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Nematodes and human therapeutic trials for inflammatory disease

D. E. Elliott¹ and J. V. Weinstock²

¹Division of Gastroenterology, University of Iowa, Iowa City, IA, USA

²Division of Gastroenterology, Tufts Medical Center, Boston, MA, USA

Summary

Helminth infections likely provide a protective influence against some immune-mediated and metabolic diseases because helminth infection dramatically decreased in developed countries shortly before the explosive rise in the prevalence of these diseases. The capacity of helminths to activate immune-regulatory circuits in their hosts and to modulate the composition of intestinal flora appears to be the mechanisms of protective action. Animal models of disease show that various helminth species prevent and/or block inflammation in various organs in a diverse range of diseases. Clinical trials have demonstrated that medicinal exposure to *Trichuris suis* or small numbers of *Necator americanus* is safe with minor, if any, reported adverse effects. This includes exposure of inflamed intestine to *T. suis*, asthmatic lung to *N. americanus* and in patients with atopy. Efficacy has been suggested in some small studies, but is absent in others. Factors that may have led to inconclusive results in some trials are discussed. To date, there have been no registered clinical trials using helminths to treat metabolic syndrome or its component conditions. However, the excellent safety profile of *T. suis* or *N. americanus* suggests that such studies should be possible.

Keywords

colitis; dendritic cells; helminths; mucosa

1 | MECHANISMS OF HELMINTH-INDUCED PROTECTION FROM INFLAMMATION

Immune dysregulation fuels development of many diseases such as IBD, multiple sclerosis, rheumatoid arthritis, type 1 diabetes and asthma/allergy. Most of these diseases are frequent in developed nations, but much less common in less developed countries. The rapidly increasing incidence of these diseases in industrializing regions of the world suggests that environmental factors are inducing the spread of these conditions around the world.¹ The “inflammatory bowel disease hygiene hypothesis” was proposed to explain the proliferation of these diseases.² It asserts that modern-day sanitation reduced exposure to organisms that

provide protection from these conditions. It also suggests that changes in the composition of our intestinal flora and fauna play a role, as one of our greatest exposures to organisms comes from within our gastrointestinal tract.

Helminth infections were considered as a potential protective factor because their occurrence dramatically decreased in developed countries shortly before the great rise in the prevalence of immune-mediated diseases. Improved sanitation and an increasingly clean food supply contributed to the demise of helminths. The inverse relationship of the prevalence of helminth infections and the frequency of immune-mediated diseases like IBD is supported by various clinical and epidemiologic studies.³ Their ability to inhabit the gut and other locations of mammalian and nonmammalian hosts probably developed many millions of years ago. The close dependency of the parasite upon its host, and the processes of co-evolution and mutualism likely created this circumstance.

Animal models of various human immune-mediated diseases show that helminth infections can prevent disease or even modulate ongoing inflammation. These models have helped to define the mechanisms of disease protection. It appears that helminth infections activate immune-regulatory circuits in their hosts and modulate the composition of intestinal flora, which appear to be the mechanisms of action. As there are many different helminth species living in different regions of their host, some of these species likely developed distinct approaches to modulate host immunity.

The following discussion reviews what has been learned regarding mechanisms through which helminthic control inflammation in IBD. Space limitations do not allow a similar discussion for other conditions. However, pathways of regulation trend to echo among the various disease states and likely apply to effects on metabolism.

2 | REGULATORY DENDRITIC CELLS AND CONTROL OF MUCOSAL INFLAMMATION

In animal models of IBD, helminths induce regulatory dendritic cells (DCs) that in turn can prevent or control intestinal inflammation. In one such model, recombination-deficient (Rag) mice that cannot make functional T or B cells are reconstituted with IL-10^{-/-} T cells. They subsequently develop colitis because their intestinal regulatory T cells (Tregs) cannot make IL-10.⁴ *Heligmosomoides polygyrus bakeri* (*Hpb*) is a murine helminth that resides in the proximal small bowel. Rag mice infected with *Hpb* are protected from this⁵ and other forms of colitis.

One of the mechanisms of protection involves induction of regulatory DCs in the intestinal mucosa.⁵ The process of induction does not require the interplay of T or B cells. DCs isolated from the intestines or mesentery lymph nodes (MLN) of *Hpb*-infected Rag mice and transferred into other uninfected Rag mice will render these animals resistant to colitis and impede intestinal mucosa production of colitogenic cytokines such as IFN- γ and IL-17.⁶ This suggests that these DCs have an important role in protecting mice from the disease.

The regulatory DCs limit intestinal T-cell responses. Colitis probably results from inappropriate effector T-cell activation in the gut that drives the pathology. Through a cell contact-dependent mechanism, the regulatory DCs probably induce T-cell anergy preventing pro-inflammatory DCs from successfully activating the effector T cells that produce colitogenic cytokines. This regulatory process does not require Tregs, IL-10, TGF- β or IL-4.⁶ However, these cells do not prevent effector T cells from populating the gut or MLN.

Hpb infection in the upper small bowel profoundly affects gene expression in DCs residing in distal regions of the gut. There is substantial downregulation of many components of the MHC antigen-presenting complex including CD80, CD86 and CD40. There also is reduced expression of several intracellular signalling pathways such as Jak1/2 and Syk, which drive production of proinflammatory cytokines that promote effector T-cell differentiation and activation.⁷

Hpb infection also diminishes the expression of many of the germ line-encoded pattern recognition receptors displayed on intestinal DCs.⁷ These receptors, which include the Toll-like (Tlr) and C-type lectin (CLEC) receptors (CTLR) among others, bind molecular motifs on bacteria, fungi, viruses or stressed host cells. Ligation of these receptors triggers intracellular signalling that can determine whether DCs will vigorously present antigen to effector T cells inducing their differentiation, proliferation and function, or perhaps render them inert.

Many of the CLECs induce phosphorylation of Syk to stimulate DC activation promoting adaptive immunity and strong Th1/Th17 responses.⁸ Subsequent studies revealed that *Hpb* infection inhibited Syk expression in intestinal DCs, which proved to be a profoundly important observation.

Inactivation of Syk is a critical event that leads to the formation of regulatory DCs in the gut. Transgenic DCs with a block in Syk expression, harvested from mice with no prior worm infection, will block colitis if they are transferred into a colitic mouse. *Hpb* produces a molecule(s) that quickly and selectively inhibits DC Syk and CLEC expression suggesting that these parasites directly target this pathway.⁹

It remains uncertain how inactivation of the Syk signalling pathway in intestinal DCs blocks colitis and mucosal antigen-induced, T-cell responses. Syk-mediated downstream signalling leads to activation of proinflammatory pathways such as NF- κ B, NFAT and CARD9.¹⁰ The Syk signalling pathway in DCs is important for inducing Th1/Th17 responses.¹¹ In many murine models of IBD, poorly regulated effector T cells located in the gut respond to luminal antigens and release pro-inflammatory molecules such as IFN- γ and IL-17 that drive the colitis.¹² These two cytokines commonly are expressed strongly in patients with either ulcerative colitis or Crohn's disease¹³ and appear to have a role in the disease process. Thus, down-modulation of Syk may decrease Th1/Th17 responsiveness leading to inhibition of IBD.

Other mechanisms also must be considered. Blocking of Syk in WT DCs in vitro reduces their display of costimulatory markers (CD80 and CD86) and MHC class II as well as secretion of pro-inflammatory cytokines (eg IL-12p40).¹⁴ Regulatory DCs from the gut of

Hpb-infected mice have a similar phenotype.^{5,7} A known effect of regulatory DCs with impaired MHC complex expression is induction of T-cell anergy due to delivery of defective costimulation to T cells.¹⁵ Thus, induction of T-cell anergy could be one of the mechanisms through which these regulatory DCs inhibit T-cell responses that induce colitis.

Bone marrow-derived DCs pulsed with antigen from *Hymenolepis diminuta* and then transferred into mouse recipients will inhibit dinitrobenzene sulphonic acid (DNBS)-induced colitis. This protection by bone marrow-derived rather than gut or MLN-derived DCs appears to require animals that can express Th2 cytokines and IL-10.¹⁶

3 | THE IMPORTANCE OF REGULATORY T CELLS

In mice, Tregs in the gut produce IL-10, which helps to prevent aberrant intestinal inflammation.^{17,18} In the distal bowel of normal healthy mice, about 25% of the lamina propria CD4⁺ T cells express Foxp3, a marker of Tregs.^{19,20} These cells likely restrain the host immune response to the normal intestinal flora. Two highly studied murine models of IBD, the Rag IL-10^{-/-} T-cell transfer model and the Rag/CD25-CD4⁺ T-cell transfer model develop colitis because of Treg dysfunction. These models illustrate the importance of Tregs for maintenance of normal mucosal immune homeostasis.

