CHAPTER 8

Global worming or deworming? Epidemiological, immunological and clinical perspectives

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– Manuscript in preparation –

Summary

Helminth infections still affect billions of people worldwide. Deworming is advocated to prevent the morbidities induced by helminths. At the same time, in resource-rich settings, the possibility of infecting patients with different life cycle stages of worms is being explored, specifically for those suffering from a range of inflammatory diseases. It has been recognized that helminths have immunomodulatory properties, which can impair not only parasite-specific immune responses but also responses to third party antigens. By using these properties, parasitic helminths allow their long-term survival within their human host but at the same time curtail overt immunological responses that lead to allergic, inflammatory bowel or autoimmune diseases. However, this would also suggest that deworming could lead to the development of inflammatory conditions, due to the removal of regulatory mechanisms associated with helminths. This can result in unfavorable situations in countries that are not prepared for the emergence of such pathological conditions. This review summarizes the consequences of deworming versus helminthic therapy.

Background

Parasitic worms have accompanied us throughout human history[1]. The fact that mortality due to helminth infections is rare, and that worm infections are often asymptomatic, with only a minority of infected individuals having intense infections[2], suggests that this is as a result of evolutionary co-adaptation between parasitic worms and man. A key component of this partnership is the immunological interaction between helminths and their mammalian hosts. Helminths modulate immunological processes: multiple mechanisms of helminth-induced immunological tolerance and regulatory pathways have been revealed over the past 50 years, that may explain chronicity of these infections[3]. Helminths also appear to fundamentally affect the host's genetic composition; studies into the recent evolutionary history of human interleukin genes reveal that pathogens have driven selection of certain genetic adaptations and that helminths have had a stronger influence than other classes of pathogens in this process[4]. Pressure from helminth infections seems to have promoted the selection of alleles likely to protect against infection, while predisposing humans to immune-mediated diseases such as allergies.

In the twentieth century, great effort was put into the worldwide control of infectious diseases by improved hygiene, vaccination programs and drug treatment. However, the decline in parasitic and other infectious diseases was associated with a marked increase in prevalence of chronic inflammatory disorders such as asthma, auto-immune disease (type 1 diabetes, multiple sclerosis) and inflammatory bowel disease[5]. Although the prevalence of asthma and allergic disorders now seems to have stabilized in developed countries[6,7], the prevalence is on the increase in developing countries[7,8]. These epidemiological observations accord with the Hygiene Hypothesis[9], which suggests that removal of the regulatory effects of pathogens such as helminths (from populations genetically adapted to live with them) tends to lead to an imbalance in the immune system followed by a number of pathological conditions.

Consequently, the question arises whether helminths should be considered as harmful pathogens or as beneficial commensals. While in low-resource settings deworming is advocated to prevent worm-associated morbidity, several research groups in the Western world are currently investigating the therapeutic potential of worms and their secreted products in inflammatory diseases. Whereas deworming may result in an increase of inflammatory disorders, introduction of helminths experimentally can potentially be harmful. Experimental therapeutic infections using worms and their products may lead to unforeseen immunological and pathological consequences. This review aims to summarize current knowledge on the immunological effects of worms, the beneficial aspects of chronic helminth infections, the possible consequences of global deworming, and the current

evidence as to whether the controlled use of worms or their products for treating patients is beneficial.

T cell responses in infectious and inflammatory diseases

The immune system is equipped with different cell types involved in recognition of pathogens and their elimination. So far, a number of T-cell subsets have been identified that appear to be key to the control of distinct classes of incoming pathogens. T-helper (Th1) cells are mainly involved in defence against intracellular pathogens, Th2 are there to combat helminths and ectoparasites and Th17 cells appear to be important for defence against extracellular bacteria and fungi[10]. However, it is also clear that these cells can inflict damage to tissues and organs if uncontrolled. Thus whereas Th1 and Th17 cells that release pro-inflammatory cytokines are involved in recruitment and activation of macrophages and neutrophils that can attack bacteria, viruses, protozoa and fungi, their overt activation is associated with autoimmune and inflammatory diseases. Th2 cells trigger responses that disable, degrade and dislodge parasites, as recently reviewed[11]. Interestingly, Th2 cytokines seem to be involved in tissue repair as well[12,13], which may encapsulate the parasite and prevent excessive inflammation[14]. However, an overactivated Th2 immune response can lead to allergies and allergic diseases. Therefore it is not surprising that an important component of the immune system is the regulatory network, spearheaded by the regulatory T cells, that are capable of controlling activated effector T cells through expression of inhibitory molecules[15]. It should be noted that additional components of the regulatory network are the so-called modified Th1 and Th2 cells which express the signature cytokines, IFN-γ and IL-4/IL-5/IL-13, respectively, along with IL-10[16]. It is thought that over time, effector Th1 and Th2 cells start to express IL-10 to control the damage that might otherwise be inflicted by these cells.

