



Review Article

Immunology of experimental and natural human hookworm infection

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SUMMARY

Human hookworm infection is one amongst the most prevalent of the neglected tropical diseases. An informative experimental animal model, that is, one that parallels a human infection, is not available for the study of human hookworm infection. Much of our current understanding of the human immune response during hookworm infection relies on the studies from experimental infection of hookworm-naïve individuals or the natural infections from individuals residing in hookworm-endemic areas. The experimental human infections tend to be acute, dose-controlled infections, often with a low larval inoculum so that they are well tolerated by human volunteers. Natural hookworm infections usually occur in areas where hookworm transmission is constant and infection is chronic. In cases where there has been drug administration in an endemic area, re-infection often occurs quickly even amongst those who were treated. Hence, although many of the characteristics of experimental and natural hookworm infection differ, both models have elements in common: mainly an intense Th2 response with the production of total and specific IgE as well as elevated levels of eosinophilia, IL-5, IL-10 and TNF. While hookworm infection affects millions of individuals worldwide, much of the human immunology of this infection still needs to be studied and understood.

Keywords experimental infection, hookworm, human, natural infection

INTRODUCTION

Hookworms are blood-feeding nematodes whose infections are usually species specific. The hookworm species infecting humans are *Necator americanus* and *Ancylostoma duodenale* (1, 2). The World Health Organization (WHO) estimates that more than 700 million people are infected with these hookworm species, including approximately 44 million pregnant women. While infection by one of these species is amongst the most common of the neglected tropical diseases (NTDs), transmission mostly occurs amongst the bottom billion, that is, individuals resident in poor rural area of tropical and subtropical regions (3, 4), who do not have access to adequate health care and readily available chemotherapy. In individuals with poor nutritional background, blood loss from hookworm infection can lead to iron-deficiency anaemia (IDA) and protein malnutrition (5–12). During pregnancy, the infection has been shown to increase the risk of a low birth weight, decreased lactation and mother/infant mortality (13).

Over the decades, the efforts to control hookworm transmission have been multiple and varied, with mainly mass drug administration (MDA), improved sanitation, and health education (3, 14–16), amongst the most popular. To date, the most successful eradication programme was conducted at the start of the 20th century, when the Rockefeller Sanitary Commission for Eradication of Hookworm Disease introduced a 5-year programme to defeat hookworm infection in United States (3). Although most strategies have resulted in a significant decrease in prevalence, none have resulted in the elimination of the disease. Relying only on the use of single drug treatment provides a short-term solution and the high transmission of hookworm often results in re-infection almost immediately after treatment (3). The problems in regard to control and the elimination of hookworm include socio-economics, the ecology of the endemic areas and the

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Disclosures: None

Received: 1 July 2013

Accepted for publication: 30 October 2013

biology of host–parasite relationship (16, 17). Until now, poverty reduction and urbanization have proven to be the most effective means for reducing hookworm transmission (18).

Life cycle

The life cycles of both species of human hookworms are considered excellent examples of direct transmission. The simplicity in the life cycle, plus poor sanitation, contributes to the endemicity of hookworm infection and the rapid re-infection after chemotherapy (19). Eggs are evacuated with human faeces, deposited in the soil, and hatch after 24–48 h, given the proper humidity and ambient temperature. The first stage or L1 are referred to as rhabditiform larvae and after a brief growth period moult into the L2 stage. Both larval stages are free-living (i.e. are not dependent on a host for food) and live by feeding on organic debris and bacteria present in the soil. The L2 later develop into the infective L3 stage, which measure approximately 600 µm in length. Unlike the previous stages, the L3 are in a state of arrested development and do not feed; nonetheless, they can survive for weeks in the environment using energy accumulated during the previous two larval stages (3). To increase the chances of an encounter with the host, the L3 seek higher ground to be more exposed, finding their target through a combination of positive thermotropism (movement towards higher temperatures) and positive thigmotropism (movement towards an object in response to touch or contact stimuli) (20). After contact with the human host, L3 penetrate through the skin, often through hair follicles, and make their way to the capillaries, migrating through the vessels to arrive at the heart and then later passing into the lungs. Once in the lungs, L3 larvae ascend to the trachea, where they are coughed up and swallowed, starting their migration through the digestive tract. The transformation into L4 larvae occurs in the lumen, where they develop into the adult form (21). The prepatent period (i.e. the time it takes for larvae penetrating the skin to reach the intestine and become sexually mature egg-laying adult worms) for both human hookworm species lasts between 5 and 8 weeks. Mature males and females copulate in the lumen and generate eggs, which pass out with the faeces into the environment, restarting the life cycle. Female adult worms can produce up to 1000 eggs per day (3, 22).

