The intestinal epithelium: sensors to effectors in nematode infection

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The role of the intestinal epithelium as part of the physical barrier to infection is well established alongside its central roles in food absorption, sensing nutrients, and water balance. Nematodes are one of the most common types of pathogen to dwell in the intestine. This article reviews recent data that have identified crucial roles for intestinal epithelial cells in sensing these kinds of pathogens and initiating innate responses, which qualitatively influence adaptive immune responses against them. Moreover, it is now clear that the epithelium itself—in addition to the cells that lie within it—are key to many of the protective mechanisms that result in expulsion of these large multicellular parasites from the intestine. An understanding of the IEC and intraepithelial leukocyte response is crucial to both development of mucosal vaccines, and the mechanisms that underlie the emerging use of intestinal dwelling helminths for therapeutic treatments of inflammatory and autoimmune disease.

INTRODUCTION

Nematode parasites that inhabit the mammalian gastrointestinal tract remain one of the most prevalent groups of infectious microorganisms of humans.[1] In geographical areas in which nematode parasites are endemic, immunity to infection in previously exposed individuals is associated with expression of T-helper type-2 (T_H2) cytokines, while persistent heavy infections can result in overproduction of proinflammatory cytokines and development of severe intestinal inflammation.[2–5] Murine models of intestinal nematode infection provide a powerful *in vivo* tool to interrogate the cellular and molecular basis for resistance or susceptibility to infection. Over the last 15 years, studies on a number of murine nematode infection models have shown that $CD4^+$ T_H1 cells expressing IFN-γ promote parasite persistence and host susceptibility, while resistance to infection is dependent on $CD4^+$ T_H2 cells and T_H2-associated cytokines, including IL-4, IL-9, IL-13, IL-25, and IL-33[6–17] (**Figure 1**).

Despite these advances in delineating cytokine regulation of host resistance and susceptibility, the questions of how protective anti-nematode T_H2 cytokine responses are initiated and what effector mechanisms T_H2 cytokines elicit to mediate host protection have remained two areas of intense research. This review will focus on recent studies that have identified intestinal epithelial cells (IECs) as a key cell population in anti-nematode immune responses. First, we will discuss the influence of IECs on the recognition of nematode parasites and initiation of innate immune responses that govern development of adaptive $CD4^+$ T_H2-cell responses required for host protective immunity. Second, we will highlight recent findings that identify a role for T_H2 cytokine-dependent regulation of the epithelial tissue—including IEC proliferation, differentiation and turnover—in expulsion of nematode parasites. Finally, we will highlight recent clinical studies suggesting that exposure to nematode parasites can be used in the treatment of chronic inflammatory diseases and discuss how IEC responses to nematode parasites may underlie these therapeutic effects.

INTESTINAL EPITHELIAL CELLS AND INITIATION OF ANTI-NEMATODE IMMUNE RESPONSES

Intestinal epithelial cells: sentinels in barrier immunity

IECs exhibit numerous physical and biochemical adaptations to maintain barrier function, including expression of elaborate tight junctions, actin-rich microvillar extensions, and secretion of a mucin and polysaccharide-rich glycocalyx.[18] In addition, IECs can express a battery of antimicrobial peptides, including defensins, cathelicidins, and calprotectins.[19] Most of these peptides are rich in hydrophobic and basic residues that confer amphiphilic properties, resulting in broad-spectrum antimicrobial properties. In addition to barrier function, there are numerous reports suggesting that IECs can influence innate and adaptive immune cell function. IECs express germ-line-encoded

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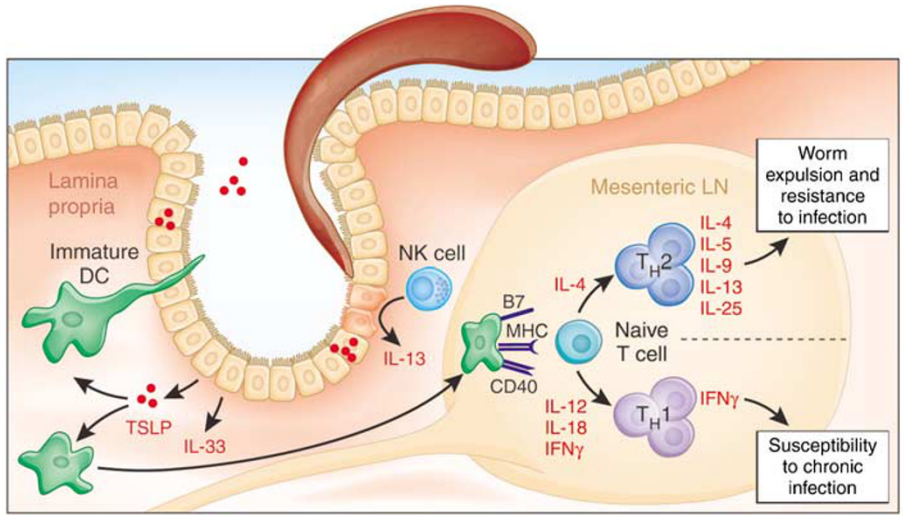

Figure 1 T_H cell-dependent regulation of resistance and susceptibility to intestinal nematode parasites. Following exposure to intestinal nematode parasites, intestinal epithelial cells express an array of immunoregulatory cytokines, including TSLP and IL-33. These factors can license intestinal DC responses potentially through regulation of their homing properties, their expression of surface molecules, including Notch ligands, and their production of proinflammatory cells. Naïve T cells are activated in the draining mesenteric lymph nodes and depending on the mouse strain, differentiate into either host protective Th2 cells that express IL-4, IL-5, IL-9, IL-13, and IL-25, or non-protective T_H1 cells that express IFN-γ.

pattern-recognition receptors, including Toll-like receptors (TLRs) and intracellular Nod-like receptors that enable microbial recognition.[20–22] Ligation of TLRs and Nod-like receptors results in activation of innate immune responses, including induction of the expression of proinflammatory cytokines and chemokines that are essential components of the anti-pathogen response. IECs express a wide range of cytokines and chemokines, including tumor-necrosis factor (TNF), transforming growth factor-β (TGF-β), IL-1, IL-6, IL-7, IL-8, IL-10, Monokine Induced by Interferon Gamma (MIG), IFN-inducible T cell α chemoattractant (ITAC), MIP-3α, CXCL9, CXCL10, CXCL11, and fracktalkine that can promote the recruitment and/or activation of immune cells. They also express major histocompatibility complex class I and class II molecules and all the machinery required for antigen processing and presentation.[23] Although their capacity to act as antigen-presenting cells remains controversial,[24–26] expression of these immune response genes supports their role as integral components of innate and adaptive anti-pathogen response in the intestinal microenvironment.

