Review article: helminths as therapeutic agents for inflammatory bowel disease

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SUMMARY
Over the last decade major advances have been made in our understanding of the mechanisms and mediators of inflammation that hold the promise of the development of new therapies for inflammatory disease. While much is to be gleaned from the application of new technologies, assessment of the age-old host–parasite relationship may also provide insights on how to counter pathological inflammatory events. In the case of inflammatory bowel disease [particularly Crohn’s disease, which is associated with T helper 1 (Th1) events] it is proposed that infection with parasitic helminths would be beneficial: the paradigm being that of immune deviation, where Th2 cytokines mobilized in response to the helminth will prevent or antagonize the disease-promoting Th1 events in the gut. The situation is unlikely to be this simple. Here we review and critique the data in support of helminth therapy for inflammatory bowel disease, drawing attention to the gaps in knowledge and presenting a view on how the field may be advanced. While the concept of helminth therapy may be superficially unappealing, this review may convince the reader of the value of more extensive analyses of the impact of helminth infection on enteric inflammation.

INTRODUCTION
Any discussion of human inflammatory bowel disease hinges mainly on three issues: the disorders are idiopathic, there is as yet no cure for either Crohn’s disease or ulcerative colitis (with the possible exception of surgery in the latter case), and current treatment relies heavily on corticosteroids and broad-spectrum immunosuppressives that have significant side-effects. The situation is unsatisfactory for both the patient and the attending physician. However, significant progress is being made on all fronts, such as the recent identification of a mutation in the NOD2 gene, which encodes an intracellular receptor for bacterial products, as a susceptibility factor in a cohort of patients with Crohn’s disease.1–3

Studies to determine the aetiology of, and a cure for, inflammatory bowel disease are complemented by extensive research efforts aimed at providing a comprehensive understanding of the mechanisms of mucosal inflammation that will facilitate the development of more efficient therapies.1 Thus, putative new treatments for inflammatory bowel disease are emerging that focus on biologicals such as anticytokine antibodies (notable success having been achieved in treating Crohn’s disease, particularly fistulizing disease, with anti-TNFα antibodies4), recombinant cytokines [e.g. interleukin (IL)-10 and IL-11], granulocyte-macrophage colony stimulating factor (GM-CSF),5–7 neutriceuticals (e.g. green-tea derived polyphenols8), and probiotics.9 The latter approach, which attempts to ‘rebalance’ the hosts’ normal commensal flora by the deliberate
introduction of beneficial micro-organisms, has been embraced with some enthusiasm based on a few intriguing clinical observations and successes in animal models of colitis. An extension of this approach is the concept that organisms other than bacteria can be used to elicit defined immune responses in the host that would antagonize or inhibit the mechanisms of the immunopathology observed in inflammatory bowel disease. Here we review the limited studies to date on the potential value of using parasitic helminths [i.e. nematodes (or roundworms) and platyhelminths (trematodes or flukes and cestodes or tapeworms)] to ameliorate colitis (Table 1).

A SIMPLE PARADIGM OF IMMUNE DEVIATION

The core of parasitology is understanding a hetero-specific relationship (Table 2), in which the parasite seeks nutrients and shelter from the host at some detriment to the host species. Both species have coevolved in an arms race in which the host attempts to recognize and destroy/eliminate the intruder, while the parasite evolves to better counter or hide from the immune response mounted by the host. Thus, immunomodulation of the host immune response is a goal of the successful parasite. Moreover, the notion of ‘harmonious parasitism’, in which an individual with a specific parasitic infection is protected from other disease conditions is not new. For instance, the iron-deficiency anaemia that accompanies intestinal hookworm infection may confer some resistance to bacterial infections, and *Heligmosomoides polygyrus* infection can protect against *Helicobacter*-induced gastritis in an animal model.

