IL-5 contributes to worm expulsion and muscle hypercontractility in a primary T. spiralis infection

Bruce A. Vallance, Patricia A. Blennerhassett, Yikang Deng, Klaus I. Matthaei, Ian G. Young, and Stephen M. Collins

Intestinal nematode infections lead to increased interleukin (IL)-5 expression, eosinophilic inflammation, and intestinal smooth muscle hypercontractility. Although eosinophils release inflammatory mediators that cause smooth muscle contraction, the role of IL-5 and eosinophils in enteric smooth muscle hypercontractility is unclear. IL-5-deficient mice and their wild-type controls were infected with the nematode Trichinella spiralis. Intestinal parasites and eosinophils were counted, and jejunal longitudinal muscle contractility was assessed. During infection, IL-5 gene expression increased significantly in wild-type mice and was accompanied by significant intestinal eosinophilia in wild-type but not IL-5-deficient mice. Although both strains developed increased muscle contractility during infection, contraction was significantly less in the IL-5-deficient mice at days 16 and 21 postinfection. In addition, parasite expulsion was transiently delayed at day 16 in IL-5-deficient mice. Thus, in the nematode-infected mouse, IL-5 appears essential for intestinal eosinophilia and contributes to, but is not essential for, the development of muscle hypercontractility. IL-5 also appears to play a minor role in expelling a primary T. spiralis infection from the gut.

Eosinophils; nematode; intestine

Blood and tissue eosinophilia are characteristic of the mammalian host response to nematode infections (40). The cytokine interleukin (IL)-5 is thought to mediate the proliferation of eosinophils during infection (51). Although there is a clear relationship between nematode infections and eosinophilia, it is uncertain whether eosinophils are actually responsible for the expulsion of nematodes from the intestine of infected hosts. Eosinophilic inflammation in general, and nematode infections in particular, are also associated with local tissue dysfunction (31, 33). We and others (6, 13, 15, 43, 46) have previously described the development of increased intestinal smooth muscle contractility during the course of enteric nematode infections. We have also recently shown (4) that the changes in the intestinal neuromuscular apparatus can persist long after a Trichinella spiralis infection has been cleared.

The changes in muscle function have been shown to be, at least in part, immune mediated and involve T lymphocytes (9, 44). Since worm expulsion is also T cell dependent, we have hypothesized that the increases in intestinal muscle contractility and the expulsion of the parasites may be linked. In this regard, attention has focused on the role of Th2 cytokines (9), including both IL-4 and IL-5. We recently demonstrated (45) that local overexpression of IL-4, caused by infecting the serosal surface of mouse small intestine with an adenoviral vector encoding the IL-4 gene, led not only to a localized eosinophilic inflammation but to an increase in the muscle contraction generated by jejunal longitudinal muscle. The prominence of eosinophils in this infection, as well as during nematode infections, led us to consider a causal role for eosinophils in the altered enteric muscle function and in worm expulsion. Supporting this hypothesis are experiments showing that eosinophil-derived mediators can cause smooth muscle contraction in tissue baths (1, 21) as well as kill T. spiralis larvae in vitro (18, 28). Therefore, worm expulsion and increased muscle function could have a common basis in the eosinophil, with eosinophil-derived products maintaining host defense not only by damaging the parasite but also by causing increased gut propulsion through their effects on muscle.

Unfortunately, little direct experimental evidence exists identifying a causal role for eosinophils in nematode expulsion or in the pathophysiology of enteric smooth muscle; however, IL-5 and eosinophils have been implicated in bronchial muscle hyperreactivity, although results appear to depend on the system used (10, 14, 20). Several studies that used neutralizing monoclonal antibodies to IL-5 to block the eosinophilia during nematode and helminth infections found little role for eosinophils in controlling these infections at either the adult or larval stage (8, 19, 34). However, these results conflict with in vitro data and may reflect an inability to completely neutralize the target IL-5 protein and its function. Added to the inherent limits of neutralizing antibodies are the often prohibitive costs associated with their chronic administration.

