Abstract and Introduction

Abstract

Purpose of Review: Inflammatory bowel disease is an emerging illness associated with socioeconomic development. The current epidemic of immune-mediated diseases may result from our loss of exposure to parasitic worms (helminths). This review summarizes some of the recent findings showing that helminths induce regulatory circuits that could prevent and treat inflammatory bowel disease.

Recent Findings: Inflammatory bowel disease appears to result from a dysregulated immune response. Although genes influence the risk of inflammatory bowel disease, it seems that critical changes in our environment have permitted its expression. One such change is the eradication of helminths. Helminths can impede interleukin-12, interferon gamma, and tumor necrosis factor α release and promote interleukin-10, transforming growth factor β, and regulatory T-cell production. Helminths can prevent and reverse intestinal inflammation in animal models of inflammatory bowel disease. In clinical studies of patients with inflammatory bowel disease, exposure to the helminth Trichuris suis reduces disease activity.

Summary: If harboring helminths protects against immune-mediated disease, then these animals must be viewed in a new light. Are there "good" helminths in addition to bad? Instead of being detestable objects marked for eradication, helminths should be viewed as useful animals that may produce important compounds helpful for therapy of human disease.

Introduction

This article summarizes some of the evidence that inflammatory bowel disease (IBD) results from an excessive immune response, reviews regulatory factors that control intestinal inflammation, shows that an environmental change permits development of IBD, and explains why the loss of exposure to helminths may be important for the emergence of IBD. The article also reviews some of our recent studies showing that helminths can inhibit or reverse colitis in animal models of IBD and that patients with IBD improve when exposed to Trichuris suis.

Etiology of Inflammatory Bowel Disease

Inflammatory bowel disease appears to result from a dysregulated immune response to intestinal contents. Inflammatory cells are always present in normal mucosa poised to protect us from
potentially harmful luminal agents. In patients with Crohn disease (CD) or ulcerative colitis, the normal tightly controlled activity of the mucosal immune system becomes excessive resulting in profound tissue damage. To prevent this tissue damage, patients with IBD are placed on potent medications (glucocorticoids, azathioprine, infliximab, cyclosporine, and/or methotrexate) that suppress cellular immunity.

Animal models of IBD show that immune dysregulation results in intestinal inflammation and provide insight into the circuits that prevent excessive activity. Immune-deficient mice develop colitis when given a segregated population of naïve CD4+ T cells (CD45RB^{High}). The colitis can be prevented by cotransfer of CD45RB^{Low}-expressing T cells. The cytokines transforming growth factor β (TGF-β) and interleukin (IL)-10 are required for this regulation.\(^1,2\) IL-10 is a regulatory cytokine that inhibits macrophage and dendritic cell activation and suppresses production of proinflammatory mediators such as tumor necrosis factor a, IL-12, IL-1, nitric oxide, and several chemokines.\(^3\) Mice that lack IL-10 develop spontaneous colitis.\(^4\) TGF-β has many actions\(^5\) including inhibition of T-cell proliferation. TGF-β prevents naïve T cells from expressing transcription factors (T-bet, Gata-3) that drive their development into effector T cells.\(^6,7\) Instead, TGF-β induces T cells to express the transcription factor Foxp3 (scurfin) that drives their development into a regulatory phenotype.\(^8^*\) Mice engineered to have T cells that cannot sense TGF-β develop spontaneous colitis.\(^9\)

In most animal models of IBD, inflammation results from an excessive T helper (Th) 1 response. Cellular immune responses often polarize into the Th1 type, characterized by cells that make IL-12, interferon gamma (IFN-γ), or tumor necrosis factor a and the Th2 type, characterized by cells that make IL-4, IL-5, and IL-13.\(^10\) When unbridled, either polarized Th1 or Th2 responses can cause disease. Diseases associated with polarized Th1 responses include CD, multiple sclerosis, insulin-dependent diabetes, rheumatoid arthritis, and psoriasis. Diseases associated with polarized Th2 responses include atopic dermatitis, allergic rhinitis, and asthma. Ulcerative colitis has features of both Th1 (IFN-γ) and Th2 (IL-4) responses.