Characteristics of immune responses in helminth infections

Helminth-induced immune regulatory network

In the late half of the 20th century, Ottesen and others started to elucidate the immunological basis of helminth-host interaction[17-19]. Interestingly, they observed that in helminth-infected individuals, the proliferative response of lymphocytes to specific antigens was lower than in uninfected subjects[17-20]. These and other studies[21] have led to the concept that cellular immune "hyporesponsiveness" induced by helminths is one of the key mechanisms used by these parasites to evade the host immune system. Numerous studies have since contributed to our understanding of a sophisticated immune regulatory network operative during helminth infections[16]. First, the presence of non-lymphocytic adherent cells was

demonstrated in the blood of patients with *Brugia malayi* microfilariae[22]. The adherent cells could suppress anti-filarial immune responses when cultured with patient-derived lymphocytes[22]. These cells are probably the precursors for regulatory antigen-presenting cells that are now studied, such as alternatively activated macrophages and monocytes or regulatory dendritic cells[23-26]. Furthermore, the existence of suppressor $CD8^+$ T cells was shown in individuals with patent *B. malayi* or *B. timori* microfilaremia in Indonesia; antibody-mediated depletion of these cells enhanced proliferative responses to parasite antigens[27]. Thereafter, the focus was shifted to a population of $CD4^+$ T cells, termed regulatory T cells (Tregs)[28]. Tregs are an essential component of the immune system, helping the immune response to reach an optimal balance between sufficiently strong anti-pathogen responses and carefully gauged control of overt and pathogenic inflammation[15]. Helminths are thought to exploit this immune suppressory mechanism to ensure their long-term survival. Natural thymus-derived Tregs are currently characterized as $CD4^+CD25^{hi}FOXP3^+$ cells, but several peripherally inducible subsets of regulatory T cells with variable phenotypes have also been described[29,30]. Several cross-sectional studies in human helminthiases have indeed provided evidence for the expansion of Tregs during infection[31-36]. Not only numbers, but also functional properties of Tregs have been studied. In individuals with patent *Wuchereria bancrofti* microfilaremia, suppressed filarial-specific proliferative T and B cell responses as well as IL-13 production *in vitro* were restored after removal of $CD4^+CD25^{hi}$ Tregs[37].

The mechanisms for Treg expansion by these parasites are not fully understood, but lately a number of helminth-derived molecules have been identified that can directly drive Treg induction. Molecules derived from *Schistosoma mansoni* can condition dendritic cells to induce IL-10 producing Tregs[38]. The excretory/secretory products of *Heligmosomoides polygyrus* as well as omega-1, a glycoprotein of *S. mansoni* eggs, can directly induce Foxp3 expression in murine $CD4^+$ T cells[39,40]. These studies indicate that there might be great potential in utilising helminth-derived molecules in the manipulation of the human immune system such that regulatory T cells are induced or enhanced.

Besides T cells, several other cells appear to be involved in immune regulation, as illustrated by emergence of regulatory B cells, a subset producing IL-10 during human helminthiases[41,42], and the importance of regulatory subsets of antigen-presenting cells as mentioned above.

Induction of type 2 responses by helminths

One of the hallmarks of helminth infections is the expansion of the Th2 responses; elevated cytokines such as IL-4, IL-5 and IL-13, along with high levels of IgE characterize helminth-infected subjects[43]. As there is evidence for a degree of counter-regulation between Th1 and Th2 responses, the strong type 2 inducing

capacity of helminths might also play a role in keeping Th1- (and Th17-) mediated diseases such as inflammatory bowel diseases (IBD) and arthritis at bay. Therefore, understanding the mechanisms whereby helminths lead to Th2 responses might, in analogy with understanding how Tregs are induced, lead to potential therapeutic interventions. Recent studies have identified helminth antigens with the ability to drive Th2 responses in murine models. Th2-inducing properties of molecules derived from *Fasciola hepatica*[44], *S. mansoni*[44] and *H. polygyrus*[45] was demonstrated by injection into mice. ES-62, a glycoprotein secreted by the filarial nematode *Acanthocheilonema viteae*, and omega-1, a molecule found in excretory secretory (ES) products of *S. mansoni* eggs, have been shown to endow DC with the ability to skew immune responses toward type 2 in a mouse model[46,47]. The Th2-inducing capacities of omega-1 may seem in contrast to the previously mentioned Foxp3-inducing activity, however in the latter NOD mice were used[40]. Some of these Th2-inducing capacities have been confirmed in experiments using human cells[46].

More recently, research into tissues at the sites of worm infection has revealed that epithelial cells, the first line of defence in the gut and lung, produce a set of cytokines, IL-25, IL-33 and thymic stromal lymphopoietin (TSLP), so-called 'alarmins'[48-50], which in turn induce innate lymphocytes to produce type 2 cytokines[51,52]. These cells, termed natural helper cells (NHC), are found in peripheral lymphoid clusters and produce IL-5 and IL-13 enhancing B and T cell function[53]; as yet very little is known about the equivalent of these cells in humans.

The association between helminth infections and inflammatory diseases

To date, human studies of the possible influence of worm infections on inflammatory diseases have included cross-sectional studies, anthelmintic trials and trials of helminth therapy. We shall now look at each in turn, discussing the potential pitfalls and advantages of each approach. We will start with summarizing the associations between helminth infections and different inflammatory diseases.