Heligmosomoides polygyrus bakeri (*Hpb*) infection expands the number of regulatory-type T cells that make IL-10 in the mesenteric lymph nodes (MLN)^{21,22} and intestinal lamina propria of its murine host.²⁰ This helminth also “activates” Tregs making them highly regulatory.²⁰ Foxp3⁺ T cells in the intestines of healthy helminth-naïve wild-type mice afford no protection from colitis when transferred into the Rag/CD25-CD4⁺ transfer model of IBD. However, Foxp3⁺ T cells isolated from the colon, terminal ileum or MLN of *Hpb*-infected WT mice populate the gut and MLNs of the Rag recipients more readily and are able to prevent the disease.²⁰

Mice treated with dextran sodium sulphate (DSS) develop colitis. Mice immunized with *Schistosoma mansoni* soluble egg antigen develop milder colitis after DSS exposure. The immunized mice display more Tregs and Th2 cytokines in the gut.²³

Hpb infection induces intestinal Tregs to express several genes including IL-10²⁰ and GATA3. GATA3 is required for Tregs to accumulate at sites of inflammation. It helps sustain high level Foxp3 and IL-10 expression, which are needed for Tregs to protect mice from colitis.^{24–26}

In the CD25-CD4⁺ T-cell transfer model of IBD, the Foxp3⁺ IL-10⁺ CD4⁺ T-cell subset is essential for controlling the disease.^{20,27–29} IFN- γ is a driver of colitis in most IBD models. In the gut, the *Hpb*-activated Tregs control colitis partly through secretion of IL-10, which inhibits production of IFN- γ from mucosal effector T cells. Other mechanisms of action are likely as well. Other helminth species such as *S. mansoni*³⁰ and *H. diminuta*³¹ also induce IL-10 secretion. *Litomosoides sigmodontis* suppresses B-cell responses in the host through induction of IL-10 and Tregs.^{32,33}

Helminths still prevent colitis in IL-10^{-/-} mice^{5,21} in which the Tregs cannot make IL-10. The worm-induced, regulatory DCs (discussed above) also block colitis using mechanisms not dependent on Tregs and IL-10. Thus, there are at least two independent immune-regulatory networks driven by *Hpb* infection that limit the expression of colitis.

Helminths stimulate TGF- β production in the intestinal mucosa.³⁴ TGF- β engagement of TGF- β receptors on mucosal T cells is essential to prevent colitis.³⁴ TGF- β can induce CD4⁺ T cells to convert to regulatory T cells that make IL-10.^{19,35} However, isolated CD4⁺ Foxp3-IL-10-T cells from the gut of uninfected healthy mice do not readily convert to their regulatory phenotype when cultured in vitro with TGF- β . Yet, intestinal CD4⁺ T cells from *Hpb*-infected mice convert to IL-10 producers and many will express Foxp3 after just 3 days in culture with TGF- β (J. V. Weinstock, manuscript in preparation). TGF- β signals through the SMAD signalling pathway, which can be blocked by expression of SMAD7.³⁶ In the normal healthy state, intestinal CD4⁺ Foxp3-IL-10-T cells express SMAD7 at high levels, which impedes TGF- β signalling and subsequent Treg development. *Hpb* infection inhibits SMAD7 expression in gut T cells. This allows strong TGF- β signalling leading to induction of IL-10 producing regulatory T cells that can block colitis (J. V. Weinstock, manuscript in preparation).

It is appreciated that many patients with IBD have mucosal effector T cells that strongly express SMAD7.³⁷ Murine Tregs have difficulty inhibiting colitis in the Rag/CD4⁺ CD25-T-cell transfer model of IBD if the effector T cells strongly express SMAD7.³⁸ Human lamina propria mononuclear cells (LPMC) isolated from patients with IBD are more susceptible to Treg inhibition in vitro following blockade of SMAD7 expression.³⁸ An oral SMAD7 antisense oligonucleotide presently is under evaluation as a treatment for patients with Crohn's disease.³⁹ Thus, the natural inhibitory effect of helminths on SMAD7 expression could be a fundamentally important process through which helminths impede the development of IBD in people.

The excretory/secretory products of *Hpb* appear to contain a molecule that can bind the TGF- β receptor. It can stimulate Foxp3 expression in splenic T cells.²²

4 | REGULATORY MACROPHAGES CAN LIMIT DISEASE

Helminths can induce alternatively activated macrophages which can help control murine IBD. The host response to helminths can generate cytokines such as IL-4, IL-5 and IL-10 that modulate macrophage activation.⁴⁰ Such alternatively activated macrophages are a source for immune modulatory molecules such as IL-10 and TGF- β that can block inflammatory responses.⁴¹

Dextran sodium sulphate administered orally to rodents damages the intestinal epithelial lining inducing gut inflammation. Infection with *S. mansoni* induces regulatory macrophages that protect mice from DSS-induced injury. The process of protection requires adult schistosome flukes, but does not require regulatory cytokines such as TGF- β and IL-10, or Tregs.⁴² A cysteine protease inhibitor (cystatin) of filarial nematodes protects mice from DSS colitis⁴³ possibly through inducing macrophages to make IL-10.^{44,45} *Taenia*

crassiceps infection also provides mice protection from DSS injury at least at least partly through induction of alternatively activated macrophages.⁴⁶

In dinitrobenzene sulphonic acid (DNBS)-induced colitis, infection with *H. diminuta* protects mice from colitis through generation of alternatively activated macrophages in the colon. These macrophages transferred into mice reduce DNBS-induced colonic inflammation once more illustrating their potential importance in limiting the disease process.^{47,48} In the DNBS model, alternatively activated macrophages work through an IL-10-dependent mechanism to control the colitis.⁴⁹

In the IL-10^{-/-} Rag model of IBD, *Hpb* infection induces regulatory macrophages in the gut of the Rag mice independent of T or B cells. These intestinal regulatory macrophages inhibit antigen-induced, IL-17 and IFN- γ secretion in the gut via a contact-dependent mechanism. Also, when transferred into Rag mice, they protect animals from colitis attesting to their importance in controlling IBD (Weinstock et al., unpublished). They develop and function independently from Tregs, IL-10 and regulatory DCs.

Hymenolepis diminuta infection induces CD19⁺ regulatory B cells in the host spleen. These cells transferred into naïve mice ameliorate DNBS-induced colitis in conjunction with regulatory macrophages and TGF- β .^{16,50}

5 | REGULATION OF IL-17 AND IL-6

IL-17 is an important proinflammatory cytokine. Colonization with *Hpb* reduces IL-17 production by lamina propria and mesenteric lymph node T cells.⁵¹ Although Th17 cells help drive immune-mediated inflammation, their role in colitis is more complex. Treatment of patients with Crohn's disease with a blocking anti-IL-17R monoclonal antibody (AMG 827) that prevents IL-17A, IL-17F and IL-25 (IL-17E) signalling resulted in worsened disease or increased disease progression in about 30% of patients given active agent as compared to 9% in those given placebo.⁵² In mice, IL-17 responses are needed for effective host defence against intestinal pathogens such as *Citrobacter rodentium* and *Salmonella enterica* serovar Typhimurium.⁵³ Mice exposed to *Hpb* have impaired resistance to enteric *Citrobacter* infection.⁵⁴ These findings show that balanced Th17 function is necessary for intestinal homeostasis. Indeed, Th17 cells are diverse with subtypes promoting an inflammatory and others a regulatory skewed response.⁵⁵ Using reporter mice, we find that *Hpb* colonization alters intestinal Th17 plasticity, decreasing the proportion of inflammatory Th17/Th1 cells and increasing that of regulatory Th17/IL-10 cells (D. E. Elliott, manuscript in preparation).

Another proinflammatory cytokine that has a complex role in intestinal homeostasis is IL-6, which promotes Th17 differentiation and also is made by Th17 cells. Patients with Crohn's disease or ulcerative colitis have increased IL-6 production in their inflamed mucosa⁵⁶ suggesting that IL-6 drives intestinal inflammation. However, systemic blockade of IL-6 signalling with anti-IL-6R monoclonal antibody can induce intestinal ulceration⁵⁷ and exacerbated disease in a patient with ulcerative colitis.⁵⁸ IL-6 signalling inhibits intestinal epithelial cell death, and loss of this cytokine may amplify mucosal injury.⁵⁹

Lamina propria and mesenteric lymph node cells from mice exposed to *Hpb* produce more IL-6 compared to those cells from helminth-naïve mice. IL-6 signals through its receptor to activate STAT3 promoting cell survival.⁶⁰ Intestinal epithelial cells from helminth-colonized mice display increased activated STAT3 expression which is dependent upon T-cell IL-6 production (D. E. Elliott, manuscript in preparation). Intestinal epithelial cells from helminth-colonized mice also have less caspase 3 activation (a measure of apoptotic response) after injury than epithelial cells from naïve mice (D. E. Elliott, manuscript in preparation). Thus, by decreasing inflammatory Th17/Th1 cells, but augmenting mucosal IL-6, helminth exposure can promote repair while regulating inflammatory responses in the gut.