Normally, the immune response is appropriately restrained by multiple homeostatic mechanisms (Fig. 1). IFN-γ made by Th1 cells inhibits development and proliferation of Th2 cells, whereas IL-4, IL-13, and IL-10 made by Th2 cells inhibit generation of Th1 cells. Thus, the Th1 and Th2 responses are counterregulatory. More recently identified are at least three phenotypes of T cells with predominant regulatory function (Treg) called the natural T regulatory, Th3, and Tr1 subtypes. Natural CD4^{+}CD25^{+} regulatory T cells, differentiated in the thymus, inhibit proliferation of other T cells by cell contact.\(^11\) Mice that lack natural regulator T cells develop autoimmune diseases. Regulatory CD4^{+}CD25^{+} T cells may also develop outside the thymus.\(^12^*\) A transcription factor that drives development of CD4^{+}CD25^{+} Tregs is FoxP3.\(^13^*,14^*\) Animals and patients lacking Foxp3 develop severe, fatal multiorgan autoimmune disease.\(^15,16\) Th3 cells make TGF-β\(^17\) that inhibits development of both Th1 and Th2 cells. Tr1 cells make copious amounts of IL-10 that inhibits both Th1 and Th2 responses.\(^3\)
What causes the immune dysregulation in IBD is unknown. A mutation in Card15 (Nod2) accounts for some of the risk of developing CD in Western populations.[18,19] Card15 is a protein expressed in Paneth cells and monocytes that senses muramyl dipeptide, a component of all bacteria.[20**,21] Although Card15 mutations confer risk, they are neither required nor sufficient for developing CD. Most patients with CD do not have Card15 mutations and most people with Card15 mutations do not have CD. This shows that the interaction of multiple genes contributes to developing CD.

Although genes contribute, environmental factors also affect the development of IBD. Studies of monozygotic (genetically identical) twins show a concordance rate for IBD in 50% of twins with CD (observed for an average of 27.4 years) and 18.8% of twins with ulcerative colitis (observed for an average of 32.2 years).[22**] Concordance rates would be much higher for identical twins if genes alone predestined IBD.

Epidemiology of Inflammatory Bowel Disease
Something about our environment appears to confer a major risk of developing IBD. The prevalence of IBD varies with time, geography, socioeconomic condition, and occupation. The incidence of IBD in North America and Europe increased dramatically during the 20th century (Fig. 1). IBD is more common among people raised in the northern latitudes. IBD is more common in urban than rural areas. IBD is less common in persons who do manual labor and are exposed to dirt. IBD is less common in military veterans if they were prisoners of war or served in combat in the tropics. IBD is common in highly developed industrialized countries but is rare in less developed tropical countries. Studies of immigrants and their children show that this is not due to genetic differences between people living in developed versus less developed countries. The offspring of people who moved to developed countries have a much higher risk of IBD than their peers in their country of origin. Furthermore, IBD emerges as countries develop.

This increase in IBD prevalence with economic development is unlikely to be due to better diagnostics or increased access to health care. Increases are seen in the same locale over time (Fig. 2) as lifestyles change. In addition, the emergence of IBD is not unique. Multiple sclerosis, insulin-dependent diabetes, allergic rhinitis, and asthma have a similar epidemiology. It is becoming clear that changing our environment increases the risk of these immune-mediated diseases.
Why worms?

Economic development leads to many changes in environmental exposures. Among these include exposure to artificial light, air conditioning, television, refrigeration, fast foods, concentrated sweets, antibiotics, and childhood vaccination, to name a few. However, most of these candidate exposures...
do not appear to confer risk of IBD on detailed investigation. Beginning in the mid-1990s, we developed the hypothesis that loss of exposure to parasitic worms (helminths) increases the risk of IBD. Helminths are transmitted most efficiently where sanitation is poor. Economic development brings running water, sewage treatment, and hygienic farming practices that prevent the spread of helminths. Hard winter freezes can impede helminth transmission accounting for north-south gradients. Today, helminth carriage is rare in locations where IBD is common, and helminths are common where IBD is rare.