Allergy & asthma

Based on the ability of helminth infections to induce a regulatory network and to modify the Th2 response, we have postulated that helminths have a special capacity to protect against allergic conditions. There is evidence from several murine models that helminth infections might be protective against allergic airway inflammation[54-57]. However, the findings from cross-sectional studies in humans regarding the association between worm infections and allergy-related conditions have so far been inconsistent (reviewed by Leonardi-Bee et al[58] and Flohr et al[59]).

For atopy, usually assessed as a positive skin prick test (SPT) response to a panel of allergens, an inverse association with worms has commonly been observed[60-65], with occasional exceptions[66]. For allergy-related clinical syndromes, results have been more mixed. Both negative[67, 68] and positive[69] associations have been reported for eczema. Negative associations have been observed for wheezing in some studies[70,71]; in others, an absence of association between worms and wheeze[71] or asthma[72], or a positive association between worms and asthma[66] have been reported. Factors that may influence the relationships between worms and allergy observed in these studies are likely to be complex but may include the timing, or chronicity of helminth infection – with recent infections having a different effect from chronic, established infections[64] – as well as the intensity of the infections, the specific parasite species and the host genetics[73, 74].

Inflammatory Bowel Diseases

Studies in murine models have shown that infection with worms protects against several forms of experimentally induced colitis[75-78] but studies in humans are hindered by the fact that inflammatory bowel disease (IBD) and parasitic infections do usually not coincide. A small study in India showed that patients with Crohn's disease had lower immune responses to different hookworm antigens compared to healthy controls, suggesting that patients had less often harbored hookworm infections, however the infection was not assessed directly[79].

Multiple sclerosis

A case-control study in 1966 already pointed to the contribution of environmental factors in MS; the presence of piped water, a flush toilet and sharing a room with 1 person or less were more often recorded as environmental factors associated with MS patients compared to healthy controls[80]. Reasoning from the Hygiene Hypothesis, the country prevalences of MS and *T. trichiura* infections were compared and shown to be almost mutually exclusive[81]. Correale and colleagues took this further by assessing a prospective cohort of MS patients. When comparing helminth-infected and -uninfected patients, the appearance of new MRI lesions was much less frequent in the infected individuals over 5 years of follow-up. This difference seemed to be associated with higher production of IL-10 and TGF-β by peripheral blood mononuclear cells (PBMC) and with suppressive activity of Tregs[82]. Furthermore, when a few of the helminth-infected patients were treated with anthelmintics due to intestinal symptoms, the number of clinical relapses and MRI lesions increased, parallel to a decrease in regulatory immune responses[83]. Using the murine model for MS, experimental autoimmune encephalomyelitis (EAE), evidence was further obtained for a protective effect of helminths or their products[84-88] on the clinical course and CNS inflammation.

Diabetes mellitus

Another autoimmune condition that has been linked to immune modulatory properties of helminths is diabetes mellitus. In the non-obese diabetes (NOD) mouse model, it was shown that several types of helminthic infections could prevent type 1 diabetes (T1D)[89-92]. In humans, an inverse association has been observed between prevalence of T1D and neglected tropical diseases, which helminth infections form a substantial part of. They showed an inverse relation between the prevalence of T1D and the access to sanitation and clean water, although this was not statistically tested[93]. Within the Chennai Urban Rural Epidemiology Study (CURES) in India, the prevalence of lymphatic filariasis was lower in T1D subjects[94]. They furthermore measured total anti-filarial IgG and IgG4 as proxies for past and current infections, respectively. Whereas total IgG levels were not different between the groups, IgG4 was higher in the non-diabetic group, indicating that current infections might lead to this inverse relationship.

For type 2 diabetes (T2D), it is more difficult to establish experimental models. This multifactorial disease is currently regarded as an inflammatory disease[95], but is also associated with genetic as well as nutritional and other life style factors. Another report from the CURES study has shown lower prevalence of LF in T2D patients[96]. Moreover, the study showed lower serum levels of pro-inflammatory cytokines in LF-positive versus LF-negative subjects with T2D, suggesting a role for anti-inflammatory properties of LF infection in protection against T2D.

Rheumatoid arthritis

Although some helminth infections can induce reactive arthritis which resembles rheumatoid arthritis[97] several studies in different rodent models have shown that helminth infections or extracts can suppress or prevent arthritis[98-100]. The filarial-derived glycoprotein ES-62 was shown to be effective as a therapy for murine collagen-induced arthritis and furthermore synovial cells from rheumatoid arthritis (RA) patients treated with ES-62 produced less pro-inflammatory cytokines after LPS stimulation[101].

Other autoimmune or inflammatory diseases

Aoyama et al. showed that *Strongyloides stercoralis* infections were less prevalent in patients with autoimmune liver diseases than in other individuals visiting the hospital[102], however there has been no further follow-up study to this. In a mouse model of Graves' disease, an autoimmune disease of the thyroid, *S. mansoni* appeared to have a protective effect[103], but this has not been examined in humans. Furthermore, it has been suggested that helminths might have therapeutic potential in atherosclerosis and other cardiovascular diseases[104], based on a study that showed *S. mansoni* to have anti-atherogenic effects in a mouse model. The

exact mechanism has not been resolved but it might be caused by the consumption of lipids by S. mansoni, rather than by immune-mediated mechanisms[105].

