Helminth Infection Enhances Disease in a Murine TH2 Model of Colitis

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Background & Aims: **There is convincing evidence from animal and human studies that infection with parasitic helminths can alleviate the histopathology and symptoms of colitis. Here the ability of the rat tapeworm *Hymenolepis diminuta* to affect the course of oxazolone-induced colitis (a TH2 model) was assessed.** ***Methods:*** **Mice were infected with *H diminuta* and 8 days later they received oxazolone (3 mg in 50% EtOH, intrarectal). On autopsy (3 or 7 days postoxazolone), disease severity was assessed by macroscopic clinical scores, histologic damage scores, myeloperoxidase and eosinophil peroxidase activity, and cytokine synthesis.** ***Results:*** **As gauged by all markers of gut function, infection with *H diminuta* caused a significant exacerbation of oxazolone-induced colitis. Indeed, while mice receiving oxazolone only began to recover ~3–4 days posttreatment, the cotreated group continued to deteriorate. Helminth infection, independent of oxazolone administration, enhanced IL-4, IL-5, IL-10, and IL-13 production from in vitro stimulated immune cells and evoked increases in colonic eosinophil peroxidase of cotreated mice. Finally, while knockout of natural killer (NK) and NK-T cells by administration of a neutralizing NK1.1 antibody reduced the inflammation in oxazolone and oxazolone + *H diminuta*-treated animals, mice in the latter group still displayed significant colitis.** ***Conclusions:*** **We have shown that *H diminuta* infection is beneficial in other models of colitis. The current data is presented as a caveat to the position that parasitic helminths in general can be considered as a therapy for heterogeneous inflammatory disorders without careful analysis of the immunologic basis of the condition.**

BASIC–ALIMENTARY TRACT

There has been a rapid increase in interest in biologicals as new therapies for inflammatory bowel disease (IBD), with the most notable, at least to date, being the effectiveness of antitumor necrosis factor alpha (TNFα) treatment.[1] Moreover, other candidate molecules such as recombinant interleukin (IL)-10 and granulocyte-macrophage colony-stimulating factor, antiadhesion molecule antibodies, and probiotics are being tested for clinical efficacy.[2,3] In this context, provocative data have arisen from animal models of gut inflammation[4–7] and clinical observations by Weinstock and colleagues[8,9] in support of the hypothesis that infection with parasitic helminths can ameliorate IBD. The premise for the consideration of parasitic helminths as an anticolitic strategy stems from 2 observations: a general lack of Crohn's disease in global areas where helminth infections are epidemic,[10] and the postulate that the increase in T helper cell (TH)2-type cytokines that accompanies helminth infection would ablate disease characterized by TH1 cytokines, such as Crohn's disease.[11] Thus far, data from animal studies support this postulate, and although the number of individuals with Crohn's disease that have received ova of the nematode *Trichuris suis* as a therapy is small, their response to this treatment has been promising.

Based on this immunologic argument, it follows that infection with parasitic helminths should exacerbate disorders that are mediated by TH2-type cytokines such as atopy and asthma. However, epidemiologic observations do not support this.[12,13] Indeed, infection with the nematode, *Heligmosomoides polygyrus*, was protective in a murine model of asthma.[14] Moreover, ulcerative colitis may be a TH2-type condition, and yet patients receiving *T suis* ova reported symptomatic relief.[9] In light of such data, a newer concept has evolved in which helminth infection may prevent, or reduce, the severity of other diseases not by simply skewing the cytokine spectrum toward TH2-type cytokines but by generating an immunoregulatory environment, in which IL-10, transforming growth factor beta (TGFβ), and regulatory T cells predominate.[15–17] We have contributed to this view, and as an adjunct to analyses of the mechanism of helminth (specifically the rat tapeworm *Hymenolepsis diminuta*) amelioration of murine colitis associated with TH1 cytokines,[4,18] the present investigation was designed to assess the impact of helminth infection on colitis evoked by the hapteniz-

Abbreviations used in this paper: AB, antibodies; AMT, 3-amino-1,2,4-triazole; ELISA, enzyme-linked immunosorbent assay; EPO, eosinophil peroxidase; IBD, inflammatory bowel disease; IL, interleukin; IR, intrarectal; MLNs, mesenteric lymph nodes; MPO, myeloperoxidase; NK, natural killer; RT-PCR, reverse transcriptase polymerase chain reaction; SEM, standard error of the mean; TGFβ, transforming growth factor; TH, T helper; TNFα, tumor necrosis factor alpha.

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ing agent oxazolone, the administration of which increases IL-4 and subsequently IL-13 (ie, TH2-dominated events).[19–21]

The data herein demonstrate, contrary to our expectation, that mice cotreated with *H diminuta* and oxazolone develop significantly more severe colonic disease than those receiving oxazolone only. Although we support the potential value of helminth therapy for specific patients with IBD (and indeed other inflammatory disorders), we present these data as a cautionary note. Thus, careful screening of the immunologic basis of a patient's disease should be paired with the selection of the appropriate parasitic helminth to maximize the benefit of helminth therapy and avoid the possibility of disease exaggeration.

