Tapeworm Infection Reduces Epithelial Ion Transport Abnormalities in Murine Dextran Sulfate Sodium-Induced Colitis

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The rat tapeworm Hymenolepis diminuta was used to test the hypothesis that helminth infection could modulate murine colitis. Mice were infected with five H. diminuta cysticercoids, and colitis was evoked via free access to 4% (wt/vol) dextran sulfate sodium (DSS)-containing drinking water for 5 days. BALB/c mice were either infected with H. diminuta and 7 days later exposed to DSS (prophylactic strategy) or started on DSS and infected with H. diminuta 48 h later (treatment strategy). Naive and H. diminuta-only-infected mice served as controls. On autopsy, colonic segments were processed for histological examination and myeloperoxidase (MPO) measurement or mounted in Ussing chambers for assessment of epithelial ion transport. Cytokines (gamma interferon [IFN-γ], interleukin 12 [IL-12], and IL-10) were measured in serum and colonic tissue homogenates. DSS treatment resulted in reduced ion responses (indicated by short-circuit current [Isc]) to electrical nerve stimulation, the cholinergic agonist carbachol, and the adenylyl cyclase activator forskolin compared to controls. H. diminuta infection, either prophylactic or therapeutic, caused a significant (P < 0.05) amelioration of these DSS-induced irregularities in stimulated ion transport. In contrast, the histopathology (i.e., mixed immune cell infiltrate, edema, and ulcerative damage) and elevated MPO levels that accompany DSS colitis were unaffected by concomitant H. diminuta infection. Similarly, there were no significant differences in levels of IFN-γ, IL-12, or IL-10 in serum or tissue from any of the treatment groups at the time of autopsy. We suggest that abolishment of colitis-induced epithelial ion transport abnormalities by H. diminuta infection provides proof-of-principle data and speculate that helminth therapy may provide relief of disease symptoms in colitis.

The dichotomous split of helper T lymphocytes into type 1 (Th1) or type 2 (Th2) cells, as originally proposed by Mosmann and colleagues (24), has provided a convenient conceptual framework for characterizing T-cell responses and immunological processes. While the Th1-Th2 paradigm does not hold true for all situations and is certainly a simplified view of in vivo T cell responses (23), it has nevertheless had a profound impact on the approach to understanding host responses to antigen, infection, and disease processes in general.

The T-cell response of rodents infected with parasitic nematodes is typically skewed towards a Th2-dominated cytokine profile (i.e., elevated levels of interleukin 4 [IL-4], IL-5, and IL-10) (13), and experiments that block or enhance the Th2 response result in predicted increases in susceptibility to or rejection of the helminth parasite, respectively (14). Fewer data are available for cestode infections (28); nevertheless, the increases in mast cells, immunoglobulin E, eosinophils, and goblet cell mucin production are consistent with a Th2 host response (21). The reciprocal cross-regulation between Th2 and Th1 cells suggests that helminth infection could prevent or reduce the effects of diseases that are characterized by Th1 responses (i.e., IL-12-driven production of gamma interferon [IFN-γ], tumor necrosis factor alpha [TNF-α], and lymphotoxin). Indeed, preliminary data have been presented showing that concomitant Schistosoma mansoni or Trichuris muris infection reduced the histopathology of colitis in mice (D. E. Elliott, J. Li, C. Crawford, A. Blum, A. Metwali, K. Qadir, J. Urban, and J. V. Weinstock, Abstract, Gastroenterology 116: A706, 1999; D. E. Elliott, C. Crawford, J. Li, A. Blum, A. Metwali, K. Qadir, J. F. Urban, and J. V. Weinstock, Abstract, Gastroenterology 118:A863, 2000).

The present study tested the hypothesis that cestode infection could reduce the severity of a chemically induced murine colitis. Mice were infected with Hymenolepis diminuta (noting that this is a nonpermissive system and the worm burden is immunologically rejected within 10 to 14 days of infection [21]) either before or after exposure to the procolitic agent dextran sulfate sodium (DSS) (9, 27). In both prophylactic and treatment regimens, H. diminuta infection significantly reduced the epithelial ion transport abnormalities that characterize DSS colitis, while unexpectedly, it did not have an impact on the DSS-induced histopathology in the colon.