Activation of IECs following exposure to viral or bacterial pathogens has been well documented (reviewed by Shibolet and Podolsky,[27] Gewirtz,[28] Abreu *et al.*,[29] and Kelly and Conway[30]). For instance, following infection with the Gram-negative, enteropathogenic bacterium, *Salmonella typhimurium*, components of the flagellum bind to TLR5 on IECs and activate nuclear factor-κB (NF-κB).[31–33] In addition, transcription of multiple immune response genes is initiated, including cytokines and chemokines, which recruit dendritic cells (DCs) and other inflammatory cells, and initiate innate and adaptive immunity. The importance of IECs in the *in vivo* response to intestinal bacteria was highlighted in studies using bone marrow chimeric mice in which the non-hematopoietic cell compartment, including IECs, were specifically targeted. For example, IECs deficient in single immunoglobulin domain-containing IL-1R-related protein, a negative regulator of TLR/IL-1R signaling, were more susceptible to intestinal inflammation driven by inappropriate immune responses to commensal bacteria.[34–36] In addition, deletion of TLR4, Nod1, or MyD88 within non-hematopoietic cells resulted in impaired control of bacterial infections, implicating IEC-mediated recognition of bacteria as a key component of protective immunity in the gastrointestinal microenvironment.[37–39]

How do IECs respond to intestinal nematode infection?

The role of IECs in innate recognition of bacterial pathogens and development of T_H1 or T_H17 cell-dependent immunity is becoming clear. As discussed above, immunity to nematode parasites requires development of pathogen-specific T_H2 cytokine responses in the gut-associated lymphoid tissues. However, whether IECs become activated following exposure to nematode parasites, and their potential influence on nematode-induced T_H2 cytokine responses and host protective immunity, has received less attention.

Two murine models of intestinal nematode infection, *Trichuris muris* and *Trichinella spiralis*, have been used to dissect the response of IECs following exposure to infection. Although their biologies differ, these parasites share a number of common features, including the capacity to invade IECs and elicit polarized T_H2 cytokine responses in resistant strains (see **Box 1**; **Figure 2**). *In vitro* studies demonstrated that IECs become activated following exposure to nematode parasites or their products. For example, following exposure to *T. muris*-derived antigens, IEC lines exhibit activation of the NF-κB pathway and expression of major histocompatibility complex class II, that is under the control of NF-κB signaling (**Figure 3**). IEC-intrinsic NF-κB activation was also observed *in vivo* following *T. muris* infection and this was associated with elevated expression of NF-κB-dependent thymic stromal lymphopoetin (TSLP)(see below).[40] IECs are also a potent source of IL-33, which has been

Box 1 The biology of *Trichuris muris* and *Trichinella spiralis* infections

T. muris

Following ingestion, infective eggs hatch in the distal small intestine and cecum to liberate the L1 larvae. L1 larvae migrate toward the base of the crypts of Leiberkühn. Here they invade IECs and move through the epithelium in a syncytial tunnel. Thus, in one sense despite their size, these worms can be considered "intracellular" dwelling. As the parasite matures, it moves to cells higher up the crypt axis and by day 21 post infection, can be seen embedded within the epithelium at the crypt table (see **Figure 2**). The worms continue to grow and molt, becoming adult parasites by day 33 post infection. Eventually, the posterior of the male and female worms emerge free into the intestinal lumen to facilitate feeding, mating, and subsequent egg deposition.[128,152–156]

T. spiralis

The intestinal phases of infection begins on ingestion of striated muscle containing so-called "nurse cells"—modified myocytes—containing L1 larvae. The nurse cell capsule is digested under the influence of pepsin and acid in the stomach, and following activation from bile salts the freely moving L1 larvae locate and invade IECs at the base of the villi in the jejunum in a manner similar to those of *T. muris*. They move through the epithelial layer "pushing" through IECs displacing the epithelial membrane, forming a syncytial tunnel through several cells. *T. spiralis* undergoes a rapid series of four molts over a 31-hour period to become dioecious adults. These are sexually mature and mate promiscuously (which involves multiple invasions of the epithelial layer) with the female worms releasing L1 larvae into the mucosa by 5 days post infection. These L1 larvae migrate to striated muscle via lymphatics and blood circulation, locate a striated muscle fiber, and encyst, developing the nurse cell.[157,158]

IEC, intestinal epithelial cell.

termed a "Th2 accelerator".[41] IL-33 mRNA is upregulated early following *T. muris* infection and exogenous IL-33 delivery upregulates TSLP expression in the intestine of mice.[16] Similar activation of IECs has been observed in the *T. spiralis* model. A panel of human IEC lines was activated following *in vitro* infection with *T. spiralis*. Bile-activated infective larvae successfully migrated into IEC monolayers and provoked expression of IL-1β, IL-8, and epithelial neutrophil-activating peptide 78.[42] Interestingly, there was no expression of TNF or TGF-β following invasion by *T. spiralis*, suggesting that IECs are selectively activated following nematode infection. A key question regarding these findings is how nematode parasites activate IECs. It is possible that analogous to studies with bacterial and viral pathogens, IECs possess pattern-recognition machinery such as TLRs or Nod-like receptors, which could specifically recognize molecular motifs common to nematode parasites. Although helminth-derived antigens have been shown to signal through TLRs[43–45] and activate NF-κB,[45,46] no nematode-specific pattern recognition receptors have been identified to date. It is interesting to note that MyD88-null mice generate strong protective type-2 responses to *T. muris*, suggesting that this signaling pathway is not critical to protection.[47] TRIF-null mice also remain resistant to *T. muris* infection (JL Pennock, unpublished observations). However, mucosal epithelial cells express other innate recognition receptors, including C-type lectins such as dectins, that recognize β-glucans that are components of fungal pathogens,[48–51] although their functions in innate recognition of nematode parasites *in vivo* has not been examined. Alternatively, nematode parasites may indirectly activate IECs via induction of the expression of endogenous activating factors such as TNF, or through physical damage resulting from the burrowing action of the parasites that could elicit stress responses. Notwithstanding this, it appears that IECs exhibit elaborate, and perhaps specific, patterns of activation following exposure to nematode parasites.