6 | INTERACTIONS BETWEEN HELMINTHS AND THE INTESTINAL MICROBIOTA HAVE A ROLE

Bacteria in the intestines readily interact with intestinal epithelium, mucosal DCs and other cells of the mucosal immune system. *Hpb* infection modifies the distribution and abundance of some intestinal bacteria.⁶¹ There is an increase in *Lactobacillaceae* and *Enterobacteriaceae species*.^{62,63} There also is an increase in *Lactobacillaceae* family members during *Trichuris muris* infection.^{64,65} Various bacterial species within this group inhibit intestinal inflammation in models of colitis.^{66,67}

A genetic loss in functional *NOD2 (CARD15)* gene expression renders humans and mice more susceptible to Crohn's disease. *NOD2-deficient* mice display a small intestinal goblet cell defect that compromises the mucosal layer allowing overgrowth of *Bacteroides* species which drive intestinal inflammation only in NOD2-deficient animals.⁶⁸ Infection with *T. muris* or *Hpb* restores the goblet cell defect via the type 2 immune response favouring the expression of protective *Clostridiales* in deference to harmful *Bacteroides*. This change in flora protects the host from disease. People undergoing deworming of helminth species like *Trichuris trichiura* display a similar shift in microbiota. This suggests that deworming promotes Crohn's disease in NOD2-deficient individuals via loss of helminthic effects on the composition of intestinal flora.⁶⁸ *Clostridium* species also are inducers of intestinal Tregs.¹⁹

Rhesus monkeys develop colitis. *Trichuris trichiura* infection results in a milder colitis associated with reduced bacterial attachment to the epithelial surface and changes to the composition of microbial communities attached to the intestinal mucosa.⁶⁹

7 | HELMINTH EXPOSURE AND METABOLIC DISEASE

Helminths can regulate inflammation and chronic low-grade inflammation is associated with obesity, insulin resistance and other components of the metabolic syndrome.^{70–73} The metabolic syndrome is defined as the occurrence of central (abdominal) obesity and at least two of the following four conditions: (i) elevated triglyceride level (≥ 150 mg/dL (1.7 mmol/L)), (ii) low HDL cholesterol [<40 mg/dL (1.03 mmol/L*) in males and <50 mg/dL (1.29 mmol/L*) in females], (iii) elevated resting blood pressure (systolic BP ≥ 130 or diastolic BP ≥ 85 mm Hg) and (iv) elevated fasting plasma glucose [(FPG) ≥ 100 mg/dL (5.6 mmol/L)].

Metabolic syndrome or its component conditions likely underlay many diseases of industrialized highly developed countries such as cardiovascular disease,⁷⁴ type 2 diabetes,⁷⁵ chronic kidney disease⁷⁶) and nonalcoholic fatty liver disease.⁷⁷ If helminths suppress inflammation, and inflammation is associated with metabolic disease, could loss of helminth exposure help fuel the emergence of metabolic disease? Recent studies indicate this may be the case.

Populations in areas with prevalent helminth exposure provide opportunity to test if infection impacts obesity and other components of the metabolic syndrome. In Chennai, India, the prevalence of lymphatic filariasis is reduced in patients with diabetes (5.7%) compared to nondiabetic subjects (10.4%) suggesting that carriage confers protection.⁷⁸ Similarly, individuals with type 2 diabetes in Aboriginal communities of Northern Australia were much less likely to have a positive ELISA test for *Strongyloides stercoralis* infection (24.4%) compared to nondiabetic subjects (46.9%).⁷⁹ Furthermore, a cross-sectional study in Flores, Indonesia, showed that individuals that harbour soil-transmitted helminths (*T. trichiura*, *Ascaris lumbricoides*, *Necator americanus*, *Ancylostoma duodenale* and/or *Strongyloides stercoralis*) had lower body mass index (BMI), total blood cholesterol and LDL-cholesterol levels compared to uninfected subjects.⁸⁰ Higher intensity of infections correlated with lower BMI and cholesterol levels. Similarly, an autopsy study of 319 cadavers in Khanty-Mansiisk Russia, a region with endemic *Opisthorchis felinus* infection, showed decrease in total cholesterol and diminished aortic atherosclerosis that correlated with increase in infection intensity.⁸¹

Studies of animal models of metabolic disease show protection by helminth exposure. Infection of high-fat-diet-fed obese mice with *Nippostrongylus brasiliensis* or *Schistosoma mansoni* resulted in weight loss and increased insulin sensitivity (reduced insulin resistance).^{82–84} Treatment with soluble schistosome egg antigens (SEA) or recombinant T2 RNase ω 1 (an immunologically active component of the egg that induces Th2 responses) produced a similar result.⁸⁵ Similarly, obese mice treated with LNFPIII, a Lewis X-containing immunomodulatory glycan found in eggs of *S. mansoni*, had improved insulin sensitivity and reduced hepatic fat accumulation in association with increased IL-10 production.⁸⁶

Infection of atherosclerosis prone apolipoprotein E-deficient mice with *S. mansoni* reduced blood cholesterol and atherosclerotic lesions in the aortic arch and brachiocephalic artery by about 50%.⁸⁷ Like apolipoprotein E-deficient mice, mice rendered deficient in low-density lipoprotein receptor (LDLR) and fed a high cholesterol diet are hyperlipidemic and readily develop atherosclerotic lesions. LDLR-deficient mice treated with SEA had lower cholesterol (~18% lower) and developed smaller aortic root plaques.⁸⁸ ES-62, a phosphorylcholine containing secretory product of the filaria *Acanthocheilonema viteae*, reduced atherosclerotic lesion area by 62% in lupus-prone FAS-ligand deficient (*gld*) apoE-deficient mice.⁸⁹ Thus, like autoimmunity and immune-mediated inflammatory disease, the recent meteoric rise of metabolic disease in industrialized highly developed countries may be fuelled by loss of previously ubiquitous helminth exposure.

8 | CLINICAL TRIALS OF HELMINTHIC THERAPY FOR IMMUNE-MEDIATED DISEASE

Much of this review focuses on the effects of helminth exposure on intestinal immunity and how this may protect from immune-mediated pathologic intestinal inflammation. A successful parasite must acquire the ability to manipulate their host's immune system. Such manipulation could result in prevention or attenuation of many different immune-mediated diseases. This effect is well demonstrated in several different animal models of immune-mediated disease. These findings have prompted clinical trials employing helminth exposure (Table 1).

Inflammatory bowel disease is a category of illnesses that includes Crohn's disease and ulcerative colitis. Crohn's disease is a chronic ulcerating inflammation that can involve any part of the gut and can result in severe abdominal pain, malnutrition, intestinal fibrosis and obstruction as well as fistulization and abscesses. Ulcerative colitis is a chronic ulcerating inflammation restricted mostly to the colon. It can induce symptoms similar to that of Crohn's disease, but is not associated with intestinal fibrosis, strictures or fistulization. Both can display relapsing/relapsing behaviour particularly early in the disease course.

Inflammatory bowel disease has been studied in clinical trials using *Trichuris suis* (pig whipworm)^{90–94} or *N. americanus* (human hookworm)⁹⁵ as therapeutic agents. Early studies showed that use of intestinal helminths in patients with active intestinal inflammation was safe and suggested that such exposure reduced symptoms. A double-blind placebo-controlled study of patients with long-standing (~8 years) ulcerative colitis and lengthy exacerbations (~11.5 months) showed statistically significant disease improvement in patients given 2500 *T. suis* ova every 2 weeks for 12 weeks.⁹⁴ That study included a blinded crossover limb for patients with continued disease activity.⁹⁰ More patients who were initially on placebo improved when crossed over to *T. suis* (56.3%) than patients who were initially on *T. suis* and then switched to placebo (13.3%, $P=0.02$). No adverse side effects were recorded. An open-label study using *T. suis* (2500 ova every 3 weeks for 24 weeks) in patients with long-standing Crohn's also demonstrated safety and suggested efficacy with 72% achieving clinical remission.⁹³

Two larger clinical trials using *T. suis* for active Crohn's disease focused on enrolling patients with early disease with the expectation it would be easier to influence the immune process in these test subjects compared to that of patients with long-established disease. However, those studies were plagued by very high placebo response rates that obscured any potential clinical effect. In addition, the parasite ova were prepared using different methodology and used at different dosages than that of the earlier studies which complicate interpretation of the results.

The European trial (Trust 2, NCT01279577) interim analysis suggested a dose-response with the lowest of the three doses used (250 ova) being ineffective. That low-dose limb was discontinued. Enrolment criteria then were relaxed to hasten completion of trial. A high placebo rate occurred. The US study (Trust 1, NCT01279577) enrolled many patients with early disease that were mostly anti-TNF α treatment unresponsive. This study also had an

unexpectedly high placebo response rate. Both studies again confirmed the safety of *T. suis* in this patient population. However, the extraordinarily placebo rates of remission left the outcome of the studies indeterminate.

