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# Immune System Investigation Using Parasitic Helminths

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## Keywords

helminth, transgenesis, neuroimmunology, animal models, lymphocytes, innate immunity

## Abstract

Coevolutionary adaptation between humans and helminths has developed a finely tuned balance between host immunity and chronic parasitism due to immunoregulation. Given that these reciprocal forces drive selection, experimental models of helminth infection are ideally suited for discovering how host protective immune responses adapt to the unique tissue niches inhabited by these large metazoan parasites. This review highlights the key discoveries in the immunology of helminth infection made over the last decade, from innate lymphoid cells to the emerging importance of neuroimmune connections. A particular emphasis is placed on the emerging areas within helminth immunology where the most growth is possible, including the advent of genetic manipulation of parasites to study immunology and the use of engineered T cells for therapeutic options. Lastly,

we cover the status of human challenge trials with helminths as treatment for autoimmune disease, which taken together, stand to keep the study of parasitic worms at the forefront of immunology for years to come.

## INTRODUCTION

Evidence of human parasitism with helminths traces back to ancient Egypt, with roundworm and blood fluke ova recovered from Egyptian mummies dated as early as 3200 BCE (1, 2). Genetic evidence indicates that helminth parasites have shaped the human genome (3). An analysis of 52 human populations across the globe indicated that when all other variables have been taken into account (e.g., diet, age, latitude), worms and ectoparasites have driven the greatest degree of polymorphisms in interleukin and interleukin receptor genes (4). This intimate coexistence between helminth and human highlights coevolutionary adaptation toward complex host mechanisms that damage, contain, and/or expel worms while also resolving inflammation and restoring affected tissues. In turn, many worm parasite species engage countermeasures that redirect, suppress, and evade host immunity such that human helminthiases are often chronic, and they affect over one billion individuals globally (5). Curiously, the reduced prevalence of helminth infection in regions of the world with optimal sanitation and health care access correlates with a high prevalence of allergy and autoimmunity (6). This presents a challenging conundrum. While there is a dire need for anthelmintic treatments and prophylactics, there is also an opportunity to harness helminth immunomodulatory capabilities as treatments for chronic inflammatory diseases. One could envision that the solution to both sides of this issue is an expanded understanding of how helminths impact their hosts' immune system. The purpose of this review is to highlight recent advances and future opportunities in helminth-host interactions that could potentially satisfy these needs.

## PHYLOGENETIC PERSPECTIVE

Nearly 300 species of helminths infect humans, and these species vary greatly in their genetics, body plans, and infectious cycles (3). Taxonomically, parasitic helminths comprise nematodes (roundworms) that include soil-transmitted helminths and filarial worms and platyhelminths (flatworms) that include trematodes like schistosomes and cestodes such as tapeworms (7). *Schistosoma* spp. and soil-transmitted helminths, including *Ascaris lumbricoides*, *Necator americanus*, *Ancylostoma duodenale*, *Trichuris trichiura*, and *Strongyloides stercoralis*, are the most common causes of helminthiasis worldwide; each infects hundreds of millions of people per year (8). Chronic parasitism with these organisms often results in cognitive and developmental impairment, fatigue, anemia, liver fibrosis, and overall debilitation. For example, lymphatic filariasis, characterized by impaired lymphatic function and debilitating lymphedema, and elephantiasis are caused by *Wuchereria bancrofti*, *Brugia malayi*, or *Brugia timori*, which collectively affected roughly 68 million individuals globally in 2019 (9). The filarial nematode *Onchocerca volvulus* establishes infection in the eye and is the causative agent of river blindness (5). While the anatomical niches inhabited by filaria pose unique challenges to the development of successful anthelmintic drugs and vaccines, study of these organisms also provides opportunity to discover novel pathways for host immunity and immunomodulation.

While mice serve as adequate hosts for some helminth species (e.g., *Schistosoma* spp.), many human helminths have a relatively narrow host specificity and only a portion of their life cycle can be studied in mouse models (10). Fortunately, many rodent-specific helminth species of filaria

**Table 1 Parasitic helminths of mice and humans**

| Common name | Human | Mouse | Experimental system |
|---|---|---|---|
| **Soil-transmitted helminths** | | | |
| Hookworm | *Ancylostoma duodenale* | *Heligmosomoides polygyrus* | + |
| | *Necator americanus* | *Nippostrongylus brasiliensis* | + |
| Roundworm | *Ascaris lumbricoides* | *Ascaris suum* | +/− |
| Whipworm | *Trichuris trichiura* | *Trichuris muris* | + |
| Threadworm | *Strongyloides stercoralis* | *Strongyloides stercoralis* | +/− |
| | | *Strongyloides ratti* | + |
| Trichina | *Trichinella spiralis* | *Trichinella spiralis* | + |
| **Filarial nematodes** | | | |
| Filarial worm | *Wuchereria bancrofti* | *Litomosoides sigmodontis* | + |
| Filarial worm | *Brugia malayi* | *Brugia malayi* | +/− |
| Filarial worm | *Brugia timori* | | − |
| Filarial worm | *Onchocerca volvulus* | | +/− |
| Eye worm | *Loa loa* | | +/− |
| **Schistosomes** | | | |
| Blood fluke | *Schistosoma mansoni* | *Schistosoma mansoni* | + |
| | *Schistosoma japonicum* | *Schistosoma japonicum* | + |
| | *Schistosoma haematobium* | *Schistosoma haematobium* | +/− |
| **Cestodes** | | | |
| Tapeworm | *Taenia* spp. | | +/− |

+ indicates suitable host for life cycle completion; +/− indicates partial life cycle or improper host tissue niche; − indicates unknown.

and gastrointestinal roundworms that have been adapted for laboratory maintenance share key features with human parasitic helminths. These rodent models of infection have been crucial for uncovering the basic tenets of mammalian immunity. A summary of these species and their human analogs is provided in **Table 1**.

Parasitic helminths have been instrumental in resolving scientific debates that have raged since the twentieth century over the critical components of immunity. Initial studies focused on B cell responses first described IgE production in the context of *Nippostrongylus brasiliensis* and *Schistosoma mansoni* infections in mice and humans, respectively (11), and the importance of IgG1 was firmly established in the context of nematode and cestode mouse models (12–14). Helminth models have solidified the role of the B-1 subset in protective IgM responses and provided convincing evidence for an IL-10-producing regulatory B cell (Breg) population. In fact, many of the major immunological discoveries of novel cell lineages and host effector pathways have come from parasitic helminth research (13, 15–18). *N. brasiliensis* and *S. mansoni* have been instrumental for the identification and/or confirmation of critical biological roles for group 2 innate lymphoid cells (ILC2s) (19–22); Foxp3$^+$ T regulatory cell (Treg) subsets; alternatively activated macrophages (M2) (23); T helper 2 (Th2), Th9, and Tr1 cells; and tuft cells (24–30). The discoveries from helminth model systems have certainly not plateaued, given the present trajectory of rapidly expanding computational investigative systems. In particular, the long-standing notion that nervous and immune systems are mechanistically intertwined is becoming manifest through the employment of helminth model systems.

## NEUROIMMUNOLOGY OF HELMINTH INFECTION

Neuroimmunology is an emerging field that has considerably advanced in recent years. The central nervous system comprises the brain and spinal cord, and the peripheral nervous system comprises autonomic and somatosensory neurons. While it has been recognized for some time that this peripheral innervation may interact with the immune system, the nature of these interactions has long been unclear. Recent studies have revealed soluble and cell-associated mediators of communication between immune cells and neurons during type 2 inflammation (31).