There are many types of helminths (Table 1) that are classified as nematodes (roundworms), trematodes (flukes), and cestodes (tapeworms). Helminths are complex multicellular organisms that make foreign proteins, lipids, and metabolites recognized by the immune system. Helminths are unique in their capacity to induce Th2 immune responses. Th2 responses serve the host by limiting the degree of helminthic colonization. To survive, worms have developed methods to evade immune-mediated destruction. Furthermore, helminths may actually benefit from the host's immune response. For example, Schistosoma mansoni depends on the host's immune response to help its eggs escape from the body. People have had helminths for many millennia. After eons of coevolution, it becomes difficult to say whether the immune system controls the helminth or the helminth controls the immune system.

Helminths alter immune responses beyond those directed against the worms. People carrying helminths show immune bias away from the Th1 response normally elicited with tetanus vaccination or in vitro mitogen stimulation. Mice harboring helminths have depressed Th1 and augmented Th2 responses to test antigens and mycobacteria.

Helminths regulate their host's immune system. They appear to accomplish this task through multiple mechanisms including release of compounds that trigger IL-10 or preventing IL-12 and tumor necrosis factor α production, release of actual mediators such as prostaglandin E2, and release of cytokine mimics such as TGF-β-like molecules. Helminth induction of immune regulatory circuits impedes Th1 and possibly even aberrant Th2 responses. This makes our recent loss of helminth exposure a strong environmental candidate to explain the increased prevalence of IBD.

Helminths and Inflammatory Bowel Disease: Animal Studies

In addition to altering systemic immune responses, helminths can change mucosal immune responses in mice. Heligmosomoides polygyrus is a murine roundworm that only colonizes the duodenum of its host. Intestinal lamina propria mononuclear cells from H. polygyrus -colonized mice make less IL-12 and IFN-γ but more IL-4, IL-13, and IL-10 than lamina propria mononuclear cells from worm-free mice (Elliott et al., submitted, 2004). This ability to influence mucosal immune responses may permit helminths to inhibit excessive intestinal inflammation. Indeed, this effect is demonstrated with various helminths in several different colitis models.

Some strains of mice and rats develop severe colitis after transrectal challenge with trinitrobenzene sulfonic (TNBS) or dinitrobenzene sulfonic (DNBS) acid. The mucosa of challenged animals is infiltrated with CD4+ IFN-γ-producing T cells. Treatment of mice with anti-IL-12, anti-tumor
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necrosis factor α[53] monoclonal antibodies, or with recombinant IL-10[54] inhibits TNBS colitis. This suggests that the colitis results from a dysregulated immune response. We and others showed that exposure to helminths prevents TNBS[55,56] and DNBS[56,57] colitis. Mice exposed to *Trichinella spiralis* or *Schistosoma mansoni* eggs made less IL-12 and IFN-γ but more IL-4 and immunoregulatory IL-10.[55,56] The protection afforded by schistosome egg exposure required intact IL-4 and Stat6 signaling.[55,56] Exposure of mice to *Trichuris muris* or *H. polygyrus* also protects mice from developing TNBS colitis (Elliott, unpublished observation, May 2004).[58]

IL-10-deficient mice have a dysregulated immune system that causes fatal colitis in response to normal gut flora. The colitis is characterized by infiltration of the mucosa with CD4⁺ IFN-γ-producing T cells.[4] Previous colonization of IL-10-deficient mice with *T. muris* or *H. polygyrus* reduces spontaneous colitis.[35] In addition to protecting from developing colitis, helminths can treat ongoing active colitis in this model. It is well established that IL-10-deficient mice that receive *H. polygyrus* after colitis improve dramatically.[59] The reduction in colitis is associated with inhibition of IFN-γ and IL-12p40 production by intestinal lamina propria mononuclear cells. Importantly, transfer of mesenteric lymph node T cells from *H. polygyrus* -colonized IL-10-deficient mice to colitic IL-10-deficient mice also reverses intestinal inflammation. This suggests that helminths activate regulatory T cells even in the absence of IL-10. Indeed, worm colonization enhances expression of mRNA for a transcription factor that drives regulatory T-cell activity (Foxp3) in mesenteric lymph node T cells. Exposure to *S. mansoni* eggs also inhibits previously established colitis in IL-10-deficient mice (Elliott, unpublished observation, May 2004).