Pioneering research by Mossman and colleagues in the mid-1980s defined the T helper 1 (Th1)–T helper 2 (Th2) paradigm, by showing that murine CD4+ T helper cell clones could be classified on the basis of their cytokine profile: stimulated Th1 cells produced predominately IL-2, IFNγ and TNFα, whereas activated Th2 cells produced IL-4, IL-5, IL-10 and IL-13. While the universe of T cell subtypes has expanded, and many immune responses may span the Th1–Th2 spectrum, the paradigm has provided a useful framework for investigating disease. Importantly, Th1 and Th2 cytokines reciprocally down-regulate the other T helper cell types. Thus, it follows that promoting Th2 events (i.e. increasing IL-4 and IL-10 levels) would be expected to inhibit diseases that are mediated by a Th1-type response. Analyses of murine responses to helminth infection has produced universal agreement that the immune reactions are characterized by elevated Th2 cytokines and appropriately so since they drive humoral immune events aimed at combating extracellular organisms (Figure 1). The human response to infection with parasitic helminths has a phenotype typical of Th2-dominated events (e.g. eosinophilia, increased IgE), although serum levels of cytokines and the response of immune cells stimulated in vitro reveals a less convincing skewing towards Th2-type cytokine production; there certainly is increased IL-10 production that would act to down-regulate Th1 responses and, as we note below, would be expected to contribute to a generalized immuno-regulatory/immunosuppressive state.

Theoretically, individuals with a helminth infection, and consequently increased levels of Th2 cytokines, should be protected from, or less vulnerable to,

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Table 1. Examples of helminth species that can parasitize humans

<table>
<thead>
<tr>
<th>Nematode (roundworms)</th>
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<tbody>
<tr>
<td>Hook worms: <em>Necator americanus, Ancylostoma duodenale</em></td>
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<tr>
<td>Pinworm: <em>Enterobius vermicularis</em></td>
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<tr>
<td>Filarial worms: <em>Wucharia bancrofti, Brugia malayi, Onchocerca volvulus</em></td>
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<tr>
<td>Guinea worm: <em>Dracunculus medinensis</em></td>
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<tr>
<td>Others: <em>Ascaris lumbricoides, Trichuris trichuria, Trichinella spiralis, Strongyloides stercoralis</em></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Platyhelminth (flatworms)</th>
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<tr>
<td>Trematode (flukes)</td>
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<tr>
<td><em>Schistosoma mansoni, Schistosoma japonicum, Schistosoma haematobium, Paragonimus sp., Opisthorchis sinensis, Echinostoma sp., Fasciola hepatica, Dicrocoelium dendriticum, Heterophyes sp.</em></td>
<td></td>
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<tr>
<td>Cestode (tapeworms)</td>
<td></td>
</tr>
<tr>
<td><em>Taenia saginata, Taenia solium, Diphyllobothrium sp.</em></td>
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Th1-related disorders: although some variability has been reported, many consider Crohn’s disease a Th1-related disorder. While modulation of the Th1–Th2 balance may be the basis of the therapeutic benefit of helminths, it is also likely that the mechanism(s) of action extend(s) beyond simple promotion of a Th2 environment and skewing away from Th1 events. In this context, ulcerative colitis may in part be an autoimmune condition, which raises the question as to whether or not helminth infection could confer any benefit in treating this inflammatory bowel disease.

EPIDEMIOLOGICAL EVIDENCE IN SUPPORT OF HELMINTH THERAPY

If helminth infection exerts an anticolitic effect, then one would expect to find a negative correlation between the geographical distribution of pandemic helminth infections and the incidence of inflammatory bowel disease: there are epidemiological data in support of this postulate. Generally, the prevalence of both Crohn’s disease and ulcerative colitis is highest (and continues to rise) in industrialized nations, while the lowest
reported incidences are in less developed countries such as Africa, South America and parts of Asia. The map of human helminthiasis is virtually the mirror image of this, showing minimal occurrence in industrialized countries and widespread occurrence throughout Africa, Asia and South America, where infection with multiple helminth species is not uncommon. Since inflammatory bowel disease is idiopathic one could conjecture that the lower incidence in, for example, Africa is due to a genetic resistance gene or, conversely, lack of a susceptibility gene. However, this is not consistent with data showing that the prevalence of inflammatory bowel disease among the African-American and Caucasian populations in the United States is similar.

