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The yin and yang of human soil-transmitted helminth infections

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Abstract

The major soil-transmitted helminths that infect humans are the roundworms, whipworms and hookworms. Soil-transmitted helminth infections rank among the most important neglected tropical diseases in terms of morbidity, and almost one billion people are still infected with at least one species. While anthelmintic drugs are available, they do not offer long term protection against reinfection, precipitating the need for vaccines that provide long-term immunologic defense. Vaccine discovery and development is in advanced clinical development for hookworm infection, with a bivalent human hookworm vaccine in clinical trials in Brazil and Africa, but is in its infancy for both roundworm (ascariasis) and whipworm (trichuriasis) infections. One of the greatest hurdles to developing soil-transmitted helminth vaccines is the potent immunoregulatory properties of these helminths, creating a barrier to the induction of meaningful long-term protective immunity. While challenging for vaccinologists, this phenomenon presents unique opportunities to develop an entirely new class of anti-inflammatory drugs that capitalise on these immunomodulatory strategies. Epidemiologic studies and clinical trials employing experimental soil-transmitted helminth challenge models, when coupled with findings from animal models, show that at least some soil-transmitted helminth-derived molecules can protect against the onset of autoimmune, allergic and metabolic disorders, and several natural products with the desired bioactivity have been isolated and tested in pre-clinical settings. The yin and yang of soil-transmitted helminth infections reflect both the urgency for effective vaccines and the potential for new immunoregulatory molecules from parasite products.

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Keywords

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1. Biology and epidemiology

The soil-transmitted helminths (STHs) is a group of parasitic nematodes of humans that are mostly restricted to the world's tropical and subtropical climates, especially in low- and middle-income countries (LMICs), where they cause infection through contact with parasite eggs or infective larval stages (Bethony et al., 2006). The adult developmental stages of STHs are found in the gastrointestinal (GI) tract where they establish chronic infections in which individual worms can live for 1–10 years. The STHs of particular worldwide importance that we will address herein are the roundworms, whipworms and hookworms.

Of the roundworms, the major species to infect humans is *Ascaris lumbricoides*, the largest (up to 400 mm in length) and most prevalent of the STHs (Elkins et al., 1986). Humans become infected after ingesting embryonated eggs, which hatch to release larvae that penetrate the intestinal mucosa to commence an obligatory extra-intestinal migratory phase in the liver and lungs, whereupon they migrate up the trachea and are swallowed, thus re-entering the GI tract. In the small intestine they develop into dioecious adult worms which mate and the female releases hundreds of thousands of eggs per day.

The major whipworm to infect humans is *Trichuris trichiura* (Else et al., 2020). People become infected by ingesting eggs, whereupon first stage larvae (L1) hatch and penetrate the epithelial cells at the base of the crypts of the large intestine (colon) where they create a multicellular epithelial tunnel. Larvae grow and moult multiple times before becoming 30–50 mm adult males and females with the characteristic whip like appearance. Zoonotic infections with other *Trichuris* spp. occur, primarily *Trichuris suis* from pigs and *Trichuris vulpis* from dogs, but neither species completes its development in humans nor are they considered to be pathogenic. Indeed, experimental human infection with *T. suis* has been reported in numerous clinical trials due to the immunoregulatory nature of the infection (see subsequent sections).

The third major group within the STHs is the human hookworms (Hotez et al., 2005). Adult hookworms reside for many years in the small intestine where they feed on blood and can cause iron deficiency anaemia (IDA) when present in high numbers, particularly in individuals who are malnourished or have compromised iron stores. Children and women of reproductive age (especially pregnant women) with low underlying iron stores are especially prone to develop hookworm IDA. The three main hookworm species to infect humans are *Necator americanus*, *Ancylostoma duodenale* and *Ancylostoma ceylanicum*. The most predominant human hookworm is *N. americanus*, which is particularly common in southern China, Southeast Asia, the Americas and most of the African continent. *Ancylostoma ceylanicum* was thought to be primarily a parasite of dogs, but has recently been identified as a significant human species throughout the Asia-Pacific region where it is sometimes co-endemic with *N. americanus* (Inpankaew et al., 2014; Bradbury et al., 2017;

Colella et al., 2021). Humans become infected with *N. americanus* through percutaneous penetration of infective third-stage larvae (L3) whereas the ancylostomatids can infect via both percutaneous and oral (and even trans-colostral) routes. A human experimental challenge model has been established for *N. americanus* (Loukas et al., 2016; Diemert et al., 2018a; Hoogerwerf et al., 2021) and it has been shown to be safe and well tolerated in doses exceeding 100 topically applied L3 (Hoogerwerf et al., 2021). This has enabled the establishment of a platform to test anthelmintic drugs and vaccines as well as assessing the therapeutic potential of experimental hookworm infections for treating diseases that result from a dysregulated immune system (Ryan et al., 2020).

Human strongyloidiasis is caused primarily by *Strongyloides stercoralis*, a STH with a unique life cycle that entails alternative free-living and parasitic developmental pathways in response to environmental stimuli (reviewed in Krolewiecki and Nutman, 2019). Unlike the other STHs, eggs shed by the female worm hatch in the small intestine and it is the L1 rhabditiform larvae that are passed in the faeces and are the diagnostic stage detected by microscopy. Due to the presence of larvae in the duodenum, autoinfection is a risk if larvae reach the infective L3 stage before being shed from the host. This can lead to hyperinfection syndrome where larvae are mostly restricted to the gut and lungs, or disseminated strongyloidiasis where larvae can invade other organs. Both conditions are common with subjects receiving high doses of corticosteroids, such as post-organ transplant patients, and can be fatal (Cappello and Hotez, 1993). Human to human infection is typically via percutaneous penetration by L3s but oral transmission is thought to be possible. Dogs can also harbour *S. stercoralis* infection and are thought to be a source of human infection in some areas (Jaleta et al., 2017). Once larvae penetrate the skin they rapidly migrate through subcutaneous tissue and sometimes leave pruritic linear streaks along the lower trunk and buttocks, often referred to as *larva currens*.