Materials and Methods

Helminth Infection and the Induction of Oxazolone Colitis

Male BALB/c mice (7–9-weeks-old, Harlan Animal Suppliers, Indianapolis, IN) were housed in filter-topped cages in the McMaster University Central Animal Facility. Each mouse received 5 infective *H diminuta* cysticercoids in 100 μL of 0.9% NaCl by oral gavage,[22] and 8 days later received an intrarectal (IR) instillation of oxazolone (3 or 4 mg in 100 μL of 50% ethanol; Sigma Chemical Co, St. Louis, MO) via a polyethylene catheter delivered 3 cm into the colon[19] (oxazolone was dissolved by rocking in a covered tube overnight). Mice were autopsied 3 or 7 days postoxazolone or humanely sacrificed if they reached a predetermined experimental end point (ie, loss of $\geq$20% body weight and significant deterioration of body condition). To check for worm infectivity, blood smears were stained using the Hema3 stain set (Fisher Scientific, Mississauga, ON), and successful infection was set at $\geq$3% eosinophils (based on a cell count of 300). Controls consisted of naïve, *H diminuta*-infected, and oxazolone-only treated animals. All experiments complied with the Canadian guidelines for animal welfare and the regulations specified by the animal care committee at McMaster University.

Macroscopic Assessment

Mice were examined daily for signs of colitis, including wet anal area, diarrhea, bleeding, weight loss, and/or behavioral changes. At autopsy, the entire colon was removed and examined for signs of damage, soft or loose stool, the accumulation of fluid and bleeding, and a clinical score was determined using a 5-point scale.[4] Due to shortening of the colon in colitic animals, the colon was divided for further assessment based on total length: the distal most 20% was discarded, the adjacent 20% was snap frozen in liquid nitrogen for use in myeloperoxidase (MPO) and eosinophil peroxidase (EPO) assays, and the next 10% fixed in formalin for histologic analysis. In an additional experiment, 1-cm sections of distal colon were excised and snap frozen for subsequent reverse transcriptase polymerase chain reaction (RT-PCR) analyses.

Histologic Assessment

Segments of the colon were fixed in 10% neutral-buffered formalin, dehydrated in graded alcohols, and cleared in xylene before embedding in paraffin wax. Sections (3 μm) were collected on coded slides, stained with hematoxylin and eosin, and examined for damage. The histology damage score was calculated on a 12-point scale.[4] Additional sections were stained with Congo red and hematoxylin for the enumeration of eosinophils: positive cells were defined on the basis of a red granular cytoplasm and a bilobed nucleus and were enumerated per histologic section of colon examined under the high power (40$\times$) microscope objective.

Myeloperoxidase (MPO) and Eosinophil Peroxidase (EPO) Assay

MPO activity was assayed according to an established protocol.[23] In brief, the presence of MPO, an enzyme found in the granulocytes, was assessed using a kinetic assay where H_2O_2 is broken down by the MPO released from the samples of colon. The data are presented as units per milligram of tissue, where 1 unit is equal to the amount of MPO required to degrade 1 μmol/L of H_2O_2 per minute. This assay was repeated on duplicate sample aliquots with the addition of 50 mmol/L 3-amino-1,2,4-triazole (AMT; Sigma Chemical Co) to inhibit EPO.[24] EPO activity was calculated by subtracting the MPO + AMT value from MPO values.

Cytokine Analysis

Spleens and mesenteric lymph nodes (MLNs) were removed, placed in RPMI media containing 5% bovine fetal serum (Sigma Chemical Co), and mashed through a 200-μm mesh nylon screen. Pelleted cells were treated with lysis buffer (0.15 M NH_4Cl, 10 mmol/L $KHCO_3$, and 0.1 mmol/L Na_2EDTA) to remove erythrocytes. Isolated cells (5×10^6 cells/mL) were cultured with 2 μg/mL concanavalin A (conA: Sigma Chemical Co) for 48 hours, the supernatants collected, and the levels of IL-4, IL-5, IL-10, IL-13, and TGFβ determined by enzyme-linked immunosorbent assay (ELISA) following the manufacturer's guidelines (R&D Systems, Minneapolis, MN). For samples in which TGFβ was to be measured, cells were switched to serum-free media with 4 changes in media over a 12-hour period to reduce the background level of TGFβ provided by the cell culture serum. Samples were treated with 1 N HCl (then neutralized with 1.2 N NaOH/0.5 M HEPES) to activate latent TGFβ, and hence, the data presented reflect total TGFβ in the samples.

RNA was extracted from colonic tissue samples using the TRIZOL extraction method (Invitrogen Life Technologies, Burlington, ON) and cDNA synthesized from 1 μg