Studies of anthelmintic treatment

Cross-sectional and observational studies are inherently flawed due to difficulty in ascertaining cause and effect, issues of confounding and reverse causation. Therefore, evidence from such studies need to be complemented by other study designs. Since remote areas still suffer from a high burden of helminthic infections[106], mass deworming programs are currently advocated[107]. This creates opportunities to investigate prospectively whether deworming paves the way for the increased prevalence of allergic and other inflammatory diseases.

Effect of deworming on atopic diseases

Anthelmintic trials are designed to study the influence of worms on allergic conditions indirectly, by studying the effects of treating the worms. Such trials have been conducted among school-going children from worm-endemic areas using different anthelmintic drugs for various periods of follow-up. An initial non-randomized study in Venezuela compared treated children with children who declined treatment, and suggested that anthelmintic treatment was associated with increased prevalence of atopy[108]. The first randomized trial was carried out among 317 Gabonese school children using open-label three-monthly praziquantel and mebendazole, versus no treatment, over 30 months; it found that anthelmintic treatment was associated with an increased rate of developing positive skin responses to house dust mites[109]. A cluster-randomized trial among 1632 children from Ecuador using two-monthly albendazole versus placebo over 12 months found no effect on SPT responses[110]. An individually randomized trial, among 1566 rural children from Vietnam using initially three-monthly mebendazole versus placebo but later three-monthly albendazole versus placebo over 12 months, found an increased risk of positive SPT responses to allergens[111]. The latest trial in Indonesia, assessed the effect of three-monthly albendazole treatment compared to placebo on SPT reactivity to different allergens. A significant increase in cockroach reactivity was observed after 21 months, but overall SPT responses were not altered[112]. None of these four trials showed any effects of anthelmintic treatment on clinical allergy outcomes. Given the fact that clinical allergy is relatively rare in these areas, it should be considered that the power of these studies might have been not sufficient to detect significant effects. Moreover, it has to be noted that differences in species of prevalent helminths, co-prevalence of other immunomodulating infections (such as oro-faecal infections[113,114] or malaria[115]), exposure to environmental pollutants and duration and timing of treatment, can have major impact on trial outcomes.

Pertinent to the duration of treatment, a study in Ecuador compared SPT reactivity and allergy-related symptoms among school-age children from communities that had received 15-17 years of periodic ivermectin treatment to school-age children from adjacent communities that had not received treatment[116]. This study observed that the prevalence of skin reactivity to allergens among children from the treated communities was double that for the children from untreated communities. The children from the treated communities also had a higher prevalence of recent eczema symptoms, but not any other allergy-related symptoms[116]. While the design of this study is again limited by the fact that the communities were not randomized relative to the intervention, the results do suggest that long-term intervention against helminths may be required to alter responsiveness to allergens and to influence allergy-related clinical outcomes.

Regarding the timing of treatment, a trial among 2507 pregnant women in Uganda using single doses of albendazole and praziquantel versus matching placebos (in a 2x2 factorial design) found that albendazole during pregnancy was associated with an increased risk of eczema in infancy[117] and in the first five years of life, whether the mother had hookworm infection or not[118]. Praziquantel during pregnancy was associated with an increased risk of eczema in infancy among children whose mothers were infected with *S. mansoni* infection[117]. In this same trial, children themselves were randomized to three-monthly albendazole versus placebo from fifteen months to five years of age; this treatment was not associated with an increased risk of eczema in early childhood[118]. These results suggest that in-utero events may be more important in priming or programming the child's immune system (thereby influencing the risk of eczema, and perhaps other allergy-related conditions) than events in early childhood. Surprisingly, anthelmintic treatment with albendazole and praziquantel during pregnancy had no beneficial effects on the immune responses to BCG, tetanus and measles vaccines in early childhood and none of the anticipated benefits for birth weight, resistance to infectious diseases, or improved child development[119,120], that would have compensated for the observed adverse effect on eczema.

All the above studies examined effects of anthelmintics in general populations. Another approach has been to examine effects of anthelmintic treatment in people already suffering from allergy-related disease. For example, a recent small trial conducted among individuals aged five to fifty years with a history of asthma in the last twelve months from a schistosomiasis-endemic area in Brazil has been published[121]. Study participants received single dose albendazole and praziquantel or placebos. No differences in asthma severity between the two treatment arms were observed over three months. When all participants were treated with both drugs after about three months of follow-up, the study observed the worsening of clinical asthma symptoms at fifteen months[121]. However, this worsening cannot be confidently attributed to anthelmintic treatment since there was no comparison

arm in the second part of the study. Larger studies investigating the effect of treating worms in people with established allergy-related diseases would be warranted.