Increasing attention is also being paid to the zoonotic roundworms, *Toxocara canis* and *Toxcara cati*, and their importance to public health (Ma et al., 2018). *Toxocara* spp. are primarily parasites of dogs and cats, but are thought to infect tens of millions of people based on seropositivity rates. *Toxocara* does not fully mature in humans but undergoes various forms of larva migrans, where larvae migrate through the liver and lungs (visceral larva migrans), eyes (ocular larva migrans) or neurological tissues (neurotoxocariasis). The term covert toxocariasis has been used to report on the asymptomatic or low-grade *Toxocara* infection, which can result in chronic eosinophilia, asthma, or developmental delays.

2. Disease burden, pathogenesis, and current control efforts

The STH infections rank among the most common neglected tropical diseases. Practically all children and many adults (including millions of pregnant women) who live in extreme poverty are affected by at least one of these infections. However, while STH infections are typically chronic and debilitating conditions, they are not considered major causes of global mortality. We therefore assess their public health impact through global and regional prevalence estimates or a metric used by the Global Burden of Disease Study (GBD) and known as the disability-adjusted life year (DALY).

The latest iteration for the year 2019 (GBD 2019) has determined that 909 million people suffer from these infections (IHME, 2020a). Ascariasis is the most common STH infection with 446 million prevalent infections (IHME, 2020b), followed by trichuriasis, currently estimated at 360 million prevalent infections (IHME, 2020c), and hookworm infection at 173 million (IHME, 2020d). Together these STH infections result in approximately 2,000 deaths annually, and almost 2 million DALYs (IHME, 2020a). However, some investigators feel that even these numbers may fail to fully account for all of the chronic morbidities (Herricks et al., 2017). Currently, the GBD2019 estimates that hookworm infection accounts for almost one-half of the DALYs lost (Fig. 1), mostly due to moderate and severe anaemia in children and pregnant women (IHME, 2020d).

Although the GBD2019 does not provide estimates for other STH infections, both strongyloidiasis and toxocariasis are highly prevalent diseases. One independent estimate reported 386 million people with strongyloidiasis in 2020 (Fleitas et al., 2020), although their method relied on hookworm infection prevalence estimates that far exceeded those released by the GBD2019. An earlier estimate for strongyloidiasis from 2017 was higher still (Buonfrate et al., 2020). In some estimations, human toxocariasis has emerged as one of the most prevalent STH infections. However, human toxocariasis is assessed by measuring antibodies in patient sera rather than by faecal examination. A 2019 systematic review and meta-analysis estimate puts the global seroprevalence of toxocariasis at 19%, but with far higher estimates in tropical regions, especially Africa and South-East Asia (Rostami et al., 2019).

The pathogenesis of disease caused by STH infections is often considered in aggregate, although in fact, each type of infection is unique in terms of pathogenesis and clinical outcome. However, there are some common features. For ascariasis, trichuriasis, and toxocariasis, typically the severest illness occurs in children because they harbour higher worm loads than adults (Else et al., 2020). Hookworms are an important exception as high hookworm burdens are found in both children and adults, including pregnant women (Ness et al., 2020). It has been estimated that approximately 7 million pregnancies are complicated by hookworm infection in Sub-Saharan Africa (Brooker et al., 2008). In general, heavier worm burdens in children lead to chronic impairments in physical growth and cognitive and intellectual development through underlying mechanisms that may be linked to malnutrition and/or host inflammatory responses (Else et al., 2020).

Each type of STH also induces unique pathologic effects. For example, large *Ascaris* roundworms cause intestinal obstruction, especially in the ileum of small children (Else et al., 2020), and this finding accounts for a high percentage of the 2,000 or more annual deaths. In addition, *Ascaris* roundworms can mechanically obstruct the bile and pancreatic ducts, especially in adults. Still another aspect is the finding that in their migration through the lungs, *Ascaris* larvae can induce severe allergic responses that produce a mixed picture of eosinophilic asthma and chronic obstructive lung disease (Weatherhead et al., 2018). In contrast, *Trichuris* whipworms inhabit the large intestine where they cause colitis and inflammatory bowel disease, and in heavy infections, a *Trichuris* dysentery syndrome which can lead to rectal prolapse (Else et al., 2020). The mechanisms of colitis are due primarily to the ability of adult *Trichuris* whipworms to release chemically-active compounds from

their stichosome, a unique parasite organ located in the anterior portion of the worm embedded in the host gut mucosa. The release of small and macro-molecules from the parasite stichosome causes biochemical changes to host epithelia, allowing the anterior end of the parasite to burrow in cellular tunnels to induce inflammation and intestinal blood loss (Else et al., 2020). However, hookworms cause far greater blood loss due to the ability of the adult stage parasites to ingest a plug of host tissue causing direct mechanical and enzymatic damage (Loukas et al., 2016). The red blood cells are ingested and lysed by the adult hookworm. The gut of the adult hookworm is lined with proteolytic enzymes, many of which are specific for cleaving haemoglobin and operate in a cascade of hydrolytic cleavages and protein breakdown (Ranjit et al., 2009). This allows the adult hookworm to digest haemoglobin and absorb host peptides and amino acids. For that reason, the major consequence of heavy hookworm blood ingestion is moderate and severe anaemia, as well as protein malnutrition and hypoalbuminaemia (Loukas et al., 2016). Severe hookworm anaemia together with the foetal iron demands in pregnancy can produce serious deleterious effects for both mothers and newborns.