IECs license intestinal DC function following nematode infection

An *in vivo* role for IECs in regulating anti-nematode immune responses was recently shown using IEC-specific knockout mice. IEC-intrinsic NF-κB activation through the classical pathway was blocked by Villin-Cre-mediated deletion of the gene encoding IKKβ to create mice in which IKKβ was specifically deleted in IECs (*ikk*βΔ^{IEC} mice).[40,52] Naive *ikk*βΔ^{IEC} mice exhibited no basal defects in intestinal development or function; however, disruption of NF-κB activation exclusively within IECs resulted in defective development of T_H2 cytokine responses and susceptibility to *T. muris* infection.[40] While control mice developed protective T_H2 cytokine responses, mesenteric lymph node cells isolated from infected *ikk*βΔ^{IEC} mice exhibited exaggerated production of parasite-specific IFN-γ and IL-17 that was associated with development of severe intestinal inflammation. Dysregulated $CD4^+$ T_H responses in *T. muris*-infected *ikk*βΔ^{IEC} mice was associated with aberrant DC responses in the gut-associated lymphoid tissues. Specifically, $CD11c^+$ $CD11b^+$ intestinal DCs exhibited heightened expression of TNF and IL-12/23p40,[40] suggesting IECs have the capacity to license DC responses and maintain intestinal immune homeostasis following parasitic nematode infection.

Previous *in vitro* studies implicated IECs, through secretion of cytokines, in conditioning of DCs to limit their production of proinflammatory cytokines. For example, secretion of the immunoregulatory cytokine *tslp* by IECs has been shown to influence human DC responses.[53] *tslp* is highly expressed in the epithelia at most mucosal surfaces, including the skin, airway, and the intestine. Within IECs, *tslp* mRNA is constitutively expressed and can be upregulated in response to a range of stimuli, including infection, inflammation, and tissue injury.[53–57] Related to intestinal nematode infection, *T. muris* infection of genetically resistant mouse strains results in a marked upregulation in *TSLP* expression in IECs.[40] *In vitro* studies by Rescigno and co-workers showed that IEC-derived TSLP could limit expression of IL-12 in human DCs and inhibit their capacity to promote T_H1-cell differentiation. Moreover, IEC conditioning promoted IL-10 production by activated DCs and enhanced their capacity to promote regulatory and T_H2 cytokine responses.[53,58] Consistent with these findings, TSLP-enriched culture supernatants from a murine IEC line could inhibit IL-12/23p40 expression in bone marrow-derived DCs.[40] Deletion of TSLPR in mice resulted in constitutive overexpression of IL-12/23p40 expression in intestinal DCs and the

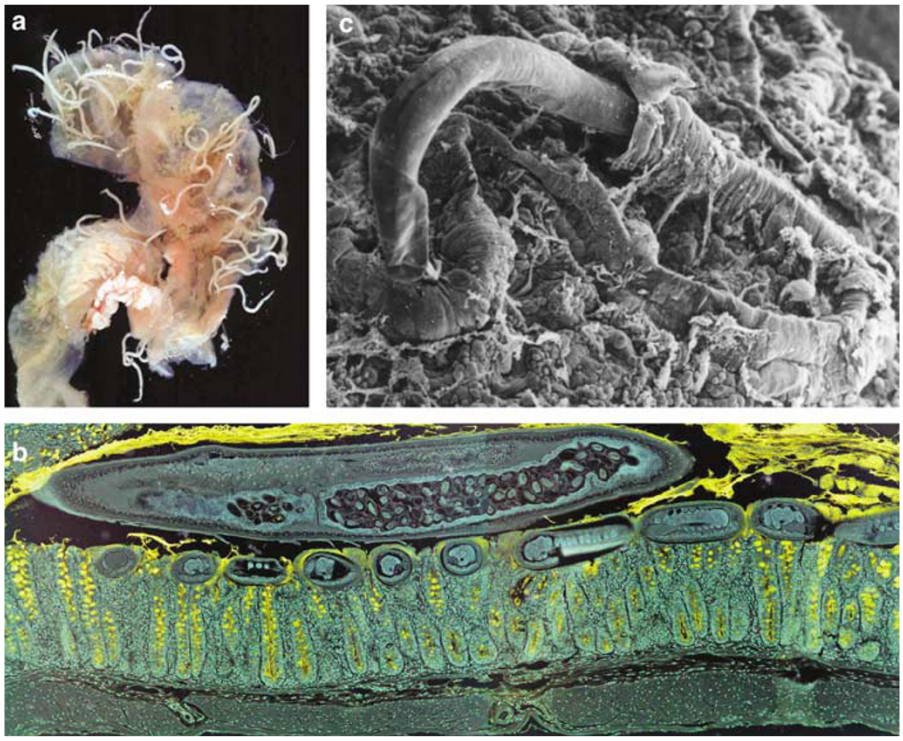

Figure 2 Intimate association of *T. muris* with host intestinal epithelial cells. Adult *T. muris* in infected mouse cecum. Mucosal surface of mouse cecum day 33 post infection (**a**). The posterior of multiple worms can be seen protruding out of the epithelial layer. Light micrograph of adult female *T. muris* day 42 post infection (**b**). Multiple transverse sections of anterior worm can be seen embedded within the epithelium at the crypt table, with transverse section of the posterior worm free in the lumen. Note eggs within the worm uterus (courtesy of N Humphreys). Scanning EM of adult worm highlighting syncytial epithelial tunnel (**c**).

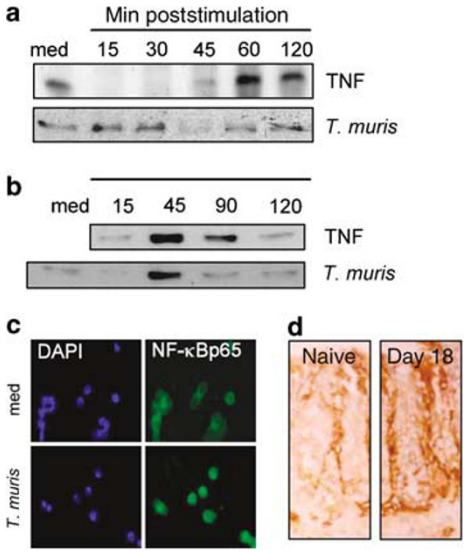

Figure 3 Intestinal epithelial cells are activated following exposure to *T. muris* . The IEC line, HT29, was stimulated with either rTNF or *T. muris* antigen, and whole-cell or nuclear extracts prepared. Whole-cell IκBα degradation (**a**) and nuclear NF-κB1 localization (**b**) were demonstrated by western blotting. Immunofluorescence also demonstrated that *T. muris* antigen induced nuclear translocation of NF-κBp65 in HT29 cells (**c**). IECs were also activated *in vivo* following *T. muris* infection, as determined by major histocompatibility complex class II expression (**d**).