Coeliac disease is a common intestinal inflammation caused by delayed-type hypersensitivity to peptide antigens present in wheat, rye and barley gluten. Coeliac disease can cause abdominal discomfort, diarrhoea, skin rash, malnutrition, intestinal ulceration and intestinal lymphoma. Coeliac disease has been studied in clinical trials of *N. americanus*.^{96,97}

Helminth exposure altered mucosal adaptive and innate immune responses, but there was no significant attenuation of a response to gluten. These studies used a gluten challenge of 16 g each day for 5 days which was clinically relevant, but immunologically quite aggressive. As little as 50 mg of gluten can reliably precipitate a response in patients with coeliac disease.⁹⁸ A second study to examine whether helminth exposure can help desensitize patients to gluten has recently been completed.⁹⁹ This trial showed that the combination of hookworm exposure and low-dose gluten exposure promoted mucosal tolerance defined as decreased intestinal T-cell IFN- γ expression and increased CD4+Foxp3+ T-cell frequency compared to baseline. The combination of helminth and gluten challenge also enhanced the diversity (operational taxonomic units) of the intestinal flora as compared to baseline challenge.¹⁰⁰

Allergic rhinitis (AKA seasonal allergy, hay fever) is a common illness that has minimal morbidity, but has high socio-economic impact because it afflicts large numbers of people. Allergic rhinitis has been studied in clinical trials of *T. suis*¹⁰¹ or *N. americanus*.¹⁰² Patients given *T. suis* had less use of medications, but otherwise no change in symptoms. The timing of exposure to helminths in these studies was close to the seasonal onset of symptoms which may have limited their efficacy. The studies showed that medicinal exposure to either of these helminths was safe with minimal adverse effects.^{102,103}

Peanut allergy is an immediate-type (type 1) hypersensitivity response to oral peanut exposure. Patients with peanut allergy can develop itching, urticaria, vomiting/diarrhoea and severe potentially fatal anaphylaxis upon ingestion of even small amounts of antigen. Peanut or tree nut allergy has been studied in an open-label clinical safety trial of *T. suis*.¹⁰⁴ The agent was well tolerated. Overall, they found no change in skin prick sensitivity except one patient lost reactivity to peanut allergen. Four patients reported improvement in seasonal allergy symptoms.

Asthma is a chronic remitting and relapsing inflammatory response that constricts the lung airways. Asthma exacerbations can cause wheezing, chest tightness, shortness of breath and death due to hypoxia. Asthma has been studied in a clinical trial using *N. americanus*.¹⁰⁵ Sixteen weeks after initial dermal exposure, there was a trend for decreased airway responsiveness to adenosine monophosphate challenge in patients exposed to helminths vs placebo which did not reach statistical significance. Other measures of asthma control were not changed but the exposure to a helminth which passes through the lung parenchyma was well tolerated in patients with underlying asthma.

Plaque psoriasis is a common chronic patchy skin rash usually involving the extensor surface areas of the limbs and back. It can be associated with arthritis and other organ involvement such as metabolic syndrome and heart disease. Psoriasis can cause severe itching, pain and secondary skin infections. Clinical trials of *T. suis* for plaque psoriasis have been initiated. One study (NCT01948271) was terminated due to lack of efficacy. The other studies were suspended due to lack of available study agent.

Rheumatoid arthritis is a chronic inflammation of the membranes (synovium) that line joints, but can also involve other organs such as the skin, lungs and kidneys. Rheumatoid arthritis can cause painful swelling and eventual destruction of afflicted joints. A clinical trial of *T. suis* for rheumatoid arthritis was initiated, but study status or results are not available.

Multiple sclerosis is a chronic progressive, although often with relapsing and remitting periods, inflammatory demyelinating disease of the central nervous system. Multiple sclerosis causes dysfunction of the involved areas of the central nervous system resulting in focal loss of sensory, motor or autonomic capacity. Multiple sclerosis shares many epidemiologic features with inflammatory bowel disease being much more common in temperate highly industrialized countries where helminth infection is rare. Also, IBD occurs with higher frequency in families with multiple sclerosis than in that of the general population. Case studies suggest that patients with concurrent helminth infection had reduced severity of multiple sclerosis¹⁰⁶ and eradication of helminths exacerbated disease severity.¹⁰⁷ Multiple sclerosis has been studied in clinical trials of *T. suis*^{108–112} or *N. americanus*. A initial small safety trial of *T. suis* in five patients with remitting/relapsing multiple sclerosis showed a decrease in new brain lesions and increase in serum IL-4 and IL-10.¹⁰⁹ The open-label extension of that trial has completed (Fleming personal communication). The interim analysis showed a modest reduction (36%) in active brain lesions vs observation period although this improvement may be explainable by natural regression towards the mean.¹¹⁰ A small trial of four patients with secondary progressive multiple sclerosis treated with *T. suis* found that the frequency of peripheral T cells producing IL-2 decreased and producing IL-4 increased with helminth exposure.¹⁰⁸ Patients also had mild increases in peripheral eosinophil counts. Another safety study involving 10 patients with remitting/relapsing multiple sclerosis did not find clinical efficacy although they were unable to perform MRI with gadolinium contrast due to mistiming of the contrast injections.¹¹² Eight of the 10 patients developed eosinophilia suggesting helminth effect, but gene profiling of unstimulated peripheral blood did not show evidence of immune modulation. There is concern that this study was too small and brief to conclude lack of efficacy. Also, the patients were continued on various immune modulatory agents that could have affected the efficacy and vitality of the worms. Other studies are ongoing, or if completed the results are not yet available.

Autism is a neurodevelopmental disorder typified by repetitive behaviours, restricted interests and impaired social understanding, social interaction and ability to communicate. Autism is not a classical immune-mediated disease, but shares many epidemiologic features with those illnesses.^{113,114} Clinical trials of *T. suis* for treatment of autism were initiated. One small trial was completed and showed a trend towards improvement with *T. suis*

exposure that did not reach statistical significance in the small study of 10 patients. There were no significant adverse effects. The other trials have been terminated due to lack of available study agent.

Clinical trials to date have demonstrated that medicinal exposure to *T. suis* or small numbers of *N. americanus* is safe with minor, if any, reported adverse effects. This includes exposure of inflamed intestine to *T. suis*, asthmatic lung to *N. americanus* and in patients with atopy. Efficacy has been suggested in some small studies, but is absent in others. However, for relapsing and remitting disease that characterizes many of the immune-mediated illnesses, it can take a large number of participants to clearly demonstrate efficacy. Many of the negative studies to date have been too small to exclude a beneficial effect. This is particularly problematic when there is a high placebo response rate. Furthermore, the proper timing of exposure and number of repeated exposures prior to measuring immunologic and clinical effects remain in question. What we have learned is that helminth treatment is well tolerated and is acceptable to many patients as a therapeutic option.

9 | CONSIDERATIONS FOR CLINICAL TRIALS OF HELMINTHIC THERAPY FOR METABOLIC DISEASE

To date, there have been no registered clinical trials treating metabolic syndrome or its component conditions with helminths. However, if preclinical studies continue to suggest the exposure to helminths may protect from metabolic syndrome and current clinical trials continue to show that exposure to helminths such as *T. suis* or low-dose *N. americanus* has a high safety profile, then it would be reasonable to propose such a trial incorporating lessons learned from clinical trials that have been performed.

For all clinical trials, it is important to clearly define the study population, primary and secondary endpoints, comparator therapy (eg placebo, best practices), treatment design, randomization/stratification process, oversight governance and data evaluation method.¹¹⁵ Preclinical studies of helminth exposure in animal models of metabolic disease will help guide selection of the study population and study endpoints. An initial important criterion will likely be central obesity. The determination of central obesity varies by agency and population group (ethnicity) results in different measures. Central obesity is defined by the Adult Treatment Panel III guidelines in the USA as having a waist circumference 102 cm (40.2 inches) for men and 88 cm (34.6 inches) for women. Other agencies such as the International Diabetes Federation (IDF) define central obesity as having a waist circumference of 80 cm (31.5 inches) for all women and 94 cm (37 inches) for Europid men but 90 cm (35.4 inches) South Asian, Japanese or Chinese men. Due to ethnic variation, determination of central obesity by simple waist circumference becomes problematic and best determination is probably made by having a waist circumference to height ratio of greater than 0.5 in men and women.¹¹⁶ Additional enrolment criteria will likely focus on the other components of the metabolic syndrome such as elevated triglycerides, low HDL cholesterol, elevated resting blood pressure or insulin resistance (elevated fasting plasma glucose). Each of these components has complex homeostatic regulation that can be transiently effected by emotional or physical stressors. Therefore,

study design will need to minimize contribution by potential confounders. Double-blind placebo-controlled studies mitigate confounder effects by distributing them randomly into all study limbs. Placebo-controlled studies with either *T. suis* or *N. americanus* have been successfully performed although this is easier with orally ingested *T. suis* (which can cause transient loose stools) than dermally applied *N. americanus* (which can cause transient itching at inoculation site and abdominal discomfort). Neither agent can be easily identified by patients in their stools.

Treatment design will be constrained by the expected rapidity of response. *T. suis* began to influence ulcerative colitis disease severity (as measured by simple index) after 6 weeks of repeated exposure.⁹⁰ To elicit change in components of the metabolic syndrome, it is likely that patients will need repeated inoculation over several months. If the endpoint condition is usually relatively stable over time, then this is achievable. However, if the condition waxes and wanes, then patient numbers may need to be increased to accommodate variance over time. The longer the study, the more difficult it is to enrol patients, the more patients become lost to follow-up and the more expensive the study becomes. Cumbersome treatment and testing procedures also negatively impact participation.

We did not find that use of helminths or “parasitic worms” deterred participation. The vast majority of patients were not apprehensive about becoming colonized with helminths. Patients are often treated with medications that have long lists of adverse effects. The central postulate of the hygiene hypothesis that our environment has become “too hygienic” is familiar to the general public. Furthermore, we live in a “probiotic” era where patients are urged to ingest health-promoting organisms. Instead, a greater concern is that patients are so enthusiastic about the potential of helminthic therapy that it augments placebo responses.