To overcome these difficulties, recent advances in recombinant DNA technology have permitted the development of mice that specifically lack a functional IL-5 gene (26). Using these mice, as well as their IL-5 expressing controls, we examined the role of IL-5 in intestinal muscle pathophysiology as well as host defense during T. spiralis infection. Our results demonstrate a critical role for IL-5 in the recruitment of eosinophils to the intestine throughout a primary T.
spiralis infection. There was also a transient delay in the expulsion of worms from the intestine of IL-5-deficient mice during the late stages of the infection. However, IL-5 expression had no effect on the number of muscle-stage larvae recovered. Finally, although IL-5 was not critical for the initiation of intestinal smooth muscle hypercontractility, it contributed to the increased tension development seen at the later stages of the infection and after its resolution. These studies therefore indicate that IL-5 contributes to, but is not essential for, controlling the intestinal phase of a T. spiralis infection and in the development and maintenance of enteric smooth muscle hypercontractility.

MATERIALS AND METHODS

Mice. C57BL/6 mice lacking IL-5 (IL-5−/− mice) were originally produced by targeted gene mutation as described by Kopf et al. (26). Breeding pairs of IL-5−/− and +/− mice were obtained from the John Curtin School of Medical Research (Australian National University, Canberra, Australia) and were kept and bred under specific pathogen-free conditions at the animal facilities of McMaster University (Hamilton, ON, Canada). Animals were kept in sterilized, filter-topped cages, handled in tissue culture hoods, and fed autoclaved food. Also, sentinel animals were routinely tested for common pathogens. The protocols employed were in direct accordance with guidelines drafted by the McMaster University Animal Care Committee and the Canadian Council on the Use of Laboratory Animals.

Trichinella infection. The T. spiralis parasites used in this study originated in the Department of Zoology at the University of Toronto, and the colony was maintained through serial infections alternating between male Sprague-Dawley rats and male CD1 mice. The larvae were obtained from infected rodents 60–90 days postinfection by a modification of the technique described by Castro and Fairbairn (7). Mice were infected by administration of 0.1 ml of PBS containing 375 T. spiralis by gavage. To minimize differences between infections, both IL-5−/− and +/− mice were infected with the same T. spiralis preparations.

DNA extraction. For analysis of IL-5 mRNA expression within the external muscle layers, control uninfected IL-5−/− and +/− mice were infected with the same T. spiralis preparations.

Detection of IL-5 by RT-PCR. For analysis of IL-5 mRNA expression within the external muscle layers, control uninfected IL-5−/− and +/− mice were infected with the same T. spiralis preparations.

Histology, tissue eosinophil, and peripheral basophil counts. Mice were killed and jejunal tissue, skeletal muscle from the hind legs, and tissue from the diaphragm were fixed in 10% neutral buffered Formalin. Sections (3 µm) were cut, and sections were stained with hematoxylin-eosin or were stained with Congo red and lightly counterstained with hematoxylin. Intestinal tissue eosinophil counts were performed by counting the number of polymorphonuclear leukocytes that stained intensely with Congo red, found within 20 villus crypt units (vcu), including the underlying muscle, per mouse (53). The number of eosinophils was then referred to as eosinophils per vcu. Photomicrographs were taken with a Zeiss camera. Basophils in the peripheral blood were also counted, with mice periodically bled from the tail vein, and the resulting peripheral blood smears stained with Diff Quik (Dade Behring, Newark, DE) for differential cell counts. Granulocytes possessing large dark-staining granules (42) were counted, with a minimum of 350–450 leukocytes examined per slide.

Adult worm counts. The entire length of the small intestine was removed and opened longitudinally, and all adult worms within the small intestine were then counted by a modification of the method described by Castro and Fairbairn (7). Briefly, the mucosa was separated from the underlying muscularis by scraping with a glass microscope slide and was mixed with 1 ml of PBS. The worms were then counted with the use of a scored petri dish and an inverted microscope. In accordance with established practices, worm rejection was considered complete when at least 98% of the infective dose had been expelled from the gut (47).

