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Review Article

The immunology of human hookworm infections

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SUMMARY

Hookworms are one of the most prevalent parasites of humans in developing countries, but we know relatively little about the immune response generated to hookworm infection. This can be attributed to a lack of permissive animal models and a relatively small research community compared with those of the more high-profile parasitic diseases. However, recently, research has emerged on the development of vaccines to control hookworm infection and the use of hookworm to treat autoimmune and allergic disorders, contributing to a greater understanding of the strategies used by hookworms to modulate the host's immune response. A substantial body of research on the immunobiology of hookworms originates from Australia, so this review will summarize the current status of the field with a particular emphasis on research carried out 'down under'.

Keywords *autoimmunity, hookworm, hygiene hypothesis, immune response, immunoregulation, vaccine*

Hookworms are one of the most common parasites of humans, with around 740 million people infected worldwide. Although they cause little mortality, heavy infections can cause iron-deficiency anaemia, growth retardation and low birth weight (1). Hookworms are most prevalent in South America, sub-Saharan Africa and East Asia; however, up until the second half of the 20th century, they were also common in the southern states of USA, Europe (2) and Australia, where they still affect some remote aboriginal communities (3). The two major anthropophilic hookworm species are *Necator americanus* and *Ancylostoma duodenale*. The more common parasite, on which the majority of studies have consequently been carried out, is *N. americanus*.

Hookworms are soil-transmitted helminths: infective larvae burrow through the skin and are activated in the process, after which they migrate through the heart and lungs to the gut, where they mature to adults, feed on host blood and produce eggs which are deposited in the faeces. Deposited eggs then develop to infective larvae, completing the life cycle (1). The host must therefore mount an immune response against a number of different parasite stages during a hookworm infection, and the parasite in turn has a number of opportunities to manipulate the host immune system. We will not dwell on the life cycle of the parasite in this review – for more detail, see (4).

The immunology of human hookworm infection has not received as much focus as that of other helminth parasites of humans, such as schistosomes and filariae. The reasons for this include the relatively low mortality caused by hookworms, the difficulty/expense in maintaining the life cycle in a suitable animal model and the inability of any of the major species of hookworms to reach maturity in mice. This has especially been a problem in Australia where the best laboratory model, the hamster, is not permitted to be maintained in the country because of quarantine regulations. Consequently, Australian hookworm research has focussed on human immunology, and especially experimental or zoonotic human infections.

The importance of hookworm infection has been highlighted by calculating the disability adjusted life years (DALYs) of the tropical communicable diseases. Hookworm, because of its high prevalence but relatively low mortality, causes a greater burden of DALYs (1·83 million) than schistosomiasis (1·76 million) or trypanosomiasis (1·60 million) (2). Two recent events have reinvigorated immunological studies on hookworms – the funding of the Human Hookworm Vaccine Initiative by the Bill and Melinda Gates Foundation (http://www.sabin.org/vaccine-development/vaccines/hookworm), and the discovery that

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parasitic helminths, and hookworms in particular, can suppress inflammation associated with autoimmune and allergic diseases – a phenomenon that is embodied by the Hygiene Hypothesis. Recent and past contributions to these and other aspects of hookworm immunology have involved talented researchers from many different countries, but in this review, we will focus particularly on the work of Australian researchers.

ANTIBODY RESPONSES TO HOOKWORM

Antibodies of the isotypes IgG1, IgG4, IgM, IgD, IgA and IgE from hookworm-endemic (both the human hookworms *N. americanus* and the zoonotic dog hookworm *Ancylostoma caninum*) populations have all been shown to bind to hookworm antigens (5). In experimental hookworm infections, parasite-specific IgM is detectable 6 weeks after infection, with parasite-specific IgG detectably increased 8 weeks after infection (6–9). IgE responses in experimental human infections appear to develop slowly over a number of exposures, and the IgE response is generally undetectable in primary infections (8,9). As a result of its protective role in many helminth infections, IgE has been of particular interest to researchers. In the 1970s, David Grove and colleagues studied the role of IgE in *N. americanus* infections in the highlands of Papua New Guinea. They were the first to show that IgE, whether it be parasite specific or polyclonal, afforded protection against hookworm infection (10,11). Further evidence of the protective role of IgE in hookworm infection comes from vaccine studies, where levels of IgE against the vaccine candidate antigen *Na*-ASP-2 (ancylostoma secreted protein-2) in endemic populations from Brazil negatively correlate with infection intensity, while IgG4 against ASP-2 positively correlates with infection intensity (12). In filariasis and schistosomiasis, parasite-specific IgG4 correlates with a suppressed 'modified TH2' response, able to be differentiated from the parasite-killing (but often more pathogenic) IgG1 or IgE immune responses (13). A similar paradigm may exist in hookworm infection, and indeed, IgG4 specific to hookworm antigens is the best serological predictor of infection (14,15), implying a modified TH2 response is almost universal in hookworm infection. Therefore, if the immune response to hookworm is skewed away from the modified TH2 IgG4 response to a protective TH2 IgE response, immunity to the parasite may be possible.