While the divergence in the distribution patterns of the incidence of inflammatory bowel disease and helminth infection is striking, caution must be exercised in attributing a cause and effect relationship between these two variables, as several factors can influence the interpretation of epidemiological data. Variations in methodology (e.g. inclusion criteria, sampling, power calculations), population diversity, under-reporting or misdiagnosis of inflammatory bowel disease, life-expectancy, nutritional status and lifestyle of the patients surveyed can all introduce bias to a study. We should also be mindful that helminth infection could be merely an element of the recently emergent hygiene hypothesis. Simply put, the immune system has evolved, by in large, to cope with microbes and pathogens. As sanitation has improved we find ourselves in environments very different from that which provided the selective pressure for the development of our immune system, and as a result of this has been an increase in autoimmune-type disorders. Thus, anecdotal evidence that can be cited in support of helminths exerting an anticolitic effect could also be tendered in support of the hygiene hypothesis: as examples, (1) the incidence of inflammatory bowel disease is on the rise in nations moving to more urbanized lifestyles; (2) Crohn’s disease is more common in subjects whose infancies were spent in houses with hot water (suggesting increased sanitary conditions in their surroundings); and (3) there is a lower incidence of Crohn’s disease in American soldiers who served in the field in Vietnam, which could represent a period of poor sanitation. This highlights the need for careful interpretation of epidemiological and socio-economic data relating to helminth infection being protective against colitis.

So while the epidemiological evidence does indicate that reduced exposure to helminthic infection may be a risk factor for developing inflammatory bowel disease, evidence in the form of controlled experiments is needed to substantiate this hypothesis.

EVIDENCE FROM ANIMAL STUDIES IN SUPPORT OF HELMINTH THERAPY

A variety of excellent animal models of colitis have been developed that display many of the features and characteristics of human inflammatory bowel disease. While none of the models fully recapitulates the human disorder, they have nevertheless been immensely useful in advancing awareness of inflammatory mechanisms in the gut. To our knowledge, the first full publication on helminth modulation of a murine colitis came from our laboratory, in which we showed that aspects of dextran sodium sulfate (DSS)-induced colitis were ameliorated in animals infected with the tapeworm Hymenolepis diminuta (Figure 2). DSS-induced colitis is associated with wasting, histopathology and physiological disturbances such as altered epithelial ion transport (the driving force for directed water movements). DSS-induced colonic histopathology was unaffected by H. diminuta infection. However, the diminished electrogeneric chloride secretory responses evoked by nerve stimulation, the cholinomimetic, carbachol or direct adenylate cyclase activation by forskolin that accompany DSS-colitis, were all significantly inhibited in mice infected with H. diminuta, given either as a prophylactic or a treatment strategy.