Strongyloidiasis produces its most severe form of illness in patients who receive high doses of corticosteroids, or in some cases other immunosuppressive therapies or co-infection with HTLV-1 (Mejia and Nutman, 2012). This results in a severe hyperinfection syndrome associated with gut microperforation, bacteraemia, and even bacterial meningitis (Mejia and Nutman, 2012). Human toxocariasis results from larval migration through host tissues, eliciting eosinophilia and lung allergic responses similar to those observed for larval *Ascaris* infection in the lungs, or in the liver leading to hepatitis (Ma et al., 2018). In addition, *Toxocara* larval migration through the brain may produce a subclinical cerebritis leading to intellectual declines and developmental delays (Hotez, 2014; Ma et al., 2018).

Such widespread pathology caused by STH has demanded much greater efforts to control infections. In 2001, the World Health Assembly pledged to deworm up to 100% of all school-aged children by the year 2010. The approach relied on studies conducted during the 1990s that found a single dose of an anthelminthic drug, typically a single dose of albendazole or mebendazole, could reduce the bioburden of STH infections. This allowed paediatric catch-up growth and improvements in childhood cognition and intellectual capacity (Savioli et al., 1992). Indeed, early on, there was evidence of not only improvements in child development, but also in educational attainment and long-term economic returns (Migel and Kremer, 2004). Countering this were systematic reviews and Cochrane analyses that questioned these benefits (Taylor-Robinson et al., 2009, 2015), which led to a vigorous disagreement in the medical parasitology community. One analysis suggested that such “worm wars” might be resolved by recognising the differences between each of the STH rather than lumping them together as a single STH entity, in which the significant effects in one species would be negated by lack of effects in the others. In addition, it is important to control for the differential effects of drug treatment on each of the major helminth species, and compare light versus moderate or severe helminth infections of children, particularly with respect to cognitive improvements (Majid et al., 2019).

Currently, hundreds of millions of both school-aged children and pre-school children receive annual deworming in low- and middle-income countries. Less clear is whether this approach

actually can lead to the elimination of STH infections, or whether additional selective pressure should be applied in the form of simultaneously treating adults on an annual basis (Ásbjörnsdóttir et al., 2018). A “deworm3” project is underway to assess whether more aggressive deworming approaches could lead to gradual declines in parasite prevalence and intensity (Ásbjörnsdóttir et al., 2018) or whether new and improved technologies might be required, such as evaluating existing anthelmintic drugs in combination, for example albendazole combined with ivermectin (Palmeirim et al., 2018; Moser et al., 2019). In addition, efforts are in progress to explore new chemical entities (NCEs) and new classes of anthelmintic drugs (Elfawal et al., 2019), as well as anthelmintic vaccines. With regards to the former, a novel screening pipeline has been proposed and is starting to yield promising NCEs, while anthelmintic vaccines are discussed in more depth below. In parallel, expanded studies are in progress to evaluate whether mass drug administration could induce parasite drug resistance to albendazole or mebendazole (Vlaminck et al., 2018). This new Starworms project examines single nucleotide polymorphisms in the genes encoding β-tubulin, the major drug target of albendazole and mebendazole (Vlaminck et al., 2018).

3. Vaccines for STHs – human trials, animal models and challenges to development

There is a general absence of reliable protective immunity to STH infections among those living in endemic areas of poverty. That said, a status quo is achieved in many infected subjects, where worm burdens that contribute to morbidity (particularly in the young and malnourished) are frequently encountered, but uncontrolled heavy intensity infections are less common. Age- and exposure-acquired immunity to hookworms (reviewed in (Loukas et al., 2016a) and whipworms (reviewed in (Else et al., 2020)) is slow to develop, if at all, however *Ascaris* burdens tend to peak in early adolescence and decrease with age along with a robust T helper 2 (Th2) immune response (Turner et al., 2003).

Given the absence of naturally acquired sterilizing immunity in STH infections, the goal of current vaccination strategies is to attain partial immunity that minimises the impact of moderate to heavy intensity infections. To date, the most compelling strategy for developing helminth vaccines (not just STH) is the irradiated parasite approach. Many studies have shown in animal models of human helminths as well as with helminths of livestock and companion animals that vaccination with live radiation-attenuated larvae confers strong protection against challenge infection. Indeed, in the 1960s Miller showed that subcutaneous vaccination of dogs with infective larvae of the canine hookworm *Ancylostoma caninum* that were attenuated with X-rays resulted in protection of pups against challenge infection (Miller, 1964). Protection was attributed to distinct but interrelated factors including reduction in larval infectivity, reduced pathogenicity, and the sterilising effect of radiation on female worm fecundity (manifesting as reduced egg burdens in vaccinees). Subsequent studies showed that gamma radiation was successful, and these criteria guided the development, manufacture and licensing of a gamma-irradiated *A. caninum* L3 vaccine in the early 1970s (Miller, 1978). Despite high efficacy, animals showed residual levels of infection that dented confidence in its value, and the vaccine was removed from the market. Irradiated STH vaccines require a ready supply and industrial scale methods for producing

the attenuated immunogen (larvae), and thus pose a number of challenges for regulatory approval and wide-scale implementation. As such, there were no efforts to develop and clinically test an attenuated larval vaccine for any human STH infection until recently when Chapman and colleagues undertook a phase 1 randomised, double-blind, placebo-controlled, challenge study to assess the safety and tolerability of ultraviolet light C (UVC)-irradiated *N. americanus* in healthy hookworm-naive adults in Australia (Chapman et al., 2021). The vaccine was well tolerated, safe and despite the small cohort sizes, significantly reduced larval burdens were recovered from hatched eggs of vaccinated participants compared with controls after challenge infection with non-attenuated larvae. While an ultimate hookworm vaccine is unlikely to take the form of irradiated larvae due to the logistical challenges outlined above, this trial nonetheless set a benchmark against which to compare subunit vaccines that are currently in development (see below), and was the first study to prove that partially protective immunity could be induced in humans by vaccination. Similar studies with attenuated forms of other STHs have yet to be undertaken, but we urge researchers to perform these trials as both proof-of-concept studies and to generate valuable reagents (eg. sera) that can be utilised in the search for protective antigens.