A considerable focus has been placed on how ILC2s communicate with neurons to orchestrate physiological changes in the intestinal and lung tissues during helminth infection (32) (**Figure 1**). Bidirectional communication between ILC2s and sensory nerve fibers in the skin, lung, and intestine is now apparent. Other cells types, including B cells (33) and macrophages (34), interact with neurons during infection, and a connection between mast cell activities and neurological function in the context of type 2 inflammation has been recognized (35). Thus, finely tuned circuits exist in which neurons promote or suppress immune responses, and immune cell–derived factors act on neurons to control neurological function (34).

### Neuronal Regulation of Immune Activation

Direct stimulation of sensory nerve fibers upon exposure to helminth-derived excretory-secretory (ES) products and alarmins like IL-25, IL-33, and thymic stromal-derived lymphopoietin (TSLP) elicits the release of an array of neuropeptides from neurons, including neuromedin U (NMU), vasoactive intestinal peptide (VIP), and calcitonin gene–related peptide (CGRP) (36–42). ILC2s express the receptors for such factors (43), and a variety of neuropeptides have been implicated in the regulation of ILC2 function during infection. Given the spatial arrangement of poised ILC2 populations relative to conventional $CD4^+$ T cell subsets in tissue niches pertinent to parasitism (e.g., skin, lung, intestine, bladder, and liver), it is perhaps not surprising that diverse ILC2 populations seem particularly tuned for neuronal communication.

Neuropeptides and their receptors can either activate or suppress ILC2 functions. In particular, activation of STAT5, STAT6, NF-κB, AP-1, and NFAT pathways in ILC2s has recently been shown to partly rely upon sensory neuron input (44). Mice lacking the NMU receptor Nmur1 have impaired ILC2 responses and decreased host protective immunity against *N. brasiliensis* infection. In this context, NMU induced a Gαq-dependent calcium influx and NFAT signaling cascade for optimal ILC2 activation (32, 44). Conversely, the $\beta_2$-adrenergic receptor ($\beta_2$AR) agonist epinephrine, CGRP, and nicotinic acetylcholine receptor agonists suppress ILC2 responses, with mice deficient in either $\beta_2$AR or CGRPR (CGRP receptor) having elevated ILC2 responses and lower worm burdens than wild-type controls (41, 42, 45, 46). In contrast, nicotinic acetylcholine receptor agonists suppress IKKα/β-NF-κB signaling to negatively regulate ILC2 effector responses (47), and epinephrine and CGRP act predominantly through Gαs to induce a cAMP-responsive gene module that promotes IL-5 production and suppresses proliferation and IL-13 production in ILC2s (37, 41, 42, 45).

ILC2s are only part of the immune cell–neuron cross talk. A variety of other immune cells are regulated by neuropeptides and by release of other neuronal factors. The neuropeptide substance P is required for cytokine responses and granuloma formation during murine infection with *Taenia crassiceps*, and it also promotes seizure during neurocysticercosis through actions on lymphocytes and mononuclear cells (48, 49). The neuron-derived mediator somatostatin can also have negative regulatory effects on the type 1–skewed responses necessary for parasite clearance during cysticercosis by regulating lymphocytes that express somatostatin receptors. Factors derived from

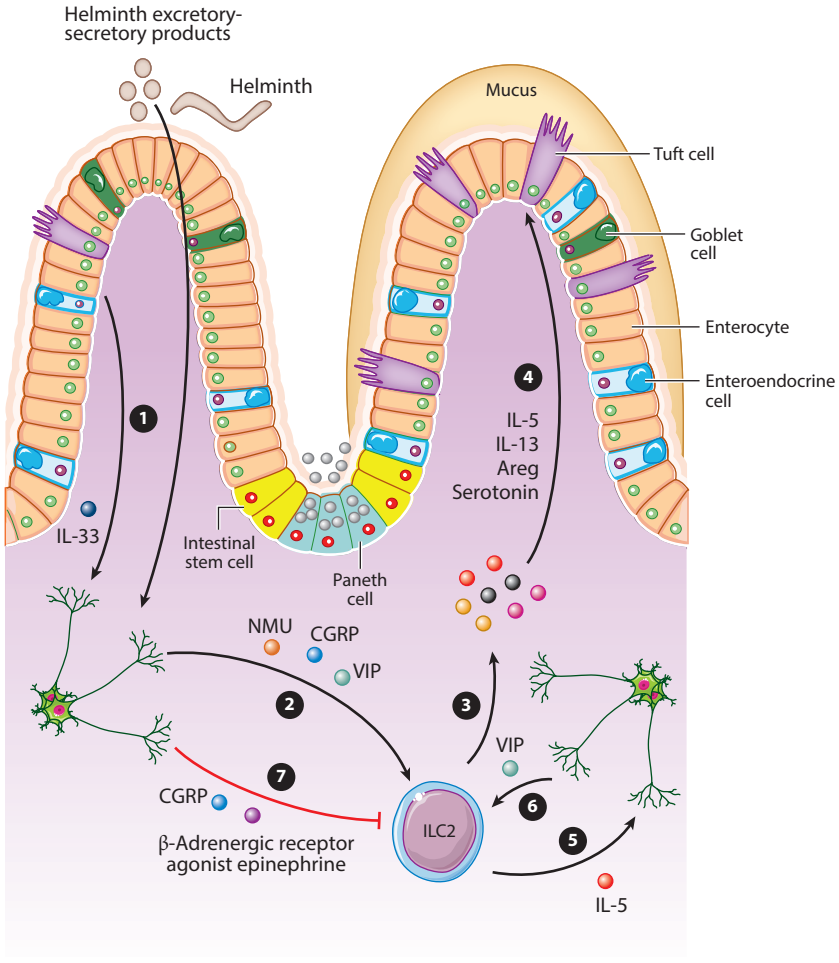


**Figure 1**

Neuroimmunology of helminth infection. During infection, helminth excretory-secretory products and associated alarmins like IL-33 can stimulate neurons (❶) to release a variety of regulatory factors such as neuromedin U (NMU), vasoactive intestinal peptide (VIP), and calcitonin gene–related peptide (CGRP) (❷). NMU, VIP, and CGRP can activate group 2 innate lymphoid cells (ILC2s) to produce factors such as IL-5, IL-13, amphiregulin (Areg), and serotonin (❸), which are critical for worm clearance and tissue repair (❹). Furthermore, these effector cytokines (❺) can also act on neurons to promote release of more proinflammatory factors like VIP (❻) that help to amplify the type 2 immune response. On the other hand, other neuronal factors like the β2-adrenergic receptor agonist epinephrine, CGRP, and nicotinic acetylcholine receptor agonists can also suppress ILC2s, thereby modulating type 2 inflammatory immune responses (❼). Therefore, the interaction between ILC2s and neurons during helminth infection is mostly bidirectional and can be either proinflammatory or anti-inflammatory. Figure adapted from an image created using Servier Medical Art and licensed under a Creative Commons Attribution 3.0 Unported License.

neurons can also influence macrophage responses through growth factors such as CSF-1 that promote macrophage survival (50), immunoregulation, and tissue protection (51). Substance P, VIP, and CGRP can also act on their cognate receptors on mast cells to stimulate degranulation during inflammation (52–54). Furthermore, neuronal factors can also influence T and B cell responses (55, 56), with neuropeptides like epinephrine and norepinephrine binding to receptors on the

surface of B cells (46) to influence the antibody response (57) and cellular migration and retention of lymphocytes from the lymph node (58).