Skeletal muscle larva counts. Skeletal muscle larva numbers were determined by homogenizing skinned, exsanguinated mice, with a mixture of PBS, HCl, and pepsin in a kitchen blender, again by a modification of the method described by Castro and Fairbairn (7). The homogenate was transferred to 1,000-ml flasks and bubbled with 95% O2-5% CO2 for 2 h. The digestate was then strained through several layers of gauze, and the worms were allowed to settle at unit gravity. After tissue digestion, the supernatant was removed, and the worms were resuspended in PBS, pH 7.4. The larvae were then washed, collected, and counted under an inverted dissecting microscope.

Muscle function. The preparation of the sections of jejunal longitudinal muscle-for-muscle contractility analysis and the analysis of the length-tension relationships have been described previously (4, 43). In brief, the jejunum was removed and placed in oxygenated (95% O2-5% CO2) Krebs solution, and 1-cm sections of whole gut were cut from the jejunum, beginning at the ligament of Treitz and proceeding 4 cm distally. Total cellular RNA was isolated with the use of a previously described guanidium isothiocyanate method (24). The concentration of RNA was determined by measuring absorbance at 260 nm, and its purity was confirmed with the use of the ratio of absorbency at 260 nm to that at 280 nm. RNA was stored at −70°C until used for RT-PCR. mRNA was then reverse transcribed as previously described to yield cDNA. Aliquots (2 µl) of cDNA (0.1 µg) were then mixed with 20 pmol of sense (5'-ATG AGA AGG ATG CTT CTG CAC TTG-3') and anti-sense (5'-GGT TCA ACT TGC GGT CAT CTT AGG C-3') primers for IL-5 (5, 37). The positive control used in the PCR reaction was the housekeeping gene hypoxanthine phosphoribosyl transferase (HPRT), and 20 pmol of each sense primer (5'-GGT GGA TAC AGG CCA GAC TTG G-3') and anti-sense primer (5'-GAT TCA ACT TGC GGT CAT CTT AGG C-3') were used to detect it (37, 38). PCR was performed in 50-µl volumes containing dNTP (200 µM), Mg2+ (1.5 mM), and Taq polymerase (2.5 units; Gibco BRL, Burlington, ON) with corresponding buffer and distilled water. IL-5 and HPRT were coamplified for 45 cycles with the use of the following cycle parameters: denaturation, 94°C for 30 s; annealing, 55°C for 30 s; and extension, 72°C for 60 s. PCR products were loaded onto a 2.5% agarose gel and then visualized after ethidium bromide staining. The 366-bp product corresponds to IL-5, and the 164-bp product corresponds to HPRT.

Histology, tissue eosinophil, and peripheral basophil counts. Mice were killed and jejunal tissue, skeletal muscle from the hind legs, and tissue from the diaphragm were fixed in 10% neutral buffered Formalin. Sections (3 µm) were cut, and sections were stained with hematoxylin-eosin or were stained with Congo red and lightly counterstained with hematoxylin. Intestinal tissue eosinophil counts were performed by counting the number of polymorphonuclear leukocytes that stained intensely with Congo red, found within 20 villus crypt units (vcu), including the underlying muscle, per mouse (53). The number of eosinophils was then referred to as eosinophils per vcu. Photomicrographs were taken with a Zeiss camera. Basophils in the peripheral blood were also counted, with mice periodically bled from the tail vein, and the resulting peripheral blood smears stained with Diff Quik (Dade Behring, Newark, DE) for differential cell counts. Granulocytes possessing large dark-staining granules (42) were counted, with a minimum of 350–450 leukocytes examined per slide.