Studies in hookworm-endemic areas have shown that levels of most isotypes of antigen-specific antibodies drop after drug cure, apart from circulating IgD which increases (16), implying that hookworm infection mediates the suppression of this antibody isotype. The function of circulating IgD has been debated for some time, but it was recently shown to bind to an unknown receptor on basophils, and cross-linking of IgD on the basophil surface leads to the production of inflammatory anti-microbial products and IL-4 (17). IL-4 from basophils was also recently shown to be crucial in the initiation and maintenance of TH2 responses (18–20). Therefore, it is tempting to speculate that hookworm suppresses the IgD response in infected individuals to suppress the development of a potentially host-protective TH2 response.

All data on humoral responses to hookworms in humans have come from blood serum studies. However, in the context of a parasite that resides in the gut lumen, such as hookworm, the mucosal and faecal antibody titres may be important in immunity. A recent study in the hamster model of *Ancylostoma ceylanicum* infection showed detectable levels of parasite-specific IgA in the faeces of multiply infected hamsters, associated with resistance to re-challenge (21). Further studies in human hookworm-endemic populations are needed to see whether the mucosal IgA response is important in resistance, as this may have implications for vaccine design.

CYTOKINE RESPONSES TO HOOKWORM INFECTION

Studies on the cytokines produced in hookworm infections show variable results: experimental and endemic (chronic) infections result in different cytokine profiles, indicating that repeated infection in endemic areas may induce a qualitatively and quantitatively different response (5,22). However, differences in techniques used may also have a role here: many studies use whole blood culture rather than PBMC purified cultures, which can result in lower concentrations of some cytokines (23), possibly leading to levels falling below the limits of detection. In addition, some groups have stimulated cell cultures with antigens derived from the dog hookworm, *A. caninum*, rather than antigens from human hookworms because of the difficulty in obtaining the latter (24–26).

Gastrointestinal parasitic infections have been long regarded to induce polarized TH2 responses, with production of IL-4, IL-5, IL-13 and IgE, which are necessary for their expulsion (27). TH2 responses have been shown to be somewhat effective against controlling hookworm infections, with elevated IL-5 positively correlating with resistance to reinfection after drug cure in humans (28).

In recent years, evidence has mounted that the immune response to hookworms may not be as simple as a polarized TH2 response. As mentioned previously, immune responses differ between experimental primary infection and responses in presumably multiply exposed endemic populations. Restimulation of PBMCs from experimentally

infected individuals with hookworm products leads to the production of TH2 cytokines (22,25); however, some studies have shown that in endemic areas IFN-γ production (a TH1 cytokine) is also evident (5,29).

In a study in Papua New Guinea, a negative correlation between infection intensity and IFN-γ production was detected, but there was no association between IFN-γ production and reinfection intensity after drug cure (28). IFN-γ production to mycobacterial antigens was also negatively correlated with egg burden, implying systemic suppression of IFN-γ production, but no protection from hookworm-specific TH1 responses. In a similar study in Brazil, individuals from a hookworm-endemic area were drug-cured and 6 months later divided into three groups – those that became reinfected after drug cure ('reinfected'), those that did not ('cured') and those that were not infected before or after drug cure ('endemic controls'). The endemic controls had higher production of IFN-γ, IL-5 and IL-13 to hookworm antigens, indicating a protective role of these cytokines in a mixed TH1/TH2 response. Also spontaneous (not antigen specific) production of IL-10 was the highest in the reinfected individuals (24). This study implies that the reinfected group may be the most susceptible to hookworm infection because of up-regulation of the regulatory cytokine IL-10 and down-regulation of the protective TH2 (or mixed TH1/TH2) response. The 'cured' group showed intermediate levels of both the effective IL-5 response and the suppressive IL-10 response, thus may represent a moderately susceptible group.