### Immune Cell–Mediated Regulation of Neuronal Function

Sensory and other neurons express a range of receptors that allow them to respond to immune cell–derived factors, including neurotransmitters and cytokines. A recent study has shown that ILC2s can produce serotonin via the enzyme tryptophan hydroxylase 1 (59), though how ILC2-derived serotonin regulates neuron function remains to be fully explored. However, more is known regarding how cytokines affect neuronal function. For example, IL-5 derived from ILC2s can act on neurons to regulate their release of proinflammatory neuropeptides such as VIP, thereby amplifying the type 2 immune response (36, 39). Receptors for cytokines including IL-7, IL-12, IL-10, and IL-4 are expressed by sensory neurons such as the nodose ganglion that innervate the upper airway and lung tissues (60). Similar to the cross talk seen with other immune cell types, mast cell mediators like histamines and 5-HT can also interact with neurons to induce excitability of these cells, leading to the characteristic pain, itch, and irritability seen during inflammation (50, 61). Overall, how cytokines regulate neuronal-specific worm clearance mechanisms remains largely unexplored territory and demands further investigation.

### Opportunities and Future Directions

Helminth model systems could be used to attain novel insights into how immune cells govern the development, activation, and growth of peripheral neurons and biological functions of the central nervous system. In support of this idea, epilepsy is a known neurological comorbidity of neurocysticercosis (caused by the tapeworm *Taenia solium*) (62), and studies of this phenomenon could result in a better understanding of the immunological mechanisms that regulate epilepsy more generally. In addition, while much of the recent literature has focused on ILC2-neuronal interactions, there is a dearth of knowledge regarding how neurons interact with the expansive immune cell populations that monitor tissue- and organ-specific environments. Even less appreciated are the immune-stromal-neuronal networks that likely serve key roles for integrating signals from the mesenchyme. Such networks could be particularly relevant in chronic helminth infection model systems with parasite species like *S. mansoni*, where parasite eggs cause extensive tissue remodeling in diverse organs, including the liver, gastrointestinal tract, and lungs (63). Thus, models of helminth infection could be maximally leveraged to dissect networks of cellular communication across the host, the involved neurons and immune cells, and a variety of other cellular and molecular players currently unknown in humans.

## ILC2s AND HELMINTH IMMUNITY

Helminth parasites affecting different organs and mucosal barrier sites induce type 2 as well as type 1 and type 17 responses and both tissue-specific and systemic responses, depending on the helminth species (64). Systemic integration of these responses is often critical for host protection (65). In this context, ILC2s have emerged as hallmark cells that connect type 2 immune responses in different tissues across the body that are directly or indirectly exposed to helminths and their products.

ILC2s express various receptors, allowing them to respond to cytokines, lipid mediators, neuronal factors, and metabolites that are specific for tissue niches (66–69). For example, lung ILC2s express high levels of ST2, at least in the steady state, and respond preferentially to IL-33; skin

ILC2s express the receptors for and preferentially respond to TSLP and IL-18; and small intestine ILC2s are enriched for IL-25R expression and respond strongly to IL-25 (69). Lung and adipose stromal cells are considered key sources of IL-33 (70–72), and tuft cells in the intestine are critical producers of IL-25 during the response to hookworm infection (29, 73–75). Recent work shows that intestinal tuft cells are also sources of cysteinyl leukotrienes that promote ILC2 activity (76). Thus, ILC2 responses can be compartmentalized in different tissues during infection.

How we think about the functions of B and T cell subsets and ILC2 populations has been considerably impacted by the late William Paul and colleagues (77). Notably, Paul and colleagues provided the first demonstration that helminth infection induces interorgan trafficking of ILC2s, in which an activated KLRG1$^+$ inflammatory population in the blood and other tissues migrates systemically to provide host protection (78). In addition, *Trichinella spiralis*, a human gastrointestinal nematode, lacks a lung migratory stage but still induces a systemic mucin response that confers immunity in the lung to subsequent infection with lung migratory *N. brasiliensis* (79). This mucin response is absent in *Rag2*$^{-/-}\gamma c^{-/-}$ mice but not *Rag2*$^{-/-}$ controls, and S1P blockade during infection decreases the accumulation of KLRG1$^+$ ILC2s in the lung and blood, suggesting that migratory ILC2s drive protective mucin responses (79). Recently, Locksley and colleagues used reporter mice and fate-mapping approaches to show that locally activated ILC2s can disseminate systemically (80). Thus, while there are tissue-specific ILC2 functions, helminths initiate systemic ILC2 redistribution. Furthermore, while cytokines or eicosanoid lipids may play key roles in regulating this process (81, 82), the soluble and cellular mediators that control ILC2 migration require further study.

## IMMUNOMETABOLISM AND HELMINTH INFECTION

Mounting evidence indicates that helminth infections can enhance host metabolic fitness via regulation and cross talk between immune and metabolic pathways. In this context, normal metabolic processes governed by immune cells are repurposed during infection to protect the host. Helminth-elicited IL-33 and IL-25 responses promote ILC2s to maintain adipose tissue in a lean state (83–86) and support homeostatic eosinophil populations (87). Protection against obesity is mediated by an integrated mechanism wherein ILC2s elicit eosinophil and M2 macrophage responses, and the host repurposes this function during helminth infection to prevent or ameliorate metabolic disease (87, 88). Such mechanisms likely make tissue niches that house parasites less hospitable while promoting a high metabolic output to support cellular respiration to fight the infection.

Studies in animal models generally conclude that helminth infection is metabolically protective for the host (87–90), and a growing number of epidemiologic studies suggest that helminths may protect against development of type 2 diabetes and metabolic disease progression (91–93). A recent meta-analysis showed that individuals with a previous or current helminth infection were 50% less likely to have metabolic dysfunction (94), and an ever-expanding number of deworming studies suggest that this protection against metabolic dysfunction may be caused by worm infestation (95–99). The mechanisms by which worms may protect against metabolic diseases are not fully understood, but they may involve secretion of bioactive molecules that promote type 2 responses (88, 100) and/or alterations in the microbiome (101). One human clinical trial is being conducted to determine whether treatment with hookworms is beneficial in people at risk of type 2 diabetes (102). Of note, the recent finding that the type 2 cytokine IL-13 is central for muscle metabolic health and exercise performance opens up even further interesting avenues for research into potentially beneficial associations between helminth infections and host health (103).

Helminth infections in humans may also protect against chronic inflammatory disease susceptibility. This effect may be due to long-term reprogramming of immune responses that can occur during helminth infection. For example, we know that helminth-infected humans can have systemic, sustained ILC2 responses after the infection has been cleared (104), which may support resistance to reinfection (105). Conversely, modulation of ILC2s has been shown in Zimbabwean children infected with *Schistosoma haematobium*, where proportions of ILC2s were reduced during infection compared to uninfected controls but were restored following anthelmintic drug treatment (106). Thus, while helminths and their secreted products are touted as potential therapies for inflammatory and allergic diseases due to immunomodulatory and suppressive effects (107–109), further studies are required to fully understand how worms affect human immune responses and physiology long-term, particularly in tissues. Although a limited number of studies have assessed helminth-induced responses within the human intestinal mucosa (110), the ethical and technical difficulties surrounding invasive sample collection of human mucosal tissue emphasize the importance of studying accessible tissue from the skin, buccal and nasal cavities, and bodily fluids like urine and bronchoalveolar lavage fluid. By maximally leveraging mass cytometry and advanced flow cytometry to analyze these specimens, it will be possible to investigate how helminth infection programs tissue-specific immune responses and affects susceptibility to metabolic, allergic, and inflammatory diseases in humans.