inability to generate protective T_H2 cytokine responses following exposure to *T. muris*.[40] In addition to TSLP, IECs are known to express Notch receptors.[59,60] T_H2 cytokine-dependent immunity to nematode parasites is dependent on the Notch pathway;[61] however, whether there is cross-regulation between the Notch and TSLP pathways is at present unknown. Notwithstanding this, it is clear that IECs—in part via secretion of TSLP—play an essential role in regulating innate DC responses and development of protective T_H2 cytokine responses following intestinal nematode infection. IECs can also directly influence multiple cell lineages, including macrophages, granulocytes, and lymphocytes. Given recent studies that implicate mast cells, basophils, and eosinophils in the innate responses that promote expression of T_H2 cytokines,[62–67] future analysis of how IECs interact with these cell populations, and perhaps govern their functions, following nematode infection could provide new insights into how anti-nematode responses are initiated and maintained.

THE INTESTINAL EPITHELIUM: AN EFFECTOR TISSUE IN THE EXPULSION OF NEMATODE PARASITES

In addition to influencing innate immune responses following intestinal nematode infection, recent studies have highlighted the role of IECs in the effector response required for expulsion of nematode parasites. The overarching view that protective immunity to gastrointestinal dwelling nematodes is specific in

its induction, but non-specific in its action, is not new,[68] but definition of the precise mechanisms involved have taken considerable time to be elucidated. The observations that resistance to intestine-dwelling nematodes was associated with many of the hallmarks of allergic disease, led to much work exploring the role of classical allergic mechanisms in worm expulsion; but it became clear that although many facets of allergic type responses were involved, they did not necessarily operate as they did in allergic responses and indeed, other mechanisms played important roles. In particular, the role of the intestinal epithelium as an integral component of the gut's array of immune effector mechanisms is only now beginning to be appreciated. As discussed above, it is now apparent that the intestinal epithelium is much more than a simple barrier in terms of host protective immunity. It is also becoming increasingly clear that it can function as an effector tissue under the regulation of the adaptive immune system. This is either via the cells that are present within the epithelial layer – including the intra epithelial leukocytes (IEL) or indeed the epithelial cells themselves. This is particularly true of pathogens inhabiting enterocytes for a part or all of their existence. Studies employing *Trichuris* spp. and *Trichinella* spp., together with those where the parasites inhabit other intestinal niches such as the intestinal lumen, are helping to define the role of the epithelium as an immune effector tissue, identifying mechanisms that may well operate against many types of intestinal pathogen.

Unusually for parasitic nematodes, *T. spiralis* has broad host specificity and readily infects laboratory rodents. Much work has been carried out on the mouse and it is clear that the intestinal phases of infection vary in length between inbred strains of mouse, although in almost all strains worms are expelled within 14–21 days post infection.[69] The rate of worm expulsion in immunocompetent animals varies in relation to generation of adaptive immunity. Interestingly, the adult worms are not killed by the immune response. Surgical transplantation of adult parasites undergoing expulsion indicates they can recover and thrive until subsequent expulsion in the recipient.[70] Indeed, it is a consistent observation for most intestinal-dwelling nematodes that worm loss is associated with alteration of habitat making the local environment unsuitable for optimum survival rather than a direct cytotoxic activity by immune cells or secreted molecules.

The intraepithelial mast cell in resistance

The mechanisms of immune-mediated worm expulsion of *T. spiralis* have been investigated extensively over many years (reviewed by Grencis[71]). Similar to other intestinal nematodes, worm expulsion is dependent upon generation of a strong $CD4^+$ T_H-cell response in the draining mesenteric lymph node. Cytokine analysis has shown that a dominant T_H2 cytokine response is generated and influences the development of a variety of immune and immunopathological changes in the infected intestine and systemically. These include a strong peripheral IgE response, peripheral and intestinal eosinophilia, goblet cell hyperplasia, villus atrophy and crypt hyperplasia, changes in intestinal muscle contractility, and mucosal mast cell hyperplasia. Dissection of the components of this broad type-2 response have highlighted the intraepithelial mast cell as a key player in worm expulsion.[72] In mice and rats, the mast cell hyperplasia observed by traditional metachromatic staining occurs temporally alongside worm expulsion. Moreover, secretion of mucosal specific mast cell proteases (particularly mouse mast cell protease 1, MMCP1) into the intestinal tissue and circulation also occurs alongside worm expulsion.[73,74] Mast cell-deficient mice (WW^v/WW^v) show delayed worm expulsion,[75] and depletion of mast cells *in vivo* in infected mice using anti-ckit or ant-stem cell factor antibody significantly delays worm expulsion.[76,77] The use of MMCP1-null mice convincingly demonstrated that this protease played a critical role in worm expulsion, with such animals exhibiting much delayed worm loss from the intestine.[78] *T. spiralis*-infected mice also show considerable loss of epithelial integrity during the intestinal phases of infection with increased permeability.[79] Decreased intestinal epithelial cell resistance and sodium-linked glucose absorption is also observed following infection. All these changes have been shown to involve IL-4 and IL-13 operating through a STAT6-dependent pathway as have secretory response to mediators such as PGE_2.[80] The increased permeability is also associated with alteration in tight junction molecules such as occludin and the claudins.[79,81] An important role for mast cell proteases in this response was demonstrated by experiments using *T. spiralis*-infected MMCP1-null mice, which did not show increase in intestinal permeability, which was coincident with delayed worm expulsion.[79]

The intestinal mastocytosis observed during intestinal helminth infection is dependent upon the interplay between hemopoeitic growth factors such as stem cell factor and cytokines produced predominately from $CD4^+$ T_H2 cells, including IL-3, IL-4, and IL-9.[77,82–84] Interestingly, stem cell factor plays a number of different roles, including promotion of proliferation and differentiation in addition to directing migration.[85–87] It is known that mast cell precursors are released from the bone marrow following *T. spiralis* infection and express the gut homing integrin αEβ7.[87,88] The chemokine CCL2 is expressed on the epithelium of nematode-infected mice and is known to induce migration of derived mucosal like mast cells[89,90] as is leukotriene –B4.[91] Once in the epithelium, TGF-β1 is critical for activation of mast cell proteases, particularly MMCP1, and expression of the αE-subunit of αEβ7 integrin, which tethers them to the epithelium.[92–94] The inactive TGF-β1 latency-associated peptide can be activated by another epithelial expressed integrin, αVβ6. Interestingly αVβ6-null mice showed reduced migration of mast cells to the epithelium, which was coincident with delayed worm expulsion.[92,95,96] Thus, following infection movement of mast cells to the epithelium and activation by the epithelium is critical for their host protective role against nematode infection.