Effect of deworming on other inflammatory diseases

The effect of deworming on other chronic inflammatory diseases has not extensively been studied, partly because naturally (and inherent to our hypothesis) the presence of helminth infections and these conditions may not overlap. An interesting study by Bager and colleagues did assess the effect of mebendazole treatment retrospectively in a population cohort in Denmark[122]. 14% of the more than 900.000 children had been prescribed mebendazole, for probable infection with *Enterobius vermicularis*, the pinworm that is still endemic in the US and in Europe[123]. The incidence rate ratios for the chronic inflammatory diseases asthma, T1D, juvenile arthritis and IBD were not significantly higher in treated children. However, mebendazole was prescribed based on presumption and not diagnosis of pinworm infection. Moreover, enterobiasis in these children might not have been sufficiently chronic to induce immune regulation[122] and thus the treatment might have masked the possible benefits.

The main drawback of anthelmintic trials in studying the influence of worms on inflammatory diseases is that they are based on a number of assumptions whose validity is currently unknown. For instance, the trials assume that the effect of worms is immediately removed following treatment and that development of allergy symptoms follows soon after anthelmintic treatment. What if the protective effects of worms persist long after anthelmintic treatment? What if the development of clinical allergy and other conditions is not immediate or could only be observed in the next generation? This concern might be answered by longer follow-up and different study designs, such as the Ugandan trial[117], which showed that the effect of treating worms in the mother could be observed in the next generation as increased risk of childhood eczema. Follow-up for this Ugandan study is currently ongoing to determine whether the risk of asthma is also increased as the children grow older. The second drawback in using anthelmintic drugs to study the possible influence of worms on allergy is that any observed effect might be due to the anthelmintic drug itself, or to broader spectrum effects, and not due to the elimination of worms. This seems to be the most likely explanation for the observed effect of albendazole treatment in pregnancy on the increased risk of eczema in the Ugandan trial described above. Albendazole acts by binding to tubulin thereby interfering with the formation of microtubules in the cytoskeleton[124] and hence can affect protozoa[125, 126], fungi[127] and mammalian cells. Whether maternal albendazole increased the risk of childhood eczema through a direct effect or through acting on other organisms, was not established in the

Ugandan study. Therefore, results from anthelmintic trials should be interpreted with caution and it might be helpful to examine effects of a variety of drugs in different trials.

Immunological consequences of deworming

A number of studies have been able to assess immune responses after anthelmintic treatment. Helminth-specific responses have been examined in Vietnamese school children in the context of the larger study on allergy[111]. Cytokine responses were only evaluated in hookworm-positive children, in which albendazole treatment led to lower IL-10 responses to hookworm antigens. A similar effect was seen in *Ascaris* responses in children treated with albendazole in Ecuador, although this study was not placebo-controlled[128]. A decrease in IL-10 might be expected after clearance of immunoregulatory helminth infections, however in earlier studies, treatment of schistosomiasis and STH infections with praziquantel and mebendazole, respectively, had been associated with enhanced IL-10 responses[129,130]. Also a placebo-controlled trial of praziquantel treatment of pregnant women resulted in higher *S. mansoni*-specific IL-10 production[131]. This latter study also found a treatment-induced increase of Th1 and Th2 responses to schistosomal antigens, similar to the enhanced Th1 and Th2 cytokines in response to *Ascaris* after albendazole treatment of school children in Ecuador[128].

Overall, immune responses tend to increase after anthelmintic treatment, which is in line with the results of our randomized controlled trial (RCT) in Indonesia, showing enhanced pro-inflammatory cytokine responses to *Ascaris* and malaria antigens as well as mitogen after albendazole treatment (Wammes et al. manuscript in preparation). However, it is not known how long this immune stimulatory effect may persist and whether this predisposes to hyperinflammatory conditions.

Very few studies have looked at allergen-specific immune responses after anthelmintic treatment. The trial in Ecuador found no differences in cytokine production in response to cockroach and house mite *Dermatophagoides pteronyssinus* antigens after repeated treatment with albendazole[128]. More trials might be needed to confirm this observation.

Helminth therapy in humans

A more direct approach, which avoids the pitfalls described above, is to study the direct effects of helminths through clinical trials using whole helminths or helminth-derived molecules.

The first scientists to undertake clinical evaluation of infecting patients with helminths were Joel Weinstock and David Elliott in the 1990s, who have recently

summarized the progress in translational studies on helminthic therapy in a review[132]. Up to now, *Trichuris suis* and *Necator americanus* worms have been used in a clinical trial setting, as discussed below.