Clinical trials of helminths or helminthic products are definitely doable. Regulatory agencies are acquainted with *T. suis* and *N. americanus*. Both organisms have good safety profiles in the dosages used in previous trials. Preclinical studies are beginning to identify helminth products (SEA, ω 1, LNFPIII, ES-62) that affect metabolic parameters. These purified products will also be amendable to clinical study but will need Phase 1 safety trials and may require more difficult treatment protocols if they need to be frequently injected.

10 | CONCLUSION

Animal models show that various helminth species prevent and/or block inflammation in various organs in a diverse range of diseases. Many helminths use common approaches to accomplish this task. These include stimulation of regulatory cytokine production, manipulation of DC function, Treg activation and modulation of macrophage activity. The abrogation of one regulatory pathway will not necessarily lead to loss of protection from disease, because some of these mechanisms of immune regulation function independently and in tandem.

Helminths have lived in close association with their mammalian hosts throughout the eons. Because of this close association, they have evolved the ability to manipulate immune responses to promote their survival often without harm to the host. The study of helminth-

host immune interactions is revealing important immune-regulatory circuits. These circuits may be susceptible to pharmacological manipulation, leading to new and safe treatments for immune-mediated diseases. Indeed, clinical trials to date have confirmed the safety of medicinal exposure to *T. suis* and *N. americanus* and have suggested that helminth exposure may be efficacious. However, many of the limited trials to date have yielded indeterminate results.

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Abbreviations

APC	antigen-presenting cells
CLEC	C-type lectin
CTLR	C-type lectin receptor
DCs	dendritic cells
DNBS	dinitrobenzene sulphonic acid
Hpb	<i>Heligmosomoides polygyrus bakeri</i>
IBD	inflammatory bowel disease
MLN	mesenteric lymph node
TLR	Toll-like receptor
TNBS	trinitrobenzene sulphonic acid

References

1. Molodecky NA, Soon IS, Rabi DM, et al. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology*. 2012; 142:46–54. e42. quiz e30. [PubMed: 22001864]
2. Elliott DE, Urban JJ, Argo CK, Weinstock JV. Does the failure to acquire helminthic parasites predispose to Crohn's disease? *FASEB J*. 2000; 14:1848–1855. [PubMed: 10973934]
3. Weinstock JV, Elliott DE. Translatability of helminth therapy in inflammatory bowel diseases. *Int J Parasitol*. 2013; 43:245–251. [PubMed: 23178819]
4. Berg DJ, Zhang J, Weinstock JV, et al. Rapid development of colitis in NSAID-treated IL-10-deficient mice. *Gastroenterology*. 2002; 123:1527–1542. [PubMed: 12404228]
5. Hang L, Setiawan T, Blum AM, et al. *Heligmosomoides polygyrus* infection can inhibit colitis through direct interaction with innate immunity. *J Immunol*. 2010; 185:3184–3189. [PubMed: 20702728]
6. Blum AM, Hang L, Setiawan T, et al. *Heligmosomoides polygyrus bakeri* induces tolerogenic dendritic cells that block colitis and prevent antigen-specific gut T cell responses. *J Immunol*. 2012; 189:2512–2520. [PubMed: 22844110]

7. Weinstock JV. Do we need worms to promote immune health? Clin Rev Allergy Immunol. 2015; 49:227–231. [PubMed: 25326880]
8. Kerrigan AM, Brown GD. Syk-coupled C-type lectins in immunity. Trends Immunol. 2011; 32:151–156. [PubMed: 21334257]
9. Hang L, Blum AM, Kumar S, et al. Downregulation of the Syk signaling pathway in intestinal dendritic cells is sufficient to induce dendritic cells that inhibit colitis. J Immunol. 2016; 197:2948–2957. [PubMed: 27559049]
10. Mocsai A, Ruland J, Tybulewicz VL. The SYK tyrosine kinase: a crucial player in diverse biological functions. Nat Rev Immunol. 2010; 10:387–402. [PubMed: 20467426]
11. LeibundGut-Landmann S, Gross O, Robinson MJ, et al. Syk-and CARD9-dependent coupling of innate immunity to the induction of T helper cells that produce interleukin 17. Nat Immunol. 2007; 8:630–638. [PubMed: 17450144]
12. Maloy KJ, Powrie F. Intestinal homeostasis and its breakdown in inflammatory bowel disease. Nature. 2011; 474:298–306. [PubMed: 21677746]
13. Kobayashi T, Okamoto S, Hisamatsu T, et al. IL23 differentially regulates the Th1/Th17 balance in ulcerative colitis and Crohn's disease. Gut. 2008; 57:1682–1689. [PubMed: 18653729]
14. Morrison VL, James MJ, Grzes K, et al. Loss of beta2-integrin-mediated cytoskeletal linkage reprogrammes dendritic cells to a mature migratory phenotype. Nat Commun. 2014; 5:5359. [PubMed: 25348463]
15. Anderson AE, Sayers BL, Haniffa MA, et al. Differential regulation of naive and memory CD4+ T cells by alternatively activated dendritic cells. J Leukoc Biol. 2008; 84:124–133. [PubMed: 18430785]
16. Matisz CE, Leung G, Reyes JL, Wang A, Sharkey KA, McKay DM. Adoptive transfer of helminth antigen-pulsed dendritic cells protects against the development of experimental colitis in mice. Eur J Immunol. 2015; 45:3126–3139. [PubMed: 26332354]
17. Izcue A, Coombes JL, Powrie F. Regulatory lymphocytes and intestinal inflammation. Annu Rev Immunol. 2009; 27:313–338. [PubMed: 19302043]
18. MacDonald TT, Monteleone I, Fantini MC, Monteleone G. Regulation of homeostasis and inflammation in the intestine. Gastroenterology. 2011; 140:1768–1775. [PubMed: 21530743]
19. Atarashi K, Tanoue T, Shima T, et al. Induction of colonic regulatory T cells by indigenous Clostridium species. Science. 2011; 331:337–341. [PubMed: 21205640]
20. Hang L, Blum AM, Setiawan T, Urban JP Jr, Stoyanoff KM, Weinstock JV. Heligmosomoides polygyrus bakeri infection activates colonic Foxp3+ T cells enhancing their capacity to prevent colitis. J Immunol. 2013; 191:1927–1934. [PubMed: 23851695]
21. Elliott DE, Setiawan T, Metwali A, Blum A, Urban JF Jr, Weinstock JV. Heligmosomoides polygyrus inhibits established colitis in IL-10-deficient mice. Eur J Immunol. 2004; 34:2690–2698. [PubMed: 15368285]
22. Grainger JR, Smith KA, Hewitson JP, et al. Helminth secretions induce de novo T cell Foxp3 expression and regulatory function through the TGF-beta pathway. J Exp Med. 2010; 207:2331–2341. [PubMed: 20876311]
23. Hasby EA, Hasby Saad MA, Shohieb Z, El Noby K. FoxP3+ T regulatory cells and immunomodulation after *Schistosoma mansoni* egg antigen immunization in experimental model of inflammatory bowel disease. Cell Immunol. 2015; 295:67–76. [PubMed: 25766778]
24. Shoemaker J, Saraiva M, O'Garra A. GATA-3 directly remodels the IL-10 locus independently of IL-4 in CD4+ T cells. J Immunol. 2006; 176:3470–3479. [PubMed: 16517715]
25. Wang Y, Su MA, Wan YY. An essential role of the transcription factor GATA-3 for the function of regulatory T cells. Immunity. 2011; 35:337–348. [PubMed: 21924928]
26. Wohlfert EA, Grainger JR, Bouladoux N, et al. GATA3 controls Foxp3(+) regulatory T cell fate during inflammation in mice. J Clin Invest. 2011; 121:4503–4515. [PubMed: 21965331]
27. Rubtsov YP, Nies RE, Josefowicz S, et al. Stability of the regulatory T cell lineage in vivo. Science. 2010; 329:1667–1671. [PubMed: 20929851]
28. Schmitt EG, Haribhai D, Williams JB, et al. IL-10 produced by induced regulatory T cells (iTregs) controls colitis and pathogenic ex-iTregs during immunotherapy. J Immunol. 2012; 189:5638–5648. [PubMed: 23125413]