There is no robust experimental animal model that parallels the course of hookworm infection in humans. Human hookworm species do not mature in mice (23). Hamsters are (at best) a semi-permissive host to these species and, although they can be infected with *N. americanus*, the infection time is much shorter and the worm

burden much lower than in humans (24, 25). Moreover, as with other forms of animal hookworm infection, the hamsters become refractory to the infection, removing the infection through as yet unknown mechanisms much like canines and the dog hookworm *A. caninum*. As such, the hamster model of infection is considered an acute model of infection as the adult worms are usually expelled a few weeks after they reach the small intestine. In contrast, human hookworms can be established for years (if not a lifetime), with the disease progressing with the accumulation of hookworms (24, 26–28).

IMMUNOLOGICAL RESPONSE DURING HUMAN EXPERIMENTAL INFECTION

Experimental human hookworm infection has made a major contribution to our understanding of the immunological response that occurs with this parasitic nematode (29). One of the first experimental studies published consisted of a self-infection with 250 L3 using four inoculums and followed for over 2 years (30). At each infection, the presence of eggs in faeces remained constant, appearing approximately 47 days post-infection. The production of eggs also remained constant throughout the four infections averaging at 2150 eggs per gram (EPG) of faeces per infection. Although during the first infection, the presence of gastrointestinal disturbances was severe, during the second infection these symptoms dissipated, with the two last inoculums resulting in no gastrointestinal discomfort at all (30).

However, a pronounced eosinophilia was observed in all four infections, although there was no change in red blood cell parameters. Specific immunoglobulin (Ig) G antibodies against the soluble excretion/secretion (ES) antigens from the adult worm were detected 6–7 weeks after the first infection. During the second infection, these antibodies appeared after only 5 weeks and, during the last two infections, they appeared even earlier. The levels of ES-specific IgG antibodies remained high in all four infections and rapidly decreased after treatment. The level of total IgE, on the other hand, increased significantly only after the second infection and remained high throughout the rest of the experiment, even after treatment for the fourth infection, although the levels were low in comparison with those seen in naturally infected individuals from endemic areas. Nonetheless, specific IgE levels to ES significantly increased after the third and fourth infections (30).

Pioneering experimental studies conducted by Maxwell *et al.* (31) followed the infection of human participants infected with *N. americanus* for 10 weeks. The primary outcome of this study was the determination that an infectious dose of 50 L3 is well tolerated by individuals, as it

did not cause anaemia nor other adverse events, although participants did report some flatulence and abdominal pain during the 5th week of the infection – most likely due to the establishment of the worms in the gut (i.e. patency) (31). The infection also caused mucosal erythema in the lungs as measured by bronchoalveolar lavage. Finally, these participants presented with an elevated blood eosinophilia that peaked 38–64 days post-infection. Only a few individuals showed a small increase in total IgE and total IgG antibody levels (31).