MATERIALS AND METHODS
Induction of colitis and helminth infection. Male BALB/c mice, 6 to 8 weeks old (Harland Animal Suppliers, Indianapolis, Ind.), were housed under conventional conditions for ≥1 week prior to examination. Colitis was induced by allowing the mice free access to 4% (wt/vol) DSS (40 kDa; ICN Biomedicals Inc., Aurora, Ohio) in their drinking water for 5 days (9). Mice were infected with five cysticercoids of H. diminuta by oral gavage in 100 μl of sterile phosphate-buffered saline (21). Two experimental protocols were employed. In the prophylactic protocol, mice were first infected with H. diminuta, and 7 days later they were treated with 4% DSS in drinking water for 5 days and then sacrificed. In the treatment protocol, mice were exposed to 4% DSS for 2 days and then infected with H. diminuta; the DSS-water was replaced with normal water 3 days later, and the animals were sacrificed after a subsequent 4 days (i.e., 5 days of DSS exposure overlapped with a 7-day helminth infection). Controls consisted of time-matched naive mice and animals given 4% DSS or H. diminuta only. As an independent check on worm infectivity, segments of small intestine from mice given H. diminuta by gavage were formalin fixed and embedded in paraffin, and
sections were stained with periodic acid-Schiff's reagent to stain mucopolysaccharides and allow identification of goblet cells (21). In all experiments, initial and final animal body weights were recorded and total water intake was noted at the end of the 5-day exposure to DSS-containing drinking water. All experiments conformed to the Canadian guidelines for animal welfare and were in compliance with the regulations specified by the Animal Care Committee at McMaster University.

Macroscopic assessment. Throughout the experimental period, mice were examined daily for signs of immune activation and intestinal dysfunction, including behavioral and postural changes, fur ruffling, wet and/or feces-stained anal area, and anal bleeding. On the day of autopsy, mice were anesthetized, blood was collected by orbital bleeding, and the animal sacrificed by cervical dislocation. The abdomen was opened, and the colon was exposed and examined for signs of fluid accumulation, fecal content, and bleeding (i.e., occult blood, hyperemic appearance, and ulceration). The entire colon from ileal-cecal junction to anus was excised and measured at rest without stretching. Colonic shortening occurs in DSS-induced colitis (9), and so the colon was divided based on percentage of total colon length: the proximal 30% was discarded, the next 30% was utilized in Ussing chamber studies, the adjacent 10% was fixed for histological examination, and the final 30% was snap-frozen in liquid N2 for determination of myeloperoxidase (MPO) activity.

Analysis of colonic epithelial ion transport. A single, whole-thickness segment of mid-distal colon (exposed surface area = 0.6 cm²) from each mouse was mounted in an Ussing chamber, and ion transport was assessed. As previously described (18), each side of the Ussing chamber was filled with 10 ml of Krebs buffer containing 10 mM glucose that was maintained at 37°C by a surrounding heated water jacket and circulated by an oxygenating gas lift. The spontaneous potential difference across the tissue was maintained at 0 V by an automated voltage clamp (World Precision Instruments, Mississauga, Ontario, Canada), and the required short-circuit current (Isc; in microamperes per square centimeter) was continuously monitored as a measure of net active ion transport across the preparation. Baseline Isc was recorded after a 15-min equilibrium period. Subsequently, each tissue was treated with three prosecretory stimuli, in the following order, and the maximal change in Isc that occurred within 10 min of treatment was recorded: (i) transmural electrical field stimulation (EFS; 10 Hz, 10 M; Sigma Chemical Co., St. Louis, Mo.); and (ii) the cholinergic agonist carbachol (CCCh; 10⁻⁷ M; Sigma Chemical Co., St. Louis, Mo.); and (ii) the adenylate cyclase-activating agent forskolin (FSK; 10⁻⁵ M; Sigma Chemical Co.). Previous studies have shown that these stimuli, at these doses, predominantly elicit a long-lasting directed Cl⁻ efflux (5).