## CONTROLLED HUMAN HELMINTH INFECTIONS

Experimental human infection is an emerging paradigm for studying the immune responses generated by exposure to a specific pathogen under controlled conditions and has the potential to expose novel tenets of human immune activation, immunoregulation, and development of immune memory (111). Controlled human infection (CHI) protocols for helminths are standardized (111) at various research institutions worldwide and are a valuable tool for studying host-pathogen interactions and pathogen-specific immune mechanisms (112). Only a few CHI studies with gastrointestinal helminths have been conducted with delineating immunology as a key objective. They have been limited predominantly to measuring infection-induced cytokines, antibodies, and peripheral blood cellular expansion (110, 113, 114). CHI studies have been used primarily for three objectives: (*a*) to improve mechanistic understanding of immunity and infection (110), (*b*) to develop candidate drugs and vaccines and test their efficacy for regulatory approval (112, 115), and (*c*) as an experimental treatment for chronic inflammatory diseases (116). Major CHI models with helminths are summarized in **Table 2**, specifically models for hookworm and pig whipworm challenge that have been used since the 1970s (113, 117). Other CHI studies involve self-infection and have been recently reviewed (109).

## HELMINTH THERAPY

CHI in helminth therapy clinical trials is useful for interrogation of the potential clinical utility of live helminth species for treatment of human disease. Helminth therapy has been used for chronic inflammatory diseases, including inflammatory bowel disease (IBD) (118, 119), celiac disease (120, 121), multiple sclerosis (MS) (122, 123), allergic asthma (124), and allergic rhinitis (125), with varying degrees of efficacy depending on the helminth species, study design, and target condition (**Table 2**). Helminth therapy studies in humans will provide unique opportunities to analyze clinical therapeutic outcomes alongside exploratory immunological studies. This will advance the translational potential of worms and their secreted products and facilitate cutting-edge immunological analyses in carefully controlled clinical settings (**Figure 2**).

**Table 2 Experimental models of human helminth infection**

| Model details | Clinical uses (example references) | Active or recently completed clinical trials, location (identif. code) |
|---|---|---|
| **Hookworm (*Necator americanus*)** | | |
| Infection initiated by percutaneous administration of 10–50 L3 larvae. Infection terminated by chemotherapy with mebendazole or albendazole. | Kinetics and variability (207)<br>Immunology (110, 113, 114, 119)<br>Vaccine development (112, 115)<br>Helminth therapy for celiac disease (120, 121, 136, 208), allergic asthma (124), Crohn's disease (102), and metabolic disease (209) | Baylor College of Medicine, George Washington University (NCT01940757)<br>Baylor College of Medicine, George Washington University, NIAID (NCT03172975)<br>Leiden University Medical Center (NCT03702530)<br>Leiden University Medical Center (NCT03126552)<br>James Cook University (multicenter study) (NCT02754609)<br>James Cook University (ACTRN12617000818336)<br>QIMR Berghofer Medical Research Institute (ACTRN12617001007325)<br>Malaghan Institute of Medical Research (ACTRN12619001129178) |
| **Pig whipworm (*Trichuris suis*)** | | |
| Repeated (eight times) oral administration of ~2,500 fertilized eggs. Infections clear without intervention. | Pilot studies (143, 144)<br>Helminth therapy for allergic rhinitis (210), ulcerative colitis (122), Crohn's disease (122, 131), multiple sclerosis (123), and autism spectrum disorder (155) | No currently active trials |
| **Schistosomiasis (*Schistosoma mansoni*)** | | |
| Infection initiated by percutaneous administration of 10–30 male cercariae in water. Infection terminated by chemotherapy with praziquantel. | Pilot studies (211) | No currently active trials |

Abbreviation: NIAID, National Institute of Allergy and Infectious Diseases.

## INFLAMMATORY BOWEL DISEASE AND CELIAC DISEASE

IBD comprises inflammatory conditions of the gastrointestinal mucosa, including ulcerative colitis (UC) and Crohn's disease (CD) underpinned by immunologically distinct mechanisms. $CD4^+$ Th1 and Th17 responses effectuate CD, while UC is associated with aberrant Th2 responses (126). The pathogenesis of IBD includes impaired tight-junction formation and intestinal epithelial barrier function, dybiosis of the gut microbiota, and mucus production (127, 128). The first clinical trials involving helminths in CD or UC patients used pig whipworm eggs [*Trichuris suis* ova (TSO)] because of the perceived safety due to attenuated survival in humans. Administration of TSO to CD ($n = 4$) and UC ($n = 3$) patients in an open-label study provided temporary clinical benefits that could be sustained with repeated administration (129). This was substantiated in a larger ($N = 54$) follow-up placebo-controlled study (TSO treatment $n = 30$, placebo $n = 24$) (130). However, a more recent study of 252 CD patients found no clinical benefit of

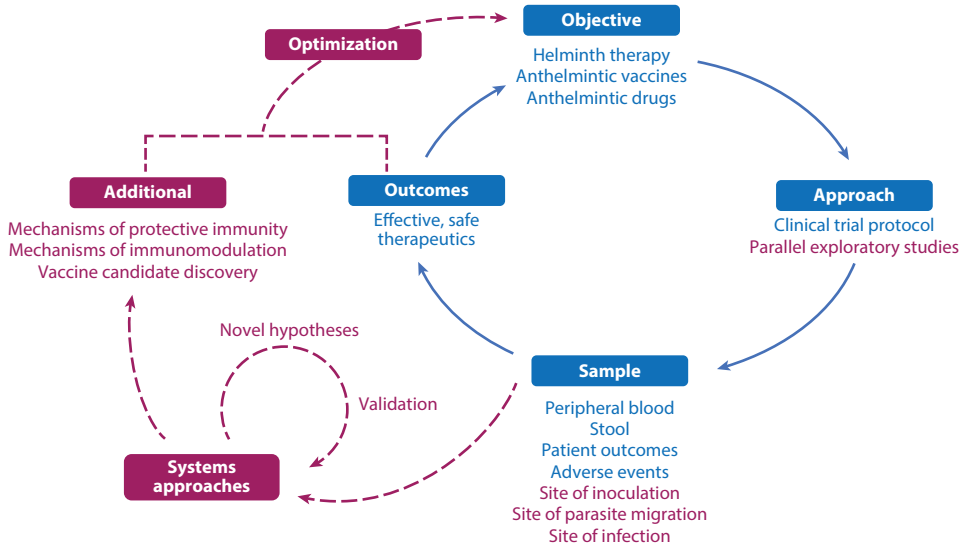


**Figure 2**

Applications and potential for experimental helminth infection. The principal objective for controlled human infection (CHI) trials with helminth infection is to develop anthelmintic vaccines and drugs as well as helminth therapies for chronic-inflammation-associated disorders (CIADs). The current approach for CHI trials is shown in blue. Typically, samples and data collected from CHI trials include peripheral blood, stool, patient outcomes, and adverse events. These are used to generate data on the safety and efficacy of the vaccine or drug being tested or on the use of helminth infection to treat the CIAD in question. There is significant capacity to increase the potential of CHI trials with helminth infection by performing parallel exploratory studies. Shown in purple, exploratory studies require additional sample collection. Sample sites include the site of inoculation (e.g., sampled by skin biopsy), the site of parasite migration (e.g., sampled by bronchiolar lavage), and the site of infection (e.g., sampled by duodenal biopsies). An unbiased systems approach to generate new hypotheses followed by validation studies will yield additional advancements, including mechanistic understanding of protective immunity and immunomodulation as well as vaccine candidate discovery. This progress toward our principal objectives will inform the rational development of anthelmintic therapeutics and helminth therapies.