In a double-blind, dose-escalating study using hookworms as a form of therapeutic infection with *N. americanus* in adults followed for 12 weeks, Mortimer *et al.* (32) confirmed that infection with more than 100 L3 is not well tolerated, resulting in the withdrawal of a participant from the study due to an extensive maculopapular eruption at the site of larval entry and gastrointestinal disturbances. The participants who received 10, 25 or 50 L3 tolerated the infection well, although a participant who received 50 L3 withdrew due to the development of diarrhoea and abdominal pain. In the remaining participants, the average white blood cell count increased, peaking at 5–9 weeks post-infection, mostly due to changes in eosinophils as seen in the above-mentioned studies. Eggs in faeces appeared 4–6 weeks after infection, with the highest egg counts in participants who received the highest doses of L3. Although total IgE levels increased slightly 2–6 weeks after infection, the difference between the dose groups was not significant. Specific IgG levels to ES antigen increased gradually, peaking at 10 weeks in participants who received 10 L3 and 12 weeks in participants that received 25 or 50 L3 (32). Based on the above-mentioned dose-escalating study, Feary *et al.* (33) conducted a randomized placebo-controlled trial in asthma-infected individuals with either 10 *N. americanus* L3 or a placebo (a histamine solution) and followed them for 16 weeks. Again, all of the hookworm-infected individuals presented with eosinophilia, which appeared at 21 days post-infection and peaked on days 42 and 84. Eggs in faeces appeared at week 6 in some volunteers and at week 8 on others, with EPGs between 95 and 213 (33).

In another double-blind study conducted by Blount *et al.*, (34) participants were randomized to receive either 10 *N. americanus* L3 or a placebo (a histamine solution) and followed over 12 weeks. As in the previous study conducted by Mortimer *et al.* (32), the infected participants showed no change in haemoglobin or haematocrit levels, although the levels of ES-specific IgG increased at weeks 8, 10 and 12. All the infected participants presented with eosinophilia, which peaked at 6 weeks post-infection. The levels of total IgE were not significantly elevated, and the levels of ES-specific IgE were also not significantly

elevated compared with controls. Eggs were detected in faeces after 6–8 weeks post-infection (34).

In a study conducted with one participant who had no previous history of hookworm infection (35), the infection was followed for a longer period of time. After cutaneous infection with 50 L3, eggs were found in the stool 53 days after infection, with the peak occurring at 145 days with the production of 600–900 EPG of faeces. The number of white blood cells peaked on day 42 post-infection and was 2.5 times higher than pre-infection levels, with eosinophil counts five times higher than at pre-infection levels. This study also evaluated the cellular immunology of a primary infection with *N. americanus*. White blood cells cultured with soluble adult worm extract (AE) produced in supernatants interleukin (IL)-5 with <20 days of infection, while the levels of IL-13 in the same cultures peaked 84 days after the infection. A decrease in serum IL-5 was correlated with the appearance of eggs in stool, while the production of serum IL-13 had no influence on eosinophil levels or the presence of eggs. Serum interferon (IFN)- γ and IL-10 were also reported, although at low levels. AE-specific IgG and IgE antibody titres increased during the infection and remained high even 3 months after treatment (35). This participant was experimentally re-infected 815 days after the previous first infection, with the time to patency decreasing to 14 days post-infection, peak egg output decreasing to 128 days post-infection, and egg output greater than during the first infection. The blood parameters measured, and the cytokine and acute phase proteins responses were also less marked than during the first infection (35).

Another study evaluated the immune response to crude hookworm antigen extracts after infection with *Necator* focusing on the three most common extracts: soluble antigens from L3 (L3), somatic adult extract (AE) and adult ES products (ES) (Table 1) (36). The authors cultured peripheral mononuclear cells (PBMCs) of two experimentally infected individuals with no previous history of hookworm infection with these three crude antigen extracts. Results showed increased proliferation during the first 20 days of infection to both L3 and AE. Levels of tumour necrosis factor (TNF)- α increased when PBMCs were cultured in the presence of L3, AE or ES crude antigen extracts before and during patency. During prepatency, a decrease in the proliferation of PBMCs was observed, but an increase in TNF- α production was maintained. Production of the chemokine CCL17 also increased in PBMCs cultured with L3 crude antigen extract, while production of IL-10 was increased in PBMCs cultured with adult ES extract (36).