Although intestinal *T. spiralis* infection is associated with prominent IgE response and mast cells within the infected small intestine are IgE positive,[97] the role of IgE in host protection, particularly during primary infections, is controversial. Worm expulsion is delayed considerably in animals lacking mast cells or MMCP1,[78] although worm expulsion and mastocytosis are still evident in *T. spiralis*-infected animals that do not possess

a functional high-affinity IgE receptor.[71] *T. spiralis* infection is also accompanied by a significant increase in intestinal muscle contractility, which is controlled by T cells secreting IL-4 and IL-13 through STAT6.[98] Interestingly, IL-18, which is known to be produced in the gut, both in the epithelium and lamina propria mononuclear cells,[99] has been shown to have definite, but varied, responses on intestinal mastocytosis induced by intestinal nematode infection;[100]. It is, thus, relatively easy to appreciate the crucial role of the epithelial cell/mast cell interaction in the generation of intestinal inflammation, in rendering the interface between host and worm unsuitable for intestinal nematodes and affecting worm reproduction and survival. The altered environment in combination with the increased muscle contractility results ultimately in worm expulsion via the so-called "weep and sweep" response.

Macrophage–IEC interactions in expulsion of nematode parasites

It is also abundantly clear, however, that intestinal mast cell-driven inflammation is not effective against all species of intestine-dwelling nematodes at all stages of infection. *Heligmosomoides polygyrus* is a natural parasite of wild mice and animals become infected following ingestion of L3 larvae from the soil. Recently, Gause and co-workers[101] have elegantly shown that alternatively activated macrophages (AAMacs) can effectively control *H. polygyrus*. This has, however, to date only been demonstrated during challenge or secondary infections. Following priming, in this case by an artificially abbreviated primary infection, AAMacs "trap" the larval stages of the parasite, which undergo a short developmental period in the submucosa prior to emerging into the intestinal lumen to develop into adults that live coiled around the villi of the small intestine. This mechanism is, therefore, well suited to parasites that are not particularly active (that is, during developmental stages) or spend time in the tissue, particularly outside of the intestinal epithelium, that is, at sites where recruitment of macrophages is possible. It would be of great interest to determine whether this mechanism of immunity operates after trickle infections (repeated low-dose infections, as experienced in nature).[102] Normally, *H. polygyrus* infections are chronic in nature and a single primary infection lasts for many months in most inbred strains of mouse. It is noteworthy that a chronic primary infection of *H. polygyrus* is associated with downregulation of intestinal mast cell hyperplasia.[103] Moreover, in mice, which have artificially elevated intestinal mast cell responses, such as those overexpressing the IL-9 gene, a primary infection of *H. polygyrus* is expelled efficiently,[104] suggesting that if potent enough, mastocytosis may be effective in removing even lumen-dwelling parasites. Some strains of mouse naturally expel a primary infection of *H. polygyrus* (which can occur after several weeks),[105] and it is unlikely that the AAMac mechanism described above plays a role against the lumen-dwelling stages of infection, although their contribution in other ways to parasite loss has not been explored. Equally, the influence of the cross talk between IECs and AAMacs on the expulsion of nematode parasites awaits investigation.

Intestinal epithelial cell differentiation in expulsion of nematode parasites: the role of goblet cells

Each crypt within the intestine is an individual proliferative unit, with pluripotent stem cells located near the base of the crypt dividing by asymmetric division to give rise to a number of cell lineages, including Paneth cells, enteroendocrine cells, and goblet cells.[106,107] Goblet cells secrete mucus, trefoil peptides, and other bioactive molecules, and are a well-characterized signature of intestinal nematode infection. In the steady state, goblet cell-derived mucus is known to create a physical barrier and provide buffering function at the mucosal surfaces of the intestine. A number of studies have demonstrated that intestine-dwelling nematodes either do not establish, are impeded from doing so, or can be physically trapped in secreted mucus gel.[108,109] Mucins form the structural core of the secreted mucus gel, but can also be membrane bound. There are relatively little data concerning the functional role of mucins during intestinal nematode infection, although a number of studies have identified that Muc-2 (secreted) and Muc-3 (membrane bound) are upregulated in the small intestine following infection by *T. spiralis*.[110,111] Transcripts for Muc-2 are also upregulated in the large intestine following infection by *T. muris*[112] as is the protein (see **Figure 4**). Glycosylation changes of mucins also occur following nematode infection, although mostly described through differential histological staining of goblet cells.[113]

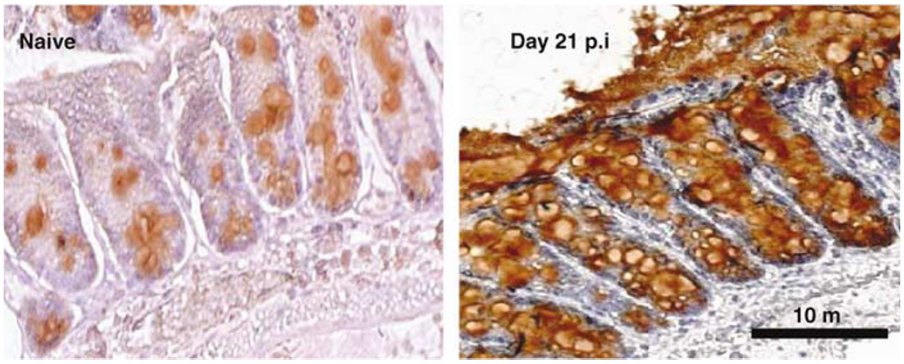

Figure 4 Elevated expression of goblet cell-derived MUC2 following exposure to *T. muris*. Muc 2 protein expression in the cecum of naïve BALB/c mice and on day 21 post infection. BALB/c mice normally expel their parasites by day 21 post infection. (courtesy of S Hussein).