Trichuris eggs

Most clinical trials so far were performed with *Trichuris suis* ova (TSO) in patients with IBD. *T. suis* is a pig whipworm, which is able to colonize the human gut for a short period of time, but without overt pathology. After two open-label trials assessing the safety of *T. suis* infection in IBD patients and showing promising results (about 70% remission in Crohn's disease[133,134]), Summers and colleagues set out to study the effect of TSO in a first placebo-controlled double blind randomized trial including 54 ulcerative colitis (UC) patients[135]. The UC Disease Activity Index (UCDAI) improved more in the TSO group compared to the placebo group, however numbers of remissions were not significantly different. The group of P'ng Loke, working on characterization of the local immune responses surrounding the location of *Trichuris* worms, studied a patient who infected himself with *T. trichiura*[136]. In this patient, during colitis, T cells only producing IL-17 were abundant, while after *Trichuris* infection more multifunctional T cells were induced, mainly producing IL-22. At the same time goblet cell hyperplasia and enhanced mucus production was seen, which suggests that IL-22 is involved in the tissue repair response, possibly together with the canonical Th2 cytokines[136]. In addition to the previous observations of helminth-associated *de novo* induction of regulatory cells, it appears that *Trichuris* worms are able to modify the cytokine signature of local inflammatory cells. In rhesus macaques with idiopathic chronic diarrhoea, which resembles UC in intestinal inflammation, the Loke group further examined the effects of *T. trichiura* treatment[137]. Interestingly, four of the five monkeys showed clinical improvement. Colonic T cells produced more IL-4 but had less Tregs after treatment, however, no data could be generated on IL-17 and IL-22 responses, which makes it difficult to correlate these clinical effects to the previously reported immunological alterations. However, IL-22 might be a promising candidate as correlate of protection and this may be further explored in helminthic therapy trials, with *T. trichiura* or other helminths.

Recently, the results of a safety trial of TSO in MS patients were reported, as a starting point for a planned phase 2 trial (trial identifier NCT00645749). The trial followed 5 patients with relapsing-remitting MS after inoculation with TSO[138]. Although the majority experienced mild gastrointestinal symptoms, the number of new lesions revealed by MRI was lower during TSO treatment than the number before treatment, or after treatment had been discontinued. This was not accompanied by a change in circulating Tregs or monocytes expressing typical molecules of alternatively activated macrophages, raising the question whether it

is difficult to detect these cells in peripheral blood of patients rather than in the intestine.
A relatively large RCT in 100 allergic rhinitis patients showed the induction of gastrointestinal symptoms and immunological response to TSO, but no effect in reducing symptom scores, medication use or skin prick test reactivity[139]. However, this trial has been criticized due to the fact that infection with TSO was too short before the hay fever season started and therefore might not have allowed sufficient time for the treatment to work[140].
As of December 2012, there are a total of 11 clinical trials registered assessing safety and/or efficacy of TSO in IBD, MS, allergies and even autism (Table 1).

Necator americanus larvae
Relatively new is the therapeutic potential of worms known to establish chronic infections in humans. An advantage of this could be that administration is only needed once, whereas TSO should be administered at two- or three-weekly intervals. The safety and efficacy of *Necator americanus* larvae has been assessed in three trials. Inoculation with 50 larvae or more was shown to cause considerable gastrointestinal symptoms in healthy volunteers[141-143]. A dose-ranging trial to establish the dose which would achieve an infection intensity of 50 eggs per gram of faeces in healthy individuals showed that inoculation with 10 larvae was sufficient. This dose also induced a modest immunological response, as measured by eosinophil counts, IgE and hookworm-specific IgG levels[143].
Before starting a RCT of *N. americanus* infection in asthma patients, two other issues were addressed, which could potentially affect the safety of these studies. It was shown in allergic rhinitis patients that the lung passage of hookworm larvae did not cause deterioration in airway reactivity[144] and that hookworm-induced type 2 responses did not potentiate an allergen-specific IgE response[145]. In an asthma trial that followed, although infection using 10 larvae was well tolerated, it did not show any beneficial effect against asthma symptoms[146]. One RCT has been reported on *N. americanus* infection in celiac disease patients under restricted diet[147]. Wheat challenge was performed to assess the effect that helminthic immune modulation would have, however no differences in mucosal T cell counts, gluten-specific IFN-γ production by PBMC or clinical responses to challenge were observed comparing groups of 10 patients. Currently five clinical trials are registered to use *N. americanus* larvae for celiac disease, allergic rhinitis, asthma and MS (Table 1).

Table 1. Overview of registered clinical trials on helminthic therapy

Trial identifier	Sponsor	Phase	Status	Condition	Intervention
ACTRN12608000241336	Asphelia Pharaceuticals	1	not yet recruiting	Crohn's disease	TSO
EUCTR2007-006099-12-DK	Statens Serum Institut, Denmark	2	not recruiting	Allergic rhinitis	TSO
NCT00645749	University of Wisconsin, Madison, US	2	recruiting	MS	TSO
NCT01006941	Rigshospitalet, Copenhagen, Denmark	2	completed	MS	TSO
NCT01040221	Montefiore, New York, US	1	not yet recruiting	Autism	TSO
NCT01070498	Beth Israel,Boston, US	1	completed	Food allergy	TSO
NCT01279577 / EUCTR2006-000720-13-DE	Dr. Falk Pharma, Frankfurt, Germany	2	recruiting	Crohn's disease	TSO
NCT01413243 / EUCTR2009-015319-41-DE	Charite, Berlin, Germany	2	recruiting	MS	TSO
NCT01433471	NYU, New York, US	?	recruiting	Ulcerative colitis	TSO
NCT01434693	Coronado Biosciences, US	1	ongoing	Crohn's disease	TSO
NCT01576471	Coronado Biosciences, US	2	recruiting	Crohn's disease	TSO
NCT01734941	Hadassah Medical Organization, Jerusalem, Israel	2	not yet recruiting	Autism	TSO
NCT00232518	University of Nottingham, UK	1	completed	Allergic rhinoconjunctivitis	N.a. larvae
NCT00469989	University of Nottingham, UK	1	completed	Asthma	N.a. larvae
NCT00671138	Brisbane, Australia	2	unknown	Celiac Disease	N.a. larvae
NCT01470521 / EUCTR2008-005008-24-GB	University of Nottingham, UK	2	recruiting	MS	N.a. larvae
NCT01661933	Brisbane, Australia	1&2	recruiting*	Celiac Disease	N.a. larvae

