *T. trichiura* infection[77,81,82]. In addition to eosinophils, increases in mast cells in the subepithelial region of children infected with *T. trichiura* from rectal biopsy of lesions from parasitized individuals indicate that the release of histamine plays a role during *T. trichiura* infection in humans[83]. TNF-α is another cytokine that could synergize with type 2 cytokines to mediate resistance to *Trichuris* parasites[84]. Immunohistochemistry staining of the lamina propria of children with whipworm infection shows an increase in TNF-α positive cells. There is also increased secretion of TNF-α in cultures of colonic biopsies, as well as more TNF-α in plasma levels of infected children[85]. Foxp3+ T cell abundance is increased in inflamed tissues during *T. trichiura* infection[72], and in regions inflamed from colitis symptoms, but is not associated with infection above what is already observed in the context of ulcerative colitis[71]. Increased abundance of Foxp3+ T cells is often associated with any type of inflammation in the intestines but the regulation and function of different types of intestinal regulatory T cells is complex[86,87]. It should be emphasized that such studies on self-infected individuals are limited by the uncertain dosage and source of parasite material for these uncontrolled observations, as well as ethical considerations. Hence, the alternative of experimental human helminth infection studies is considered below.

IMMUNE RESPONSES DURING EXPERIMENTAL HELMINTH INFECTION CHALLENGE

Human helminth challenge infections with *Necator americanus*[88] and *S. mansoni*[89] are being conducted in countries where helminth infections are no longer endemic as a platform for testing vaccines and drugs, but also to understand the pathogenesis and immune responses to helminth infections. In addition, the pig whipworm *T. suis* has also been tested in human subjects as a therapeutic for inflammatory bowel disease and other autoimmune diseases[90]. For safety reasons, either low dosage of larvae for *N. americanus*, or male-only *S. mansoni* cercariae that do not produce pathogenic eggs[89] are utilized. Study volunteers are then cured with anthelminthic drug treatment after the studies, although some choose not to be treated. Since *T. suis* does not establish patent infection in humans, no treatment is necessary for studies with *Trichuris suis* ova (TSO). Many of these studies have been done in the context of autoimmune disorders[71,91–93] and many of these challenge infection studies have been recently reviewed[94–96]. As expected, a general feature is increased type 2 cytokines such as IL-4, IL-5, and IL-13 being made from cultured PBMCs from infected individuals compared to uninfected individuals[97,98] as well as increased eosinophilia in the peripheral blood[72,98,99].

There have been the most controlled human infection studies with the hookworm *N. americanus*, which provides opportunities to characterize biopsy specimens from the duodenum in a few examples. As expected, challenge infection is associated with eosinophilia[98–100], production of type 2 cytokines[97,98,100], but also other pro-inflammatory and regulatory cytokines like IL-2, IL-10, IFN-γ as well as IL-17A following stimulation of PBMCs isolated infected volunteers[97]. Consistent with these peripheral responses, mucosal responses at the infected tissue site in the gut mucosa has a mixed response made up of a strong type 2, regulatory and some type 1 responses[97]. An increase in mucosal cytokines of IL-23 and IL-22 was also observed[97] and intense eosinophilic

infiltration into the mucosa similar to natural infections[100,101]. Controlled infection studies were performed in the context of Celiac Disease, whereby infection was associated with a reduction in IFN gamma and IL-17 producing T cells and cytokines and the expansion of CD4+ FoxP3+ T cells expressing CTLA-4 with the production of IL-10 in the mucosal tissue sites[91,102]. This was also associated with duodenal eosinophilia[100].

For *S. mansoni*, controlled human infection is still at an early stage, with a study among naive volunteers in the Netherlands[89]; hence, the immunological analysis has only been done in this study. Despite the lack of pathogenic egg production because of infection with male cercariae only[103], some study subjects still experienced an acute inflammatory response known as Katayama syndrome[104], especially with a higher dose of parasites[89]. Nonetheless, there is interest in implementing this strategy to accelerate vaccine development in endemic countries such as Uganda[105]. As expected, there is seroconversion for IgM and IgG over time after infection and an interesting trend toward higher IgG1 levels in subjects with Katayama syndrome[89]. Indeed, volunteers that develop this acute symptom were associated with higher immune responses overall, including both Th2 cytokines, but also Th1 cytokines such as IFNg, IP-10, and MIP-1B. It is still unclear why some volunteers develop acute symptoms while others do not[89].