The reason for this divergence between gut form and function is not clear, though it has been noted that patients with inflammatory bowel disease can be symptom-free despite endoscopic evidence of inflammation (S.M. Collins, personal communication). Also, given the patchy nature of DSS-colitis, it is possible that the uninvolved tissue in H. diminuta-infected mice responds more like normal tissue because of differences in the colonic milieu compared to tissue from DSS-only treated animals. Moreover, this was not a simple matter of altered cytokine levels, since there were no differences in the levels of IL-10, IL-12 or IFNγ when DSS and DSS + H. diminuta colonic tissues and sera were compared on the day of autopsy. DSS-induced colitis is somewhat reminiscent of human ulcerative colitis, suggesting that the benefits of helminth therapy may stretch beyond Th1–Th2 modulation, and might reflect
We presented these data from the DSS model as proof-of-concept evidence in support of tapeworm infection exerting an anticolitic effect. Co-treatment with *H. diminuta* significantly reduced colitis-induced bleeding (B) but was not associated with an improvement in either histological damage score (arbitrary units) or colonic myeloperoxidase activity (units/mg wet wt.), which was actually increased in the *H. d. + DSS*-treated mice (C) ( insets ci and cii are representative H&E stained sections of colon from DSS and *H. d. + DSS* mice, respectively; M, muscle; E, epithelium; L, gut lumen; U, ulcer; original magnification $\times 400$). In contrast, *H. diminuta* infection resulted in functional improvement in the colon as determined by the change in short-circuit current ($\Delta$Isc, i.e. net active ion transport) evoked by the cholinergic agonist, carbachol (CCh, $10^{-4}$ M) or forskolin (FSK, $10^{-5}$ M), which mobilizes cAMP and elicits chloride secretion (D) ($n = 8–9$ mice from three separate experiments; mean $\pm$ S.E.M.; * and $\dagger$, $P < 0.05$ compared to control and DSS, respectively; data adapted from ref. 36).

Collectively these studies show that nematode, cestode and trematode (i.e. *S. mansoni* eggs) helminth parasites can effectively reduce colitis in four different murine model systems. It is important to note that in these models the mouse is a nonpermissive host and spontaneously expels the worm burden (the exception being *S. mansoni* egg antigen, which does elicit a vigorous immune response); a point we will return to later.
EVIDENCE FROM HUMAN STUDIES IN SUPPORT OF HELMINTH THERAPY

The most provocative data in support of pursuing helminth infection as a therapeutic option for human inflammatory bowel disease have been provided by Weinstock and colleagues. In their initial investigation three patients with active, steroid-refractory Crohn’s disease were given an oral dose of 2500 viable eggs of the pig whipworm, *Trichuris suis*. The patients showed no adverse reactions to the helminth treatment, and remarkably all three experienced substantial improvement in their disease as quantified by the Crohn’s disease activity score (CDAI) and the inflammatory bowel disease quality-of-life questionnaire. Follow-up studies currently being conducted by the same team appear to be repeating these findings, although it must be emphasized that the data are preliminary, involve a very small number of patients, and will require verification in a controlled, large-scale randomized clinical trial.

Equally intriguing is the preliminary finding that patients with ulcerative colitis, which has an immune profile that can be dominated by a Th2 cytokine spectrum, also responded positively to oral administration of *T. suis* ova (an observation that lends credence to our postulate based on the DSS–*H. diminuta* murine model). Validation of these data and the assumption that *T. suis* ova evoke a host IL-10 response suggest that the concept of helminth therapy may have to be readdressed to extend beyond the modulation of the Th1–Th2 balance.

INSIGHTS ON THE MECHANISM OF ACTION OF HELMINTH THERAPY

Recognizing that the interaction between host and parasite is immensely more complex than a simple antigen-driven response, how might a parasitic helminth infection protect against colitis?

Changes in the gut lumen

Increases in enteric goblet and mast cell numbers are hallmarks of intestinal helminth infection. The goblet cell response results in increased mucus production, and activated mast cells release a variety of mediators that lead to increased vascular and epithelial permeability (i.e. the leak hypothesis), easing the movement of phagocytic cells, antibodies and complement into the gut lumen. In addition, mast cell mediators will evoke active epithelial ion transport and consequently water efflux into the lumen. The appearance of increased water, immune factors and mucus in the gut lumen would be expected to result in reduced contact with any lumen-derived pro-colitic agent.

In this context, helminth infection also results in altered muscle function, and any increase in rostral–caudal peristalsis would again limit contact time between the luminal contents and the epithelium.

A wealth of data have been presented in favour of active participation of a component of the gut flora in the aetiology of inflammatory bowel disease. A few studies have shown that helminth infection can affect the composition of the gut flora, and in theory this could be part of the anticolitic effect of helminths. Other reports have noted that helminth infection can enhance gut disease associated with bacterial infection, and may promote septicemia. These contrasting studies highlight the need to consider helminth therapy in the context of the pathophysiology of the patients’ disease and any known or recent infections (Figure 3).