TSO *Trichuris suis* ova; N.a. *Necator americanus*; *enrolling by invitation only

Helminth-derived molecules

Regarding the fact that helminth species currently used or planned to be used in trials are able to colonize the human intestine or could have clinical and pathological consequences, there has been a shift on focusing on helminth-derived molecules to substitute whole parasite treatment approach (reviewed by William and Margaret Harnett[148]).

As already alluded to earlier, several helminthic products with immune modulating properties have been defined. For several of these, cellular immunological responses induced have been investigated and some have been tested in disease models, however none has been administered to humans. Currently, ES-62 is the most well-characterized candidate molecule for therapeutic trials. This phosphorylcholine-coupled glycoprotein was first identified in 1994[149] and, as discussed, has been used to treat arthritis in a murine model[101]. Furthermore, through inhibition of mast cell histamine release, ES-62 may protect against allergic diseases[150].

The *Heligmosomoides* excreted-secreted (ES) products[76], characterized by the group of Rick Maizels, have been shown to suppress allergic airway inflammation[55]. AvCystatin, another molecule secreted by *A.viteae*, inhibits the development of allergic airway inflammation and acute colitis[151]. Moreover, it has been shown that *in vitro* Th2 responses of PBMC from grass pollen allergic patients are markedly reduced by adding AvCystatin to cultures[152]. Furthermore, there is data indicating that soluble products from *S. mansoni*, *T. suis* and *Trichinella spiralis* can suppress clinical signs of EAE by modulation of DC[88].

In murine colitis, extracts from *S. mansoni* adult worms and ES products of the canine hookworm *Ankylostoma caninum* have displayed beneficial effects[153]. Although treatment with *S. mansoni* extracts did not improve the clinical score, it diminished local inflammation and myeloid cell infiltration in colonic tissue. In parallel, lower mucosal Th1 and Th17 responses and enhanced expression of IL-10 and TGF-β in T cells was found[153]. These results illustrate that altered immune responses do not always lead to clinical improvement, and a longer time period may be needed for a detectable clinically beneficial effect. Lastly, Lewis^{X}-containing glycan from *S. mansoni* eggs, lacto-N-fucopentaose III (LNFPIII), was tested in mouse models of inflammatory conditions. LNFPIII suppressed EAE by enhancement of IL-10 and Th2 cytokines[154] and was also shown to be beneficial in psoriasis[155]. Taken together these results in animal models encourage further studies, and possibly clinical trials, to evaluate their beneficial effects.

Challenges in helminth immunotherapy

There are some drawbacks in the use of helminthic therapy as currently proposed. As mentioned before, infection with *N. americanus* but possibly also *T. suis* could have pathogenic effects in humans. Patients undergoing helminth infection should

be monitored closely for infection intensity and possible extra-intestinal manifestations of the infection[156]. The long-term consequences of this approach have not been assessed; the trials with the longest follow-up time were for 24 weeks. The advantage for introducing hookworm infections would be that only single inoculation is needed, whereas TSO should be provided every two to three weeks. However, this also implies that hookworm infections are less controllable, as they lead to chronic infestations. The timing of infection is another issue, since under natural conditions, the protective effects of helminthic infections might have been acquired in early life. Moreover, possible relevant immunomodulatory effects could need substantial length of time to fully develop.

Another issue is the helminth-induced immune modulation itself. Immune regulatory responses are desirable to counteract inflammatory disorders, but could be detrimental for other immune-associated conditions. Not only defence against incoming pathogens may be impaired, but also anti-tumor immune responses may be compromised. Immunosuppression is a strategy for tumor cells to evade host immune responses and efforts are being made to inhibit Tregs in cancer by immunotherapy[157]. Therefore, non-specific blanket immune suppression, although mild, should be considered critically.

Emerging area – Immunometabolism

Immunometabolism is an emerging concept, which explores the interaction between nutrients, metabolism and the immune system. Since metabolic disorders such as T2D and obesity are associated with immune alterations, they can be regarded as inflammatory diseases[95]. It has become clear that some of the pathways involved in pathogen sensing are also involved in the induction of inflammation that leads to metabolic disorders. Pathogen-associated molecules are known to induce pro-inflammatory cytokine production, such as TNF, through toll-like receptor (TLR) signaling[158]. Large amounts of TNF are found in adipose tissue of obese mice[159]. The intracellular signaling pathways of TNF involve serine kinases, which have been shown to phosphorylate serine residues in insulin receptor substrates [160,161]. As serine phosphorylation is the inhibitory counterpart of tyrosine phosphorylation usually induced by insulin binding to the receptor, the signaling events of TNF lead to inhibition of insulin signaling, resulting in insulin resistance[161].