STHs produce a diverse array of secreted molecules and vesicles that interact with surrounding host tissues where they orchestrate various parasitism processes such as tissue penetration, somatic migration, feeding and immune modulation. Proteins involved in these processes are prime candidate antigens to target with subunit vaccines. Along those lines, a Human Hookworm Vaccine Initiative was established in 2000 to reproduce the effects of irradiated larvae but using recombinant protein subunit vaccines (Hotez et al., 2003). Based on earlier findings of two predominant larval secreted proteins known as *Ancylostoma* secreted protein-1 (ASP-1) (Hawdon et al., 1996) and ASP-2 (Hawdon et al., 1999) released upon host stimulation, and which were also found in *N. americanus* (*Na*-ASP-2) (Goud et al., 2005), the HHVI focused on these two molecules. It was subsequently found that ASP-2 was an immunodominant macromolecule associated with irradiated vaccines (Bethony et al., 2005). Although an alum formulation of yeast-expressed recombinant ASP-2 was shown to be safe and immunogenic in helminth-naïve human volunteers (Bethony et al., 2008), follow up phase 1 studies in an endemic area of Brazil revealed that the vaccine was allergenic due to the presence of pre-vaccination host IgE among those chronically exposed to the parasite (Diemert et al., 2012). For that reason, larval antigen vaccines were abandoned in favour of adult hookworm antigens.

As a second and more successful strategy for human hookworm vaccines (Hotez et al., 2010), recombinant forms of two parasite-derived enzymes involved in blood-feeding and detoxification are in clinical development - the haemoglobin-degrading aspartic protease, *Na*-APR-1 (Pearson et al., 2009), and the haem-detoxifying glutathione-S-transferase, *Na*-GST-1 (Asojo et al., 2007; Hotez et al., 2010). Both vaccines have been tested independently in phase 1a safety trials (https://clinicaltrials.gov/ct2/show/NCT01717950) (Diemert et al., 2017), and more recently in a *N. americanus* endemic region of Gabon as a combination of co-administered vaccines adjuvanted with alhydrogel (Adegnika et al., 2021). Both vaccines were shown to be immunogenic and safe, and now await efficacy testing using the controlled hookworm infection model. Both *Na*-APR-1 and *N*a-GST-1 are expressed in the gut of the adult stage parasite. Moreover, both of these adult hookworm antigens appear to circumvent

past issues with inducing allergic responses. In general, STH and other helminth vaccine antigen selection approaches now have rigorous criteria for down-selecting proteins that drive IgE responses in infected individuals (Diemert et al., 2018b).

There is an equally real need for a vaccine that reduces infection intensity and transmission in trichuriasis. Despite mass anthelmintic drug administration programs to school-age children, *T. trichiura* infection continues to be a burden, notably due to the low efficacy of current drugs and high rates of post-treatment re-infection. To our knowledge, there has never been a clinical trial with a *Trichuris* vaccine of any sort. However, a comprehensive review on trichuriasis vaccines with a major emphasis on candidate antigens tested in the *Trichuris muris* mouse model was recently published (Hayon et al., 2021). Vaccination of susceptible mice with Freund's adjuvanted *T. muris* excretory/secretory (ES) products conferred complete protection against challenge infection (Dixon et al., 2010). Many of these ES antigens originate from the *Trichuris* stichosome organ which is embedded in the host colonic mucosa (Briggs et al., 2018). While this highlights the value of the murine model for pre-clinical discovery and development of a subunit vaccine for human whipworm infection, it should be noted that susceptibility and immunologic resistance/clearance of the infection is mouse strain- (median histocompatibility complex) and sex-dependent, and is influenced by the nature of exposure, notably trickle versus bolus infection (Yousefi et al., 2021). One subunit vaccine that is showing particular promise is the *Trichuris* stichosome secreted antigen known as whey acidic protein (WAP) (Briggs et al., 2018). Still another contains a fragment spanning the catalytic domain of serine/threonine phosphatase 2A fused to a self-adjuvanting synthetic oleic-vinyl sulfone, which when administered to mice intra-nasally provided almost complete protection against *T. muris* challenge infection. Indeed, similar self-adjuvanting mucosally-delivered vaccines based on peptides/subunits of protective protein antigens from hookworms also confer high protection in animal models (Bartlett et al., 2020). These findings, while preliminary in nature, highlight the potential of this vaccine platform and the advantages it confers for distribution of vaccines to remote areas.

Vaccines against ascariasis are proposed to reduce the parasite burden and, consequently, infection-induced morbidity and transmission (Else et al., 2020). Mouse models, while not allowing complete development of these large parasites in a small animal, have proven useful in assessing anti-larval responses in the lungs. Multiple exposures of mice to eggs of the pig whipworm *Ascaris suum* conferred high levels of protection against accumulation of larvae in the lungs (Nogueira et al., 2016). Moreover, vaccination of mice with different *A. suum* extracts derived from adult and larval parasites provided partial protection against larvae reaching the lungs, and passive transfer of IgG from vaccinated mice conferred similar levels of protection (Gazzinelli-Guimarães et al., 2018). Infection of pigs with *A. suum* has been used as a permissive model of human ascariasis, and oral vaccination of pigs with radiation-attenuated eggs conferred 94% protection against challenge infection (Urban and Tromba, 1984). While ascariasis vaccines have not yet entered clinical development or testing, a number of subunit vaccines have been assessed in the mouse model, including As37 which is conserved amongst STH and represents a potential pan-STH vaccine candidate (Versteeg et al., 2020), as well as another antigen known as As16 (Wei et al., 2017). A chimeric antigen consisting of peptides from multiple antigens was recently shown

to confer robust protection against establishment of larvae in the lungs (de Castro et al., 2021). In parallel with each STH vaccine are efforts to combine these antigens in a universal or multivalent pananthelmintic vaccine, in addition to studies to identify common consensus antigens against roundworms, whipworms and hookworms (Zhan et al., 2014).