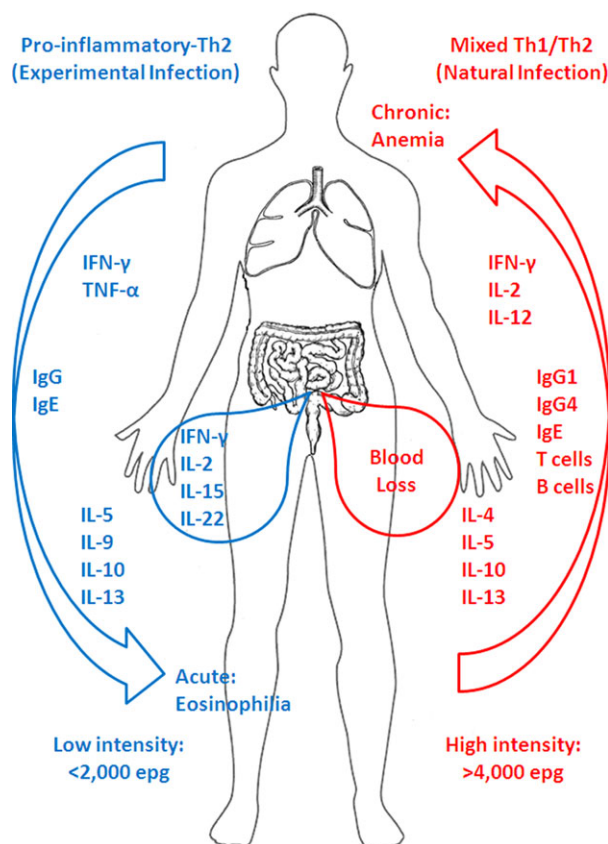
A randomized clinical trial was conducted using participants with coeliac disease who were in remission and infected with 15 L3 to study the human immunology of

Table 1 Cytokines produced in the presence of the main crude antigens from *Necator americanus*: soluble antigens from L3 (L3), somatic adult extract (AE) and soluble excretion/secretion (ES) antigen from the adult worm, in human experimental and natural infections

Type of Infection	L3	AE	ES	
Experimental	CCL17 (35) TNF (35)	IL-5 (34) IL-13 (34) TNF (35)	IL-4 (36) IL-5 (36) IL-9 (36)	IL-10 (35,36) IL-13 (36) TNF (35)
Natural	IFN- γ (44) IL-5 (44) IL-10 (44)	IFN- γ (49) IL-2 (52) IL-5 (49,52)	IL-10 (44,49,52,59) IL-12 (49) IL-13 (49)	IL-10 (44,50) TNF (50)

hookworm disease and its effect on this autoimmune disease (37). A systemic Th2 response was observed, with an increase in the levels of serum IL-5 and serum IL-13 starting from week 4 and peaking at week 12 post-infection. One of the main advantages of this study was access to biopsies from the mucosa of the small intestine both pre- and post-infection. The intestinal biopsies showed increased levels of IFN- γ mRNA after the infection compared with pre-infection (37). As a Th1 profile was not observed in these infected individuals, the presence of IFN- γ was considered to most likely be from an innate source. This is in keeping with data from a previous study by this same group in which ES products from *N. americanus* were shown to bind to host natural killer (NK) cells and trigger them to produce IFN- γ (38, 39). The study using coeliac patients also showed that mRNA levels of ALDH1A2 and IL-15 were increased after infection, although there was a decrease in IL-23 mRNA levels post-infection. The biopsies of the patients with coeliac were re-stimulated with ES and a strong Th2 response to this crude antigen extract was observed, with increased levels of IL-4, IL-5, IL-13, IL-9 and IL-10 mRNA (Figure 1) (37). This effect was also shown in PBMCs under the same re-stimulation conditions. Levels of IL-15, transforming growth factor (TGF)- β and IL-22 mRNA were also increased in biopsies subsequently cultured with ES (37). Thus, it was hypothesized that hookworm infection can decrease the levels of pro-inflammatory cytokines and immune cell activation by inducing a strong Th2 and regulatory response environment that contributes to worm survival for years in the host.