Thus, it may be that a mixed TH1/TH2 response is induced in hookworm infection, but as only the TH2 cytokine IL-5 correlates with protection (28), only the TH2 response appears effective against the parasite. Mixed TH1/TH2 responses are also seen in schistosome and filarial infections and are associated with an effective immune response against these parasites (30). This was elegantly demonstrated in mouse studies using an irradiated schistosome cercaria vaccine, where mice deficient in either the TH1 or the TH2 arm of the immune response had heightened susceptibility to infection (31). If it is the case that only the TH2 response is effective against hookworm, the difference between anti-hookworm responses and responses to schistosomes and filariae may be in the niche that each parasite occupies within the host. Schistosomes and filariae are blood- and lymphatic-dwelling parasites, respectively, and are therefore exposed to the full force of the cellular immune response, where TH1 effector mechanisms, such as nitrogen and oxygen radicals from macrophages, may be as effective at eliminating parasites as TH2 effector mechanisms, such as toxic eosinophil products. Hookworms, by contrast, live for the vast majority of their lives in the host as adults in the lumen of the gut, where inflammatory TH1 responses may cause more harm to the host than to the parasite.

Although mixed TH1/TH2 responses have been reported in endemic populations, only a polarized TH2 response has ever been reported in experimental human hookworm infection (8,22). It may be that repeated infection in endemic areas is required for the stimulation of a TH1 response to hookworm; however, a study using repeated experimental infection (50 larvae followed by another 50 larvae 27 months later) showed negligible levels of IFN-γ to hookworm antigen at all time points (22). A further possibility is that other pathogens common in helminth endemic areas (e.g. malaria) may skew immune responses towards a TH1 phenotype. In mouse models of coinfection with hookworm (*Nippostrongylus brasiliensis*) and TH1-inducing protozoa or bacteria, although a suppression of helminth-specific TH2 responses has been seen (32–34), to our knowledge, no induction of helminth-specific TH1 responses has been reported in mice or humans. Thus, it is possible that reports citing anti-hookworm IFN-γ responses are actually because of endotoxin contamination of the stimulating antigen, particularly given that adult and larval hookworms are derived from the intestine or faecal culture, respectively. This possibility is difficult to exclude without data from uninfected, unexposed control subjects, which is often absent from these studies. For instance, a recent study showed the highest production of IFN-γ to larval antigens at week 0 of an experimental infection, prior to exposure to the parasite (25).

CELLULAR RESPONSES TO HOOKWORM

Only a small number of studies have characterized the T- and B-cell immune response to hookworm *ex vivo*. Two studies show a small decrease in proportions of circulating $CD4^+$ T cells and $CD19^+$ B cells in hookworm-infected individuals from an endemic area (26,35), with increased levels of the activation markers CD69 and HLA-DR on T cells (26). Other studies have shown similar results with other parasitic (36) and bacterial (37,38) infections, indicating this is most likely an effect of long-term inflammation, resulting in the activation of T cells and movement of T cells from the circulation to the effector site or draining lymph node.

Hookworm infection also causes changes to the cells of the innate immune system, most obviously blood eosinophilia. In both experimental and endemic infections, eosinophilia is evident within 4 weeks after exposure (7,8,22,25,39,40). Eosinophils from hookworm-infected individuals also show increased expression of activation

markers compared to uninfected individuals (41). It is now recognized that eosinophils are competent antigen-presenting cells as well as effector cells, as they have been shown to process and present antigen on MHC class II molecules and stimulate T cells (42). Thus, eosinophils may be important cells in initiating or maintaining the immune response during hookworm infection.