Although very high levels of protection have been observed in rodent models of STH infections, a lower bar has been set for efficacy of human vaccines due to the limitations of these animal models. Moreover, modelling studies have shown that such high levels of protection are not required to substantially reduce morbidity due to the correlation between infection intensity and pathogenesis in hookworm infection at least (Bartsch et al., 2016). A caveat that must be considered with all helminth vaccine programs is the issue of administering vaccines to individuals who are already infected, often chronically so, and therefore under the influence of helminthiasis-driven immunoregulation (see next section). At the very least, subjects should be dewormed with an anthelmintic drug prior to vaccination, but the long-lasting immunomodulatory effects of STH infections are well documented (Loukas et al., 2016; Else et al., 2020), and could result in reduced vaccine efficacy in affected individuals. Finally, such vaccines are predicted to be highly cost-effective and cost-saving due to the poverty promoting effects of these parasites (Bartsch et al., 2016). For that reason, they are sometimes referred to as ‘antipoverty vaccines’ (Hotez et al., 2011).

4. Next generation integrated control and elimination

The current approaches emphasizing mass treatment or deworming have so far not succeeded in promoting STH elimination unless there is a commensurate rise in living standards and economic development. Efforts to optimize mass treatment by expanding access to anthelmintics for the entire community and combining traditional and new anthelmintics drugs – including ivermectin or moxidectin (where these drugs are already deployed for onchocerciasis and lymphatic filariasis control programs) – to create added synergies for trichuriasis and hookworm, will help. But even these measures may be insufficient. For instance, anthelmintic drug resistance monitoring needs to be better and fully integrated into global deworming programs. We also have an opportunity to introduce new anthelmintic vaccines for STH infections and potentially integrate them in vaccine-linked chemotherapy approaches (Zawawi and Else, 2020). Adding vaccinations to deworming could reduce the amplitude of post-treatment reinfections to a point in which STH transmission is no longer sustained, a goal aided by the absence of significant animal reservoirs for these infections (except possibly for ascariasis in pigs). A similar approach has been proposed for schistosomiasis in Africa and the Americas. Currently, the global policymakers have not prioritized vaccine-linked chemotherapy approaches, choosing instead to rely exclusively on deworming despite major questions regarding its sustainability (Lin and Addiss, 2018).

5. Immunoregulatory strategies of STH

Protective immunity to STH infections takes on different forms depending on the individual species and the route of infection. *Necator americanus* for example first enters the human

host via the skin, whereas the whipworms and ascarids are orally infective. While we have a general understanding of the protective mechanisms at play from rodent models, such as *N. brasiliensis* and *T. muris* (Allen and Sutherland, 2014), the protective response in humans is less well understood, and indeed for hookworms at least, there is no clear-cut evidence of protective immunity (Loukas et al., 2016). Nonetheless, a robust type 2 response is initiated early with IgE-armed basophils trapping larvae in the skin, and further clearance of STH larvae occurs in the lungs, where M2 macrophages damage and orchestrate the clearance of parasites (Loukas et al., 2016; Else et al., 2020). Once adult worms are lodged in the gut, secretion of type 2 cytokines and IL-22 in intestinal mucosal tissue of experimentally infected subjects has been detected, and induction of goblet cell hyperplasia and mucus production (Broadhurst et al., 2010; Gaze et al., 2012), all of which culminate in worm expulsion. Moreover, the production of IL-25 by intestinal tuft cells has been shown to be a key early event in triggering protective T_H2 responses in animal models (Gerbe et al., 2016).

In the face of this robust modified type 2 response, it is remarkable how ineffective our natural defenses against STH are, reflecting the ability of these parasites to modulate and disable host immune mechanisms (McSorley and Maizels, 2012), and contributing to the difficulty in developing highly efficacious subunit vaccines. In particular, acquired immunity to repeated STH infections is poorly expressed, with rapid re-infection following anthelmintic chemotherapy (Jia et al., 2012), and no obvious decline in worm burdens (notably for hookworm infections) observed as populations age (Anderson and May, 1982). STHs combine short-term tactics to minimise immune stimulation with a longer-term strategy to exploit host immune regulatory networks, in order to mute host reactivity and inactivate expulsion mechanisms.

In the first instance, parasites that enter through the skin (eg hookworms, *Strongyloides*) encounter different barriers to those taking the oral route (*Ascaris*, *Trichuris*, *Toxocara*). Barrier tissues release alarmins, such as IL-33 and TSLP, to alert the immune system and prime antigen-presenting cells (dendritic cells, DCs); in animal models at least, helminths release products that block the IL-33 pathway (Osbourn et al., 2017; Vacca et al., 2020), thus neutralising the ability of epithelial cells to kick-start the immune response. Similarly, secretions of *Trichuris suis* reduce TSLP release by intestinal epithelial cells (Hiemstra et al., 2014), as well as interfere with DC activity as discussed below.

Live larvae are generally sheathed with a redundant cuticle that can be rapidly discarded on entry into the host (Kumar and Pritchard, 1992). The sheath is now recognised not only as a protective layer, but as a decoy. Thus, skin-penetrating *N. americanus* larvae attract lectin-dependent DC adherence to their sheath, allowing the parasites to migrate away from immune cells immobilised on the cast material (Hassan et al., 2018). Similarly, *Toxocara* larvae which can migrate through somatic tissues express a surface coat that attracts antibody and granulocyte binding, but is readily shed to facilitate immune evasion (Fattah et al., 1986; Page et al., 1992).