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Muscle function. The preparation of the sections of jejunal longitudinal muscle-for-muscle contractility analysis and the analysis of the length-tension relationships have been described previously (4, 43). In brief, the jejunum was removed and placed in oxygenated (95% O2-5% CO2) Krebs solution, and 1-cm sections of whole gut were cut from the jejunum, beginning at the ligament of Treitz and proceeding distally. The lumen of each segment was flushed with Krebs buffer before the insertion of short (2–3 mm) lengths of Silastic tubing (0.065 in. OD, 0.030 in. ID; Dow Corning, Midland, MI) into the open ends of the gut segments. Tubing was then tied in place with surgical silk. The insertion of the tubing was found to maintain patency of the gut segments over the course of the experiments. Segments were then hung in the longitudinal axis and attached at one end to a Grass FT03C force transducer (Grass, Quincy, MA), and responses were recorded on a Grass 7D polygraph. Tissues were equilibrated
for 30 min at 37°C in Krebs solution, oxygenated with 95% O2-5% CO2, before starting the experiment. Experiments were then conducted to examine the length-tension characteristics of the muscle before and after infection. Segments were stretched by applying tension equivalent to 0–1,250 mg of weight, and contraction was assessed after stimulation with 1 µM carbachol (Sigma Chemical, St. Louis, MO). Initial experiments indicated that this tension range was sufficient to determine the maximal responsiveness of both control and inflamed tissues. After each application of tension, the length of the tissue and the contractile response were recorded. At the end of the experiment, the tissue was removed, blotted, and weighed, and the optimal tension (T0) and the tissue length that gave the maximum contractile response were used to calculate the cross-sectional area of the tissue.

Carbachol dose-response curve. The previously identified T0 for a tissue was applied in carbachol dose-response experiments before the addition of the first dose of carbachol. Gut segments were then exposed to noncumulative final bath concentrations of 1 nM–1 mM carbachol by addition of microliter aliquots to 20-ml tissue baths. After the maximal response to each dose was obtained, tissues were rinsed twice and equilibrated in fresh Krebs solution for 15 min before addition of the next agonist dose.

Data presentation and statistical analysis. Responses to carbachol were recorded from tracings, followed by the calculation of contractile activity, which was expressed as milligrams of tension per cross-sectional area as described previously (43). For each mouse, the mean tension was calculated from at least three segments. All the results are expressed as means ± SE, and n refers to the number of mice tested. Statistical significance was calculated with the Student’s t-test for comparison of two means or a one-way ANOVA for the comparison of three or more means. Multiple comparisons were performed with the Neuman–Keuls multiple comparison test. P < 0.05 was considered significant.

RESULTS

T. spiralis infection: macroscopic. Both strains behaved similarly after infection, during the course of which they developed the normal changes in behavior associated with T. spiralis infection, including the adoption of a hunched appearance, reduced movement, and piloerection by day 4–6 of infection. Both strains lost similar amounts of body weight (15–20%) by day 12, recovering the weight over the following days (not shown). On killing, however, it was consistently noted that tissue edema, as well as the redness of the intestinal tissue, appeared to be reduced in the IL-5 +/- mice, compared with the IL-5 +/+ mice.

IL-5 gene expression. Because IL-5 can act as an eosinophil chemoattractant, and eosinophils are seen infiltrating the muscle layers as well as the mucosa during T. spiralis infection, we wished to determine whether IL-5 was expressed within the neuromuscular layers and whether it increased during infection. To examine IL-5 expression within the enteric longitudinal muscle layer, we semiquantitatively measured IL-5 mRNA expression by performing RT-PCR on total RNA extracted from the LMMP of the jejunum from uninfected mice and IL-5 +/- mice infected 6 days before with T. spiralis. As can be seen in Fig. 1, the predicted 366-bp-sized PCR product for IL-5 was detectable in all three uninfected mice but was significantly increased in tissues from the three infected mice. Note that the housekeeping gene HPRT did not significantly change expression during infection. As expected, IL-5 mRNA was not detected in IL-5 +/- mice in the absence or presence of infection (data not shown).