Modulation of the immune response

Much of the experimental data in support of an anticolitic effect of helminths has been provided by studies of nonpermissive systems (i.e. the worm is spontaneously expelled from the host) which, intriguingly, used small intestinal parasites but noted a benefit in the large intestine. These studies indicate involvement of the host immune system, and circulating factors or cells that move from the small intestine or associated lymph nodes into the large intestine. A logical explanation (and the starting premise of these studies) was that the parasitic infection elicited a Th2 response, thus preventing or overcoming the Th1 events responsible for the pathology. For example, *S. mansoni* was found to reduce the secretion of IFN-γ induced by exposure to tetanus toxoid. Furthermore, a beneficial Th2 response may extend beyond inhibition of Th1-driven events. Th2 cytokines promote the development of goblet and mast cells, which would alter the gut environment (see above), and these cytokines are responsible, at least in part, for antibody isotype switching to IgE and IgA production: antibodies important in mast cell activation and in mucosal immunity, respectively. However, a helminth-driven IL-5 response would generate an anti-helminthic eosinophilia and,
contrary to the proposed protective role of helminth infection, eosinophils have been implicated in the pathogenesis of inflammatory bowel disease. Many changes in the human gut evoked by helminth infection could either enhance or dampen the severity of colitis. The examples given illustrate this point, as a comprehensive review of this area would be quite speculative and is beyond the scope and focus of this article.

We suggest that the benefits of helminth therapy may not be completely explained by the reciprocal nature of Th1–Th2 cross-inhibitory responses. Concomitant with helminth infection may be the generation of an anti-inflammatory or immunoregulatory environment characterized by the presence of IL-10, TGFβ and a variety of regulatory T cell populations (Figure 3). Indeed, the suggestion that helminth infection can protect against non-Th1 cytokine-dominated disease is compatible with the generation of a generalized immunosuppressed or regulated anti-inflammatory state (see below).

Modulation of the neuroendocrine response

The fact that helminths that inhabit the small intestine can modulate colonic disease allows for the possibility of neuroendocrine involvement. Several changes in the enteric nervous system circuitry, neurotransmitter content and the number of enteroendocrine cells are associated with helminth infection. Similarly there are abundant data describing neurone–immune cell juxta-position, an association that is increased in the intestine of helminth-infected rodents and ample evidence of neuroimmune bidirectional communication. One could hypothesize that changes in, for example, the synthesis of vasoactive intestinal peptide (VIP) that accompany some helminth infections could impact on colitis. Neuroendocrine mediators have been assessed as candidates for the induction and amelioration of colonic inflammation; however, involvement of the neuroendocrine system in mediating the beneficial effect helminth infection has not been examined, a gap in the field that needs to be addressed.

Helminth-derived immunosuppressive factors

There is no doubt that parasites influence their environments, and one would intuitively accept that the successful parasite would suppress the host immune response; there is evidence of this. Moreover, recent studies have shown that a carbohydrate-rich fraction of the tapeworm *Echinococcus granulosus* and an *S. mansoni*-derived lysophosphatidylserine induces host synthesis of IL-10, supporting the promotion of a Th2 or immunoregulatory environment (Figure 3). *Nippostronglus brasiliensis* produces a VIP-like molecule, indicating that helminths have the potential to manipulate neuroimmune events in the host. If helminth-derived immunosuppressive/immunomodulatory factors are a significant component of the anti-colitic effect, then the identification and characterization of these molecules has the potential to produce...
new therapeutics that could negate the need for helminth infection.\textsuperscript{61}

From an analysis of the available data it is clear that infection with helminth parasites has the ability to ameliorate a Th1-dominated colitis. However, it is equally apparent that how this benefit of helminth infection is achieved is not known: an unfortunate situation in the authors’ opinion. We have tendered a number of possible mechanisms of helminth modulation of colitis, all of which are feasible but many of which are largely speculative and need to be assessed.