In summary, human experimental studies show an acute, dose-controlled infection with no development of anaemia. Infections of 50 L3 or more result in gastrointestinal and pulmonary discomfort, with increasing discomfort, as the infective inoculum increases. Eosinophilia is present throughout the infection and peaks at 35–84 days post-infection, which coincides with the appearance of eggs in faeces (4–6 weeks post-infection). There is a positive correlation between the infective inoculum and the amount of eggs per gram of faeces. ES-specific IgG levels in serum

**Figure 1** Diagram summarizing the major characteristics of human natural and experimental hookworm infection.

are increased, peaking at 6–7 weeks post-infection and then decrease after treatment, while AE-specific IgG levels remain high even 3 months after treatment. There is a slight increase in ES- and AE-specific IgE levels in serum as well as total IgE, and this increase seems to be related to the infection dose or the prolonged exposure to the parasite (multiple hookworm infections). Multiple hookworm infections also resulted in the amelioration of the symptoms observed during initial hookworm infection, indicating an as yet unknown accommodation of the host to hookworm infection as seen in natural hookworm

transmission. The production of IL-5 and IL-13 increases in PBMCs cultured with AE in the first 20 days of infection and the levels of IL-13 peak at 12 weeks both in culture and in serum. The level of IL-5 in culture, on the other hand, decreases with the appearance of eggs in faeces. Levels of TNF- α increase in culture with L3, AE and ES before and during patency and the production of this same cytokine also increases in the gut after infection, which could be due to the binding of worm ES to NK cells.

IMMUNOLOGICAL RESPONSE DURING HUMAN NATURAL INFECTION

There are several important limitations when investigating natural hookworm infections in individuals resident in hookworm-endemic areas: (i) most of the studies are based on samples from chronically infected individuals, with no indication of the time of primary infection; (ii) worm burden is assessed by the surrogate marker of EPG and as such indicates only presence of mating worms and the many factors that can influence egg deposition, including the constant exposure to infective larvae in areas of high transmission; and (iii) transmission of other parasitic diseases, including infection with other helminths or protozoans, often results in co-infection, thus modulating the immunological response to hookworm infection (3). In this review, we will emphasize the immunological parameters observed in individuals mono-infected with hookworm under natural conditions, that is, studies that report observations obtained from individuals infected only with *N. americanus* in hookworm-endemic areas.

Most of the information on the immunology of natural human hookworm infection comes from epidemiological studies in hookworm-endemic areas, most of which initially centred on the systemic and specific humoral response to infection. Antibody responses to crude antigen extracts could be observed for all five isotypes: IgA, IgD, IgE, IgG and IgM (40, 41). Some of these antibody responses correlated with different aspects of the infection: for example, IgG to adult ES products was shown to correlate with parasite burden measured by EPG and titres of the antibody response decreased with the age of the host (42). Moreover, the majority of the studies conducted in endemic areas occurred in either Papua New Guinea or Brazil, both of which have *N. americanus* transmission (43, 44). These studies usually centre on different aspects of the natural infection, with studies from Papua New Guinea focusing on the antibody response in relationship to the intensity of the infection (42, 43, 45–48) and the studies in Brazil focusing more on the cell-mediated response to hookworm infection (39, 44, 49, 50). A study

conducted in infected individuals from an endemic area of Papua New Guinea demonstrated an age-specific humoral response with a significant correlation between IgG against crude L3 antigen extract and age, a significant negative correlation between IgG against adult ES products and pretreatment burdens in individuals aged 33–48 years and a significant negative correlation between anti-ES IgG and IgE in this same age group (42). In another study conducted in this same area, total IgE levels during hookworm infection increased compared with pre-infection levels and were significantly correlated with an increase in pretreatment worm burden (45). Another study also showed that levels of total IgE and eosinophils were negatively correlated with the weight and fecundity of the worms, that is, the higher the levels of total IgE and eosinophils, the lower the weight and fecundity of worms recovered from the hosts after treatment (46). This group also showed that the levels of IFN- γ were negatively correlated with egg burden, that is, the higher the level of IFN- γ , the lower the egg burden (45). The suggestion that this cytokine might act as a protective mechanism against the infection is still unclear (45, 51).

