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Immunopathology of intestinal helminth infection*

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SUMMARY

The relationship between intestinal pathology and immune expulsion of gastrointestinal nematodes remains controversial. Parasite expulsion is associated with intestinal pathology in several model systems and both of these phenomena are T cell dependent. However, while immune expulsion of gastrointestinal helminth parasites is usually associated with Th2 responses, the effector mechanisms directly responsible for parasite loss have not been elucidated. In contrast, the intestinal pathology observed in many other disease models closely resembles that seen in helminth infections, but has been attributed to Th1 cytokines. We have used infection with the nematode Trichinella spiralis *in mice defective for cytokines to demonstrate that although parasite expulsion is indeed IL-4 dependent, contrary to expectations, the enteropathy is also regulated by IL-4. Furthermore, abrogation of severe pathology in iNOS deficient and TNF receptor defective animals does not prevent parasite expulsion. TNF and iNOS are therefore involved in intestinal pathology in nematode infections, apparently under regulation by IL-4 and Th2 mediated responses. Therefore, it appears that the IL-4-dependent protective response against the parasite operates by a mechanism other than merely the gross degradation of the parasite's environment brought about by the immune enteropathy. However, it remains important to elucidate the protective mechanisms involved in parasite expulsion, which are still unclear.*

Keywords *helminth parasite, IL-4, intestinal pathology, mast cell, goblet cell*

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INTRODUCTION

Gastrointestinal (GI) nematode infections in humans continue to cause significant morbidity and mortality throughout the developing world, yet the existence and nature of protective mechanisms against these parasites remain unclear. This is despite a long history of experimental studies in laboratory rodents, which, although it has provided a detailed knowledge of the immunology of protective responses against GI nematodes, has yet to define the precise mechanisms that bring about loss of worms from the gut. One theme that has run through many experimental studies is that protective responses are dependent upon the pathological changes that infection induces, first formulated in 1975 by Larsh and Race (1) in the concept of allergic inflammation. It is crucial to know whether or not this hypothesis is correct, for if vaccines against intestinal nematodes are to be developed, it will be important to ensure that they provide protection without inducing harmful pathological responses.

Nematodes occupy a variety of niches within the intestine: luminal (e.g. *Ascaris* sp., *Nippostrongylus brasiliensis);* mucosal surface (e.g. hookworms); intraepithelial (e.g. *Trichinella spiralis*, *Trichuris* sp.) tissue penetrating (e.g. *Heligmosomoides polygyrus*, Strongyles), and the pathology associated with them varies according to the particular host-parasite combination. The pathology (enteropathy) may have several causes, including direct damage from the attachment, migration/burrowing and feeding activities of the worm or secondary damage resulting from opportunistic bacterial infections, or the host's immune response to the parasite or bacteria. The relative contributions of parasite and immune response to enteropathy remain unclear in many infections, as does the requirement for intestinal inflammation in protection. The immune response may in fact provide no benefit to the host in terms of limiting parasite survival or fecundity, instead resulting in damaging pathological reactions. Thus, as summarized in Table 1, there are several scenarios; the parasite may be expelled by an immune response which is

Table 1 Possible relationships between protection and pathology in gastrointestinal helminth infection

Protection	Pathology	Example
Yes	Yes	*T. spiralis* in most strains of mice (7,28)
No	Yes	*Ascaris suum* in pigs (87)
		Hookworms in hamsters and man (88,89)
		T. spiralis-infected hamsters (90)
Yes	No	*T. muris* in high-responder mice (91,92)
		H. polygyrus in responder mice (C.E.Lawrence unpublished observation)
No	No	Low level *Trichuris* spp. in humans and low-responder mice (91–93)
		H. polygyrus in low responder mice (C.E.Lawrence unpublished observation)

temporally associated with a mucosal lesion, the parasite may survive in the face of such a reaction, the parasite may be expelled with little or no apparent pathology, or the parasite may survive without an obvious intestinal lesion. While much effort has concentrated on dissecting the protective immune response to a variety of GI nematodes, there has been relatively little attention paid to the mechanisms controlling the accompanying intestinal pathology, and whether or not this is an important and necessary component of protection.