TSO compared to control treatment (131), suggesting that the protective effect of TSO is modest and may not be sufficient to limit pathology in CD patients where the disease etiology and intestinal inflammatory milieu may differ between patients. Further, TSO is not a natural human pathogen and thus may not have evolved strategies to suppress human inflammatory responses in IBD. The lack of immunological investigation and reliance solely upon clinical scoring of disease severity in these TSO trials preclude strong conclusions on why some patients did not respond to the treatment. Detailed immune characterization has been performed in two studies. In the first, a UC patient infected himself with the human gastrointestinal nematode *T. trichiura* (132), and in the second a healthy person treated himself with TSO (133). The former landmark study involving self-infection with *T. trichiura* showed clinical improvements associated with expansion of IL-22-producing $CD4^+$ T cells, mucus expression, and increased intestinal mRNA transcripts for carbohydrate and lipid metabolism genes (132). In the case study with TSO, treatment resulted in increased mRNA expression of *IL4*, *IL10*, *IL17*, *TGFB*, *FOXP3*, *GATA3*, and *RORC* in the intestine, consistent with induction of mixed Th responses (133). However since this study involved a healthy volunteer, no associations between helminth-evoked immune responses and clinical outcomes can be established.

The human hookworm *N. americanus* possesses distinct advantages for CHI due to its ability to induce infections lasting multiple years following a single low-dose inoculation that causes limited pathology in well-nourished people (134). In parallel with the original TSO IBD study, an open-label study in patients with CD treated with *N. americanus* showed similar positive outcomes; however, the study was small and involved cotreatment with other IBD therapeutics (119). Perhaps the most impactful studies on this topic are those that focus on people with the gluten-driven gastrointestinal autoimmune condition celiac disease (135). These clinical studies have been partnered with immunological investigations at the nidus of infection that have advanced understanding of helminth-mediated immunomodulation (110, 120, 121, 136). Initial placebo-controlled studies revealed that hookworm-infected patients have decreased production of the inflammatory cytokines IFN-γ and IL-17 from intestinal immune cells (120) but do not show reduced clinical symptoms if given a high-dose gluten challenge (120). On the other hand, a small follow-up open study ($N = 12$) that involved gradual dose escalation of gluten in the diet revealed highly significant protection against clinical symptoms accompanied by expansion of mucosal $FOXP3^+$ Tregs and reduction of intestinal T cells expressing IFN-γ (121). A larger randomized controlled trial was recently completed (NCT02754609) (**Table 2**) and found modest improvements in symptoms in hookworm-infected participants; however, hookworm infection was not better than placebo for protecting against intestinal pathology following moderate gluten challenges (137).

## ALLERGIC DISEASES

Epidemiological studies in human populations have suggested that infections with helminths are associated with lower rates of allergic diseases (138–140), which has been validated in deworming studies (141, 142). Consequently, helminth therapy for allergic airway inflammation, including asthma (124) and allergic rhinitis (hay fever) (143, 144), has been trialed using TSO or hookworm. In these trials, helminth therapy did not have any significant therapeutic effect compared to the relevant placebo control treatment, although there were trends toward improvements in some parameters in the hookworm asthma study. In each of these studies, infections did result in increased eosinophilia (124, 143), and infection of allergic rhinitis patients with TSO resulted in increased parasite-specific humoral responses (143). Given the striking similarity between prototypical type 2 inflammatory responses generated by allergens and those generated by helminths, the mechanistic explanation for this ameliorative effect of worms on allergic disease may not be readily apparent, unless one focuses on the strong immunoregulatory effect involving myeloid suppressor cells and Breg and Treg subsets. Indeed, animal models of helminth-allergy interactions suggest that the mechanism may involve immunoregulatory cell expansion (107), changes in the microbiome (145), or direct suppression of proallergic responses by helminth-secreted molecules (146–148). Future efforts should further explore the development of novel worm-secreted molecules that exhibit functional activity against human immune cells, with a view to human clinical trials with these molecules. Immunomodulatory soluble mediators derived from helminth ES products include a vast array of microRNAs, glycoproteins, and other types of extracellular vesicle cargo. While the biology of extracellular vesicles from hosts and parasites is a rich, unexplored area of scientific inquiry with considerable potential for immunological advancement, this topic has been thoroughly reviewed elsewhere (108). Further, the exploration of the helminth-altered microbiome for specific bacterial communities or metabolites that are associated with protection against allergy may lead to novel probiotic therapies (149). Lastly, the allergic march toward diseases such as asthma and food allergy occurs early in childhood; however, all of the human clinical studies have involved only adults. It is plausible that helminth-based therapies are most effective

during childhood; therefore, some consideration should be given to the possibility of conducting helminth challenge trials in children.

## MULTIPLE SCLEROSIS

MS is a chronic neurodegenerative disease characterized by dysregulation of the blood-brain barrier and formation of focal plaques, areas of demyelination with lymphocyte infiltration and inflammation (150). Naturally acquired helminth infections of MS patients living in endemic regions led to reduced pathological score and frequency of disease exacerbations accompanied by reduced production of inflammatory cytokines (IL-12 and IFN-γ) and increased production of regulatory cytokines (IL-10 and TGF-β) by myelin basic protein–specific T cells (151). Follow-up work demonstrated that these beneficial effects in MS patients are ablated following anthelmintic drug treatment (152). Three phase 1 studies have employed TSO therapy for MS where eosinophilia was elevated (122, 153, 154). In one study, serum levels of the type 2 cytokines IL-4 and IL-10 were increased in 80% of trial participants (122); however, a different study did not reveal significant changes in gene expression of cytokines and T cell lineage–specific transcription factors (153). In the most recent study, *T. suis*–specific IgG1 and IgE were evident during treatment, as well as an increase in $FOXP3^+$ Tregs (154). While TSO has an excellent safety profile and is associated with promising trends of clinical improvement, the interindividual variation, small sample sizes, and lack of placebo controls preclude strong conclusions. A recent placebo-controlled study involving hookworm treatment for relapsing MS had similarly inconclusive results, but infected individuals tended to have fewer relapses, which was associated with expanded peripheral Treg populations (123). Nonetheless, these studies highlight an unchartered frontier: the use of helminths and/or their ES products for therapeutic interventions that operate through endogenous immunoregulatory mechanisms. Some recent progress has been made with a proof-of-concept TSO trial for autism, a related inflammatory condition of the nervous system (155). In future studies, researchers should consider exploring the potential benefit of helminths for neuropsychiatric disorders with an immunological basis, such as depression, anxiety, and schizophrenia, where systemic low-grade type 1 inflammatory responses are implicated in disease progression (156). Considerable investigation is warranted to identify the precise parasite-disease combination.