Recently, basophils have gained regard as a key cell type in TH2 immune responses. The importance of basophils early in TH2 responses was shown in mice, where depletion of basophils prior to gastrointestinal parasite infection resulted in increased parasite burden and decreased TH2 responses (18). Basophils from individuals experimentally infected with hookworm are activated by *N. americanus* antigen from 8 weeks after infection, and this effect was retained as long as 5 years after infection (9). Basophils are potently activated by cross-linking of surface-bound IgE; however, as mentioned previously, increases in polyclonal or antigen-specific IgE are often undetectable in experimental infections, including in this study. Thus, basophil activation by *N. americanus* antigen within weeks of primary infection may be via either cross-linking of undetectably low levels of surface-bound parasite-specific IgE or cross-linking of *N. americanus* antigen-specific surface-bound IgG. Human basophils were recently found to express the low-affinity IgG receptors CD16 and CD32 (43), although some evidence shows that cross-linking of IgG receptors on basophils may be inhibitory rather than stimulatory (44). Thus, it will be interesting to see if basophil activation during early hookworm infection is dependent on IgE receptors and whether basophils can be activated by cross-linking of surface-bound IgG.

Another mechanism of basophil activation during hookworm infection may be by protease activation [via an as yet unknown mechanism (45)], as naïve human basophils exposed to *N. americanus* excretory secretory products (NaES) produce IL-4 and IL-13, and this production was inhibited by protease inhibitors (46). Basophils were recently shown to be necessary and sufficient to induce TH2 responses *in vitro* and *in vivo* to protease allergens, as they are activated by proteases, act as antigen-presenting cells and induce a TH2 response by releasing IL-4 and thymic stromal lymphopoietin (19). Thus, basophils may be extremely important both in the initiation and in the maintenance of the TH2 response to hookworm infection.

When studying the effects of hookworm infection on dendritic cell (DC) differentiation, a Brazilian study saw that DCs derived from hookworm-infected patients' monocytes show defective differentiation, with decreased CD11c (and residual expression of CD14) compared to uninfected controls. These DCs also show defective expression of CD86 and Class I and II MHC molecules, resulting in defective antigen presentation (41). Interestingly, a dog hookworm product, *A. caninum* Tissue inhibitor of Metalloproteases-1 (*Ac*-TMP-1), was recently shown to affect mouse DC maturation such that they could promote $CD4^+$ and $CD8^+$ regulatory T-cell differentiation (47). It will be interesting to see if the same mechanism takes place with human hookworm TMP-1 and human DCs.

Hookworm infection also affects NK cells, with a larger number of NK cells in the circulation of infected individuals. These NK cells appear activated as they spontaneously produce IFN-γ in culture (48). NaES acts as a chemoattractant for NK cells and also binds to a subset of NK cells, directly inducing IFN-γ release (49). Interestingly, this effect is lost in individuals from hookworm-endemic areas (48), implying saturation of the NK cell receptor. Thus, hookworms may release molecules that actively attract and expand NK cells during infection and stimulate IFN-γ release through an undefined NK receptor. This has been proposed as an immune evasion strategy as the IFN-γ released could cross-regulate the otherwise protective TH2 response.

HOOKWORM VACCINES

The first hookworm vaccine was developed in 1965 against the dog hookworm *A. caninum* and consisted of irradiated larvae (50). Although this vaccine gave good protection against experimental and field challenge, it was withdrawn from veterinary use after concerns with efficacy and shelf life were raised. In the 1980s, David Grove and Simon Carroll switched their focus from human immunity to vaccines using *A. ceylanicum* infection in dogs as a model for the human disease. They showed that dogs that were chronically infected then treated with an anthelmintic were resistant to reinfection (51), highlighting for the first time that immunity to reinfection could occur, at least in the *A. ceylanicum*/dog relationship. Carroll and Grove then went on to explore the protective efficacy of hookworm extracts and showed that protection against *A. ceylanicum* infection in dogs by vaccination with adult worm aqueous somatic extracts when formulated with Freund's adjuvants (52), kicking off efforts to develop vaccines based on soluble molecules rather than whole parasites.