While in the first instance, STH evasion mechanisms vary widely according to their route of entry and subsequent migratory tropism, all helminth species exert profound effects on DCs (White and Artavanis-Tsakonas, 2012). DCs are the pivotal population which

presents parasite antigens to T cells, triggers the activation of these cells, and drives them toward the Th2 mode that is necessary for parasite destruction. Thus, blocking DC function is of critical importance in STH infection. For STHs with limited options for *in vivo* models, investigations have primarily been conducted in vitro, using ES products from congeners (eg *A. suum* and *T. suis* for their human counterparts). These ES products abrogate DC responses to bacterial stimuli (eg lipopolysaccharide), minimise inflammatory cytokine production, and reduce their ability to stimulate T cells. In the case of *Ascaris*, an abundant body constituent (pseudocoelomic fluid, PCF) was found to mediate these effects (McConchie et al., 2006). More recently, *Ascaris* PCF has been shown to strongly interfere with the central pathway of toll like receptor (TLR) signaling in DCs through MyD88 (Arora et al., 2020). A close parallel exists with the ES products of *T. suis* which not only down-modulate human DCs in a similar manner (Klaver et al., 2013), but are able to interfere with macrophage TLR signaling (Ottow et al., 2014).

Having negotiated the innate immune system, STHs then need to create a new balance in the immune system to minimise local inflammation and permit their continued tenure. If this balance is not struck, parasites may be expelled but the host may also suffer collateral immunopathology from an over-zealous immune response. To some extent, this new host–parasite accord resembles a form of immunological tolerance mediated at two levels. First, immune cells may be intrinsically hyporesponsive or down-regulated, as occurs in the modified type 2 response (Maizels and Yazdanbakhsh, 2003). Secondly, immune reactivity to STHs may be actively suppressed by the regulatory T cell (Treg) population that normally protects the body from autoimmunity and food allergy through soluble regulatory cytokines (IL-10 and TGFβ) and surface inhibitory receptors such as CTLA-4 (White et al., 2020). Consistent with this concept, children exposed to high levels of *Ascaris* and *Trichuris* showed poor immune reactivity (IL-4 and IFNγ production) and high levels of regulatory cytokines in *in vitro* lymphocyte assays (Turner et al., 2008; Figueiredo et al., 2010).

Effector T cell hyporesponsiveness is commonly observed across many human helminth infections, including STHs (Figueiredo et al., 2010), and may result from aberrent DC signalling, Treg modulation, or both. Paradoxically, hyporesponsive individuals can display very high levels of IgG4 isotype antibodies rather than IgE; indeed, in human ascariasis, the IgG4:IgE ratio correlated with intensity of infection (Turner et al., 2005). An interesting parallel was drawn with the “Modified Type 2” phenotype observed during desensitization of allergic individuals, in which the IgE isotype is displaced by a dominant IgG4 antibody response (Platts-Mills et al., 2004). Subsequently, IgG4 was found to be promoted by IL-10 and TGF-β (Satoguina et al., 2008), suggesting that this isotype may be a reflection of potent Treg activation during STH infection.

The Treg pathway is emerging as a central feature in human STH infections, and is tracked by expression of the canonical transcription factor FOXP3 (Logan et al., 2018; de Ruiter et al., 2020). In some animal models of STH, parasites secrete proteins that induce Foxp3 expression and functional Tregs (Johnston et al., 2017; White et al., 2021), including in human peripheral blood T cells (Cook et al., 2021). Direct evidence for Treg expansion, or Treg-driving secreted products, in human STH infections is relatively limited, although in natural *N. americanus* infections there are greater frequencies of $FOXP3^+$ Tregs,

associated with elevated CTLA-4 as well as IL-17 (Ricci et al., 2011). A similar rise in FOXP3$^+$ Tregs was observed in coeliac disease patients given experimental therapy with live hookworms (Croese et al., 2015). In *Strongyloides* patients, elevated Tregs are associated with suppression of the Th2 effector cytokine IL-5 (Montes et al., 2009), while in a remarkable study in Argentina, multiple sclerosis patients acquiring intestinal helminth infections (not exclusively STHs) showed raised FOXP3$^+$ Treg numbers and were protected from disease relapse (Correale and Farez, 2007). As well as Treg frequency, their function may also be altered in STH infection, as following albendazole treatment of children in Indonesia, Treg numbers did not decline but their CTLA-4 expression was abated (Wammes et al., 2016).

6. Use of STH and their molecular derivatives to treat inflammatory diseases

The safety and tolerability of experimental human helminth infections have resulted in their use as a novel therapeutic modality for the treatment of a range of diseases that result from a dysregulated immune response, notably allergic and autoimmune diseases (Garg et al., 2014; Zuo et al., 2018; Ryan et al., 2020). Two helminths, both STHs, have been used – the zoonotic *T. suis* and the anthropophilic *N. americanus*. The therapeutic efficacy and tolerability of orally administered *T. suis* ova (TSO) has been assessed in phase 1 trials in patients with IBD – Crohn's disease and ulcerative colitis - and despite promising phase 1 trials (Summers, 2005), subsequent phase 2 trials failed to reach their clinical endpoints in both IBD (Schölmerich et al., 2016) and multiple sclerosis (Voldsgaard et al., 2015; Fleming et al., 2019).