Worm counts. Evaluation of the parasite load in the small intestine of T. spiralis infected IL-5 +/- and IL-5 +/- mice revealed a time-dependent decrease in worm numbers from the small bowel of both mouse genotypes over the course of a primary infection (Fig. 2). Although a larger number of worms were recovered from the small bowel of IL-5 +/- mice at both days 12 and 16 postinfection, the difference between IL-5 +/- and IL-5 +/- mice was only significant on day 16. By day 21, both IL-5 +/- and +/- mice had expelled their parasite load from the small bowel. However, a few (5–10) adult T. spiralis worms were also found in the cecum and...
colon of IL-5−/− mice but not in IL-5+/+ mice at day 21. During the infection, eosinophils identified in histological sections from IL-5+/+ mice were often seen in close proximity to T. spiralis parasites (Fig. 3A). However, parasites found in IL-5−/− mice had few inflammatory cells in their vicinity (Fig. 3B), with the eosinophils replaced by neutrophils, macrophages, and lymphocytes.

Eosinophil counts. The number of eosinophils identified microscopically, in the jejunum of IL-5−/− and +/+ mice, was evaluated per vcu both before and during a primary T. spiralis infection (see Fig. 4). Before infection, the number of intestinal eosinophils was similar in IL-5+/+ (0.8 eosinophils/vcu) and IL-5−/− (0.5 eosinophils/vcu) mice. After infection, eosinophil numbers rose significantly by day 8 (14.0 eosinophils/vcu) in the IL-5+/+ mice. After this peak, eosinophil numbers dropped as of day 12 (6.3 eosinophils/vcu) and day 16 (5.8 eosinophils/vcu) and continued to decrease until reaching 3.5 eosinophils/vcu at day 21. In contrast, only a small increase in the number of intestinal eosinophils was seen in the IL-5−/− mice over the course of the infection, with numbers ranging from 0.4 to 1.0 eosinophils/vcu.

Eosinophil distribution. During infection of IL-5+/+ mice, eosinophils made up a significant fraction of the inflammatory cells infiltrating the intestine, with eosinophils seen both in the tips of the jejunal villi as well as at the base of the villi and in the crypt regions. Eosinophils were also seen infiltrating the external muscle layers and myenteric plexus (see Fig. 3C). In contrast, the cellular infiltrate was reduced in the IL-5−/− mice and was composed predominantly of mononuclear cells, rather than granulocytes. Again, this was found both in the mucosa and in the external jejunal muscle layers (see Fig. 3D). Although an occasional eosinophil was seen infiltrating the intestine of infected IL-5−/− mice, they were extremely uncommon.

Basophil counts. We examined peripheral blood smears taken from mice both before and during infection (days 12 and 16) for the presence of basophils. Previous studies in the rat (30) showed that a peripheral basophilia was present at these times during a T. spiralis infection. We were, however, unable to identify any basophils in uninfected mice of either genotype, and basophils were still very rare.
leukocytes) during T. spiralis infection. However, similar small numbers were found in both IL-5 +/+ and IL-5 −/− mice, suggesting that mouse basophils do not require IL-5 and that they do not appear to play a prominent role in this model.

Skeletal muscle larvae counts. During infection, T. spiralis larvae migrated from the intestine to the skeletal muscle and, 40–60 days after infection, were completely encysted within their nurse cells in the skeletal muscle. Digestion of the bodies of previously infected mice recovered ~25,000 muscle larvae per body, with no significant difference found between IL-5 +/+ and IL-5 −/− mice (Fig. 5). A mixed inflammatory cell infiltrate was seen surrounding the encysted muscle larvae, containing numerous eosinophils (Fig. 6A) in IL-5 +/+ mice. The inflammatory response against the encysted larvae was smaller in IL-5 −/− mice, with eosinophils again replaced by mononuclear cells (Fig. 6B).

