Helminths and the IBD Hygiene Hypothesis

Joel V. Weinstock, MD, and David E. Elliott, MD

Abstract: Helminths are parasitic animals that have evolved over 100,000,000 years to live in the intestinal track or other locations of their hosts. Colonization of humans with these organisms was nearly universal until the early 20th century. More than 1,000,000,000 people in less developed countries carry helminths even today. Helminths must quell their host's immune system to successfully colonize. It is likely that helminths sense hostile changes in the local host environment and take action to control such responses. Inflammatory bowel disease (IBD) probably results from an inappropriately vigorous immune response to contents of the intestinal lumen. Environmental factors strongly affect the risk for IBD. People living in less developed countries are protected from IBD. The "IBD hygiene hypothesis" states that raising children in extremely hygienic environments negatively affects immune development, which predisposes them to immunological diseases like IBD later in life. Modern day absence of exposure to intestinal helminths appears to be an important environmental factor contributing to development of these illnesses. Helminths interact with both host innate and adoptive immunity to stimulate immune regulatory circuitry and to dampen effector pathways that drive aberrant inflammation. The first prototype worm therapies directed against immunological diseases are now under study in the United States and various countries around the world. Additional studies are in the advanced planning stage.

(Inflamm Bowel Dis 2009;15:128–133)

Key Words: helminths, hygiene hypothesis, IBD

Helminths are worm-like animal parasites. Helminths infect humans in all areas of the world, but they are particularly common in warmer regions. Depending on the parasite species, these organisms are spread through contact with contaminated water, soil, or food. More than one-third of humans presently carry these organisms, with the highest frequency found in children subject to poor sanitation. Children and adults in the United States before the 1940s often had helminths, which were particularly common in poor city dwellers and residents of the rural South.[1] Although less common today, they occasionally are detected in poor populations living in underserved regions of the US[2] and in recent immigrants from less developed countries.[3]

The first helminths date back at least to the time of the dinosaurs.[4] Examination of petrified human waste on the floor of caves and inspection of mummies documents that these organisms have colonized humans for many thousands of years. Most helminths are highly restricted in host selection, attesting to the closeness of this host–parasite association. Also, depending on the worm species, they have preferences for living in various locations of their host like the intestinal lumen, bile ducts, lungs, blood stream, and elsewhere. To accomplish this, they must evade and control the host's immune system. This has been achieved through millions of years of coevolution allowing time for both the parasite and its host to gradually adjust to this relationship. Many worms seem invincible to human defense. Worms produce factors and receptors with homology to molecules of the human immune system as revealed by recently decoded worm genomes. It is likely that helminths sense hostile changes in the local host environment and take action to quell these responses. Many worms also have a peculiar outer integument that can absorb host molecules and which may assist in cloaking the parasitic organism from host detection.

Unlike bacteria and viruses, worms are large organisms with complex tissues and fully developed organs. The 2 groups of worms that inhabit the interior of humans are the nematodes (roundworms) and the platyhelminths (flatworms), which have distinct evolutionary histories.[5] Some of the common human roundworms include *Ascaris lumbricoides, Necator americanus* (hookworm), *Trichuris trichiura* (whipworm), *Enterobius vermicularis* (pinworm), *Strongyloides stercoralis*, and filariae species like *Wuchereria bancrofti* and *Brugia malayi*. Most of the common roundworms (excluding filariae) live in the gut.

There are 2 classes of human flatworms called trematodes (flukes) and cestodes. The trematodes live predominantly in the venous system (e.g., schistosome species), biliary system (e.g., *Clonorchis*), gut (e.g., *Fasciolopsis*), or airway (e.g., *Paragonimus*). The cestodes include the intestinal tapeworms like *Diphyllobothrium latum* (fish tapeworm), *Taenia saginata* (beef tapeworm), and *Taenia solium* (pig tapeworm).

Humans also are exposed to various helminths of do-

mesticated or wild animals through agricultural practices, hunting, and animal husbandry. Most of these helminths, however, fail to sustain human colonization, presumably because of genetic differences in the human host, and have no association with human illness (e.g., *Trichuris suis*). Other animal helminths like *Toxocara canis* (dog ascarid), *Dirofilaria immitis* (dog heartworm), and *Trichinella spiralis* infrequently cause pathology in humans.