Trichuris suis is a parasite of pigs and is expelled from the human body rapidly, thereby requiring frequent dosing. *Necator. americanus* however is primarily anthropophilic and survives for many years in infected people (Loukas et al., 2016). Experimental *N. americanus* infection in human volunteers is safe and well-tolerated (Blount et al., 2009; Feary et al., 2009; Daveson et al., 2011). Croese et al. assessed the safety of low-dose percutaneously administered *N. americanus* L3 in patients with Crohn's disease (one of the two major forms of IBD), and found the infection to be well tolerated; moreover, despite being on open label trial, all patients who remained in the trial for 1 year were in disease remission (Croese et al., 2006). A randomised controlled trial (RCT) is underway in New Zealand to comprehensively assess the efficacy of *N. americanus* as a maintenance therapy in patients with ulcerative colitis (Australian New Zealand Clinical Trials Registry ACTRN12620000956909). *Necator americanus* has also been assessed for efficacy in a RCT in coeliac disease, where subjects on a gluten-free diet received *N. americanus* L3 or topical chilli sauce (placebo) (Croese et al., 2020). Hookworm infection did not restore tolerance to sustained moderate consumption of gluten but was associated with improved symptom scores after intermittent consumption of lower gluten doses, and infection has been shown to promote Treg responses in the gut of human subjects (Croese et al., 2015).

An increasing body of literature supports a role for helminths in combatting inflammation in type 2 diabetes (de Ruiter et al., 2017; Gao et al., 2021). Animal studies with model STHs

have shown that hookworms can prevent diet-induced obesity and insulin resistance in mice (Yang et al., 2013; Khudhair et al., 2021). Epidemiologic studies have revealed a negative association between STH infections and metabolic syndrome, particularly for *Strongyloides* infection (Tracey et al., 2016). Following on from this review, the first clinical trial assessing experimental helminth infection (*N. americanus*) in metabolic syndrome is underway in Australia (Pierce et al., 2019). Most of these early-phase clinical trials with experimental STH infections were impaired by the absence of current good laboratory/manufacturing protocols for infective stage parasites. Methods for the production of cGMP hookworms were recently reported (Diemert et al., 2018) and are essential if helminth therapy is to be widely adopted and commercially developed in the future.

A rapidly growing array of helminth-derived immunomodulatory molecules is being defined and evaluated in pre-clinical models of inflammatory diseases (Maizels et al., 2018; Ryan et al., 2020). ES products from STHs, as frequently found across the helminth groups, can dampen inflammation in mouse models (Ebner et al., 2014). To date, relatively few individual mediators have been defined from STHs (Fig. 2), but notable among them are members of the TIMP-like hookworm Anti-Inflammatory Protein family, AIP-1 (Buitrago et al., 2021) and AIP-2 (Navarro et al., 2016), the latter of which acts through DCs to expand Tregs and protect against inducible asthma and colitis. The cystatin family of cysteine protease inhibitors are abundantly secreted by parasitic nematodes and have been shown to have anti-inflammatory properties (Schierack et al., 2003), including those secreted by *Ascaris* (Coronado et al., 2019) and the rodent hookworm *N. brasiliensis* (Dainichi et al., 2001). These proteins suppress antigen-specific responses by inhibiting the catalytic activity of proteases involved in antigen processing, and the former has proven efficacious in a mouse model of house dust mite airway allergy. STH secrete immunoregulatory molecules that act directly on T cells, typified by the hookworm homologues of sea anemone ShK toxins that block Kv1.3 voltage-gated potassium channels and are crucial for the activation of effector memory T cells (Smallwood et al., 2021). STH also secrete proteins that mimic cytokines and cytokine receptors, including the major secreted protein of *T. muris*, p43, which has homology to the IL-13 receptor α2, and binds to IL-13, inhibiting its function *in vitro* and *in vivo* (Bancroft et al., 2019). Finally, the SCP/TAPS family of cysteine-rich proteins is massively expanded in the genomes and secretomes of STHs, particularly hookworms (Cantacessi et al., 2009; Tang et al., 2014), and two members of this family have been shown to have immunomodulatory properties. Neutrophil Inhibitory Factor (NIF) from *A. caninum* binds to CD11b/CD18 and inhibits neutrophil accumulation at sites of tissue injury (Moyle et al., 1994), and progressed to phase 2 clinical trials for treating ischemic stroke (Krams et al., 2003). The major larval secreted protein released upon tissue invasion by *N. americanus*, *Na*-ASP-2, is a SCP/TAPS which binds to CD79A on B cells and suppresses expression of genes that regulate B cell signalling (Tribolet et al., 2014).

In addition to proteins, STHs secrete small molecules (Wangchuk et al., 2019a, 2019b; Yeshi et al., 2020). *Ancylostoma caninum* metabolites can ameliorate colitis in mice and suppress inflammatory cytokine secretion from human peripheral blood mononuclear cells (Wangchuk et al., 2019a, 2019b). There is also an emerging role for microRNAs contained within extracellular vesicles of related animal parasites in suppressing inflammation (Coakley et al., 2017; White et al., 2020), including prevention of inducible colitis

(Eichenberger et al., 2018), and this is an exciting field that is rapidly gaining momentum (Drurey and Maizels, 2021). Finally, STHs have been shown to modulate inflammation indirectly through manipulation of the host microbiome, a topic that has been extensively reviewed elsewhere (Giacomin et al., 2015; Brosschot and Reynolds, 2018).

7. Concluding remarks

The morbidity attributable to STH infections is substantial, and uncontested. Vaccines are sorely needed in the absence of major progress towards elimination based on mass drug administration alone. The absence of vaccines for any human helminth infection can be attributed to a lack of financial investment in this area, but it is not just a money problem. Helminths deploy a sophisticated array of strategies to divert and subvert the human host's best attempts to immunologically terminate them. While frustrating to researchers and devastating to infected subjects who suffer the pathologic sequelae, the immunomodulatory properties of these helminths have revealed hitherto unexplored pathways to suppress inflammation and other physiological processes. Coevolution of vertebrates with their invertebrate parasites has resulted in an arms race that must hold a treasure trove of untapped opportunities for modern medicine. The safety and tolerability profile of relatively high numbers of *N. americanus* in healthy volunteers (Hoogerwerf et al., 2019) and the tantalising pre-clinical studies revealing therapeutic moieties in animal models of inflammatory disorders set the scene for exciting and fruitful endeavours ahead. We look forward to the next major breakthroughs in the field, whether they be aimed at eliminating or promoting these infections under the right circumstances. Of course, there remains the question that in a worm-free world, will we suffer from an even greater burden of non-communicable diseases of modernity? That subject poses moral and ethical dilemmas, and as such warrants an entire article unto itself.