## CHIMERIC ANTIGEN RECEPTOR T CELLS

Can chimeric antigen receptor T (CAR-T) cell technology be used to understand chronic parasitism or even redirect it toward favorable outcomes for the host? CAR technology involves molecularly fusing the variable heavy ($V_H$) and variable light ($V_L$) domains of an antibody in a single-chain variable fragment (scFv) format to the intracellular signaling domains of molecules involved in T cell activation and costimulation. Typically introduced into T lymphocytes, CAR molecules are fusion proteins that combine parts of T cell receptor activation machinery (CD3 ζ chain) and costimulatory molecules (such as CD28 or 4–1BB) with the antigen-binding domains of antibodies or receptor ligands (157). CAR-T cells redirect the specificity of T lymphocytes toward new targets, specified by the extracellular antigen-recognition domains, and produce potent inflammatory reactions in response to target cells, including cytokine secretion, proliferation, and cytolysis; these are, importantly, independent of MHC.

T cells are the primary vector for CAR technology, and CAR technology has predominantly been used as a treatment option for cancer. However, it has also been evaluated in other effector immune cells, such as natural killer cells (158), macrophages (159), and Tregs (160, 161). CAR-Tregs specific for $HLA\text{-}A2^+$ MHC-I are currently under clinical investigation as a means

to prevent HLA-A2$^+$ donor kidney rejection in HLA-A2$^-$ recipients (162). In addition, recent advances have broadened the application of CAR-T cell therapies to target antibody-mediated autoimmune disease (163), cellular senescence (164), HIV-1 infection (165–167), and opportunistic fungal infections (168). Therefore, the umbrella of CAR technology is broadening to additional indications, including infectious disease. Considerations for expanding this application include the type of immune response required to treat a particular disease (proinflammatory or anti-inflammatory), the antigen(s) to be targeted and available targeting reagents, the required durability of the response, and the potential for off-target toxicities. Perhaps the design of CAR-T cells specific for conserved parasite epitopes will allow expansion of durable Treg subsets (e.g., GATA3$^+$ Tregs) for treatment of autoimmunity in the absence of intact parasites.

## NEW PROSPECTS FOR DISCOVERY—GENE SILENCING, EDITING, AND TRANSGENESIS IN HELMINTHS

Genetic gain-of-function and loss-of-function studies have been fundamental for understanding host immunological mechanisms, and tools for the genetic manipulation of various vertebrate and invertebrate species have seen radical advances in recent years. Unfortunately, many of these approaches have remained untenable for use in parasitic worms. Parasitic helminths typically require passage through a mammalian host involving multiple tissue-specific cues for proper development. Some helminths (e.g., filarial nematodes, schistosomes) require additional intermediate hosts to generate the infective stages that enter the definitive mammalian host where sexual reproduction occurs. Such life cycle complexity creates unique challenges for genetic manipulation through introducing novel genetic material for stable transgene expression. This is further complicated by the need for drug-mediated selection pressure to isolate successfully targeted clones. If achieved, however, successful manipulation of the parasitic helminth genomes and transcriptomes would open the door to multiple discoveries, including (*a*) novel host cell lineages and immunological pathways, (*b*) pathogen-derived virulence factors, (*c*) immunomodulatory molecules, (*d*) ligands for host pattern recognition receptors, and (*e*) how to use helminths for drug delivery. This section outlines recent advances in parasitic helminth genetic modification and discusses avenues of innovation and investigation.

Among the many challenges to genetic manipulation of parasitic helminths is the delivery of a sufficient amount of genetic material through the helminth body wall without causing worm mortality. One way that this obstacle has been overcome is through microinjection, a technique that was first developed for gene delivery into the free-living nematode *Caenorhabditis elegans* (169). Microinjection has since been adapted to parasitic helminths of mammals, including *Strongyloides* and *Parastrongyloides* (170, 171). While this method has proven useful, it requires tremendous investment and specialized equipment. Electroporation, a more accessible approach, has been effective at delivering genetic material into helminths. This method has been used to introduce plasmid DNA, mRNAs, and small interfering RNAs that can mediate gene silencing in *S. mansoni* (172–174) and the sheep parasite *Trichostrongylus colubriformis* (175).

The ability to deliver and transiently express nucleic acids in parasitic helminths has significantly enhanced our understanding of the roles of various genes in development and physiology. Some of the earliest studies to utilize RNA interference (RNAi) targeted *SmCB1*, the gene encoding cathepsin B, to discern its role in *S. mansoni* biology (176). While cathepsin B was hypothesized to be crucial for hemoglobin digestion, suppression of its expression by electroporation of a *SmCB1* double-stranded RNA revealed that this enzyme has little impact on hemoglobin catabolism but is crucial for larval development (173). RNAi- and DNA-mediated expression of dominant-negative mutations in *S. mansoni* has also revealed a role for SmMef2 as a regulator of

the Wnt pathway and for the schistosome CBP/p300 homolog *cpb1* in cell turnover and tissue homeostasis (177).

Another application of RNAi has been to resolve the mechanism of action of anthelmintic drugs. The drug oxamniquine was previously used to treat *S. mansoni* infections, until resistance to this drug evolved in the 1970s, but the gene(s) mediating susceptibility versus resistance remained unknown for some time. Through genetic and biochemical analyses, Anderson's group (178) identified a sulfotransferase enzyme (SmSULT-OR) that activates oxamniquine in its proform and that is mutated in a resistant *S. mansoni* strain. Using RNAi, this group demonstrated that knockdown of SmSULT-OR in susceptible *S. mansoni* is sufficient to confer resistance to oxamniquine treatment (178). Even more recently, RNAi screens have been employed to identify new genes in an unbiased manner, for example, those involved in tegumentary development (179). These applications, in addition to advancing our knowledge of parasitic helminth biology, have elucidated novel targets for new anthelmintic drugs and therapeutics, but clearly these technologies could be adapted to learn more regarding antigen-specific immunity using model antigen systems.

While transient transformation of parasitic helminths is a powerful tool, the ability to generate stable lines that maintain germline transmission of transgenes would be a tremendous boon for infection studies. This goal has been especially challenging, as it requires a method for integrating genetic material into the parasite genome. Two methods for genome integration into parasitic helminths were developed in 2012: one involving retroviral vectors in *S. mansoni* (180) and the second employing random, high-copy-number genome insertion into *Strongyloides ratti* using the *piggyBac* transposase (181). However, the advent of CRISPR/Cas9 technology stands to further increase the feasibility of this process and allow for more targeted gene insertions, deletions, and mutations. Heritable mutagenesis via CRISPR/Cas9 was first reported in *S. stercoralis* and *S. ratti*, where it was used to disrupt the twitchin gene *unc-22*, a gene that has been successfully mutagenized with an overt motility phenotype in *C. elegans* (182). More recently, this technique has been applied in *S. mansoni* and *Opisthorchis viverrini*. Cas9-mediated deletion of *S. mansoni* T2 ribonuclease ω1, an egg-secreted protein known to induce Th2 responses, significantly reduced the ability of schistosome egg antigen (SEA) to induce IL-4 and IL-5 production by Jurkat cells cocultured with SEA-pulsed macrophages (183). In the liver fluke *O. viverrini*, growth factor granulin (*Ov*-GRN-1) has been shown by RNAi to mediate enhanced host wound closure in biliary duct epithelial cells. Cas9-mediated knockout of *Ov*-GRN-1 reduced the capacity of *O. viverrini* ES products to enhance wound closure in a monolayer scratch assay and ameliorated periductal fibrosis during infection (184). Importantly, both studies introduced Cas9 and plasmid DNA or guide RNA via electroporation, and neither report stated whether transfectants were capable of germline transmission. Moreover, in both models, the efficiency of deletion and frequency of off-target effects stand to be more thoroughly evaluated and optimized. However, these innovations constitute a major advancement for helminth transgenesis and have already demonstrated the utility of the CRISPR/Cas9 system for studying host-parasite interactions.