Worms may alter other "nonimmunologic" reactions in the gut. For example, mice develop intestinal epithelial cell injury in response to dextran sodium sulfate ingestion.[60] Colonization with *Hymenolepis diminuta* reduced the abnormal epithelial ion transport evoked by dextran sodium sulfate without grossly changing the toxin-induced histopathology.[61] In individuals, these types of changes may decrease symptoms from intestinal inflammation.

**Helminths and Inflammatory Bowel Disease: Clinical Studies**

Epidemiologic evidence, case control observations, and animal studies all suggest that helminths may afford protection from or even treat intestinal inflammation. When our animal studies began to show that helminths could reduce colitis, we started exploring their potential use in IBD. There are many types of helminths, and several were judged good candidates for therapeutic application. Others have enough potential to cause disease that their application to human IBD was considered impractical at this time. After comparing many helminths, we focused on whipworm as a potential agent.

The human whipworm is *Trichuris trichiura*. Individuals acquire whipworm by ingesting mature eggs. The egg hatches in the small bowel, and the helminth remains confined to the intestine, residing in the right colon. Adult worms lay immature eggs that pass with the stool. Eggs require 4 to 6 weeks of incubation in moist, warm soil to mature. Because of this, the worm cannot multiply in the host or directly spread to others. Modern hygienic practices block transmission of *T. trichiura*.

People have had *T. trichiura* for eons; whipworm eggs are found in mumified remains and
fossilized stools dating back more than 10,000 years.[39] Whipworm infections were common in North America and Europe before the 1940s. Although *T. trichiura* has become rare in these locales, it remains common in less developed tropical countries. Currently, approximately 800 million people carry whipworm, and the vast majority of these infestations are asymptomatic. However, very heavy colonization with *T. trichiura* can cause a dysentery-like syndrome. Trichuriasis is curable with readily available antihelminthic drugs.

Like most helminths, *Trichuris* sp cannot survive outside their hosts. Therefore, there is no readily available source of human whipworm. The porcine whipworm (*Trichuris suis*) is closely related to *T. trichiura*. *T. suis* can colonize in humans,[62] but such colonization is self-limited. There are no human illnesses attributed to *T. suis*. Importantly, *T. suis* eggs are available because we can obtain whipworms from pigs grown in a specific pathogen-free environment. Characteristics that make *T. suis* a favorable therapeutic candidate are listed in Table 2.

We first investigated the effect of colonization with *T. suis* on active IBD in a small open-label trial with four patients with CD and three with ulcerative colitis.[63**] The primary goal of the initial trial was to determine whether helminth therapy would be safe in people with intestinal inflammation. The patients ingested 2500 mature *T. suis* ova and were observed. The patients with CD were followed using the CD Activity Index (CDAI).[64] Three of the four patients with CD achieved remission, and the fourth had a 151-point improvement in CDAI. The patients with UC were followed using the Simple Clinical Colitis Activity Index (SCCAI).[65] All three showed improvement in SCCAI, achieving scores of 4 or less. Our conclusion was that exposure to *T. suis* appeared to be safe in patients with IBD. We proceeded to larger trials.

We performed a larger open-label trial testing repeated dosing of *T. suis* in active CD.[66] Twenty-nine patients with baseline CDAI scores between 220 and 450 entered the study. Most had disease that was refractory to standard therapy. They received 2500 *T. suis* ova every 3 weeks for 24 weeks. Their disease activity was monitored at weeks 12 and 24 using the CDAI. At week 12, 75.9% of the patients responded with a decrease in CDAI of 100 or more points and 65.5% achieved remission with a CDAI score less than 150. At week 24, 79.3% experienced a response and 72.4% were in remission. There were no side effects or complications attributable to *T. suis* colonization. This was an open-label study without a placebo arm, but it suggests that patients with CD improve with exposure to *T. suis* and that this helminth has a high safety profile.