Colon histology and MPO levels. Colon sections were fixed in 10% neutral buffered formalin, dehydrated through graded alcohols, cleared in xylene, and embedded in paraffin wax. Sections (3 μm thick) were collected onto coded slides, stained with hematoxylin and eosin, and examined by two investigators (C.R. and D.M.M.). A damage score was calculated using the criteria outlined by Appleyard and Wallace (2), where a score of 11 is considered maximum tissue damage. MPO activity was measured following a published protocol (33), and data are presented as units per milligram (wet weight) of tissue, where one unit of activity is defined as the amount of MPO required to degrade 1 μM H2O2 per min at room temperature.

Serum and tissue cytokine levels. Levels of IFN-γ, IL-12, and IL-10 in serum were measured by an enzyme-linked immunosorbent assay (developed and validated at Michigan University [30]) that had detection limits of 25 pg/ml. In an additional experiment, the region of colon designated for physiological studies was instead weighed, homogenized in sterile phosphate-buffered saline containing a cocktail of protease inhibitors (leupeptin [2 μg/ml], pepstatin A [2 μg/ml], aprotinin [10 μg/ml], phenylmethylsulfonyl fluoride [100 μg/ml]; all from Sigma Chemical Co.), and centrifuged at 1,500 rpm (MSE MicroCentaur; Sanyo) for 10 min, and the supernatant was saved for measurement of IFN-γ, IL-12, and IL-10 levels. All cytokine determinations were performed by a single investigator (C.M.H.), who was unaware of the treatment groups.

Data presentation and analysis. Experiments were repeated three times, and data from all mice are presented as means ± standard errors of the means (SEM). Data were compared by one-way analysis of variance (WINKS software; Tersoft, Cedarhill, Tex.) followed by post hoc pairwise comparisons with the Newman-Keuls test, where a P value of <0.05 was accepted as a level of statistically significant difference.

RESULTS

(i) Prophylactic protocol. As previously described (21), H. diminuta infection resulted in a clear increase in the number of goblet cells in the small infection (data not shown). Daily average water or DSS-water intake was not significantly different between any of the groups (milliliters per mouse per day: control, 4.3 ± 0.4; H. diminuta, 4.3 ± 0.6; DSS, 3.8 ± 0.1; and H. diminuta plus DSS, 4.0 ± 0.2 [data from three experiments]), indicating that any effect of H. diminuta infection (see below) was not due to a general feeling of malaise causing reduced intake of the procolitic DSS-containing water. Similar water intake patterns have been reported (1). Mice receiving DSS with or without H. diminuta displayed a significant weight loss (Table 1). On autopsy, colons from control mice were normal in appearance, contained hard fecal pellets, and measured 103 ± 3 mm (n = 9); colons from H. diminuta-infected mice were indistinguishable from those of controls (Table 1). Colons from DSS-treated mice were significantly shortened, pale, and distended; ~50% of the colons showed fluid accumulation, and eight of nine animals had soft, blood-tinted stools. In contrast, the colons of H. diminuta-plus-DSS-treated mice showed macroscopic improvement, with distinct fecal pellets and with blood being present in the stool of only one of nine mice. Colons were significantly shorter than those of controls, however (Table 1).

(ii) Epithelial ion transport. Neither baseline colonic Isc nor ion conductance, an indicator of passive ion transport (as calculated by the ohmic relationship from Isc and the spontaneous potential difference measured under open-circuit conditions), was consistently altered by infection with H. diminuta or exposure to DSS (Table 1). Assessment of the responses to prosecretory stimuli revealed no significant differences between tis-
The concept that parasitic infection can modulate the course of nonparasitic disease is not unprecedented, and Desowitz has documented a number of provocative, albeit anecdotal, examples of “harmonious parasites” (8). More recently, coinfection with the parasitic nematode Heligmosomoides polygyrus was found to counter Helicobacter felis-induced murine gastric atrophy (16). The reciprocal inhibition (or antagonism) of Th1-type (i.e., IL-12 and IFN-γ) and Th2-type (i.e., IL-4 and IL-10) cytokines supports the hypothesis that helminth infection would ameliorate disease where a Th1 profile predominates, such as Crohn’s disease. Indeed, Elliott et al. (12) have collated epidemiological data illustrating a reduced incidence of diagnosed Crohn’s disease in areas of endemic helminthiasis. The same investigators presented preliminary data showing that infection with parasitic nematodes can reduce the severity of Crohn’s disease and the histopathology that accompanies trinitrobenzenesulfonic acid-induced colitis in mice (Elliott et al., Gastroenterology 116: A706; R. W. Summers, J. Urban, D. Elliott, K. Qadir, R. Thompson, and J. Weinstock, Abstract, Gastroenterology 116: A828, 1999). Here, we provide additional support in favor of this concept by showing that tapeworm infection reduced the colonic epithelial ion transport irregularities observed in murine DSS-induced colitis.