More recently, recombinant vaccines have been found to exert partial efficacy in the dog hookworm model using *A. caninum*, stimulating human trials with orthologous *N. americanus* antigens presently underway. The first recombinant vaccine to show efficacy against hookworm was *ancylostoma* secreted protein-1 (*Ac*-ASP-1), which conferred partial protection in mice challenged with *A. caninum* (53,54). ASPs are a large family of proteins,

which are the most highly expressed products of *in vitro* activated larvae (55), with the related ASP-2 protein discovered shortly after ASP-1 (56). However, mice are not a permissive host for hookworms, and ASP-1 did not confer protection in permissive hosts including hamsters (57) and dogs (58). ASP-2, by contrast, appeared to show similar protection to that of irradiated larvae (57), and in human hookworm-endemic populations, IgE specific to ASP-2 negatively correlated with hookworm burden, thus highlighting its potential as a vaccine candidate in animal models and endemic regions (12). An *Na*-ASP-2 vaccine is currently in development: it has been shown to raise effective and safe immune responses in unexposed individuals (59) and is currently in phase I clinical trials in Brazil being conducted by researchers (including ourselves) at The Human Hookworm Vaccine Initiative – see http://www.sabin.org/vaccine-development/vaccines/hookworm. The mechanism of protection of the ASP-2 vaccine appears to be against the larval stage of the hookworm: ASP-2 is transcribed in all life-cycle stages of hookworms, but the protein is released only after the larvae become activated by penetrating host skin (56). Most vaccine strategies have focussed on the larval stage of the hookworms; however, there is some evidence that resistance to later stages is possible (60).

In repeated experimental hookworm infections, it could be seen that although the majority of the newly infected larvae migrated from the skin to the gut, only a small number could attach successfully to the gut wall (60). The total number of worms attached (previously patent plus new arrivals) seemed dependent on levels of eosinophilic inflammation of the gut wall, and so it appears that resistance to the later gut feeding stages of the parasite is possible. Interestingly, in human enteric infection with dog hookworm in an Australian community (see later), much more pronounced inflammation was seen than that with human hookworm (61). High levels of eosinophil infiltration in the gut wall caused inflammation and pathology. This inflammatory allergic response has been cited as the cause of dog hookworm ejection from humans, and its absence in human hookworm infection (and dog hookworm infection in dogs) argues for active and species-specific suppression of the anti-hookworm response (62). Thus, eosinophilic attack of adult worms in the gut may lead to ejection of the parasite, but at the cost of inducing a destructive eosinophilic enteritis.

Other vaccine strategies to attack the adult parasite are being developed, which may not cause damaging inflammation. One approach is to target the gut of the adult worm to prevent it from successfully feeding. Hookworms ingest blood from ruptured capillaries in the host gut wall, where the blood is digested in the hookworm's own gut and absorbed. A cascade of proteolytic enzymes carries out the digestion of host blood, and these enzymes can be considered 'cryptic' antigens – they are never exposed to the host immune system, and so an immune response is never raised against them. During the course of feeding, however, the hookworm gut is exposed to antibodies in the host blood, a phenomenon of which we are targeting in our vaccine development strategy (63).

A vaccine candidate, aspartic protease-1 (APR-1), has been identified from the adult blood-feeding stage of the parasite; a vaccine targeting APR-1 is aimed primarily at preventing effective nutrient uptake in the gut of the adult hookworm, effectively starving it to death (64). APR-1 is a protease involved in the haemoglobin digestion cascade within the gut of hookworms (65). It has been shown to be effective against both *A. caninum* infection in dogs (64,66) and *N. americanus* in hamsters (67). Indeed, the proposed mechanism by which APR-1 vaccines protect the host is via the induction of antibodies that neutralize the enzymatic activity of the protease, thus rendering it unable to digest haemoglobin and other blood proteins (Figure 1). APR-1 proved difficult to express in a manner that renders it easily produced in large quantities, so the neutralizing epitopes of APR-1 were identified and fused to other hookworm and even schistosome vaccine antigens to develop chimeric vaccines that target multiple antigens and even multiple blood-feeding helminth species (68). A strategy for a successful hookworm vaccine may use a cocktail of potential vaccine candidates, including APR-1, targeting both the larval and blood-feeding stages (63).