Muscle response to carbachol. The contractile response by jejunal longitudinal muscle to 10−6 M carbachol was similar between IL-5 −/− and wild-type mice before infection. As can be seen in Fig. 7, eight days after T. spiralis infection, there was a substantial and significant (P < 0.01) increase in the tension generated by longitudinal muscle from both IL-5 +/+ and −/− mice. Generated tension increased approximately threefold over that of controls, with no significant difference between the two mouse strains. The contractile response generated by longitudinal muscle from IL-5 +/+ mice at day 8 (2,300 mg/mm²) increased even further over control data at day 16, to 2,900 mg/mm² and decreased thereafter to 1,700 mg/mm² at day 21. In contrast, the tension obtained from the intestines of IL-5 −/− mice peaked at 2,350 mg/mm² at day 8, had already begun to decrease (2,150 mg/mm²) at day 16, and was only 1,200 mg/mm² on day 21. The tension generated by muscle from IL-5 +/+ mice was significantly greater than that obtained from the IL-5 −/− mice at both days 16 and 21. Even so, muscle contraction had not returned completely to normal in the IL-5 −/− mice on day 21, still remaining significantly elevated over that of uninfected IL-5 −/− mice.

The dose-response relationships for carbachol-induced contraction in uninfected and day 21-postinfected IL-5 +/+ and −/− mice are shown in Fig. 8. In noninfected mice, the response to carbachol was maximal between 10−5 and 10−4 M, and there was no significant difference between control IL-5 +/+ and IL-5 −/− mice in their dose response to carbachol. However, by day 21 the contraction generated by muscle from IL-5 +/+ mice was significantly greater than that generated by IL-5 −/− mice, over a dose...
range of $10^{-6}$ M to $10^{-3}$ M carbachol. The maximum response to carbachol remained at a dose range of $10^{-5}$ and $10^{-4}$ M for both mouse strains on day 21.

**DISCUSSION**

Gastrointestinal roundworm infections result in both tissue and blood eosinophilia, which develop as one arm of a tripartite host response to infection, in concert with elevated serum levels of IgE (47, 48, 50) and mucosal mast cell hyperplasia (29, 48). IL-5 is thought to be particularly important for the proliferation of eosinophils in vivo (39, 49), as well as their growth (32), particularly important for the proliferation of eosinophils in vivo (39, 49), as well as their growth (32), activation (32), and survival (35, 36). IL-5 is one of the first cytokines expressed in the intestine during nematode or helminth infections. Svetic et al. (38) described elevated IL-5 mRNA expression in the Peyer’s patches of mice during Heligmosomoides polygyrus infection, and high levels of IL-5 protein are produced by cells isolated from the mesenteric lymph nodes within a few days of a T. spiralis infection (17). The presence of infiltrating eosinophils within the jejunal neuromuscular layers raised the question of whether IL-5 expression also increased in these layers. Compared with uninfected mice, we found a significant tripling of IL-5 expression within the LMMP of wild-type C57BL/6 mice on day 6 postinfection. This was accompanied by a strong intestinal eosinophilia affecting both the muscle layers and mucosa. Thus local expression of IL-5 may be important in recruiting eosinophils to the intestinal muscle layers. Although the cellular source of the IL-5 within the muscle layers of infected mice is unclear, IL-5 can be expressed by several cell types, including eosinophils and T lymphocytes, which have been previously shown to quickly infiltrate the jejunal muscle layers during a nematode infection (12).