In assessing the impact of helminth infection on colitis, we used H. diminuta for three main reasons. First, this worm has
no hooks or teeth and so causes no abrasive damage in the gut; it is a noninvasive parasite. Second, the mouse is a nonpermissive host for *H. diminuta*, expelling a primary infection within 10 to 14 days via an immunological mechanism requiring thymus-dependent T cells. Third, the impact of tapeworms on colitis has not hitherto been examined, and if the worm is to be considered a novel biological anticolitic therapy, it can also be readily eradicated (i.e., via praziquantel therapy) at a patient’s request. The ability of anti-TNF-α, anti-IFN-γ, anti-IL-1, and recombinant IL-10 to ameliorate DSS colitis (3, 25, 26, 31) suggests that at least part of the pathophysiology of this model is mediated by a Th1 response, allowing us to test the paradigm that helminth infection may reduce Th1-dominated enteric inflammation.

FIG. 2. (A) Change in Isc in colonic tissue in response to CCh (10^{-4} M). Mice were infected with five cysticercoids of *H. diminuta* with or without a subsequent 5-day exposure to 4% (wt/vol) DSS-containing drinking water (a negative value indicates a drop in Isc; means ± SEM; * and #, P < 0.05 compared to other groups; n = 7 or 8 mice from three separate experiments). (B) Four representative Isc responses to CCh (arrow): a, control; b, *H. diminuta* infection; c, 4% DSS treatment; d, *H. diminuta* plus DSS (prophylactic protocol).
The results from six separate experiments (three using a prophylactic and three using a treatment regimen) revealed that tapeworm infection led to a significant improvement in the macroscopic appearance of the colon compared to those of DSS-only-treated mice. This subjective finding was supported by the observation that H. diminuta infection reduced the ion transport abnormalities that accompany DSS-induced colitis. Vectorial, electrogenic ion transport creates the driving force for water movement and thus regulates water balance in the intestine, the extremes of which can result in debilitating diarrhea or constipation. Assessment of tissues from other rodent models of colitis and examination of tissue resections from patients with inflammatory bowel disease consistently reveal altered ion transport, typically reduced responses to prosecretory stimuli (4, 7, 17). Thus, the ability of H. diminuta infection to normalize, at least in part, the colonic ion transport in the face of colitis supports the contention that helminth infection is of benefit in preventing or treating some forms of colitis.

Analysis of colonic structure revealed that tissues excised from DSS-only- and DSS-plus-H. diminuta-treated mice were not appreciably different. This is a somewhat paradoxical finding given the clear improvement in colonic function (i.e., epithelial ion transport) elicited by helminth infection. Also, MPO levels were significantly higher in colonic tissues from DSS-H. diminuta-treated mice than in those from DSS-only-treated mice. In this context, chemokine (specifically, CCR2 and CCR5)-deficient mice developed less colonic damage in response to DSS but had similar numbers of macrophages, CD4+ T cells, and neutrophils (as determined by MPO levels) in their colons (1). Collectively, these data suggest that while MPO measurement is a good marker for infiltrating polymorphonuclear cells, it may not reflect leukocyte activity—i.e., cells are recruited to the gut but may not receive the proper stimuli to allow full activation. Currently, the mechanism underlying this divergence in gut form and function is unclear, but it could be due to the fact that the damage in this model is patchy and so the unaffected tissue may remain normal because of differences in the colonic milieu in DSS-only- versus DSS-plus-H. diminuta-treated mice. It is noteworthy that there are instances in which patients with inflammatory bowel disease report a lack of disease symptoms but do have endoscopic evidence of inflammation (S. M. Collins, personal communication).