Challenge infection with pig whipworm eggs, TSO, which is well tolerated in humans with some success in treatment of inflammatory bowel disease has shown variable immunological outcomes in patients[106–111]. Sometimes, modulation of the Th1/Th2 balance and innate response is seen[107] and sometimes not[106]. In the most recent report, during infection with TSO, changes in the B cell responses and activation of the T cell pool were seen; however, no significant changes in the proportion of other innate cell population, and the balance between Th1/Th2 cell were noticeable following immunophenotyping of PBMCs by mass spectrometry[106].

Although epithelial cell changes such as goblet cell hyperplasia clearly occur during challenge infection, with the recent knowledge of epithelial cell responses critical in murine model of helminth infection[112], it will be interesting to determine whether tuft cell responses in humans exhibit similar biology. In addition to the small number of study subjects investigated, the systemic and mucosal immune response during challenge helminth infection is also complicated by heterogeneity of the underlying disease conditions that are being studied. Nonetheless, detailing the host response in great depth for a small number of subjects can still provide substantial insights into unique features of helminth infection on the human intestinal immune response.

NEW APPROACHES FOR STUDYING HUMAN MUCOSAL RESPONSES TO HELMINTHS

There are currently multiple technological improvements that have the potential to be transformative for our understanding of human mucosal responses. Here we highlight several that could be applied to helminth infections. Analysis of mucosal biopsies has been difficult partly because it requires the processing of fresh tissues that is time consuming and dependent on the time of clinical procedures. Hence, the development of approaches to immediately cryopreserve mucosal biopsy tissues[113,114] greatly expands the capacity for detailed immunophenotyping of tissue-resident cells, as well as the generation of intestinal organoids from individual patients for mechanistic experiments[115]. Being able to study epithelial cells in organoid systems close to a native state should enable investigating immune cell interactions with these cells in co-culture systems.

While single-cell sequencing technologies have yet to be fully applied to helminth infections in human subjects, it has already enabled the identification of many new cell populations in the intestinal mucosa during inflammatory bowel diseases and changed our understanding of complexity and heterogeneity of this disease[116]. As this technology continues to improve, sequencing does not just provide transcriptional profiles by RNA-seq, but can validate protein expression of molecules expressed on the cell surface by CITE-seq, as well as epigenetic states of the cells by ATAC-seq. Characterization of epigenetic state and transcription in the same cells will further enable dissection of transcriptional regulation at a molecular level. In addition, V(D) J sequencing enables the assessment of clonal expansion and BCR and TCR usage; hence, allowing us to gain unprecedented insights into immune response of different organ systems.

The intestinal immune system has been well characterized at a single cell level[117]. This includes samples from in utero intestinal developmental tissues[118], but has primarily centered on steady-state conditions and inflammatory bowel diseases[117]. There have been studies examining the response to the gut microbiota[119]; however, intestinal immune responses against eukaryotic pathogens remain to be explored by single-cell sequencing. Hence we have a good view of the cellular landscape of the human intestinal immune system at steady state and during IBD, but not for other types of intestinal infections including helminths. Nonetheless, the detailed atlas of immune and non-immune cells in the intestine already available at steady state, including the interactions with the neuronal system[120], will facilitate mapping any new data generated during human challenge infection studies onto a well-established road-map to identify novel signatures that are helminth specific. In mouse models of helminth infection, intestinal CD4+ T cells[121] and epithelial cells[122] have been examined, indicating interesting signatures of goblet cell, tuft cell, and T_H2 cell expansion and phenotypes, which would be important to confirm in a human setting.

Organoid technology is already improving our understanding of epithelial cell responses to helminth infections in mouse models[123], including the ability of helminths to regulate or inhibit appropriate epithelial cell responses[124], in combination with scRNA-seq[122]. The revolution in understanding of tuft cell biology driven by helminth infections has been driven by mouse intestinal organoids[5–7,125], but this has not yet transferred over to the human setting. Recently, a role for macrophage migration inhibitory factor in expanding intestinal tuft cells[126] has also been investigated using a combination of in vivo mouse models and organoid systems. As another example, caecaloids have been developed in mice[127] that can be used to examine early infection events by the mouse whipworm *T. muris*, which could potentially be replicated in the human system to study *T. trichiura* in an in vitro setting.