About 1–2 million people in the US have Crohn's disease (CD) or ulcerative colitis (UC), which usually begins during the second to third decade of life. Inflammatory bowel disease (IBD) probably results from an inappropriately vigorous immune response to contents of the intestinal lumen. Evidence supporting this contention includes the effectiveness of immune suppressants at controlling the disease and the higher than expected occurrence of IBD with some inherited defects in immune regulation. Also, in support of this hypothesis are experimental data derived from mice who are prone to IBD because of naturally acquired or genetically engineered defects in immune regulation.[6,7] In most of these murine models the inflammation is driven by Th1 and/or IL17 circuitry and by substances in the intestinal lumen.

Environmental factors affect the risk for IBD.[8] Appendicitis followed by appendectomy lowers the incidence of UC,[9–11] whereas cigarette smoking enhances the chance for CD.[12] Some enteric infections can trigger IBD like cytomegalovirus and amebic (*Entameba histolytic*) colitis. A major observation that points to the importance of environment in IBD is the striking geographic variations in IBD frequency. Early studies showed that IBD was more common in northern versus southern regions of the US and Europe.[13,14] This north–south gradient is still present in some areas today.[15] Migration studies suggest that children born to individuals who relocate from regions of low CD or UC frequency to areas of high disease prevalence acquire greater disease risk.[16–18] IBD prevalence has increased in Western Europe and North America and now affects about 1 in 250 people in some affluent regions.[19,20] IBD now is spreading throughout many Asian nations.

In the 1990s we proposed the "IBD hygiene hypothesis." It states that raising children in extremely hygienic environments negatively affects immune development, which predisposes them to immunological diseases like IBD later in life.[1] Moreover, we proposed that the modern day absence of exposure to intestinal helminths is an important environmental factor contributing to IBD. Helminths regulate their host's immune system and prevent excessive immune responses.

There is expanding epidemiological data pointing to a protective role for helminth infections in various immunological diseases. For instance, helminth infections inversely correlate with allergic disorders. A case–control study in Ethiopia showed that people infected with hookworm have a low frequency of asthma,[21] an observation supported by a similar study conducted in Vietnam.[22] A randomized, controlled prospective study demonstrated that repeated anti-helminthic therapy of helminth-infected Gabonese children increases the frequency of allergic sensitivity to house dust mites.[23] Infection with *Schistosoma hematobium* induces a parasite-specific IL10 response in the host and suppresses atopic reactivity in Gabonese children.[24]

Multiple sclerosis is another disease displaying a north–south gradient with an increasing prevalence in developed countries.[25] This rise in multiple sclerosis correlates with diminished carriage of *T. trichiura* (whipworm) in various locales.[26] Multiple sclerosis patients who developed helminthic infection during the course of their disease displayed fewer disease exacerbations and develop fewer new brain lesions, as shown by magnetic resonance imaging, compared to the uninfected multiple sclerosis control group.[27]

Epidemiological data supporting a role for helminth infection in protection from IBD is more circumstantial. There is a clear inverse correlation between the frequency of helminth infections and the prevalence of IBD. Direct proof of the concept that helminths acquired naturally protect humans from IBD remains sparse. Reports have now appeared showing IBD control after natural exposure to intestinal helminths.[28]

There have been several clinical trials using helminths to treat IBD. Results from these trials suggest that infection with at least some human or animal helminths improves clinical outcome, which supports the premise that natural helminth infection is protective. There was clinical improvement in a double-blind clinical study in UC and an open-label study in CD. These studies used live ova from porcine whipworm (*Trichuris suis*) as an oral therapeutic intervention.[29–31] Another study in CD showed efficacy using live human hookworm administered via skin application.[32]

Scientists frequently turn to animal models designed to mimic aspects of human disease to understand basic pathophysiology of human illness. While there are no true models of human IBD, many of the current animal models afford understanding of the processes that protect the gut from unwanted immune attack and are useful for the initial testing of new candidate therapies. Using such animal models it is readily evident that helminths suppress the ongoing pathology of murine IBD and prevent disease onset.