HUMAN ENTERIC INFECTION WITH THE DOG HOOKWORM, *ANCYLOSTOMA CANINUM*

In the early 1990s, gastroenterologist John Croese and medical parasitologist Paul Prociv identified a series of cases of eosinophilic gastroenteritis in Caucasian residents of North Queensland (69). Initially, the disease was of unknown aetiology but as more cases were diagnosed, solitary adult hookworms were identified from a few patients and were subsequently identified as the canine hookworm, *A. caninum*, a parasite that was previously thought not to reach maturity in the human gut (69) (Figure 2). As awareness spread amongst clinicians, cases were diagnosed in other areas where *A. caninum* was prevalent, including southern Queensland (70) and the south of the USA (71). While solitary adult *A. caninum* were identified in only a handful of patients, infection was suspected in many more, so we developed assays to detect circulating IgG and IgE antibodies to adult *A. caninum* excretory/secretory proteins and confirmed that many of

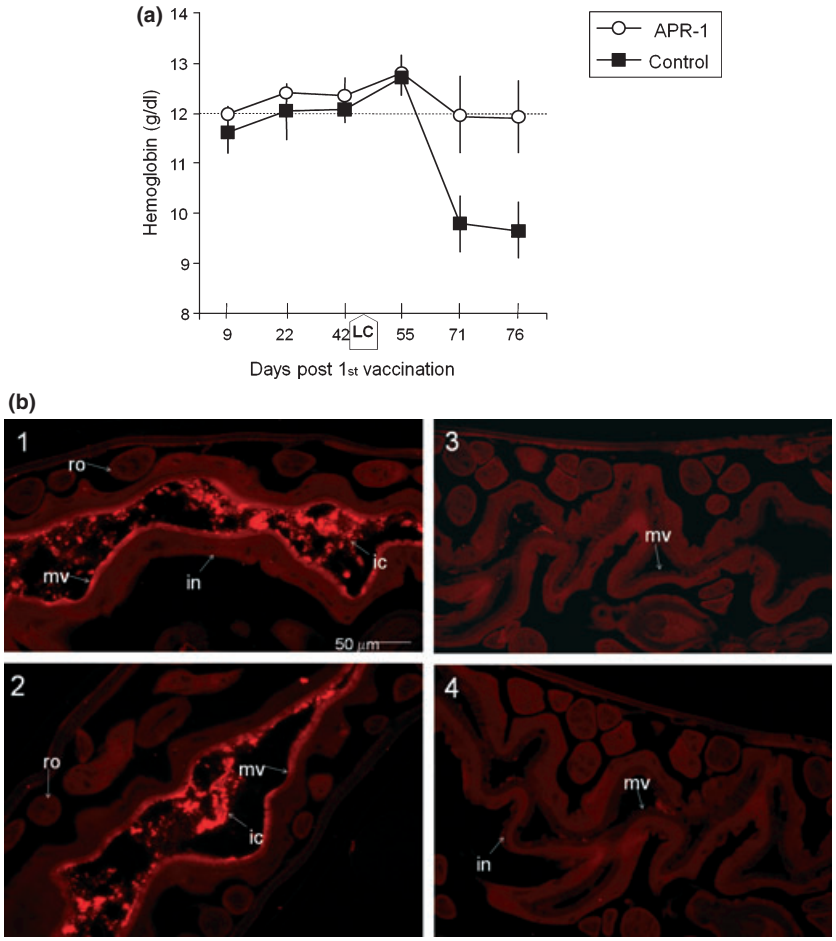

Figure 1 Haemoglobinase vaccines interrupt blood feeding. (a) The *Ac*-APR-1 haemoglobinase vaccine reduces anaemia in vaccinated but not control dogs after challenge with infective hookworm larvae. LC = larval challenge. (b) Antibodies from dogs vaccinated with recombinant *Ac*-APR-1 (frames 1 and 2) but not antibodies from control dogs (frames 3 and 4) bind to and damage the microvillar surface of the gut when they are ingested during the parasite's blood meal. Reproduced from reference 64. in = Intestine; mv = microvilli of intestinal cells; ro = reproductive organs.

the suspected cases of eosinophilic gastroenteritis where there was no parasitologic evidence of infection (i.e. no detection of adult worms or faecal eggs) were likely caused by occult infection with *A. caninum* (70,72). It is also noteworthy that in at least one patient, an adult *A. caninum* was observed in the absence of any overt pathology or symptoms (70). These findings pose an intriguing scenario whereby human enteric infection with the zoonotic *A. caninum* might be far more common than appreciated, and many of these infections might go unnoticed because of mild to no detectable pathology/symptoms.