How these changes relate to host protection is unclear, although infection of mice with the nematode *Strongyloides venezuelensis* suggests that recognition of carbohydrate moieties on IECs can profoundly influence where the nematodes establish themselves in the intestine.[114]

Goblet cells are also the source of a number of other molecules involved in host defense, including intestinal trefoil factor-3.[115] Its function is unclear, but a number of studies suggest a protective role in colitis[116] and control by IL-4 and IL-13.[117] It is also upregulated following infection by a number intestine-dwelling nematodes,[111] but a role in host protection is undefined. It has been suggested that intestinal trefoil factor-3 may interact with Muc-2 in the protection of the mucosa against damage.[116]

Chloride channel, calcium-activated 3 (mCLCA3, Gob5)[118] is also expressed exclusively by goblet cells and upregulated following infection by *T. muris* and *T. spiralis*,[40,111,112] although, again, its function is unclear. In a similar vein, intelectins were molecules originally described in mouse intestinal Paneth cells and mooted to have antimicrobial actions via their ability to bind a variety of non-mammalian sugars or through their ability to bind to lactoferrin and regulating its antimicrobial properties[119] (reviewed by Nair *et al.*[120]). Interestingly, intelectin-1, which shows little change in BALB/c mice upon infection in the small intestine with *T. spiralis*, is upregulated following in BALB/c mice infected with *T. muris* around the time of worm expulsion.[112,120] A variant of intelectin-1 (intelectin-2), which is undetectable in naïve BALB/c mice, is strongly upregulated during *T. spiralis* infection and secreted into the mucus gel.[121] Its protective role, if any, remains to be determined.

Goblet cells are also now known to be a source of one of the members of the resistin-like family of molecules, RELMβ (FIZZ2).[120,122–125] Expression is believed to be under the control of IL-13 and recent data have demonstrated it secretion into the intestinal lumen associated with worm loss following infection with *Nippostrongylus brasiliensis*, *T. spiralis*, and *T. muris*.[123] Rapid expression of RELMβ is also a hallmark of T_H2 memory responses following secondary challenge with *T. muris*.[126] Interestingly, the site-specific expression transcription factor Cdx2, which is involved in intestine-specific expression of RELMβ in response to commensal bacteria, is not required for expression of RELMβ in response to intestinal nematode infection.[124] The fact that RELMβ was highly upregulated in the intestine during the period of worm expulsion, implied that there may be a causal relationship. The functional activity of RELMβ has been hypothesized to act via interference with nematode sensing leading to "disorientation" of the worm, contributing to a reduced ability to maintain its niche within the intestine and reproduce effectively.[120,123,127] However, RELMβ-null mice are still able to expel *T. muris* (MG Nair and D Artis, unpublished observations), again highlighting the redundant nature of the effector responses mounted against these parasites.

The epithelial escalator: elevated IEC turnover in expulsion of intestinal nematodes

One feature that is particularly consistent throughout all protective mechanisms against intestinal nematodes is the involvement of IL-13. As highlighted above, IL-13 regulates a number of epithelial responses, including goblet cell differentiation and maturation. Recent work involving the *T. muris* system has identified a role for IL-13 in a novel effector mechanism against intestinal nematodes mediated by IECs. As described above, infection of *T. muris* in the mouse (as with all species of *Trichuris*) is entirely confined to the epithelium of the cecum and colon (see **Box 1**). At all stages of the life cycle, the parasites must maintain their optimum position within the constantly moving epithelium, and avoid being shed into the intestinal lumen alongside the normal renewal of the intestinal enterocytes.

The majority of experimental studies on *T. muris* infection have used a moderate-to-large dose of eggs to follow infection. In most inbred strains of mouse, worms are expelled as larval stages, usually before the L3 stage that occurs between days 17–21 post infection. This response is dependent upon $CD4^+$ T cells and a variety of studies have highlighted contributory roles for a variety of type-2 cytokines in the resistant response, including IL-4, IL-9, IL-10, IL-25, and IL-33, with dominant role for IL-13 (see above). Many type-2 immunopathological features accompany resistance, including elevated IgE levels, intestinal mastocytosis, goblet cell hyperplasia, and intestinal eosinophilia (reviewed by Cliffe and Grencis[128]). Worm expulsion proceeds normally in animals depleted of mast cells or eosinophils, and adoptive transfer of $CD4^+$ T cells to severe combined immunodeficient mice is sufficient to induce worm expulsion, indicating that B-cell responses and antibody are not necessary in this system.[129–132] Goblet cell hyperplasia has been correlated with resistance under the control of IL-13, and is concordant with the secretion of RELMβ as described above.[123,125]

Another feature of *T. muris* infection is a change in the proliferative capacity of the intestinal epithelium.[133] Profound changes in the rate of intestinal cell turnover occur in mice during the period of worm expulsion. In mice expelling their parasites, almost a doubling of the rate of turnover occurs, whereas in animals that do not expel their worms, only a slight elevation in turnover is apparent.[134] Most notably, in IL-13-null mice (which do not expel) the rate of turnover is similar to that seen in naturally susceptible wild-type mice. In mouse strains that naturally do not expel their parasites, a type-1 cytokine response is elicited, characterized by production of IFN-γ, IL-12, and IL-18.[13,135–137] The production of IFN-γ serves not only to counter-regulate the potential protective T_H2 response, but also increases proliferation of the intestinal epithelium and, through induction of CXCL10 in the infected intestine, slows down the turnover rate.[134] Accordingly, a slow-moving epithelium is established, presumably ideal for worm survival. Most notably, blocking CXCL10 *in vivo* raises the turnover rate, which causes worm expulsion, without changing the ongoing type-1 cytokine response normally seen in susceptible animals.[134] In this way, the intestinal epithelium itself acts as an "epithelial escalator" moving the parasite out of its optimum niche and expelling it into the lumen (**Figure 5**). Interestingly, in animals naturally harboring a chronic infection, once the crypts in the infected intestine become very large, an increase in apoptotic cells is observed in the stem cell region of the cecum and colon.