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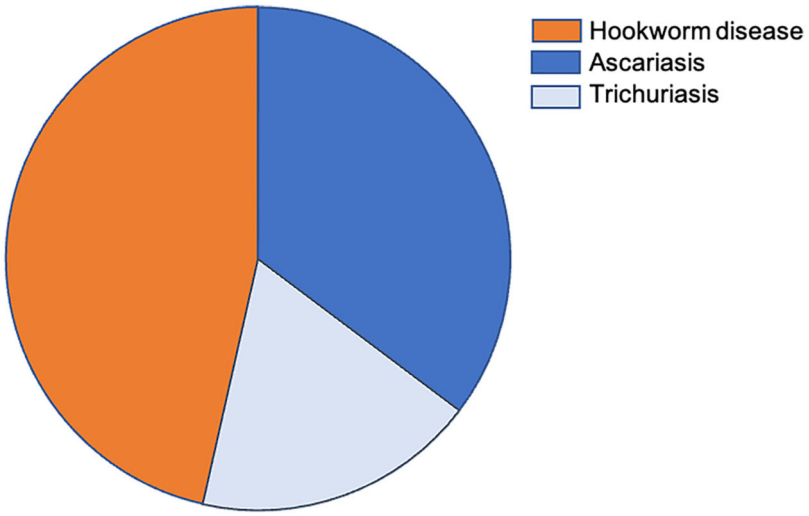

Fig. 1.

Composition of disability adjusted life years by constituent Level 4 causes for both sexes combined, 2019. Adapted from IHME, 2020a.

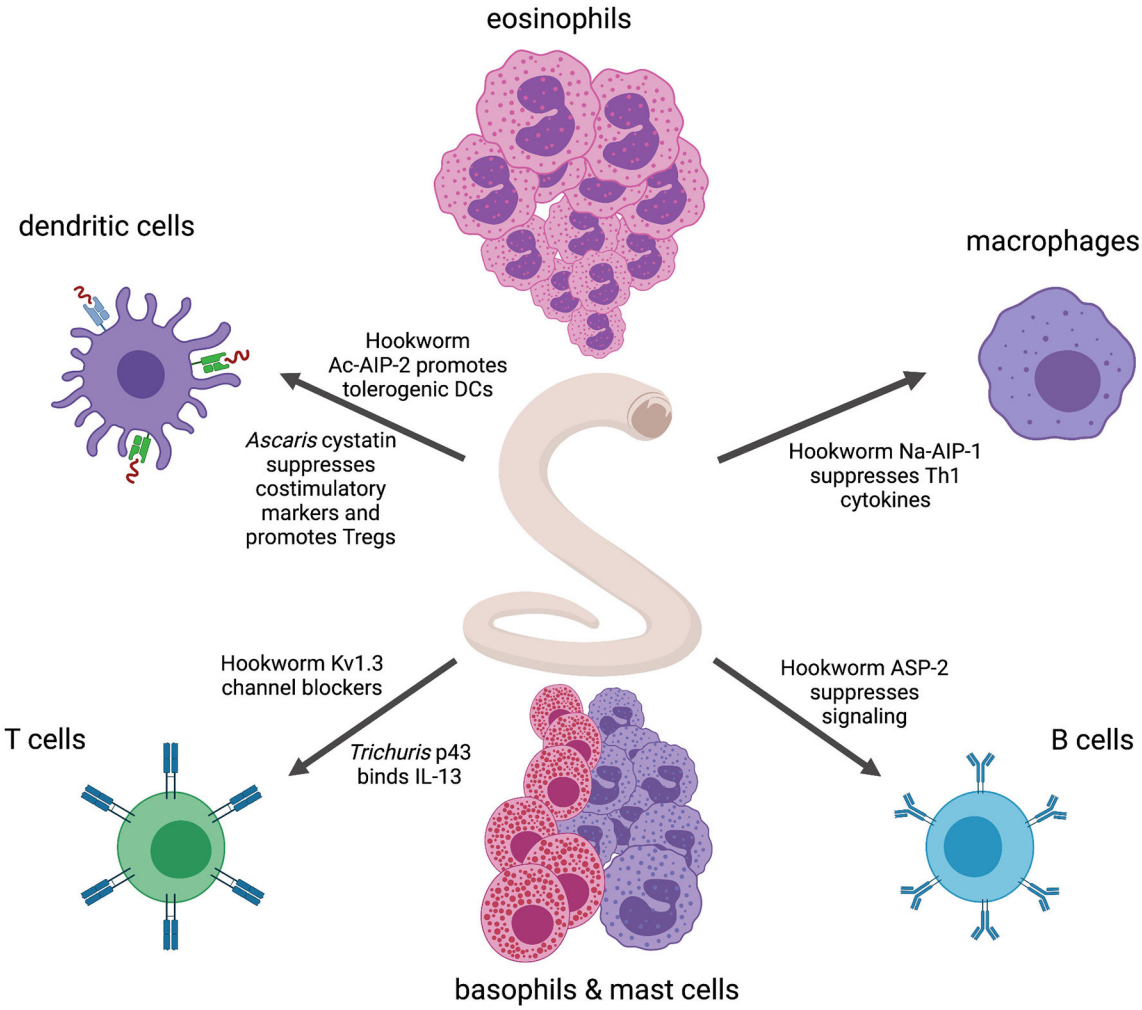

Fig. 2.

Mechanisms by which soil transmitted helminth defined recombinant proteins modulate the host immune system.