HOOKWORM AND THE HYGIENE HYPOTHESIS

The Hygiene Hypothesis states that as populations become more hygienic and therefore virtually eliminate childhood parasitic infections (which have been constant partners through human evolution), there has been a concurrent increase in immune dysregulatory syndromes, such as autoimmunity, allergy and inflammatory bowel diseases. Diseases such as these are substantially less common in parts of the world with high helminth endemicity, and within endemic areas, the prevalence of allergic atopy is significantly lower in individuals with chronic worm infections (73–78). In epidemiologic studies, there is a good case for hookworm infection suppressing immune dysregulation. Hookworm appears to protect against asthma more than any other parasite investigated; a meta-analysis of studies of asthma in parasite endemic and nonendemic areas showed that *Ascaris lumbricoides* may have an asthma inducing effect, yet hookworm significantly protects against asthma, despite larvae of both parasites migrating through the lungs (79).

Interventional studies show that after drug cure, allergy may increase at the population level (80,81). Chemotherapy to remove intestinal helminths results, in some studies, in aggravated allergic responsiveness. In a recent double-blinded placebo-controlled interventional trial in an area of Vietnam where hookworm is the most common

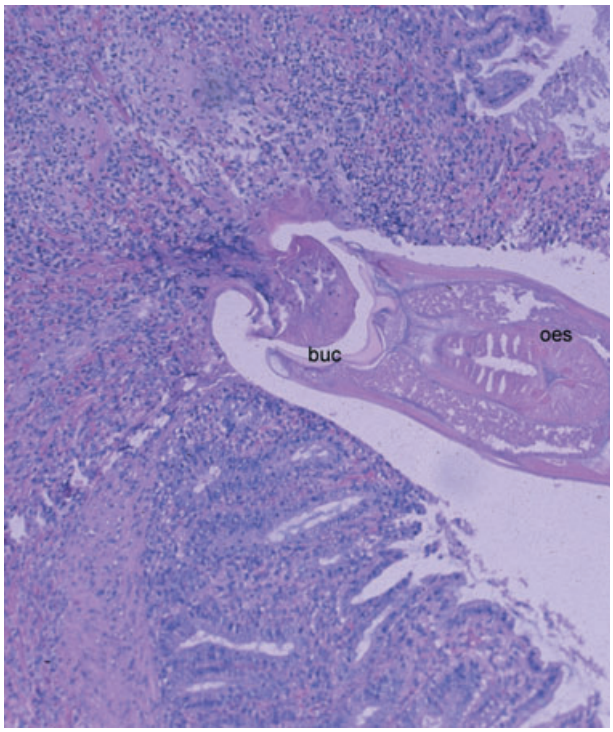

Figure 2 Adult *Ancylostoma caninum* in the canine gut. Transverse histological section through the duodenum of a dog experimentally infected with *A. caninum*. Note the anterior end of the adult hookworm showing the muscular oesophagus (oes) and the buccal capsule (buc) grasping a plug of duodenal mucosa.

infection, the anthelmintic-treated group had a significantly increased incidence of skin allergy sensitivity to house dust mite or cockroach allergens. This protection correlated with significantly higher levels of baseline IL-10 production to hookworm antigen, with a trend for decreased production of IL-10 after treatment (82).

The idea that worm-induced immunomodulation could be used to treat immune dysregulation in the developed world has been gathering support in recent years. A turning point was a clinical trial in the USA, where *Trichuris suis*, the pig whipworm, was used to treat inflammatory bowel disease. The results of the trial were very encouraging, and the majority of treated patients went into remission (83,84). However, the same therapy was ineffective against allergic rhinitis in humans (85). Humans are not a fully permissive host for *T. suis*, so the infection had to be boosted with larvae every 3 weeks to ensure continual presence of larvae in the gut (86). As a treatment for immune dysregulatory diseases, hookworm may be an attractive prospect – it is virtually asymptomatic in low-level experimental infections (40), it poses no risk of transmission in modern sanitary environments and it survives for years within the human host, thus making continual reinfection unnecessary. British and Australian researchers have used hookworm in seasonal hayfever, Crohn's disease and coeliac disease, with varying success. The British trials showed that hookworm infection, despite the migratory stage through the lungs, does not exacerbate airway reactivity in allergic individuals; however, no suppression of allergic responses was detected (8,39). No suppression of inflammatory immune responses, as measured by production of IFN-γ or TNF-α, or induction of immunoregulatory mechanisms, as measured by levels of circulating $CD4^+CD25^{hi}Foxp3^+$ Tregs or polyclonal $CD4^+$ T-cell production of IL-10, was seen either (8).