We also discovered that the intestinal eosinophilia that developed in IL-5+/+ mice during T. spiralis infection was completely abolished in the absence of IL-5. Only a small population of eosinophils was found within the jejunal of IL-5−/− mice throughout the infection, similar in number to that seen in uninfected mice. Thus, although IL-5 production is not a requirement for the recruitment of eosinophils to the small bowel under physiological conditions, IL-5 does appear essential for the increased production and recruitment of eosinophils to the gut during infection. The other eosinophil growth and chemotactic factors that are presumably active during the infection are thus unable to compensate in the absence of IL-5. We did not characterize the numbers of circulating eosinophils in this study because the presence of peripheral blood eosinophilia during nematode infections has been well documented, as has its abrogation in the face of IL-5 neutralization (8, 19) or deficiency (26). It is also possible that IL-5 mediates the proliferation of other inflammatory cell types, possibly contributing to the peripheral basophilia that has been documented to occur in the rat during nematode infections (30). However, mice in general, and the C57BL/6 strain in particular, appear to be unsuitable for evaluating the mechanisms of basophilia because they possess very few circulating basophils (22, 42) even during a T. spiralis infection.

In addition, we found that worm burdens were greater in the small bowel of IL-5−/− mice than in wild-type mice, but the difference was only significant at the late stages of infection (day 16). These results suggest that IL-5 and eosinophils contribute to the protective process against T. spiralis infection in the mouse, but they are not critical for its success, as worm expulsion still occurred in the absence of IL-5 and tissue eosinophilia. It is of note that there was a significant delay between the time that eosinophils infiltrated the infected bowel and the expulsion of the parasite. A significant intestinal eosinophilia was seen within the infected gut by day 8 postinfection, while the absence of IL-5 and eosinophils had no effect on worm expulsion until 8 days later. Because adult Trichinella possess a thick tegument, the delay may indicate the difficulty that even eosinophils have in damaging the parasites enough to induce their expulsion. Alternatively, it may indicate a delay between the arrival of eosinophils to the infected intestine and their activation to an antiparasitic role. Eosinophils have previously been shown to require additional activation signals, such as IgE, to effectively damage parasites in vitro (28), and significant elevations in IgE levels during T. spiralis infection take approximately 2 wk to develop (27). Curiously, no difference was found in the number of skeletal muscle larvae recovered from IL-5−/− and +/- mice. This was surprising considering the number of eosinophils found in the inflammatory infiltrate surrounding the encysted T. spiralis larvae in the IL-5+/+ mice. However, unlike the adult worms that are continually exposed to the host's immune response, the larvae are only exposed for a few hours, during their migration from the intestine, after which the larvae are protected by the nurse cells they invade and eventually by the cysts that form around them.
Intestinal neuromuscular dysfunction has already been described in rodents after infection by several gastrointestinal parasites, including Nippostrongylus brasiliensis (15), H. polygyrus (16, 41), and T. spiralis (43, 46), and studies from our laboratory have characterized longitudinal smooth muscle hypercontractility during T. spiralis infection in both rats (46) and mice (4, 43). Just as IL-5 and eosinophils appeared to have no significant effect on adult worm survival in the early stages of infection, their absence also had no impact on the induction of intestinal smooth muscle hypercontractility. Both mouse strains developed a similar threefold increase in muscle contraction on day 8 postinfection, similar in magnitude to the levels seen in other immunocompetent mouse strains during infection (43).

However, we also recently described that T. spiralis infection can lead to sustained changes in intestinal muscle function, which may persist at least 1 mo after the resolution of the infection, in certain strains of mice (4). The persistence of these changes appears to be genetically regulated and corresponds to the strength of the immune response against the parasite. C57BL/6 mice have already been well defined in terms of their immune responsiveness to T. spiralis Infection, being intermediate responders (47). Thus we suspected that the increased muscle function seen in the IL-5+/+ mice would not be as sustained as in other, stronger-responding mouse strains (4, 43). We therefore elected to study the muscle hypercontractility after 16 and 21 days of infection, rather than, as previously done, at day 28 or 42 (4). Interestingly, although the early phase of muscle contraction was similar in IL-5−/− and +/+ mice, we found that the changes in muscle function that persist in the wild-type mice, even after the infection has been resolved, were significantly reduced in the IL-5−/− mice. Recent studies examining the mechanisms of the persistent muscle dysfunction in the strong-responding NIH Swiss mouse strain found that the increased muscle function could be partially attenuated by postinfectious treatment with corticosteroids (3). This suggests that the state of muscle hypercontractility is being actively maintained through a steroid-sensitive and presumably inflammatory process. Whether this involves eosinophils and eosinophil-derived products requires further evaluation.