The morbidity and mortality that is often associated with human GI helminth infections reflect in part the nutritional effects of diarrhoea and malabsorption, and the resulting malnutrition that can accentuate the effects of infection by suppressing the protective immune response as well as compromising intestinal repair (2–4). In experimental animal models, the pathology associated with infection is characterized by villus atrophy, crypt hyperplasia, goblet cell hyperplasia and infiltration of the mucosa by a variety of inflammatory cells, of which eosinophils and mast cells are prominent (5–7). A requirement for gross intestinal pathology in the expulsion of nematodes has been widely accepted not only because the two phenomena are usually concident in immunologically normal hosts, but it has been shown in concurrent infections that the expulsion of one species of nematode can bring about the loss of antigenically unrelated species (8). It is also true that, in concurrent infections, *H. polygyrus*, which is known to downregulate intestinal changes, can suppress the expulsion of *T. spiralis* (9,10). Two explanations for the association between expulsion and pathology have been proposed. First, that pathological changes create an unfavourable environment for the parasites, which are then forced from their preferred niches, or that the local inflammatory response (through increased permeability) results in increased exposure to components of the systemic immune system (11). Studies in nude, thymectomized or cyclosporin A-treated mice have established the T cell dependency of parasite expulsion and much of the accompanying intestinal pathology (6,7). Second, cell transfer studies further demonstrated that protection against gastrointestinal nematodes is mediated by $CD4^+$ T helper cells (12,13). More recently, protective responses have been associated with production of Th2 cytokines, including IL-3, IL-4, IL-5, IL-9 and IL-13 and antiparasite IgE and IgG1 antibody responses (14–17). Th1-mediated events appear unimportant or antagonistic in terms of protection (14,18,19).

The pathology associated with immune rejection of many intestinal nematodes shows similarities to a number of immunologically mediated enteropathies such as graft-vs-host disease (GvHD) and inflammatory bowel disease (IBD). Cytokine and T cell receptor-deficient mice have proved useful in determining the role of the immune response in such changes (20–23). For example, in GvHD, small intestinal damage appears to progress through a proliferative phase, comprising crypt hyperplasia, enhanced NK activity, increased expression of MHC class II antigens, mucosal mast cell (MMC) hyperplasia, lymphocytic infiltration and goblet cell hyperplasia, to a destructive phase characterized by villus atrophy, immunosupression, malabsorption and diarrhoea. However, whereas immune rejection of the majority of gastrointestinal nematodes is a Th2-dependent process, in GvHD and IBD intestinal pathology is usually associated with Th1-type cytokines and particularly TNF-α (24–27). Since many pathological features are common to parasite-induced lesions and Th1-mediated enteropathies, there may be mechanisms in common. This has been studied by using mice defective for cytokines or their receptors to investigate cytokine regulation of both immunopathology and parasite expulsion.

Our work with the nematode *T. spiralis* has shown that, consistent with previous findings, parasite expulsion is Th2- and specifically IL-4-dependent. However, contrary to expectations, enteropathy (assessed by changes in villus-crypt ratios) was regulated by IL-4 and not IFN-γ (28). The detailed kinetics of the events in intact mice were not entirely consistent with local inflammation being the sole cause of expulsion, in that resolution of the some components

of the enteropathy begins before the main phase of parasite loss. Moreover, abrogation of severe pathology in p55 TNF receptor (TNF-R1) gene deficient mice did not prevent parasite expulsion. The results of this study have two main conclusions that prompt re-examination of both the function of immunopathology in the expulsion of gastrointestinal nematode parasites and the role of IL-4 in enteropathies. First, whilst expulsion of *T. spiralis* was temporally associated with severe intestinal pathology in normal mice, immune expulsion occurred with minimal pathology in TNF-R1-deficient mice. Second, a novel role for IL-4 was indicated by its regulation of the enteropathy accompanying *T. spiralis* infection. Thus, although protection against the parasite is dependent upon an IL-4- and Th2-mediated response, the accompanying pathology is TNF-mediated but also IL-4-dependent.