Despite the lack of improvement in colonic histopathology, the presence of H. diminuta or the response to helminth infection did lead to improved ion transport parameters. The simplest explanation for the beneficial effect of H. diminuta in this system is that a helminth-evoked Th2 response is opposing a procolitic Th1-dominated event. Measurement of Th1 and Th2 characteristic cytokines in serum or colonic tissue homogenates revealed no significant differences in IL-12, IFN-γ, or IL-10 levels between the experimental groups. However, changes in the Th1-versus-Th2 cytokine balance early in the infection or during the induction of colitis are likely important and would have been missed in this study. Indeed, mitogenic stimulation of mesenteric lymph node lymphocytes from H. diminuta-infected BALB/c mice resulted in preferential production of Th2-type cytokines (28).

Other putative consequences of H. diminuta should be considered, such as changes in levels of other immune mediators, for all of which a cogent case can be made for modulation of gut function (29). Indeed, the H. diminuta-mouse system is a nonpermissive model of enteric parasitosis, and the immune response directed against the worm (e.g., increased goblet cell activity [21]) would impinge on other infections or immune events. Also, H. diminuta is a small-intestine-dwelling worm and enters the colon only when being expelled from the host. We have shown that H. diminuta-infected mice have increased levels of substance P and serotonin-positive enteroendocrine cells and decreased vasoactive intestinal peptide levels in their small intestines at the peak time of worm rejection (20, 22).

### TABLE 2. Cytokine levels

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum (ng/ml)</th>
<th>Tissue (pg/mg)</th>
<th>Level of cytokine in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IFN-γ IL-12</td>
<td>IL-10 IFN-γ</td>
<td>IL-12 IFN-γ IL-10</td>
</tr>
<tr>
<td>Control</td>
<td>0.17 ± 0.11</td>
<td>2.59 ± 0.38</td>
<td>ND</td>
</tr>
<tr>
<td>H. diminuta</td>
<td>1.11 ± 0.32</td>
<td>3.98 ± 0.31</td>
<td>109 ± 44  42 ± 4  7 ± 3</td>
</tr>
<tr>
<td>4% DSS</td>
<td>0.46 ± 0.27</td>
<td>2.48 ± 0.68</td>
<td>186 ± 11  26 ± 5  10 ± 3</td>
</tr>
<tr>
<td>H. diminuta +</td>
<td>0.86 ± 0.25</td>
<td>1.99 ± 1.27</td>
<td>210 ± 30  45 ± 4  10 ± 5</td>
</tr>
</tbody>
</table>

*Values are means ± SEM; n = 4 for serum and n = 5 for tissue. ND, not detected.*
Thus, in the context of neuroimmunodulation of gut function (6, 19), it is feasible that cestode-induced changes in neuropeptides and neural communication between the small and large bowels account for the reduced epithelial ion transport abnormalities seen in DSS-plus-H. diminuta treated mice. Another intriguing possibility is that the benefit of H. diminuta infection is via modulation of enteric bacterial populations, as has been shown in infected rats (11). In this context, DSS colitis is at least partially driven by the gut microflora (32). Finally, helminths can modulate their environment by the release of immunosuppressive molecules and analogues of mammalian neuropeptides (10, 15). Thus, the possibility that H. diminuta is actively involved in regulating gut function should not be overlooked; however, given the fact that the worms never achieve a large biomass in the mouse, the relevance of molecules released from the worm in this particular model system is debatable. Indeed, this study raises many questions pertinent to defining the precise mechanism by which H. diminuta infection ameliorated the ion transport abnormalities that occur in the colon of DSS-treated mice and to understanding the lack of impact on DSS-evoked colonic histopathology. These issues are the subject of current laboratory investigations.

In summary, this is, to our knowledge, the first demonstration that cestode infection given before or after exposure to a procolitic stimulus can ameliorate the epithelial ion transport irregularities observed in colitis, serving as a proof-of-principle demonstration of the therapeutic benefit of helmint infection in a putative Th1-type model of colitis. While the thought of using a parasite as a therapeutic modality will appear distasteful to many, this may be countered by the development of a biological therapy that brings relief from intestinal inflammatory disease symptomatology with minimal side effects.

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REFERENCES