However, organoid structures for other tissues and organs have yet to be deeply exploited to study host interactions with helminth infections. Tumor organoids for example[128], have become sophisticated models of the tumor microenvironment, which can be combined with fibroblasts and immune cells to examine these complex interactions. There are many similarities between the immunoregulatory features of certain tumor microenvironments and the consequences of helminth infections[129], especially in the tissues. In combination with CRISPR-based gene modification[130], the functions of specific molecules in shaping the host-helminth interaction not just in the intestinal epithelium but in other organ systems can be examined genetically. In the rapidly expanding field of immune-oncology, tumor organoids are being used as models to optimize T cell responses against cancers, when combined with other cells from the tumor microenvironment[131]. There are exciting opportunities to apply such experimental strategies to study human responses to helminth infections, as such in vitro systems can now be generated from human biopsy materials and immune cells.

From the perspective of personalized medicine, biopsies obtained to produce patient-specific organoids for analysis could

potentially predict helminth mediated effects on inflammatory bowel disease patients, which have very heterogeneous responses to different treatment options. While such studies are still hypothetical, it is clear that the combination of reductionist in vitro systems such as the organoids, coupled with complex single-cell sequencing analysis of ex vivo patient samples, will have the potential to help us understand heterogeneity in human intestinal immune responses to helminth infections.

EFFECTS OF HELMINTHS ON MICROBIOME DURING NATURAL INFECTION

The microbiota plays a critical role in regulating the mucosal immune system and during intestinal helminth infection, these worms must interact with the gut bacteria and their metabolites. These complex interactions likely determine the establishment of helminth colonization, expulsion of the parasites, disease severity, and host immune-modulation. As we discuss the heterogeneity of human responses to helminth infection, it is important to discuss the role that interactions between the gut microbiota and helminths may play in this process. Understanding these interactions may provide new strategies for alleviating human morbidity resulting from helminth infections in endemic regions and emerging challenges such as anthelminthic drug resistance[132].

Most of the work in this area involves describing associations between intestinal helminths and fecal samples by 16S ribosomal sequencing analysis in endemic populations. These include cross-sectional ($n = 12$)[53,133–147] and longitudinal studies ($n = 10$)[148–159], but the conclusions are often divergent, due to differences in the prevalence of helminths and study population for each analysis (see below). A common feature observed from some cross-sectional studies is increased gut microbial diversity in helminth-infected individuals[133,138,142,144–146,151,153,159]; however, some other studies showed either reduced diversity[146] or no significant differences in microbial diversity in infected individuals[134,135,139,140,143,148,154]. A recent effort to conduct a meta-analysis suggested helminths that colonized the large intestine, i.e., *T. trichiura* and *Enterobius vermicularis* are more likely to increase microbial diversity and alter microbial composition[160]. While the bacteria associated with helminth infections varies between studies, some commonly reported organisms fall in the order Clostridiales[136,139,155], order Bacteroidales[136,138,155], family *Paraprevotellaceae*[144,151], family *Lachnospiracaea*[140,153], and *Bacteroides* enterotype[141,142].

As the gut microbiota varies across geography, ethnicity and mode of subsistence[161], in studies conducted in various continents including South America[53,141,157], North America[136,147], Africa[133,138,139,148,153,154,156], Europe[151], and Asia[134,135,140,142–146,149,150,152,153,155,158,159], it is not surprising to have divergent findings. In addition, studies have focused on different parasites, based on the study population including *T. trichiura*[133,144,149,155,157], *A. lumbricoides*[147], *S. haematobium*[139,156], *S. mansoni*[154] *S. japonicum*[134], *Strongyloides stercoralis*[151], *Clonorchis sinensis*[142], *Haplorchis taichui*[146], *E. vermicularis*[159], or mixed infections[136,138,140,141,143,145,148,150,152,153,158]. Differences in lifecycle and physical location in the intestine would likely affect the gut bacterial interactions[160]. Analyzing the composition of mucosal bacteria may be more informative for helminths (e.g., *A. lumbricoides* and hookworm) living in the small intestine.

Longitudinal studies to examine the effect of deworming on gut microbiota may provide more insight into cause and effect relationships, but are also potentially confounded by direct impact of anthelmintic treatment on the gut microbiota. While some studies observed differences in gut microbial diversity following deworming treatment[148,151,155], others observed no impact from treatment[152,154,157]. The microbiota may also affect treatment efficacy, as the treatment outcome of the combination of albendazole and ivermectin was associated with pre-treatment enterotype, with increased egg reduction in Enterotype 3, which is enriched with *Ruminococcus torques* & *Eubacterium coprostanoligenes*[158].