In order to understand fully the relationship between protective and pathological Th2 responses in GI helminth infection, it will be necessary to identify the sources of the mediators that have been implicated in each phenomenon. One cell that may provide a link is the mucosal mast cell (MMC). Mast cells are thought to be crucial effector cells in expulsion of nematode parasites and this role has been well documented in infections with *N. brasiliensis*, *Strongyloides venezuelensis* and *T. spiralis* (29–33). However, there is also evidence suggesting that these cells are not essential for worm expulsion even in some of these species, and appear unimportant in responses against other species such as *T. muris* (34). Infection of mast cell-deficient W/W^v mice or the use of antibodies against IL-3 or IL-4, which block mastocytosis, have failed to prevent expulsion of *N. brasiliensis* (35,36). Conversely, STAT6 knockout mice do generate mucosal mastocytosis but fail to expel *N. brasiliensis* (37). Furthermore, because mast cells are a potent source of pro-inflammatory cytokines, including TNF (38–41), it may be that the eosinophilia and mastocytosis in the infected mucosa represent an immunopathologic rather than a protective response, contributing to intestinal inflammation through the production of proteinases, cytokines and other inflammatory mediators such as histamine, leukotrienes and prostaglandins. Our results support this hypothesis, because in TNF-R1-deficient mice infected with *T. spiralis*, parasite expulsion occurred with minimal pathology in the apparent absence of an intestinal mastocytosis plus a comparatively decreased IgG1 and IgG2b response. Examination of pathology and protection in other helminth infections has also suggested that Th2 responses may primarily be pathological. For example, IL-4-deficient mice infected with *Onchocerca volvulus* show reduced corneal damage (42). In murine *Schistosoma mansoni* infections where TNF-α plays a crucial role in the pathology associated with pulmonary granulomata and Th2 responses are protective, IL-4-deficient mice have significant reduced pathology (43).

The reason for the long standing association between intestinal pathology and protection against GI nematodes, and the apparent discrepancy between Th2-mediated protection and Th1-mediated pathology, can be rationalized through a central role of IL-4 in both processes. Our data (28) show that in *T. spiralis* infections pathology is regulated by IL-4, although TNF is also necessary. Interestingly, there is evidence of a role for Th2 cytokines in enteropathies of other aetiologies; elevated levels of IL-4, IL-5, IL-6 and IL-10 and decreased levels of IFN-γ and IL-2 having been demonstrated in a number of intestinal pathologies (44–46).

Another potential role for TNF in GI helminth infection is the induction of Th2 responses. Although some components of the Th2 response appear to be reduced in TNF-R1-deficient mice infected with *T. spiralis* (e.g. mucosal mastocytosis and Th2-dependent antibodies), the response appears, nonetheless, adequate to induce a protective but not pathological response. The increased crypt cell turnover in TNF-R1-deficient mice, accompanied by changes in intestinal physiology, may be sufficient to expel *T. spiralis*; indeed, it has been suggested previously that there is considerable redundancy in the mechanisms leading to worm expulsion (47,48). This emphasizes the finding that not all components of intestinal pathology are necessary for protection against *T. spiralis*, but the relative roles of TNF and IL-4 and the mechanisms regulated by each require further study. It is interesting to note that TNF-R1 or TNF-R2 deficient mice infected with *T. muris,* a parasite expelled by an IL-13-dependent mast cell-independent mechanism (34,49), were unable to expel the parasite and the Th2 response was substantially reduced (50). *In vivo* blockade of TNF-α although not impairing the Th2 response could also delay worm expulsion. It was therefore suggested that TNF-α played a role in regulating Th2 effector activity, possibly by regulating IL-4 and IL-13 receptor expression on cells in the intestinal microenvironment.

TNF may also mediate its pathological effects in GI helminth infections via the production of inducible nitric oxide synthase (iNOS), as has been shown in other parasite infections (51). Neutralization of iNOS in other models of intestinal pathology can ameliorate enteropathy (52,53) and it has been demonstrated that NO can protect against a number of helminth infections (54–56). Interestingly, it has recently been demonstrated that NO can inhibit mast cell activation, possibly through the induction of expression of c-kit (57). Preliminary studies in which iNOS-deficient mice were infected with *T. spiralis* (58) indicate that, similar to TNF, iNOS is involved in the pathogenic but not protective response to *T. spiralis*.