Rodents administered trinitrobenzene sulfonic acid (TNBS) in alcohol intrarectally develop Th1-type colitis. The intestinal roundworm *T. spiralis*[33] or the rat intestinal tapeworm *Hymenolepis diminuta* protect rodents from this disease. There is similar protection by infecting mice with the intestinal roundworm *Heligmosomoides polygyrus* or *T. muris* or with the vascular fluke *Schistosoma mansoni*[34] or by treatment rodents with schistosome ova.[35] However, oxazolone-induced colitis appears to worsen with *H. diminuta* infection.[36] Thus, not all models of IBD show protection.

The immune system can be roughly divided into 2 parts. The innate immune system comprises the cells and mechanisms that immediately defend the host from infection in a nonspecific manner through the pattern recognition of molecules foreign to the host. The adaptive immune system, which employs T and B cells, recognizes specific antigens and confers long-lasting protective immunity. However, the adaptive immune system takes several days to respond to an initial challenge by an invading organism. In the gut, innate immunity usually suffices to protect us from our intestinal flora. Cells of the immune system make molecules called cytokines that drive, modulate, or suppress immune responses. Aberrant expression of various cytokines by the adaptive immune system underlie the basic pathophysiology of IBD. As demonstrated in many murine models of colitis, IFNγ, IL17, IL12, and IL25 are some of the cytokines important for driving intestinal inflammation. TGFβ and IL10 are important immune modulatory cytokines that limit immune reactivity. There are also cells of the immune system that help drive, modulate, or suppress inflammation. These include effector T cells (Th1, Th2, Th17), regulatory T cells (Treg, Tr1, Th3), dendritic cells, and alternatively activated macrophages among others.

Various studies have shown that worm infection can suppress intestinal T-cell responsiveness to various mitogens and antigens. This can include the Th1, Th2, and Th17 pathways of inflammation. The mechanisms of regulation are quite varied (Fig. 1).

Intestinal helminths protect mice from TNBS-induced, Th1-type colitis by restraining the IFNγ/IL12 p40 response in the colon. Worm infection also downmodulates Th1 pathways in the gut of healthy noncolitic wildtype mice. Several regulatory mechanisms mediate this protection. Worm infection induces mucosal lamina propria mononuclear cells to produce Th2 cytokines (e.g., IL4, IL13) and large quantities of regulatory factors (e.g., IL10, TGFβ). In TNBS colitis, abrogation of the Th2 pathway blocks worm protection, showing the importance of Th2 cytokines in this IBD model.[35] IL10 is a critical immune regulatory cytokine. In worm-infected mice, blocking the IL10 receptor partly restores IFNγ and IL12 production in lamina propria mononuclear cells from TNBS colitis or from healthy wildtype mice, suggesting that IL10 also helps regulate the Th1 pathway. The rat tapeworm *H. diminuta* also ameliorates a Th1 colitis via an IL10 mechanism.[37] Mice that cannot make IL10 develop a chronic colitis driven by cytokines associated with Th1/Th17 responses (e.g., IFNγ, IL17, IL12, IL23), attesting to the importance of this cytokine for mucosal immune homeostasis. However, helminth infection (*T. muris* or *H. polygyrus*) or injection of nonviable schistosome ova still prevents disease and reverse ongoing intestinal inflammation as well as curtail intestinal IFNγ and IL12p40 production in this
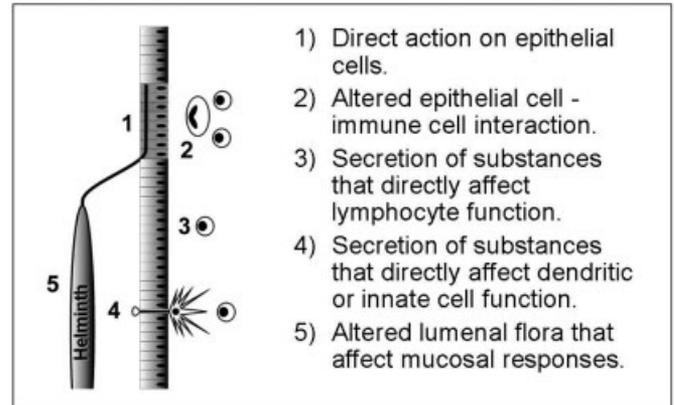
FIGURE 1. Helminths potentially induce multiple regulatory pathways. Helminths are large multicellular organisms that can influence multiple host responses. Helminths can interact directly with epithelial cells (1) possibly influencing mucous production, defensin release, and barrier function. Either by direct contact or soluble mediators, helminths may alter epithelial-immune cell (macrophage/T cell/NK cell) interaction (2). Helminths can release factors (3) such as TGFβ that can directly affect lymphocyte function to downregulate inflammation. Helminths can release substances that alter the function of dendritic cells, mast cells, NK cells, and/or macrophages (4). In addition, helminths may release factors that alter bacterial flora and indirectly influence mucosal responses (5). Various helminth products may mediate these effects and work in concert to reduce intestinal inflammation.