Combatting microorganisms has high energy demand and moreover, worms and other parasites use host nutrients for their long-term survival. As a consequence, parasitic worms might need to tightly co-regulate immune responses and nutrient metabolism, to avoid depriving their host from resources necessary for survival. Therefore, these metabolic properties of parasitic infections could be of interest. Recently the effects of LNFPIII, which is not only present on *S. mansoni* eggs but

also found in human breast milk[162], was assessed on immune metabolism. LNFPIII was already shown to induce an immunoregulatory phenotype in macrophages[163] but in addition, Bhargava and colleagues reported that this glycoconjugate improves insulin signaling and thereby sensitivity in white adipose tissue in high fat diet-fed mice, in part through IL-10[164]. Furthermore, some detailed studies of the interaction of Th2 cytokines and metabolic homeostasis have shown that IL-4 shifts cellular energy resources from fatty acids to glucose oxidation, by enhancing the activity of insulin[165]. These studies highlight the possible metabolic advantages of harboring helminth infections.

Final remarks

In summary, there is an apparent contrast between efforts to deworm populations in remote areas suffering from helminth-associated morbidities and initiatives to test effects of helminthic therapy on patients with hyperinflammatory diseases (Figure 1).

Murine models have supported the hypothesis that helminths or their products may be beneficial for inflammatory conditions. Human studies in poor-resource settings have been less consistent, which may be explained by the presence of other modulating infections and by the fact that the clinical effects of deworming may take longer to establish. Further deworming trials should take these issues into account and plan for longer follow-up periods. Human studies of the therapeutic use of helminths in resource-rich settings may shed more light on the role of helminths in human physiology and immunology. Although some positive results have been obtained in IBD and MS, not much benefit was seen in treatment of asthma and allergy. Further trials should be less modest in numbers of patients included. Furthermore, assessment of locally – and importantly – systemically induced immune (regulatory) responses deserves further attention, since the underlying mechanisms and consequences of voluntary helminth infection could influence other health outcomes in patients.

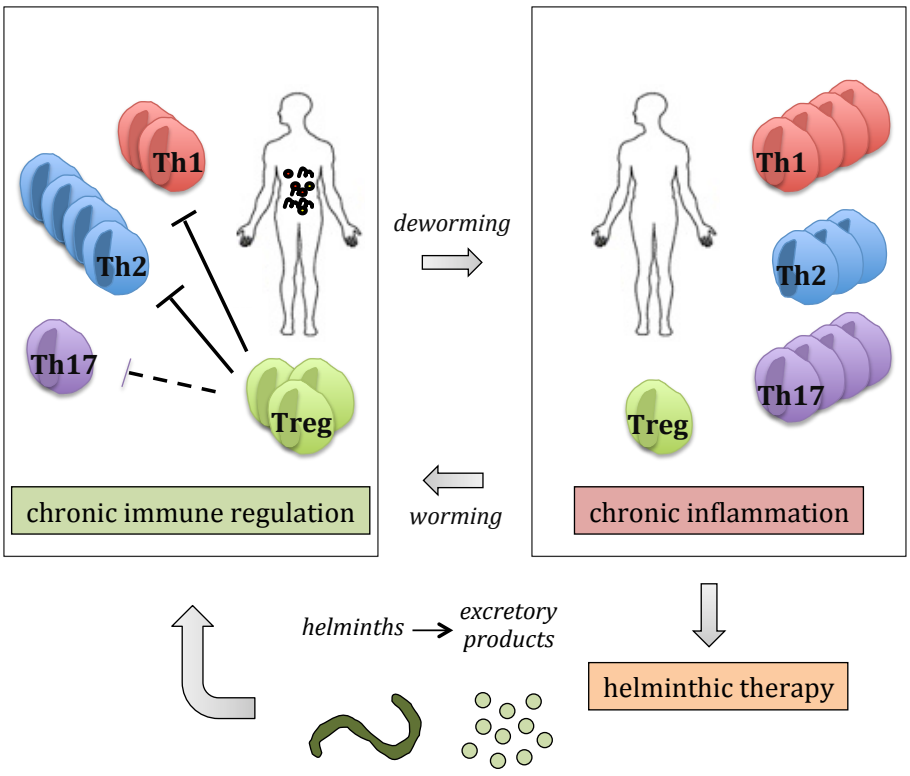

Figure 1. The immunological effects of deworming and worming. Helminth-infected individuals (left panel) harbor enhanced Th2 responses. The number and immunosuppressive capacity of Tregs may also be increased during infection. After deworming, removal of immune suppression could lead to over-inflammation, characteristic of several inflammatory diseases (right panel). Th1, Th2 or Th17 responses are more abundant, whereas Tregs are less in number and function. Treatment with experimental helminth infection or with helminth-derived immunomodulatory molecules could restore the immune regulation as observed during natural chronic helminth infections. The resulting suppression of inflammatory T-cell responses may curtail symptoms of several immune-mediated diseases.

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