In contrast to other enteropathies, there is no evidence that IFN-γ plays an important role in either protection or pathology in *T. spiralis* infections. Infections in mice treated with anti-IFN-γ antibody or in IFN-γR-deficient mice (28,59) were comparable in time course and pathology to those in controls. Immunodepletion of IFN-γ in SCID mice infected with *T. muris* reduced the degree of crypt hyperplasia although, because the parasites are not expelled in these mice, no assessment of effects on protection could be made (60). It was not shown whether these effects were directly or indirectly mediated via a decrease in the levels of iNOS or TNF.

Despite our experiments with TNF-R1 deficient mice showing that severe IL-4 regulated enteropathy is not required for immune expulsion of *T. spiralis*, it is still conceivable that Th2 cytokines are acting in a direct fashion to create an environment unfavourable for intestinal parasites; however, it remains to be directly shown whether these effects are sufficient to expel parasites. There is considerable evidence to support a variety of pathophysiological effects of IL-4 and/or TNF on the gut. These effects may be mediated by factors including cytokines and mast cells products such as leukotrienes and 5-hydroxytryptamine. Mast cell products have also been shown to induce epithelial injury at villus tips and this may be one mechanism by which villus atrophy is induced (61) (the absence of a mastocytosis in TNF-R1 and IL-4 deficient animals may explain why there is no villus atrophy despite a crypt hyperplasia in TNF-R1 deficient mice). *T. spiralis* infections result in increased fluid and mucus secretion into the lumen as well as increased intestinal propulsive activity and more rapid intestinal transit (62–67). The increased contractility of radial and longitudinal muscle is greater in high-than slow-responder mice and is ameliorated in SCID and $CD4^+$ deficient mice, suggesting a role for $CD4^+$ T lymphocytes. More recently, it has been suggested that both IL-4 and IL-5 may play a role in the intestinal neuromuscular dysfunction in *T. spiralis* infection (68,69). These alterations have also been shown to be mediated by TNF and iNOS in other models of intestinal motility dysfunction (53,70). However, these changes may be independent of the hyperplasia and hypertrophy of the muscularis, since we have observed no substantial differences in the thickening of the muscularis in infected wild-type, TNF-R1-or IL-4-deficient mice (28). The effects of infection on smooth muscle contractility in IFN-γR, TNF-R1, IL-4 and iNOS gene deficient mice therefore require examination. The role of mast cells in the induction of these responses could also be determined by analysing the effects of infection on intestinal neuromuscular function in mast cell deficient W/W^v mice.

Increased numbers of goblet cells (GC) and qualitative changes in mucus secretion follow infection with a number of nematode parasites and it has been proposed that mucin proteins mediate this response by enveloping the parasites and/or interrupting adhesion (71). However, the role of GC and mucus in the generation of a protective response versus its role in resolving intestinal inflammation following infection with GI nematode parasites remain unresolved. Although goblet cell hyperplasia is mediated by a $CD4^+$ Th2 immune response, it appears to be IL-4 independent. The most convincing role for GC in the expulsion of parasites is seen in *N. brasiliensis* infection. Using IL-13-deficient mice, it was demonstrated that, unlike wild-type and IL-4-deficient mice, IL-13-deficient animals fail to clear *N. brasiliensis* efficiently, despite developing a Th2 response. Importantly IL-13-deficient animals also failed to generate the goblet cell hyperplasia that is coincident with worm expulsion. It was concluded that IL-13 may induced the production of GC hyperplasia and intestinal mucus which facilitates expulsion of *N. brasiliensis* (72). However, GC do not appear to play a role in the expulsion of other nematode parasites, such as *T. spiralis,* where IL-13 is not important in expulsion of the parasite. In our studies wild-type, IFN-γR-deficient, TNF-R1-deficient and IL-4-deficient mice infected with *T. spiralis* demonstrated significant goblet cell hyperplasia and increased mucin glycoprotein (Shekels *et al*., unpublished observations). Intestinal mucin protein levels, Muc2 and Muc3 and mucin mRNA were coordinately upregulated in response to *T. spiralis* infection and may form the basis of an innate mucosal response independent of specific IFN-γ, TNF and IL-4 cytokines. Importantly, this study also demonstrated that goblet cell hyperplasia and upregulated mucin secretion are not essential components of the protective immune response to all GI helminths.