disease state. Thus, IL10 may be helpful, but is not necessarily essential, for helminth control of IBD.[1,38]

Colitis resulting from IL10 deficiency is driven in part through the Th17 pathway. IL12p40 is a molecule shared by IL12 and IL23. IL23 and other cytokines induce and sustain the mucosal IL17 response. Worm infection blocks mucosal IL23 and IL17 secretion, suggesting that regulation of IL17 is another important mechanism of colitis control (in press). How helminths limit IL17 (Th17) responses is not yet fully understood. However, blockade of IL23 secretion and stimulation of IL4 production at the mucosal surface plays a partial role (submitted: Elliott, Metwali and Leung).

Gram-negative bacteria frequently express lipopolysaccharides (LPS). LPS interacts with receptors of the innate immune system (TLR4) on host cells, driving production of protective proinflammatory molecules. The normal intestinal mucosa usually is relatively unresponsive to LPS, since the normal intestinal flora produces large amounts of this molecule and uncontrolled responses to LPS would be detrimental to the mucosa. *H. polygyrus* infection induces display of TLR4 on a subset of mucosal T cells. Engagement of this receptor promotes production of regulatory (TGFβ, IL10) rather than proinflammatory molecules.[39] Thus, under the influence of helminths, damage to the mucosa allowing LPS penetration may provoke activation of IL10/TGFβ producing

regulatory T cells, helping to quell excessive adaptive immunity.

Mucosal T cells must be able to sense TGFβ for normal mucosal immune homeostasis. Mice with defective TGFβ signaling restricted just to T cells have difficulty limiting both Th1 and Th2 responsiveness in the intestinal mucosa and spontaneously develop colitis. Infection with *H. polygyrus* cannot prevent this colitis or dampen mucosal Th1 responsiveness in this mouse, suggesting that TGFβ signaling via mucosal T cells is an important requirement for worm protection from IBD (submitted: Ince, Setiawan and Metwali.).

T cells that regulate rather than incite mucosal immune responses are important for restraining inflammation in various animal models of IBD.[40] There are several regulatory T-cell phenotypes, including IL10-producing Tr1 cells, TGFβ-producing Th3 cells, and IL4/IL10-secreting Th2 cells. Also, there are special classes of regulatory T cells that express a transcription factor called Foxp3, which instructs the T cells to differentiate into a regulatory phenotype.

Helminths induce various regulatory T-cell subsets within the gut mucosa of their hosts. Worm infection induces within the gut the appearance of regulatory T cells that make IL10 and TGFβ. These cells help limit Th1 responsiveness. However, helminths can inhibit mucosal inflammation in the absence of IL10. T cells from the mesenteric lymph node (MLN) of *H. polygyrus*-infected IL10-deficient mice stop colitis when transferred into colitic IL10 deficient recipients.[38] Transfer of MLN T cells from worm-naïve IL10-deficient mice does not inhibit colitis, suggesting that helminth exposure induces the regulatory activity. Helminth colonization induces FoxP3 expression by MLN and LP T cells. LP T cells from *H. polygyrus*-infected mice, in contrast to LP T cells from worm-naïve controls, strongly suppress proliferation of both CD4+ and CD8+ effector T cells. Foxp3+ CD8+ regulatory T cells mediate this effect. This regulatory activity functions independently of IL10 and TGFβ, but requires Class I MHC interactions and cellular contact to mediate their regulation.[41] Transfer of CD8+ T cells into an IL10KO Rag transfer model of colitis downmodulates disease activity, suggesting that CD8+ T cells limit IBD. Regulatory CD8+ T cells may have a role in several immune-mediated diseases, as shown in an animal model of multiple sclerosis.[42] When pharmacological intervention eliminates helminths from the gut, regulatory-type T cells linger in the intestine, suggesting that worm exposure early in life may lead to prolonged resistance to IBD.