In contrast, the Australian Crohn's disease trial led by John Croese showed a strong trend for suppression of Crohn's disease symptoms after infection (87). However, caveats of this trial include a lack of blinding or a placebo control group, and continued and variable use of immunosuppressants. This trial is currently being extended by Croese and our group, to use hookworm to treat coeliac disease, a gluten-induced enteropathy dependent on a TH1/TH17 response (ms submitted). Twenty coeliac sufferers (all of whom were on a long-term gluten-free diet and therefore had an essentially healthy gastrointestinal tract) were recruited and split into double-blinded hookworm-infected and control groups. After 20 weeks of infection, all participants were given an oral gluten challenge to induce coeliac pathology. Again, a nonsignificant trend for less pathology was seen in the hookworm-infected group. Because of the coeliac status of the participants, endoscopy was carried out to check for pathology and also allowed for the assessment of the hookworm response in the mucosa. Spontaneous production of IL-5 from duodenal biopsies was detected in the hookworm group, with highest levels in biopsies taken immediately adjacent to the hookworm bite site. Interestingly, no other TH2 cytokines (IL-4 or IL-13) were spontaneously produced by duodenal biopsies in the hookworm group. These data may give more credence to the hypothesis that eosinophil recruitment, dependent on IL-5, is directly responsible for the degradation of the hookworm bite site, forcing the parasite to select a new feeding area (60). The source of this IL-5 in the mucosa is not known but could be mast cells rather than TH2 cells, especially when considering the lack of other TH2 cytokines (88). TH1 and TH17 inflammatory cytokines from the mucosa were suppressed during hookworm infection, showing immunomodulation by the parasite at the site of infection and (coeliac) inflammation. Some systemic suppression was also seen, with a trend for less gluten-specific TH1 cells in the blood. This trial gives strong evidence that hookworm infection can suppress inflammatory responses.

The differences between the British study (8) and our own may be because of a number of factors. The British study was designed to investigate suppression of allergic airway responses, whereas ours investigated a TH1/TH17 gut enteropathy. Although there is good epidemiological data to support hookworm suppression of allergic responses, allergy may be more difficult to assess in an experimental setting: the time and dose of antigen are uncontrolled, the pathology is physically separated from the adult parasites and the TH2 nature of the immune response may be harder to suppress in this system. Coeliac disease is well established as a TH1-mediated pathology, with recent articles showing a role for TH17 also (89,90). Hookworms induce a strong TH2 response, and TH2 responses are known to cross-regulate TH1 and TH17 responses (91). Thus, in our coeliac disease trial, two mechanisms could be suppressing pathology – the regulatory responses which control immune dysregulation in endemic populations and also cross-regulation by a TH2 response of an inflammatory TH1/TH17 response occurring in the same physical location.

CONCLUSION

Human coevolution with hookworms has reached a stage where humans are relatively asymptomatic when harbouring low-intensity infections, assuming reasonable nutritional status of the host. Evidence is gathering that the hookworm manipulates the human immune system such that the infection is tolerated with minimal pathology to either the worm or the host. It seems likely that during our coevolution, the immune system has adjusted to compensate for the continual suppression by hookworm infection. Thus, in the absence of these parasites, our immune responses have become 'hyperactive', resulting in an increase in the prevalence of immune dysregulatory illnesses in the developed world. Future studies will show whether we can use hookworms, or preferably molecules derived from them, to correct this imbalance. Indeed, if vaccines and other control measures aimed at reducing the prevalence of hookworm (and other neglected tropical diseases) are implemented en masse, the resulting effect on the prevalence of autoimmunity and allergy in these countries is of potential concern.

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