29. Uhlig HH, Coombes J, Mottet C, et al. Characterization of Foxp3+CD4+CD25+ and IL-10-secreting CD4+CD25+ T cells during cure of colitis. *J Immunol.* 2006; 177:5852–5860. [PubMed: 17056509]
30. Elliott DE, Li J, Blum A, et al. Exposure to schistosome eggs protects mice from TNBS-induced colitis. *Am J Physiol Gastrointest Liver Physiol.* 2003; 284:G385–G391. [PubMed: 12431903]
31. Hunter MM, Wang A, Hirota CL, McKay DM. Neutralizing anti-IL-10 antibody blocks the protective effect of tapeworm infection in a murine model of chemically induced colitis. *J Immunol.* 2005; 174:7368–7375. [PubMed: 15905584]
32. Haben I, Hartmann W, Specht S, et al. T-cell-derived, but not B-cell-derived, IL-10 suppresses antigen-specific T-cell responses in *Litomosoides sigmodontis*-infected mice. *Eur J Immunol.* 2013; 43:1799–1805. [PubMed: 23529858]
33. Hartmann W, Haben I, Fleischer B, Breloer M. Pathogenic nematodes suppress humoral responses to third-party antigens in vivo by IL-10-mediated interference with Th cell function. *J Immunol.* 2011; 187:4088–4099. [PubMed: 21900178]
34. Ince MN, Elliott DE, Setiawan T, et al. Role of T cell TGF-beta signaling in intestinal cytokine responses and helminthic immune modulation. *Eur J Immunol.* 2009; 39:1870–1878. [PubMed: 19544487]
35. Round JL, Mazmanian SK. Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. *Proc Natl Acad Sci USA.* 2010; 107:12204–12209. [PubMed: 20566854]
36. Malhotra N, Kang J. SMAD regulatory networks construct a balanced immune system. *Immunology.* 2013; 139:1–10. [PubMed: 23347175]
37. Monteleone G, Kumberova A, Croft NM, McKenzie C, Steer HW, MacDonald TT. Blocking Smad7 restores TGF-beta1 signaling in chronic inflammatory bowel disease. *J Clin Invest.* 2001; 108:601–609. [PubMed: 11518734]
38. Fantini MC, Rizzo A, Fina D, et al. Smad7 controls resistance of colitogenic T cells to regulatory T cell-mediated suppression. *Gastroenterology.* 2009; 136:1308–1316. e1301–1303. [PubMed: 19192480]
39. Monteleone G, Neurath MF, Ardizzone S, et al. Mongersen, an oral SMAD7 antisense oligonucleotide, and Crohn's disease. *N Engl J Med.* 2015; 372:1104–1113. [PubMed: 25785968]
40. Kreider T, Anthony RM, Urban JF Jr, Gause WC. Alternatively activated macrophages in helminth infections. *Curr Opin Immunol.* 2007; 19:448–453. [PubMed: 17702561]
41. Reyes JL, Terrazas LI. The divergent roles of alternatively activated macrophages in helminthic infections. *Parasite Immunol.* 2007; 29:609–619. [PubMed: 18042168]
42. Smith P, Mangan NE, Walsh CM, et al. Infection with a helminth parasite prevents experimental colitis via a macrophage-mediated mechanism. *J Immunol.* 2007; 178:4557–4566. [PubMed: 17372014]
43. Schnoeller C, Rausch S, Pillai S, et al. A helminth immunomodulator reduces allergic and inflammatory responses by induction of IL-10-producing macrophages. *J Immunol.* 2008; 180:4265–4272. [PubMed: 18322239]
44. Klotz C, Ziegler T, Figueiredo AS, et al. A helminth immunomodulator exploits host signaling events to regulate cytokine production in macrophages. *PLoS Pathog.* 2011; 7:e1001248. [PubMed: 21253577]
45. Van Dyken SJ, Locksley RM. Interleukin-4-and interleukin-13-mediated alternatively activated macrophages: roles in homeostasis and disease. *Annu Rev Immunol.* 2013; 31:317–343. [PubMed: 23298208]
46. Ledesma-Soto Y, Callejas BE, Terrazas CA, et al. Extraintestinal helminth infection limits pathology and proinflammatory cytokine expression during DSS-induced ulcerative colitis: a role for alternatively activated macrophages and prostaglandins. *Biomed Res Int.* 2015; 2015:563425. [PubMed: 26090422]
47. Hunter MM, Wang A, Parhar KS, et al. In vitro-derived alternatively activated macrophages reduce colonic inflammation in mice. *Gastroenterology.* 2010; 138:1395–1405. [PubMed: 20044996]

48. Johnston MJ, Wang A, Catarino ME, et al. Extracts of the rat tapeworm, *Hymenolepis diminuta*, suppress macrophage activation in vitro and alleviate chemically induced colitis in mice. *Infect Immun*. 2010; 78:1364–1375. [PubMed: 20028812]
49. Leung G, Wang A, Fernando M, Phan VC, McKay DM. Bone marrow-derived alternatively activated macrophages reduce colitis without promoting fibrosis: participation of IL-10. *Am J Physiol Gastrointest Liver Physiol*. 2013; 304:G781–G792. [PubMed: 23494123]
50. Reyes JL, Wang A, Fernando MR, et al. Splenic B cells from *Hymenolepis diminuta*-infected mice ameliorate colitis independent of T cells and via cooperation with macrophages. *J Immunol*. 2015; 194:364–378. [PubMed: 25452561]
51. Elliott DE, Metwali A, Leung J, et al. Colonization with *Heligmosomoides polygyrus* suppresses mucosal IL-17 production. *J Immunol*. 2008; 181:2414–2419. [PubMed: 18684931]
52. Targan SR, Feagan BG, Vermeire S, et al. Mo2083 A randomized, double-blind, placebo-controlled study to evaluate the safety, tolerability, and efficacy of AMG 827 in subjects with moderate to severe Crohn's disease. *Gastroenterology*. 2012; 143:e26.
53. Geddes K, Rubino SJ, Magalhaes JG, et al. Identification of an innate T helper type 17 response to intestinal bacterial pathogens. *Nat Med*. 2011; 17:837–844. [PubMed: 21666695]
54. Chen CC, Louie S, McCormick B, Walker WA, Shi HN. Concurrent infection with an intestinal helminth parasite impairs host resistance to enteric *Citrobacter rodentium* and enhances *Citrobacter*-induced colitis in mice. *Infect Immun*. 2005; 73:5468–5481. [PubMed: 16113263]
55. Basu R, Hatton RD, Weaver CT. The Th17 family: flexibility follows function. *Immunol Rev*. 2013; 252:89–103. [PubMed: 23405897]
56. Mitsuyama K, Sasaki E, Toyonaga A, et al. Colonic mucosal interleukin-6 in inflammatory bowel disease. *Digestion*. 1991; 50:104–111. [PubMed: 1804734]
57. Iwasa T, Nakamura K, Ogino H, et al. Multiple ulcers in the small and large intestines occurred during tocilizumab therapy for rheumatoid arthritis. *Endoscopy*. 2011; 43:70–72. [PubMed: 21108178]
58. Atreya R, Billmeier U, Rath T, et al. First case report of exacerbated ulcerative colitis after anti-interleukin-6R salvage therapy. *World J Gastroenterol*. 2015; 21:12963–12969. [PubMed: 26668517]
59. Jin X, Zimmers TA, Zhang Z, Pierce RH, Koniaris LG. Interleukin-6 is an important in vivo inhibitor of intestinal epithelial cell death in mice. *Gut*. 2010; 59:186–196. [PubMed: 19074180]
60. Battle TE, Frank DA. The role of STATs in apoptosis. *Curr Mol Med*. 2002; 2:381–392. [PubMed: 12108949]
61. Reynolds LA, Finlay BB, Maizels RM. Cohabitation in the intestine: interactions among helminth parasites, bacterial microbiota, and host immunity. *J Immunol*. 2015; 195:4059–4066. [PubMed: 26477048]
62. Reynolds LA, Smith KA, Filbey KJ, et al. Commensal-pathogen interactions in the intestinal tract: lactobacilli promote infection with, and are promoted by, helminth parasites. *Gut Microbes*. 2014; 5:522–532. [PubMed: 25144609]
63. Walk ST, Blum AM, Ewing SA, Weinstock JV, Young VB. Alteration of the murine gut microbiota during infection with the parasitic helminth *Heligmosomoides polygyrus*. *Inflamm Bowel Dis*. 2010; 16:1841–1849. [PubMed: 20848461]
64. Holm JB, Sorobetea D, Kiilerich P, et al. Chronic *Trichuris muris* infection decreases diversity of the intestinal microbiota and concomitantly increases the abundance of Lactobacilli. *PLoS ONE*. 2015; 10:e0125495. [PubMed: 25942314]
65. Houlden A, Hayes KS, Bancroft AJ, et al. Chronic *Trichuris muris* infection in C57BL/6 mice causes significant changes in host microbiota and metabolome: effects reversed by pathogen clearance. *PLoS ONE*. 2015; 10:e0125945. [PubMed: 25938477]
66. Satish Kumar CS, Kondal Reddy K, Reddy AG, et al. Protective effect of *Lactobacillus plantarum* 21, a probiotic on trinitrobenzene sulfonic acid-induced ulcerative colitis in rats. *Int Immunopharmacol*. 2015; 25:504–510. [PubMed: 25727887]
67. van Baarlen P, Wells JM, Kleerebezem M. Regulation of intestinal homeostasis and immunity with probiotic lactobacilli. *Trends Immunol*. 2013; 34:208–215. [PubMed: 23485516]