‘First do no harm’

Parasitism \textit{per se} is a malevolent condition, and in presenting the case in favour of helminths it would be remiss not to draw attention to the potential hazards of their use.

Therapeutic use of helminths and the promotion of a Th2 environment raise the spectre of creating a predisposition toward allergic/atopic disease. However, there is little evidence to support this, and, paradoxically, epidemiological and laboratory-based research indicates that helminth infection may actually protect against allergic-asthmatic conditions,\textsuperscript{52} autoimmune disorders (e.g. multiple sclerosis (as mimicked by experimental allergic encephalomyelitis in mice)),\textsuperscript{72} diabetes mellitus,\textsuperscript{73} and perhaps even transplant rejection.\textsuperscript{74} These observations are in fact consistent with helminth induction of an anti-inflammatory environment as discussed above. However, we remind the reader that there is evidence in support of helminth infection aggravating disease conditions.\textsuperscript{53–55, 75}

Within the parasitological community the kinetics of the parasite–host relationship are recognized as key aspects of this association, and similar questions must be asked within the framework of helminth therapy for inflammatory bowel disease. Will the nutritional status of the patient influence the outcome of helminth therapy, and should helminth therapy be avoided in immunocompromised individuals and suspended during pregnancy? Intuitively, one would answer yes to this question. Is an acute, spontaneously cured infection more or less beneficial than a chronic infection? Reflection on the murine model systems and the limited human data available indicate that helminth rejection and the immunological events that accompany this are a major component of the anticolitic effect, but this does not negate the putative prophylactic effect of a low-burden, chronic infection. Would use of killed larvae be as effective as viable ones? We surmise that this will not be the case (preliminary personal observation), and suggest that it is the intricacies of the host–parasite interaction that are important and not merely an antigen-driven response, which could be elicited by, for instance, systemic delivery of a foreign protein with a Th2-promoting adjuvant such as cholera toxin.

Which helminth should be prescribed? The ‘therapeutic helminth’ must be as innocuous as possible, and this precludes the use of many species such as filarial worms, schistosomes and auto-infective species. Weinstock and colleagues see significant anticolitic benefit with the nematode \textit{T. suis},\textsuperscript{46} and it is our bias that intestinal cestodes (i.e. hymenolepids) are interesting candidates as therapeutic helminths.\textsuperscript{36, 44} Also, if repeat therapy is desired, should the same species of parasite be used or a different helminth considered? Finally, the individual patient’s perception of helminth therapy and their adjustment to the concept would be a pivotal decision branch in any logarithm of potential treatment approaches to colitis (Figure 3).

CONCLUDING COMMENTS

The starting point for considering parasitic helminths as a therapeutic option in inflammatory bowel disease is the modulation of the Th1–Th2 cytokine balance. It is our contention that while such an explanation will be confirmed under specific circumstances, the impact of helminths will extend beyond this paradigm and should be considered in the context of a more global immunoregulatory model. We have only begun to assess the intricacies of the hetero-species relationship in which parasitic helminths are used as an anti-inflammatory therapy. There is much to learn, and the potential of helminth therapy is deserving of rigorous assessment. The caveat with helminth therapy is that the target patient population (e.g. cohorts of patients with Crohn’s disease) needs to be well defined and, at least in the immediate future, is likely to comprise individuals that have failed traditional treatments. The issue of palatability of helminth therapy is a concern, but one which may be negated if the treatment consistently induces disease remission or substantive symptom relief. Indeed, very effective antihelminthics are available should the patient have difficulty in adjusting to helminth therapy. We suggest that analyses of multiple parasitic helminth–murine colitis models will yield information...
relevant to the modulation and management of human colitis, and echo the sentiments of Desowitz ‘that there is a (therapeutic) lesson to be learned from a more tolerant view of host–parasite relationships’.14

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