It should be noted that although a deficiency in IL-5 and eosinophils inhibited both the persistence of muscle hypercontractility and the expulsion of the parasites, the inhibition was only partial compared with that seen in T cell-deficient mice (44). This suggests that other cell types and other T cell-dependent factors, such as IL-4 (45), may be involved in these effects, perhaps in cooperation with IL-5. It is also interesting that roles in IL-5 in both worm expulsion and in the changes in muscle function were only seen at the late stages of infection. While this may simply indicate that both functions rely on the same mediators, it may also reflect a closer and possibly causal relationship between the two. The increased longitudinal muscle contractility is thought to contribute to increased intestinal propulsive activity during infection. Although immunologically mediated damage to the parasite may be an initial requirement, the increased propulsion in concert with water and mucus secretion into the lumen should act to dislodge the damaged parasite and flush it from the bowel. This theory is supported by the observation that nematode parasites are often damaged but alive (23, 48) when they are expelled from their hosts. An eviction rather than a neutralization of the parasite is also supported by the finding that, by day 21 postinfection, a small population of T. spiralis worms was recoverable from the cecum and colon of some infected IL-5−/− mice.

In this study we have exclusively attributed the differences in worm expulsion and muscle function to the effects of IL-5 on eosinophils. IL-5 does have other functions, including acting as a B cell growth (2, 25) and IgA-enhancing factor (52). However, IL-5−/− mice have already been shown to have normal antibody responses as well as cytotoxic T cell development during helminthic infections (26). Thus the primary activity of IL-5 during T. spiralis infections is most likely on eosinophils. Not only can it act as an eosinophil growth and differentiation factor (32, 33, 49), but it can also attract them to a site of inflammation or infection and, once there, delay their apoptosis and induce them into a more active, hypodense state (33, 35, 36, 49). If IL-5 does act through eosinophils in both worm expulsion and the maintenance of muscle hypercontractility as seen in this study, there are several possible mechanisms of action. Mediators such as major basic protein and eosinophil cationic protein, when released by eosinophils, have been shown to cause significant tissue damage (49). These plus other mediators, including leukotrienes (33, 49) and other products of the arachidonic pathway, are released by activated eosinophils and individually, or in combination, have been shown to contract muscle strips (11, 21) and kill T. spiralis larvae in vitro (1, 18, 28). Determining the specific role of these mediators in both host defense and tissue dysfunction in vivo remains a considerable challenge for the future.

Thus IL-5 appears not only to be necessary for the development of the intestinal eosinophil response to T. spiralis infection, but mice deficient in IL-5 production and the resulting eosinophil response suffered a transient delay in expelling a primary T. spiralis infection compared with their wild-type controls. Also, although IL-5 and eosinophils appear to not play any crucial role in the initiation of intestinal smooth muscle hypercontractility, they do appear to be involved in sustaining the changes in this model. These findings thus have bearing on the frequent clinical finding that functional motility disorders often follow a bout of enteric infection, persisting long after the infection has been resolved (9). This is termed postinfectious irritable bowel syndrome (9). Together these results suggest that, although the eosinophil is an important component of host defense within the gastrointestinal tract, attention should also be focused on the potential role of IL-5 and eosinophils in the pathophysiology of enteric muscle
and motility disturbances following inflammation of the gastrointestinal tract.

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Address for reprint requests and other correspondence: S. M. Collins, Rm. 4W8, McMaster Univ. Medical Centre, Hamilton, ON, Canada L8N 3Z5 (E-mail: scollins@fhs.csu.mcmaster.ca). Received 8 September 1998; accepted in final form 26 April 1999.

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