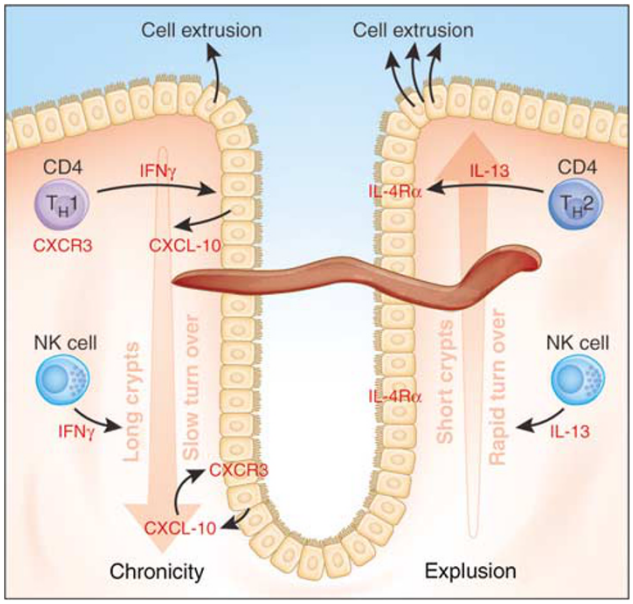

Figure 5 T_H cytokines control intestinal epithelial cell proliferation, differentiation, and turnover. During *T. muris* infection in resistant mice, IL-13 released from T_H2 cells or intraepithelial NK cells increase the turnover of cecal epithelium. Worms are unable to maintain their optimum niche within the epithelium and are extruded into the gut lumen along with epithelial cells as part of the normal tissue renewal—"the epithelial escalator". In susceptible mice, IFN-γ released from T_H1 cells (or NK cells) increases epithelial proliferation and through induction of CXCL10, slows down epithelial turnover and the parasite is provided with a slow-moving, growing epithelium, conducive to worm survival.

Again, this appears to be under immunological control of via TNF and IFN-γ.[138]

The epithelial escalator is under the control of IL-13, although whether this operates directly through epithelial cells themselves, or through other cell types such as myofibroblasts lying underneath the crypts, is unknown. Certainly, intestinal epithelial cells are known to express IL-4Rα.[139] Also, TNF-α is involved in the protective response to *T. muris* infection,[9] although its precise role remains to be defined. Recent work has suggested that TNF-α acts as a global enhancer of ongoing cytokine responses during infection.[140,141] Moreover, local cellular sources of IL-13 are yet to be defined precisely. It is known that $CD4^+$ T cells capable of adoptively transferring immunity to severe combined immunodeficient mice are required to home back to the intestine to mediate protection, and that blocking intestinal homing with antibodies prevents worm expulsion,[142] and these are a likely source of the cytokine. Recent data have, however, highlighted other populations of cells within the epithelium of nematode infected mice that may, at least in part, fulfill this role. Following infection with the related nematode *T. spiralis*, a population of intraepithelial natural killer (NK) cells become capable of producing IL-13, which mediates a number of changes, including villus atrophy crypt hyperplasia and goblet cell hyperplasia. Interestingly, this cell population is also generated in athymic and severe combined immunodeficient mice and induces similar changes to those seen in wild-type mice.[143] It is surprising that in wild-type animals other cell types ($CD3^+$, $CD4^+$, $CD8^+$ cells) present in this compartment do not appear to express IL-13 following infection. The capacity to produce IL-13 is coincident with the expression of IL-4Rα on epithelial cells. Intra-epithelial leukocytes are particularly well placed to act upon the epithelium as it moves up and around this strategically positioned cell population. The mechanism of NK cell activation during intestinal nematode infection is presently unknown. With regards to the *T. muris* system, recent work by Gause and co-workers has observed that NK cells are present in the mesenteric lymph node following *T. muris* infection and that these cells are capable of producing IL-13.[144] The role of these cells in immune protection to *T. muris* has been investigated by MR Hepworth *et al.* (unpublished observation) who has shown that NK cells play an increasingly important role as a source of protective IL-13 when the $CD4^+$ T-cell compartment is limiting. These cells appear to be distinct from basophils, which have recently been described as a key cell type producing early IL-4 (and IL-13) and helps polarize responses toward the T_H2 phenotype.[65,66] Thus, it appears that there are a variety of cell types producing IL-13 following intestinal nematode infection. A common feature of them all is that they act on IECs to induce a variety of immunological and physiological changes that ultimately are not conducive to worm survival. Taken together, studies in diverse nematode systems have demonstrated multiple T_H2 cytokine-dependent effector mechanisms, including mastocytosis, eosinophilia, and recruitment of AAMacs. In addition, there are profound changes in IEC function, including altered permeability, proliferation, turnover, and differentiation. Therefore, as discussed above, parasite-specific CD4 T-cell responses elicit nonspecific "modular" type-2 responses (**Figure 6**). Depending on the parasite in question, distinct components of this modular response have differing degrees of effectiveness in precipitating worm expulsion.

Another feature of many intestinal nematode infections is weight loss, particularly during the acute phases of infection. Intuitively, it is reasonable to suggest that this is an adaptive response for the host and the result of the combination of reduction in food intake (appetite suppression and avoidance of further infectious stages) and possibly cytokine mediated anorexia. TNF-α (cachectin) is known to be produced in response to nematode infection (see **Figure 3**),[9,140,141] although does not appear to play a significant role in the weight loss during the intestinal phase. Weight loss following infection is particularly evident following *T. spiralis* infection, with a period of hypophagia occurring during the period of worm expulsion. This is associated with an increase in enteroendocrine cell numbers in the epithelium of the small intestine and secretion of the satiety factor cholecystokinin into the circulation.[145] Remarkably, this change is mediated through $CD4^+$ T cells and secretion of IL-4 and IL-13. Again, this adds a further complexity to the battery of epithelial responses under the control of infection-induced immunity.

THE CONSEQUENCES OF INTESTINAL NEMATODE INFECTION; REGULATION—A THERAPEUTIC OPPORTUNITY?

The aforementioned discussion clearly shows that intestinal nematodes regardless of where they live within the intestine,