In a re-infection study from the same endemic area, Pritchard *et al.* (47) showed that the mean worm burden, assessed by worm expulsion after chemotherapy, was reduced after treatment followed by 2 years of re-exposure to infection. This was also true for the faecal egg output during the first-year post-treatment, with an increase during the second year. Working in the same area as above, this group also reported rates of re-infection in children <3 years after treatment as high as 97% (45). The kinetics of the antibody response were also evaluated in the re-infection study (47) and the following results were obtained: (i) a significant decrease in the levels of ES-specific IgG and IgM during the first year, with no change in the second year; (ii) no change in the levels of ES-specific IgA in the first year and a significant increase during the second year; (iii) the maintenance of the high levels of ES-specific IgE during the first year, with a slight decrease in the second year; (iv) an increase in the levels of ES-specific IgD following treatment and maintained in the second year; (v) a decrease in the levels of L3-specific IgG throughout the 2 years; (vi) a drop in the levels of L3-specific IgM, IgA and IgE after treatment with a recovery during the second year; and (vii) the levels of L3-specific IgD were unchanged during the first year and increased significantly in the second year (47).

Pritchard *et al.* (43) also studied basophil competence during hookworm infection in individuals in Papua New Guinea. In this study, they observed the presence of IgG anti-IgE autoantibodies, an increase in eosinophils and no change in the number of basophils between infected and

uninfected individuals. In an *in vitro* assay using basophils from infected individuals, they observed that anti-human IgE and normal and heat-inactivated ES from *Necator*-induced histamine release from the basophils in a dose-dependent manner. In contrast, basophils from uninfected volunteers released significantly less histamine after exposure to anti-IgE. There was also a difference in the levels of total and ES-specific IgE between infected and uninfected volunteers. Therefore, they concluded that basophils remain competent in hookworm-infected individuals from an endemic area, suggesting that hookworm down-regulation of immune responses is not due to the blockade of the IgE receptor FcεR1 (43).

The studies conducted in endemic areas of Brazil are from an area where co-endemicity with other parasitic helminths is common, and therefore, we focus on the results obtained only from individuals mono-infected with hookworm. In a study conducted in school-aged children, the comparison of unstimulated PBMCs of cured children (verified to be cured during the study using the Kato-Katz technique) and hookworm-infected children (those who remained positive after treatment) yielded higher baseline levels of TNF- α than negative endemic controls (proven to be negative during 6 months prior to enrolment in the study). Interestingly, the PBMCs stimulated with hookworm AE of the egg-negative control group showed higher levels of IL-12, IFN- γ , IL-5 and IL-13 than the hookworm-infected children. The only cytokine level observed to be higher in the AE-stimulated PBMCs of infected children compared with egg-negative controls was IL-10 (49) (Figure 1, Table 1).

In another study conducted in adults and children from a *Necator*-endemic area in Brazil, only individuals with hookworm infection, as determined by the presence of eggs in faeces, were included in the study and stratified by age. The study showed correlations between egg burden and cytokines produced by stimulated PBMCs of infected people (50). The egg burden in people mono-infected with *N. americanus* was negatively correlated with IL-10 production from PBMCs cultured with the crude antigen extracts L3, AE or ES. Moreover, TNF- α levels produced by these cells in culture with ES also correlated negatively with egg burden (50). This agrees with a previously published study on cellular responses during *N. americanus* infection, which demonstrated that hookworm-infected individuals had less T cells (CD3 and CD4) and B cells (CD19) than the noninfected control group, as measured through lymphocyte phenotyping of unstimulated whole blood samples (44). Although the mean percentages of CD4 were lower in *Necator*-infected individuals compared with egg-negative individuals, the levels of activated CD4 (HLA-DR+) were elevated. The number of activated T cells (CD3+CD69+,

CD8+HLA-DR+) and B cells (CD19+CD27+) was also higher than in the noninfected individuals. In keeping with other studies, the cytokine response to the crude *N. americanus* antigen extracts was mixed. This mixed response (Th1/Th2) was lower in infected individuals than in egg-negative individuals, especially in response to AE and ES, indicating a modulatory effect of *N. americanus* infection. This study also showed a positive correlation between the levels of IFN- γ and IL-5 produced by PBMCs stimulated with L3 that contradicts the idea that IFN- γ has an antagonistic effect on Th2 responses. Contrary to the previous studies from this same group, the production of IL-10 from PBMCs stimulated with AE, ES and L3 was higher in egg-negative compared with infected individuals (44). This discrepancy could be due to the fact that in the previous study (49), the cured and infected individuals were treated prior to blood collection.