There may also be technical factors explaining the variability seen in these studies. For example, the bioinformatic approach used for the microbiota analysis can introduce technical limitations. Most studies utilize 16S rRNA sequencing[133–158], while a few have utilized shotgun metagenomics[138,140,153,158]. 16S sequencing only provides a portion of the gut microbiota profile[162] and does not provide genome data to infer microbial functional, which can be generated by metagenomics. However, shotgun metagenomic analyses are limited by available reference databases, which often do not include data from underrepresented groups that are helminth infected. Metagenomic data can provide insights into other eukaryotes, which is understudied. Such transkingdom interactions were investigated by Partida-Rodriguez et al. in a parasite-infected mother-child cohort study in a semirural community in Mexico[136]. Although intestinal helminth infection was not associated with other eukaryotes, there was a positive correlation between bacterial and other eukaryotic taxa (e.g., fungus *Candida* with *Bacteroides* and *Actinomyces*, *Bifidobacterium* and *Prevotella copri*)[136]. Questions surrounding interactions between helminths with viruses (virome), fungi (mycobiome), and archaea (archaeome) remain to be characterized and would be of potential interest in the field in future studies.

Despite the importance of helminths and the microbiota in regulating immune responses, there are just a few human studies that have examined this three-way relationship[53,138,149,150,159]. In Indonesia, Martin et al. observed that higher microbial diversity was associated with greater IFN-γ responses to PHA in helminth-negative individuals when compared to helminth-positive individuals[150]. There was also a negative association between proportions of Bacteroidetes and IL-10 responses to LPS in uninfected individuals and helminth infection diminished this effect[150]. Among an indigenous population in Malaysia, we found blood transcriptional profiles that were associated with *T. trichiura* infection[149]. In this study, serum zinc and iron levels were affected by helminth infection status, independent of dietary metal intake, and these serum zinc and iron levels were also associated with an abundance of specific microbial taxa[149]. A study in Cameroon also identified associations between cytokine responses, intestinal helminths, and the gut microbiota[138], whereas a study in Columbia identified circulating TGF-β as the strongest predictor of the *T. trichiura* egg burden in a cross-sectional study[53]. There has also been one study examining secreted IgA levels in *E. vermicularis* infected schoolchildren in Taiwan[159].

Overall, our understanding of the three-way interaction between helminths, the microbiota, and immune responses remains poor and most studies are restricted to the analyses of peripheral blood and stool samples from study participants. Metabolites are an important regulator of the crosstalk between the immune system and the microbiome[163,164], and while many mouse studies have uncovered a role for microbial metabolites in regulating responses to helminthes[27],this area remains understudied in human infection. For example, helminth infection is associated with increased short-chain fatty acid production and Clostridiales organisms that have important immunoregulatory properties[165,166], and additional studies are needed to firmly establish this relationship in human populations.

EFFECTS OF HELMINTHS ON MICROBIOME DURING CHALLENGE INFECTION

Controlled experimental challenge infections may be an approach to minimize confounding factors and determine more direct effects of helminths on the microbiome and mucosal responses. The hookworm, *N. americanus* infection studies have been