While helminth exposure induces regulatory cytokines and T cells that have a major impact on mucosal adaptive immunity, they also directly modulate innate immunity. Rag mice do not produce T cells or B cells, but remain healthy without colitis in appropriate animal care facilities. However, transfer of IL10KO T cells into these mice makes them susceptible to colitis driven by both Th1 and Th17 T cells and

their associated cytokines. Exposure of Rag mice to helminths (*H. polygyrus*) for a brief interval before the introduction of adaptive immunity (IL10KO T cells) renders the animals resistant to IBD. Also, antigen-responsive Th1 and Th17 T cells that reside in the gut have difficulty responding to antigens after such an exposure. Isolated LPMC from T- and B-cell-deficient mice previously exposed to worms mixed with LPMC from colitic mice block antigen-specific responses in the responding colitic T cells. The cells mediating this regulation are not yet defined but could involve dendritic cells, alternatively activated macrophages, or even regulatory NK cells.

Oral dextran sodium sulfate (DSS) induces intestinal inflammation in rodents. This model of IBD is distinct, since inflammation is independent of adaptive immunity. Schistosomiasis protects BALB/c, but not C57BL/6 mice from DSS enteritis. The schistosome worms mediate the protection. Protection from colitis is macrophage-dependent. There is no apparent role for regulatory T cells or the usual regulatory cytokines (e.g., IL10 or TGFβ) in this model system.[43]

Helminths also protect rodents with immunological diseases that simulate conditions like multiple sclerosis (experimental autoimmune encephalomyelitis,[44,45] Graves' hyperthyroidism,[46] asthma,[47,48] rheumatoid arthritis,[49] food allergy,[50] and type 1 diabetes.[51,52] For instance, infection with 1 of several nematode parasites can decrease the susceptibility of rodents to allergic disease.[47,48,50,53] Protection in animal models of asthma also may involve IL10, regulatory-type T cells, and alternatively activated macrophages.[54] Thus, helminths activate several immune regulatory pathways capable of limiting deviant inflammation. Helminths also induce other immune modulators like arginases, resistins (RELM family), chitinase, and intelectins. These lesser-studied molecules help regulate Th2 cytokines, and macrophages and epithelial cells function at sites of mucosal inflammation.[55] Their role in helminth immune protection from immunological disease deserves further study.

CONCLUSION

Worm-based therapies are under development at several pharmaceutical companies. Some laboratories are isolating agents from helminths that could prove useful as therapeutic agents.[56–58] Others have developed pharmaceutical grade helminths that currently are under review at both the US and European food and drug administrations. The first prototype worm "vaccine" studies against immunological diseases like CD, UC, multiple sclerosis, asthma, food allergy, and even allergic rhinitis are now under way or in the advanced planning stage in the US and various countries around the world.

Use of all new experimental therapies must proceed with caution. The vast majority of mild to moderate worm infestations rarely manifest as disease. While many helminths hold little pathogenic potential, others carry risk. For in-

The <u>You</u> Ecosystem

1) You are more than just your genetic self.

2) You are a community of interactive organisms.

3) Removing key players can unbalance the system.

FIGURE 2. Over many thousands of years, our genetic selves have evolved in close proximity to the many organisms that live within and on our bodies, and that we encounter through every day life. Due to the process of mutual co-evolution, some of these interactions have become important for proper development and maintenance of our immune systems, intestinal lining and perhaps other organs within our bodies. In the 20th Century, changes in our life-styles have been progressively removing us from our natural environment. This may have eliminated key microbial, viral and helminthic interactions resulting in system dysfunction predisposing us to immunologic and perhaps other diseases.

stance, *S. mansoni* can cause liver fibrosis and portal hypertension, while *Strongyloides* can multiply within the gut and become invasive in the immune-compromised host. Some studies using murine and larger animals suggest that worm infection can interfere with vaccine efficacy, which could have important implications for human health.[59] Acute infection with a helminth could leave some animals more susceptible to enteric infections with pathogenic bacteria.[60,61] Modern-day therapeutics for IBD have limited efficacy and are not without their danger. There may be little therapeutic risk using properly selected and administered helminths or their derivatives in our hygienic societies.