Another study in Brazil evaluated the role of regulatory T cells (Tregs) during human hookworm infection. Infected individuals showed greater levels of Tregs identified using CD4, CD25 and FoxP3 as markers than noninfected controls (48). These Tregs also showed higher expression of CTLA4 and GITR surface receptors as well as IL-10, TGF- β and IL-17. Interestingly, the only hookworm crude antigen extract that showed regulatory capacity through a decrease in the production of IL-17 was AE. Finally, after the depletion of CD4+CD25+ cells, proliferation of CD4 was increased in cells cultured with AE and ES and proliferation of CD8 was increased in cells cultured with ES, showing that the Tregs were functional (48).

In a study conducted in Togo, Africa, the immunological parameters of hookworm were measured using umbilical cord cells from neonates and PBMCs from their mothers who were infected with hookworm. The results showed that cells from both of these sites naturally produced Th1 (IL-2, IFN- γ and IL-12) as well as Th2 and modulatory cytokines (IL-5 and IL-10, respectively). When umbilical cord cells from neonates and PBMCs from mothers were stimulated with crude AE antigen extract, both cell sources produced similar levels of IL-2, IL-5 and IL-10, but the umbilical cells produced more IL-12 and less IFN- γ than the PBMCs from the mothers (52). These results suggest that offspring of infected mothers were sensitized in the uterus. However, this study did not obtain data on the immunological response of these children after birth or determine whether their response differed from that seen in hookworm-infected children whose mothers were not infected during pregnancy. Another study conducted in adults from a hookworm-endemic area in Zimbabwe assessed the relationship between the production of *N. americanus* AE-specific antibodies and the age, sex and infections status of the participants. Serum

levels of AE-specific IgG, IgG2, IgG3, IgG4 and IgE increased as the eggs in faeces increased, while the level of AE-specific IgG4 also positively correlated with worm load (measured as the number of worms expelled after treatment). The relationship between age and isotype responses also showed a positive correlation with serum AE-specific IgG, IgG3, IgG4 and IgA (53).

In summary, natural hookworm infection is an uncontrolled infection (with respect to the inoculum) that tends to be chronic in nature, often increasing in intensity with the age of the infected individual (54). Studies conducted in naturally infected populations show that individuals present the following antibodies in response to hookworm infection: L3-specific IgG and IgM, ES-specific IgG, IgM and IgE, AE-specific IgG, IgG2, IgG3, IgG4 and IgE, and increased levels of total IgE. Levels of total IgE and AE-specific IgG4 are positively correlated with worm burden, while total IgE is negatively correlated with the weight and fecundity of the worms. AE-specific IgG, IgG2, IgG3, IgG4 and IgE are positively correlated with EPG of faeces. The age of the individuals is also positively correlated with the levels of AE-specific IgG, IgG3, IgG4 and IgA. Infected individuals also present eosinophilia and, although they do not present basophilia, their basophils remain competent despite continuous contact with the parasite. The increase in eosinophils is negatively correlated with the weight and fecundity of the worms.

However, these studies show conflicting results for the production of cytokines, probably due to differing immunological methods and study populations. Higher levels of TNF- α and lower levels of IL-12, IFN- γ , IL-5 and IL-13 were observed in PBMCs stimulated with AE of hookworm-infected compared with egg-negative individuals. When PBMCs from infected individuals were cultured with L3, there was a positive correlation between IFN- γ and IL-5, although there was no difference between the groups. The production of IL-10 from PBMCs stimulated with AE, ES and L3, on the other hand, was shown to be lower in infected than in egg-negative individuals in one study and higher in another study where the individuals were treated prior to blood collection. Nonetheless, the IL-10 produced in the presence of L3, AE and ES is negatively correlated with the amount of eggs in faeces, which is also true for IFN- γ . Also, infected participants had a greater number of functional Tregs than uninfected individuals. Finally, during natural human infections with hookworm, a mixed Th1/Th2 response is observed.

CO-INFECTION

The presence of other soil-transmitted and/or intestinal helminth (e.g. *Ascaris*, *Trichuris* and *Schistosoma mansoni*)

infecting the same individual is not uncommon in hookworm endemic areas. Co-infection studies have shown that the immunology of human hookworm infection can vary depending on the type of co-infection when compared with hookworm mono-infections.