We recently published the results of a double-blind, placebo-controlled trial of *T. suis* in patients with UC.[67**] This trial included 54 patients with active UC as defined by a Ulcerative Colitis Disease Activity Index (UCDAI)[68] of 4 or higher. The mean initial UCDAI of the participants was 8.7. Patients received either placebo or 2500 *T. suis* eggs every 2 weeks for 12 weeks. The primary end point was improvement signified by a decrease in UCDAI of 4 or more. The patients were randomly assigned: 30 to *T. suis* and 24 to the placebo arm. Analyzed using the intention-to-treat principle, 43.3% of the patients exposed to *T. suis* improved compared with 16.7% given placebo ( *P* < 0.04). The patients were also followed with the SCCAI. Patients given placebo showed no improvement in SCCAI, whereas patients who received *T. suis* had a significant decrease in scores. The SCCAI was determined every 2 weeks and permitted evaluation of time to response. The SCCAI for patients who improved by a decrease in UCDAI of 4 or more is shown in Figure 3. Patients had significant SCCAI...
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**Figure 3.** Time to Improvement for Trichuris Suis -Responsive Patients With Ulcerative Colitis as Measured by the Simple Clinical Colitis Activity Index (SCCAI)
The study also included a crossover phase. Patients who were given placebo for the first 12 weeks were switched to *T. suis* for a second 12-week interval. Patients who initially received *T. suis* were changed to placebo. The blind was maintained. Patients with a UCDAI score of 4 or more at crossover were analyzed. In the second 12 weeks, 56.3% of the patients who received *T. suis* improved compared with 13.3% of patients given placebo (*P* = 0.02). When the two study periods were combined, 47.8% of patients given *T. suis* improved compared with 15.4% of patients on placebo (Fig. 4) (*P* = 0.002) as analyzed by the intention-to-treat method. If we exclude the rare patients who did not complete the study due to protocol violations, 50% of patients given *T. suis* improved compared with 15.8% on placebo (*P* = 0.002). This study shows that helminth colonization can effectively reduce symptoms and inflammation caused by ulcerative colitis. This was the first double-blind, placebo-controlled study of the therapeutic use of helminths. It used a single-dose regimen. Future studies using other doses, timings, or helminths will likely show even greater efficacy.
Conclusion

One of the factors leading to the emergence of IBD may be lack of exposure to helminths. This exposure normally begins early in childhood, setting up immune response patterns that continue for the life of the individual. In addition to IBD, other immune-mediated diseases have emerged with establishment of modern hygiene.[34] Exposure to schistosomes can protect animals from developing insulin-dependent diabetes[69] or experimental autoimmune encephalitis (a model of multiple sclerosis).[70**,71] Exposure to helminths may protect from allergy and asthma.[51] This suggests that helminths may have served as a lid on a "Pandora's box" of immune pathology. Future study of helminths will identify mechanisms of immune regulation, will potentially uncover novel compounds that alter inflammatory responses, and will address the myriad of questions surrounding their potential for clinical application.

Table 1. Helminthic Parasites That Colonize the Human Gastrointestinal System
Table 2. Favorable Characteristics of Tricharis Suis

Closely related to human whipworm Trichuris trichiura
Produces a self-limited colonization in humans
Has no known pathogenic potential
Remains confined to the intestine
Does not multiply in the host
Cannot be directly spread to close contacts
Eggs can be obtained from pigs grown in specific pathogen-free environment

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Abbreviation Notes

CD Crohn Disease
IBD Inflammatory Bowel Disease
IFN -γ Interferon Gamma
IL Interleukin
TGF -β Transforming Growth Factor β
Th T Helper (1, 2, 3)

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Trichuris Suis patent source