68. Ramanan D, Bowcutt R, Lee SC, et al. Helminth infection promotes colonization resistance via type 2 immunity. *Science*. 2016; 352:608–612. [PubMed: 27080105]
69. Broadhurst MJ, Leung JM, Kashyap V, et al. IL-22+ CD4+ T cells are associated with therapeutic trichuris trichiura infection in an ulcerative colitis patient. *Sci Transl Med*. 2010; 2:60ra88.
70. Bastard JP, Maachi M, Lagathu C, et al. Recent advances in the relationship between obesity, inflammation, and insulin resistance. *Eur Cytokine Netw*. 2006; 17:4–12. [PubMed: 16613757]
71. McNelis JC, Olefsky JM. Macrophages, immunity, and metabolic disease. *Immunity*. 2014; 41:36–48. [PubMed: 25035952]
72. Caillon A, Schiffrin EL. Role of inflammation and immunity in hypertension: recent epidemiological, laboratory, and clinical evidence. *Curr Hypertens Rep*. 2016; 18:21. [PubMed: 26846785]
73. Tedgui A, Mallat Z. Cytokines in atherosclerosis: pathogenic and regulatory pathways. *Physiol Rev*. 2006; 86:515–581. [PubMed: 16601268]
74. Mottillo S, Filion KB, Genest J, et al. The metabolic syndrome and cardiovascular risk a systematic review and meta-analysis. *J Am Coll Cardiol*. 2010; 56:1113–1132. [PubMed: 20863953]
75. Aschner P. Metabolic syndrome as a risk factor for diabetes. *Expert Rev Cardiovasc Ther*. 2010; 8:407–412. [PubMed: 20222818]
76. Singh AK, Kari JA. Metabolic syndrome and chronic kidney disease. *Curr Opin Nephrol Hypertens*. 2013; 22:198–203. [PubMed: 23340215]
77. Wainwright P, Byrne CD. Bidirectional relationships and disconnects between NAFLD and features of the metabolic syndrome. *Int J Mol Sci*. 2016; 17:367. [PubMed: 26978356]
78. Aravindhan V, Mohan V, Surendar J, et al. Decreased prevalence of lymphatic filariasis among diabetic subjects associated with a diminished pro-inflammatory cytokine response (CURES 83). *PLoS Negl Trop Dis*. 2010; 4:e707. [PubMed: 20559443]
79. Hays R, Esterman A, Giacomini P, Loukas A, McDermott R. Does *Strongyloides stercoralis* infection protect against type 2 diabetes in humans? Evidence from Australian aboriginal adults. *Diabetes Res Clin Pract*. 2015; 107:355–361. [PubMed: 25656764]
80. Wiria AE, Wammes LJ, Hamid F, et al. Relationship between carotid intima media thickness and helminth infections on Flores Island, Indonesia. *PLoS ONE*. 2013; 8:e54855. [PubMed: 23365679]
81. Magen E, Bychkov V, Ginovker A, Kashuba E. Chronic *Opisthorchis felinus* infection attenuates atherosclerosis—an autopsy study. *Int J Parasitol*. 2013; 43:819–824. [PubMed: 23792298]
82. Husaarts L, Garcia-Tardon N, van Beek L, et al. Chronic helminth infection and helminth-derived egg antigens promote adipose tissue M2 macrophages and improve insulin sensitivity in obese mice. *FASEB J*. 2015; 29:3027–3039. [PubMed: 25852044]
83. Yang Z, Grinchuk V, Smith A, et al. Parasitic nematode-induced modulation of body weight and associated metabolic dysfunction in mouse models of obesity. *Infect Immun*. 2013; 81:1905–1914. [PubMed: 23509143]
84. Wu D, Molofsky AB, Liang HE, et al. Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. *Science*. 2011; 332:243–247. [PubMed: 21436399]
85. Hams E, Bermingham R, Wurlod FA, et al. The helminth T2 RNase omega1 promotes metabolic homeostasis in an IL-33-and group 2 innate lymphoid cell-dependent mechanism. *FASEB J*. 2016; 30:824–835. [PubMed: 26490658]
86. Bhargava P, Li C, Stanya KJ, et al. Immunomodulatory glycan LNFPIII alleviates hepatosteatosis and insulin resistance through direct and indirect control of metabolic pathways. *Nat Med*. 2012; 18:1665–1672. [PubMed: 23104131]
87. Doenhoff MJ, Stanley RG, Griffiths K, Jackson CL. An anti-atherogenic effect of *Schistosoma mansoni* infections in mice associated with a parasite-induced lowering of blood total cholesterol. *Parasitology*. 2002; 125:415–421. [PubMed: 12458825]
88. Wolfs IM, Stoger JL, Goossens P, et al. Reprogramming macrophages to an anti-inflammatory phenotype by helminth antigens reduces murine atherosclerosis. *FASEB J*. 2014; 28:288–299. [PubMed: 24043262]

89. Aprahamian TR, Zhong X, Amir S, et al. The immunomodulatory parasitic worm product ES-62 reduces lupus-associated accelerated atherosclerosis in a mouse model. *Int J Parasitol.* 2015; 45:203–207. [PubMed: 25666929]
90. Elliott DE, Summers RW, Weinstock JV. Helminths and the modulation of mucosal inflammation. *Curr Opin Gastroenterol.* 2005; 21:51–58. [PubMed: 15687885]
91. Sandborn WJ, Elliott DE, Weinstock J, et al. Randomised clinical trial: the safety and tolerability of *Trichuris suis* ova in patients with Crohn's disease. *Aliment Pharmacol Ther.* 2013; 38:255–263. [PubMed: 23730956]
92. Summers RW, Elliott DE, Qadir K, Urban JF Jr, Thompson R, Weinstock JV. *Trichuris suis* seems to be safe and possibly effective in the treatment of inflammatory bowel disease. *Am J Gastroenterol.* 2003; 98:2034–2041. [PubMed: 14499784]
93. Summers RW, Elliott DE, Urban JF Jr, Thompson R, Weinstock JV. *Trichuris suis* therapy in Crohn's disease. *Gut.* 2005; 54:87–90. [PubMed: 15591509]
94. Summers RW, Elliott DE, Urban JF Jr, Thompson RA, Weinstock JV. *Trichuris suis* therapy for active ulcerative colitis: a randomized controlled trial. *Gastroenterology.* 2005; 128:825–832. [PubMed: 15825065]
95. Croese J, O'Neil J, Masson J, et al. A proof of concept study establishing *Necator americanus* in Crohn's patients and reservoir donors. *Gut.* 2006; 55:136–137. [PubMed: 16344586]
96. Croese J, Gaze ST, Loukas A. Changed gluten immunity in celiac disease by *Necator americanus* provides new insights into autoimmunity. *Int J Parasitol.* 2013; 43:275–282. [PubMed: 23291460]
97. Daveson AJ, Jones DM, Gaze S, et al. Effect of hookworm infection on wheat challenge in celiac disease—a randomised double-blinded placebo controlled trial. *PLoS ONE.* 2011; 6:e17366. [PubMed: 21408161]
98. Catassi C, Fabiani E, Iacono G, et al. A prospective, double-blind, placebo-controlled trial to establish a safe gluten threshold for patients with celiac disease. *Am J Clin Nutr.* 2007; 85:160–166. [PubMed: 17209192]
99. Croese J, Giacomini P, Navarro S, et al. Experimental hookworm infection and gluten microchallenge promote tolerance in celiac disease. *J Allergy Clin Immunol.* 2015; 135:508–516. [PubMed: 25248819]
100. Giacomini P, Zakrzewski M, Croese J, et al. Experimental hookworm infection and escalating gluten challenges are associated with increased microbial richness in celiac subjects. *Sci Rep.* 2015; 5:13797. [PubMed: 26381211]
101. Bager P, Arved J, Ronborg S, et al. *Trichuris suis* ova therapy for allergic rhinitis: a randomized, double-blind, placebo-controlled clinical trial. *J Allergy Clin Immunol.* 2010; 125(123–130):e121–e123.
102. Blount D, Hooi D, Feary J, et al. Immunologic profiles of persons recruited for a randomized, placebo-controlled clinical trial of hookworm infection. *Am J Trop Med Hyg.* 2009; 81:911–916. [PubMed: 19861631]
103. Bager P, Kapel C, Roepstorff A, et al. Symptoms after ingestion of pig whipworm *Trichuris suis* eggs in a randomized placebo-controlled double-blind clinical trial. *PLoS ONE.* 2011; 6:e22346. [PubMed: 21829616]
104. Jouvin MH, Kinet JP. *Trichuris suis* ova: testing a helminth-based therapy as an extension of the hygiene hypothesis. *J Allergy Clin Immunol.* 2012; 130:3–10. quiz 11–12. [PubMed: 22742834]
105. Feary JR, Venn AJ, Mortimer K, et al. Experimental hookworm infection: a randomized placebo-controlled trial in asthma. *Clin Exp Allergy.* 2010; 40:299–306. [PubMed: 20030661]
106. Correale J, Farez M. Association between parasite infection and immune responses in multiple sclerosis. *Ann Neurol.* 2007; 61:97–108. [PubMed: 17230481]
107. Correale J, Farez MF. The impact of parasite infections on the course of multiple sclerosis. *J Neuroimmunol.* 2011; 233:6–11. [PubMed: 21277637]
108. Benzel F, Erdur H, Kohler S, et al. Immune monitoring of *Trichuris suis* egg therapy in multiple sclerosis patients. *J Helminthol.* 2012; 86:339–347. [PubMed: 21838960]
109. Fleming JO, Isaak A, Lee JE, et al. Probiotic helminth administration in relapsing-remitting multiple sclerosis: a phase 1 study. *Mult Scler.* 2011; 17:743–754. [PubMed: 21372112]