Moving forward, there are a number of immunological questions that can be addressed using helminth gene manipulation, as is summarized in **Figure 3**. Gene deletion and mutagenesis can be used to better understand the role of known helminth products in virulence, host sensing of helminths, and immunoregulation. Additionally, CRISPR/Cas9 or RNAi-mediated screens should be employed to identify new helminth products involved in these processes. Further, transgenesis could be used in helminth immunology, as it has been employed in other infectious disease fields (185–187) to study mechanisms used by antigen-specific lymphocytes to function in tissue-specific niches. By engineering pathogens to transgenically express known model antigens such as ovalbumin or the Eα peptide variant 2W1S, we can identify and study antigen-specific $CD4^+$ T cells and B cells during helminth infection. The ability to perform targeted mutagenesis

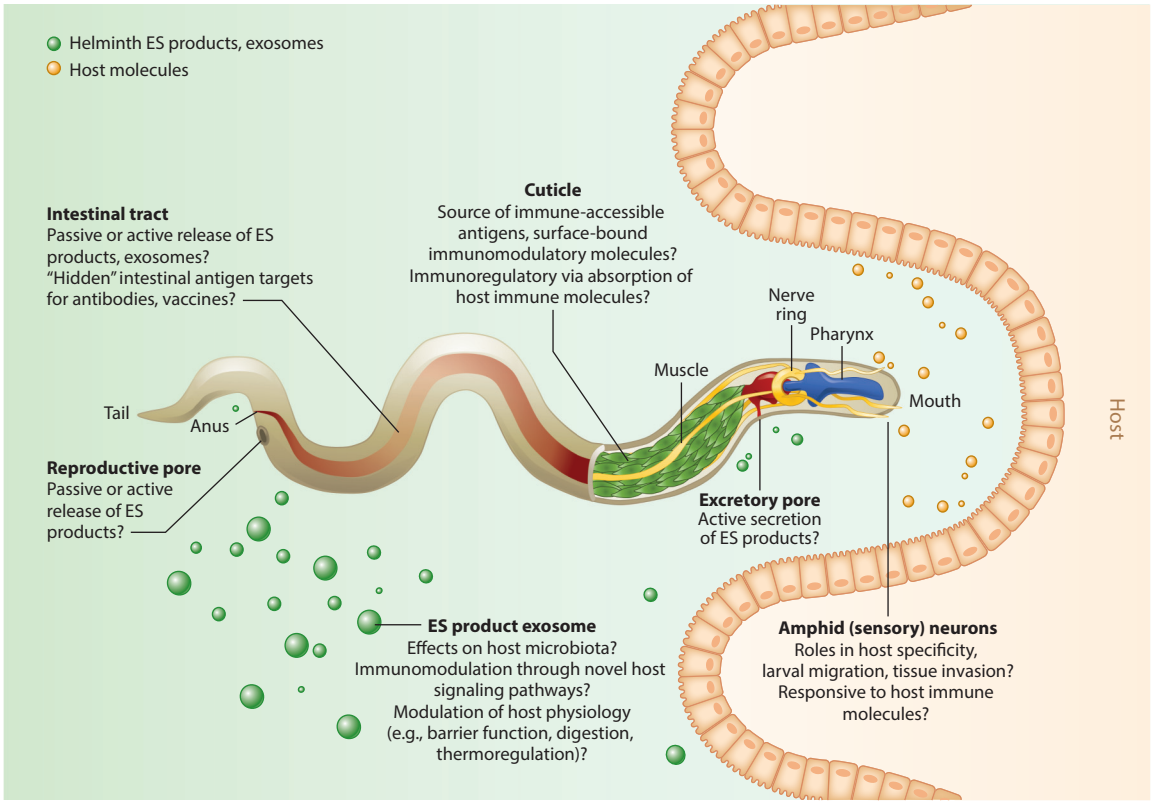


**Figure 3**

Anatomic considerations for targeted transgenesis in parasitic nematodes. Directed transgenesis of parasitic nematodes using tissue-specific promoters would allow for the interrogation of the role of different host immune cells in host-parasite interactions during infestation of tissue-specific niches. Diagram shows regions within gastrointestinal nematodes where tissue-specific promoters exist and the unanswered immunological questions that could be addressed. Abbreviation: ES, excretory-secretory.

with CRISPR/Cas9 also introduces the possibility of targeting model antigen expression to different helminth compartments or life cycle stages, which would present the host immune system with an evolving context-specific antigen pool in tissue-specific microenvironments. Even the generation of transgenic, fluorescent protein–expressing parasitic helminths would establish a powerful approach for visualizing how helminths interact with their hosts in vivo using live imaging or ex vivo using tissue-clearing methods and confocal microscopy. Altogether, these advancements in helminth transgenesis are just beginning to influence immunological studies and stand to completely revolutionize the way immunologists understand host-pathogen interactions.

## NEW FRONTIERS FOR MODELING HELMINTH IMMUNOLOGY

Much of what we understand about helminth immunity has been uncovered in mammalian models of helminth infection, particularly rodent models. Understandably, these models have been widely used because of their genetic, molecular, and cellular similarities to humans and the availability of species-specific reagents and genetic tools. However, helminth infections are widespread throughout the animal kingdom, affecting other vertebrate as well as invertebrate species. Moreover, many features of mammalian immunity are conserved in nonmammalian

vertebrate and invertebrate model organisms, and different tools exist to study specific questions in greater detail (**Figure 4**). Therefore, we propose that studying host responses across a wider evolutionary range will facilitate our understanding the immune system in greater detail.

## Insect Models of Helminth Infection

Compared with rodent models, invertebrate insect model systems have a number of advantages: They are cheaper to maintain, have short life cycles, and rapidly reproduce, and protocols involving insects currently do not require the approval of an animal care and use committee. Additionally, many insect models (e.g., *Drosophila melanogaster*, *Aedes aegypti*) are amenable to RNA-mediated gene silencing, making them useful for genetic studies of immune mediators. The cells of the insect innate immune system are called hemocytes, and they circulate freely in the hemolymph (188). Hemocytes have been classed into different cell types in different insect species, but there are three broad classes defined in the fruit fly *D. melanogaster*: plasmacytes, phagocytes that comprise a majority of hemocytes; crystal cells, which are involved in antimicrobial responses and wound repair; and lamellocytes, which are only readily seen upon infection or injury and are responsible for encapsulating pathogens (188). In addition to cellular immunity, insects also have well-described humoral immune responses involving antimicrobial peptides (AMPs) (189, 190) as well as thioester-containing proteins similar to those of the vertebrate complement pathway, which recognize and opsonize pathogens and mediate microbial phagocytosis (191). Some signaling pathways involved in innate recognition of pathogens in vertebrates are conserved in insects, including the Toll signaling pathway that was first described in *D. melanogaster* and the immune deficiency pathway that regulates NF-κB (192).