Modern hygienic practices have greatly decreased deaths in the US and Europe from acquisition of enteric pathogens. Humans and helminths and their immune systems evolved closely together over many thousands of years. To promote their own survival, helminths stimulate immune regulatory pathways within their hosts to control immune reactivity. Clean water and food, paved streets and sidewalks, and modern sanitation systems have reduced exposures to helminths and perhaps other microbial organisms. This could be leading to immune dysregulation and increased susceptibility to immunological diseases (Fig 2). Controlled reintroduction of such exposures during childhood and perhaps beyond may help reestablish immune balance and lower the risk for immunological diseases.

REFERENCES

1. Elliott DE, Urban JF Jr, Argo CK, et al. Does the failure to acquire helminthic parasites predispose to Crohn's disease? *FASEB J.* 2000;14:1848–1855.
2. Healy GR, Gleason NN, Bokat R, et al. Prevalence of ascariasis and amebiasis in Cherokee Indian school children. *Public Health Rep.* 1969;84:907–914.
3. Salas SD, Heifetz R, Barrett-Connor E. Intestinal parasites in Central American immigrants in the United States. *Arch Intern Med.* 1990;150:1514–1516.
4. Poinar G Jr, Boucot AJ. Evidence of intestinal parasites of dinosaurs. *Parasitology.* 2006;133(Pt 2):245–249.
5. Rolff J. Why did the acquired immune system of vertebrates evolve? *Dev Comp Immunol.* 2007;31:476–482.
6. Mayer EA, Collins SM. Evolving pathophysiologic models of functional gastrointestinal disorders [Review]. *Gastroenterology.* 2002;122:2032–2048.
7. Boismenu R, Chen Y. Insights from mouse models of colitis [Review]. *J Leukocyte Biol.* 2000;67:267–278.
8. Loftus EV Jr, Sandborn WJ. Epidemiology of inflammatory bowel disease [Review]. *Gastroenterol Clin North Am.* 2002;31:1–20.
9. Andersson RE, Olaison G, Tysk C, et al. Appendectomy and protection against ulcerative colitis. *N Engl J Med.* 2001;344:808–814.
10. Derby LE, Jick H. Appendectomy protects against ulcerative colitis. *Epidemiology.* 1998;9:205–207.
11. Russel MG, Dorant E, Brummer RJ, et al. Appendectomy and the risk of developing ulcerative colitis or Crohn's disease: results of a large case-control study. South Limburg Inflammatory Bowel Disease Study Group [see Comments]. *Gastroenterology.* 1997;113:377–382.
12. Calkins BM. A meta-analysis of the role of smoking in inflammatory bowel disease. *Dig Dis Sci.* 1989;34:1841–1854.
13. Sonnenberg A, McCarty DJ, Jacobsen SJ. Geographic variation of inflammatory bowel disease within the United States [see Comments]. *Gastroenterology.* 1991;100:143–149.
14. Shivananda S, Lennard-Jones J, Logan R, et al. Incidence of inflammatory bowel disease across Europe: is there a difference between north and south? Results of the European Collaborative Study on Inflammatory Bowel Disease (EC-IBD). *Gut.* 1996;39:690–697.
15. Kappelman MD, Rifas-Shiman SL, Kleinman K, et al. The prevalence and geographic distribution of Crohn's disease and ulcerative colitis in the United States [see Comment]. *Clin Gastroenterol Hepatol.* 2007;5:1424–1429.
16. Carr I, Mayberry JF. The effects of migration on ulcerative colitis: a three-year prospective study among Europeans and first- and second-generation South Asians in Leicester (1991-1994). *Am J Gastroenterol.* 1999;94:2918–2922.
17. Jayanthi V, Probert CS, Pinder D, et al. Epidemiology of Crohn's disease in Indian migrants and the indigenous population in Leicestershire. *Q J Med.* 1992;82:125–138.
18. Probert CS, Jayanthi V, Hughes AO, et al. Prevalence and family risk of ulcerative colitis and Crohn's disease: an epidemiological study among Europeans and south Asians in Leicestershire. *Gut.* 1993;34:1547–1551.
19. Bernstein CN, Blanchard JF, Rawsthorne P, et al. Epidemiology of Crohn's disease and ulcerative colitis in a central Canadian province: a population-based study. *Am J Epidemiol.* 1999;149:916–924.
20. Loftus EV Jr, Schoenfeld P, Sandborn WJ. The epidemiology and natural history of Crohn's disease in population-based patient cohorts from North America: a systematic review. *Aliment Pharmacol Ther.* 2002;16:51–60.
21. Scrivener S, Yemaneberhan H, Zebenigus M, et al. Independent effects of intestinal parasite infection and domestic allergen exposure on risk of wheeze in Ethiopia: a nested case-control study [see Comment]. *Lancet.* 2001;358:1493–1499.
22. Flohr C, Tuyen LN, Lewis S, et al. Poor sanitation and helminth infection protect against skin sensitization in Vietnamese children: a cross-sectional study. *J Allergy Clin Immunol.* 2006;118:1305–1311.
23. van den Biggelaar AH, Rodrigues LC, van Ree R, et al. Long-term treatment of intestinal helminths increases mite skin-test reactivity in Gabonese schoolchildren. *J Infect Dis.* 2004;189:892–900.
24. van den Biggelaar AH, Lopuhaa C, van Ree R, et al. The prevalence of parasite infestation and house dust mite sensitization in Gabonese schoolchildren. *Int Arch Allergy Immunol.* 2001;126:231–238.
25. Fleming J, Fabry Z. The hygiene hypothesis and multiple sclerosis [see Comment] [Review]. *Ann Neurol.* 2007;61:85–89.
26. Fleming JO, Cook TD. Multiple sclerosis and the hygiene hypothesis [Review]. *Neurology.* 2006;67:2085–2086.