110. Fleming JO, Weinstock JV. Clinical trials of helminth therapy in autoimmune diseases: rationale and findings. *Parasite Immunol.* 2015; 37:277–292. [PubMed: 25600983]
111. Rosche B, Wernecke KD, Ohlraun S, Dorr JM, Paul F. *Trichuris suis* ova in relapsing-remitting multiple sclerosis and clinically isolated syndrome (TRIOMS): study protocol for a randomized controlled trial. *Trials.* 2013; 14:112. [PubMed: 23782752]
112. Voldsgaard A, Bager P, Garde E, et al. *Trichuris suis* ova therapy in relapsing multiple sclerosis is safe but without signals of beneficial effect. *Mult Scler.* 2015; 21:1723–1729. [PubMed: 25698173]
113. Becker KG. Autism, asthma, inflammation, and the hygiene hypothesis. *Med Hypotheses.* 2007; 69:731–740. [PubMed: 17412520]
114. Siniscalco D, Antonucci N. Possible use of *Trichuris suis* ova in autism spectrum disorders therapy. *Med Hypotheses.* 2013; 81:1–4. [PubMed: 23597946]
115. Umscheid CA, Margolis DJ, Grossman CE. Key concepts of clinical trials: a narrative review. *Postgrad Med.* 2011; 123:194–204. [PubMed: 21904102]
116. Ashwell M, Gunn P, Gibson S. Waist-to-height ratio is a better screening tool than waist circumference and BMI for adult cardiometabolic risk factors: systematic review and meta-analysis. *Obes Rev.* 2012; 13:275–286. [PubMed: 22106927]

TABLE 1

Clinical trials testing effects of helminth exposure on immune-mediated disease

Disease	Study/ID	Agent/dose	Description
Inflammatory bowel disease (Crohn's and ulcerative colitis)	Open-label pilot, University of Iowa	<i>Trichuris suis</i> , 2500 embryonated ova single oral dose (repeated dose in two patients)	Open-label study of seven patients to determine safety of intestinal helminth exposure in patients with active intestinal inflammation. Most patients experienced clinical improvement. No adverse effects
Crohn's disease	Open-label trial, University of Iowa, USA	<i>T. suis</i> , 2500 embryonated ova orally every 3 wks for 12 wks	29 patients, most with longstanding disease. 79% of patients had significant clinical improvement. No adverse effects
Ulcerative colitis	Double-blind placebo-controlled trial, University of Iowa, USA	<i>T. suis</i> , 2500 ova orally every 3 wks for 12 wks	54 patients, most with longstanding disease. Significant improvement in 43% of helminth-exposed patients and 17% of patients given placebo. Statistically significant difference. No adverse effects
Crohn's disease	Open-label trial, James Cook University, AUS	25–100 <i>Necator americanus</i> L3 by dermal exposure	9 patients (5 reinoculated between weeks 27–30). Clinical improvement occurred in the majority with active disease but did not reach statistical significance. Mild adverse effects including itching at site of inoculation and painful transient enteropathy occurred in reservoir donors
Crohn's disease	Dose escalation study NCT01434693	<i>T. suis</i> single dose of 500, 2500, 7500 ova orally vs placebo	36 patients. Single dose well tolerated. Adverse effects no different than placebo
Crohn's disease	Trust 1: Double-blind placebo-controlled study NCT01576471	<i>T. suis</i> 7500 ova orally every 2 wks for 12 wks	250 patients with early Crohn's disease. Study failed due to high placebo response and remission rates (~50%)
Crohn's disease	Trust 2: Double-blind placebo-controlled study NCT01279577	<i>T. suis</i> at 3 dose levels 250, 2500, 7500 ova orally every 2 wks for 12 wks	212 patients with early Crohn's disease. Improvement in patients with most severe disease but did not reach statistical significance due to high placebo response/remission rates (~50%). Only minor adverse effects
Ulcerative colitis	Double-blind placebo-controlled crossover study NCT01433471	<i>T. suis</i> 2500 ova orally every 2 wks for 12 wks	18 patients (immunologic analysis also planned) enrolling
Ulcerative colitis	Double-blind placebo-controlled study NCT0195354	<i>T. suis</i> 7500 ova orally every 2 wks for 12 wks NCT0195354	120 patients with extensive immunologic analysis. Study halted due to slow recruitment from need for extensive testing
Celiac disease	Double-blind placebo-controlled study NCT00671138	<i>N. americanus</i> 10 L3 by dermally at week 0 with additional 5 L3 at week 12. Five day 16 g gluten challenge at week 20	20 patients with documented celiac disease. Exposed patients had alterations in innate and adaptive immune responses but no protection from aggressive gluten challenge
Celiac disease	Open label pilot testing gluten desensitization in patients with celiac disease NCT01661933	<i>N. americanus</i> 10 L3 by dermally at week 0 with additional 10 L3 at week 10. Daily 10 mg gluten exposure from week 8 to week 16 followed by daily 50 mg gluten exposure to week 24	12 patients with documented celiac disease. The combination of hookworm exposure and gluten microchallenge promoted mucosal tolerance
Allergic rhinitis	Double-blind placebo-controlled study EUCTR2007 006099-12	<i>T. suis</i> 2500 ova orally vs placebo every 3 wks for 24 wks	100 patients mild diarrhea but no significant adverse effects. Patients in active limb used less medications but otherwise no significant difference between study groups ^{88,103}
Allergic rhinitis	Double-blind placebo-controlled study NCT00232518	<i>N. americanus</i> 10 L3 by dermal exposure	30 patients no significant adverse effects. Exposure induced eosinophilia without potentiation of anti-allergen IgE. Efficacy not evaluated

Disease	Study/ID	Agent/dose	Description
Peanut or Tree Nut Allergy	Open label safety study NCT01070498	<i>T. suis</i> 100 to 2500 (depending on age of patient) ova orally every 2 wks for 12 wks	18 patients with peanut or tree nut food allergy. No significant adverse effects. No change in skin prick sensitivity except one patient lost reactivity to peanut allergen
Asthma	Blinded, randomized placebo-controlled trial NCT00469989	<i>N. americanus</i> 10 L3 by dermal exposure. Airway reactivity tested at 16 wks	32 patients. No significant adverse effects. No improvement in asthma control but trend for improvement in measures of airway reactivity
Plaque psoriasis	Open label safety study NCT01836939	<i>T. suis</i> 2500 or 7500 ova orally every 2 wks for 10 wks	8 patients. Study suspended due to loss of availability of study agent
Plaque psoriasis	Open label pilot study NCT01948271	<i>T. suis</i> 7500 ova orally every 2 wks for 16 wks	16 patients with moderate to severe plaque psoriasis. Study terminated due to lack of efficacy
Plaque psoriasis	Double-blind placebo-controlled study NCT02011269	<i>T. suis</i> 7500 or 15 000 ova orally every 2 wks for 10 wks	25 patients. Study suspended due to loss of availability of study agent
Rheumatoid arthritis	EUCTR2011-006344-71-DE	<i>T. suis</i> 2500 ova orally every 2 wks for 24 wks	50 patients. Study status not available
Multiple sclerosis	Open label safety study (HINT 1) NCT00645749	<i>T. suis</i> 2500 ova orally every 2 wks for 12 wks	5 patients with relapsing remitting multiple sclerosis. No significant adverse effects. Favorable MRI and immunologic effects suggesting potential efficacy
Multiple sclerosis	Open label efficacy study (HINT 2) NCT00645749	<i>T. suis</i> 2500 ova orally every 2 wks for 40 wks	15 patients. No significant adverse effects. Interim analysis showed a modest reduction in active brain lesions vs observation period
Multiple sclerosis	Open label safety study EUCT2009-015319-41	<i>T. suis</i> 2500 ova orally every 2 wks for 24 wks	4 patients with secondary progressive multiple sclerosis. No significant adverse effects. Systemic evidence for slight immunomodulatory impact
Multiple sclerosis	Open label safety study NCT01006941	<i>T. suis</i> 2500 ova orally every 2 wks for 12 wks	10 patients with relapsing remitting multiple sclerosis. No significant adverse effects. No clinical, MRI or immunologic evidence for efficacy
Multiple sclerosis	Placebo-controlled trial (TRIOMS), EUCTR2009-015319-41-DE	<i>T. suis</i> 2500 ova orally every 2 wks for 48 wks	50 patients with relapsing remitting multiple sclerosis. Study results not available
Multiple sclerosis	Placebo-controlled trial (WIRMS), NCT01470521 EUCT2008-005008ZY-GB	<i>N. americanus</i> 25 L3 by dermal exposure. Efficacy evaluated at 9 mos after exposure	72 patients. Study is completed but results not yet available
Autism	Double-blind placebo-controlled cross-over study NCT01040221	<i>T. suis</i> 2500 ova or placebo orally every 2 wks for 12 wks, 4 week washout, then crossover	10 patients. No significant adverse effects. Trend for treatment limb to be more beneficial than placebo but did not reach statistical significance
Autism	Double-blind placebo-controlled cross-over study NCT02140112	<i>T. suis</i> 2500 ova or placebo orally every 2 wks for 4 wks followed by 7500 ova every 2 wks for 12 wks, 4 week washout, then crossover	20 patients. Study terminated prior to enrollment.
Autism	Double-blind placebo-controlled study NCT01734941	<i>T. suis</i> 2500 or 7500 ova or placebo orally every 2 wks for 16 wks	60 patients. Study terminated due to lack of available study agent

Please see text for relevant references.