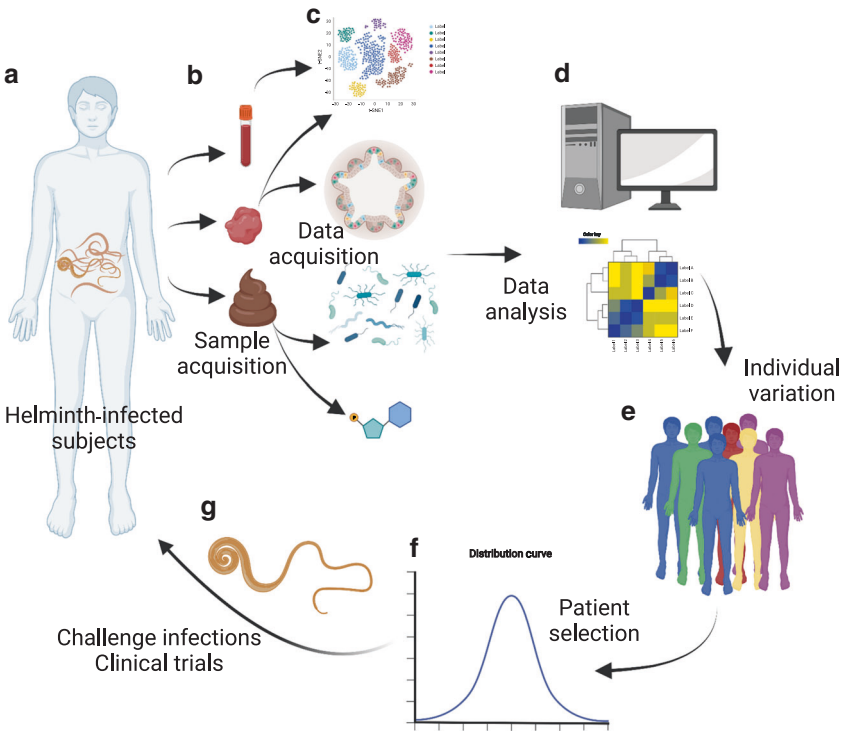

Fig. 1 Understanding the human immune response to helminth infections. A systems immunology approach toward understanding inter-individual immune variation to helminth infection can uncover new mechanisms for therapy and vaccination. Clinical research (**a**) on helminth infected subjects from well-characterized study cohorts with careful documentation of environmental metadata provides the basis for infrastructure required for appropriate sample acquisition (**b**). The development of successful freezing protocols has enabled biobanking blood and fecal samples, as well as more recently, tissue biopsy material, which can now by analyzed at a later stage (**c**) under state-of-the-art laboratory conditions. Spectral flow cytometry (>40 parameters), as well as single-cell-sequencing analysis, of blood and tissue samples enables detailed phenotyping of cellular composition and function but can also enable understanding of transcriptional regulation at a molecular level in combination with whole genome sequencing. Fecal samples can undergo metagenomic and metatranscriptomic sequencing analyses to determine microbial composition and function, as well as metabolomic analyses to identify metabolites that may influence the immune system of the subjects. **d** Data analyses by bioinformatics and computational biology approaches utilizing new algorithms for calculating effect sizes and visualizing large data sets can enable insights into drivers of inter-individual immune variation (**e**). Immune responses to helminth infections typically fall within a normal distribution curve (**f**) with individuals who fall at both tails of the distribution curve having the potential for pathology resulting from super infection or immune pathology. Understanding this immune distribution may enable better selection of subjects for clinical trials or challenge infection studies (**g**) that will minimize unexpected adverse events and maximize potential therapeutic benefits. Created with BioRender.com.

conducted on healthy individuals[167,168], participants with relapsing multiple sclerosis[169], and coeliac disease[170]. A study on 8 healthy volunteers infected with 20 L3 larvae found no major impact on the gut microbiota at 8 weeks post infection[167]. A more recent study on 20 healthy young volunteers with higher dosages (i.e., 50, 100, or 150) of L3 found that the bacterial richness increased significantly during the established infection phase (week 8–20), but not during the acute infection phase (trial week 0–8)[168]. As hookworm resides in the small intestine, these results are consistent with deworming studies indicating that these worms may not alter the gut microbiota significantly, especially in the first 8 weeks after the infection.

One of the goals for developing challenge infection models is to determine if helminth infection can have therapeutic benefits for autoimmune diseases[171]. A study on 24 relapsing multiple sclerosis patients infected with 25 L3 larvae and 26 patients on placebo treatment observed greater gut microbial diversity in infected individuals compared with the placebo group[169]. There were also significant differences in some bacterial taxa with putative immune-modulatory between groups[169]. In a rare study with access to biopsy material, 12 patients with coeliac disease were challenged with 20 L3 larvae and the microbiota of the duodenum tissue was analyzed[170]. There was greater microbial richness and diversity of tissue-adherent microbiota after exposure to *N. americanus*. Overall, infection with *N. americanus* may perhaps restore some microbial diversity to patients with inflammatory conditions, even if it does not have a large effect on the microbiota of healthy study participants.

SUMMARY

Helminths and the microbiota have co-evolved with their mammalian hosts to exist primarily under homeostatic asymptomatic conditions. Pathogenesis and disease morbidity arises when this homeostasis fails, which could be a result of heterogeneity in immune responses directed against the worms, composition of the microbial communities present, or genetic diversity in the worm population. We hypothesize that under steady-state conditions, the regulatory and the type 2 response, as well as some type 17 cytokines such as IL-22, helps maintain the mucus barrier to prevent bacterial translocation and increased inflammation. Conditions that result in either too strong or too weak a response against the parasites will result in aberrant inflammatory responses that are damaging to the host, either from an overwhelming parasite burden or collateral tissue damage. A better understanding of immune mechanisms that regulate this balance in humans is limited by our ability to access tissue samples, as peripheral blood is more easily characterized. However, recent advances in technology and clinical studies with challenge infections should begin to fill in many of the gaps in understanding

the differences between mechanisms at play in the human population, compared to what has been learnt from mouse models of infection. In addition, understanding how heterogeneity in human immune responses to helminth infections could be modulated by the gut microbiota will also be critical (Fig. 1).

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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