For example, individuals co-infected with the helminth *Oesophagostomum bifurcum* and *Necator* had a high level of total IgE and although specific IgE response to adult *O. bifurcum* antigen clearly distinguished between co-infected individuals and those infected solely with *N. americanus*, some cross-reactivity was observed (55). The production of IgG and IgG4 antibodies specific to the adult antigens of both helminth parasites showed a high degree of cross-reactivity. There was no Th1 or Th2 dominance in co-infected individuals; however, the co-infected cohort showed higher levels of TNF- α and IFN- γ than mono-infected individuals, while levels of IL-5 and IL-10 did not differ between the groups. Taken together, these results indicate that this type of co-infection induces a Th1 permissive cytokine response to favour the parasites (55). On the other hand, co-infection between a filarial helminth and *N. americanus* decreased the levels of IL-4 and IL-5 compared with mono-infection in children from Papua New Guinea. Furthermore, co-infection between malaria and *N. americanus* induced lower levels of IL-4 and hookworm-specific IgE compared with mono-infection in the same endemic site (45).

In the previously mentioned cross-sectional study from an endemic area of Brazil (50), the authors also compared the immune response from PBMCs obtained from mono-infected individuals and individuals co-infected with *Necator americanus*, *Ascaris lumbricoides* and/or *Schistosoma mansoni*. Mono-infected individuals showed less IgG3, IgG4 and IgE to L3 antigen extract than co-infected individuals; less IgG1, IgG4 and IgE to AE; and less IgG1 and IgG4 to ES. Co-infected individuals showed a negative correlation between the chemokine CXCL10 produced by PBMCs cultured with L3 antigens or ES and EPG. Co-infection with *Ascaris* and/or *S. mansoni* also induced a lower level of TNF- α and led to a decrease of hookworm-specific IFN- γ and the chemokine CXCL10 production. There was no change observed in the production of IL-10 or Th2 cytokines under the same circumstances (50).

CONCLUSION

Experimental human infections have provided major contributions to our understanding of primary hookworm infection. The immunology of this infection is a strong eosinophil presence during the first days after L3 skin penetration, followed by a strong pro-inflammatory response, with the presence of IFN- γ , IL-2, IL-15 and

IL-22 but no IL-17A or IL-23 cytokines. The source of the Th1 cytokine IFN- γ is thought to be from innate cells (e.g. NK cells). Experimental hookworm infections also present a Th2 and modulatory responses with the presence of IL-5, IL-13, IL-9 and IL-10 cytokines, even when the number of infective larvae is low (15 L3). On the other hand, natural infections show a mixed Th1/Th2 response in the production of cytokines and chemokines, a strong T and B cell activation, and a strong humoral response with the presence of IgG, IgM and IgE antibodies specific to all crude antigen extracts from different stages of the life cycle. Therefore, both types of studies in humans, natural and experimental, contribute to different aspects to the immunology of hookworm infection. Experimental infections contribute to the understanding of a primary infection, and studies conducted in endemic areas can add information on chronic infections. Thus, by comparing both strategies, more knowledge is obtained to understand the immunological events that occur during hookworm infection, which may also lead to better intervention strategies to control this highly prevalent neglected tropical disease.

FINAL REMARKS

Although the studies reviewed here have contributed greatly to our understanding of human hookworm infection, there remain gaps in our understanding of this host–pathogen relationship. For example, the few studies on the mucosal immune response in humans have studied this response in experimental infection. Currently, there are no studies on the mucosal immune response to a natural infection. Also, in most studies, both experimental and immunoepidemiological, there has been a strong focus on the antibodies induced during the infection and hardly any investigation on the cells that produce them (i.e. B cells). Although many researchers have conducted experimental infections in humans, the sample sizes have been small and infection interrupted early. Finally, a standardization of cell culture during these studies is needed to obtain comparable results between different research groups as the methodology for crude antigen production, the concentration of cells and antigens used as well as the time of cell culture incubation will greatly impact the results.

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