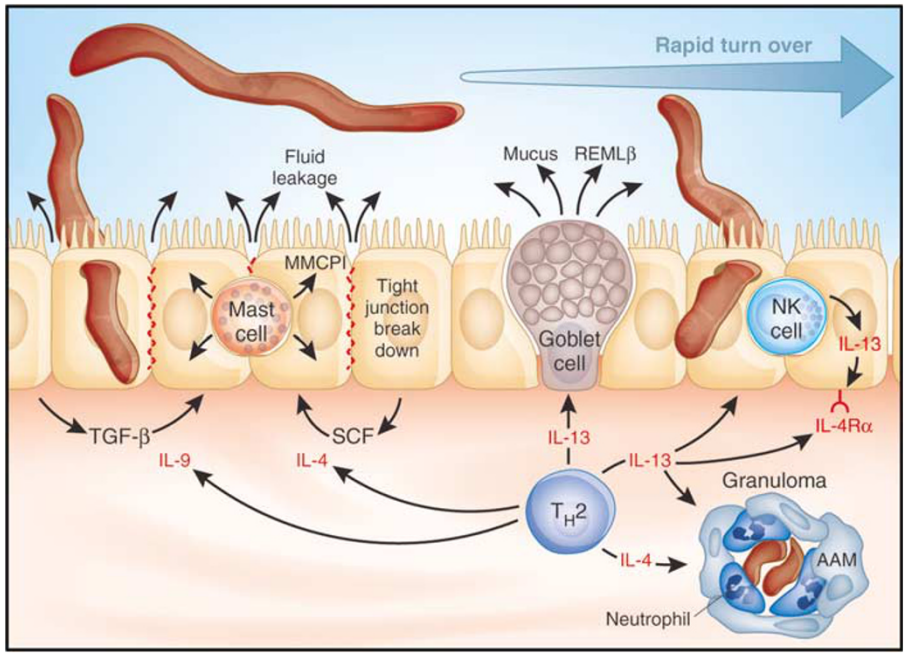

Figure 6 T_H2 cytokines control intestinal epithelial cell effector mechanisms against intestinal nematode parasites. T_H2 cells release IL-4, IL-9 (and IL-3), which act in concert with stem cell factor and TGF-β1 to cause differentiation, maturation, and activation of intraepithelial mucosal mast cells, inducing release of mmcp-1. This alters tight junction integrity, leading to increased intestinal permeability. IL-13 from T_H2 cells (and intraepithelial NK cells) induces goblet cell differentiation, expression of RELMβ, and is associated with mucus release into the gut lumen. IL-13 from T_H2 cells increases epithelial cell turnover and in conjunction with IL-4, induces alternatively activated macrophages that can "entrap" parasites within the submucosa.

induce strong innate and adaptive response—they are not the "silent serpents" they were originally thought to be. The relationship between parasite and host is an active dynamic one irrespective of whether the infection is acute or chronic. The host responds with the intention of removing the infection and generates a coordinated type-2 response comprising of a variety of effector cells and molecules. It is now appreciated that this has to extend beyond the conventional "antibody and cells" response to be effective and is, in part, no doubt due to evolution of immune evasion mechanisms employed by the parasites. It would appear that the host cannot distinguish between the different species of nematode/helminth that infects *per se*, but rather, certain signatures associated with this type of pathogen are sensed and a generic or modular T_H2 response is generated with the hope that one or more of the type-2 controlled effector mechanisms generated will be effective. The fact that under natural conditions protective immunity takes considerable time to develop, reflects the effectiveness of the immune evasion mechanisms employed by the parasite and the natural history of infection (that is, low repeated dose of infection). The potential for damage and inflammation following infection by these pathogens is, therefore, considerable and is evident in the well-documented pathology and disease that accompanies infections of this type as worldwide with estimates of 39 million DALYS associated with intestinal nematode infection.[146]

At first glance, therefore, it is somewhat paradoxical that intestine-dwelling nematodes are currently being assessed and used in clinical trials to control a variety of inflammatory diseases, including inflammatory bowel disease and some autoimmune conditions. Of particular relevance to the present review is the use of *Trichuris suis* as a therapeutic agent in the clinic.[147–149] As with all *Trichuris* species, *T. suis* inhabits the epithelial niche of the cecum and colon. The basis for its therapeutic use is that in order for the host and parasite to survive, part of the immune evasion strategy used by the parasite involves regulation of any potentially damaging intestinal pathology it induces. Indeed there is considerable evidence emerging from experimental studies of *T. muris* infection to show that without regulation, the colitis and inflammation that occurs following infection can be severe and even life threatening.[150] The cytokine IL-10 is critical to this regulatory process and is coincident with an increase in the production of regulatory T cells (NE Humphreys, unpublished observations). A detailed treatise is beyond the scope of this review, but a number of studies have shown that T regulatory cells are induced following nematode infection and can control any potential pathology associated with infection through cytokines such as IL-10 or TGF-β.[151] Moreover, these regulatory cells can influence unrelated pathological responses distant from the site of infection (Humphreys, unpublished observations). It is thus proposed that, as most species of intestinal nematodes are species specific, patent (chronic) infections of *T. suis* cannot establish in humans and only a limited and curtailed infection occurs—enough, however, to generate immunoregulatory mechanisms that can also control concurrent autoimmune or aberrant inflammation such as inflammatory bowel disease. As discussed above, IECs become activated following exposure to nematode parasites and secrete a wide range of bioactive molecules, including regulatory cytokines.

Therefore, it likely that at least a portion of nematode-induced immunoregulation in the intestine is dependent on IECs, perhaps through interactions with DCs.

The literature reviewed above clearly shows that the host epithelium responds to nematode parasite infections, particularly by invasive species such as *Trichuris*, and a vigorous, coordinated response is generated against them. This includes a battery of effector mechanisms, which vary in effectiveness depending upon the species of the nematode. That if these responses are effective and the host expels their worms via an adaptive mediated immunity, then in common with responses to other pathogens, the host is primed, establishes immunological memory, and is able to expel future challenge infections. If, however, protective immunity is not established, by whatever means (infection dose, evasion mechanisms), then the potential for host damage is considerable. Chronic infection then ensues and to prevent potentially damaging immunopathology and inflammation, regulation is required, primarily for host survival. The influence of this parasite-induced immunoregulation is not parasite specific and can influence other ongoing immune responses, including those that can cause disease. How this fits in with the current helminth therapeutic regimes being used in the clinic is unclear. One either has to speculate that repeated abbreviated infections are sufficient to induce immunoregulation—which is counter to the majority of the literature, as it should induce resistance to future infection—or that some parasites establish themselves, lasting longer than anticipated. These would potentially induce pathology, which would subsequently lead to activation of the regulatory mechanisms highlighted above. Regardless of whatever scenario, it is clear that intestine-dwelling nematodes present a considerable immunological challenge to the host intestinal immune system. They are undoubtedly pathogens, not commensals, and as such will be recognized as antigenic and potentially dangerous. As such the aim of the body is to remove them as efficiently as possible. If this does not happen, then a precarious and dynamic relationship is established between the host and parasite, with the epithelium playing a crucial role in the etiology of the infection, ultimately directing the immune response and influencing the degree of disease that ensues.

CONCLUDING REMARKS

In summary, it is clear IECs exhibit robust innate immune responses during intestinal nematode infection that can influence both the initiation and effector phases of the anti-parasite response. Given the increasing recognition of the role of IECs in eliciting and regulating intestinal immune responses against pathogens, new approaches harnessing the natural immunoregulatory properties of IECs may offer greater success in the design of oral vaccines or worm-based immunotherapies.

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DISCLOSURE

The authors declare no conflict of interest.

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