27. Correale J, Farez M. Association between parasite infection and immune responses in multiple sclerosis. *Ann Neurol.* 2007;61:97–108.

28. Buening J, Homann N, von Smolinski D, et al. Helminths as governors of inflammatory bowel disease. *Gut.* 2008;57 (in press).

29. Summers RW, Elliott DE, Urban JF Jr, et al. Trichuris suis therapy in Crohn's disease [see Comment]. *Gut.* 2005;54:87–90.

30. Summers RW, Elliott DE, Urban JF Jr, et al. Trichuris suis therapy for active ulcerative colitis: a randomized controlled trial [see Comment]. *Gastroenterology.* 2005;128:825–832.

31. Summers RW, Elliott DE, Weinstock JV. Is there a role for helminths in the therapy of inflammatory bowel disease? *Nat Clin Pract Gastroenterol Hepatol.* 2005;2:62–63.

32. 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.

33. Khan WI, Blennerhasset PA, Varghese AK, et al. Intestinal nematode infection ameliorates experimental colitis in mice. *Infect Immun.* 2002; 70:5931–5937.

34. Moreels TG, Nieuwendijk RJ, De Man JG, et al. Concurrent infection with Schistosoma mansoni attenuates inflammation induced changes in colonic morphology, cytokine levels, and smooth muscle contractility of trinitrobenzene sulphonic acid induced colitis in rats [see Comment]. *Gut.* 2004;53:99–107.

35. Elliott DE, Li J, Blum A, et al. Exposure to schistosome eggs protects mice from TNBS-induced colitis. *Am J Phys Gastrointest Liver Physiol.* 2003;284:G385–G391.

36. Hunter MM, Wang A, McKay DM. Helminth infection enhances disease in a murine TH2 model of colitis. *Gastroenterology.* 2007;132:1320–1330.

37. Hunter MM, Wang A, Hirota CL, et al. 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.

38. Elliott DE, Setiawan T, Metwali A, et al. *Heligmosomoides polygyrus* inhibits established colitis in IL-10-deficient mice. *Eur J Immunol.* 2004;34:2690–2698.

39. Ince MN, Elliott DE, Setiawan T, et al. Heligmosomoides polygyrus induces TLR4 on murine mucosal T cells that produce TGFbeta after lipopolysaccharide stimulation. *J Immunol.* 2006;176:726–729.

40. Mottet C, Uhlig HH, Powrie F. Cutting edge: cure of colitis by CD4+CD25+ regulatory T cells. *J Immunol.* 2003;170:3939–3943.