Mucins are thought to act in cooperation with trefoil proteins in the protection and repair of the epithelium (73). Trefoil factors are expressed along the GI tract and increased levels are noted near sites of inflammation and ulcerative lesions (74). Furthermore, it has been demonstrated that mouse intestinal trefoil factor may play a role in the alteration of the physicochemical nature of goblet cell mucins during *N. brasiliensis* infection (75). Thus, perhaps in GI nematode parasite infection, mucins are not aiding in the host's protective expulsion of the parasite but rather functioning in the repair of the damaged intestinal epithelium.

T cell activation in the lamina propria is associated with epithelial cell shedding and loss of villi and this has been postulated to be mediated by increased production of matrix metalloproteases (MMP), which, by degrading the lamina propria matrix, represent a major pathway by which T cells cause injury in the gut (76). Production of MMPs also facilitates movement of cells out of the vasculature into sites of inflammation and contributes substantially to the

degradation of connective tissue during inflammatory disease (77). Furthermore, MMPs are required for the release of soluble TNF-α from its membrane precursor form (78) and elevated concentrations of MMP in inflamed intestine may contribute by increasing levels of TNF in the intestinal tissue. More recently, it has been demonstrated that TNF may cause tissue injury in the gut by stimulating mucosal mesenchymal cells to secrete MMPs, as the neutralization of TNF inhibited the production of MMPs (79). Therefore, it will be pertinent to analyse MMP levels in the intestine of *T. spiralis* infected mice and the role that cytokines and mast cells play in the production of these enzymes.

The intestinal pathology seen in infections with GI parasites may not necessarily be wholly mediated by the parasite but also by populations of commensal bacteria in the gut. Disruption of epithelial integrity, either by the direct actions of the parasite feeding or invading epithelial cells, or indirectly by cytokines induced in response to the parasite infection, may permit entry of bacteria or bacterial products such as LPS to intestinal tissue. This hypothesis is supported by data in which conventionally reared (CR), antibiotic-treated, specific pathogen free or gnotobiotic pigs have been infected with *Trichuris suis* and the effects on enteropathy examined. These studies demonstrated that absence or removal of bacteria prevented pathology in the colon except in the area immediately surrounding adult worms. Furthermore, bacteria were found in large numbers in lymphoglandular complexes, enterocytes, submucosa and muscularis of CR pigs infected with *T. suis*, whereas this was not observed in normal uninfected pigs (80–82). We therefore propose that disruption of epithelial integrity by parasite infection, either by the direct actions of the parasite or indirectly by induction of a Th2 cytokines response, allows bacteria to invade tissue leading to enhanced pathology possibly through induction of the TNF and iNOS responses. However, there is also recent evidence that certain bacterial infections may ameliorate the pathological effects of GI parasite infection, mice coinfected with *Lactobacillus casei* and *T. spiralis* showed reduced enteropathy with enhanced resistance to the parasite (83). Conversely, parasite infection has also been shown to ameliorate the pathology induced by *Helicobacter felis* infection (84). Therefore the mediators of pathology in GI parasite infections may be multifactorial and thus warrant further investigation.

In conclusion, studies with a variety of genetically modified mice have shed new light on the complex relationship between the protective and pathological immune responses controlling parasite infections. TNF and NO are important components of the pathological response accompanying the expulsion of a gastrointestinal nematode parasite. In the absence of TNF-R1 or iNOS, mice do not develop the severe villous atrophy and mucosal mastocytosis which accompany infection with *T. spiralis* in wild-type controls, but this does not inhibit the ability of the animals to expel the parasite. TNF and iNOS may therefore promote parasite expulsion by promoting a Th2 response, other components of which are then effective. In biological terms, limitation of excessive TNF and/or NO production and the resulting tissue damage, without loss of protective function, would no doubt of general benefit to the host, but this option is not normally available *in vivo*. Selective inhibitors of TNF or iNOS, currently under consideration for the treatment of inflammatory bowel diseases, may prove beneficial for both Th1 and Th2-mediated enteropathies without compromising Th2 protective responses (85,86) and may therefore have potential in manipuating host responses against GI nematodes. It remains important to elucidate the protective mechanisms involved in the expulsion of GI helminths, which are still unclear. Allergic inflammation does not appear to induce parasite expulsion but may reflect the extreme activation of the immune system in the gut in response to a complex pathogen. Research into this relationship is still relevant in understanding mucosal inflammation in both parasitic and inflammatory bowel disorders.

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