Invertebrates have been particularly useful in understanding host-helminth interactions, and the development of axenic helminth lines that lack bacteria has helped to uncouple host immune responses against helminths versus bacterial symbionts (193, 194). Interestingly, some axenic nematodes like *Steinernema carpocapsae* have similar infectivity and lethality as their symbiotic counterparts (193), while others like *Heterorhabditis bacteriophora* show attenuated lethality when they are derived axenically (195). Indeed, prior bacterial infection, but not prior *B. malayi* infection, of the *A. aegypti* mosquito upregulates transcription of AMP defensin, which reduces the intensity of subsequent *B. malayi* infections (196). The use of axenic nematodes for infection studies in insects has and will continue to resolve which aspects of the immune response are directed specifically to bacteria and/or helminths. Development of such axenic or germfree mammalian helminths could also be useful in addressing this question as well as in identifying which, if any, pattern recognition receptors are specifically engaged by helminth products.

As is true for mammalian helminths, some insect helminths can enforce immune suppression via ES products, but evasion and suppression mechanisms distinct from those described for mammalian helminths have also been uncovered in entomopathogenic helminths. One such mechanism is cuticle absorption by hemolymph effector proteins. This mechanism has been reported for *Steinernema feltiae* during infection of *Galleria mellonella*: Epicuticular lipids from *S. feltiae* are capable of binding host-interacting proteins present in *G. mellonella* hemolymph and subsequently suppressing AMP synthesis, phenoloxidase activity and melanization, and phagocytosis (197, 198). Whether this mechanism is also used by helminths that infect mammalian hosts is not well appreciated, but it is likely that the cuticle is an active player in host immunosuppression.

## Zebrafish as Models of Helminth Infection

Whereas insect models are limited by their lack of an adaptive immune system, zebrafish are an attractive model for the study of helminth immunity because they have both an innate immune

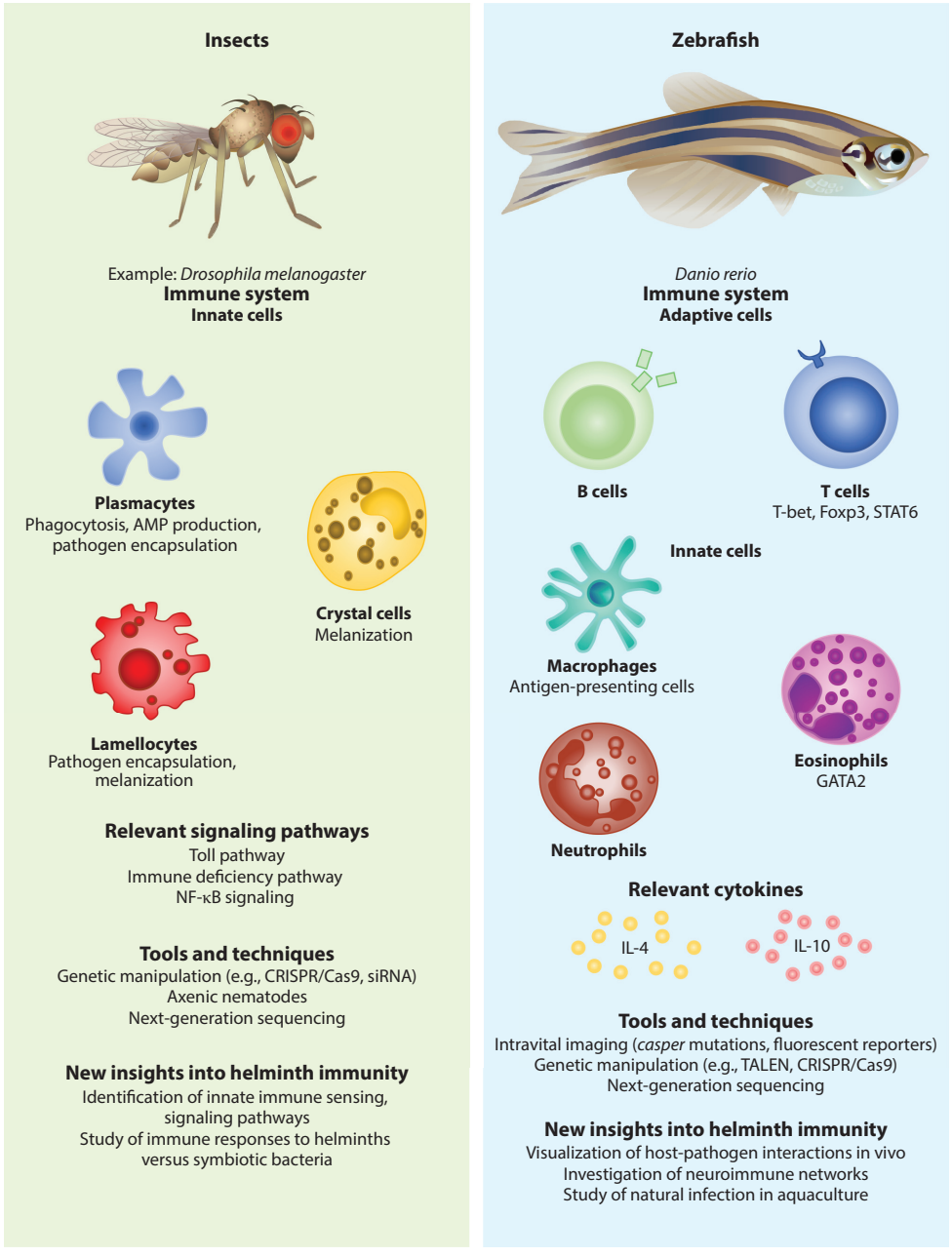


**Figure 4**

Nonmammalian models of helminth immunity. The amenability to genetic manipulation and imaging techniques such as intravital imaging makes *Drosophila melanogaster* and *Danio rerio* attractive systems in which to study evolutionarily conserved elements of helminth immunity and discover novel host defense mechanisms. Abbreviations: AMP, antimicrobial peptide; siRNA, small interfering RNA; TALEN, transcription activator–like effector nuclease.

system and an adaptive immune system and are host to over 20 helminth species in the wild (199). There are few publications on type 2 immunity in zebrafish, which has limited the study of helminth infections in this model. However, recent work has demonstrated that zebrafish possess cells, characterized by expression of GATA2, that are phenotypically and transcriptionally similar to eosinophils and that degranulate in response to *Heligmosomoides polygyrus* extract (200). Further, homologs of transcription factors necessary for the development of different Th cell subsets (e.g., T-bet, STAT6, and Foxp3) are expressed in zebrafish (201), and evidence for the requirements of both IL-4 and IL-4Rα interactions in immunity is present (202), suggesting that zebrafish are an underappreciated model system for exploring vertebrate immunity.

The use of zebrafish as a model for helminth infection is particularly appealing given the genetic and imaging tools available for this system. Genetic modification in zebrafish has been performed using zinc-finger nucleases, transcription activator–like effector nucleases, and the CRISPR/Cas9 system (199) and has been used to generate a number of fluorescent reporter lines that label various immune cells, including macrophages and neutrophils (203). Transparent zebrafish (with *casper* mutations) have also been developed to facilitate live imaging of biological processes over long periods (204). Used together, these tools would be phenomenal for visualizing immune responses to parasitic helminths in live tissues. Further, given the number of transgenic reporters that label cells of the nervous system (205, 206), this system could even be used to study the interactions of immune cells with neuronal cells during helminth challenge in a way that is not currently possible in mice and other mammals.

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