41. Metwali A, Setiawan T, Blum AM, et al. Induction of CD8+ regulatory T cells in the intestine by Heligmosomoides polygyrus infection. *Am J Physiol Gastrointest Liver Physiol.* 2006;291:G253–259.

42. Jiang H, Zhang SI, Pernis B. Role of CD8+ T cells in murine experimental allergic encephalomyelitis. *Science.* 1992;256:1213–1215.

43. 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.

44. La Flamme AC, Ruddenklau K, Backstrom BT. Schistosomiasis decreases central nervous system inflammation and alters the progression of experimental autoimmune encephalomyelitis. *Infect Immun.* 2003;71: 4996–5004.

45. Sewell D, Qing Z, Reinke E, et al. Immunomodulation of experimental autoimmune encephalomyelitis by helminth ova immunization. *Int Immunol.* 2003;15:59–69.

46. Nagayama Y, Watanabe K, Niwa M, et al. *Schistosoma mansoni* and α-glaactosylceramide: prophylactic effect of Th1 immune suppression in a mouse model of Graves' hyperthryroidism. *J Immunol.* 2004;173: 2167–2173.

47. Wilson MS, Taylor MD, Balic A, et al. Suppression of allergic airway inflammation by helminth-induced regulatory T cells. *J Exp Med.* 2005; 202:1199–1212.

48. Kitagaki K, Businga TR, Racila D, et al. Intestinal helminths protect in a murine model of asthma. *J Immunol.* 2006;177:1628–1635.

49. McInnes IB, Leung BP, Harnett M, et al. A novel therapeutic approach targeting articular inflammation using the filarial nematode-derived phosphorylcholine-containing glycoprotein ES-62. *J Immunol.* 2003; 171:2127–2133.

50. Bashir ME, Andersen P, Fuss IJ, et al. An enteric helminth infection protects against an allergic response to dietary antigen. *J Immunol.* 2002;169:3284–3292.

51. Zaccone P, Fehervari Z, Jones FM, et al. Schistosoma mansoni antigens modulate the activity of the innate immune response and prevent onset of type 1 diabetes. *Eur J Immunol.* 2003;33:1439–1449.

52. Saunders KA, Raine T, Cooke A, et al. Inhibition of autoimmune type 1 diabetes by gastrointestinal helminth infection. *Infect Immun.* 2007;75: 397–407.

53. Mangan NE, van Rooijen N, McKenzie AN, et al. Helminth-modified pulmonary immune response protects mice from allergen-induced airway hyperresponsiveness. *J Immunol.* 2006;176:138–147.

54. Erb KJ. Helminths, allergic disorders and IgE-mediated immune responses: where do we stand? [Review]. *Eur J Immunol.* 2007;37:1170–1173.

55. Nair MG, Guild KJ, Artis D. Novel effector molecules in type 2 inflammation: lessons drawn from helminth infection and allergy [Review]. *J Immunol.* 2006;177:1393–1399.

56. Melendez AJ, Harnett MM, Pushparaj PN, et al. Inhibition of Fc epsilon RI-mediated mast cell responses by ES-62, a product of parasitic filarial nematodes [see Comment]. *Nat Med.* 2007;13:1375–1381.

57. Schnoeller C, Rausch S, Pillai S, et al. A helminth immunomodulator reduces allergic and inflammatory response by induction of IL-10-producing macrophages. *J Immunol.* 2008;180:4265–4272.

58. MacDonald AJ, Libri NA, Lustigman S, et al. A novel, helminth-derived immunostimulant enhances human recall response to hepatitis C virus and tetanus toxoid and is dependent on CD56+ cells for its action. *Clin Exp Immunol.* 2008;152:265–273.

59. Sabin EA, Araujo MI, Carvalho EM, et al. Impairment of tetanus toxoid-specific Th1-like immune responses in humans infected with Schistosoma mansoni. *J Infect Dis.* 1996;173:269–272.

60. Chen CC, Louie S, McCormick BA, et al. Helminth-primed dendritic cells alter the host response to enteric bacterial infection. *J Immunol.* 2006;176:472–483.

61. Weng M, Huntley D, Huang IF, et al. Alternatively activated macrophages in intestinal helminth infection: effects on concurrent bacterial colitis. *J Immunol.* 2007;179:4721–4731.